CONTENTS

Study on the Features of Universal Citizen and the Level of Attention to Its Components in the Social Studies of Primary Schools from the Viewpoint of Lesson-planning Experts, Principals and Teachers Employed in the Ministry of Education & Training at Elementary Level in Tehran, Iran

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Abstract: It is more than two decades that globalization has turned into an important political, economic, social and cultural subject in many countries. Many commentators have optimistic and positive approach to this phenomenon; whereas, many others look at it pessimistically, and some critics use the term 'globalization'. On the other hand, the concept of citizenship aims at propagating the main principles of democracy among people, so as most of these principles play vital role in the formation of a nation and strengthening the pillars of democracy. Due to the undeniable need in training citizenship, and necessity of being informed of science and technology and paying heed to this point that globalization is not a decision, but an already-occurring inevitable process; it seems indispensable to understand globalization to be able to use its advantages and avoid its deficiencies. Therefore, the aim of this research is studying on the features of universal citizen and the level of attention to its components in the social studies of primary schools from the viewpoint of lesson-planning experts, principals and teachers employed in the ministry of education & training at elementary level in Tehran during the school-year 2010-2011. The sample size is calculated based on Morgan table that consists of 427 lesson planners, principals and teachers of primary level using the hierarchal random sampling method. In the present research, three topics have been scrutinized as the components of citizenship known as the criteria of training, used in social studies textbooks in primary schools. They are 'wisdom', 'assuming responsibility', 'commitment', and 'criticism'. The methodology employed in this research is 'field finding', and a twenty-six questions questionnaire is used for data collection. Descriptive statistic methods (abundance & percentage) and deductive statistics, (chie square) are used for the analysis of the data. The research shows that the textbooks on social studies taught at levels 3, 4, and 5 in elementary schools have paid an average level of attention (and higher) to the fore-cited topics in the training of global citizens.
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Keywords: Globalization; Citizenship; Universal citizen; Social studies

1. Introduction

It can be said that globalization is the procedure of compactness of increasing time and space through which all people in the world – more or less – are almost being mixed consciously in the universal community. Mochida reminds that despite the fact that many books and articles have been written on globalization; there is still no fixed, unified and comprehensive definition on it (Mochida, 2005). In general, one can distinct five thorough definitions on globalization from each other, as stated by Mochida quoted from Scholte . They are as follows:

1. Globalization as being internationalization, emphasizing on the growth of exchange and mutual international dependence.
2. Globalization as meaning emancipation and liberation, that here, it underlines removing the restrictions imposed by the government for making an open and borderless universal economy.
3. Globalization as meaning universalization and becoming public that emphasizes on expanding various goals and experiences for all people round the world.
4. Globalization as meaning to become western or modern, mostly into American form that is a kind of dynamism on which social structures of modernity including capitalism, logicality, industrialization and bureaucracy are extended in the whole world. Consequently, native and already-existing cultures will be deteriorated in this process.
5. Globalization as meaning deterritorialization that requires a new geographical form.

According to Carnoy (1999), Tofler in his well-known book entitled, *Education and Training for Tomorrow* states: "Nowadays the existing educational systems round the world have got two significant responsibilities. First, they should make attempt to make the youth familiar to the experience gained by previous generation, so as to enrich and accumulate the experiences obtained through subsequent generations, so that they can be presented to the young citizens as priceless capitals of civilization to be used in their social and individual lives. Secondly, educational systems should train their products – that is the same students and the potential future citizens of national and international societies – in a way that they can be equipped with special knowledge and skills after graduation to be able to solve both their own problems, and also be able to challenge their issues at local, national and universal levels (Carnoy, 1999).

Tofler also believes that at the age of globalization of education and training, educational systems need to train citizens in a way to be able to present their own professional services both in their hometowns, and in other societies based on their industrial and economic needs. To achieve such a goal, Tofler believes it is indispensable for all developed or underdeveloped nations to add universal, regional and national objectives to their training aims and purposes, so that the man of future can gain adequate efficiencies and capabilities (Soudien, 2001).

One of the most important objectives is that all children enjoy primary education, so that all of them pass primary education by 2015. It is also important to disregard any racial and sexual discrimination in this regard (Debkin, 2009). The significance of education and training citizens is not merely caused by rapid social changes, extremist ideologies, racism, sectarian differences, and equalization of people psychologically, and it is not just for making people familiar to their responsibilities; but the ultimate aim of such education is to help people think liberally and move toward democracy. Education of citizens is a plan for the creation of such society in which individuals are trained to act responsibly, consciously and faithfully.

This plan is not merely restricted to the experts of humanities or just for those working in the field of educational sciences, but it also includes policy-makers and planners interested in social institutions (Kerr, 1999). Democratic values should be deeply stabilized and fixed in people's daily lives. Therefore, political democracy will not be preserved safely unless these values are permanently kept in people's minds (Dewey, 1937).European council of training democratic citizens points out that: "Education for democratic citizens is not basically inculcation of democratic forms, but expansion and reinforcement of innovative activists, upgrading the capability of their active participation and enabling them to ask question (Buttery, 2003).

One of the fundamental purposes of training citizens is enabling them to think critically to take part in political debates (Helestead & Pike, 2006). As stated by Maksakovsky, any change for globalization requires the preparedness of social and cultural grounds. Definitely, the same paradigm used in developed countries pertaining to education and training cannot be used in a culturally, socially and economically-retarded society to modify its education and training principles drastically (Maksakovsky, 2006).

Researchers have made a thorough examination regarding the education of citizens and the necessity of corresponding with the principles of such education to the universal criteria, including those in Iran: As a previous study done by Sheikhzadeh , (2008) under the title of Content Analysis of Textbooks on Theology, Civil Laws, and Persian in Grade 4 at Elementary Level with Due Consideration to the Concept of Globalization. The research proves that Persian literature, as a concept of citizenship, encourages students to thinking and meditation, more than theology and civil laws. The topic of assuming social and individual responsibility is taught in the book Civil Laws more than the two other books. Concepts of inter-cultural education are less emphasized in the textbooks of grade 4 elementary level, so it is necessary for the textbook authors to pay special attention to the foregoing concepts.

- Karamkhani, (2007&2008) made a research under the title of Content Analysis of Elementary Level Textbooks regarding the reinforcement of critical thinking as a necessity of globalization. In this research the contents of the questions in the books, Persian literature and social studies have been analyzed based on critical thinking at three grades of (3, 4, and 5) in primary schools. Unlike the students of four at elementary level who paid more attention to this skill, students of grade three were not so much interested in this regard. Generally speaking, all books in these three grades pay less attention to the training of this skill as is expected to be.
- Shahseni, (2007) analyzed the textbooks on humanities in elementary period using dialogue critical analysis technique. He presented it in form of his PhD dissertation entitled Clarification of Components of Sociability in the textbooks of Humanities in Elementary

Period in the Framework of Dialogue Critical Analysis. According to the results, religious and moral values in all books have the maximum of abundance, and that the values of globalization have been omitted from the content of the textbooks.

The components scrutinized in this research are as flows: Wisdom-basis component, loving one's country, law-basis component, assuming responsibility, commitment, participation, and criticism.

2. Material and Methods

The present research is in form of research field paying attention to the ideas of exerts in lesson planning, principals and teachers already working in elementary schools. In this study, 27 experts of lesson planning, 37 principals, and 363 teachers are involved. The sample size is calculated based on Morgan table, and is chosen according to the hierarchal random sampling. The researcher's questionnaire is used for data collection. Therefore, a questionnaire containing 26 questions is provided by the researcher, arranged based on Likert's 5 degree scale.

3. Results

3.1. The Information related to the concept of 'wisdom' are summarized in Table 1

Table 1: The range of sample selection based on job in the 'wisdom-basis' component, divided into 5 levels

		Teacher	Principal	Expert
Too many	%	1.4	2.7	0
Too many	abundance	5	1	0
Many	%	20.1	21.6	11.1
Many	abundance	73	8	3
Average	%	42.4	40.5	48.1
Average	abundance	154	15	13
Few	%	34.4	35.1	33.3
Few	abundance	125	13	9
Very few	%	1.7	0	7.4
Very few	abundance	6	0	2

Degree of freedom = 8 Chie square = 7.387 Risk of error vulnerability at level 0.05 = 0.495

Considering the fact that calculated chie square, having degree of freedom 8, at the error vulnerability level of %5 is less than chie square of the table, it can be inferred that there is no meaningful difference among the three occupational groups. According to the contents of table no. 1, approximately more than %50 of groups believe that paying heed to the 'wisdom-basis' component in social studies, as a lesson in primary school, is average and higher. On the other hand, based on the obtained results approximately 50% of the research participants also believe that enough heed to this matter is less than the average level. The finding verifies Shahseni's views upon which he believes globalization values have been omitted from the discourse of the primary school textbooks. According to the obtained average results in Table 1, additional research needs to be conducted for further significant results.

3.2 The information related to the concept of 'responsibility & commitment' are summarized in Table 2.

Considering the fact that calculated chie square, having degree of freedom 8, at the error vulnerability level of %5 is less than chie square of the table, it can be inferred that there is no meaningful difference among the three occupational groups. According to the contents of table no. 2, approximately more than %80 of groups believe that paying heed to 'assuming responsibility & commitment' in social studies, as a lesson in primary school, is average and higher.

As the findings indicate in this table, a significant number of the research participants believe that the matter has not received enough heed among the school textbooks. Unlike to Shahseni' views who believes that globalization values have been omitted from the discourse of the primary school books, the finding verifies Sheikhzadeh' views who believes that the subject of assuming individual and social responsibility in civil study textbook at primary level has received more heed compared to other books.

3.3. The Information related to the concept of 'criticism' are summarized in Table 3

Table 2: The range of sample selection based on job division in the component of 'assuming responsibility & commitment', classified into 5 levels ranging from 'very few' to 'too many'

		Teacher	Principal	Expert
Too many	%	7.4	13.5	0
Too many	abundance	27	5	0
Many	%	36.6	37.8	25.9
Many	abundance	133	14	7
Average	%	41.3	43.2	55.5
Average	abundance	150	16	15
Few	%	13.2	5.4	14.8
Few	abundance	48	2	4
Very few	%	1.4	0	3.7
Very few	abundance	5	0	1

Degree of freedom = 8 Chie square = 9.124 Risk of error vulnerability at level 0.05 = 0.332

Table 3: The range of sample selection based on job division in the component of 'criticism' classified into 5 levels ranging from 'very few' to 'too many'

		Teacher	Principal	Expert
Too many	%	5.2	2.7	0
Too many	abundance	19	1	0
Many	%	25.9	37.8	18.5
Many	abundance	94	14	5
Average	%	41.4	35.1	48.1
Average	abundance	153	13	13
Few	%	20.7	24.3	14.8
Few	abundance	75	9	4
Very few	%	6.7	0	14.8
Very few	abundance	22	0	4

Degree of freedom = 8 Chie square = 11.452 Risk of error vulnerability at level 0.05 = 0.177

Considering the fact that calculated chie square, having degree of freedom 8, at the error vulnerability level of %5 is less than chie square of the table, it can be inferred that there is no meaningful difference among the three occupational groups.

According to the contents of table no. 3, approximately more than %75 of groups believe that paying heed to the component of 'criticism' in social studies, as a lesson in primary school, is average and higher.

As the findings indicate a significant number of the research participants believe that the matter has received enough heed among social study textbooks at primary level. This is unlike to Karamkhani's views that has analyzed the findings of the study and concluded that training the critical thinking skill has received little attention in the third grade of primary school while it has received an average attention in the fourth and fifth grades. However, the entire textbooks among all three grades have paid less attention to the training of the skill than the average level.

According to the findings indicated in these three tables it seems that further related complementary studies are required to make a more significant difference among the findings of the study.

4. Discussion

The findings of research indicate that the research participants believe that textbooks of social studies in grade 3, 4, and 5 at elementary level have had the following different attention regarding the topics on training global citizens:

1. The topic of wisdom is paid less than the average attention
2. Topics such as 'loving one's country' and flexibility to 'criticism' are paid attention at an average level
3. Topics such as, 'commitment to law', 'assuming responsibility' and 'participation' are paid attention more than the average level.

These findings, unlike Shahseni's belief, prove that universal values of dialogue have not been omitted from the textbooks in primary schools. On the other hand, identical to Karamkhani's findings, this research also verifies that concepts as 'criticism' has been paid attention at an average level in such books.Moreover, the present research

substantiates and confirms Shaikhzadeh's ideas concerning responsibility and commitment who believes these two concepts have been emphasized in civil study textbooks in grade 4 elementary schools more than the other grades.

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References

1. Bottery,M. (2003). The End of Citizenship The Nation State, Treats to Its Legitimacy and Citizenship Education in the Twenty-First Century. Cambridge Journal of Education, 33 p. 116
2. Carnoy, M. (1999). Globalization and Education Reform. UNESCO, Paris, p. 48
3. Dabkain, G. (2009). Globalization and Trade in Third Worlds. Blackwell, London. p. 63
4. Dewey, J. (1937). The Public and Its Problems. Athens, Ohio: Swallow Press.
5. Grain S. (2001). Tenth World Congress of WCCES: Education Equity and Transformation, Cape Town University, Southern African, p. 32-34
6. Helestead, J. Mark & Pike, A. Mark (2006). Citizenship and Moral Education (Values in Action). Routledge, p. 38
7. Karamkhani, Z. (1387/2008). Content Analysis of Primary School Lesson Books Based on Teaching Critical Thinking Skill as a Necessity for Globalization. The Eight Conference of Iran Lesson Schedule Study, Globalization and Keeping Native the Lesson Schedule: Challenges & Opportunities.
8. Kerr, D. (1999). International Review of Curriculum and Assessment Frameworks Citizenship Education in the Curriculum Authority (QCA), p. 4
9. Maksakovsky, V. (2006). What is Hampering the Development of Our Education? The New Economy and the New Education. Journal of Russian Education & Society, p. 18-30.
10. Mochida, K. (2005). From Competition to Collaboration in Education: A Shift Away from the Neoliberal Agenda*?* Kyushu University: Research Bulletin Education, 8, p. 1-22.
11. Shahseni, Sh. (1386/2007). Clarification of Components of Sociability in the Books of Humanities in Elementary Period in the Framework of Dialogue Critical Analysis. PhD Thesis, Faculty of Training & Psychology, Shiraz University.
12. Sheikhzadeh,M. (1385/2006).The Approach of Combining Primary School Lesson Schedules with Emphasis on Critical Thinking. The Conference of Innovation in Primary Schools' Lesson Schedules. Iran Lesson Schedule Studies Society, Shiraz University.

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Occult Hepatitis B Infection among Egyptian Chronic Hepatitis C Patients and its Relation with Liver Enzymes and Hepatitis B Markers

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Abstract: Background: Hepatitis B (HBV) and hepatitis C (HCV) viruses are the most common causes of chronic liver disease. Coinfection with HBV and HCV is not uncommon among individuals in HBV endemic areas. Occult HBV (OHB) infection is characterized by detection of HBV DNA in the serum or liver tissue of patients who test negative for HBsAg. This study aimed to evaluate the frequency of OHB infection among Egyptian patients with chronic HCV infection and its relation with liver function tests and HBV markers. **Methods**: Serum of 50 chronic HCV patients who tested negative for HBsAg and anti-HBc-IgM were analyzed for liver function tests and HBV markers using micro particle enzyme immunoassay kit (Axsym), in addition to quantitative detection of HCV RNA and HBV DNA by real time PCR. **Results:** HBV-DNA was detectable in serum of 10/50 patients (20%) with a mean of 374.8 IU/ml. Among OHB positive and negative groups, anti-HBc was detected in 100% and 80% (P=0.289), anti-HBs was detected in 20% and 57.5% (P=0.034) and HBeAg was detected in 60% and 0% (P=0.000), respectively. Also, the mean level of ALT and AST showed significant elevation in OHB positive group when compared to negative group, (p=0.000 for both). There was no significant correlation between the level of HBV-DNA and the levels of ALT and AST. **Conclusions:** OHB with low serum levels of HBV-DNA was observed in 20% of chronic HCV patients in Egypt and was associated with elevation in ALT and AST. HBeAg could be a useful maker for OHB prediction but a negative result doesn't exclude OHB infection, whereas negative anti-HBc-IgG may exclude such infection.
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Key words: Occult hepatitis B, chronic HCV, liver enzymes, anti-HBc, anti-HBs, HBeAg.

1. Introduction

Hepatitis B virus (HBV) belongs to the Hepadnaviridae family of animal viruses, and its genome consists of a circular partially double-stranded DNA molecule of 3.2 kb in length which contains four overlapping reading frames that code for surface proteins (HBsAg), core proteins (HBc/HBeAg), the viral polymerase, and the transcriptional transactivator X protein (HBx) [1]. Hepatitis C virus (HCV) is classified in the Hepacivirus genus of the Flaviviridae family, and its genome is a positive-stranded RNA of 9.6 kb in length that encodes a large polyprotein that undergoes proteolytic processing by cellular and viral proteinases to generate the individual viral proteins [2]. Hepatitis B and hepatitis C viruses are the most common causes of chronic liver disease world-wide. Both viruses induce chronic hepatitis, which may progress to cirrhosis and eventually to hepatocellular carcinoma [3]. Due to shared routes of transmission, coinfection with HBV and HCV is not uncommon among individuals in HBV endemic areas [4].

Due to a lack of large scale population-based studies the exact number of HBV/HCV coinfected patients is unknown. Moreover, there may be underestimation of the true number of people with HBV/HCV coinfection as there is a well-known entity of occult HBV (OHB) infection in patients with chronic hepatitis C [5]. Occult hepatitis B infection is generally defined as the detection of HBV-DNA in the sera or tissues of subjects who have negative tests for HBsAg, with or without anti-HBc or antibody to HBV surface antigen (anti-HBs), outside the pre-seroconversion window period [6]. Occult hepatitis B virus infection is most frequently seen in patients with hepatitis B core antibody (anti-HBc) as the only HBV serological marker [7]. However, it is also reported in patients with hepatitis B surface antibody (anti-HBs) alone or even in those without any HBV serological marker [8,9].

Different scenarios of infection have been described with HBV/HCV coinfection. Some patients may be inoculated with both viruses simultaneously and will present with acute hepatitis due to both viruses. In addition, HBV superinfection in patients with chronic hepatitis C, and HCV superinfection in patients with chronic hepatitis B have both been reported [10,11].

HBV or HCV can play the dominant role, HBV and HCV can inhibit each other simultaneously and they can alternate their dominance. Both viruses have the ability to induce seroconversion of the other. The chronology of infection may have a role in determining the dominant virus. However, the overall effect appears to be HCV suppression of HBV [12].

Occult HBV has been associated with more advanced fibrosis/cirrhosis and with a poor response to interferon [5], but not in all studies. Cirrhosis is considered to be the most important risk factor for HCC. Therefore, besides having a possible direct oncogenetic effect, occult HBV infection may increase neoplastic transformation in HCV-infected patients [13]. The oncogenicity of occult HBV infection is related to the transactivating role of the HBx protein and to the ability of HBV to integrate the host genome [14,15]. Indeed, some studies have shown that HBV DNA could be detected in HBsAg-negative patients with HCC [16,17].

Many patients with chronic HCV infection have fluctuating levels of serum alanine aminotransferase (ALT); it remains unclear whether co-infection with occult HBV may contribute to liver inflammation and ALT flare [18].

The aim of this study is to evaluate the frequency of OHB infection among Egyptian patients with chronic HCV infection and its relation with liver function tests and hepatitis B markers.

2. Patients and methods

Out of 220 chronic hepatitis C patients examined, fifty Egyptian patients with HCV infection (38 males, 12 females, with mean age of 38.70 ± 9.98 yr.) were recruited from Virology Outpatient Clinic, Assiut University Hospital from April 2010-June 2010. Criteria for inclusion were (1) HCV antibody and HCV RNA positive, (2) negative HBsAg and anti-HBc IgM, and (3) absence of signs for decompensation (ascites, encephalopathy, or gastrointestinal bleeding). Patients were excluded from the study according to the following exclusion criteria: (1) evidence for coexisting liver disease; (2) previous antiviral treatment; (3) serological evidence of concurrent infection with HIV; (4) presence of hepatocellular carcinoma; (5) patients on hemodialysis; (6) severe systemic disease. The participants were subjected to detailed clinical history, full physical examination and abdominal ultrasonography. The study was approved by the Ethics Committee of Faculty of Medicine, Assiut University and informed consent was obtained from all the participants.

Sample collection, serological markers assay

Laboratory investigations were performed for every subject. Five ml of venous blood were aseptically collected in plain tubes. The samples were centrifuged within 30 minutes at 3000rpm for 10 minutes, then the serum samples were collected, divided into aliquots and stored at -70^{o}C for further analysis. Liver function tests (serum ALT, AST and albumin) were performed using a chemical analyzer Hitachi 911 (Boehringer Mannheim, Germany). Serological markers of HBV [HBsAg, anti-HBs, anti-HBc IgM and IgG, and hepatitis B e antigen (HBeAg)], anti-HCV and anti-HIV were tested using commercially available micro particle enzyme immunoassay kits (AXSYM, Abbott Laboratories, Germany).

DNA and RNA extraction: DNA was extracted from 200 µl of serum with DNA extraction kit, catalog no.57704, and RNA was extracted from 140 µl of patient serum with viral RNA Mini Kit, catalog no.52904, according to manufacturer's procedure, (QIAGEN, GmbH, Germany).

Quantitative detection of HCV RNA and HBV DNA by real time PCR: In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e. in real-time) allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run [19].

Detection of HBV DNA was performed on 7500 fast real time PCR system (Applied Biosystems) using ready to use PCR kit supplied by artus® HBV TM PCR Kit (24), Version 1, catalog no. 4506163, QIAGEN GmbH, Germany. The HBV RG/TM Master contains reagents and enzymes for the specific amplification of a 134 bp region of the hepatitis B virus genome. The amplicon is detected by measuring the FAM® fluorescence. In addition, the artus HBV TM PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an Internal Control by measuring the JOE™ fluorescence.

Detection of HCV RNA was performed on 7500 fast real time PCR system (Applied Biosynthesis) using ready to use PCR kit supplied by artus® HCV RG RT-PCR Kit 24, Version 1, catalog no. 4518263, QIAGEN GmbH, Germany. The HCV RG Master A and B contain reagents and enzymes for the reverse transcription and specific amplification of a 240 bp region of the HCV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green. In addition, the artus HCV RG RT-PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control in fluorescence channel Cycling Orange.

Statistical analysis

Data were collected and analyzed by computer program SPSS "version 17" (The Statistical Package for the Social Science Program), Chicago, USA). All data were expressed as mean ± SD and percentages. Comparisons between two groups were analyzed by Student t-test and Chi square. Correlation between studied parameters was performed by Spearman rank correlation coefficient. P value < 0.05 was considered significant.

3. Results

Out of 220 chronic hepatitis C patients examined for HBsAg and anti-HBc IgM, 50 patients were negative for both markers and were included in this study. The demographic and laboratory data of the 50 patients are shown in table 1.

Rates of hepatitis B markers and HBV-DNA in total patients (Table 2)

Anti-HBc IgG was detected in 42/50 patients (84%), anti-HBs was detected in 25/50 patients (50%) and HBeAg was detected in 6/50 patients (12%). HBV-DNA was detected in 10/50 patients (20%) and its levels ranged from 133-722 IU/ml with a mean ± SD of 374.8 ± 216.3 IU/ml. Out of the 50 studied patients, eight patients (16%) were negative for all markers, nine patients (18%) were positive only for anti-HBc IgG, 23 patients (46%) were positive for anti-HBc IgG and anti-HBs, four patients (8%) were positive for anti-HBc IgG and HBV-DNA, four patients (8%)were positive for anti.HBc IgG, HBeAg and HBV-DNA and two patients (4%) were positive for all markers. According to presence or absence of HBV-DNA, patients were classified into OHB positive (10 patients) and OHB negative (40 patients) groups.

Demographic and laboratory data of OHB positive and negative groups

As shown in Table 3, there was no significant difference in the males to females ratio and the mean age of the two groups of patients. ALT and AST levels were significantly higher in the OHB positive group (P =0.000 for both). All OHB positive patients had ALT and AST levels ≥ 40 IU/L, while in the OHB negative group ALT and AST levels ≥ 40 IU/L were only observed in 10% and 22.5% of patients, respectively. There was no significant correlation between the level of HBV-DNA and the levels of ALT (r = 0.63, p = 0.051) or AST (r = 0.585, p = 0.075).

Hepatitis B markers in OHB positive and negative groups

Anti-HBc IgG was detected in all OHB positive patients (10/10) and 80% (32/40) of OHB negative patients. There was no significant difference between the rates of anti- HBc IgG in OHB positive and negative patients (P = 0.289). Anti-HBs was detected in 20% (2/10) of OHB positive patients and 57.5% (23/40) of OHB negative patients. There was a significant difference between the rates of anti-HBs in OHB positive and negative patients (P = 0.034). HBeAg was detected in 60% (6/10) of OHB positive patients and not detected in any OHB negative patient (0/40). There was a significant difference between the rates of HBeAg in OHB positive and negative patients (P = 0.000) (Table 4).

The levels of HBV-DNA in HBeAg positive patients ranged from 212-722 IU/ml (mean ± SD = 462 ± 227.11) compared to 133-356 IU/ml (mean ± SD = 244 ± 128.17) in those negative for HBeAg (p = 0.123).

Relation between HCV-RNA levels and OHB infection

As shown in table (5), there was no significant difference between the mean level of HCV-RNA in the OHB positive and negative groups (P = 0.114). None of the OHB positive and 7.5% (3/40) of OHB negative patients had levels of HCV-RNA ≤10.000 IU/ml. HCV-RNA levels ≥ 500.000 IU/ml were observed in 20% (2/10) and 5% (2/40) of OHB positive and negative patients, respectively. There was also no significant correlation between the levels of HBV-DNA and HCV-RNA (r = 0.304, p = 0.393).

Table 1: Demographic and laboratory data of 50 HCV studied patients

Gender	
Male	38 (76%)
Female	12 (24%)
Age (years)	
Range	22- 48
Mean ± SD	38.70 ± 9.98
ALT (IU/L)	
< 40	36 (72%)
≥ 40	14 (28%)
Mean ± SD	37.54 ± 28.25
AST (IU/L)	
< 40	31 (62%)
≥ 40	19 (38%)
Mean ± SD	44.02 ± 29.87
Albmin (gm/dl)	
< 3.5	15 (30%)
≥ 3.5	35 (70%)
Mean ± SD	3.63 ± 0.60
HCV-RNA level (IU/ml)	
Range	9.000-790.000
Mean ± SD	191.105 ± 210.138

Table 2: Rates of HBV markers and HBV-DNA in total 50 studied patients

No of patients	Anti-HBc IgG	Anti-HBs	HBeAg	HBV-DNA
2 (4%)	+	+	+	+
23 (46%)	+	+	-	-
4 (8%)	+	-	+	+
4 (8%)	+	-	-	+
9 (18%)	+	-	-	-
8 (16%)	-	-	-	-
Total positive No (%)	**42 (84%)**	**25 (50%)**	**6 (12%)**	**10 (20%)**

Table 3: Demographic and laboratory data of occult hepatitis B (OHB) positive and negative patients

Criteria	OHB +ve (10 patients)	OHB –ve (40 patients)	*P* value
Gender			
Male	8 (80%)	30 (75%)	0.741
Female	2 (20%)	10 (25%)	
Age (years)			
Range	23-61	22-61	0.139•
Mean ± SD	43.75 ± 9.37	42.74 ± 9.61	
ALT (IU/L)			
< 40	0 (0%)	36 (90%)	0.000*
≥ 40	10 (100%)	4 (10%)	
Mean ± SD	78.80 ± 36.91	27.22 ± 11.95	0.000*•
AST (IU/L)			
< 40	0 (0%)	31 (77.5%)	0.000*
≥ 40	10 (100%)	9 (22.5%)	
Mean ± SD	92.60 ± 33.34	31.88 ± 10.39	0.000*•
Albumin (gm/dl)			
< 3.5	2 (20%)	13 (32.5%)	0.700
≥ 3.5	8 (80%)	27 (67.5%)	
Mean ± SD	3.75 ± 0.57	3.60 ± 0.61	0.486•

Chi-square test • Independent samples t-test * Statistical significant difference (P<0.05)

Table 4: The rates of hepatitis B markers in occult hepatitis B (OHB) positive and negative patients

Hepatitis B marker	OHB +ve (10 patients)	OHB –ve (40 patients)	*P* value
Total anti-HBc			
+ve (n=42)	10/10 (100%)	32/40 (80%)	0.289
-ve (n=8)	0/10 (0%)	8/40 (20%)	
HbeAg			
+ve (n=6)	6/10 (60%)	0/40 (0 %)	0.000*
-ve (n=44)	4/10 (40%)	40/40 (100%)	
Anti-HBs			
+ve (n=25)	2/10 (20%)	23/40 (57.5%)	0.034*
-ve (n=25)	8/10 (80%)	17/40 (42.5%)	

Chi-square test * Statistical significant difference (P<0.05)

Table 5: HCV-RNA levels in occult hepatitis B (OHB) positive and negative patients

HCV-RNA level (IU/ml)	OHB +ve (10 patients)	OHB –ve (40 patients)
≤10.000	0 (0%)	3 (7.5%)
>10.000- 500,000	8 (80%)	35 (87.5%)
>500,000	2 (20%)	2 (5%)
Mean ±SD•	285.280 ± 290.263	167.562 ± 182.195

Chi-square test (not applicable) • Independent samples t-test (P=0.114)

4. Discussion

The frequency of OHB in HCV patients varies greatly, ranging from 0%-52% [20,21]. In our study, 20% of patients with chronic HCV had OHB. 20% of this group were females and 80% were males, comparable to 25% females and 75% males in the OHB negative group. There was no significant difference in the mean age of the two groups. Similar results were obtained by **Shavakhi *et al.*** [22] who detected OHB in 19.4% of chronic HCV patients; 25% of them were females and 75% were males. Out of the non-infected subjects, 19.3% were females and 80.7% were males. The two groups were not significantly different in respect of sex and age. **Fujiwara *et al.*** [23] have also detected HBV-DNA in 19.5% chronic HCV patients. Comparable results were reported by **Kanbay *et al.*** [24] and **Ramia *et al.*** [25] who detected HBV-DNA in 14.2% and 16.3% of HCV positive patients.

On the other hand, higher frequency was obtained by **Shetty *et al.*** [26] who detected OHB in 28% of patients with HCV cirrhosis. **Torbenson *et al.*** [27] have also detected OHB in 45% of injection drug users with chronic HCV. Lower percentage was indicated by **Ismail *et al.*** [28] who found that 6.3% of hemodialysis patients with HCV infection had occult HBV. Another study was conducted by **Emara *et al.*** [29] who detected OHB in 3.9% of Egyptian chronic HCV patients under pegylated interferon/ribavirin therapy (using COBAS® TaqMan®HBV Test) and this lower frequency was attributed to the effect of treatment on the HBV.

Occult HBV is characterized by the presence of ongoing viral replication with very low levels of viremia (<200 IU/ml), and negativity for HBsAg, while the so-called 'false' OHB with higher levels of HBV-DNA that are negative for HBsAg are usually due to the occurrence of mutations of the HBsAg sequence that may alter the recognition by some immunoassays [30]. In our study, the mean level of HBV-DNA in serum was low (374.8 ± 216.3 IU/ml). **Noborg *et al.*** [31] reported that blood HBV-DNA level in HBsAg-positive subjects was high (10^4 to 10^8 copies/mL), but in those with OHB it was below 10^2 copies/ml. This suggests that many OHB patients would be serum PCR-negative and the use of liver tissue may be more helpful in detecting OHB infection. Recent definitions of OHB infection included liver HBV DNA positivity as a prerequisite for considering OHB infection [32], but examination of liver tissue is not always applicable in clinical practice and that is why highly sensitive PCR assays with low detection limit should be used in diagnosis of OHB infection [33].

The reasons for persistence of low levels of HBV-DNA in the absence of detectable HBsAg remain largely undefined, both host and viral factors are important in suppressing viral replication and keeping the infection under control. Low levels of viral replicative activity may result from the presence of defective interfering particles or to mutations in transcription control regions or the polymerase domain leading to inefficient replication in conjunction with the discordant release of HBsAg by the hepatocytes [34]. Additional mechanisms include (i) formation of immune complexes; [35] (ii) mutations affecting the '*a*' epitope of the S gene that encodes amino acid residues within the HBsAg coding region rendering the virus undetectable; or (iii) coinfection with hepatitis delta virus or HCV resulting in downregulation of HBV replication and a reduction in antigen synthesis [36].

In standard practice, the best diagnostic test to assess liver inflammation is liver biopsy, but ALT has been used as a surrogate marker for liver inflammation. In chronic HCV patients with OHB, fluctuation of HBV-DNA might directly affect the ALT level, and such an accumulation might increase the severity of liver disease in OHB infected patients [37]. In this study, ALT and AST levels ≥40 IU/L were detected in all OHB positive patients compared to 10% and 22.5% of the OHB negative group, respectively. The mean levels of ALT and AST were significantly higher in the OHB positive group (P =0.000 for both). In accordance with our results, **Shavakhi *et al.*** [22] found that OHB was observed in a considerable number of HCV patients in Tehran and was associated with elevation in liver enzymes. Serum AST and ALT were higher in patients with OHB and this finding may have clinical implication. In some studies, there was an elevation in transaminase in OHB patients, and it was associated with progression to cirrhosis [38,21]. However, in other studies on HCV patients with OHB, no elevation in ALT and ALT was found, and surprisingly, histological changes and cirrhosis in the OHB group were the same as HCV only group [39-41]. **Selim *et al.*** [18] have also found that HBV DNA was detected in 13.3% of patient with normal or slightly high ALT, while in those with ALT flare, HBV DNA was detected in 63.3% of patients (p<0.001). They have concluded that presence of OHB, with its added deleterious effect, must always be considered in chronic HCV patients, especially those with flare in liver enzymes.

As regard HBV markers in OHB positive and negative patients, this study shows that anti-HBs was detected in 20% of OHB positive and 57.5% of OHB negative patients (p = 0.034). Anti-HBc IgG was detected in all OHB positive and 80% of OHB negative patients (p = 0.289). Therefore, positive anti-HBc IgG can not be a predictor for OHB infection but a negative result may exclude it as none of the OHB positive group was anti-HBc IgG negative. In agreement with our findings, concerning anti-HBc, **Fujiwara *et al.*** [23], **Marusawa *et al.*** [42] and **Fukuda *et al.*** [21] found that all the occult HBV-positive patients were positive for anti-HBc. Different results were observed in other studies; **Shetty *et al.*** [26] reported that (63%) of OHB positive patients were positive for anti-HBc, whereas only 42% of patients without OHB tested positive for anti-HBc. In a study conducted by **Emara *et al.*** [29], OHB could not be predicted by serological markers of HBV infection, where only 2 out of the 6 patients with detectable HBV DNA had anti-HBc antibodies, and none had anti-HBs antibodies. **Selim *et al.*** [18] did not find any association between the presence of HBV DNA and various serology markers of HBV infection. **García-Montalvo** and **Ventura-Zapata** [43] detected anti-HBs in 62.5% of samples from OHB positive blood donors, and no significant difference was observed between HBV DNA positivity and anti-HBs levels.

Our study shows that HBeAg was detected in six patients, all of them had detectable HBV-DNA. Six of ten OHB positive patients (60%) and none of the OHB

negative patients were HBeAg positive (p = 0.000). Therefore, positive HBeAg could be a predictor for OHB infection but a negative result doesn't exclude it. **Yuan *et al.*** [44], revealed that HBeAg was not detected among 30 blood donors with OHB. **García-Montalvo** and **Ventura-Zapata** [43] detected HBeAg in 1/24 (4.16%) blood donors with OHB. These contradictory results could be attributed to the difference in studied subjects as they were blood donors and not chronic HCV patients. The positivity for HBeAg could be attributed to a superinfection in a naturally immune subject or to reactivation of a latent infection; the mutated virus had a reduced fitness and was therefore able to replicate only at low levels, resulting in a mild form of OHB infection.

In our study, the mean levels of HBV-DNA in HBeAg positive patients (462 ± 227.11) was higher (but not statistically significant) than in those negative for HBeAg (244 ± 128.17).

In this study, there was no significant difference between the mean levels of HCV-RNA in the OHB positive and negative groups (P = 0.114) and there was also no significant correlation between the levels of HBV-DNA and HCV-RNA (r = 0.304, p = 0.393). Discrepancies in results were observed in other studies. **Rodrı´guez-I´n˜igo *et al.*** [45] revealed that serum HCV RNA concentration was significantly lower in the six patients with OHB infection than in patients with chronic HCV without HBV infection. To the contrary, **Liu *et al.*** [46] reported that patients with HCV/HBV dual infection were noticed to have high HCV RNA load than those with HCV mono-infection. This seems to be applicable to genotype 4, where HBV DNA positive patients showed higher baseline HCV viral load than HCV monoinfected patients. Suppression of the dominant virus -usually HCV predominates over HBV- may be associated with flares of the non-dominant virus.

OHB with low serum levels of HBV-DNA was observed in 20% of chronic HCV patients in Egypt. It was associated with elevation in ALT and AST. HBeAg was detected in 60 % of OHB patients and negative in all OHB negative patients. Hence, it could be a useful maker for OHB prediction but a negative result doesn't exclude OHB infection, whereas negative anti-HBc-IgG may exclude such infection.

Recommendations:

Testing for HBeAg should be added to other markers that are routinely screened to detect OHB infection. In HCV patients, the likelihood of a HBV DNA test increases with elevated AST and ALT levels. Hence, screening for HBV-DNA at a time of elevated liver enzymes using highly sensitive PCR assays, with low detection limit, may be a good focal point for detection of OHB.

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References:

[1] Ganem D, and Varmus HE (1987): The molecular biology of the hepatitis B viruses, Annual Review of Biochemistry, 56:651-693.
[2] Rosenberg S (2001): Recent advances in the molecular biology of hepatitis C virus, Journal of Molecular Biology, 313:451-464.
[3] Ke-Qin KE, (2002): Occult hepatitis B virus infection and its clinical implication, Journal of Viral Hepatitis, 9:243-257.
[4] Fattovich G, Tagger A, Brollo L, Giustina G, Pontisso P, Realdi G, Alberti A, Ruol A (1991): Hepatitis C virus infection in chronic hepatitis B virus carriers, Journal of Infectious Diseases, 163 :400-402.
[5] Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G (1999): Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease, England Journal of Medicine, 341:22-26.
[6] Jafarzadeh A, Arababadi K, Mirzaee M, Pourazar A (2008): Occult Hepatitis B Virus Infection Among Blood Donors with Antibodies to Hepatitis B Core Antigen, Acta Medica Iranica, 46:27-32.
[7] Chemin I, Zoulim F, Merle P, Arkhis A, Chevallier M, Kay A, Cova L, Chevallier P, Mandrand B, Trépo C (2001): High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology, Journal of Hepatology,34 :447-454.
[8] Fukuda R, Ishimura N, Niigaki M, Hamamoto S, Satoh S, Tanaka S, Kushiyama Y, Uchida Y, Ihihara S, Akagi S, Watanabe M, Kinoshita Y(1999): Serologically silent hepatitis B virus coinfection in patients with hepatitis C virus-associated chronic liver disease: clinical and virological significance, Journal of Medical Virology, 58:201–207.
[9] Torbenson M, Thomas DL, Occult hepatitis B (2002): Lancet Infectious Diseases,2 :479–486.
[10] Liaw YF (2002): Hepatitis C virus superinfection in patients with chronic hepatitis B virus infection, Journal of Gastroenterology, 37:65-68.
[11] Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM (2004): Impact of acute hepatitis C virus superinfection in patientswith chronic hepatitis B virus infection, Gastroenterology, 126:1024-1029.
[12] Liaw YF (2001): Concurrent hepatitis B and C virus infection: Is hepatitis C virus stronger?, Journal of Gastroenterology and Hepatology, 16: 597-598.

[13] Tamori A, Nishiguchi S, Kubo S, Enomoto M, Koh N, Takeda T, Shiomi S, Hirohashi K, Kinoshita H, Otani S. (2003): Sequencing of human-viral DNA junctions in hepatocellular carcinoma from patients with HCV and occult HBV infection, Journal of Medical Virology, 69:475-481.

[14] Shiota G, Oyama K, Udagawa A, Tanaka K, Nomi T, Kitamura A, Tsutsumi A, Noguchi N, Takano Y, Yashima K, Kishimoto Y, Suou T, Kawasaki H. (2000): Occult hepatitis B virus infection in HBs antigen-negative hepatocellular carcinoma in a Japanese population: involvement of HBx and p53, Journal of Medical Virology, 62:151-158.

[15] Ding X, Park YN, Taltavull TC, Thung SN, Jin X, Jin Y, Trung NS, Edamoto Y, Sata T, Abe K (2003): Geographic characterization of hepatitis virus infections, genotyping of hepatitis B virus, and p53 mutation in hepatocellular carcinoma analyzed by *in situ* detection of viral genomes from carcinoma tissues: comparison among six different countries, Japanese Journal of Infectious Diseases, 56:12-18

[16] Tamori A, Nishiguchi S, Kubo S, Narimatsu T, Habu D, Takeda T, Hirohashi K, Shiomi S (2003) : HBV DNA integration and HBV-transcript expression in non-B, non-C hepatocellular carcinoma in Japan, Journal of Medical Virology, 71:492-498.

[17] Ohba K, Kubo S, Tamori A, Hirohashi K, Tanaka H, Shuto T, Nishiguchi S, Kinoshita H (2004): Previous or occult hepatitis B virus infection in hepatitis B surface antigen-negative and anti-hepatitis C-negative patients with hepatocellular carcinoma, Surgery Today, 34:842-848.

[18] Selim HS, Abou-Donia HA, Taha HA, El Azab GI, Bakry AF (2011): Role of occult hepatitis B virus in chronic hepatitis C patients with flare of liver enzymes, European Journal of Internal Medicine, 22:187-190.

[19] Mackay IM (2004): Real-time PCR in the microbiology laboratory, Clinical Microbiology And Infection, 10:190-212.

[20] De Maria N, Colantoni A, Friedlander L, Leandro G, Idilman R, Harig J, Van Thie D (2000):The impact of previous HBV infection on the course of chronic hepatitis C, American Journal of Gastroenterology, 95:3529-3536.

[21] Fukada R, Ishimura N, Niigaki M, Hamamoto S, Satoh S, Tanaka S, Kushiyama Y, Uchida Y, Ihihara S, Akagi S, Watanabe M, Kinoshita Y (1999): Serologically silenthepatitis B virus coinfection in patients with hepatitis C virus-associated chronic liver disease: clinical and virological significance, Journal of Medical Virology, 58:201-207.

[22] Shavakhi A, Norinayer B, Esteghamat FS, Seghatoleslami M, Khodadustan M, Somi MH, Masoodi M, Zali MR (2009): Occult hepatitis B among Iranian hepatitis C patients, Journal of Research in Medical Sciences, 14: 13-17.

[23] Fujiwara K, Tanaka Y, Orito E, Ohno T, Kato T, Sugauchi F, Suzuki S, Hattori Y, Sakurai M, Hasegawa I, Ozasa T, Kanie F, Kano H, Ueda R, Mizokami M (2004): Lack of association between occult hepatitis B virus DNA viral load and aminotransferase levels in patients with hepatitis C virus-related chronic liver disease, Journal of Gastroenterology and Hepatology, 19:1343-1347.

[24] Kanbay M, Gur G, Akcay A, Selcuk H, Yilmaz U, Arslan H, Boyacioglu S, Ozdemir FN (2006): Is Hepatitis C Virus Positivity a Contributing Factor to Occult Hepatitis B Virus Infection in Hemodialysis Patients?, Digestive Diseases and Sciences, 51: 1962- 1966.

[25] Ramia S, Sharara AI, El-Zaatari M, Ramlawi F, Mahfoud Z (2008): Occult hepatitis B virus infection in Lebanese patients with chronic Hepatitis C, Journal of Clinical Microbiology& Infectious Diseases, 27:217-221.

[26] Shetty K, Hussain M, Nei L, Reddy KR, Lok ASF (2008): Prevalence and Significance of Occult Hepatitis B in a Liver Transplant Population With Chronic Hepatitis C, Liver transplantation, 14: 534-540.

[27] Torbenson M, Kannangai R, Astemborski J, Strathdee SA, Vlahov D, Thomas DL (2004): High Prevalence of Occult Hepatitis B in Baltimore Injection Drug Users, Hepatology, 39:51-57.

[28] Ismail H, Soliman M, Ismail N (2010): Occult hepatitis B virus infection in Egyptian hemodialysis patients with or without hepatitis C virus infection, Pathology and Laboratory Medicine International, 2:113-120.

[29] Emara MH, El-Gammal NE, Mohamed LA, Bahgat MM (2010): Occult Hepatitis B Infection in Egyptian Chronic Hepatitis C Patients: Prevalence, Impact on Pegylated Interferon/Ribavirin Therapy, Virology Journal, 7:324-331.

[30] Paparella C, De Rosa F, Longo R, Cappiello G, Ursitti A, Rosa M, Morosetti M,Spanò A (2010): Appearance of HbeAg in an occult persistent hepatitis B virus infection, Intervirology,53:173-175.

[31] Noborg U, Gusdal A, Horal P, Lindh M (2000): Levels of viraemia in subjects with serological markers of past or chronic hepatitis B virus infection, Scandinavian Journal of Infectious Diseases, 32:249-252.

[32] Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M,Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D,Smedile A, Squadrito G, Trépo C, Villa

E, Will H, Zanetti AR, Zoulim F (2008): Statements from the Taormina expert meeting on occult hepatitis B virus infection, Journal of Hepatology, 49:625-657.

[33] Carreno V, Bartolome J, Castillo I, Quiroga J (2008): Occult hepatitis B virus and hepatitisC virus infections, Reviews in Medical Virology, 18:139-157.

[34] Hollinger FB (2008): Hepatitis B virus infection and transfusion medicine: science and the occult, Transfusion, 48:1001-1026.

[35] Hu KQ (2002): Occult hepatitis B virus infection and its clinical implications, Journal of Viral Hepatitis, 9:243–257.

[36] Weinberger KM, Bauer T, Bohm S, Jilg WG (2000): High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum, Journal of General Virology, 81:1165-1174.

[37] Alfonso-Urbis HJ and Que E (2009): Prevalence of occult hepatitis b infection among kidney donors with antibodies to hepatitis b core antigen, Philippines Journal of Internal Medicine, 47:203-206.

[38] Uchida T, Kaneita Y, Gotoh K, Kanagawa H, Kouyama H, Kawanishi T, Mima S (1997): Hepatitis C virus is frequently coinfected with serum marker-negative hepatitis B virus: probable replication promotion of the former by the latter as demonstratedby *in vitro* cotransfection, Journal of Medical Virology, 52:399-405.

[39] Hui CK, Lau E, Wu H, Monto A, Kim M, Luk JM, Lau GK, Wright TL (2006): Fibrosis progression in chronic hepatitis C patients with occult hepatitis B co-infection, Journal of Clinical Virology, 35:185-192.

[40] Kazemi-Shirazi L, Petermann D, Muller C, (2000): Hepatitis B virus DNA in sera and liver tissue of HBsAg negative patients with chronic hepatitis C, Journal of Hepatology, 33:785-790.

[41] Jang JY, Jeong SW, Cheon SR, Lee SH, Kim SG, Cheon YK, Kim YS, Cho YD,Kim HS, Jin SY, Kim YS, Kim BS (2011): Clinical significance of occult hepatitis B virus infection in chronic hepatitis C patients, The Korean Journal of Hepatology, 17:206-212.

[42] Marusawa H, Uemoto S, Hijikata M, Ueda Y, Tanaka K, Shimotohno K, Chiba T (2000): Latent hepatitis B virus infection in healthy individuals with antibodies to hepatitis B core antigen, Hepatology, 31:488-495.

[43] García-Montalvo BM and Ventura-Zapata LP, (2011): Molecular and serological characterization of occult hepatitis B infection in blood donors from Mexico, Annals of Hepatology, 10:133-141.

[44] Yuan Q, Ou SH, Chen CR, Ge SX, Pei B, Chen QR, Yan Q, Lin YC, Ni HY, Huang CH, Yeo AET, Shih JWK, Zhang J, and Xia NS (2010): Molecular Characteristics of Occult Hepatitis B Virus from Blood Donors in Southeast China, Journal of ClinicalMicrobiology, 38:357-362.

[45] Rodrı´guez-I´n˜igo E, Bartolome´ J, Ortiz-Movilla N, Platero C, Lo´pez-Alcorocho JM, Pardo M, Castillo I, and Carren˜o V (2005): Hepatitis C Virus (HCV) and Hepatitis BVirus (HBV) Can Coinfect the Same Hepatocyte in the Liver of Patients with Chronic HCV and Occult HBV Infection , Journal of Virology, 79:15578–15581.

[46] Liu CJ, Chen PJ, Chen DS (2009): Dual chronic hepatitis B virus and hepatitis C virusInfection, Hepatology International,3:517-525.

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Folding and Differential Equations of Some Curves in Minkowski Space

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Abstract*:* In this paper we will introduce a new connection between folding and differential equations of some curves in Minkowski space. The concept of folding on some curves in Minkowski space will be characterized by using differential equations. New types of linear ordinary differential equations are introduced. Theorems governing this connection are obtained.

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1. Introduction and definitions

As is well known, the theory of folding is always one of interesting topics in Euclidian and Non-Euclidian space and it has been investigated from the various viewpoints by many branches of topology and differential geometry [2, 3, 4, 5, 6, 8,14, 19]. Minkowski space represents one of the most intriguing and emblematic discoveries in the history of geometry. Although if it were introduced for a purely geometrical purpose, they came into prominence in many branches of mathematics and physics. This association with applied science and geometry generated synergistic effect: applied science gave relevance to Minkowski space and Minkowski space allowed formalizing practical problems[15, 16, 17, 18, 20].

Most folding problems are attractive from a pure mathematical standpoint, for the beauty of the problems themselves. The folding problems have close connections to important industrial applications. Linkage folding has applications in robotics and hydraulic tube bending. Paper folding has application in sheet-metal bending, packaging, and air–bag folding. Also, used folding to solve difficult problems related to shell structures in civil engineering and aero space design, namely buckling instability. Isometric folding between two Riemannian manifold may be characterized as maps that send piecewise geodesic segments to a piecewise geodesic segments of the same length. For a topological folding the maps do not preserves lengths, i.e. A map $\Im: M \to N$, where M and N are c^{∞} –Riemannian manifolds of dimension m, n respectively is said to be an isometric folding of M into N, iff for any piecewise geodesic path $\gamma: J \to M$, the induced path $\Im \circ \gamma: J \to N$ is a piecewise geodesic and of the same length as γ. If $\Im$ does not preserve length, then $\Im$ is a topological folding [1, 7, 9, 10, 11, 12, 13].

2. Main results

Our aim in this position is to establish types of theorems which describe different new types of folding and differential equations of some curves in three and four dimensional Minkowski space.

Let $L = p + tv$, where $p, v \in \mathbb{R}^3$ be the straight line in E_1^3, and $f_1: L \to L$ be a folding from L into itself such that $f_1(L) = (p_1 + t^* v_1, p_2 + t^* v_2, p_3 + t^* v_3)$, $a \le t^* < \infty$, the point $(p_1 + av_1, p_2 + av_2, p_3 + av_3)$ is a singular point of L or the set of points of L where f_1 fails to be differentials. The system of differential equations of this folding will be

$\dot{x} + \dot{y} + \dot{z} + k = 0, \ddot{x} + \ddot{y} + \ddot{z} = 0$

Which are defined on (a, ∞). Also, if $f_2(L) = (p_1 + t^* v_1, p_2 + t^* v_2, p_3 + t^* v_3)$, $a \le t^* \le b$, $a < b$, then, f_2 is a type of folding of $f_2(L)$ into itself with two singular points$(p_1 + av_1, p_2 + av_2, p_3 + av_3)$, $(p_1 + bv_1, p_2 + bv_2, p_3 + bv_3)$.

Moreover, if $f_3(L) = (p_1 + tv_1, p_2 + tv_2, p_3 + tv_3)$, then, f_3 is a type of folding of $f_3(L)$ into itself without singular points.

By changing the value of $t \in (-\infty, \infty)$, we have an infinite types of foldings with a singular point, with two singular points, or without singular point like f_1, $f_2$and f_3 respectively. This foldings preserve the curvature.

From the above discussion we obtain the following theorem.

Theorem 1. Let f_i be a folding from L into itself by changing in the value of $t \in (-\infty, \infty)$ then f_i, $\forall$ $i \in \mathbb{N}$, have the system of linear ordinary differential equations.

If $g: L \to L$ be the folding of L into itself such that $g(L) = (p_1 + tv_1, p_2 + t^n v_2, 0)$, $n > 1$, $n \in \mathbb{N}$, then this folding doesn't preserve the curvature $K(g(L)) \neq 0$, since $\ddot{y} \neq 0$. The system of differential equations of $g(L)$ will be

$\dot{x} + \dot{y} + \dot{z} - v_1 \cdot (y - p_2) \cdot \frac{n}{t} = 0$,
$\ddot{x} + \ddot{y} + \ddot{z} \cdot (y - p_2) \cdot \frac{n(n-1)}{t^2} = 0, \ldots,$
$x^{(n)} + y^{(n)} + z^{(n)} - n! \cdot v_2 = 0,$
$x^{(n+1)} + y^{(n+1)} + z^{(n+1)} = 0$

Thus, the above result can be formulated in the following theorem.

Theorem 2. Let $g(L) = p + t^n v$, $n > 1$, $n \in \mathbb{N}$, be a folding from L into itself and any folding homeomorphic to this type of folding, then $g(L)$ produce the $(n+1)$ - order system of ordinary differential equations.

Theorem 3. If H: $L \to L$ be the folding of L into itself such that $H(L) = p + t^{\frac{1}{n}} v$, $n > 1$, $n \in \mathbb{N}$, and any folding homeomorphic to this type of folding , then the corresponding system of ordinary differential equations is given by

$$\left.\begin{array}{l} x^{(m)} = \frac{(1-n)(1-2n)(1-3n)\cdots(1-(m-1)n)}{n^m}((x - p_1)\cdot t^{-m}) \quad y^{(m)} = \frac{(1-n)(1-2n)(1-3n)\cdots(1-(m-1)n)}{n^m}((y - p_2).t^{-m}), \\ z^{(m)} = \frac{(1-n)(1-2n)(1-3n)\cdots(1-(m-1)n)}{n^m}((z - p_3)\cdot t^{-m}) \end{array}\right\}$$

, $m \in \mathbb{N}$.

Now, we consider the following types of folding on a circle $S^1 = p + r(cost, sint, 0)$, $r \neq 0$, in 3- dimensional Minkowski space E_1^3 .

Let f_1: $S^1 \to S^1$ be a folding from S^1 into itself such that $f_1(S^1) = p + r(cost^*, sint^*, 0)$, $0 \leq t^* < \pi$, with one singular point. Also, let f_2: $S^1 \to S^1$ where $f_2(S^1) = p + r(cost^*, sint^*, 0)$, $\frac{\pi}{2} \leq t^* \leq \pi$, then f_2 is a kind of a folding from S^1 into itself with two singular points.

Again, f_3: $S^1 \to S^1$ is a folding from S^1 into itself such that $f_3(S^1) = p + r(cost, sint, 0)$, $f_3$is a folding without singular point. Then by changing in the value of $t \in (0, 2\pi)$, we get an infinite number of foldings which can be represented by this differential equations:

$\dot{x} + \dot{y} + \dot{z} - x + p_1 + y - p_2 = 0$
$\ddot{x} + \ddot{y} + \ddot{z} + x + y - p_1 - p_2 = 0$
$\dddot{x} + \dddot{y} + \dddot{z} + x - y + p_2 - p_1 = 0$
So we can state the following theorem.

Theorem 4. All types of the foldings of the circle S^1 into itself given by changing in the value of $t \in (0, 2\pi)$ have the system of linear ordinary differential equations.

Now, let g_1: $S^1 \to S^1$ where $g_1(S^1) = p + r(cos\frac{t}{n}, sin\frac{t}{n}, 0)$, $n \in \mathbb{N}$, is folding from the circle S^1 into itself. Then the system of differential equations of $g_1(S^1)$ is:

$\dot{x} = \frac{-y}{n} + \frac{p_2}{n}$, $\dot{y} = \frac{x}{n} - \frac{p_1}{n}$, $\dot{z} = 0$
$\ddot{x} = \frac{-x}{n^2} + \frac{p_1}{n^2}$, $\ddot{y} = \frac{-y}{n^2} + \frac{p_2}{n^2}$, $\ddot{z} = 0$
$\dddot{x} = \frac{y}{n^3} - \frac{p_2}{n^3}$, $\dddot{y} = \frac{-x}{n^3} + \frac{p_1}{n^3}$, $\dddot{z} = 0$
$x^{(4)} = \frac{x}{n^4} - \frac{p_1}{n^4}$, $y^{(4)} = \frac{y}{n^4} - \frac{p_2}{n^4}$, $z^{(4)} = 0$

If g_2: $S^1 \to S^1$ be a folding from S^1 into itself such that $g_2(S^1) = p + r(cos\frac{t}{n}, -sin\frac{t}{n}, 0)$, $n \in \mathbb{N}$, This folding can be represented by this linear ordinary differential equations:

$\dot{x} = \frac{y}{n} - \frac{p_2}{n}$, $\dot{y} = \frac{-x}{n} + \frac{p_1}{n}$, $\dot{z} = 0$
$\ddot{x} = \frac{-x}{n^2} + \frac{p_1}{n^2}$, $\ddot{y} = \frac{-y}{n^2} + \frac{p_2}{n^2}$, $\ddot{z} = 0$
$\dddot{x} = \frac{-y}{n^3} + \frac{p_2}{n^3}$, $\dddot{y} = \frac{x}{n^3} - \frac{p_1}{n^3}$, $\dddot{z} = 0$
$x^{(4)} = \frac{x}{n^4} - \frac{p_1}{n^4}$, $y^{(4)} = \frac{y}{n^4} - \frac{p_2}{n^4}$, $z^{(4)} = 0$

Generally, we will arrive the following theorem.

Theorem 5. If $g(S^1)$ be a folding from S^1 into itself such that $g(S^1) = p + r(cos\frac{(-1)^m t}{n}, sin\frac{(-1)^m t}{n}, 0)$, $n, m, q \in \mathbb{N}$, and m is odd or even then, $(x^{(2q-1)}, y^{(2q-1)}, z^{(2q-1)})$ is the same as $(-x^{(2q-1)}, -y^{(2q-1)}, -z^{(2q-1)})$ and, $(x^{(2q)}, y^{(2q)}, z^{(2q)})$ is the same as $(x^{(2q)}, y^{(2q)}, z^{(2q)})$.

Again, if, $h_1 : S^1 \to S^1$ is a folding from S^1 into itself defined by $h_1(S^1) = p + m\, r(cos\, t, sin\ t, 0)$, $m \in \mathbb{N}$. This folding can be represented by:

$\dot{x} = -y + p_2$, $\dot{y} = x - p_1$, $\dot{z} = 0$
$\ddot{x} = -x + p_1$, $\ddot{y} = -y + p_2$, $\ddot{z} = 0$
$\dddot{x} = y - p_2$, $\dddot{y} = -x + p_1$, $\dddot{z} = 0$
$x^{(4)} = x - p_1$, $y^{(4)} = y - p_2$, $z^{(4)} = 0$

Also, if $h_2 : S^1 \to S^1$ where $h_2(S^1) = p + \frac{1}{n} r(cos\, t , sin\ t, 0)$, $n \in \mathbb{N}$, is a folding from S^1 into itself. Then the system of linear ordinary differential equations of h_2 (S^1) is:

$\dot{x} = -y + p_2$, $\dot{y} = x - p_1$, $\dot{z} = 0$
$\ddot{x} = -x + p_1$, $\ddot{y} = -y + p_2$, $\ddot{z} = 0$
$\dddot{x} = y - p_2$, $\dddot{y} = -x + p_1$, $\dddot{z} = 0$
$x^{(4)} = x - p_1$, $y^{(4)} = y - p_2$, $z^{(4)} = 0$

If h_3: $S^1 \to S^1$is a folding from S^1 into itself such that $h_3(S^1) = p - m\, r(cos\, t, sin\ t, 0)$, $m \in \mathbb{N}$. Also, the linear ordinary differential equations of h_3 (S^1) is:

$\dot{x} = -y + p_2$, $\dot{y} = x - p_1$, $\dot{z} = 0$
$\ddot{x} = -x + p_1$, $\ddot{y} = -y + p_2$, $\ddot{z} = 0$
$\dddot{x} = y - p_2$, $\dddot{y} = -x + p_1$, $\dddot{z} = 0$
$x^{(4)} = x - p_1$, $y^{(4)} = y - p_2$, $z^{(4)} = 0$

Now, if $h_4 : S^1 \to S^1$ such that $h_4(S^1) = p - \frac{1}{n} r(\cos t, \sin t, 0)$, $n \in \mathbb{N}$, is a folding from S^1 into itself. Then $h_4(S^1)$ have the linear ordinary differential equations:

$$\dot{x} = -y + p_2 \ , \quad \dot{y} = x - p_1 \ , \quad \dot{z} = 0$$
$$\ddot{x} = -x + p_1 \ , \quad \ddot{y} = -y + p_2 \ , \quad \ddot{z} = 0$$
$$\dddot{x} = y - p_2 \ , \quad \dddot{y} = -x + p_1 \ , \quad \dddot{z} = 0$$
$$x^{(4)} = x - p_1 \ , \quad y^{(4)} = y - p_2 \ , \quad z^{(4)} = 0$$

So we can state the following theorem:

Theorem 6. If $h(S^1) = p + l\, r(\cos t, \sin t, 0)$ were $l \in (\mathbb{Q}^+ \cup \mathbb{Q}^-) - \{0\}$ is a folding from the circle S^1 into itself then $h(S^1)$ have the system of linear ordinary differential equations. Also, the system of differential equations of $h(S^1)$when $l \in \mathbb{Q}^+$ is the same as the system of differential equations of $h(S^1)$when $l \in \mathbb{Q}^-$.

Now, let $f_1 : H \to H$ be a folding of hyperbola $H = p + r\ (0, sinht, cosht)$, $r > 0$, $p \in \mathbb{R}^3$, in Minkowski 3-space E_1^3 into itself such that $f_1(H) = p + r\ (0, sinht^*, cosht^*)$, $c \le t^* < \infty$, this folding induces singular point $(p_1, p_2 + r\ sinhc, p_3 + r\ coshc)$.

Also, $f_2 : H \to H$ is a folding from H into itself such that $f_2(H) = p + r\ (0, sinht^*, cosht^*)$, $a \le t^* \le b$, $a < b$, then $f_2(H)$ is a kind of a folding from H into itself with two singular points $(p_1, p_2 + rsinha, p_3 + rcosha)$ and $(p_1, p_2 + r\ sinhb, p_3 + r\ coshb)$. The system of linear ordinary differential equations are:

$$\dot{x} = 0 \ , \quad \dot{y} = z - p_3 \ , \quad \dot{z} = y - p_2$$
$$\ddot{x} = 0 \ , \quad \ddot{y} = y - p_2 \ , \quad \ddot{z} = z - p_3$$

Then we get the following theorem:

Theorem 7. Let f be the folding of H into itself by changing in the value of $t \in (-\infty, \infty)$ then f produce the 2 -order system of ordinary differential equations.

Now, if $g : H \to H$ is the folding of H into itself such that $g(H) = p + r\ (0, -sinht, cosht)$, then the system of differential equations of $g(H)$is:

$$\dot{x} = 0 \ , \quad \dot{y} = -z + p_3 \ , \quad \dot{z} = -y + p_2$$
$$\ddot{x} = 0 \ , \quad \ddot{y} = y - p_2, \quad \ddot{z} = z - p_3$$

Also, let $M_1 : H \to H$ where $M_1(H) = p + lr\ (0, sinht, cosht)$, $l \in \mathbb{Q}^+$, is the folding from H into itself, then the system of linear ordinary differential equations of $M_1(H)$is:

$$\dot{x} = 0 \ , \quad \dot{y} = z - p_3 \ , \quad \dot{z} = y - p_2$$
$$\ddot{x} = 0 \ , \quad \ddot{y} = y - p_2, \quad \ddot{z} = z - p_3$$

Again, let $M_2 : H \to H$ be the folding from H into itself such that $M_2(H) = p - lr\ (0, sinht, cosht)$, $l \in \mathbb{Q}^+$, This folding can be represented by this system of differential equations:

$$\dot{x} = 0 \ , \quad \dot{y} = z - p_3 \ , \quad \dot{z} = y - p_2$$
$$\ddot{x} = 0 \ , \quad \ddot{y} = y - p_2, \quad \ddot{z} = z - p_3$$

Thus, the above result can be formulated in the following theorem:

Theorem 8. If $M(H) = p + (-1)^m\ lr\ (0, sinht, cosht)$, $m = 1, 2$, is a folding from H into itself and any folding homeomorphic to this type of folding, then the system of ordinary differential equations at m = 1 is the same as the system of ordinary differential equations at m = 2.

In this position, consider the hyperhelix in Minkowski 4-space defined as $H^2 = (a\cos\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right), a\sin\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right), b\cos\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right), b\sin\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right))$. Now, we introduce types of foldings of the hyperhelix H^2

Let $f_1 : H^2 \to H^2$ where $f_1(H^2) = (a\cos\left(\frac{r}{\sqrt{a^2r^2+b^2}}t^*\right), a\sin\left(\frac{r}{\sqrt{a^2r^2+b^2}}t^*\right), b\cos\left(\frac{1}{\sqrt{a^2r^2+b^2}}t^*\right), b\sin\left(\frac{1}{\sqrt{a^2r^2+b^2}}t^*\right))$, $0 \le t^* < \sqrt{a^2r^2+b^2}\,\pi$, is a folding from H^2 into itself.

Also, let $f_2 : H^2 \to H^2$ such that $f_2(H^2) = (a\cos\left(\frac{r}{\sqrt{a^2r^2+b^2}}t^*\right), a\sin\left(\frac{r}{\sqrt{a^2r^2+b^2}}t^*\right), b\cos\left(\frac{1}{\sqrt{a^2r^2+b^2}}t^*\right), b\sin\left(\frac{1}{\sqrt{a^2r^2+b^2}}t^*\right))$, $\sqrt{a^2r^2+b^2}\,\frac{\pi}{2} \le t^* \le \sqrt{a^2r^2+b^2}\,\frac{3\pi}{2}$, be a folding from H^2 into itself. This type of folding with singular points and this folding can be represented by:

$$\dot{x_1} = -\frac{r}{\sqrt{a^2r^2+b^2}}x_2, \quad \dot{x_2} = \frac{r}{\sqrt{a^2r^2+b^2}}x_1 \ ,$$
$$\dot{x_3} = -\frac{1}{\sqrt{a^2r^2+b^2}}x_4 \ , \quad \dot{x_4} = \frac{1}{\sqrt{a^2r^2+b^2}}x_3,$$
$$\ddot{x_1} = -\frac{r^2}{a^2r^2+b^2}x_1, \quad \ddot{x_2} = -\frac{r^2}{a^2r^2+b^2}x_2 \ ,$$
$$\ddot{x_3} = -\frac{1}{a^2r^2+b^2}x_3, \quad \ddot{x_4} = -\frac{1}{a^2r^2+b^2}x_4$$

Then we get the following theorem

Theorem 9. Let f be the folding of H^2 into itself by changing in the value of t, $n \in \mathbb{N}$, then the corresponding system is given by:

$$x_1^{(2n-1)} = (-1)^n \frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}}x_2 \ ,$$
$$x_2^{(2n-1)} = (-1)^{n+1}\frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}}x_1,$$
$$x_3^{(2n-1)} = (-1)^n \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}}x_4,$$

$x_4^{(2n-1)} = (-1)^{n+1} \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_3.$

$x_1^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_1,$

$x_2^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_2 ,$

$x_3^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_3,$

$x_4^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_4 .$

Now, Let $g: H^2 \to H^2$ be a folding from H^2 into itself such that $g(H^2) = (a\cos\left(\frac{r}{m\sqrt{a^2r^2+b^2}}t\right)$,$a\sin\left(\frac{r}{m\sqrt{a^2r^2+b^2}}t\right)$, $b\cos\left(\frac{1}{m\sqrt{a^2r^2+b^2}}t\right)$, $b\sin\left(\frac{1}{m\sqrt{a^2r^2+b^2}}t\right)$), where $m, n \in \mathbb{N}$. Then the system of linear ordinary differential equations of $g(H^2)$ is:

$\dot{x_1} = -\frac{r}{m\sqrt{a^2r^2+b^2}} x_2,$ $\dot{x_2} = \frac{r}{m\sqrt{a^2r^2+b^2}} x_1 ,$

$\dot{x_3} = -\frac{1}{m\sqrt{a^2r^2+b^2}} x_4,$ $\dot{x_4} = \frac{1}{m\sqrt{a^2r^2+b^2}} x_3,$

$\ddot{x_1} = -\frac{r^2}{m^2(a^2r^2+b^2)} x_1,$ $\ddot{x_2} = -\frac{r^2}{m^2(a^2r^2+b^2)} x_2 ,$

$\ddot{x_3} = -\frac{1}{m^2(a^2r^2+b^2)} x_3,$ $\ddot{x_4} = -\frac{1}{m^2(a^2r^2+b^2)} x_4, \ldots,$

$x_1^{(2n-1)} = (-1)^n \frac{r^{2n-1}}{m^{2n-1}(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_2,$

$x_2^{(2n-1)} = (-1)^{n+1} \frac{r^{2n-1}}{m^{2n-1}(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_1,$

$x_3^{(2n-1)} = (-1)^n \frac{1}{m^{2n-1}(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_4,$

$x_4^{(2n-1)} = (-1)^{n+1} \frac{1}{m^{2n-1}(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_3,$

$x_1^{(2n)} = (-1)^n \frac{r^{2n}}{m^{2n}(a^2r^2+b^2)^n} x_1,$

$x_2^{(2n)} = (-1)^n \frac{r^{2n}}{m^{2n}(a^2r^2+b^2)^n} x_2,$

$x_3^{(2n)} = (-1)^n \frac{1}{m^{2n}(a^2r^2+b^2)^n} x_3,$

$x_4^{(2n)} = (-1)^n \frac{1}{m^{2n}(a^2r^2+b^2)^n} x_4.$

Generally, we will arrive the following theorem

Theorem 10. If g is the folding of H^2into itself by the change in the angles, then $g(H^2)$ produce the n - order system of ordinary differential equations.

Also, let $M: H^2 \to H^2$ be a folding from H^2 into itself defined as $M(H^2) = (c\cos\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right)$, $c\sin\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right)$, $d\cos\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right)$, $d\sin\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right)$), where $c \neq a$ and $d \neq b, n \in \mathbb{N}$. This folding can be represented by this system of differential equations:

$\dot{x_1} = -\frac{r}{\sqrt{a^2r^2+b^2}} x_2,$ $\dot{x_2} = \frac{r}{\sqrt{a^2r^2+b^2}} x_1 ,$

$\dot{x_3} = -\frac{1}{\sqrt{a^2r^2+b^2}} x_4,$ $\dot{x_4} = \frac{1}{\sqrt{a^2r^2+b^2}} x_3,$

$\ddot{x_1} = -\frac{r^2}{a^2r^2+b^2} x_1,$ $\ddot{x_2} = -\frac{r^2}{a^2r^2+b^2} x_2 ,$

$\ddot{x_3} = -\frac{1}{a^2r^2+b^2} x_3,$ $\ddot{x_4} = -\frac{1}{a^2r^2+b^2} x_4, \ldots,$

$x_1^{(2n-1)} = (-1)^n \frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_2,$

$x_2^{(2n-1)} = (-1)^{n+1} \frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_1 ,$

$x_3^{(2n-1)} = (-1)^n \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_4,$

$x_4^{(2n-1)} = (-1)^{n+1} \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_3,$

$x_1^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_1 ,$

$x_2^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_2 ,$

$x_3^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_3,$

$x_4^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_4.$

Again, let $Q: H^2 \to H^2$ be a folding from H^2 into itself such that $Q(H^2) = (a\cos\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right)$, $-a\sin\left(\frac{r}{\sqrt{a^2r^2+b^2}}t\right)$, $b\cos\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right)$, $-b\sin\left(\frac{1}{\sqrt{a^2r^2+b^2}}t\right)$), $n \in \mathbb{N}$. The system of linear ordinary differential equations of $Q(H^2)$ is:

$\dot{x_1} = \frac{r}{\sqrt{a^2r^2+b^2}} x_2 ,$ $\dot{x_2} = -\frac{r}{\sqrt{a^2r^2+b^2}} x_1 ,$

$\dot{x_3} = \frac{1}{\sqrt{a^2r^2+b^2}} x_4 ,$ $\dot{x_4} = -\frac{1}{\sqrt{a^2r^2+b^2}} x_3,$

$\ddot{x_1} = -\frac{r^2}{a^2r^2+b^2} x_1,$ $\ddot{x_2} = -\frac{r^2}{a^2r^2+b^2} x_2 ,$

$\ddot{x_3} = -\frac{1}{a^2r^2+b^2} x_3,$ $\ddot{x_4} = -\frac{1}{a^2r^2+b^2} x_4, \ldots,$

$x_1^{(2n-1)} = (-1)^{n+1} \frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_2 ,$

$x_2^{(2n-1)} = (-1)^n \frac{r^{2n-1}}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_1 ,$

$x_3^{(2n-1)} = (-1)^{n+1} \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_4,$

$x_4^{(2n-1)} = (-1)^n \frac{1}{(a^2r^2+b^2)^{\frac{2n-1}{2}}} x_3.$

$x_1^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_1,$

$x_2^{(2n)} = (-1)^n \frac{r^{2n}}{(a^2r^2+b^2)^n} x_2 ,$

$x_3^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_3,$

$x_4^{(2n)} = (-1)^n \frac{1}{(a^2r^2+b^2)^n} x_4.$

Thus, the above result can be formulated in the following theorem:

Theorem 11. Let $Q(H^2)$ be a folding from H^2 into itself where $Q(H^2) = (a\cos\left(\frac{(-1)^m r}{\sqrt{a^2r^2+b^2}}t\right)$, $a\sin\left(\frac{(-1)^m r}{\sqrt{a^2r^2+b^2}}t\right)$, $b\cos\left(\frac{(-1)^m}{\sqrt{a^2r^2+b^2}}t\right)$, $b\sin\left(\frac{(-1)^m}{\sqrt{a^2r^2+b^2}}t\right)$), $m, q \in \mathbb{N}$, and m is odd or even,

then $(x_1^{(2q)}, x_2^{(2q)}, x_3^{(2q)}, x_4^{(2q)})$ is same as $(x_1^{(2q)}, x_2^{(2q)}, x_3^{(2q)}, x_4^{(2q)})$.

Now, let $\alpha(t) = (at, sinht, cosht)$, $a \neq 0$, be the curve in Minkowski space E_1^3 and $\acute{\alpha}(t) = (a, cosht, sinht)$. Consider the following types of folding on $\alpha(t)$. Let $f_1: \alpha(t) \to \alpha(t)$ be a folding from $\alpha(t)$ into itself such that $f_1(\alpha(t)) = (at^*, sinht^*, cosht^*)$, $c_1 \leq t^* < \infty$, $c_1 \in \mathbb{R}$. Then the point $(ac_1, sinhc_1, coshc_1)$ is a singular point. Also, $f_2: \alpha(t) \to \alpha(t)$ were $f_2(\alpha(t)) = (at^*, sinht^*, cosht^*)$, $c_1 \leq t^* \leq c_2$, $c_1, c_2 \in \mathbb{R}$, then f_2 is a kind of a folding from $\alpha(t)$ into itself with two singular points $(ac_1, sinhc_1, coshc_1)$ and $(ac_2, sinhc_2, coshc_2)$. Again, if $f_3: \alpha(t) \to \alpha(t)$ be defined as $f_3(\alpha(t)) = (at, sinht, cosht)$ then f_3 is a folding from $\alpha(t)$ into itself and f_3 give1-manifold without singular point. Then by changing in the value of $t \in (-\infty, \infty)$, we get types of folding can be represented by this differential equations:

$\dot{x} = a$, $\dot{y} = z$, $\dot{z} = y$,
$\ddot{x} = 0$, $\ddot{y} = y$, $\ddot{z} = z$.

So we can state the following theorem.

Theorem 12. All the folding of $\alpha(t)$into itself by changing the value of $t \in (-\infty, \infty)$ have the same linear ordinary differential equations.

Moreover, if $g: \alpha(t) \to \alpha(t)$, $g(\alpha(t)) = (at, sinh^n t, cosh^n t)$, $n \neq 0$, g is a folding from $\alpha(t)$ into itself. The system of linear ordinary differential equations of $g(\alpha(t))$ is:

$\dot{x} = a$, $\dot{y} = n\, y^{\frac{n-1}{n}} z^{\frac{1}{n}}$, $\dot{z} = n\, z^{\frac{n-1}{n}} y^{\frac{1}{n}}$

$\ddot{x} = 0$, $\ddot{y} = n(n-1)\, y^{\frac{n-2}{n}} z^{\frac{2}{n}} + ny$, $\ddot{z} = n(n-1)\, z^{\frac{n-2}{n}} y^{\frac{2}{n}} + nz$

$\dddot{x} = 0$,

$\dddot{y} = n(n-1)(n-2)\, y^{\frac{n-3}{n}} z^{\frac{3}{n}} + 2n(n-1)\, y^{\frac{n-1}{n}} z^{\frac{1}{n}} + n^2\, y^{\frac{n-1}{n}} z^{\frac{1}{n}}$,

$\dddot{z} = n(n-1)(n-2)\, z^{\frac{n-3}{n}} y^{\frac{3}{n}} + 2n(n-1)\, z^{\frac{n-1}{n}} y^{\frac{1}{n}} + n^2\, z^{\frac{n-1}{n}} y^{\frac{1}{n}}$.

Then we get the following theorem.

Theorem 13. Under the defined folding and any folding homeomorphic to this type of folding the system of linear ordinary differential equations of $g(\alpha(t))$ is an infinite system.

Let $M: \alpha(t) \to \alpha(t)$ be a folding from $\alpha(t)$ into itself such that $M(\alpha(t)) = (at^n, sinht, cosht)$. This folding can be represented by the differential equations:

$\dot{x} = ant^{n-1}$, $\dot{y} = z$, $\dot{z} = y$
$\ddot{x} = an(n-1)t^{n-2}$, $\ddot{y} = y$, $\ddot{z} = z$
$\dddot{x} = an(n-1)(n-2)t^{n-3}$, $\dddot{y} = z$, $\dddot{z} = y$, ...,
$x^{(n)} = a(n!)$, $y^{(n)} = y^{(n-2)}$, $z^{(n)} = z^{(n-2)}$
$x^{(n+1)} = 0$, $y^{(n+1)} = y^{(n-1)}$, $z^{(n+1)} = z^{(n-1)}$

Generally, we will arrive the following theorem:

Theorem 14. The folding of $M(\alpha(t))$ and any folding homeomorphic to this type of folding produce the (n+1) - order system of ordinary differential equations.

Now, if $E: \alpha(t) \to \alpha(t)$ where $E((at, sinht, cosht)) = (ct, bsinht, b\, cosht)$ is a folding from $\alpha(t)$ into itself, then the system of linear ordinary differential equations of $E(\alpha(t))$ is:

$\dot{x} = c$, $\dot{y} = z$, $\dot{z} = y$
$\ddot{x} = 0$, $\ddot{y} = y$, $\ddot{z} = z$
$\dddot{x} = 0$, $\dddot{y} = z$, $\dddot{z} = y$

Also, let the folding F: $\alpha(t) \to \alpha(t)$, $w \in \mathbb{N}$, be given by $F((at, sinht, cosht)) = (at, \sinh(wt), \cosh(wt))$, $w \in \mathbb{N}$, from $\alpha(t)$ into itself without singular point. Then, $F(\alpha(t))$ has the system of linear ordinary differential equations is:

$\dot{x} = a$, $\dot{y} = wz$, $\dot{z} = wy$
$\ddot{x} = 0$, $\ddot{y} = w^2 y$, $\ddot{z} = w^2 z$
$\dddot{x} = 0$, $\dddot{y} = w^3 z$, $\dddot{z} = w^3 y$.

Also, If $K: \alpha(t) \to \alpha(t)$ such that $K((at, sinht, cosht)) = \left(at, sinh\frac{t}{w}, cosh\frac{t}{w}\right)$, $w \in \mathbb{N}$, is a folding from $\alpha(t)$ into itself. Then the system of differential equations of $K(\alpha(t))$ is:

$\dot{x} = a$, $\dot{y} = \frac{1}{w} z$, $\dot{z} = \frac{1}{w} y$
$\ddot{x} = 0$, $\ddot{y} = \frac{1}{w^2} y$, $\ddot{z} = \frac{1}{w^2} z$
$\dddot{x} = 0$, $\dddot{y} = \frac{1}{w^3} z$, $\dddot{z} = \frac{1}{w^3} y$.

Let $Z: \alpha(t) \to \alpha(t)$ be a folding from $\alpha(t)$ into itself such that $Z(\alpha(t)) = (at, -\sinh(wt), \cosh(wt))$, $w \in \mathbb{N}$. This folding can be represented by the ordinary differential equations:

$\dot{x} = a$, $\dot{y} = -wz$, $\dot{z} = -wy$
$\ddot{x} = 0$, $\ddot{y} = w^2 y$, $\ddot{z} = w^2 z$
$\dddot{x} = 0$, $\dddot{y} = -w^3 z$, $\dddot{z} = -w^3 y$.

Also, if $W: \alpha(t) \to \alpha(t)$ is a folding from $\alpha(t)$ into itself such that $W((at, sinht, cosht)) = \left(at, -sinh\frac{t}{w}, cosh\frac{t}{w}\right)$, $w \in \mathbb{N}$. The system of differential equations of $W(\alpha(t))$ is:

$\dot{x} = a$, $\dot{y} = \frac{-1}{w} z$, $\dot{z} = \frac{-1}{w} y$
$\ddot{x} = 0$, $\ddot{y} = \frac{1}{w^2} y$, $\ddot{z} = \frac{1}{w^2} z$
$\dddot{x} = 0$, $\dddot{y} = \frac{-1}{w^3} z$, $\dddot{z} = \frac{-1}{w^3} y$.

Thus, the above result can be formulated in the following theorem:

Theorem 15. Let $V(\alpha(t)) = (at, \sinh(ct), \cosh(ct))$, $c \in (\mathbb{Q}^+ \cup \mathbb{Q}^-) - \{0\}$ be a folding from

$\alpha\ (t)$ into itself and any folding homeomorphic to this type of folding . Then the corresponding system of $V(\ \alpha\ (t))$ is given by:

$\dot{x} = a \quad , \quad \dot{y} = cz \quad , \quad \dot{z} = cy$

$x^{(i)} = 0 \quad , \quad y^{(i)} = c^i y \quad , \quad z^{(i)} = c^i z$,

were $i > 1$, i is even

$x^{(j)} = 0 \quad , \quad y^{(j)} = c^j z \quad , \quad z^{(j)} = c^j y,$

were $j > 1$, j is odd

Now, let $L = p + tv$, $S^1 = p + r(cost, sint, 0)$, $r \neq 0$ be the straight line and the circle in Minkowski space E_1^3 and $\mathcal{H}$ be a folding defined as $\mathcal{H}: L \to S^1$, where $\mathcal{H}(L) = p + (bcos\, nt,\ bsin\, nt,\ 0)$, then $\mathcal{H}(L)$ is a covering space of S^1 and the system of differential equations of $\mathcal{H}(L)$ is:

$\dot{x} = -ny \quad , \quad \dot{y} = nx \quad , \quad \dot{z} = 0$

$\ddot{x} = -n^2 x \quad , \quad \ddot{y} = -n^2 y \quad , \quad \ddot{z} = 0$

$\dddot{x} = -n^3 y \quad , \quad \dddot{y} = -n^3 x \quad , \quad \dddot{z} = 0$

Also, let $\Im: S^1 \to S^1$, be a type of folding of a circle into itself without singular point defined as $\mathcal{H}(L) = p + (rcos\, nt\ , rsin\, nt,\ 0)$, then $\Im(\ S^1)$ is a covering space of S^1.

So we can state the following theorem.

Theorem 16. The system of linear ordinary differential equations of a covering space of the circle S^1 is an infinite.

Theorem 17. If $\mathcal{H}: L \to S^1$, where $\mathcal{H}(L) = (bcos\, nt\ , bsin\, nt,\ 0), \forall\ \ i \in \mathbb{N}$ be a folding of $\mathcal{H}(L)$ into S^1, then the corresponding system of a covering space of S^1 is given by:

$x^{(2i-1)} = (-1)^i\, n^{2i-1}\, y,\ y^{(2i-1)} = (-1)^{1+i}\, n^{2i-1}\, x$, $z^{(2i-1)} = 0$

$x^{(2i)} = (-1)^i\, n^{2i}\, x \quad , \quad y^{(2i)} = (-1)^{2+i}\, n^{2i}\, y$, $z^{(2i)} = 0$

3. Conclusion

In this paper we achieved the approval of the important of the foldings and differential equations of some curves in Minkowski space. The relations between foldings and types of linear ordinary differential equations are introduced. Theorems which governs these relations are presented.

References

1. El-Ahmady A. E, The variation of the density on chaotic spheres in chaotic space-like Minkowski space time, Chaos, Solitons and Fractals, 31 (1272-1278) (2007).
2. El-Ahmady A. E, Folding of fuzzy hypertori and their retractions, Proc. Math. Phys. Soc. Egypt, Vol.85, No.1 (1- 10) (2007).
3. El-Ahmady A. E, Limits of fuzzy retractions of fuzzy hyperspheres and their foldings, Tamkang Journal of Mathematics, Volume37, No. 1 (47-55) Spring, (2006).
4. El-Ahmady A. E, Fuzzy folding of fuzzy horocycle, Circolo Matematico di Palermo Serie II ,Tomo L III (443-450) (2004).
5. El-Ahmady A. E, Fuzzy Lobachevskian space and its folding, The Journal of Fuzzy Mathematics, Vol. 12 No. 2, (609-614) (2004).
6. El-Ahmady A. E, The deformation retract and topological folding of Buchdahi space, Periodica Mathematica Hungarica Vol. 28 (19-30) (1994).
7. El-Ahmady A. E. and Rafat H, Retraction of chaotic Ricci space, Chaos, Solutions and Fractals, 41 (394-400) (2009).
8. El-Ahmady A. E. and Rafat H, A calculation of geodesics in chaotic flat space and its folding, Chaos, Solutions and Fractals, 30 (836-844) (2006).
9. El-Ahmady A. E. and Shamara H. M, Fuzzy deformation retract of fuzzy horospheres, Indian J. Pure Appl. Math. 32 (10), 1501-1506, October (2001).
10. El-Ahmady A. E. and El-Araby A, On fuzzy spheres in fuzzy Minkowski space ,Nuovo Cimento Vol.125B(2010) .
11. El-Ahmady A. E, Retraction of chaotic black hole, The Journal of Fuzzy Mathematics Vol.19, No. 3, (2011).
12. El-Ahmady A. E. and Al-Hesiny E, The topological folding of the hyperbola in Minkowski 3-space, The International Journal of Nonlinear Science, Vol. 11, No. 4,(451-458) (2011).
13. El-Ahmady A. E, The geodesic deformation retract of Klein Bottle and its folding, The International Journal of Nonlinear Science, Vol. 12, No. 3, (2011).
14. Demainel E. D, Folding and unfolding ,Ph. D. Thesis, Waterloo University ,Canada (2001).
15. Walrave J , Curves and surfaces in Minkowski space ,Doctoral Thesis ,K .U .Leuven ,Fac .Sci . ,Leuven , (1995) .
16. Catoni F, Boccaletti D, Cannata R , Catoni V, Nichelatti E, Zampetti P, The Mathematics of Minkowski Space -Birkhauser Verlag, Basel,Boston, Berlin ,(2008).
17. Naber G. L, Topology, Geometry and Gauge fields: Foundations, Texts in Applied Mathematics 25,2^{nd} Edition, Springer-Verlage New York, Berlin ,(2011).
18. Naber G. L, Topology, Geometry and Gauge fields: Interactions, Applied Mathematics Sciences, 2^{nd} Edition, Springer-Verlage New York, Berlin ,(2011).
19. DI-Francesco P, Folding and coloring problem in Mathematics and Physics, Bulliten of the American Mathematics Society, Vol. 37, No. 3, (251-307) (2000).
20. Lopez R ; Differential geometry of curves and surfaces in Lorentz –Minkowski space, Instituto de Matematica e Estatistica ,University of Sao Paulo ,Brazil ,(2008).

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Effect of Establishment of Treatment Guidelines on Antibiotic Prescription Pattern for Children with Upper Respiratory Tract Infection

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Abstract: Background: Upper respiratory tract infection in childhood is a common cause of antibiotic description which increases the likelihood for emergence of antibiotics-resistant microorganisms leading to increased illness, deaths, and substantial economic loss. **Objective:** To evaluate antibiotics prescription pattern for children and to establish clinical practical guidelines for judicious antibiotic use in upper respiratory tract infection. **Methodology:** Randomized controlled trail, Pediatric cases of upper respiratory tract infection were studied for overall antibiotics and disease specific prescription rate and their relation to several risk factors. **Result**: Significant reduction of overall antibiotic prescription between intervention and control groups was 0.008 with odd ratio 1.2 and confidence interval CI 0.62-2.39. By using logistic regression models for antibiotic prescription as dependant outcome variable showed to be significant and influenced by; assignment to intervention and control groups, primary diagnosis, associated symptoms were cough, sputum and pain also discussion with parent before prescription. **Conclusion:** Using standardized guidelines for pediatric antibiotic prescription in upper respiratory tract infection as intervention method caused reduction in antibiotics' prescribing rates for some upper respiratory tract diseases, while maintaining a high level of prescription in others.

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Key words: Antibiotics, upper respiratory tract, pediatric, guidelines.

1. Introduction

Children are particularly challenging group of patients when trying to ensure the safe use of medication [(1)]. Upper respiratory tract infection in children is a common cause of health care visits and antibiotic prescription [(2)] that increases the likelihood for emergence of antibiotics-resistant microorganisms leading to increased illness, deaths, and substantial economic loss [(3)].

Antibiotic over prescribing is a major health problem worldwide, which contributes to antibiotic resistant bacteria. Antibiotics are frequently prescribed for the management of upper respiratory tract infections, in spite of the fact that the majority of these infections are viral in origin [(4)].

Children receive a significant proportion of the antibiotics prescribed each year and represent an important target group for effort aimed at reducing unnecessary antibiotic use [(5)]. Approximately 75% of all outpatient antibiotic prescriptions given to children are for upper respiratory tract conditions, such as viral upper respiratory tract infections (URTIs), bronchitis, pharyngitis, sinusitis and otitis media [(6)] which are mostly of viral origin, they resolve by their own, and antibiotics need not to be prescribed [(7)]. The main reasons for physician overprescribing of antibiotics for URTIs are due to diagnostic uncertainty in ill-appearing, febrile child and anxious parents, and fear of physicians to offer only symptomatic therapy. If the physicians were more certain that the infection is viral in origin, they may not prescribe broad spectrum antibiotics [(8)]. Other factors that affect prescription of antibiotics are physician characteristics, such as location of medical training, specialty, and number of years in practice, hospital affiliation and place of practice private or general settings [(9-10)]. Socioeconomic status and level of knowledge of the parents are factors for influencing physician prescribing of antibiotics [(11)]. the Centers for Disease Control and Prevention (CDC) established principles for the use of antimicrobial agents in children, which were later endorsed by the American Academy of Pediatrics [(12,13)]. Several studies have suggested interventions to improve the implementation of these recommendations, such as physician education by written guidelines, parental education, point-of-care evidence, and use of public media.[(14,15)]

Study objectives:

1. To evaluate the pattern of antibiotics prescribing for children younger than 18 years with upper respiratory tract infection.

2. To establish and implement evidence-based clinical practical guidelines for judicious antibiotic use in upper respiratory tract infection and train physician on its use.
3. To measure effectiveness of clinical practical guidelines in children with upper respiratory tract infection in term of changing diagnostic criteria, prevalence and pattern of antibiotic prescription.

2. Patients and Methods:

Subjects:

1- Four pediatric outpatient clinics affiliated to governmental health care center in Byreda city, Qassim Governorate, KSA were contracted to participate in this study all study done through four months. Each pediatric clinic had one full time pediatric specialist.
2- Pediatric cases: 6months to 18 years with primary diagnosis upper respiratory tract infection only.

Study design:

Randomized Intervention pre and after study:

1- Pre intervention measurement for antibiotic prescription rate in pediatric cases of upper respiratory tract infection in four pediatric outpatient clinics before randomization.
2- Assignments of pediatric clinics randomly into control and intervention groups, 2 control (B) and 2 interventions (A).
3- Intervention phase start when the two intervention clinics' physicians attended educational seminar provided by senior pediatrician –professor-and senior pharmacist-professor about how to develop standardized evidence based treatments guidelines, followed by adopted readymade ,evidence based, upper respiratory tract infection guidelines for pediatric population provided by center for disease and prevention CDC I2 , 13 then Post intervention measurements for antibiotic prescription rate in pediatric cases of upper respiratory tract infection in both control and intervention group.
4- Comparison between pre and post intervention measurements.

Measuring variables:

The main measurable variables for both intervention and control groups are:

1- The overall antibiotics' prescription rate for children with upper respiratory tract infection.
2- Upper respiratory disease specific antibiotics prescription rate.
3- Association between several risk factors as disease category, signs and symptoms such as fever, cough, sputum, pain and general condition, parent educational level, presence of treatment guidelines and parents' discussion with physician.

Intervention method:

Physician training work shop on how to develop standardized evidence based acceptable clinical guidelines, at the end of workshop pediatrician agreed on adopted standardized guidelines and apply it on their daily practice CDC/AAP [12,13]

Study time limit:

Four months: one month for pre intervention measurement, two months for 4 intervention seminar sessions and one month for post intervention measurements.

Sample size:

A total of 370 cases: 185 before and 185 after implementation of clinical practical guidelines, 110 cases intervention group (A)before intervention and 110 cases for same group after intervention, 75 cases control group (B) before intervention and 75 for same group after no intervention.

Sample selection:

Pediatric cases visiting pediatric clinics 6 month to 18 year age according to CDC guidelines [(12,13)] during study period complaining of upper respiratory tract infection which defined as presence of one of the following: Otitis Media, rhinitis, sinusitis, pharyngitis, non specific cough illness/bronchitis.

Total number of upper respiratory tract infection cases visited the four contracted clinics were 831 cases only 370 cases agree to participate in this study.

Exclusion criteria:

Patients had other acute or chronic health problems, other than upper respiratory tract infection and patient who had more than one diagnosis of upper respiratory tract infection diseases like otitis media plus pharyngitis or patient with upper respiratory tract disease associated with complication such as presence of pharyngeal abscess, perforated drum etc…

Data was collected using*:*

Variable measurement designed checklists measuring:

1- Socio demographic data: age, gender, father and mother occupation, education.
2- Clinical history, sign and symptoms: general condition, primary diagnosis, pain, fever, cough, sputum, rash, illness duration, presence of any complication
3- Treatment prescription: antibiotic prescription, type, duration, first or second time prescription, discussion before prescription

Statistical analysis:

Statistical Package for social science **S**PSS 11 was used. Comparison made using appropriate

statistical test. Logistic regression we used block entry with removal of non significant variables.

Ethical consideration:

Patient parents who shared in study were asked for consent, patient' parent who refused participation not considered in sample size, all study data considered highly confidential.

3. Result:

Table I: shows mean age of patients under study in pre intervention period was 4.58± 2.83 years and 96% of their parents were employed and 58.4% were male patients.

Table II : shows categories of patients according to their diagnosis, most of patient in intervention group (A) before and after intervention were diagnosed with acute pharyngitis 33 and 35 from total of 110 orderly, they were 10 cases before intervention and 17 cases after intervention period in control group (B).Common cold cases were 37 and 35 respectively in control group (B) before and after intervention compared to 4 and 7 cases in group (A) before and after intervention respectively but in control group (B) most frequent diagnosis was common cold 37 and 35 from 75 totals for both pre and after intervention period.

Table III, shows that overall antibiotic prescription before intervention in group (A) it was 42.7 % compared to 27.2% post intervention with significant difference. In group (B) the control group the overall rate in pre-intervention period was 30.66 which increased to 37.3% in post intervention period. Most frequent diagnosis categories showed 100% prescription was acute tonsillitis in both intervention and control groups before intervention but decrease to 50%in intervention group (A) after intervention with high significant difference and acute pharyngitis showed 60.6% prescription in intervention group (A) before intervention but decrease to 51.42% without significant difference considering control groups (B) in pre-intervention period it was 50% increased to 64.7% in post - intervention period significant difference.

Common cold showed 0% prescription in both groups in pre-intervention period, it still 0% in intervention group after intervention and increased to 5.7% in control group in post intervention period. Running nose showed 0% prescription rate in group (A) before and after intervention compared to 57.14% in group (B) in pre-intervention period which increased to 100% in post intervention period with significant difference, while non specific URTI showed prescription rate 11.4% which decreased to 3.4% for intervention group (A) pre and post intervention respectively with significant difference and in group (B) non specific URTI showed prescription rate was 12.5% increased to 33.3% in post intervention period with significant difference.

Table IV, comparing overall antibiotics prescription in post intervention period which showed no significant difference in-between intervention and control groups 0.09 with odd ratio 1.84 and confidence interval CI (0.94-1.43).

Table V showed risk model using logistic regression models for antibiotic prescription as dependant with assignment to intervention and control groups, primary diagnosis ,associated symptoms were cough, sputum and pain also discussion with parent before prescription of antibiotics which all founded to be significantly influence dependant variable.

Table I: Basic data for pediatric patients before intervention

variables	Total population 175
Age Mean ±SD	4.5869 ± 2.839 years
Father occupation	Manual worker :20.5% Employee :46.5% Professional: 28.1% Not working:4.9
Gender	Male : 58.4% Female: 41.6%

Table II: Numbers of diagnosed cases at pediatric clinic before and after intervention in both intervention and control groups.

Diagnosis	Intervention group A before	Control group B before	Intervention group A after	Control group B after	Total
Acute pharyngitis	33	10	35	17	95
Nonspecific upper respiratory tract infection (non specific cough)	35	8	28	9	80
Otitis media	5	6	6	4	21
Common cold	4	37	7	35	83
tonsillitis	18	7	17	6	48
Running nose	15	7	17	4	43
Total	110	75	110	75	370

Table III: Rate of antibiotic prescription before and after intervention

	group (A) total 110	Intervention group(A) Total 110	Significance*	Group(B) total 75	Control group (B) Total 75	Significance*
Acute pharyngitis	60.6%	51.42%	0.17	50%	64.7%	0.068
Non specific URTI	11.4%	3.4%	0.02	12.5%	33.3%	0.001
Otitis media	100%	100%	1	100%	75%	0.000
Common cold	0%	0%	1	0%	5.7%	0.128
Tonsillitis	100%	50%	0.000	100%	100%	1
Running nose	0%	0%	1	57.14%	100%	0.00
Overall prescriptions	42.72%	27.2%	0.016	30.66%	37.3%	0.388

*MC Nemar test with Yates' correction

Table IV: Comparing over all antibiotic prescription after intervention in control and intervention groups

Over all Antibiotics prescription	Intervention Total 110	Control Total 75	*p*-value
yes	30	28	*0.09
no	80	47	**CI :1.16 (0.94-1.43)

*Fisher exact one tail **CI: confidence interval

Table V: Risk models for prediction of antibiotic prescription considering some risk variables

Variables	B	S.E	Wald	Significance	Exp(B) RR CI 95%
Group	-2.117	.513	17.0 12	.000	.120 (.044 -.329)
Primary diagnosis	.307	.133	5.335	.021	1.359 (1.04-.329)
sputum	-3.041	.633	23.0 38	.000	.0489(.041-.165)
Pain	1.274	.389	10.694	.001	3.574 (1.66-7.66)
Discussion before prescribing with patient	2.244	.771	8.473	.004	9.432(2.081-42.73)

4. Discussion

Over-prescribing of antibiotics in health care settings has brought along the worldwide problem of resistant pathogens, [14] that the pharmaceutical industry is struggling to overcome by producing newer antibiotics. The existent recommendation that antibiotics are only indicated in bacterial infection is frequently not complied with. Physicians diagnose URTIs upon clinical findings but often disregard the fact that URTIs could be of viral origin and antibiotic treatment is not indicated [16]. It has been previously documented that clinicians pre-scribe antibiotics not only to relieve symptoms, but also to prevent disease transmission, prevent secondary infections and to satisfy patients demand for antibiotics[17, 18].Several research teams have attempted to reduce antibiotic prescribing for respiratory tract infections by an educational intervention[16] .

As shown in table I studied subjects were children whom were all had upper respiratory tract patients went to pediatric clinics shared in this study with mean age 4.58±2.83years, most of their parent were worked as employee 46.5% and 28.1 were professionals. In our study male children were 58.4%.

Most assessed overall antibiotic use for respiratory tract infections in general, and reported a drop in prescription rates.[19,20] however, only a few studies assessed the changes with regard to specific diagnoses[21]. As table II describes the most frequent diagnosed upper respiratory tract infection among study subject were acute pharyngitis, non specific upper respiratory tract, common cold, tonsillitis, running nose and otitis media respectively.

This study provided us with a picture of antibiotics description in four pediatric clinics in governmental sector of Qassim state KSA, after an educational intervention through establishment of standardized treatment guidelines for URTI in pediatric population and we convinced that assessing changes in antibiotic prescribing for specific diseases, make us better able to

pinpoint areas where further intervention might be needed.

There was a significant reduction in antibiotic prescription as showed In table III for the rate of antibiotic prescription for tonsillitis from 100% to 50% and non specific URTI from 11.4% to 3.4% in intervention group (A) while in control group which no intervention was done at all the rate of antibiotic prescription increased from 12.5% to 33.3% in cases diagnosed with non specific URTI and prescription rate remain 100% in cases diagnosed with tonsillitis. Yaron *et al.,* [14] studied effect of educational intervention on pattern of antibiotic prescription in children and experienced no change between pre and post intervention rate considering tonsillitis. In contrast to Melendar *et al.,* [22] which studied effect of medical audit on antibiotic prescription for children which experienced reduction of antibiotic prescription rate for tonsillitis from 94% to 77% and for non specific URTI from 13% to 8%. Acute pharyngitis showed decrease in prescription rate from 60.6%to 51.42% for intervention group (A) but in control group (B) the rate increased from 50% to 64.7%. In contrast to Yaron *et al.,*[14] otitis media showed reduction in prescription rate from 93% to 87.4%. In our study otitis media prescription rate in intervention group (A) showed no change and remained as high as 100% same as control group (B).

Cases diagnosed with common cold showed 0% prescription rate in both pre and post intervention periods in group 9 (A) but in control group (B) it increased from 57.14 to 100%.

Janothen *et al.,* [23] studied effect of outreach educational approach for physician and families on antibiotic prescription rate for pediatric upper respiratory tract infection founded that intervention group showed decrease in overall prescription rate by 15% in intervention group and 9.8% in control group which matched with our study result in which antibiotics prescription rate was decreased from 42.72 % pre intervention to 27.2% post intervention in intervention group (A) with high significant difference 0.016 while in control group (B) the rate increase from 30.66% pre intervention to 37.3% post intervention.

Table IV compare over all antibiotic prescriptions between intervention and control group after intervention by developing standardized treatment guidelines and its application on daily work in intervention clinic only group (A), group (B) or control group receive no intervention guidelines and we found that number of children receiving antibiotics in intervention group was 30 from 110 and in group (B) control group they were 28 from 75 with no significant difference, that findings didn't match with Finkelstein *et al.,* [24] who experienced decrease in overall antibiotics prescription in intervention group (18.6%) compared with (11.5%) in control practices, he related that to the study effect on both groups rather than intervention effect and our study result matched with Yaron *et al.,*[14] who found reduction in overall prescription for all URTI diagnosis after intervention but he couldn't relate all reduction to intervention effect because his study has no control group.

Using regression model on outcome variable which was antibiotic prescription as table V showed significance regarding comparisons groups as intervention and control group which enhance significant test result, also the type of primary diagnosis was significantly regress on antibiotic prescription which agreed with Janothen *et al*., [23] who found that rate of antibiotic prescription differ according to primary diagnosis. Fahima *et al.*, [25] found that patients experienced yellowish discharge and pain responded more to intervention method which were guidelines and patient pamphlet and showed reduction of antibiotic prescription but in our study presence of sputum, pain and discussion with patient father or mother before prescribing are factors which affected antibiotic prescription rate in our intervention group (A).

Conclusion & recommendation:

Using standardized guidelines for pediatric antibiotic prescription in upper respiratory tract infection as intervention method reduced prescribing rates of antibiotics for some respiratory tract diseases while maintaining a high level of prescription in others. Further research should focus on other intervention methods and comparing effectiveness, sustainability and cost effectiveness of different interventions.

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References:

1-Conroy.S, Sweis D, Planner. C, Yeung. V, Collier. J, Hainess. L, Wong. IC (2007): Interventions to reduce dosing errors in children, a systematic review of the literatures, Drug Saf.; 30(12):1111-25.

2-Garlos. G, Grijdva (2009): Overall antibiotic prescriptions rates for respiratory tract infections. Science Daily Aug. 21.

3-Somathi Nambiar, Richard. H, Schwartz; Michael J Sheridan (2002): Antibiotic use for upper respiratory tract infection, how well do pediatric resident do; ARCH Pediatr. Adolesc. Med.; 156:621-624.

4-Badrya A ALmalki and Abdul Jamil Choudhry (2006): Knowledge and Practice Physician regarding prescription of antibiotics in the treatment of upper respiratory tract infection, Field Training Program Saudi Epidemiology Bulletin.;13(3):17-19.

5-Nyquist AG, Gonzales R, Steiner JF, Sande MA (1998): antibiotic prescribing for children with colds, upper respiratory tract infections, and bronchitis; JAMA,; 279(11):875-7.

6-Dowell SC, Marcy SM, et al. (1998): Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections. Pediatrics.; 101:163-148.

7-Michael E. Pichichero, John L. Green, Anne B. Francis, Steven M. Marsocci and Marie L. Murphy: outcome after judicious antibiotic use for respiratory tract infections seen in a private pediatric practice. Pediatrics, 105(4): 753-759.

8-Pichichero ME (1999): Understanding antibiotic overuse for respiratory tract infection in children. PEDIATRICS. 104(6): 1384-1388.

9-Manious AG, Husten WJ, love MM. (1998): Antibiotic for cold in children. Who are the high prescriber? Arch Pediatr Adolesc Med; 152:349-52.

10-Davy T, Dick PT, Munk P. (1998): Self-reported prescribing of antibiotics for children with undifferentiated acute respiratory tract infection with cough. Pediatr Infect Dis J.; 17:457-62.

11- Anita L. Kozyrskyj, Matthew E.Dahi, Dan G.Chateau,et al. (2004): Evidence –based prescribing of antibiotics for children: role of socioeconomic status and physician characterestics. CMAJ;doi:10.1503/cmaj.1031629. vol. 171 no. 2 139-145.

12- Dowell SF (1998): Principals of judicious use of antimicrobial agents for children's upper respiratory infections. Pediatrics. 1;101:165-171,www.cdc.gov/getsmart/campaign.../info.../child-approp-treatmt.pdf

13 -American Academy of Pediatrics and American Academy of Family Physicians (2004): Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. Pediatrics;113:1451-1.

14. Yaron Razon, Shai Ashkenazi, Avner Cohen, Eli Hering, Shlomo Amzel, Hanan Babilsky, Arie Bahir, Eli Gazala and Itzhak Levy (2005): Effect of educational intervention on antibiotic prescription practices for upper respiratory infections in children: a multicentre study. Journal of Antimicrobial Chemotherapy, 56: 937–940.

15-Rosenstein N, Philips WR, Gerber MA. (1998): The common cold-principles of judicious use of antimicrobial agents. Pediatrics; 101(1):181-184.

16-Avorn J, Solomon DH. (2000): Cultural and economic factors that (mis) shape antibiotic use: the nonpharmacologic basis of therapeutics. Ann Int Med.; 133(2):128-135.

17-Arroll B, Kenealy T. (2002): Antibiotics for the common cold. Cochrane Database Sys Rev. (3): CD000247. Review.PMID: 16034850 [PubMed - indexed for MEDLINE].

18-Butler CC, Rollnick S, Pill R. (1998): Understanding the culture of prescribing: qualitative study of general practitioners and patients' Perceptions of antibiotics for sore throats. BMJ.; 317(7159):637-642.

19- Trepka MJ, Belongia EA, Chyou PH et al. (2001): The effect of a community intervention trial on parental knowledge and awareness of antibiotic resistance and appropriate antibiotic use in children. Pediatrics; 107: e6.

20- Belongia EA, Bradley JS, Chyou P et al. (2001): A community intervention trial to promote judicious antibiotic use and reduce penicillin-resistant Streptococcus pneumoniae carriage in children. Pediatrics; 108: 575–83.

21- Finkelstein JA, Stille C, Nordin J et al. (2003): Reduction in antibiotic use among US children, 1996–2000. Pediatrics; 112: 620–7.

22-Melander E, Bjorgell A, Bjorgell P et al. (1999): Medical audit changes in physicians' prescribing of antibiotics for respiratory tract infections. Scand J Prim Health Care; 17: 180–4.

23- Jonathan A. Finkelstein, Robert L. DavisScott F, Dowell, Joshua P, Metlay, Stephen B. Soumerai, Sheryl L. Rifas-Shiman, Margaret Higham, Zachary MillerIrina Miroshnik, Alex Pedan, (2001): Reducing Antibiotic Use in Children: A Randomized Trial in 12 Practices . Pediatrics Vol., 108:1-7.

24-Finkelstein JA, Davis RL, Dowell SF, Metlay JP, Soumerai SB, Rifas-Shiman SL, Higham M, Miller Z, Miroshnik I, Pedan A, Platt R. (2001): Reducing antibiotic use in children: a randomized trial in 12 practices. Pediatrics. 108(1):1-7.

25-Fahima Alawadii, John Brebnerii and Zeinab Khalil (2010): A multi-intervention program to reduce antibiotic prescription for patients with upper respiratory tract infection in primary health care settings in the United Arab Emirates. Samoa Medical Journal; 2 (1):19-30.

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Assessing Characteristics of Clinical Psychologist for Effective Counseling

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Abstract: All we get are often subject to problems due to the limited knowledge and experience we need to consult with others. You know, sometimes people not immune to emotional crises. Developments and changes that occur in life. Such a pass in college, getting married, moving away from family and friends, illness and loss of our loved ones. Sometimes we experience anxiety, doubt, fear, conflict, and even makes the crisis. When adapted to the new environment and a new experience, these feelings are normal, but if these negative feelings far more than normal, which can reduce the physical, mental focus and relaxation. Effective counseling requires a good knowledge and understanding of the characteristics, needs and potential clients and situations in which they see themselves. This process is also influenced by beliefs, value system and attitude of the consultant, his worldview, psychological schools of current theoretical frameworks and assumptions are accepted. Its not counselors, clients can change lives, but they can gain a better understanding, confidence, self-efficacy and problem-solving skills to support and help.
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Keywords: Clinical Psychologist, Counseling

Introduction:

Clinical psychology is an integration of science, theory and clinical knowledge for the purpose of understanding, preventing, and relieving psychologically-based distress or dysfunction and to promote subjective well-being and personal development. Central to its practice are psychological assessment and psychotherapy, although clinical psychologists also engage in research, teaching, consultation, forensic testimony, and program development and administration. In many countries, clinical psychology is a regulated mental health profession.

The field is often considered to have begun in 1896 with the opening of the first psychological clinic at the University of Pennsylvania by Lightner Witmer. In the first half of the 20th century, clinical psychology was focused on psychological assessment, with little attention given to treatment. This changed after the 1940s when World War II resulted in the need for a large increase in the number of trained clinicians. Since that time, two main educational models have developed—the Ph.D. scientist–practitioner model (requiring a doctoral dissertation and therefore research as well as clinical expertise); and the Psy.D. practitioner–scholar model.

Clinical psychologists are now considered experts in providing psychotherapy, psychological testing, and in diagnosing mental illness. They generally train within four primary theoretical orientations—psychodynamic, humanistic, behavior therapy/cognitive behavioral, and systems or family therapy. Many continue clinical training in post-doctoral programs in which they might specialize more intensively in disciplines such as psychoanalytic approaches, or child and adolescent treatment modalities. Clinical psychologists can offer a range of professional services, including:

- Administer and interpret psychological assessment and testing
- Conduct psychological research
- Consultation (especially for multi-disclinary teams in mental health settings, such as psychiatric wards and increasingly other healthcare settings, schools and businesses)
- Development of prevention and treatment programs
- Program administration
- Provide expert testimony (forensic psychology)
- Provide psychological treatment (psychotherapy)
- Teach

Assessment:

An important area of expertise for many clinical psychologists is assessment, and there are indications that as many as 91% of psychologists utilize this core clinical practice. Such evaluations are usually conducted in order to gain insight into, and form hypotheses about,

psychological or behavioral problems. As such, the results of these assessments are often used to clarify a person's diagnosis and assist in planning treatments or arranging for services. Methods used to gather information include formal tests, clinical interviews, reviews of past records, and behavioral observations. There exist literally hundreds of various assessment tools, although only a few have been shown to have both high validity (i.e., test actually measures what it claims to measure) and reliability (i.e., consistency). These measures generally fall within one of several categories, including the following:

- **Intelligence & achievement tests** – These tests are designed to measure certain aspects of cognitive functioning (often referred to as IQ) in comparison to a group of people with similar characteristics (such as age or education). These tests, including the WISC-IV and WAIS-IV, attempt to measure traits such as general knowledge, verbal comprehension, working memory, attention/concentration, logical reasoning, and visual/spatial perception. Several of these tests have been shown to accurately predict scholastic achievement and occupational performance, and help to identify a person's cognitive strengths and weaknesses.
- **Personality tests** – These tests aim to describe patterns of behavior, thoughts, and feelings, and generally fall within two categories: objective and projective. Objective measures, such as the MMPI-2 or the MCMI-III, are based on forced-choice responses—such as yes/no, true/false, or a rating scale—and generate scores that can be compared to a normative group. Projective tests, such as the Rorschach inkblot test, use open-ended responses, often based on ambiguous stimuli, to reveal non-conscious psychological dynamics such as motivations and perceptions of the self and the world.
- **Neuropsychological tests** - Tests in this category are often used to evaluate a person's cognitive functioning and it's relationship to a person's behavior or psychological functioning. They are used in a variety of settings, for purposes such as clarifying a diagnosis (especially in distinguishing between psychiatric and neurological symptoms), better understanding the impact of a person's neurological condition on their behavior, treatment planning (especially in rehabilitation settings), and for legal questions, such as determining if a person is faking their symptoms (also referred to as malingering) or if they are capable to stand trial.
- **Clinical interviews** – Clinical psychologists are also trained to gather data by observing behavior and collecting detailed histories. The clinical interview is a vital part of assessment, even when using other formalized measures, as it provides a context in which to understand test results. Psychologists can employ a structured format (such as the SCID or the MMSE), a semi-structured format (such as a sequence of questions) or an unstructured format to gather information about a person's symptoms and past and present functioning. Such assessments often include evaluations of general appearance and behavior, mood and affect, perception, comprehension, orientation, memory, thought process, and/or communication.

Clinical Psychology - one of the Registered Practitioner Psychology Professions

Psychology is the scientific study of human thought and behaviour. Many people are interested in psychology, and in fact it is one of the most popular degrees that can be studied at university. But having a degree in psychology is not the same as being a Registered Practitioner Psychologist. A Registered Psychologist is a legally regulated professional who has a postgraduate qualification in the application of psychological science to a particular issue.

There are currently seven types of Practitioner Psychologists:

- Clinical Psychologists
- Counselling Psychologists
- Educational Psychologists
- Forensic Psychologists
- Health Psychologists
- Occupational Psychologists
- Sports & Exercise Psychologists

Clinical psychologists and psychiatrists often work in the same clinics and see people with similar problems, but there is a clear difference between them.

Clinical psychologists' key role is to consider what the science of psychology tells them about how to help with the problem. And psychiatrists' key role is to consider what the science of medicine tells them about how to help with the problem.

They are each able to do this because the first stage in training as a clinical psychologist is a degree in psychology, whereas the first stage in training as a psychiatrist is a degree in medicine.

So if you are depressed, for instance, a psychiatrist is best placed to help you think about whether a biological

treatment like antidepressant drugs may help. And a clinical psychologist is best placed to help you think about whether a psychological therapy like cognitive behavioural therapy may help.

Clinical psychologists will usually be able to help you see a psychiatrist if that would be helpful for your problem, and psychiatrists will usually be able to help you see a clinical psychologist if they would be more able to help you with your problem.

GRADUATE PROFESSIONAL PROGRAM

General Principles. The general principles which underlie the graduate program appear to us of primary importance -- in fact much more important than the details of the program. If clarity in the formulation of goals exists, there should be relatively little difficulty about agreeing on the means for implementing them. As has already been indicated, it is the opinion of the Committee that the setting up of a detailed program is undesirable. Such a step, if accepted generally, would go far in settling clinical psychology at a time when it should have great lability. Considerable experimentation with respect to the personality and background of students as well as the content and methods of courses will for a long time be essential if we are to develop the most adequate program. Our aims are rather to achieve general agreement on the goals of training and encourage experimentation on methods of achieving these goals and to suggest ways of establishing high standards in a setting of flexibility and reasonable freedom. We also hold that the goals should not be determined by special situations and special demands, but should be oriented toward the question of what is the best training for the clinical psychologist. Against this general background the principles which we consider important are the following:

1. A clinical psychologist must first and foremost be a psychologist in the sense that he can be expected to have a point of view and a core of knowledge and training which is common to all psychologists. This would involve an acquaintance with the primary body of psychological theory, research, and methods on which further training and interdisciplinary relationships can be built.

2. Preparation should be broad; it should be directed to research and professional goals, not to technical goals. Participants should receive training in three functions: diagnosis, research and therapy, with the special contributions of the psychologist as a research worker emphasized throughout. Although many will probably tend to specialize in one or another of these areas after obtaining the degree, the Committee feels strongly that them should be training in each of these areas during the graduate period. We are particularly concerned that training shall be of such a quality as to eliminate the possibility that a technician, whether in the sense of a directive or nondirective counselor, a Multiphasic specialist, a Binet tester, a Rorschach specialist, or a remedial instructor, will be turned out as a clinical psychologist, and so depended upon for a range of work he will be unable to do.

3. In order to meet the above requirements the program calls for study in six major areas: a. General psychology; b. Psychodynamics of behavior; c. Diagnostic methods; d. Research methods; e. Related disciplines; f. Therapy. Such a program should go far towards reducing the dangers inherent in placing powerful instruments in the hands of persons who are essentially technicians, persons who from the standpoint of the academic group have no real foundation in a discipline, and who from the standpoint of the clinical group have no well-rounded appreciation of the setting in which they function.

4. The specific program of instruction should be organized around a careful integration of theory and practice, of academic and field work, by persons representing both aspects. Just as there is great danger, in the natural revolt against "academic" dominance, of ending up with a "practical" program, so is there danger in the continued dominance of the academy. It is important to break down the barriers between the two types of approach and through their smooth integration impress the student with the fact that he is taking one course of training provided by one faculty.

5. Through all four years of graduate work the student should have contact, both direct and indirect, with clinical material. This can be accomplished in the theoretical courses through the constant use of illustrative case material with which the instructor has had personal contact. The student should from the first year be provided with opportunities for actual contact with human material in naturalistic, test, and experimental situations in the setting of practicum, clerkship, and internship. Throughout, the effort should be made to maintain and to build upon that most valuable quality, the naive enthusiastic interest in human beings with which the student first comes into the program.

Conclusion:

Every day clinical psychologists help a wide range of people of all ages with all sorts of problems. Some have particular emotional or mental health problems, such as depression or schizophrenia. Others have difficulties with their thinking (also known as 'cognitive' problems). These can take many forms, such as problems with memory or perception after a head injury, a learning disability or dementia. There are many more areas of life where a clinical psychologist can help. These could include helping people manage and live with health conditions such as HIV, cancer or chronic pain, assisting people who have difficulties in maintaining relationships or providing advice about how to care for a child who has been abused.

Whatever the problem, the clinical psychologist will consider what scientific research says about its probable cause and what will be likely to help. Sometimes the clinical psychologist will be the one who then provides the help. Examples of this include seeing the person for a number of sessions to provide psychological therapy or giving advice on how to manage memory problems.

And sometimes the clinical psychologist will recommend other people who can help, perhaps advising them on the best way forward for the client. Clinical psychologists are trained by the NHS, just like doctors and nurses, and most work there too.

Registered clinical psychologists have a degree in psychology plus an additional three to five years of postgraduate experience and university training in applying the science of psychology to clinical problems. It therefore takes six to eight years to qualify as a Registered clinical psychologist, and the qualification that Registered clinical psychologists now obtain is a doctorate in clinical psychology. However, not all of these people are registered to practise clinically; for example, some might be academics or teachers. If you want to check whether someone is registered to practise clinically, you need to check with the Health Professions Council.

References:

1. Alessandri, M.; Heiden, Lynda (ed.); Dunbar-Welter, M. (1995). "History and overview". Introduction to clinical psychology. New York, NY: Plenum Press. ISBN 0-306-44877-7.
2. Compas, Bruce; Gotlib, Ian (2002). Introduction to clinical psychology. New York, NY: McGraw-Hill Higher Education. ISBN 0-07-012491-4.
3. Evans, Rand (1999). "Clinical psychology born and raised in controversy". APA Monitor (American Psychological Association) **30** (11).
4. Routh, Donald (2000). "Clinical psychology training: A history of ideas and practices prior to 1946". American Psychologist **55** (2): 236.
5. Hall, John; Llewelyn, Susan (2006). What is clinical psychology (4th ed.). United Kingdom: Oxford University Press. ISBN 0-19-856689-1.
6. Henry, David (1959). "Clinical psychology abroad". American Psychologist **14** (9): 601–04.
7. Murray, Bridget. (2000). The degree that almost wasn't: The PsyD comes of age. Monitor on Psychology, 31(1).
8. APA. (2005). Guidelines and Principles for Accreditation of Programs in Professional Psychology: Quick Reference Guide to Doctoral Programs.
9. Michalski, Daniel; Kohout, Jessica; Wicherski, Marlene; Hart, Brittany (June 2011). "2009 Doctorate Employment Survey". APA Center for Workforce Studies. APA. Retrieved December 16, 2011.
10. "Match--FAQs--Applicants--Eligibility and Participation--Should I consider attending a non-accredited or non-APPIC member internship program?". appic.org. Association of Psychology Postdoctoral and Internship Centers. Retrieved January 15, 2012.
11. Widiger, Thomas; Trull, Timothy (2007). "Plate tectonics in the classification of personality disorder: Shifting to a dimensional model". American Psychologist **62** (2): 71–83.
12. Mundt, Christoph; Backenstrass, Matthias (2005). "Psychotherapy and classification: Psychological, psychodynamic, and cognitive aspects". Psychopathology **38** (4): 219.
13. Clay, Rebecca (January 2011). "Revising the DSM". apa.org. Retrieved 15 January 2012.

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Effect of intra-operative topical tetracycline versus 5-fluorouracil in prevention of post-mastectomy seroma in rabbit model

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Abstract: ***Background.*** Seroma is most common post-mastectomy and axillary dissection (AD) complication. This study aimed to evaluate and compare efficacy of tetracycline (TCN) or 5-fluorouracil (5-FU) intra-operative topical application in preventing post-mastectomy seroma development in rabbits. ***Material and methods.*** Forty adult female albino rabbits divided into 3 groups [control (n=12), TCN (n=14), 5-FU (n=14)] were used. Rabbits underwent unilateral thoracic mastectomy and AD. Immediately following operation, equal volumes of saline, TCN (5-10mg/kg b.wt), 5-FU (12mg/kg b.wt) were instilled under surgical skin flaps. On seventh post-operative day, seroma formation and wound healing processes were evaluated. Seroma fluid was aspirated and evaluated for sodium, potassium, chloride, total proteins levels. Histological examination of dissecting area was made. ***Results.*** Seroma developed in 22 rabbits (55.0%); 7 in saline (58.3%), 9 (64.3%) in TCN, 6 (42.9%) in 5-FU groups. Seroma fluid volume was lowest in 5-FU group. Seroma fluid nature was inflammatory exudate. Histopathological examination revealed lack of walling, inflammatory cellular infiltrate, blood extravasations, tissue necrosis in all groups with least changes in 5-FU group**.** ***Conclusions.*** Inflammatory cell exudate was common feature of rabbit with post-mastectomy seroma. 5-FU was best in decreasing seroma volume and minimizing histopathological changes in post-mastectomy wound healing.
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Key words: 5-Fluorouracil; Histopathology; Post-mastectomy; Rabbit; Seroma**;** Tetracycline.

1. Introduction

Seroma is most common problem occurring after mastectomy. It is accumulation of serous fluid under skin flaps following mastectomy or in axillary dead space immediate post-operative period. Seroma incidence varied from 10% to over 85% [1]. Most surgeons view it as necessary evil rather than complication, as it usually resolves within few weeks. However, excessive fluid accumulation will stretch skin and resulting in patient discomfort and prolonged hospital stay. Morbidities related to seroma include wound site infections, flap necrosis, wound dehiscence, and delay in adjuvant treatment [2].

Seroma formation prevention has become most important goal, due to lack of effective treatment. In all methods, the main preventive strategy is to reduce lymphatic leakage and obliterate dead space, including use of ultrasound scissors in performing lymphadenectomy, closed suction drainage, repeated aspirations, external compression on flaps, dead space closure by multiple-sutured flaps to underlying muscles, increasing collagen synthesis using several sclerosing agents at dissection areas, therefore fixing skin flaps to underlying muscles. Most of aforementioned methods have failed but some sclerosing agents, such as fibrin glue or fibrin sealant; have been found to be effective for seroma prevention. Nonetheless, agents have given rise to some side effects and cost-effective disadvantages [3]. Tetracycline (TCN) is antibiotic that proved to have potent sclerosing agent by enhancing fibroblast collagen synthesis. TCN was used for malignant pleural effusions [4], endometrial cysts [5], hepatic cysts [6], pericardial effusions [7], gallbladder lumen [8]. Two reports have suggested TCN efficacy, as topical sclerosing agent, in post-mastectomy seromas [9,10]. 5-Flourouracil (5-FU) is commonly used chemotherapeutic agent in breast cancer. Studies have shown that intra-peritoneal administration of 5-FU increases adhesion formation [11].

Based on these reports, an experimental trial was carried out to evaluate and compare preventive effects of intra-operative topical application of TCN and 5-FU, as adhesion-inducing agents in rabbit mastectomy model. The possible mechanisms of TCN and 5-FU actions were also investigated.

2. Material and Methods

The study protocol was approved by Medical Ethical Committee of King Fahed Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. Forty female albino rabbits (3-4 months old); weighing between 2.50-4.50 kg were used. All animals were fed standard food and water (*Ad libitum* supplied by animal care unit in KFMRC). Rabbits were operated under Ketamin [Ketalar 50mg/ml, EspecIalidad USO Hospital Ario] and Seton 2% [EspecIalidad USO Veterinario]. Unilateral pectoral

modified mastectomy procedure was done as described previously [12]. Elliptical vertical incision around nipple of pectoral breast was made. Nipple with surrounding skin was discarded. Humeral attachment of pectorals muscle was ligated with 3/0 Polyglactin [Vicryl, Ethicon J&J]. The dissected breast together with small part of pectorals muscle, part of axillary fat, and associated lymph nodes were removed. The underneath of skin flap was scraped by surgical blade blunt side to traumatize subcutaneous lymph vasculature. Then mastectomy site was washed with normal saline and dried with sterile gauze.

Operated animals were divided into three groups according to solution immediately added to post-mastectomy dead space prior to skin closure. Control group (n=12), rabbits underwent unilateral mastectomy and 3cc saline was added to post-mastectomy dead space. In TCN group (n=14), rabbits underwent unilateral mastectomy and 1.2cc TCN diluted in 1.8cc saline (5-10 mg/kg) was added to post-mastectomy dead space. In 5-FU group (n=14), rabbits underwent unilateral mastectomy and 0.8cc 5-FU diluted in 2.2cc saline (12 mg/kg) was added to post-mastectomy dead space. TCN and 5-FU doses for rabbits were calculated as **Paget and Barncs** [13].

All mastectomy wounds were vertically closed with 3/0 polyglactin [Vicryl, Ethicon J&J] continuous subcuticular stitches to prevent wound self dehiscence. Operated animals were observed post-operatively for 2 hours. Visual inspection was done daily for arm movements or wound complications.

On seventh day post-operatively, mastectomies area were inspected and palpated for swelling. Those suspected to having seroma were aspirated using (10 ml syringe) from most dependant axillary region. Dead space was opened and further aspiration of remaining fluid. Aspirated fluid was inspected for any turbidity, blood stain or suspended particles. Aspirated fluid characteristics were recorded. Biochemical analysis (total protein and electrolytes as sodium, potassium, chloride) of seroma fluid was done in biochemistry laboratory at King Abdulaziz University hospital.

Following seroma aspiration, animals were sacrificed with high-dose of ether anesthesia. Skin flaps and underlying tissues mastectomy region were dissected including axilla and thoracic wall, and fixed in 10% formalin. Formalin-fixed tissues were embedded in paraffin, cut into 5 µm thick slices and stained with hematoxylin and eosin for histopathological examination and Massion Trichrome staining of collagen fibers**.** Inflammatory cells in post-mastectomy seroma region were counted in five fields in each of five slides of serial sections using Image Pro-Express version 6 of studied photographs to confirm histological observations.

Statistical analysis. Data were analyzed by Statistical Package for Social Science (SPSS Inc., Chicago, IL) version 16.0. Quantitative data were expressed as mean +/- stander deviation (SD), and qualitative data as number & percentage. One Way ANOVA test was used for quantitative variable while Chi square test for qualitative variables. Significance (*P*) level was accepted at <0.05.

3. Results

Percentage of animals that developed seromas in saline, TCN and 5-FU groups were 58.3%, 64.3% and 42.9%, respectively with insignificance difference between them *(P* <0.727). The percentage of serous and serosanguineous seroma fluid were in saline (71.4%, 28.6%), in TCN (55.6%, 44.4%) and in 5-FU groups (54.5%, 45.5%), with insignificance difference between them *(P* <0.387). There was insignificance difference in amount of fluid aspirated from rabbits of saline, TCN, 5-FU groups (*P* >0.05). However, seroma development in 5-FU group was least in volume (Table 1).

There were insignificant difference in sodium, potassium, chloride and total proteins levels in seroma fluid of different studied groups (*P* >0.05) (Table 2).

Seroma of all groups showed marked inflammatory cellular aggregation. Dead space wall lacked fibrous sealing with dominating capillary dilation and vascular congestion in most seroma cases (Figs 1A, C, E). Extravasations of red and inflammatory cells in underlying healed tissue were observed. Inflammatory cells were mainly lymphocytes and eosinophils. All aforementioned findings were observed with lesser extent in 5-FU group (Fig 1F).

Non-seroma cases regardless of therapy type showed reduced dead space, free from cell debris or inflammatory cells. Floor was well delineated with either flat closely endothelial like cells or collagen deposit. Underlying floor tissue showed nearly mature fibrous scar with few inflammatory cells mainly lymphocytes (Figs 1B, D, F). Pro-image analysis of studied photographs confirmed histological observations where there was extremely significant decrease of inflammatory cells in post-mastectomy region of seroma rabbits treated by 5-FU when compared to saline and TCM groups (Table 3). Regards therapeutic regimen efficacy, it was found that 5-FU provided most satisfactory results.

Table (1): Comparison regarding seroma in different studied groups

Items	**Saline** (n=12)	**Tetracycline** (n=14)	**5-Fluorouracil** (n=14)	**Total** (n=40)	**Significance**
Seroma development	7 (58.3%)	9 (64.3%)	6 (42.9%)	6 (42.9%)	*P* <0.727
Aspirated fluid amount (ml)	6.64±8.06 (0.50-21.00)	6.61±7.58 (1.00-26.00)	2.17±1.51 (0.50-4.00)	-	**P* >0.993 ***P* >0.247 ****P* >0.226
Fluid type					
Serous	5 (71.4%)	5 (55.6%)	2 (33.3%)	12 (54.5%)	
Serosanguineous	2 (28.6%)	4 (44.4%)	4 (66.7%)	10 (45.5%)	*P* <0.387

Data was expressed as mean +/- stander deviation and range or number (%) as appropriate. *P:* significance between groups; **P*: significance between saline and tetracyclin; ***P*: significance between saline and 5-fluorouracil; ****P*: significance between tetracyclin and 5-fluorouracil.

Table (2): Comparison between seroma fluid components in different groups

Groups	**Sodium** (mmol/L)	**Potassium** (mmol/L)	**Chloride** (mmol/L)	**Total proteins** (g/L)
Saline (n=7)				
Mean±SD	139.57±2.88	5.11±0.95	105.71±3.77	44.86±6.82
Range	(134.00–142.00)	(4.10–6.90)	(102.00–113.00)	(36.00–53.00)
Tetracycline (n=9)				
	136.67±12.04	4.71±0.66	103.44±5.92	47.56 ±9.81
	(105.00–143.00)	(3.80–5.80)	(91.00–110.00)	(40.00–72.00)
	P >0.499	*P* >0.306	*P* >0.377	*P* >0.498
5-Fluorouracil (n=6)				
	138.17±4.91	5.03±0.64	102.50±4.59	47.50±4.28
	(129.00–141.00)	(4.40–6.20)	(94.00–106.00)	(44.00–55.00)
	P >0.766	*P* >0.850	*P* >0.260	*P* >0.547
	**P* >0.738	**P* >0.432	**P* >0.723	**P* >0.989

P: signifiance versus saline; **P*: signifiance versus tetracycline.

Table (3): Comparison between inflammatory cells numbers of seroma and non-seroma specimens in each group and between groups

Treatment	**Seroma**	**Non seroma**	**Significance**
Saline	35.00±3.00	14.00±4.00	*P* <0.001
Tetracycline	43.00±7.00	13.00±1.00	*P* <0.001 **P* <0.003
5-Fluorouracil	19.00±7.00	13.00±3.00	*P* <0.004 **P* <0.001 ***P* <0.001

P: Seroma versus non-seroma of same group.
**P*: Significance versus saline. ***P*: Significance versus tetracycline

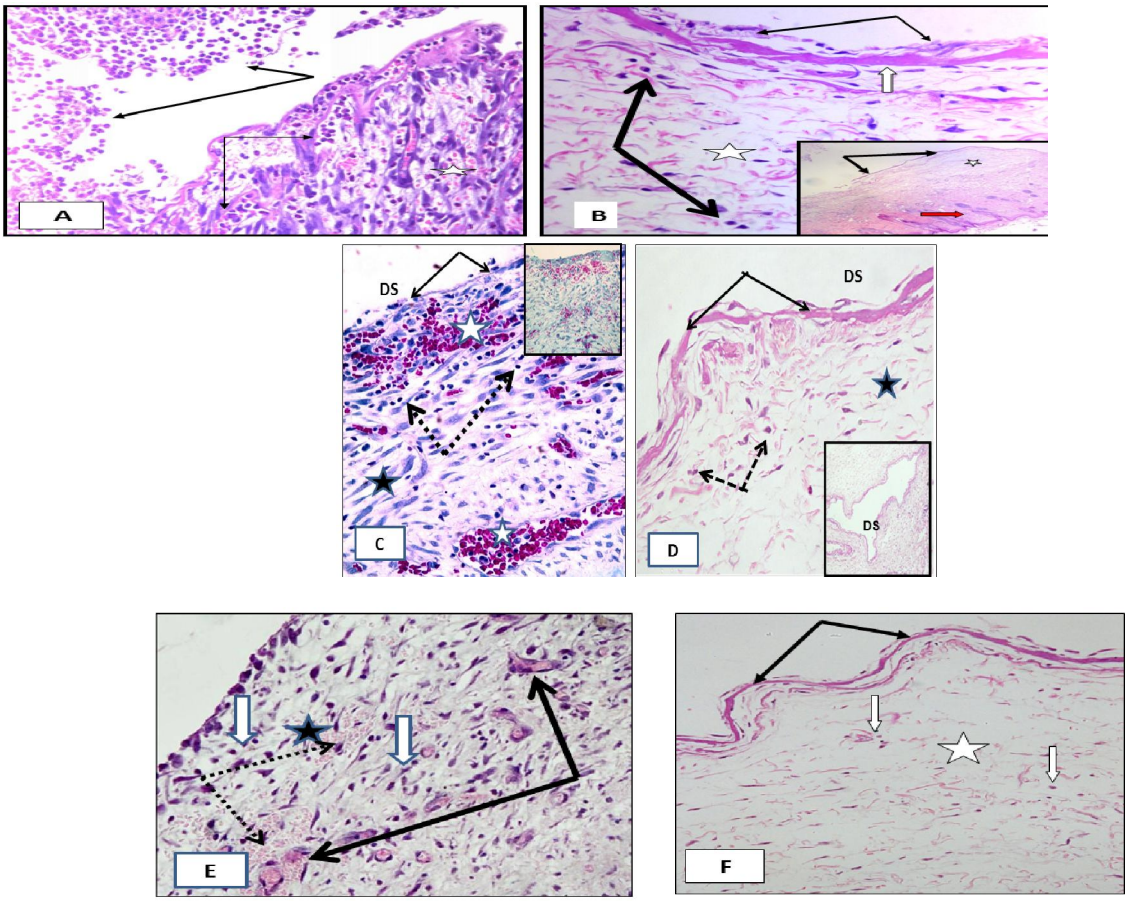

Figure (1): Histopathological changes in breast tissue
A: Saline treated seroma case showed marked cellular infiltration mainly eosinophils in dead space (thick arrows) and seroma floor (thin arrows).
B: Non-seroma case showed few cells, less granulation tissue (star) and attempt to walling (black arrows) in insert and collagen deposition (white arrow) (H&E X400).
C: Sections from tetracycline treated post-mastectomy seromas stained by Masson for collagen and H&E (insert) showed dominating of granulation tissue (black star), lymphocyte infiltrate (dotted arrows), dilated congested vessels and blood extravasations (white star).
D: Non-seromas case showed small dead space (insert), less granulation tissue (black star), few mononuclear leucocytes (dotted arrows), and collagen deposition delineates dead space (black arrows).
E: Dead space floor of post-mastectomy seroma from 5-fluorouracil group showed numerous congested capillaries (black arrows), high cellularity (star)
F: Non-seroma of same group showed fibrous sealing (black arrows), few lymphocytes (vertical white arrow) and absence of vascular congestion (H&E stain X200).

4. Discussion

Wound seromas represent significant cause of morbidity as retard wound healing, promotes wound infection and delays adjuvant therapy initiation. Management of these seroma in outpatient department causes additional burden on nursing staff and hospital resources. In this study, chemical and cellular examination of aspirated seroma fluid reveals its similarity to serum. Total protein, sodium; potassium; chloride levels were different from lymph but similar to inflammatory exudate suggesting its inflammatory origin. Similarly, **Watt-Boolsen *et al.*** [14] demonstrated that seroma is exudate resulting from acute inflammatory reaction, and concluded that seroma formation reflects increased intensity and prolongation of wound repair. **McCaul *et al.*** [15] demonstrated that drainage fluid similar to inflammatory exudate. Meanwhile, others [14] hypothesized that seroma is mostly originate from lymph.

Although several techniques have been reported for seroma formation prevention, there is no standard clinical approach in routine practice. Use of sclerosant in seroma management is not new. In humans,

seromadesis has been reported with erythromycin [16], povidone iodine [17], talc [18] and hypertonic saline [19]. Tetracycline sclerotherapy can administrate in two ways, first topically to chest wall and skin flaps prior to skin closure [20], while second is to aspirate seroma postoperatively and instill tetracycline after its dilution in 0.9% NaCL [9]. The present study revealed that at 7th day post-mastectomy, seroma developed in all studied groups (saline 58.3%, TCN 64.3%, 5-FU 42.9%). Meanwhile, volume of aspirated seroma fluid was least in 5-FU group. Wide dead space, fibrous walling lack, vascular congestion and inflammatory cellular infiltrate mainly by lymphocytes and eosinophils were most histopathological features in post-mastectomy seroma developed in TCN group. In 1986; **McCarthy *et al.*** [21] designed controlled trial to study tetracycline effects on patients with prolonged drainage after mastectomy. Trial was aborted as tetracycline was painful and not effective. In 2000, researchers at Mayo Clinic [20] used tetracycline (1g TCN in 100 cc 0.9% NaCL) during surgery (intra-operative) as prophylaxis; however, this trial was stopped because 2 weeks postoperatively, tetracycline group had more seromas than saline group (53% vs. 22%, P <0.01). Although **Sitzmann *et al.*** [9] reported good results of sclerotherapy after mastectomy; their study was retrospective and anecdotal in only 5 patients. In **Hokkam *et al.*** study [22], treatment of 49 patients with post-mastectomy seromas by aspiration and sclerosant solution instillation containing tetracycline (2g tetracycline dissolved in 100ml NaCL 0.9% +10ml lidocaine). They reported that thirty six patients (73.4%) were successfully treated with one sclerotherapy session while nine patients (18.4%) needed two sessions and four patients (8.2%) needed three sessions. Majority of patients (85.7%) had no complications after technique.

In this study, intra-operative application of 5-FU leads to reduction in number of rabbit models that developed post-mastectomy seroma compared to saline and TCN groups (42.9% versus 58.3%, 64.3%). Also, amount of aspirated seroma fluid was insignificantly lower in 5-FU compared to other groups. Histological examination of seroma area in 5-FU group reveals decrease dead space, vascular congestion, inflammatory cells infiltration, enhances healing and normal scaring process. Similarly, **Kocdor *et al.*** [23] reported that 5-FU decreased angiogenesis, vascular congestion and inflammatory cells in seroma of rat model. It has been suggested that vasodilatation is obvious during inflammation period of wound healing and event for cellular efflux and fluid extravasation [24]. Eosinophils predominance observed in present samples was mostly had role in vascular dilatation and fluid efflux. Allergic reaction to iodine was reported to be among etiological factors [25]. The decrease in cellular infiltrate and fibroblast proliferation observed in present study may be attributed to tumoricidal effect of 5-FU [2].The mechanisms underlying seroma prevention by 5-FU remain unclear. But possible explanation can be due to decrease in inflammatory cells may underline decrease in seroma volume via decreasing cytokines. **McCaul *et al.*** [15] reported that seromas contain significantly more granulocytes and monocytes than lymphocytes. It has been shown that seroma contains IgG, granulocytes, leukocytes, which are normally present in acute inflammatory exudates [26]. Another important finding of this study is decreased angiogenesis and vascular congestion in 5-FU rabbits. Decreased angiogenesis and vasodilation may reduce fluid efflux from capillary bed towards dissection surfaces. Cytokines, prostaglandins, vasoactive amines and angiogenic factors released from acute inflammatory cells play important role in vasodilation and angiogenesis [24]. Angiogenesis role on seroma formation were investigated by **Wu *et al.*** [25] who examined vascular endothelial growth factor (VEGF) and endostatin levels in wound fluid and plasma of patients mastectomy patients. Especially in early postoperative period, significantly higher VEGF and lower endostatin levels were detected in seroma fluid compared with plasma which suggested that angiogenesis is initiated immediately after surgery and wound fluid is more informative than blood. On contrary, 5-FU, as antimetabolite, is able to inhibit VEGF-mediated angiogenesis [27]. Therefore, in present study, decreased angiogenesis or vascular congestion may be contributing factor for seroma prevention. 5-FU may significantly affect some microvessel network patterns such as interconnective loop formation or microvessel branching in dose dependent fashion [28]. In present study, 5-FU was applied directly to healing tissue that may produce significant vascular cell toxicity and flap ischemia and necrosis. Thus, applications of diluted 5-FU diminish ischemic complications at wound sites.

5. Conclusion.

Post-mastectomy seroma is common complication. Seroma fluid nature was inflammatory exudate. Intra-operative application of 5-FU was superior to TCN in decreasing incidence and the amount of post-mastectomy seroma fluid. The mechanism of action could be via decreasing inflammatory cells infiltrate or decreasing vasodilatation and angiogenesis or promoting fibrous walling. Further study is going on to investigate cytokines role and vasoactive mediators in seroma formation so an appropriate antidote could be applied for its prevention.

6. References

1. Kuroi K, Shimozuma K, Taguchi T, Imai H, Yamashiro H, Ohsumi S, Saito S (2005): Pathophysiology of seroma in breast cancer. Breast Cancer; 12(4):288–293.
2. Woodworth PA, McBoyle MF, Helmer SD, Beamer RL (2000): Seroma formation after breast cancer surgery: incidence and predicting factors. Am Surg.; 66(5):444–450.
3. Jain PK, Sowdi R, Anderson ADG, MacFie J (2004): Randomized clinical trial investigating the use of drains and fibrian sealant following surgery for breast cancer. Br J Surg.; 91(1):54–60.
4. Hausheer FH, Yarbro JW (1985): Diagnosis and treatment of malignant pleural effusions. Semin Oncol; 12(1):54-75.
5. Chang CC, Lee HF, Tsai HD, Lo HY (1997): Sclerotherapy — An adjuvant therapy to endometriosis. Int J Gynaeol Obstet; 59(1):31–34.
6. Davies CW, McIntyre AS (1996): Treatment of a symptomatic hepatic cyst by tetracycline hydrochloride instillation sclerosis. Eur J Gastroenterol Hepatol; 8(2):173–175.
7. Celermajer DS, Boyer MJ, Bailey BP, Tattersall MH (1991): Pericardiocentesis for symptomatic malignant pericardial effusion: A study of 36 patients. Med J; 154(1):19–22.
8. El-Mufti M (1993): Sclerotherapy of the human gallbladder using ethanol and tetracycline hydrochloride. Br J Surg.; 80(7):916.
9. Sitzmann JV, Dufresne C, Zuidema GD (1983): The use of sclerotherapy for treatment of postmastectomy wound seromas. Surgery; 93(2):345–347.
10. Nichter LS, Morgan RF, Dufresne CR (1983): Rapid management of persistent seromas by sclerotherapy. Ann Plast Surg.; 11(3):233-236.
11. El-Malt M, Ceelen W, Van den Broecke C, Cuvelier C, Van Belle S, de Hemptinne B, Pattyn P (2003): Influence of neo-adjuvant chemotherapy with 5-fluorouracil on colonic anastomotic healing: experimental study in rats. Acta Chir Belg.; 103(3):309-314.
12. Lindsey WH, Masterson TM, Spotnitz WD, Wilhelm MC, Morgan RF (1990): Seroma prevention using fibrin glue in a rate mastectomy model. Arch Surg.; 125(3):305-307.
13. Paget GE, Barnes JM (1964): Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. In: Laurence DR, Bacharach AL (eds) Evaluation of drug activities Pharmacometrics. London and New York, Academic press: 160-162.
14. Watt-Boolsen S, Nielsen VB, Jensen J, Bak S (1989): Postmastectomy seroma. A study of the nature and origin of seroma after mastectomy. Dan Med Bull; 36(5):487–489.
15. McCaul JA, Aslaam A, Spooner RJ, Louden I, Cavanagh T, Purushotham AD (2000): Etiology of seroma formation in the patients undergoing surgery for breast cancer. Breast; 9:144–148.
16. Kafali H, Yurtseven S, Atmaca F, Ozardali I (2003): Management of non-neoplastic ovarian cysts with sclerotherapy. Int J Gynaecol Obstet.; 81(1):41–45.
17. Throckmorton AD, Askegard-Giesmann J, Hoskin TL, Bjarnason H, Donohue JH, Boughey JC, Degnim AC. (2008): Sclerotherapy for the treatment of postmastectomy seroma. Am J Surg.; 196(4):541–544.
18. Saeb-Parsy K, Athanassoglou V, Benson JR (2006): Talc seromadesis: a novel technique for the treatment of chronic seromas following breast surgery. Breast J; 12(5):502–504.
19. Gruver DI (2003): Hypertonic saline for treatment of seroma. Plast Reconstr Surg.; 112(3):934.
20. Rice DC, Morris SM, Sarr MG, Farnell MB, van Heerden JA, Grant CS, Rowland CM, Ilstrup DM, Donohue JH (2000): Intra-operative topical tetracycline sclerotherapy following mastectomy: A prospective, randomized trial. Journal of Surgical Oncology; 73(4):224–227.
21. McCarthy PM, Martin JK, Wells DC, Welch JS, Ilstrup DM (1986): An aborted, prospective, randomized trial of sclerotherapy for prolonged drainage after mastectomy. Surg Gynecol Obstet.; 162(5):418–420.
22. Hokkam E, Farrag S, El Kammash S (2009): Tetracycline sclerotherapy in treating postmastectomy seroma: A simple solution for a frequently occurring problem. Egyptian Journal of Surgery; 28:99–104.
23. Kocdor MA, Kilic Yildiz D, Kocdor H, Canda T, Yilmaz O, Oktay G, Harmancioglu O (2008): Effects of locally applied 5-fluorouracil on the prevention of postmastectomy seromas in a rat model. Eur Surg Res.; 40(4):256–262.
24. Lorenz HP, Longaker MT (2001): Wounds: biology, pathology, and management. In: Norton JA, Bollinger RR, Chang AE, et al (eds) Surgery, Basic Science and Clinical Evidence. New York: Springer,:221–239.
25. Wu FPK, Hoekman K, Meijer S, Cuesta MA (2003): VEGF and endostatin levels in wound fluid and plasma after breast surgery. Angiogenesis; 6(4):255–257.
26. Bacilious N, Kulber DA, Peters ED, Gayle LB, Chen MJ, Harper AD, Hoffman L (1995): Harvesting of the latissimus dorsi muscle: a small animal model for seroma formation. Microsurgery; 16(6):646–649.
27. Basaki Y, Chikahisa L, Aoyagi K, Miyadera K, Yonekura K, Hashimoto A, Okabe S, Wierzba K, Yamada Y (2001): Gamma-hydroxybutyric acid and 5-fluorouracil, metabolites of UFT, inhibit the angiogenesis induced by vascular endothelial growth factor. Angiogenesis; 4(3):163–173.
28. Lennernas B, Albertsson, Lennernas H, Norrby K (2003): Chemotherapy and antiangiogenesis: drug-specific effects on microvessel sprouting. Acta Oncol.; 42(11):294–303.

Genetic Diversity in Faba Bean (*Vicia faba* L.) Using Inter-Simple Sequence Repeat (ISSR) Markers and Protein Analysis

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Abstract: The present study aims to assess the genetic diversity among 10 varied faba bean cultivars, collected from two wide-ranging geographical locations of Egypt. Variability based on the DNA level was inspected through nine ISSR-PCR screening, which showed obvious differences among the various *Vicia faba (V. faba)* cvs. A total of 576 ISSR loci were detected and 398 (69.10%) of them were polymorphic, which represent a relatively high polymorphism level. Cluster analysis via ISSR markers separated three green large-seeded cvs. (*V. faba* var. major) from dry small-seeded cvs. (*V. faba* var. minor). The small-seeded cvs. were further classified into two sub-clusters according to two geographic locations. The first sub-cluster included dry small-seeded cvs. grown under clay soil conditions (Abies location). However, the second sub-cluster integrated the similar dry small-seeded cvs. but were grown under calcareous soil conditions (Fuka location). SDS-PAGE analysis of various faba bean leaf proteins reflected some variations among studied *V. faba* populations. The results clarified that ISSR markers and protein analysis were helpful to recognize genetic variation among faba bean cultivars.
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Key words: Cluster analysis, Egypt, faba bean, genetic diversity, ISSR markers, protein analysis.

1. Introduction

Faba bean, broad bean or field bean (*V. faba* L.; 2n = 12) is a major food and feed grain legume owing to the high nutritional value of its seeds, which are rich in protein 27-34% (**Link *et al*., 1995; Duc, 1997**). It is considered as one of the major sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people cannot afford meat sources of protein (**Duc, 1997; Alghamdi, 2009**). In Egypt, faba bean is among the main nutritional source of plant proteins (**El-Danasoury *et al*., 2008; Bakry *et al*., 2011**). Its consumption exceeded 440,000 t in 2001 (**FAO, 2002**). Nevertheless, the total production of this crop is still limited and falls to cover the increasing local consumption, so there is a prerequisite to enlarge the production by expansion throughout reclaimed areas which signify the scope of cultivated lands (**Khalafallah *et al*., 2008; Bakry *et al*., 2011**).

Nowadays, with increasing the number of faba bean varieties, it is difficult to differentiate these varieties based on morphological characters alone. These characters are either influenced by environmental factors and stage of plant development or reveal limited variation (**Terzopoulosa and Bebeli, 2008**). This has led to the progress of the new steady parameters like use of their genetic material (nucleic acids and proteins) as a tool for varietal identification (**Vishwanath *et al*., 2011**).

Recently, DNA-marker approaches have become gradually more utilized for taxonomic and phylogenetic analyses. They are not affected by environmental factors or by plant developmental stages. Besides, these approaches have potential for the routine testing of the genetic diversity and purity of accessions held in germplasm collections (**Gilbert *et al*., 1999**). The most commonly used polymerase chain reaction (PCR)-based marker systems for genetic diversity and relationships in faba bean species are randomly amplified polymorphic DNA (RAPD) (Link *et al*., 1995; El-Danasoury *et al*., 2008), amplified fragment length polymorphism (AFLP) (**Zeid *et al*., 2003; Duc *et al*., 2010)** and species specific repeats (SSR) (**Zeid *et al*., 2009**). The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism (**Belaj *et al*., 2003; Jabbarzadeh, *et al*., 2010**).

Inter-simple sequence repeat (ISSR-PCR) is a route that overcomes most of these technical limitations (**Reddy *et al*., 2002; Chen *et al*., 2008**). It

is a fast and simple system with a cost-efficient as well as it does not require any prior knowledge about the sequences to be amplified, being tremendously useful in genetic diversity, phylogeny, genomics and evolutionary studies (**Hu *et al*., 2003, Chen *et al*., 2008; Aguilera *et al*., 2011**). ISSR analysis has been successfully documented to determine genetic diversity and relationships in numerous economic legume species such as cow pea (**Ajebade *et al*., 2000**), common bean (**Galvan *et al*., 2003; Gonzales *et al*., 2005**), chickpea (**Sudupak, 2004**), in addition to faba bean (**Terzopoulosa and Bebeli, 2008**). The aims of this work is to: (i) identify and test ISSR-PCR markers for screening genetic diversity in the two commonly cultivated faba bean groups; dry small-seeded and green large-seeded cvs. (ii) Estimate the genetic diversity and relationships among these cvs. Under two varied geographic regions in Egypt rooted in ISSR markers and protein analysis.

2. Materials and Methods

Plant material:

Ten faba bean cvs. Collected from two different geographic locations; Abies, (Alexandria Governorate, 31° 12′ 46″ N and 29° 59′ 30″ E) and Fuka, (Matrouh Governorate, 31° 07′ 30″ N and 28° 05′ 00″ E) were used to ISSR-PCR detection markers. These cvs. represent the green large-seeded form (*V. faba* var. major), which are known as broad beans and the main vegetable type **(Rubatzky and Yamaguchi, 1997),** as well as the dry small-seeded types, (*V. faba* var. minor) which are known as field beans (Table 1).

DNA Extraction

Total DNA was extracted from 1 g of young leaves using Biospin plant genomic DNA extraction kit (Bioer Technology Co., Ltd. China). DNA quality was checked using 1.0% agarose gel electrophoresis and its concentration was determined spectrophotometrically.

ISSR-PCR

Nine random primers (Sigma, Germany) were selected for ISSR analysis (Table 2).

ISSR amplification was carried out using thermo-cycler (Eppendorf, Hamburg) in 25 µl of mixture containing 10 ng of genomic DNA, 10x Taq polymerase buffer, 50 mM $MgCl_2$, 0.2 mM each of dATP, dTTP, dCTP, dGTP, 25.0 p moles for each primer, and 0.5 U Taq DNA polymerase (Promega). Amplification conditions were: 95°C for 5 min; 45 cycles of 95°C for 1 min. 95°C for 1 min followed by annealing. The annealing temperature for PCR amplification was found to vary according to the base composition of the primers, for 1 min followed by a final extension 72°C for 10 min. Amplified products were fractionated by electrophoresis in 2% (w/v) agarose/TBE gels, visualized, and documented using a gel documentation and image analysis system (Alfa Imager M 1220, Documentation and Analysis System, Canada).

Table (1): Geographical distributions and species characteristics of the 10 faba bean cultivars.

Geographic areas	Cultivar name	Cultivar type	Seed type	Cultivar origin	Soil texture
Fuka, Matrouh					
1	ILB 450	Dry small-seeded	Minor	ICARDA	Calcareous
2	Misr I	Dry small-seeded	Minor	Egypt	Calcareous
3	Giza 843	Dry small-seeded	Minor	Egypt	Calcareous
4	Sakha 3	Dry small-seeded	Minor	Egypt	Calcareous
5	Rena Mora	Dry small-seeded	Minor	Spain	Calcareous
6	Giza 3	Dry small-seeded	Minor	Egypt	Calcareous
7	ILB 312	Dry small-seeded	Minor	ICARDA	Calcareous
8	Giza 716	Dry small-seeded	Minor	Egypt	Calcareous
Abies, Alexandria					
9	Aquadolce	Green large-seeded	Major	Spain	Clay
10	Aspany1	Green large-seeded	Major	Spain	Clay
11	Aspany2	Green large-seeded	Major	Spain	Clay
12	ILB 450	Dry small-seeded Dry small-seeded	Minor	ICARDA	Clay
13	Misr I	Dry small-seeded Dry small-seeded	Minor	Egypt	Clay
14	Giza 843	Dry small-seeded Dry small-seeded	Minor	Egypt	Clay
15	Sakha 3	Dry small-seeded	Minor	Egypt	Clay
16	Rena Mora		Minor	Spain	Clay
17	Giza 3		Minor	Egypt	Clay
18	ILB 312		Minor	ICARDA	Clay

Table (2): ISSR primers, their sequences, annealing temperature, size of amplified fragments (bp), total number of amplified fragments (TAF), number of polymorphic bands (PB), polymorphic percentage (PB%) and specific bands identified per primer used to access genetic diversity of 10 faba bean cultivars.

Primers	Sequence 5′→ 3′	Annealing temp. (°C)	Size range (bp)	TAF	PB	PB (%)
ISSR-1	GAGAGAGAGAGAGAGAC	52	200-900	92	63	68.48
ISSR-2	CACACACACACACACAG	52	150-900	99	76	76.77
ISSR-3	GTGTGTGTGTGTGTGTC	52	250-700	48	25	52.08
ISSR-4	GAGAGAGAGAGACC	40	250-700	79	48	60.76
ISSR-5	CACACACACACAGG	52	200-900	52	48	92.31
ISSR-6	GAGAGAGAGAGAGG	40	150- 400	47	31	65.96
ISSR-7	TGTGTGTGTGTGTGG	40	200-700	50	32	64.00
ISSR-8	GAGAGAGAGAGACC	40	200-700	43	30	69.76
ISSR-9	GTGTGTGTGTGTCC	40	250-800	66	45	68.18
Total 9				576	398	69.10

TAF = Total amplified fragments, PB = Polymorphic bands and PB (%) = Percentage of polymorphism.

Total protein and SDS-PAGE

Total protein was extracted from 2 g fresh weight of plant leaves. Each sample were grinded with 10 mL of Extraction buffer (0.5M Tris-HCl (pH 6.8), 10% sucrose, 2% SDS, and 5% 2-mercaptoethanol). The slurry was centrifuged at 5000 rpm for 20 min. Three milliliter of ammonium sulphate solution were added 1 mL of the supernatant to precipitate the proteins then kept overnight in a refrigerator. It was then centrifuged at 5000 rpm for 20 min. the pellet was washed two or three times in 70% acetone. SDS-PAGE was performed by the method described by **Laemmli, (1970).** Protein was analyzed on 1.5 mm thick and 15 cm long gels run in a dual vertical slab unit (Hoefer Scientific Instruments, san Francisco, CA, USA, MODEL SE 600 Series Hoefer, Pharmacia Biotech). From each sample, 50 µl of extract was loaded a polyacrylamide gel. The separation gel (12%) and staking (4%) were prepared from an acrylamide monomer solution. Electrophoresis was carried out at constant current of 35 mA through the stacking gel, and at 90 mA through the separation gel at 4°C After electrophoresis the gel was stained by Coomassie Brilliant Blue R-250 and the molecular weight (MW) of protein corresponding to each band was calculated by protein marker with kilo Daltons (kDs) molecular weights.

Data analysis

The ISSR bands were scored using the binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively for each relative position. Genetic similarity between a pair of faba bean cvs. was calculated using Nei and Li's index of similarity (**Nei and Li 1979**). Cluster analysis was conducted based on genetic similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) procedure in NTSYS-pc version 2.1 software package (**Rohlf, 2000**) in order to deduce genetic relationships among various faba bean cvs.

3. Results and Discussion

ISSR analysis

The genetic diversity and genetic relationship among 10 faba bean cultivars collected from two different geographical locations in Egypt (Table 1) was evaluated, using ISSR assay. The nine ISSR primers amplified 576 bands, 398 bands out of them were polymorphic. The percentage of polymorphism of the amplified products was 69.1%. The size of all amplified bands ranged from about 150 to 900 bp (Table 2).

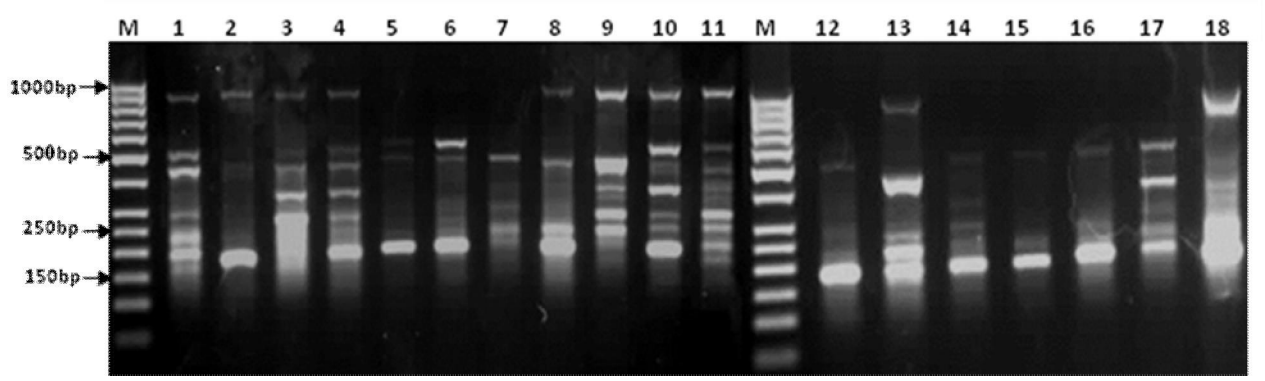
The average of the total amplified bands per studied primer was 64, ranging from 43 to 99 bands. For the polymorphic bands, the average was 44.2 amplified bands per primer, representing 69.1% of polymorphism. These results are relatively close with those of **Terzopoulosa and Bebeli, (2008)**, who found that the percentage of polymorphism revealed within the Greek faba bean populations ranged from 37.5% to 84.62% with an average of 67.48% using 11 ISSR primers.

The ISSR banding patterns of 10 faba bean populations, which were amplified by ISSR primers; ISSR-1,ISSR-2 and ISSR-5 are shown in Figs. (1 a, b and c).

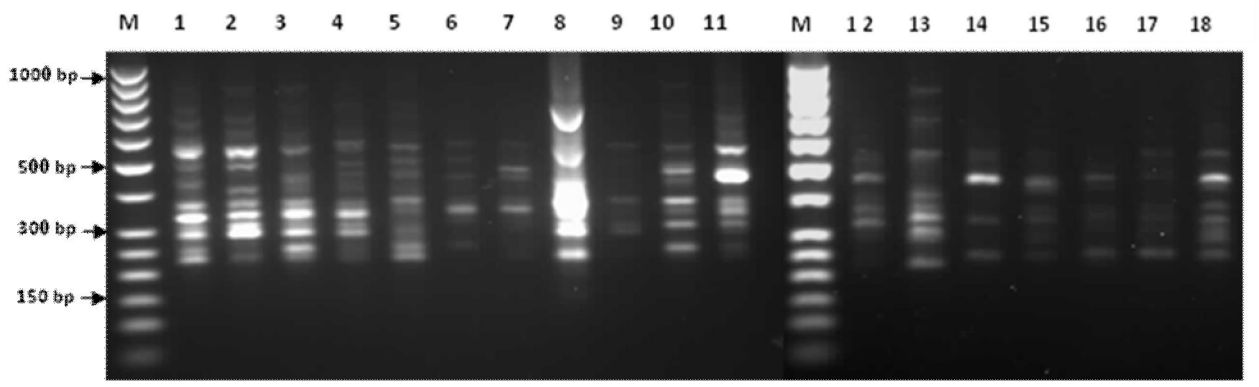
The genetic similarity among the 10 faba bean cvs. ranged from 24% to 95% (Table 3).

The highest genetic similarity was registered for two green large-seeded cvs. (Aspany1 and Aspany 2) as well as two dry small-seeded cvs. Sakha 3 and Rena Mora (95%) followed by ILB 450 and

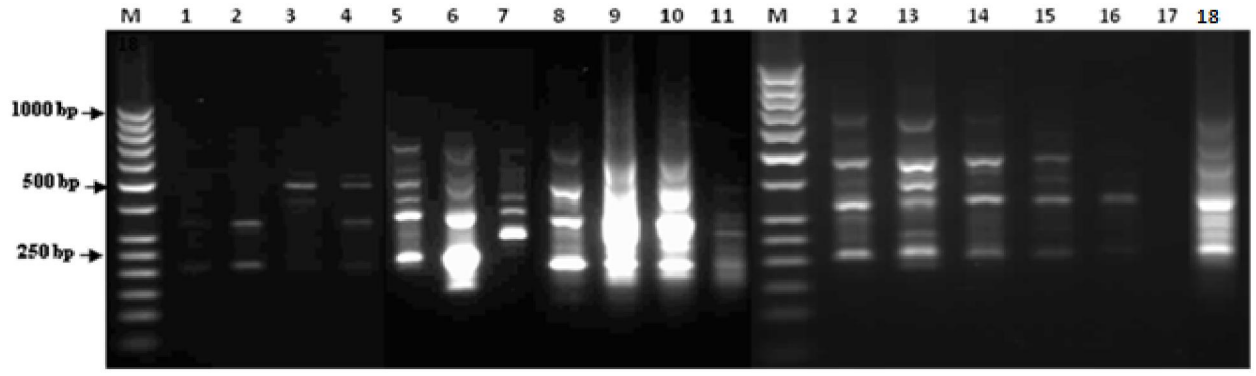
Giza 843 (94%). However, the lowest genetic similarity (24%) was observed in cvs. Sakha 3 and ILB 450 as well as green large-seeded Aquadolce and dry small-seeded Misr I cvs. (57%) followed by green large-seeded Aspany 1 and dry small-seeded Misr I (58%). Similar results were reported by **Duc *et al*. (2010)**, who found that the faba bean offered an enormous genetic variability for breeding purposes.



(a) Primer ISSR-1



(b) Primer ISSR-2



(c) Primer ISSR-5

Figure(1 a, b and c): Banding pattern of various faba bean cvs. generated by ISSR primers under two different geographical locations (Fuka and Abies). Numbers 1-18 stand for the faba bean cvs. listed in Table (1). M is a DNA molecular-size marker**.**

Table (3): Similarity matrix showing the relationship among various faba bean cvs. in the two different geographical locations in Egypt based on ISSR detection data.

Experimental location	Fuka (Matrouh Governorate)								Abies (Alexandria Governorate)									
F. bean cultivars	ILB 450	Mis I	Gi 843	Sak 3	Re Mo	Gi 3	ILB 312	Gi 716	Aqu	Asp 1	Asp 2	ILB 450	Mis I	Gi 843	Sak 3	Re Mo	Gi 3	ILB 312
ILB 450	100																	
Misr I	91	100																
Giza 843	**94**	89	100															
Sakha 3	88	89	92	100														
Rena Mo	83	82	87	85	100													
Giza 3	79	80	79	83	86	100												
ILB 312	77	76	75	79	88	88	100											
Giza 716	83	86	79	83	76	82	82	100										
Aquadolce	77	78	75	83	72	70	72	78	100									
Aspany1	72	75	70	76	69	69	69	75	91	100								
Aspany2	75	76	73	79	72	72	72	74	90	**95**	100							
ILB 450	76	77	78	**24**	83	81	77	71	63	62	67	100						
Misr I	70	69	72	68	75	73	73	67	**57**	**58**	63	86	100					
Giza 843	74	75	76	74	79	81	75	71	61	62	67	86	84	100				
Sakha 3	77	76	81	75	80	80	74	70	62	63	68	85	83	91	100			
Rena Mo	76	75	80	74	81	81	71	69	61	64	67	86	82	92	**95**	100		
Giza 3	77	74	83	75	80	76	70	66	66	69	74	79	77	83	90	89	100	
ILB 312	68	69	74	70	71	71	65	63	61	62	67	82	88	88	83	84	87	100

Cluster analysis based on Nei's genetic distances using the UPGMA method could place the 10 faba bean cvs. into two main clusters: i.e., A and B (Fig. 2). The dry small-seeded cvs. (field beans) which cultivated under two different geographical locations; Fuka and Abies were grouped in cluster A. While, the green large-seeded cvs. (broad beans) were grouped in cluster B. Cluster A was further divided into two sub-clusters: A1 and A2. Sub-cluster A1 was subsequently composed of 8 small-seeded cvs. which cultivated under Fuka region. While, sub-cluster A2 included the small-seeded cvs. that grown under Abies region. The results indicated that the 8 dry small-seeded cvs.; i.e., ILB 450, Misr I, Giza 843, Sakha 3, Rena Mora, Giza 3, ILB 312 and Giza 716 which were collected from these two different locations were joined in one cluster (A). That's might be due to the high genetic similarity among the same cvs., particularly the most of them are Egyptian varieties introduced from ICARDA **(Ghandorah and El-Shawaf, 1993).** Therefore, ISSR technique could divide the 10 examined faba bean cvs. depending on the genetic similarity among them.

SDS-PAGE protein analysis:

The protein banding patterns based on SDS-PAGE for the various faba bean cvs. grown under two different geographic locations Fuka and Abies are shown in Fig. (3). The total number of bands were 25, having molecular weights ranged from 42 KDa to 95 KDa. High number of bands ranged from 8 to 15 observed in the cvs. grown under clay soil conditions (Abies location). While the lowest number of bands is shown in faba bean cvs. grown under desert soil conditions (Fuka location). This result might be due to increasing soil fertility of Abies coupled with enhancement of N uptake under this region, which was associated with increasing protein content of faba bean cvs., comparing with the same cvs. but grown under the poor desert soil of Fuka region.

Data in Fig. (3) can concluded that, it was possible to differentiate the closely related cvs. of faba bean on the basis of gel protein analysis under wide-ranging environments. This inconsistency of protein profiles can supported by the findings of **Duc, (1997),** who observed that protein contents of faba bean were divergent depending on the genotypes and environmental conditions. Similarly, **Alghamdi, (2009),** mentioned that the total protein content of faba bean was varied greatly among genotypes and locations. Moreover, **Ghafoor and Arshad (2008**) observed a considerable amount of variation in pea based on SDS-PAGE analysis under different locations.

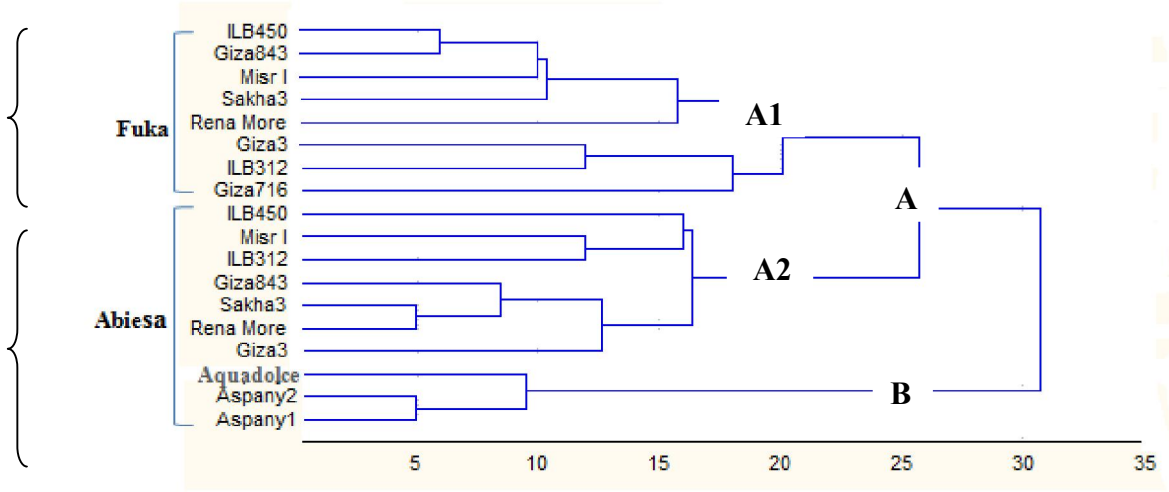


Figure (2): Dendrogram cluster analysis of various faba bean cvs. showing the genetic similarity anchored in ISSR-PCR primers data.

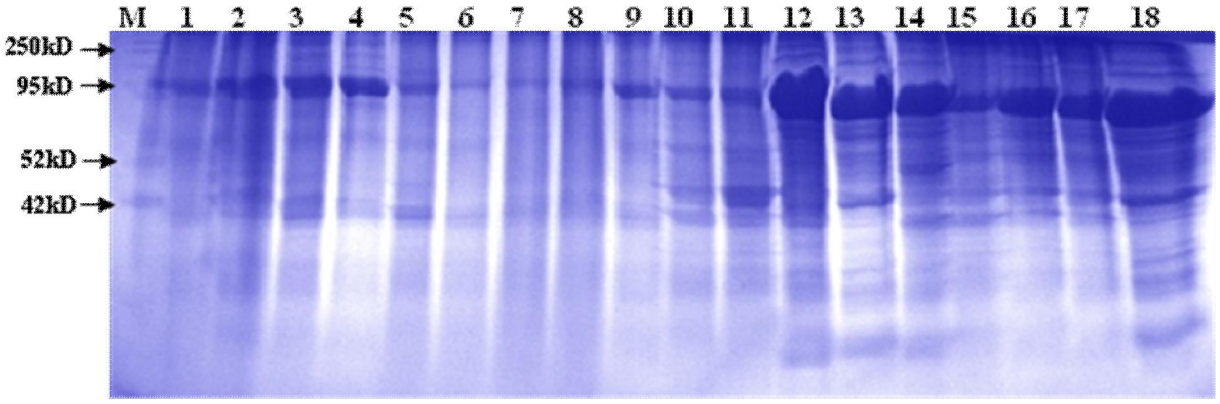


Figure (3): SDS-PAGE protein banding pattern of various faba bean cvs. grown under two different geographic locations (Fuka and Abies). M, referred to the protein standard marker with kilo Daltons (kDs) molecular weights.

Conclusion

ISSR markers are useful tool for detection the genetic diversity among *V. faba* cvs. and help in studying genetic relationships and clarifying taxa of the same species under two diverse geographic locations. Since, ISSR technique reflected enough polymorphism to distinguish among different dry small-seeded (field beans) and green large-seeded (broad beans). Also, it was possible to differentiate the closely related faba bean cvs. based on protein analysis.

References

1. Aguilera, J. G., L.A. Pessoni,G.B. Rodrigues, A.Y. Elsayed, D.J. da Silva, E.G.de Barros(2011):Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill). Revista Brasileira de Ciências Agrárias Recife 6(2): 243-252.
2. Ajebade, S. R., N.F.Weeden, S.M. Chite (2000): Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111(1): 47-55.
3. Alghamdi, S. S. (2009): Chemical composition of faba bean (*Vicia faba* L.) genotypes under various water regimes. Pakistan Journal of Nutrition 8(4): 477-482.
4. Bakry, B. A., T. A. Elewa, M. F. El karamany, M. S. Zeidan, M. M. Tawfik (2011): Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition. World Journal of Agricultural Science 7(1): 68-72.
5. Belaj, A., Z.Satovic, G.Cipriani, L.Baldoni, R.Testolin, L. Rallo , I. Trujillo (2003): Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theoretical and Applied Genetics 107: 736-744.
6. Chen, Y., R. Zhou, X. Lin, K. Wu; X. Qian, S. Huang (2008): ISSR analysis of genetic diversity in sacred lotus cultivars. Aquatic Botany 89: 311–316.

7. Duc, G. (1997): Faba bean (*Vicia faba* L). Field Crops Research 53: 99-109.
8. Duc, G; S. Bao, M. Baum; B.Redden, M. Sadiki, M. J Suso, M.Vishniakova, X. Zong (2010): Diversity maintenance and use of *Vicia faba* L. genetic resources. Field Crops Research 115: 270-278.
9. El-Danasoury, M. M., A. E. El-Ghubashy, H. Yossif , R. T. Behairy (2008). DNA fingerprinting to identify some faba bean (*Vicia faba* L.) varieties. Journal of Agricultural Science Mansoura University 33(6): 4619-4630.
10. FAO, (Food and Agriculture Organization). (2002): FAO Yearbook 2001: Production., vol. 55. FAO, Rome, Italy.
11. Galvan, M. Z., B. Bornet, P. A. Balatti, M. Branchard (2003): Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132: 297-301.
12. Ghafoor, A. , M. Arshad (2008). Seed protein profiling of *Pisum sativum* L. germplasm using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for investigation of biodiversity. Pakistan Journal of Botany 40: 2315-2321.
13. Ghandorah, M. O., I. S. El-Shawaf (1993): Genetic variability, heritability estimates, and predicted genetic advance for some characters in faba bean (*Vicia faba* L.). Journal of Kingdom of Saud University of Agricultural Science 5(2): 207-218.
14. Gilbert, J. E., R. V. Lewis, M. J. Wilkinson, P. D. S. Caligari (1999): Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theoretical and Applied Genetics 98: 1125-1131.
15. Gonzales, A., W. A. Delgado-Salinas, R Papa, P. Gepts (2005): Assessment of inter simple sequence repeat markers to differentiate sympatric wild and domesticated populations of common bean. Crop Science 45: 606-615.
16. Hu, J., M. Nakatani, A. Lalusin, T. Kuranouchi, T. Fujimura (2003): Genetic analysis of sweet potato and wild relatives using inter-simple sequence repeats (ISSRs). Breeding Science 53: 297-304.
17. Jabbarzadeh, Z., M. Khosh-khui, H. Salehi , A. Saberivand (2010): Inter simple sequence repeat (ISSR) markers as reproducible and specific tools for genetic diversity analysis of rose species. African Journal of Biotechnology 9(37): 6091-6095.
18. Khalafallah, A. A., K. M. Tawfik, Z. A. Abd El-Gawad (2008). Tolerance of seven faba bean varieties to drought and salt stresses. Research Journal of Agriculture & Biological Science 4(2): 175-186.
19. Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(5259): 680-685.
20. Link, W., C. Dixkens, M. Singh, M. Schwall , A. Melchinger (1995): Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. Theoretical and Applied Genetics 90: 27-32.
21. Nei, M. , W. H. Li (1979): Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceeding National Academy of Sciences of USA 76: 5269-5273.
22. Reddy, M. P., N. Sarla , E. A. Siddiq (2002). Inter-simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-12.
23. Rohlf, F. (2000): NTSYSpc. Numerical Taxonomy and Multivariate Analysis System-Version 2.10b. Applied Biostatistics Inc, New York.
24. Rubatzky, V. E. , M. Yamaguchi (1997). World vegetables, principles, production, and nutritive values. 2nd edition. Chapman & Hall, ITP, New York. pp. 843.
25. Sudupak, M. A. (2004): Inter- and intra-species inter simple sequence repeat (ISSR) variation in the genus *Cicer*. Euphytica 135: 229-238.
26. Terzopoulosa, P. J. , P. J Bebeli (2008):Genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers. Field Crops Research 108: 39-44.
27. Vishwanath, K., K. P. Prasanna, H. M. Pallvi, P. S. Rajendra, S. Ramegowda, P. J. Devaraju, T. V. Anantharayanan (2011): Identification of tomato (*Lycopersicon esculentum*) varieties through total soluble seed proteins. Research Journal of Agricultural Science 2(1): 8-12.
28. Zied, M., C. C. Schon, W. Link (2003): Genetic diversity in recent elite faba bean lines using AFLP markers. Theoretical and Applied Genetics 107: 1304-1314.
29. Zeid, M., S. Mitchell, W. Link, M. Carter, A. Nawar, T. Fulton, S. Kresovich (2009): Simple sequence repeats (SSRs) in faba bean: new loci from Orbanche-resistant cultivar 'Giza 402'. Plant breeding 128(2): 149-155.

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Minerals Content and Antimicrobial Efficacy of date Extracts against Some Pathogenic Bacteria

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Abstract: Dates contain many minerals, which is very important for healthy life. Sodium, potassium, calcium, manganese, cupper, cadmium, nickel, lead, ferric, zinc, cobalt and magnesium were determined in twenty samples, collected from Jeddah, Saudi Arabia, of two types of dates, Safawy (*Phoenix dactylifera*) and Chinese date (*Ziziphus zizyphus*). The mean value of sodium was 3.5 µg/g and 3.8 µg /g in Chinese and Safawy dates, respectively. The mean concentrations of potassium were 1.34 µg/g and 1.04 µg/g but the mean concentrations of the detected calcium were 12.7 µg/g and 9.1µg/g in the Chinese date and Safawy date, respectively. Manganese concentration means were 1.50 and 2.64 µg/g in Chinese and Safawy dates, respectively. Cupper, cadmium, nickel, ferric, zinc, cobalt, magnesium were detected in the two previous types of dates but lead was not detected in either Chinese or Safawy dates. The antibacterial activity of date water extracts was determined using agar well diffusion method. The water extract of Chinese date (inhibition zone ranged from 11-19 mm) was more active compared to the extract of Safawy date (inhibition zone ranged from 10-13 mm). The antibacterial activity of date water extracts was compared with that of Ampicillin (control antibiotic).
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Keywords: Minerals, Chinese date, Safawy date, antimicrobial activity.

1. Introduction

Phoenix dactylifera commonly known as the true date palm was cultivated for its edible sweet fruit (Salem and Hegazi, 1971). Due to its long history of cultivation for fruit, its exact native distribution is unknown, but probably originated somewhere in the desert oases of northern Africa and Western Asia. Fruits of date palm are a main source of stable food in the kingdom of Saudi Arabia, Middle East and south Asian countries. Dates have always played an important role in the economic and social lives of people of this area. El-Nakhal et al. (1987) added that date palm contained a high percentage of carbohydrate, fat which comprising 14 types of fatty acids, 15 salts and minerals, protein with 23 different amino acids, six vitamins and a high percentage of dietary fiber. It is highly nutritionally food product, rich in calories and many vitamins and mineral (Hasnaoui et al., 2010).

There are many different verities of *Phoenix dactylifera* L. (Al-Shahib and Marshall, 2002) including Safawy which is grown mainly in Almadina. The nutritional and functional constituents of *Phoenix dactylifera* were studied by Al-Farsi and Lee (2008) where ten minerals were reported, the major being selenium, copper, potassium, and magnesium. *Phoenix dactylifera* contained a high percentage of carbohydrate, fat, minerals, protein, vitamins and a high percentage of dietary fiber (Al-Shahib and Marshall, 2003). There are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 - 916 mg/100 g date depending on the type of mineral. In many varieties, potassium can be found at a concentration as high as 0.9% in the flesh. Other minerals and salts that are found in various proportions include boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc. Additionally, the seeds contain aluminum, cadmium, chloride, lead and sulphur in various proportions (Al-Shahib and Marshall, 2003).

Dates contain elemental fluorine that is useful in protecting teeth against decay. Selenium, another element believed to help prevent cancer and important in immune function, is also found in dates (Al-Shahib and Marshall, 2003). Its output in flesh added to its biochemical features, destine it to several potential technological transformations in the domain of food science as the moderate drying or the lyophilization in view to produce an enriched flours. The extraction pulp was carried in view to produce mash or bracing refreshing drinks, the fermentation of the juice in view of the production of vinegar and finally the treatment of the pulp in order to produce jam and other candy products. Furthermore, the fruit yields food products such as date vinegar, date chutney or sweet pickle, date paste for bakery products (Mikki et al., 1989; Gad et al., 2010; El-Sohaimy and Hafez, 2010).

Ziziphus zizyphus commonly called jujube, red date, or Chinese date, is a species of *Ziziphus* in the buckthorn family Rhamnaceae, used primarily for its fruits. Common names in Arabic are nabq, dum, tsal, sadr, zufzuuf and sidr. Although date fruit contains a

significant amount of nutrients that can be utilized by microorganisms to support their growth, date fruits can typically be stored healthily in a normal warehouse for several months without showing signs of microbial growth (Zohary and Hopf, 1993). Because of this, it has been postulated that dates may contain antimicrobial and antifungal agents that may prevent microbial contamination. At the same time, several studies have shown that water or organic extraction can be used to extract and release antimicrobial compounds from plant materials.

Little information concerning minerals and antimicrobial activity of *Ziziphus zizyphus* and *Phoenix dactylifera* were found. For this reason, the aims of this study were to determine and compare the minerals content and antimicrobial activity in two date types collected from Jeddah, Saudi Arabia.

2. Materials and Methods

Plant Materials

The plant materials used in the study, *Phoenix dactylifera* and *Ziziphus zizyphus,* were purchased from the local market of Jeddah, Saudi Arabia. Moisture content of the samples was measured according to AOAC (1990). The total solids content is a measure of the amount of material remaining after all the water has been evaporated. Thus, % total solids = (100 - % Moisture content). Total fibers contents were determined by the method described by Garcia et al. (1997). Ash content was determined according to Gan et al. (1998). Chemical analysis for the determination of total carbohydrate was adapted from the phenol–sulphuric acid method as described by Dubios et al. (1956). The total amount of carbohydrate was determined based on a standard calibration curve prepared using glucose. The total Lipid extracted from date samples according to Folch et al. (1957) using a mixture of chloroform: methanol (2:1 v/v). Protein content was determined according to Lowry et al. (1951).

Determination of minerals content

Mineral content of dates were determined both qualitatively and quantitatively using the inductively coupled plasma optic essission spectroscopy (Perkin Elmer 43 ooDV) according to Brekken and Steinnes, (2004).

Bacterial isolates and Culture medium:

The tested bacteria were collected from culture collection of Microbiology laboratory, Faculty of Science, KAU. Tested bacterial isolates were *Escherichia coli , Pseudomonas aeuroginosa, Shigella* sp., *Klebsiella* sp., *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus sp.* which were suspended in Tryptic Soy Broth (TSB, Difco, USA) and incubated at 37°C for 20 h. Mueller Hinton Agar (MHA, Difco, USA) was used for detection of the antimicrobial activity using paper disc diffusion method.

Preparation of plant extracts:

The fruits of dates used in this study were air-dried in the dark at room temperature and then ground to powders using a mechanical grinder. Approximately 50 g of the powdered materials were extracted by maceration in 200 ml of boiling water for 8 hr. The volume of the obtained extract was then reduced to 20 ml using Lypholization followed by and filtration using Whatman No. 1 filter papers and the crude extracts were stored at -20°C until required for testing.

Screening of Antibacterial Activity by the Agar Diffusion Method:

The antibacterial activities of some bacterial isolates to two plant extracts were tested using the disk-agar method standardized by the National Committee for Clinical Laboratory Standards. Six mm-diameter paper discs were bored in the agar plates and 20 µl of each extract at concentration of 400 µg/ml was dispensed into the discs. Antibacterial activity was evaluated by measuring inhibition zone diameters. Ampicillin was included as positive controls and water served as negative controls.

Statistical analysis:

Each value is the mean of three reading. Statistical analysis was carried out using student t-test.

3. Results

The two types of date fruits (*Phoenix dactylifera* and *Ziziphus zizyphus*), were collected and extracted. Moisture content, protein, carbohydrates, lipids and total solid were differed significantly in the two date types (table 1). The Chinese date contained higher protein and lower lipids than the other investigated type. The results obtained for the two date mineral contained were summarized in table 2 and figure 2. The values showed the mean value of every mineral in both Safawy and Chinese date.

The extract of the two types of date were tested for antibacterial activity against seven genera of bacteria (4 Gram -ve and 3 Gram +ve) which was characterized by their resistant to one or more of the used antibiotics. The antibacterial activity was detected using agar well diffusion method. The extract of chinese date was more active (diameter of inhibition zone ranged from 11-19 mm) compared to the activity of Safawy date (inhibition zone ranged from 10-13 mm).The antibacterial activity of date

water extracts was compared with that of Ampicillin (control antibiotic) which showed a high antibacterial activity (Table 3).

A B

Fig 1. A: Safawy (*Phoenix dactylifera*) and B: Chinese date (*Ziziphus zizyphus*).

Table 1: Chemical analysis of two cultivars of date (Safawy and Chinese dates), (n= 20)

Parameter measured (%)	Safawy date	Chinese date
Moisture	13.80%	9.2%*
Protein	2.11 %	4.7%*
Carbohydrates	73.00%	42.4%*
Lipids	2.90%	1.7%*
Crude fiber	4.8%	3.7%
Ash	4.13%	3.9%
Total Solid	76.60%	80.2%*

*: significant results

Table 2: Mineral analysis (Mean ±SE) calculated in µg/g dry weight of safawy and Chinese date (n= 20)

Mineral	Safawy date	Chinese date
Na	3.80±0.020*	3.5 ± 0.01
K	1.04 ±0.003	1.34± 0.01**
Ca	9.10 ±0.030	12.7± 0.03 **
Mg	4.60± 0.002	6.6 ± 0.001**
Mn	2.64±0.010	1.50 ± 0.01
Cu	1.13±0.020	2.43 ± 0.02**
Cd	3.07 ±0.020	3.16±0.020*
Ni	16.27±0.030	17.85±0.01**
Pb	<dL	< dL
Fe	70.22±0.030	117.2 ± 0.03**
Zn	20.41±0.12	32.21± 0.03**
Co	50.13±020**	22.12± 0.020

Values are expressed as mean of 20 samples ± SE.
*= significance at P ≤ 0.05, **= highly significant at P ≤ 0.001, <dL = under limit of detection

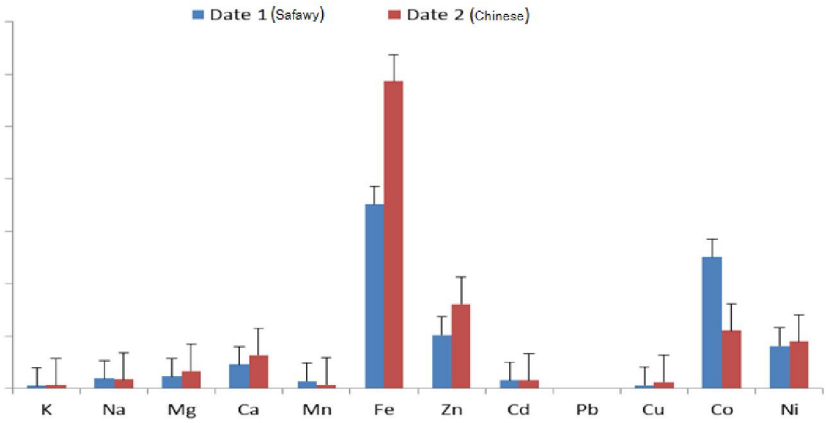

Fig 2.The mineral content of the two cultivars of date fruits

Table 3:The antimicrobial activity of the two cultivars of date fruits

Plant used / Test organisms	*Ziziphus zizyphus*	*Phoenix dactylifera*	Control antibiotic (Ampicillin)
Escherichia coli	11±4.1	10±2.1	24±0.12
Pseudomonas aeuroginosa	11±9.1	11±3.0	26±0.35
Shigella sp.	16±3.2	10±2.2	24±0.54
Klebsiella sp.	16±11.2	12±6.1	24±0.44
Bacillus subtilis	19±6.2	13±3.6	30±0.60
Staphylococcus aureus	13±4.4	11±2.4	30±0.31
Micrococcus sp.	13±2.3	12±2.2	31±0.11
Bacterial index	14.1	11.3	27

4. Discussion:

The date fruits are regarded as a popular food commodity for thousands of years in Egypt, the Arabian Gulf peninsula and its neighboring region and provide unique functional and nutritional values. Numerous health benefits beyond its nutritional value have been associated with consuming date palm fruits to enrich nutrition values of different kinds of food. (El-Sohaimy and Hafez, 2010). Date palm fruits contain a variety of minerals, which have a variety of functions that help maintain a healthy body. Magnesium and calcium are essential for healthy bone development and for energy metabolism. Iron is essential to red blood cell production. Red blood cells carry all the nutrients to cells throughout the body. Dates contained an ideal amount of sodium and were a great source of potassium, a nutrient that is great in the maintenance of a healthy nervous system and in balancing the body's nervous system. Phosphorus works with calcium to help with bone strength and growth, potassium that helps to keep your muscles working correctly and selenium is important for cell growth and repair. The date palm fruits provide unique functional and nutritional values. Numerous health benefits beyond its nutritional value have been associated with consuming date palm fruits to enriched nutrition values of different kinds of food. Dates could have an important all-round role to play in dietary health. There is every possibility that they contain other components that may have useful functional properties.

The concentrations of nitrogen, potassium, phosphorous, calcium, magnesium and iron were detected in four cultivars of date palm during 1978 and 1979 seasons. The average concentrations of these nutrient elements in the date palm cultivars ranged from 0.97 -1.36 %N. 0.11 to 0.178% P, 1.36 to 1.59% K, 1.00 to 1.29% Ca, 0.39 to 0.47 % Mg and 208 to 267 ppm Fe. Furthermore, El-Sohaimy and Hafez (2010) found that the date palm extracts contained 13.80 % moisture, 86.50 % total solid, 2.13% ash and 5.20% fibers contents but the protein, carbohydrates and lipids contents were 3.00%, 73.00% and 2.90 % respectively. They added that the low level of lipids content compared with its content of sugars means that, the date palm is save to heart and blood patients because its containing a very low level of fatty acids and cholesterol.

As illustrated in table (1), the Chinese date content in protein is higher than the Safawy date while, the later has a higher lipid than the previous one. The Safawy date content of carbohydrate

(73.00%) is higher than the Chinese date (42.4%). These characteristics may affect the taste of both of them. The moisture content of the Safawy date is higher than the Chinese date this property may affect the storage capacity that mean the Chinese date may be better in storage than the Safawy date.

From table (2) and figure (2) it is obvious to find a high content of the macronutrient in the Chinese date than the Sawafy date except for the Na+ content. However, it is clearly to see a very highly significant content of the Fe content in both types. Nevertheless, the content is high in Chinese date than the Sawafy date. Regarding the micronutrient it is clear to find a high content of Cu, Ni, and Zn in the Chinese date than the Sawafy date. However, the Sawafy date has a high content of Mn and Co than the Chinese date. For the Pb in both types was not detected while for Cd in both types is almost the same content.

One of the goals of this study was to determine inhibitory effect of palm extract on some pathogenic bacteria. Moderate antibacterial activates was recorded compared to control antibiotic. Palm kernel extract containing di peptides of different degree of hydrolysis against spore-forming bacteria *Bacillus cereus, B. circulans, B. coagulans, B. licheniformis, B. megaterium, B. pumilus, B. stearothermophillus, B. subtilis, B. thuringiensis, Clostridium perfringens;* and non-spore forming bacteria *Escherichia coli, Lisinibacillus sphaericus, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococcus aureus*. Potentially PKE peptides could be used in food preservation and developed as antibacterial agent in the pharmaceutical industry (Tan et al., 2011).

Conclusion:

This detailed information on nutritional and health promoting components of dates enhance our knowledge and appreciation for the use of dates in our daily diet and as a functional food ingredient.

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References:

1. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: a review. Crit Rev Food Sci. Nutr. 2008; 48(10):877-87.
2. Al-Shahib W, Marshall RJ. Dietary fiber content of 13 varieties of date palm (*Phoenix dactylifera L*). J. Food Sci. Technol. 2002; 37: 719-721.
3. Al-Shahib W, Marshall RJ. The fruit of the date palm: its possible use as the best food for the future?. Int J. Food Sci. Nutr. 2003; 54(4):247-59.
4. AOAC. Official methods of analysis of the association of official analytical chemists. 15th edition.Washington, DC, Association of Official Analytical Chemists.1990.
5. Brekken A, Steinnes E. Seasonal concentrations of cadmium and zinc in native pasture plants: consequences for grazing animals. Sci. Total Environ. 2004; 326: 181–195.
6. Dubois M, Gilles JK, Hamilton PA, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956; 28(3): 350-356.
7. El-Nakhal HM, El-Shaarawy MI, Messallam AS."Tamrheeb" a new protein rich product from dates. Proc. of Second Symposium on the Date Palm.1987; Saudi Arabia.
8. El-Sohaimy SA, Hafez EE. Biochemical and Nutritional Characterizations of Date Palm Fruits (*Phoenix dactylifera L.)*. Journal of Applied Sciences Research. 2010; 8:1060-1067
9. Folch, J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry.1957; 226: 497-509.
10. Gad AS, Kholif AM, Sayed AF. Evaluation of the Nutritional Value of Functional Yogurt Resulting from Combination of Date Palm Syrup and Skim Milk. American Journal of Food Technology. 2010; 5(4): 250-259.
11. Gan Z, Ellis PR, Vaughan JG, Galliard T. Some effects of non-endosperm components of wheat and of added gluten on whole meal bread microstructure. J. Cereal Science. 1998; 10: 81-91.
12. Garcia, OE, Infante RB, Rivera CJ. Determination of total soluble and insoluble dietary fibre in two new varieties of *Phaseolus vulgaris L.* using chemical and enzymatic gravimetric methods. Food Chemistry. 1997; 59(1): 171-174.
13. Hasnaoui A, Elhoumaizi MA, sehraou AA, Hakkou A. Chemical composition and microbial quality of main varieties of dates grown in figuig oasis of Morocco. Int. J. Agric. Biol. 2010; 12: 311–314.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagents. J. Biol. Chem. 1951; 193: 265-275.
15. Mikki MS, AL-Taisan SM, Abdulaziz AA. Isolation and identification of the chemical constituents of the spathe of the date palm. Al- Hassa Regional Agricultural Research Centre, Hofuf, Saudi Arabia; 1989.
16. Salem SA, Hegazi SM. Chemical composition of the Egyptian dry dates. Journal of Applied Sciences Research. 197; 6(8): 1060-1067.
17. Tan YN, Ayob MK, Osman MA, Matthews KR. Antibacterial activity of different degree of hydrolysis of palm kernel expeller peptides against spore-forming and non-spore- forming bacteria. Lett. Appl. Microbiol. 2011; 17. doi: 10.1111/j.1472-765X.2011.03137.x.
18. Zohary D, Hopf M. Date palm, *Phoenix dactylifera*. In: Domestication of plants in the old World, 2nd ed. Clarendon, Oxford.1993; 157-162.

Prevalence of Depression, Anxiety, Dementia and other Non Motor Features of a large Cohort of Egyptian Parkinson's Disease Patients

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Abstract: Background: There is a lack of awareness of the considerable disability associated with non motor symptoms (NMS) in PD among physician. The aim of the work is to estimate the prevalence of depression, anxiety, dementia and other NMS of Parkinson's disease (PD) Egyptian patients. **Material and Methods**: The study included, 112 patients with Parkinson's disease. Each individual was scored on the Unified Parkinson's Disease Rating Scale part III (UPDRS) and the the Hoehn and Yahr Scale (HY) to evaluate motor symptoms. Other symptoms were quantified with the Mini Mental State Examination (MMSE), Hamilton Depression and Anxiety Scales (HAM-D, HAM-A) and the Non-Motor Symptom Questionnaire and Scale (NMSQuest and NMSS). **Results**: According to HAM-A and HAM-D scales; anxiety and depression were noted in 78% and 54% of patients whilst dementia was recorded in 22%. According to NMSS, mood/cognition was the commonest domain (87.5%), and sleep disturbance/fatigue was the second frequent domain with a prevalence rate 78.6%, but all other non-motor symptoms also scored highly: gastrointestinal and urinary domains (76.8% for both), Sexual dysfunction (73%), cardiovascular (70.5%). Perceptual problems /hallucinations were the least frequently recorded domain (9.9 %%). There were significant correlations between UPDRS and HAM-D, HAM-A scores as well as with MMSE. UPDRS were also correlated with total NMSQuest and NMSS and each domain separately except cardiovascular and perceptual problems. Duration of illness was significantly correlated with UPDRS, depression, and dementia but not with other NMS. **Conclusions**: mood/cognition, sleep disorders, GIT, and sexual disorders were common non motor manifestations in PD patients. Patients with a longer duration of PK had higher scores on the UPDRS part 3 and were more likely to have depression, anxiety and dementia.

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Key words: Depression, Anxiety, parkinson's disease, non motor manifestations, cognitive dysfunction

1. Introduction

Parkinson's disease (PD) is a progressive neurological condition which is characterized and diagnosed by the presence of classical motor symptoms, such as tremor, rigidity, bradykinesia and gait disturbance. Although PD is primarily a movement disorder, it is accompanied by various non motor symptoms. The NMS of PD is very diverse. It includes psychiatric and behavioral problems, cognitive dysfunction, sleep disturbance, gastrointestinal, sexual dysfunction and cardiovascular symptoms. These symptoms are challenging in advanced stages of the disease, and they frequently limit the effective treatment of motor signs, leading to increased disability and poor quality of life [1,2,3]. Neuropsychiatric disorders are common in PD, but not frequently recognized by clinicians. The assessment of these conditions must be routinely conducted due to their impact on the motor signs and on the quality of life of patients and caregivers. Depression is considered the most common neuropsychiatric disorder associated with PD. The actual rate of depression in persons with PD is unknown but reported rates vary from 7 to 76%. Difficulty in diagnosing depression in PD is quite complex due, in part, to the overlap of symptoms of PD with those of depressive syndromes [4]. Up to forty percent of PD patients were clinically diagnosed with significant anxiety [5]. Nearly 80 to 90% of PD patients have some type of sleep difficulty [6], and virtually all patients with PD will experience a sleep disorder during the course of the disease [7]. Cognitive impairment is common in PD, and approximately 30 to 40% of patients with PD meet criteria for dementia. [8]. These symptoms may be also related to the effect pharmacological and non-pharmacological treatment. Early and accurate identification and appropriate management of the nature and severity of non-motor symptoms in PD will aid the holistic care of this progressive neurodegenerative illness, improve the quality of life of patient and career, and contribute to limiting the financial impact of PD. Because few studies have provided an integrated scale for the assessment of NMS in PD, NMS have not been regularly used to assess PD, especially in Egypt. Recently, the Non-motor Symptoms Scale (NMSS) for PD was developed to quantify the overall prevalence of NMS [9,10].

The primary purpose of this cross-sectional study was to estimate the frequency of depression, anxiety, dementia and other non motor manifestations among a

large cohort group of PD patients and to evaluate the influence of disease duration, severity and Motor scale on NMS.

2. Material and Methods

One hundred and twelve PD patients participated in the study. They were selected consecutively from those who attended the Department of Neurology, Assiut University Hospital, Assiut, Egypt, from December 2009 to December 2010. All patients fulfilled the UK Parkinson's Disease Brain Bank Criteria for idiopathic PD [11]. The mean age of the patients was 60.96 ± 12.7 ranging from 28- 88 years, 77 males and 35 were females, the mean duration of illness was 6.17 ± 5.9 with a ranging from 6 months to 25 years.

We rated motor and functional performance on PD patients using Unified Parkinson's Disease Rating Scale (UPDRS) part 3 [12] and the Hoehn and Yahr Scale [13].

Minimental State Examination (MMSE) [14], Hamilton Depression Scale (HAM-D) [15] and Hamilton anxiety scale [16] were applied for each patient. Patients underwent also clinical examination, including tests investigating for possible other NMS using a self reported questionnaire for non-motor symptoms (NMSQuest) [17]. NMSQuest comparises 30 common symptoms scored yes or no, and is designed to provide a rapid screen for problematic NMS as an aid for clinical management. Then Non-Motor Symptoms Scale for Parkinson's disease (NMSS) that contains nine dimensions were applied [9]. The nine relevant domains included: cardiovascular (2 items); sleep/fatigue (4 items); mood/cognition (6 items); perceptual problems/hallucinations (3 items); attention/memory (3 items); gastrointestinal tract (3 items); urinary (3 items); sexual function (2 items); and miscellaneous (4 items). Score for each item is based on a multiple of severity (from 0 to 3) and frequency scores (from 1 to 4).

During their interview, patients were usually assisted by a caregiver, in order to maximize data collection in case of cognitive impairment. All patients provided fully informed written consent. The local ethics committee of Assiut University Hospital had approved the experimental protocol.

Statistical analysis

Questionnaires and different scales were reviewed, and open-ended questions were coded and entered using a simple spread sheet. Analysis followed after data verification and correction. All data were analyzed with the aid of the SPSS ver. 16 (www.spss.com). The results were expressed as mean ± SD. Degree of dementia was classified mild (21-19), moderate (14-18) and severe dementia less than 14 for illiterate patients and 2 point above for educated patients [18].

Degree of anxiety was classified as mild (≤17), moderate (18–24) or severe (≥ 25) [16] and depression was also classified into; mild symptoms equated to a score of 7–17, moderate to 18–24 and severe to 25 or more[15]. Spearman's correlation coefficient was done between duration of illness, total UPDRS, self assessment scale, NMSS, NMSQ, HDS, HAS, and subscales.

3. Results

Of 112 PD patients, clinical and demographic data are illustrated in table 1. Seventy patients (62.5%) were illiterate and 42 patients (37.5%) were literate. The mean duration of illness was 6.17 ± 5.9 years ranging from 6 months to 25 years. Fifteen patients had an early age of onset (younger than 40 years), twenty patients (17.85%) had positive family history.

Most of the patients (107/112 or 95.5% of patients) had resting tremor, while bradykinesia and rigidity were recorded in 82.1% and 85.7% respectively. Using the Hoehn and Yahr rating, Most of the patients were presented in stage 1 to 2 (80.3%) while stage 5 was least frequent (3.6%) see table 2.

Table 3 shows the mean values of each scale and domain using in this study.

A mild degree of anxiety was commonest (52.7%), while only 17 patients (15.2%) had severe anxiety. Depression (HAM-D) was recorded in 53.6%, anxiety (HAS) in 77.7%. Comorbid depression with anxiety was observed in 60 patients (53.6%).

Dementia was recorded in 22.3% of patients, most of them having a mild degree of dementia according to MMSE (Table 4). Significant correlation between dementia and depression and anxiety (MMSE versus HAM_D r= -0.30 p = 0.001, HAM-A versus HAM-D r= 0.75 , P= 0.0001; MMSE versus HAM-A- versus r= -0.29 p = 0.001)

Table 5 illustrate the frequency and percent of each domain: Sleep disturbance/fatigue was the second commonest domain after mood/cognition domain (87.5%), with a prevalence rate of 78.6%, but all other non-motor symptoms also scored highly: gastrointestinal and urinary domains (76.8% for both), Sexual dysfunction (73.2%), cardiovascular (70.5%), Perceptual problems /hallucinations were the least frequently recorded symptoms (9.9%%). NMS were present in 108 (96.25%) patients and occurred during both on and off periods. Only 4 patients (3.75%) had no NMS. The average total frequency of symptoms noted in the NMSQuest as well as the total NMSS scores in each domain are shown in table 5.

As regards to the frequency of each symptom of NMSQuest: feeling anxious, fightened or panicky, (60.7%), getting up regularly at night to pass urine (59.8%), a sense of urgency to pass urine that makes rush to the toilet (54.5%), feeling lightheaded, dizzy or weak when standing from sitting or lying (53.6%) and

constipation (51.8%) were the most common symptoms among studied patients

Followed by unexplained pains (not due to known conditions such as arthritis (47.1%), feeling less interested in sex or more interested in sex (46.4%), and difficulty getting to sleep at night or staying asleep at night (46.4%). The least symptoms were the double vision (7.1%) and believing things and visual hallucination (9.8%) (Table 6).

There were no significant differences between males and females in scores on the different rating scales and no significant differences between scores from patients with early or late age of onset also in different rating scales. There was no significant difference between patients who had positive family history of cases versus sporadic cases.

Table 7 illustrated the correlation between age, duration of illness, and UPDRS on one hand and the different rating scales on the other hand. It was shown that: age was significantly correlated with MMSE only (P= - 0.009).

Duration of illness was significantly correlated with UPDRS, HAM-D, HAM-A, MMSE, NMSS, and NMSQuest, while only sleep/fatigue, Perceptual problems/hallucination and attention/memory domains were correlated with the duration of illness.

There was a significant positive correlation between UPDRS score and Hoehn and Yahr staging on one hand and HAM-D, HAM-A, and NMSQuest score, on the other hand, and significant negative correlation between UPDRS and MMSE) were shown in (Figure 1A, 1B, 2 and 1C) respectively.

Significant positive correlation between the UPDRS with sleep/fatigue, mood/cognition and attention/memory domain, details illustrated in Table 7.

Table 1: Demographic and clinical data of Parkinson's Disease patients

	Mean ± SD
Age (Years) Range Age of onset (≤ 40years/ < 40 years)	60.96± 12.1 28- 88 15/97
M/F	77/35
Positive family history/negative family history	20/92
Duration (years) Range	6.17 ± 5.9 0.5-25
Illiterate number (%) Literate <6 years >6years	70 (62.5%) 42 (37.5%) 13(11.6%) 29 (25.9%)
Symptoms of PK number (%) Bradykinesia Resting tremors Rigidity Postural instability	 92 (82.1%) 107 (95.5%) 96 (85.7%) 70(62.5%)

Table 2: Hoehn and Yahr Stages of Parkinson's Disease patients

Severity of Parkinson's disease	Frequency	Percent
Stage 1: Unilateral disease.	42	37.5
Stage 1.5: Unilateral plus axial involvement.	24	21.4
Stage 2: bilateral disease, without impairment of balance	24	21.4
Stage 3: Mild to moderate bilateral disease; some postural instability; physically independent.	9	8.0
Stage 4: severe disability; still able to walk or stand unassisted	9	8.0
Stage 5: Wheelchair bound or bedridden unless aided	4	3.6
Total	112	100%

TABLE 3. Score distribution of the applied measures and non-motor symptoms scale

Scales	Scores (mean ± SD)	Range
Total UPDRS part 3	18.5 ± 11.4	3 - 50
Hamilton Anxiety Score	13.9± 8.9	0 - 40
Hamilton Depression Score	11.17 ± 7.2	0 – 33
MMSE score	25.0 ± 4.5	7 – 30
NMS Scale Cardiovascular domain Sleep/fatigue domain Mood/cognition domain Perceptual problems/hallucination domain Attention/memory domain Gastrointestinal domain Urinary domain Sexual function domain Miscellaneous domain Total NMSS	 2.07 ± 1.9 3.04 ± 2.6 4.1 ± 3.3 0.18 ± 0.13 1.76 ± 2.2 3.2 ± 2.7 3.0 ± 2.5 2.3 ± 2.3 1.6 ± 1.8 20.88 ± 12.8	 0-6 0-15 0-16 0-1 0-6 0-12 0-10 0-8 0-9 0-58
NMS Questionnaire	9.9 ± 5.9	0-21

Table (4):Frequency of depression, anxiety, dementia among PD using Hamilton depression and anxiety and MMSE.

Manifestation	No manifestations	Positive manifestation	Mild degree	Moderate degree	Severe degree
Anxiety	25(22.3%)	87 (77.7%)	59(52.7%)	11(9.8%)	17(15.2%)
Depression	52(46.4%)	60 (53.6%)	21(18.8%)	22(19.6%)	17(15.2%)
Dementia	87(77.7%)	25 (22.3%)	18(16.1%)	6(5.3%)	1(0.9%)

Table 5: Frequency and percent of occurrence of each domain of NMSS

NMS Scale	Yes/No (number of cases) for each domain	Percent of each domain Yes/No Percent
Domain		
Cardiovascular	79/33	70.5/29.5
Sleep/fatigue	88/24	78.6/21.4
Mood/cognition	98/14	87.5/12.5
Perceptual problems	11/101	9.9/90.1
Attention/memory	81/31	72.3/72.7
Gastrointestinal	86/26	76.8/23.2
Urinary	86/26	76.8/23.2
Sexual function	82/30	73.2/26.8
Miscellaneous	72/40	64.3/35.7
Total NMSS	108/4	96.25/3.75

Table(6) Frequency of each symptom according to NMSQuest

Non motor symptoms	Yes (n, %)	No (n, %)
1-Dribbling saliva during the daytime.	33(31.7%)	79(55.8%)
2-Loss or change in your ability to taste or smell.	11(9.8%)	101(90.1%)
3-Difficulty swallowing food or drink or problems with chocking.	27(24.1%)	85 (75.9%)
4-Vomiting or feelings of sickness (nausea).	12(10.7%)	90(89.3%)
5- Constipation (less than 3 bowel movements a week) or having to strain to pass a stool (feces).	58(51.8%)	54(48.2%)
6- Bowel (fecal) incontinence.	5(4.5%)	107(95.5%)
7-Feeling that your bowel emptying is incomplete after having been to the toilet.	18(16.1%)	94(83.9%)
8- A sense of urgency to pass urine that makes rush to the toilet.	61(54.5%)	51(45.5%)
9-Getting up regularly at night to pass urine.	67(59.8%)	45(40.2%)
10- Unexplained pains (not due to known conditions such as arthritis).	49(47.1%)	53(52.9%)
11- Unexplained change in weight (due to change in diet).	37 (33 %)	75 (67%)
12-Problems remembering things that have happened recently, or forgetting to do things.	34(30.36%)	78(69.6%)
13- Loss of interest in what is happening around you or in doing things.	43(38.39%)	69(61.61%)
14- Seeing or hearing things that you know or told are not there.	15(13.4%)	97(86.6%)
15- Difficulty concentrating or staying focused.	47(41.96%)	65(58.04%)
16- Feeling sad, "low or blue".	53(47.3%)	59(52.68%)
17- Feeling anxious, frightened or panicky.	68(60.7%)	44(39.3%)
18- Feeling less interested in sex or more interested in sex.	52(46.4%)	60(53.6%)
19- Finding it difficult to have sex when you try.	48(42.9%)	64(57.1%)
20- Feeling lightheaded, dizzy or weak when standing from sitting or lying.	60(53.6%)	52(46.4%)
21- Falling.	44(39.3%)	68(60.7%)
22- Finding it difficult to stay awake during activities such as working, driving or eating.	44(39.3%)	68(60.7%)
23- Difficulty getting to sleep at night or staying asleep at night.	52(46.4%)	60(53.6%)
24- Intense, vivid dreams or frightening dreams.	31**(27.7%)**	81(72.3%)
25- Talking or moving about in your sleep as if you are 'acting out' a dream.	17(15.2%)	95(84.8%)
26- Unpleasant sensations in your legs at night or while resting, and a feeling that you need to move.	17(15.2%)	95(84.8%)
27- Swelling of your legs.	20(17.9%)	92(82.1%)
28- Excessive sweating.	24(21.4%)	88(88.6%)
29- Double vision.	8 (7.1%)	104 (92.9%)
30- Believing things are happening to you that other people say are not true.	11(9.8%)	101(97.1%)

Table 7: The correlation between age, UPDRS , duration, stage, on one hand and diffenet scales used in this study score

Item	Age r(p value)	Duration r(p value)	UPDRS part 3 (motor score) r(p value)	Stage Hoehn and Yahr scale r (p value)
Hamilton anxiety scale	0.05 (0.57)	0.35(0.0001)	0.57(0.0001)	0.52(0.0001)
Hamilton depression scale (HAM-D)	9.1(0.23)	0.33(0.0001)	0.53(0.0001)	0.48(0.0001)
Mini mental state examination score(MMSE)	-0.24(0.009)	-0.19(0.04)	-0.27(0.003)	-0.29(0.003)
Non motor symptoms Questionnaire score (NMSQuest)	0.02(0.84)	0.22(0.01)	0.25(0.006)	0.18(0.52)
Total score of Non motor symptoms assessment scale (NMSS)	0.07(0.411)	0.23(0.01)	0.30(0.001)	0.71(0.066)
cardiovascular domain	0.09(0.33)	0.12(0.19)	0.18(0.055)	0.07(0.44)
sleep/ fatigue domain	0.04(0.67)	0.29(0.002)	0.33(0.0001)	0.27(0.004)
mood/cognition domain	0.13(0.156)	0.16(0.288)	0.31(0.001)	0.26(0.005)
Perceptual problems/ hallucination domain	0.08(0.382)	0.52(0.000)	-.04(0.679)	-0.12(0.208)
attention/ memory domain	0.12(0.198)	0.29(0.002)	0.35(0.0001)	0.21(0.02)
gastrointestinal domaind	0.02(0.78)	0.02(0.82)	0.15(0.10)	0.01(0.990)
urinary domain	0.06(0.53)	0.17(0.06)	0.07(0.45)	0.04(0.65)
sexual domain	-0.01(0.95)	0.09(0.95)	0.07(0.4)	0.045(0.650)
miscellaneous domain	0.13(0.167)	-0.15(0.32)	0.15(0.108)	0.02(0.815)

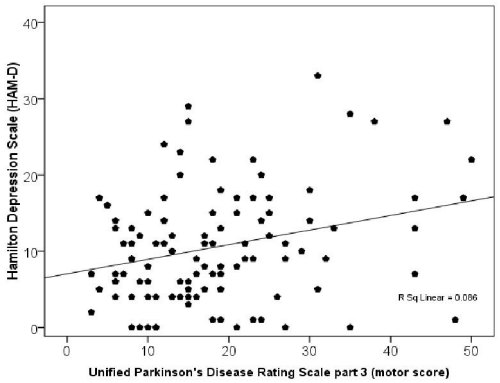
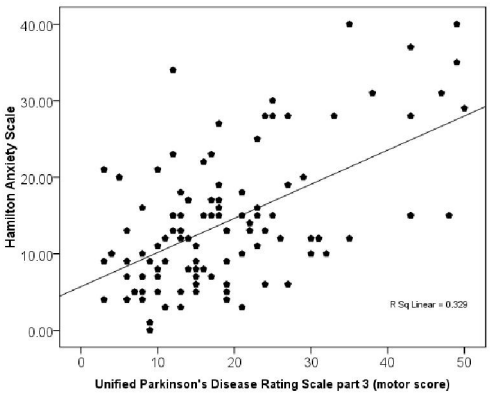


Figure: 1A



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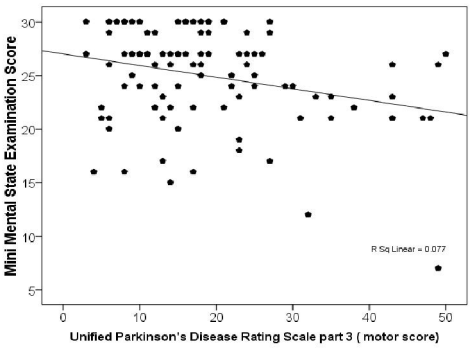

Figure: 1C

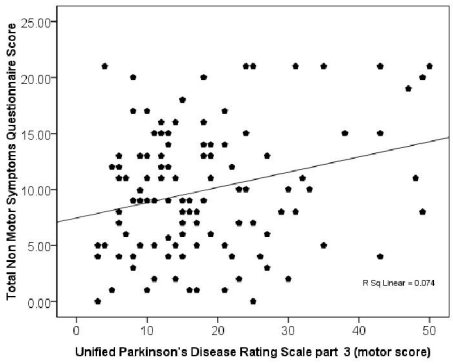

Figure 2.

4. Discussion

NMS of PD have received relatively little attention, despite diverse presentations of these conditions and their impact on the quality of life. It is increasingly clear that non-motor symptoms have a dramatic effect on the lives of both PD patients and caregivers [7]. NMS of PD are not well recognized in clinical practice, either in primary and secondary care. Some physicians have devoted significant attention to the NMS of PD [2, 19].

Depression, anxiety, fatigue and sleep disturbance are among the most troubling symptoms for PD patients, but during routine consultations, **Shulman *et al.*** [20] reported that patients with these symptoms are not identified by neurologists in over 50% of consultations and sleep disturbance in particular is not recognized in over 40% of patients. These efforts induced the Movement Disorder Society to modify the existing UPDRS to include an independent subpart to measure NMS [21].

To our knowledge, this is the first report of prevalence of non-motor symptoms among a large sample of Egyptian PD patients. Our study population included all stages of **Hoehn and Yahr** Scale [13] from 1 to 5, probably as a consequence of study participants being recruited from the outpatient clinic of Assiut University Hospital. Tremor (95.5%), rigidity (85.7%), and bradykinesia (82.1%), were the dominant motor symptoms in the present study.

In the present study the overall rate of NMS that occurred not only during the off period, but during other periods as well was 96.25% (at least one domain of NMSS), and only 3.75% had criteria of PD without any NMS. All of them seen as early as stage I of **Hoehn and Yahr**[13]. Previous studies that focused on NMS reported a wide range of prevalence, between 17% and 100%, of NMS among PD patients showing motor fluctuations. **Hillen and Sage** [22] reported that only 17% of patients with fluctuating PD had NMS. They used a single question with an open answer and likely to have underestimated the prevalence of NMS. On the other hand, **Witjas *et al.*** [23] reported that all patients had at least one type of NMS, most of which were associated with the off state. The wide variation in the prevalence for NMS may be related to severity, staging, time of assessment (off or on period) as well as tasks used to diagnose NMS.

Shulman *et al.* [20], **Sullivan *et al.*** [24] reported a low detection rate of NMS. In one study, **Sullivan *et al.*** [24] depression, anxiety, and constipation were addressed and treated in only 50% of patients, with even lower detection rates for other NMS such as fatigue (6%), memory (9%), somnolence (16%), insomnia (30%), incontinence (35%), and pain (35%). Another study, **Shulman *et al.*** [20] found a lower diagnostic accuracy for fatigue (25%), depression

(35%), anxiety (42%), and sleep disturbance (60%) by physician's interview compared to patients' response using a screening questionnaire.

Depression:

As domain Mood/cognition was the most common domain recorded in the present study 87.5% but because this domain including only 6 items concerning in NMSS depression and Anxiety we tried to apply HAM-D and HAM-A scales to assess not only the symptoms and degree of depression and Anxiety.

Depression in PD is the main factor impacting quality of life [25, 26, 27]. The actual rate of depression in persons with PD is unknown but reported rates vary from 7 to 76%. This may be due to the different criteria used to diagnose depression and to the different characteristics of the population to be screened. Higher rates are observed in subjects of outpatient neurologic clinics when compared to community setting studies [4]. **In the present study** depression was recorded in 53.6%, with variable degree of depression. The high prevalence of depression reported in this study is consistent with **Schrag *et al.*** [28] and **Kummer and Teixeira**, [29]; **Dobkin *et al.*** [30] and it may be attributable to sociocultural factors including poor social support in Egypt and the high cost of PD treatment. The significant correlation between UPDRS with Hamilton depression in the studied patients means that increasing the severity of PD associated with more depression.

Some authors claimed that depression in PD would be mild to moderate, seldom fulfilling diagnostic criteria for a major depressive disorder. However, the frequency of major depression, minor depression and dysthymia is now estimated to be 17%, 22% e 13%, respectively [31]. In the present study 18.8%, 19.6% and 15.2% had mild, moderate and severe degree of depression respectively. Age and sex had no relation with the depression while the potential risk factors for depression in PD include, comorbidity with anxiety, increased severity of the disease.

A problem regarding depression in PD is the fact that the somatic symptoms following depression may superimpose symptoms from PD itself and other co morbidities. Psychomotor slowness, decreased initiative, and blunted affect are depressive symptoms which may be confounded with bradykynesia, stooped posture and hypomania in PD. Our results showed that depressed PD patients had higher UPDRS-3 and HY scores when compared to non-depressed PD patients. There are conflicting studies on the association between depression and PD severity but the majority of them report a positive association [32, 33].

Psychiatric symptoms (hallucinations perceptual problems) were the least recorded symptoms (9.9%) among Egyptian PK patients. **Gallagher *et al.*** [34] reported that cognitive and neuropsychiatric complications of PD were generally well documented: hallucinations (82%), and delusional thought disorder (64%). The difference may be related to the use of anti-cholenergic drugs anti-parkisonian drugs.

Anxiety

The precise frequency of anxiety in PD is still uncertain. Nevertheless it is recognized that anxiety in PD is extremely common. It has been estimated that the prevalence rate of anxiety disorder in patients with PD was variable ranging from 3.6% to 40% [35,36]. It is feasible that the most frequent anxiety disorder in PD is social anxiety disorder, as nearly 50% of PD patients can be diagnosed with social phobia [37]. In the present study, the highest prevalence of the non-motor manifestations among PD patients was the anxiety. It was recorded in 77.7%, mild degree of anxiety was the commonest (52.7%), while only 15.2% had severe anxiety. The high prevalence of depression and anxiety observed in our study indicates that this problem should be addressed by clinicians managing PD patients.

We did not find any significant differences in prevalence of anxiety or depression between males and females, in contrast with the general population in which women have a higher prevalence of anxiety disorders than men [5,38, 39] . This result supports the hypothesis that anxiety and depression disorders are uniquely associated with PD, and may differ from anxiety disorders in the general population.

Comorbid depression with anxiety was observed in 53.6% of our patients. The severity and the duration of PD were also positively related to anxiety and depression. **Menza *et al.*** [38] reported 26% comorbidity, while **Nuti *et al.*** [5] reported 19% comorbidity. It is possible that anxiety worsens motor signs, which can reciprocally determine further depression.

This high prevalence of anxiety disturbances in PD underscores its prominence as a significant psychiatric co-morbidity in PD. Previous estimates have ranged from 28%- 40% [5, 40, 41, 38, 42, 43, 44] which was lower than observed in our study. This difference may be related to methodological assess, severity and staging.

Cognitive impairment and dementia

According to the NMSS the domain of attention/memory including only 3 items, the prevalence of this domain was 72.3%. However this domain didn't allowing to measure dementia and the degree of cognitive important. So we applied MMSE as screening test to measure the prevalence of dementia among our patients.

The prevalence of dementia in PD vary between 24 to 31% [45]. In the present study dementia was recorded in 22.3% of PD patients most of them had

mild degree of dementia. One of the risk factor of dementia is the educational factor which may be relevant since 62.5 % of our sample were illiterate and 11.6% were educated for less than 6 years. It is worth mentioning that patients with low educational level are particularly susceptible to the deleterious effect of depression on cognition [46].

However the prevalence of dementia among our patients was lower than that recorded in the previous study it may be attributed to many factors such as ; young age group (the mean was 60.96), shorter disease duration (the mean = 6 years), severity of motor signs as 90 patients presented in stage 1, 1.5 and 2. Anxiety and depression were also presented as risk factors for dementia in PD patients, as MMSE were correlated with the age, duration, UPDRS, depression and anxiety.

The recorded cardiovascular domain included experience light-headedness, dizziness, weakness on standing from sitting or lying positions and falling secondary to fainting or blacking out. In the present study, cardiovascular symptoms domain recorded in 70.5% of patients with PD. Dizziness was the commonest cardiovascular symptom in PD, with prevalence rate up to 58%[47]. Similar to our result, **Gallagher *et al.*** [34] recorded CV domain in 64%.

Sleep Disorders

In the present study sleep/fatigue domain was the second most prevalent non-motor symptoms in PD after anxiety. Nearly 78.6% of PD patients have one or more type of sleep disorders. The commonest type of sleep disturbances in the form of difficulty getting to sleep at night or staying asleep at night that was recorded in 46.4%. In the previous studies, 80 to 90% of PD patients have some type of sleep difficulty [6], and virtually all patients with PD will experience a sleep disorder during the course of the disease [2]. Sleep disorders may be caused by a series of factors including degeneration of sleep regulatory centers in the brainstem and thalamo-cortical pathways, or due to PD symptoms affecting the normal sleep, such as the motor impairment, depressive and anxiety disorders, and bladder incontinency.

Sleep fragmentation is the earliest and most common sleep disorder in PD, and it gradually worsens with disease progression [6]. Vivid dreaming nightmares and night terrors (27.7%), were also common in this study that nearly similar to the results reported by **Sharf *et al.*** [48].Thirty one patients vocalize during sleep recorded in our study. The vocalization content may vary from incomprehensible sounds to detailed conversations, laughing, cursing or screaming as recorded by Friedman and **Millman**,[49]. PD patients were also more prone to other sleep disorders such as restless legs syndrome, periodic limb movements that was recorded in 15% in the present study. Sleep disorders in PD are seldom diagnosed and treated.

In the present study gastrointestinal domain was also common that were recorded in 76.8% in studied patients, constipation (51.8%) and dribbling saliva (31.7%) during the daytime were the commonest symptoms. Our findings were consistent with results of **Gallagher *et al.*** [34] who reported that hyper salivation and constipation in 48% PD patients. However, the less common GIT symptoms in the present study, was that difficulty of swallowing or chocking problems with drinking (24.1%), feeling of incomplete bowel emptying after toilet (16.1%),vomiting or nausea (10.7%), loss or change in taste or smell ability (9.8%), and fecal incontinence (4.5%). Although salivary production is reduced in PD, drooling occurs partly due to reduced swallowing which may reflect involvement of cranial autonomic ganglia or brainstem salivatory nuclei [50]

These recorded GIT symptoms were partially consistent with recording data of **Sakakibara *et al.*** [51] who reported that patients with PD $\geq$ 50% report lower GIT symptoms such as constipation, diarrhea and fecal incontinence. Because in PD ; constipation occurs from decreased colonic transport and disturbed defecation as in 80% of PD patients; clonic transport time is increased and most patients cannot defecate completely [52]. However, **Cersosimo and Benarroch**, [50] stated that upper GIT symptoms including drooling, esophageal dysmotility and delayed reduced gastric emptying is secondary to involvement of cranial autonomic ganglia or brainstem salivatory nuclei .

Urinary domain

The reported prevalence of lower urinary tract symptoms (LUTS) in patients with PD ranges from 38 to 71% [53, 54, 55, 56]. However, it has been difficult to determine to what extent PD contributes to LUTS. This is because not only PD patients, but also men older than 60 years of age may have an obstruction component to their urinary symptoms brought about by benign prostate hyperplasia. Among our studied patients urinary symptoms were recorded in 76.8% including nocturia (59.8%) and urgency (54.5 %) that were consistent with findings of **Gallagher *et al.*** [34] and **Sakakibara *et al.*** [51] study. **Araki and Kuno**, [57] have shown a correlation between bladder dysfunction in patients with PD and neurological disability.

Sexual dysfunctions:

Only few previous studies have looked at sexual symptoms in PD and control subjects. The reported prevalence of sexual symptoms in patients with PD ranges from 37 to 65% [58, 59, 60, 61, 62, 63, 64]. In the present study sexual dysfunction domain was recorded in more than half percent of patients (73.2%),

either feeling less interested in sex or more interested in sex (46.4%) or difficult to have sex in 48 patients (42.9%). Our findings are nearly similar to finding of **Gallagher *et al.*** [34] study who recorded libido changed in 45% among PD patients.

Miscellaneous domain included pain, change in taste, weight and excess sweating. This domain was recorded in 64.3% in the present study. Sweating problems in PD is considered one of non motor symptoms and autonomic dysfunctions in PD [3]. **Schestatsky *et al.*** [65] study reported hyperhidrosis in PD. In the present study, we recorded excessive sweating in 21.4% of studied PD patients. It has been postulated that excessive sweating in PD may occur as a compensatory reaction to lower sympathetic function in the extremities [55].

Unlike previous studies (**Witjas *et al.*** [23]; **Hillen and Sage**, [22] ;**Raudino**, [66]; **Gunal *et al.***[67]), diffuse pain was frequent (47.1%) in our study. These discrepancies may be attributed to methodological differences for assessment of pain. Our study counted NMS occurring during off and on periods.

In this study, we found that NMS were very common in Egyptian patients with PD, with a prevalence of the whole spectrum of NMS being 96.3%, and the similarities with international studies strongly suggests the results are reliable but larger confirmatory studies may be needed.

In conclusion, neuropsychiatric and NMS are common in PD, but not frequently recognized by clinicians. The assessment of these conditions must be routinely conducted due to their impact on the motor signs and on the quality of life of patients and caregivers. The results of this study further indicated that Egyptian doctors should increase their recognition capabilities of NMS in PD. Future studies must focus on elucidating their pathophysiology these syndromes.

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References

1 – Parkinson J. An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci., 2002;14:223-36.
2- Chaudhuri KR, Yates L, Martinez-Martin P. The non-motor symptom complex of Parkinson's disease: a comprehensive assessment is essential. Curr Neurol Neurosci Rep., 2005; 5: 275-83.
3- Chaudhuri KR, Healy DG, Schapira AH; National Institute for Clinical Excellence. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol., 2006; 5(3):235-45.
4- Veazey C, Aki SO, Cook KF, Lai EC, Kunik ME. Prevalence and treatment of depression in Parkinson's disease. J Neuropsychiatry Clin Neurosci., 2005; 17(3):310-23.
5- Nuti A, Ceravolo R, Piccinni A, Dell'Agnello G, Bellini G, Gambaccini G, Rossi C, Logi C, Dell'Osso L, Bonuccelli U. Psychiatric comorbidity in a population of Parkinson's disease patients. Eur J Neurol 2004;11(5):315–320.
6- Oerlemans WG, de Weerd AW. The prevalence of sleep disorders in patients with Parkinson's disease. A self-reported, community-based survey. Sleep Med., 2002;3: 147-149.
7- Clarke C E, Zobkiw R M, Gullaksen E. Quality of life and care in Parkinson's disease. Br J Clin Pract., 1995; 49 (6): 288-93.
8- Voon V, Krack P, Lang AE, Lozano AM, Dujardin K, Schüpbach M, D'Ambrosia J, Thobois S, Tamma F, Herzog J, Speelman JD, Samanta J, Kubu C, Rossignol H, Poon YY, Saint-Cyr JA, Ardouin C, Moro E. A multicentre study on suicide outcomes following subthalamic stimulation for Parkinson's disease. Brain 2008;131:2720-2728.
9- Chaudhuri KR, Martinez-Martin P, Brown RG, Sethi K, Stocchi F, Odin P, Ondo W, Abe K, Macphee G, Macmahon D, Barone P, Rabey M, Forbes A, Breen K, Tluk S, Naidu Y, Olanow W, Williams AJ, Thomas S, Rye D, Tsuboi Y, Hand A, Schapira AH. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: Results from an international pilot study. Mov Disord .,2007;22:1901–1911.
10- Chaudhuri KR, Martinez-Martin P. Quantitation of non-motor symptoms in Parkinson's disease. Eur J Neurol., 2008;15 (suppl 2):2–7.
11- Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of Clinical-Diagnosis of Idiopathic Parkinsons-Disease - A Clinicopathological Study of 100 Cases. Journal of Neurology Neurosurgery and Psychiatry, 1992; 55(3):181–184.
12- Fahn S, Elton R, Members of the UPDRS Development Committee (1987) The Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne DB, Goldstein M, eds. Recent developments in Parkinson's disease, vol 2. Macmillan Health Care Information, Florham Park, pp 153–63, 293–304.
13- Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality.Neurology, 1967; 17: 427-42.
14- Folstein MF, Folstein SE, McHugh PR (1975) ''Mini-Mental State''. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res., 12:189–198.
15- Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry, 23:56–62.
16- Hamilton M: The assessment of anxiety states by rating. Br J Med Psychol., 1959; 32:50–55.

17- Chaudhuri KR, Martinez-Martin P, Schapira AH, Stocchi F, Sethi K, Odin P, Brown RG, Koller W, Barone P, MacPhee G, Kelly L, Rabey M, MacMahon D, Thomas S, Ondo W, Rye D, Forbes A, Tluk S, Dhawan V, Bowron A, Williams AJ, Olanow CW.International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for Parkinson's disease: the NMSQuest study. Mov Disord. 2006;21(7):916-23.

18- Farrag A, Farwiz HM, Khedr EH, Mahfouz RM, Omran SM. Prevalence of Alzheimer's disease and other dementing disorders: Assiut-Upper Egypt study. - Dement Geriatr Cogn Disord. 1998 ;9 (6):323-8.

19- Hely MA, Morris JG, Reid WG, Trafficante R. The Sydney Multicentre Study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 yr. Mov Disord., 2005; 20: 190-9.

20- Shulman L M, Taback R L, Rabinstein AA, Weiner W J. Non-recognition of depression and other non-motor symptoms in Parkinson's disease. Parkinsonism Relat Disord. 2002; 8 (3): 193-7.

21- Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease. The Unified Parkinson's Disease Rating Scale (UPDRS): status and recommendations. Mov Disord., 2003; 18: 738-50.

22- Hillen ME, Sage JI. Nonmotor fluctuations in patients with Parkinson's disease. Neurology, 1996; 47: 1180-3.

23- Witjas T, Kaphan E, Azulay JP, Blin O, Ceccaldi M, Pouget J, Poncet M, Cherif AA. Nonmotor fluctuations in Parkinson's disease: frequent and disabling. Neurology, 2002; 59: 408-13.

24- Sullivan KL, Ward CL, Hauser RA, Zesiewicz TA. Prevalence and treatment of non-motor symptoms in Parkinson's disease. Parkinsonism Relat Disord., 2007;13: 545.

25- Gulati A, Forbes A, Stegie F, *et al.* A clinical observational study of pattern and occurrence of non-motor symptoms in Parkinson's disease. Presented at Movement Disorders Congress, Rome, June 2004. Mov Disord., 2004; 19 (Supp 9): S406 (P1187).

26- Hobson P, Holden A, Meara J. Measuring the impact of Parkinson's disease with the Parkinson's Disease Quality of Life questionnaire. Age Ageing, 1999; 28 (4): 341-6

27- Karlsen KH, Larsen JP, Tandberg E, Maeland JG. Influence of clinical and demographic variables on quality of life in patients with Parkinson's disease .J Neurol Neurosurg Psychiatry, 1999; 66: 431-435.

28- Schrag A, Jahanshahi M, Quinn N. What contributes to quality of life in patients with Parkinson's disease? J Neurol Neurosurg Psychiatry 2000; 69: 308-312.

29- Kummer A, Teixeira AL. Depressive disorders in Parkinson's disease may be due to a shared immune-mediated neurodegenerative process. Med Hypotheses, 2008; 70: 201-202.

30- Dobkin RD, Allen LA, Menza M. Cognitive-behavioral therapy for depression in Parkinson's disease: a pilot study. Mov Disord., 2007; 22: 946-952.

31- Findley L, Aujla M, Bain PG, Baker M, Beech C, Bowman C, Holmes J, Kingdom WK, MacMahon DG, Peto V, Playfer JR. Direct economic impact of Parkinson's disease: a research survey in the United Kingdom. Mov Disord., 2003; 18 (10): 1139-45.

32- Piovezan MR, Teive HA, Piovesan EJ, Mader MJ, Werneck LC. Cognitive function assessment in idiopathic Parkinson's disease. Arq Neuropsiquiatr., 2007;65(4A):942-6.

33- Prado RC, Barbosa ER. Depression in Parkinson's disease. Study of 60 cases. Arq Neuropsiquiatr., 2005; 63(3B):766-71.

34- Gallagher DA, Lees AJ, Schrag A.. What are the most important nonmotor symptoms in patients with Parkinson's disease and are we missing them? Mov Disord., 2010; 25(15):2493-500.

35- Leentjens AF, Dujardin K, Marsh L, Martinez-Martin P, Richard IH, Starkstein SE, Weintraub D, Sampaio C, Poewe W, Rascol O, Stebbins GT, Goetz CG. Anxiety rating scales in Parkinson's disease: critique and recommendations.Mov Disord. 2008; 23(14):2015-25.

36- Walsh K, Bennett G. Parkinson's disease and anxiety.. Postgrad Med J. 2001 ; 77(904):89-93.

37- Kummer A, Cardoso F, Teixeira AL. Frequency of social phobia and psychometric properties of the Liebowitz social anxiety scale in Parkinson's disease. Mov Disord. 2008; 23 (12):1739-43.

38- Menza MA, Robertsonhoffman DE, Bonapace AS. Parkinsons-Disease and Anxiety – Comorbidity with Depression. Biological Psychiatry, 1993;34(7):465–470.

39- Hettema JM, Prescott CA, Kendler KS.A population-based twin study of generalized anxiety disorder in men and women. J Nerv Ment Dis. 2001; 189(7):413-20.

40- Stein MB, Heuser IJ, Juncos JL, Uhde TW. Anxiety disorders in patients with Parkinson's disease. Am J Psychiatry, 1990;147(2):217–220.

41- Lauterbach EC, Freeman A, Vogel RL. Differential DSM-III psychiatric disorder prevalence profiles in dystonia and Parkinson's disease. Journal of Neuropsychiatry and Clinical Neurosciences, 2004;16 (1):29–36.

42- Shiba M, Bower JH, Maraganore DM, McDonnell SK, Peterson BJ, Ahlskog JE, Schaid DJ, Rocca WA. Anxiety disorders and depressive disorders preceding Parkinson's disease: a case-control study. Mov Disord 2000; 15(4):669–677.

43- Henderson R, Kurlan R, Kersun JM, Como P.Preliminary examination of the comorbidity of anxiety and depression in Parkinson's disease.J Neuropsychiatry Clin Neurosci. 1992 Summer;4(3):257-64.
44- Weisskopf MG, Chen H, Schwarzschild MA, Kawachi I, Ascherio A.Prospective study of phobic anxiety and risk of Parkinson's disease. Mov Disord. 2003; 18(6):646-51.
45- Aarsland D, Zaccai J, Brayne C. A systematic review of prevalence studies of dementia in Parkinson's disease. Mov Disord., 2005;20: 1255-1263.
46- Kummer A, Harsányi E, Dias FMV, Cardoso F, Caramelli P, Teixeira AL. Depression impairs executive functioning in Parkinson's disease patients with low educational level. Cogn Behav Neurol .,2009; Sep;22(3):167-72.
47- Low PA. Prevalence of orthostatic hypotension. Clinical Autonomic Research ,2008; 18(Suppl.1): 8-13.
48- Sharf B, Moskovitz C, Lupton MD, Klawans HL. Dream phenomena induced by chronic levodopa therapy. J Neural Transm. 1978;43(2):143-51.
49- Friedman JH, Millman RP.Sleep disturbances and Parkinson's disease.CNS Spectr. 2008; 13(3 Suppl 4):12-7.
50- Cersosimo MG, Benarroch EE. Neural control of the gastrointestinal tract: implications for Parkinson disease. Mov Disord. 2008; 23(8):1065-75.
51- Sakakibara R, Uchiyama T, Yamanishi T, Shirai K, Hattori T. Bladder and bowel dysfunction in Parkinson's disease. Journal of Neural Transmission 2008; 115(3):443-60.
52- Jost WH, Schrank B. Defecatory disorders in de novo Parkinsoniansecolonic transit and electromyogram of the external anal sphincter. Wiener klinische Wochenschrift, 1998;110(15):535-7.
53- Murnaghan GF.Neurogenic disorders of the bladder in Parkinsonism. Br. J.Urol 1961;33: 403-409.
54- Hattori T, Yasuda K, Kita K, Hirayama K. Voiding dysfunction in Parkinson's disease.Jpn J Psychiatry Neurol., 1992;46: 181- 186.
55- Gray R, Stern G,Malone-Lee J. Lower urinary tract dysfunction in Parkinson's disease: changes relate to age and not disease. Age Ageing, 1995; 24: 499-504.
56- Andersen JT. Disturbances of bladder and uretheral function in Parkinson's disease. Int Urol Nephrol., 1985; 17: 35-41.
57- Araki I, Kuno S. Assessment of voiding dysfunction in Parkinson's disease by the international prostate symptom score. J Neurol Neurosurg Psychiatry. 2000 ; 68(4):429-33.
58- Brown RG, Jahanshahi M, Quinn N, Marsden CD. Sexual function in patients with Parkinson's disease and their partners. J Neurol Neurosurg Psychiatry, 1990;53:480–486.
59- Basson R. Sexuality and Parkinson's disease. Parkinsonism Relat Disord., 1996;2:177–185.
60- Welsh M, Hung L, Waters CH. Sexuality in women with Parkinson's disease. Mov Disord. 1997; 12: 923–927.
61- Jacobs H, Vieregge A, Vieregge P. Sexuality in young patients with Parkinson's disease: a population based comparison withhealthy controls. J Neurol Neurosurg Psychiatry, 2000; 69: 550–552.
62- Moore O, Gurevich T, Korczyn AD, Anca M, Shabtai H, Giladi N. Quality of sexual life in Parkinson's disease. Parkinsonism Relat Disord., 2002;8:243–246.
63- Papatsoris AG, Deliveliotis C, Singer C, Papapetropoulos S. Erectile dysfunction in parkinson's disease. Urology, 2006; 67: 447–451.
64- Bronner G, Royter V, Korczyn AD, Giladi N. Sexual dysfunction in Parkinson's disease. J Sex Marital Ther., 2004;30:95–105.
65- Schestatsky P, Valls-Sole J, Ehlers JA, Rieder CR, Gomes I. Hyperhidrosis in Parkinson's disease. Movement Disorders 2006; 21(10):1744- 8.
66- Raudino F. Non motor off in Parkinson's disease. Acta Neurol Scand., 2001; 104: 312-5.
67- Gunal DI, Nurichalichi K, Tuncer N, Bekiroglu N, Aktan S. The clinical profile of nonmotor fluctuations in Parkinson's disease patients. Can J Neurol Sci., 2002; 29: 61-4.

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Study of bis{2-(naphtha [3,4]imidazol-2-yl) quinolinato} Magnesium

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Abstract: The study of bis{2-(naphtha[3,4]imidazol-2-yl) quinolinato} Magnesium (MgNIQ) is presented in this report. It was observed the decomposition temperature is high to 577℃ but no melting transition (T_m) of MgNIQ up to 450℃. By using of MgNIQ as emitted layer exhibits a broad maximum spectrum peak at 615 nm. The color of the emitted light is in the orange-red region in the CIE coordinate of x = 0.36 y = 0.53.
[Yu-Feng Lin, Pin-Wen Cheng, Shih-Hsuan Chiu, Chen-Hao Wang, Shung-Jim Yang, Anchi Yeh. **Study of bis{2-(naphtha [3,4]imidazol-2-yl) quinolinato} Magnesium.** Life Sci J 2012;9(2):519-522]. (ISSN:1097-8135). http://www.lifesciencesite.com. 77

Keywords Electroluminescence; red light; device

1. Introduction

Luminescent organic/organometallic compounds have attracted much attention recently because of their potential applications in electroluminescnt (EL) displays [1-5]. Organic and polymer devices provide advantages over their inorganic counterparts, such as high luminous efficiency and fine-pixel formation. Luminescent chelate complexes have been shown to be particularly useful in electroluminescnt (EL) displays because of their relatively high stability and volatility. The most well-known example of such chelate compounds is $A1q_3$, not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. Via the modification of the ligand of metal chelate compound, the emission color of a metal chelate compound may be tuned. Other properties, such as thermal stability and carrier mobility, may also be improved upon. In the present work, we report the synthesis and electroluminescent (EL) property of bis{2-(naphtha[3,4]imidazol-2-yl) quinolinato} Magnesium (MgNIQ). The attachment of the naphtha[3,4]imidazol group at 2-position would allow the ligand to form stable complexes with metal ions similarly to 8-hydroxyquinoline. Therefore, the thermal stability, an important character for the practical application in the electronic fields, of this metal complex is investigated by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The organic emitting device using MgNIQ as emitting layer has been fabricated to study the electroluminescent property of this metal complex.

2. Experimental

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The dibutylmagnesium solution (0.5M in heptane, 0.6927mL, 10mmol) was slowly added to 100 ml of THF solution containing 2-(naphtha[3,4]imidazol-2-yl) quinoline [8] (2.95g, 10mmol) at 0℃ under N_2. After the resulting mixture was stirred at room temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at 5×10^{-3} Torr, and the residual solid was sublimed to purify the final product. Orange powder of MgNIQ was obtained in 85% yield. The formula of this compound has been determined by ^{1}H NMR and elemental analysis. The organic light emitting device, Fig. 1, using MgNIQ as the emitting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under 3×10^{-7} Torr. In the present work, the N,N'-bis-(1-naphthyl)-N,N'-diphenyl-1,1'-biphenyl-4,4'-diamin e (NPB) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum (Alq_3) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission International de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermogravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of 20℃/min. Glass transition temperature (T_g) and melting point (T_m) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).

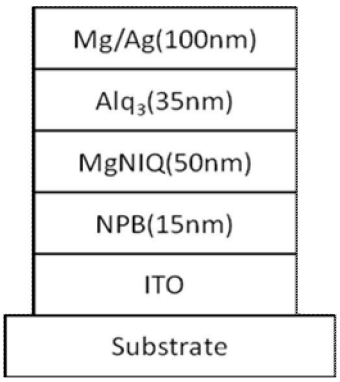
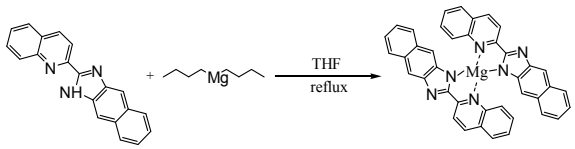


Fig. 1. Device structure of organic light emitting device (OLED) fabricated in this work

Scheme 1.



3. Results and discussion

Fig. 2 shows the TGA of MgNIQ that possesses a maximum rate of weight loss occurring at 577℃ and no weight loss was observed at the temperature lower than 465℃. Above 600℃, there is about 13 wt % of residue composed of Magnesium ash. This Magnesium complex is reasonably stable upon exposure to air and exhibited a very high thermal stability in nitrogen, which is attributed to the fact that the Mg-N (imidazole) bond is highly polarized [9, 10]. The melting temperature (T_m) of MgNIQ was not observed up to 450℃ with DSC curve. The DSC and TGA results indicate that the MgNIQ possesses a very high thermal stability, which may serve as an advantage for the fabrication of organic light emitting device because the use of the materials with high thermal stability as the active emissive layer or carrier transporting layer may provide the device with greater longevity [11, 12].

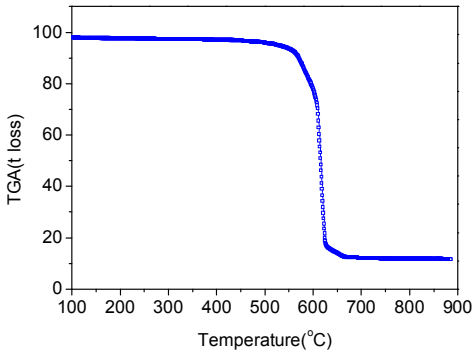


Fig. 2. TGA curve of MgNIQ.

The Photoluminescent (PL) spectra of the MgNIQ solutions and neat film, excited with 350 nm laser line, were illustrated in Figure 3. At low concentration, 1×10^{-5} M in DMF, only one emission band is observed with maximum at 489nm, corresponding to the relaxation of MgNIQ from the excited state of a single molecule into ground state. Besides the 489 nm band, a new emission band appeared while the concentration of MgNIQ increased from 1×10^{-5} to 1×10^{-3} M. This new emission band having a maximum at 565nm is observed in the spectrum of the MgNIQ neat film. We have assigned this new emission band to the excimer and higher aggregates emission [13, 14] resulting from the relaxation of collision complex into the lower energy state. The EL spectrum of organic light emitting device at the bias voltage of 13 V, Fig. 4, shows the broad emission band in the 500-700nm region with the maximum at 615nm. The emission is almost fixed in the orange-red region in the CIE coordinate of x = 0.36 y = 0.53, Fig. 5. For the small molecular organic materials, to develop the new type of material with red emission is very important because this kind of material is very seldom prepared so far, and it is very important for the fabrication of full color display panels. The change of the spectral wavelength may be achieved also by general conception of search and design of modified materials for wide band emission consists in substitution of the backside groups by electron acceptors like halogens etc. and different kind of donors [15, 16]. At the same time important role here may play electron-vibration interactions determining the spectral broadening of the emission lines. So the future strategy of the materials design may be in this way also.

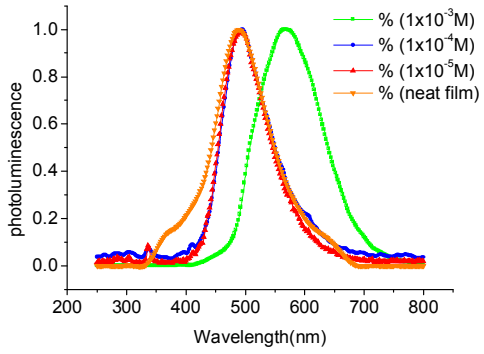


Fig. 3. Photoluminescent spectra of the MgNIQ in solutions and neat film

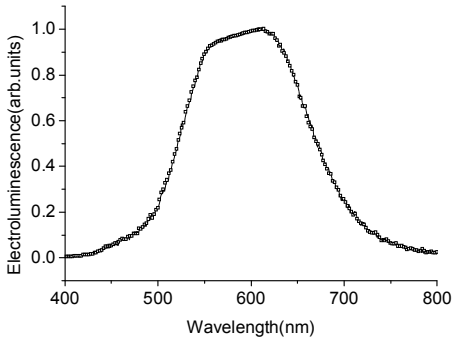


Fig. 4. EL spectrum of OLED fabricated in this work.

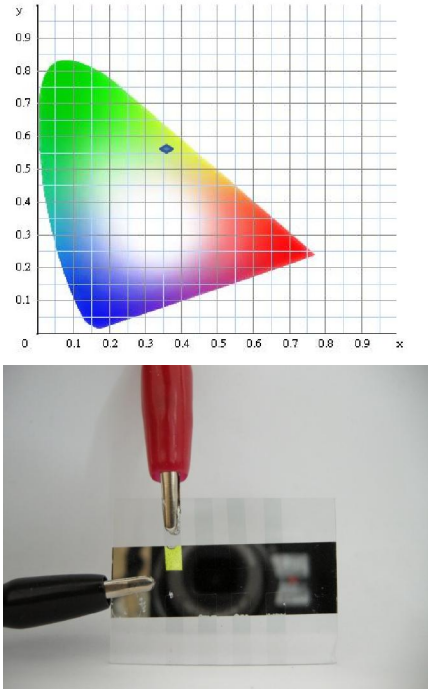


Fig. 5. CIE color coordinates (CIEx,y = 0.36，0.53) for the light emission produced by the OLED devices.

Figure 6 shows the energy level diagram of the HOMO and LUMO of the different organic materials and the work function of cathode and anode. The LUMO energy of MgNIQ is 2.6eV determined form the HOMO energy (5.8eV) obtained from the cyclic voltammetry (CV) method and the optical band gap estimated from the absorption onset. Comparing the energy level of MgNIQ with NPB, it is clear that the MgNIQ has the much higher hole injection barrier than that of NPB; in fact, it is impossible for the hole injection from ITO into MgNIQ without the assistance of NPB or some other kind of HTLs. This diagram also pointed out that the Alq_3 has the lower electron injection barrier than that of MgNIQ, so the electron injection from the MgAg into MgNIQ will be enhanced and confines the recombination zone at the interface between NPB and MgNIQ. Fig. 7 shows the current-voltage and luminance- voltage characteristics of this device having a low turn on voltage of about 6.0V for current and luminance. This device shows a brightness of 2414 cdm^{-2} at the driving voltage of 13V with current density of 334 mA/cm^2, decaying to 25 cdm^{-2} in 120 hours.

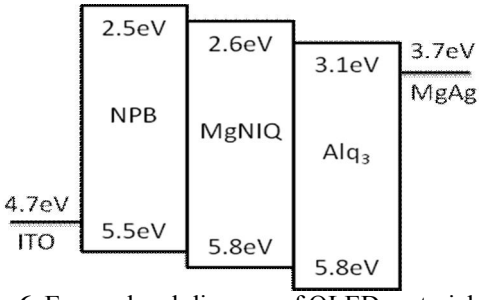


Fig. 6. Energy level diagram of OLED materials, ITO, and Mg-Ag alloy

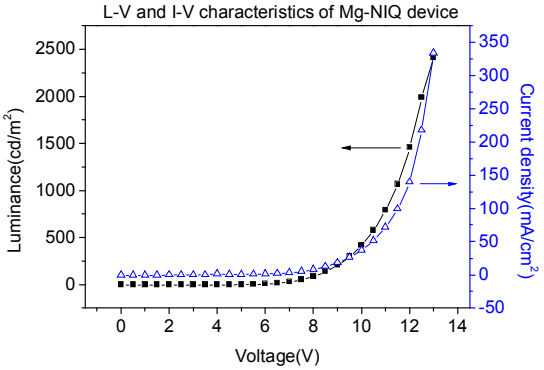


Fig.7. Current-voltage and luminance- voltage characteristics of OLED fabricated in this work.

4. Conclusion

A novel metal complex, bis{2-(naphtha[3,4] imidazol-2-yl) quinolinato} magnesium (MgNIQ), was successfully prepared by the reaction of 2-(naphtha[3,4]imidazol-2-yl) quinoline and dibutyl-magnesium. The investigation demonstrated that this compound possess charge transfer and film-forming properties and has high thermal stability.

The excimer emission resulting from the collision complex was observed. The devices composed of MgNIQ as the emitting layer can tune the emitting color via the controlling of carrier recombination region. Because of its high thermal stability and excellent electrical characteristics, MgNIQ and its related compound suggest a possible application for the use of the organic light emitting devices.

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Reference

[1] C. W. Tang, S.A. VanSlyke, Appl. Phys. Lett., 51 (1987) 913.
[2] C. Adachi. S. Tokito, J. Tsutusi, S. Saito, Jpn. J. Appl. Phys., 27 (1988) 713.
[3] J. H. Burroughes, D. D. C. Bradley, A. R. Brown, R. N. Marks, K. Mackay, R. H. Friend, P. L. Burns, A. B. Homes. Nature, 347 (1990) 539.
[4] J. R. Sheats, H. Antoniadis, M. Hueschen, W. Leonard, J. Miller, R. Moon, D. Roitman, A. Stocking, Science, 273 (1996) 884.
[5] H. Nakada, T. Tohma. Inorganic and Organic Electroluminescence, Wissenschaft-und-Technik-Verlag, Berlin, (1996) 385.
[6] S. –F. Liu, C. Seward, H. Aziz, N. –X. Hu, Z. Popovic, S. Wang, Organnometallics, 19 (2000) 5709.
[7] H. Schmidbaur, J. Lettenbauer, D. L. Wilkinson, G. Muller, O. Z. Kumberger, Naturforsch, 46B (1991) 901.
[8] T. R. Chen, A. C. Yeh and J. D. Chen, Tetrahedron Lett., 46 (2005) 1569.
[9] S. -F. Liu, Q. Wu, H. L. Schmider, H. Aziz, N. –X. Hu, Z. Popovic, S. Wang, J. Am. Chem. Soc., 122 (2000) 3672.
[10] Q.Wu, M. Esteghamatian, N. –X. Hu, Z. D. Popovic, G. Enright, S. R. Breeze, S. Wang, Angew. Chem. Int. Ed., 38 (1999).
[11] Z. –K. Chen, H. Meng, Y. –H. Lai, W. Huang, Marcromolecules, 32 (1999) 4351.
[12] S. Tokito, H. Tanaka, K. Noda, A. Okada, Y. Taga, Appl. Phys. Lett., 70 (1997) 1929.
[13] R. Aroca, T. D. Cano, Chem. Mater., 15 (2003) 38.
[14] H. Beens, A. Weller, Organic Molecular Photophysics, ed. Birks, J. B., Vol. 2, New York: Wiley, 1975, p. 159.
[15] M. Makowska-Janusik, J. Sanetra, H. Palmers, D. Bogdal, E. Gondek, I. V. Kityk, Materials Letters, 58 (2004) 555.
[16] Albert J. van Reenen, Lon J. Mathias, Liezel Coetzee, Polymer 45 (2004) 799.

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Serum Levels of Adiponectin and Ghrelin in Patients with Acute Myocardial Infarction

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Abstract: Background: Inflammation is widely known to play a key role in the development and progression of cardiovascular diseases. It has been observed that adipokines play an increasingly large role in systemic and local inflammation. Therefore, adipose tissue may have a more important role than previously thought in the pathogenesis of several disease types. We study serum levels of adiponectin and ghrelin in patients with acute myocardial infarction (AMI) with study of some of cardiovascular risk factors. **Methods:** We analyzed 64 patients with acute myocardial infarction admitted at our emergency unit and 20 age and sex matched healthy controls. Clinical parameters, glycemic, lipid profile, tumor necrosis factor- α (TNF-α), interleukin -6 (IL-6), serum insulin, insulin resistance (HOMA-IR), as well as serum adiponectin and ghrelin were assayed. **Results:** We found significantly ($P < 0.01$) increased levels of TNF-α, IL-6, insulin, and HOMA-IR in patients with AMI rather than healthy controls. Plasma adiponectin levels and ghrelin were significantly decreased ($P < 0.01$) compared to those of controls. We found significant correlations between plasma adiponectin levels and BMI, hypertension, TNF-α and IL-6. In the case of ghrelin, we found significant correlations with BMI, HDL-C, diabetes mellitus and fasting glucose. **Conclusions:** Low serum adiponectin and ghrelin level may be risk factor for AMI independent of other traditional cardiovascular risk factors and may provide a novel therapeutic target.

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Keywords: Acute myocardial infarction, adiponectin, ghrelin

1. Introduction

Inflammation is widely known to play a key role in the development and progression of cardiovascular diseases. It is becoming increasingly evident that obesity is linked to many proinflammatory and obesity associated cardiovascular conditions. It has been observed that adipokines play an increasingly large role in systemic and local inflammation. Therefore, adipose tissue may have a more important role than previously thought in the pathogenesis of several disease types (Aprahamian and Sam, 2011). Recently, it has become apparent that adipose tissue is an active endocrine and paracrine organ that releases several bioactive mediators. Those substances influence body weight homeostasis, inflammation, insulin resistance, and diabetes, but their precise role in atherosclerosis has not been elucidated fully (Shah *et al*., 2008). Adiponectin and ghrelin have emerged as novel adipokines, adipose tissue-specific protein but their role in coronary artery disease remains obscure. The adiponectin monomer (30 kDa) has a structure consisting of a globular head and a collagenous tail, and this monomer is able to multimerize to form several stable complexes of low, medium, and high molecular weight. Adiponectin shares sequence homology with collagens VIII and X as well as complement factor C1q. It has previously been referred to as ACRP30 for adipose complement related peptide of 30 kDa based upon its homology to C1q (Scherer *et al*., 1995). Ghrelin, a stomach-derived hormone, functions in multiple biological processes including glucose metabolism, adipogenesis, cell differentiation, and proliferation (Cordido *et al*., 2009). *In vitro* data suggest a dual role of ghrelin proatherogenic in the early phase and antiatherogenic in the advance stage of coronary artery disease (Kellokoski *et al*., 2009).

2. Subjects and Methods:

Subjects and study design:

The study population included 64 (52 males and 12 females) subjects, aged 40 to 66 years, who consecutively were admitted at our emergency unit from June 2010 to July 2011. It was approved by the faculty committee for research ethics. Patients who participated in this study gave informed consent. Participants were assigned to 2 groups:

1- AMI (64) on admission. The AMI diagnosis was made on the basis of typical symptoms consistent with myocardial ischemia (chest discomfort), newly developed ischemic ST-T changes (ST-elevation or ST-segment depression or prominent T-wave inversion) in at least 2 contiguous ECG leads, and elevated cardiac associated biomarkers CK-MB and troponin I.

2- Healthy controls (20). Age and sex matched individuals, without any chronic metabolic or

cardiovascular disease or overt cardiac origin symptoms. None of the controls was receiving any long term medication or was suffering from an acute infection.

The exclusion criteria: was the history of AMI within the past 6 months, the significant concomitant diseases such as autoimmune disease, infection, malignancy, chronic heart failure, and severe chronic liver or renal disease.

Methods:

Venous blood samples were collected on admission and following an overnight fasting, lipogram parameters, fasting serum glucose, liver function testes and renal function testes were analyzed on autoanalyzer Cobas c 311 (Roche/Hitachi cobas c systems). We quantified CK-MB, troponin-I and serum insulin using the two-site immunoenzymometric assay method (TOSOH, AIA 600II). The glycosylated hemoglobin (HbA_{1c}) was determined by high performance liquid chromatography (The BioRad D10 analyser). Body mass index (BMI, kg/m^2) was assessed. The insulin resistance was estimated by a homeostasis model assessment (HOMA-IR) index with the following formula: HOMA-IR = fasting insulin (μIU/ml) x fasting glucose (mg/dl)/405. Measurement of IL-6 was performed by kits from biosource Europe. Measurement of TNF-α was performed using Quantikine, R&D Systems, Inc. Serum concentrations of adiponectin performed using Quantikine R&D Systems; Inc. These assays employ the quantitative sandwich enzyme immunoassay technique. Serum levels of ghrelin were assayed using competitive enzyme immunoassay kits (DRG International Inc., USA, 3706).

Statistical analysis:

The data are presented as mean ± standard deviation (SD) and number (n). Linear relationships between variables were determined using Pearson's correlation coefficient. The differences between groups were compared by T-test and ANOVA test. Statistical significance is considered a value of $P < 0.05$. All statistical analyses were performed using SPSS software, version 10.0.

3. Results:

Table 1, show age, sex and frequencies of each risk factor of study group. The subjects consisted of 52 men and 12 women with a mean age of 55±11 years. Thirty-seven subjects had hypertension and dyslipidemia was present in 35 subjects. Cigarette smoker was present in 49 subjects and 23 diabetic one. Table 2, depicts clinical characteristics and laboratory variables. We found significantly ($P < 0.01$) increased levels of TNF-α, IL-6, insulin, and HOMA-IR in patients with AMI rather than healthy controls. AMI groups had inadequate glycemic control. Plasma adiponectin levels were significantly decreased ($P < 0.01$) compared to those of controls. Serum ghrelin levels significantly decreased across the patients group, compared with the healthy controls group ($P < 0.01$). Diabetic patients with AMI had significantly ($P < 0.05$) downregulated ghrelin levels than the nondiabetic patients. In the case of ghrelin, we found significant correlations with BMI, HDL-C, diabetes presence and fasting glucose. We found significant correlations between plasma adiponectin levels and BMI and hypertension (Table 3). In our study there is no significant correlation between adiponectin or ghrelin level and CK-MB or troponin-I but we found significant correlations between plasma adiponectin levels and TNF-α and IL-6 (Table 4).

Table I: Age, sex and frequencies of each risk factor of study group

Age (range)	40–66 years
Male : Female	52:12
Dyslipidemia (%)	54.7%
Diabetic : Non-Diabetic	23(35.9%): 41(64.1%)
Hypertensive : Non-Hypertensive	37(57.8%): 27(42.2%)
Smoker : Non-Smoker	49(76.6%): 15(23.4%)

Table 2: Clinical and laboratory variables of studied groups

	AMI (n=64)	Controls (n=20)	*p*-value
BMI (kg/m^2)	24.3±0.41	20.3±0.21	> 0.05
CK-MB (ng/ml)	20±5.3	1.5±0.42	< 0.01
Troponin –I (ng/ml)	2.3±0.5	0.01±0.06	< 0.01
Chol (mg/dl)	197±5.4	107±9.4	< 0.05
HDL-C (mg/dl)	48±2.7	45±3.8	> 0.05
LDL-C (mg/dl)	122.5±54.2	112.5±44.2	> 0.05
TG (mg/dl)	164±8.1	104±3.1	< 0.05
F. glucose (mg/dl)	153.5±73	85.2±23	< 0.05
HbA_{1c} (%)	7.2±1.9	5.2±0.9	< 0.05
Insulin (μIU/ml)	25±2.2	15±2.6	< 0.01

HOMA-IR	9.4±4.38	3.15±3.3	< 0.01
Adiponectin (µg/ml)	3.2±1.9	8.1±3.5	< 0.01
Ghrelin (ng/ml)	10.2±0.9	43.6±2.3	< 0.01
TNF-α (pg/ml)	39±9.8	12±2.1	< 0.01
IL-6 (pg/ml)	59±35	7±1.2	< 0.01

CK-MB, Creatine Kinase MB; Chol, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HbA_{1c}, hemoglobin A_{1c}; F.glucose, fasting glucose

Table 3: Correlation between serum adiponectin, ghrelin & other cardiovascular risk factors

	Adiponectin		Ghrelin	
	r	*p* -value	r	*p* -value
BMI	-0.360	0.02*	-0.299	0.04*
Smoking	-0.315	0.36	-0.362	0.62
Hypertention	-0.315	0.04*	-0.192	0.33
DM	-0.172	0.42	-0.245	0.04*
Chol	-0.175	0.54	-0.165	0.06
HDL-C	0.182	0.22	0.312	0.01*
LDL-C	-0.123	0.25	-0.421	0.05
TG	-0.192	0.22	-0.325	0.12
F.glucose	-0.072	0.52	-0.315	0.02*

DM, diabetes mellitus * significant: $p < 0.05$

Table 4: Correlation between serum adiponectin, ghrelin & CK-MB, troponin-I and pro- inflammatory cytokines

	Adiponectin		Ghrelin	
	r	*p* -value	r	*p* -value
CK-MB	-0.360	0.42	-0.299	0.44
Troponin-I	-0.315	0.36	-0.362	0.62
TNF-α	-0.386	0.04*	-0.192	0.33
IL-6	-0.423	0.02*	-0.245	0.64

* Significant: $p < 0.05$

4. Discussion:

The present study demonstrated significantly lower adiponectin and ghrelin serum levels in patients with acute myocardial infarction compared with healthy individuals. The most pronounced elevation of inflammatory markers, TNF-α and IL-6 levels were documented in the AMI patients group with significant negative correlations between them and adiponectin serum levels. Low plasma adiponectin has been associated with myocardial infarction in young patients independent of other conventional risk factors (Persson *et al*., 2010). Adiponectin inhibits the expression of TNF-*α* in adipocytes, and both TNF-*α* and IL-6 inhibits the production of adiponectin (Fasshauer *et al*., 2003; Maeda, *et al*., 2001)), these data suggest that the metabolic consequences observed in obesity may be related to an imbalance of pro- and anti-inflammatory cytokines. Thus, adipokines contribute to the pathophysiology of obesity linked disorders through their ability to modify proinflammatory and metabolic processes. Adiponectin also exerts anti-hypertrophic effects and protects against ischemia-reperfusion injury (Shibata *et al*., 2007; Shibata *et al*., 2004), and it mediates protective effects in obesity related metabolic and vascular disease presumably by its anti-inflammatory actions and protects the heart against ischemia reperfusion injury through its ability to suppress myocardial inflammation and apoptosis (Shibata *et al*., 2005). Adiponectin has anti-atherosclerotic, as well as anti-inflammatory properties that may play an important role in preventing the progression of coronary artery disease. Results from clinical surveys show that low adiponectin levels, while being a predictive marker for early stage atherosclerosis, are also significantly associated with coronary artery disease (Kumada *et al*., 2003). In healthy individuals, adiponectin maintains anti-inflammatory properties, but in Disease states where adiponectin levels decrease result in proinflammatory signaling and exacerbation of disease (Aprahamian and Sam, 2011). Ghrelin, has been implicated in diabetes and insulin sensitivity (Kadoglou *et al*., 2010b), but its precise role in atherosclerosis development has not been clarified fully yet (Kadoglou *et al*., 2008; Skilton and Celermajer, 2006). This study is in consistent with another study which demonstrating low ghrelin

serum levels in patients with AMI compared with the control group independent of other cardiovascular risk factors (Kadoglou et al., 2010a). Thus, our results indicate a role of ghrelin in coronary atherosclerosis development. Otherwise, there are controversial clinical data about the involvement of ghrelin in carotid atherosclerosis. Future studies will elucidate the protective role of ghrelin in atherosclerosis progression. The role of ghrelin in atherosclerosis seems quite complex, and basic research studies have documented either proinflammatory or anti-inflammatory properties (Yano *et al*., 2009).

Conclusion:

Our study suggests that the low serum adiponectin and ghrelin level may be risk factor for AMI independent of other traditional cardiovascular risk factors. They may be two promising, clinically important proteins, which link adiposity, inflammation, and atherosclerosis and provide a novel therapeutic target.

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References:

1. Aprahamian TR, Sam F. (2011): Adiponectin in Cardiovascular Inflammation and Obesity. Int J Inflam.; 2011:376909.
2. Cordido F, Isidro ML, Nemin˜a R, Sangiao-Alvarellos S. (2009): Ghrelin and growth hormone secretagogues, physiological and pharmacological aspect. Curr Drug Discov Technol.; 6:34–42.
3. Fasshauer M, Kralisch S, Klier M, *et al*. (2003): Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochemical and Biophysical Research Communications; 301(4):1045–1050.
4. Kadoglou NP, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, Kottas G. (2010a): Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease. Transl Res. ; 155(5):238–46.
5. Kadoglou NP, Sailer N, Moumtzouoglou A, *et al*. (2008) Novel markers of carotid atherosclerosis in patients with type 2 diabetes. Exp Clin Endocrin Diab; 35:661–8.
6. Kadoglou NP, Tsanikidis H, Kapelouzou A, *et al*. (2010b): Effects of rosiglitazone and metformin treatment on apelin, visfatin, and ghrelin levels in patients with type 2 diabetes mellitus. Metabolism; 59(3):373–9.
7. Kellokoski E, Kunnari A, Jokela M, Ma¨kela¨ S, Kesa¨niemi YA, Ho¨rkko¨ S. (2009): Ghrelin and obestatin modulate early atherogenic processes on cells: enhancement of monocyte adhesion and oxidizedlow-density lipoprotein binding. Metabolism; 58:1572–80.
8. Kumada M, Kihara S, Sumitsuji S, *et al*. (2003): Association of hypoadiponectinemia with coronary artery disease in men. Arteriosclerosis, Thrombosis and Vascular Biology; 23(1):85–89.
9. Maeda N, Takahashi M, Funahashi T, *et al*. (2001) PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes; 50(9):2094–2099.
10. Persson J, Lindberg K, Gustafsson TP, Eriksson P, Paulsson-Berne G, Lundman P. (2010): Low plasma adiponectin concentration is associated with myocardial infarction in young individuals. Journal of Internal Medicine; 268(2):194–205.
11. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. (1995): A novel serum protein similar to C1q, produced exclusively in adipocytes. Journal of Biological Chemistry; 270(45):26746–26749.
12. Shah A, Mehta N, Reilly MP. (2008): Adipose inflammation, insulin resistance, and cardiovascular disease. JPEN J Parenter Enteral Nutr.; 32:638–44.
13. Shibata R, Izumiya Y, Sato K, *et al*. (2007): Adiponectin protects against the development of systolic dysfunction following myocardial infarction. Journal of Molecular and Cellular Cardiology; 42(6):1065–1074.
14. Shibata R, Ouchi N, Ito M, *et al*. (2004): Adiponectin-mediated modulation of hypertrophic signals in the heart. Nature Medicine; 10(12):1384–1389.
15. Shibata R, Sato K, Pimentel DR, *et al*. (2005): Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. Nature Medicine; 11(10):1096–1103.
16. Skilton MR, Celermajer DS. (2006): The effects of obesity related peptides on the vasculature. Curr Vasc Pharmacol; 4: 79–85.
17. Yano Y, Toshinai K, Inokuchi T, *et al*. (2009): Plasma des-acyl ghrelin, but not plasma HMW adiponectin, is a useful cardiometabolic marker for predicting atherosclerosis in elderly hypertensive patients. Atherosclerosis; 204:590–4.

3/13/3012

Evaluating subcontractor performance using Evolutionary Gaussian Process Inference Model

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Abstract: " Subcontractor Evaluation" is one of the methods which general contractors use to evaluate subcontractor performance. The result is often used as a reference index for subcontractor choice during the outsourcing of activities within a project. Inappropriate subcontractor choice would have a direct impact on the duration, cost, quality, and safety of a project, leading to failure in achieving its goals and target profits. Therefore, this paper establishes a set of Evolutionary Gaussian Process Inference Model, which utilize a Gaussian Process to map the relationships between data input and output and uses Bayesian inference together with Particle Swarm Optimization to optimize the hyper-parameters of the Gaussian Process covariance function to obtain the best inference predictive ability. The model provides construction managers with quantitative measures of subcontractor performance in their selection process.
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Keywords: subcontractor performance; Gaussian process; Particle Swarm Optimization; EGPIM; Bayesian inference

1. Introduction

The construction industry is one of the key parts of a nation's economic development. The production processes in construction are constrained by contracts, design illustrations, construction specifications and site conditions. Since projects require a variety of resources to achieve final construction, contractors are unable to perform all tasks by themselves, as many of them are highly complex or specialist activities. In order to do so, they must source specialized labor from outside themselves. Therefore, except for activities that contractually cannot be outsourced, contractors usually subcontract out activities and only oversee the overall planning and management of the project [1] .

This sub-contractual form of project management is a way to reduce costs and improve efficiency and, contractors in Taiwan generally outsource a high percentage of their projects. In this context, the decision-making process for subcontractor choice to gain a better competitive advantage is a key issue of study. The evaluation of subcontractors usually happens during the construction period. Key criteria of evaluation include how contractor will cooperate with other subcontractors, both in the present and in future. However, current methods do not clearly map out the relationships between individual construction factors and a subcontractor's overall performance (a review of the total score) In order to improve such circumstance, many artificial wisdom technologies have been developed in recent years, assisting in processing large amount of data, which could analyze and find out the rules and patterns to predict future behavior and in assistance of decision making. Ulubeyli Presents a study of subcontractor selection practices of Turkish contractors in international projects[2]. Cheng was to propose support model using Evolutionary Support Vector Machine Inference model (ESIM) that would improve current subcontractor performance evaluation practices [3].

In this research establishes an Evolutionary Gaussian Process Inference Model (EGPIM) which utilizes the Gaussian Process (GP) to map out the relationship between data input and output and uses Bayesian inference together with particle swarm optimization (PSO) to optimize the hyper-parameters in covariance functions so as to obtain the best predictive ability. The objective of EGPIM learning was to map the relationships between the primary scores and the final scores with the learned results potentially being used to assess subcontractor performance directly from primary to final scores. By using the model's prediction, the expected value and variance that are needed in decision-making can establish a data confidence interval to serve as references for decisions.

This dynamic production model boasts quick training, short execution times, and accurate predictions which place it in good stead to provide managers with a practical basis for subcontractor performance evaluation.

2. Review of Approaches

2.1. Gaussian process regression

Gaussian process (GP), a widely utilized AI technique, has been widely applied in chemistry, construction, medicine and other fields[4] . GP provides a statistical advantage and is easy to learn[5] . It uses probability theorems to predict unknown input data and estimates prediction accuracies (estimation variances) to greatly improve the statistical significance of such predictions[6] [7] . GP is a combination of random variances in which capricious and limited numbers of random variances all obey Gaussian distribution:

$$F(\mathbf{X}) = \{f(X_1), f(X_2), \dots, f(X_N)\} \sim N(\mu, K) \quad (1)$$

Where **X** is the variance of the input data, and the mean function is m(**X**) = E[f(**X**)], and the covariance function is k(X,X') = E[(f(X)-m(X))(f(X')-m(X'))]. From the previous description, as GP can explain multi-dimensional Gaussian distributions, with the trait that the random process f(X) could dominate and control random variances and with the explanation of the random process with probability distribution, a flexible non-parameter probability model can be defined; μ is the mean of variances, and K is the covariance matrix of the covariance function. GP can be described via mean function m(**X**) in $f(X_i)$ and covariance function k(X,X') in the random process.

$$f(X) \sim GP\big(m(X), k(X, X')\big) \quad (2)$$

In real situations, however, data prediction is often accompanied by noise, and therefore, when the value Y is calculated by the estimation of the function, an error parameter ϵ should be considered. Likewise, ϵ also coincides with the Gaussian distribution. Y is calculated as follows:

$$\mathbf{Y} = F(\mathbf{X}) + \epsilon \quad (3)$$

Joint distribution calculated under Gaussian distribution:

$$\begin{bmatrix} \mathbf{Y} \\ Y_* \end{bmatrix} | X, \theta \sim N\left(0, \begin{bmatrix} K + \sigma^2 I & k \\ k^T & \kappa + \sigma^2 \end{bmatrix}\right) \quad (4)$$

Hence, the conditional of probability distribution can also be calculated with expected value together with noise, the X_* represents the new input data:

$$Y_* | Y, X, \theta, \sigma^2 \sim N(m(X_*), v(X_*)) \quad (5)$$

In the end, based on conditional probability distribution, the mean and variance of expected value Y_* can be calculated:

$$m(X_*) = k^T (K + \sigma^2 I)^{-1} Y \quad (6)$$

$$v(X_*) = \kappa + \sigma^2 - k^T (K + \sigma^2 I)^{-1} k \quad (7)$$

2.2. Bayesian Inference

Bayesian inference uses distribution information from unknown parameters in addition to model and data information[8]. This information (called "prior") exists prior to the experiment and is expressed by the probability distribution of unknown parameters[9][10]. Bayesian theorem uses known information to construct a posterior probability density for system status variances. It utilizes the model to predict prior estimated status variance density and then makes rectifications based on the latest observation information. Using observation information to calculate status variances increases trust in the accuracy of different values and delivers the best model estimation [11].

2.3. Particle Swarm Optimization algorithm (PSO)

The Particle Swarm Optimization (PSO) algorithm is a relatively new algorithm derived by J. Kennedy and R.C. Eberhart in 1995 from a simplified social model simulation. (8) PSO algorithms mimic mechanisms used by birds to share information in flight.(9) The particle concept requires members in groups without mass and volume and with designated speed and acceleration. The first version of PSO added neighboring speed values and considered multi-dimensional search and distance-based acceleration. Inertia weight, introduced later, enhanced the algorithm's exploitation and exploration and paved the way to form a standard version of the algorithm(10) (11).

3. Evolutionary Gaussian Process Inference Model (EGPIM)

The EGPIM is a Gaussian process combined with Particle Swarm Optimization (PSO) and Bayesian inference that is based on historical data. EGPIM uses GP to reveal the intricate relationship between variance input and output.

Bayesian inference structure gives the posterior probability for the entire function and serves as the reference for parameter optimization. PSO searches the best hyper-parameter GP and required Bayesian analysis. Figure. 1 illustrates the model structure and its three component parts.

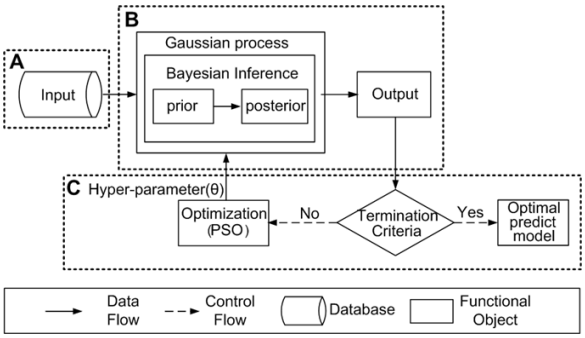

Figure 1. EGPIM structure

A. Data Input

This stage involves collecting and arranging input data **X** and output data **Y**, and establishing database D={**X**,**Y**}. **X** is the collection of data input factor of N dimensions and **Y** is the collection of m pieces of output. Thus, any Y_i is the output value of its case input value $\{X_{1i},X_{2i},\ldots,X_{Ni}\}$.When the database is coordinated, data are separated into training data and test data, with training data identifying reflection relationships between input and output data and test data used to check model prediction accuracy (5).

The corresponding function value of any input factor X_j is $f(X_j)$: $F(X)= \{f(X_1), f(X_2),\ldots, f(X_N)\}$; F(X) is the function congregation demonstrating the relationship between X and Y, with a Gaussian process used to describe function distribution. Assuming that function F(**X**) coincides with Gaussian distribution and makes work easier, the expected value m(**X**) would be 0 and probability would meet the normal distribution

$$P(F) = \frac{1}{(2\pi)^{\frac{N}{2}}|K|^{\frac{1}{2}}}\exp\left[-\frac{1}{2}F^{T}K^{-1}F\right] \sim N(0,K) \qquad (8)$$

where K is the matrix constructed from covariance function , and the equation above the probability of the set function F is regarded to be controlled by covariance matrix K.

B. Gaussian process and Bayesian inference

i. Covariance matrix and parameter

After determining a stationary pattern, the covariance function is chosen to construct the covariance matrix. Parameter model and quantity vary according to function. This study adopts the Squared Exponential, the most common covariance function.

ii. Gaussian process and Bayesian inference

A covariance function is then chosen to construct the covariance matrix. Parameter model and quantity vary according to function. As we adopted the Squared Exponential covariance function, the formula is as follows:

$$k_{SE}(X_i,X_j) = \sigma_f^2\exp\left[-\frac{1}{2}\left(\frac{X_i-X_j}{r_i}\right)^2\right] + \sigma_n^2\delta_{ij} \qquad (9)$$

In eq(8), σ_f controls overall function volatility, σ_n indicates overall function error, r_i shows the relationship between variances X_i and X_j in function space, and N represents data input dimensions. σ_f, σ_n, r_1, r_2,…,r_n represent matrix hyper-parameters and θ is their aggregate.

According to the chosen covariance function, and utilizing Bayesian theorem, we infer the posterior probability of the entire function P(F | **X**,**Y**) as:

$$P(F|\mathbf{X},\mathbf{Y}) = \frac{P(\mathbf{Y}|F,\mathbf{X})P(F)}{P(\mathbf{Y}|\mathbf{X})} \qquad (10)$$

Minimizing the Negative Log-Marginal Likelihood (NLML) and combining PSOs help maximize posterior probability in order to obtain the most likely hyper-parameter during the minimization process.

C. Hyper-parameter Optimization

Applying PSO to EGPIM optimizes the hyper-parameter in function space. This is the best model function. This step includes three steps, as follows:

i. Initial Stage

After setting up PSO parameters, particle groups, particle speed and positions are started randomly to initiate and implement iteration. These include (1) group scale m, (2) maximum speed V_{max}, (3) acceleration constant c_1 and c_2, (4) maximum inertia weight W_{max}, (5) minimum inertia weight W_{min}, (6) maximum iteration times, and (7) terminating accuracy requirement NLML (Negative Log Marginal Likelihood).

ii. Optimization stage

A fitness calculation of particles discriminates between good and bad particles. Adaptation value depends on NLML.

$$-\log P(\mathbf{Y}|\mathbf{X},\theta) = \frac{1}{2}Y^{T}(K(X,X)+\sigma^2I)^{-1}Y) + \frac{1}{2}\log|K(X,X)+\sigma^2I| + \frac{N}{2}\log 2\pi \qquad (11)$$

Particle search speed and direction are calculated as follows:

Particle speed calculation:

$$V_{id}^{t+1} = W^{t+1}\times V_{id}^{t} + c_1\times \text{rand}(\,)\times(pBest_{id}-S_{id}^{t}) + c_2\times \text{rand}(\,)\times(gBest_{id}-S_{id}^{t}) \qquad (12)$$

Particle weight

$$w = w_{max} - \frac{w_{max}-w_{min}}{iter_{max}}\times iter \qquad (13)$$

New search direction calculation

$$S_{id}^{t+1} = S_{id}^{t} + V_{id}^{t+1} \qquad (14)$$

Where V is the speed of the particle; S is the current location of the particle; pBest is the optimization found by the particle itself, i.e., body extrema; gBest is the optimization of the whole swarm, i.e., global extrema. R and ( ) are the random numbers within (0, 1); c1 and c2 are called learning factors. The search process ends once particles fly into the optimum location of the space after multiple learning sessions and renewals of location and speed. The final output, gBest, is the best optimization.

iii. Termination Stage

With gBest identified as the best solution, fitness value is compared against the global solution. If fitness value>global solution, the search continues.

The search ends only when requirement accuracy (NLML) and search Itermax are achieved.

EGPIM can be optimized through the adaption process with 3 phases (Figure 2). Each model result is evaluated using NLML. The process uses PSO to search simultaneously for optimum hyper-parameters.

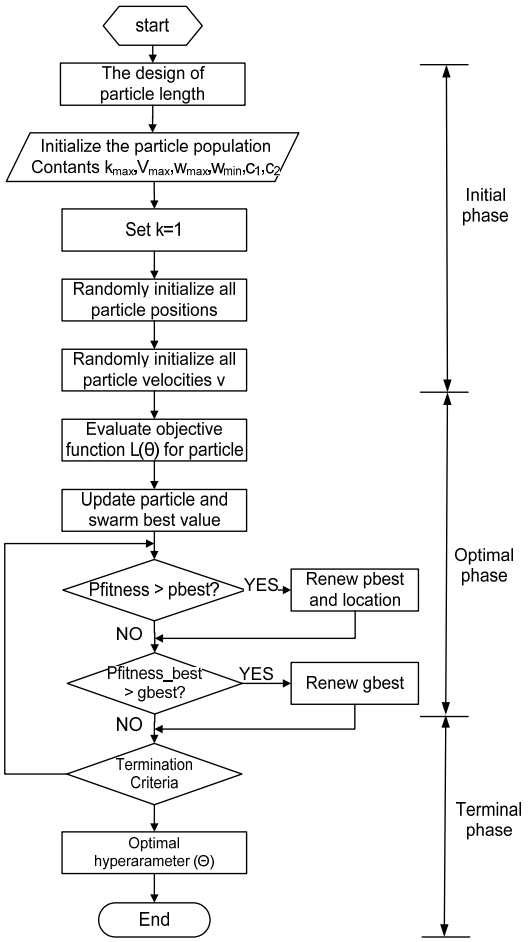

Figure 2. EGPIM Adaptation Process

4. EGPIM for subcontractor performance assessment

Performance is as an important index used by contractors to select optimal subcontractors (12). The use of this index assumes that a subcontractor's performance history can be used to predict their future performance. 'Subcontractor performance, however, can be affected by various expected and unexpected factors, such as management ability, worst conditions, such as weather, natural environment problems, or a bad workplace environment and subjective assessments [13]. This shows significant complexity in the prediction of subcontractor performance since there are uncertainties that require judgment calls based on human expert knowledge and experience [14]. Subcontractors are generally categorized into four types based on the services they provide. These are: 1) labor, 2) labor and materials, 3) materials, and 4) equipment, each with their particular characteristics. In practice, subcontractor performance is evaluated using two scores, namely a primary score and a final score with various contributing factors. Primary scores are evaluated by field superintendents. Final scores are assigned by the contractor's project management using the primary score as one point of reference. Problems that occur with this evaluation approach include: 1) there is difficulty in generalizing performance indicators for the different types of sub-contractors; 2) both the primary and final scores are independently determined by human experts based on personal knowledge and experience; and 3) the relationship between primary and final scores is not well defined.

This study gathered subcontractor data from an actual construction engineering general contractor that was established in 1956 and was valued at the time of this study to be about 11 million US dollars. Using a questionnaire survey, this study identified twelve key factors used by this general contractor to assess potential subcontractors (see Table 1). Each subcontractor was scored by the relevant field superintendent against twelve key factors. Each factor was quantified by giving a score based on qualitative ranks as follows: excellent (8 points), good (6 points), normal (4 points), poor (2 points), and bad (0 points). Final scores for subcontractor evaluation were then conducted by the general contractor's managers. The range of the final scores varied between 56 and 88 (see Table 2). The objective of EGPIM learning was to map the relationships between the primary scores and the final scores with the learned results potentially being used to assess subcontractor performance directly from primary to final scores.

Table 1. Subcontractor assessment factors.

NO.	Factors
1	Construction technique
2	Duration control abilities
3	Cooperative managers
4	Material wastage
5	Services provided after work completion
6	Collaboration with other subcontractors
7	Safe working environment
8	Self-owned tools
9	Clean working environment
10	Effective management capabilities
11	Manager personality
12	Financial condition

Table 2. Training and test data examples for subcontractor performance.

		Training Data												
subcontractor	Contract NO.	Factor NO.												Final Score
		1	2	3	4	5	6	7	8	9	10	11	12	
A	A_01	6	6	4	6	6	4	6	6	6	4	8	8	72
	A_02	6	6	6	8	6	6	4	6	6	6	6	8	76
	A_03	6	6	6	6	6	6	6	6	6	6	8	8	76
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
M	M_03	6	8	6	8	4	6	6	6	6	4	8	8	76
	M_04	8	6	6	8	6	6	4	6	6	6	8	8	76
		Test Data												
N	N_01	6	8	4	4	4	4	4	4	6	6	6	6	62
	N_02	6	6	4	6	4	6	6	4	4	4	8	6	56
	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
	N_14	8	8	6	6	8	6	6	8	6	6	8	8	86
	N_15	8	8	6	8	8	6	8	8	8	6	8	8	88

4.2. EGPIM parameter settings for subcontractor performance assessment

All the data shown in Table 2 were normalized to the range [0, 1] prior to EGPIM learning. As activities accomplished by subcontractor N were treated as testing data, 61 sets of data of the 76 were used as training data, with 15 remaining for testing. The EGPIM parameters were set to appropriate values in order to calculate optimal learning results, as in Table 3.

4.2. Subcontractor performance assessment result

The learned subcontractor performance (NLML) and the hyper-parameters of the model after 500 iterations are displayed in Table 4, while a graph of the testing results for EGPIM and other methods appears in Figure. 2. From these, it is clear that the EGPIM results were significantly more accurate than those achieved using ESIM [15] [3] and the original GP. For comparison, the testing root-mean-square error (RMSE) for ESIM was 5.35 and for GP it was 5.83, which were improved by EGPIM at 2.06. The longest time taken by the training courses for the Subcontractor Evaluation Performance was 1 minute, 51 seconds) the reliability of these EGPIM predictions of subcontractor performance can assist managers to select the appropriate subcontractors with greater precision and accuracy.

One of the main goals of this paper was to provide improvements to the existing EGPIM by using linear or non-linear relationships amongst the data. These improvements allow EGPIM to provide more accurate predictions for subcontractor performance evaluations than ESIM and GP. Ultimately, this paper proposes an EGPIM which integrates GP, Bayesian inference, and PSO to work with problems in the field of construction management.

Table 3. EGPIM parameter settings for subcontractor performance assessment.

	EGPIM parameter	Value
1	Particle group scale m	50
2	Maximum speed Vmax	0.9
3	Acceleration constant c1 and c2	2.0
4	Maximum inertia weight Wmax	0.7
5	Minimum inertia weight Wmin	0.4
6	Maximum iteration times	500
7	Terminate accuracy requirement NLML	−200

Table 4. Training results of EGPIM.

NLML	−105.712
Elapsed Time (sec)	111.8
Maximum iteration	485

5. Conclusions

This study applied the EGPIM to subcontractor performance data and utilized a model for evaluating subcontractor performance by collecting and extracting the rules from the actual measured data through a training algorithm. Knowledge of the data of the subcontractor evaluation model can have a direct impact on the quality of the model's results. We suggest that further studies try to evenly collect and collate a number of training cases for model training and testing in order to compare various model training approaches. To improve accuracy and better represent the model's results, construction managers should take care when conducting subcontractor evaluation and be more efficient in making more records in the database.

This paper builds a model for evaluating subcontractor performance by utilizing the EGPIM to effectively extract the knowledge and experience of engineering personnel to reduce the influence of subjective judgment, hence also reducing uncertainty. The EGPIM is based on regression and can obtain maps of the relationships between input and output values in a short time, so that it can build a real-time prediction model.

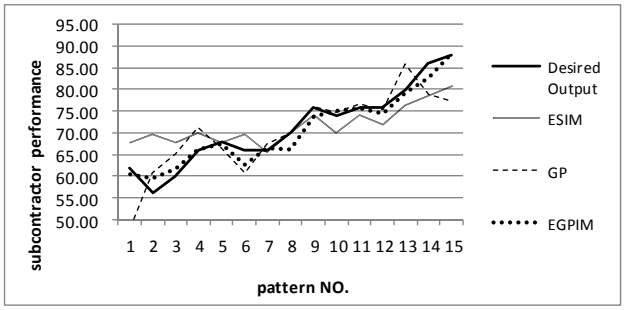

Figure. 3. A graph of the testing results for subcontractor performance using several methods.

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References

1. Thomas Ng S, Tang Z, Palaneeswaran E. Factors contributing to the success of equipment-intensive subcontractors in construction. International Journal of Project Management [Internet]. 2009;27(7):736–44.
2. Ulubeyli S, Manisali E, Kazaz A. Subcontractor selection practices in international constraction projects. Journal of Civil Engineering and Management. 2010;16(1):47–56.
3. Cheng M-Y, Wu Y-W. Improved construction subcontractor evaluation performance using ESIM. Applied Artificial Intelligence: An International Journal. 2012;26(3):261–73.
4. Frean M, Boyle P. Using Gaussian Processes to Optimize Expensive Functions. [Internet]. In: Wobcke W, Zhang M, editors. In proceeding of: AI 2008: Advances in Artificial Intelligence, 21st Australasian Joint Conference on Artificial Intelligence. Auckland, New Zealand: 2008 [cited 2012 Apr 9]. p. 258--267.
5. Snelson EL. Flexible and efficient Gaussian process models for machine learning. University College London; 2007.
6. Kocijan J, Murray-Smith R, Rasmussen CE, Irard A. Gaussian Process Model Based Predictive Control. In: American Control Conference. Boston, Massachusetts.: IEEE; 2004. p. 2214–9.
7. Rasmussen CE, Williams C. Gaussian Processes for Machine Learning. International Journal of Neural Systems [Internet]. 2006;14(2):69–106.
8. Kennedy J, Eberhart R. Particle swarm optimization [Internet]. In: Proceedings of IEEE International Conference on Neural Networks. IEEE; 1995. p. 1942–8.
9. Matsui T, Kato K, Sakawa M, Uno T, Matsumoto K. Particle Swarm Optimization for Nonlinear Integer Programming Problems. In: Proceedings of the International MultiConference of Engineers and Computer Scientists. Hong Kong: 2008.
10. Zhou Y, Zeng G, Yu F. Particle swarm optimization-based approach for optical finite impulse response filter design. Applied optics [Internet]. 2003 Mar 10;42(8):1503–7.
11. Fourie PC, Groenwold AA. The particle swarm optimization algorithm in size and shape optimization. Structural and Multidisciplinary Optimization. 2002 May 1;23(4):259–67.
12. Kale S, Arditi D. General contractors' relationships with subcontractors: a strategic asset. Construction Management and Economics [Internet]. 2001;19(5):541–9.
13. Shash AA. Subcontractors' Bidding Decisions. Journal of Construction Engineering and Management [Internet]. 1998;124(2):101–6.
14. Arditi D, Chotibhongs R. Issues in Subcontracting Practice. Journal of Construction Engineering and Management. 2005;131(8):866–76.
15. Cheng M-Y, Wu Y-W. Evolutionary support vector machine inference system for construction management. Automation in Construction. 2009;18(5):597–604.

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Effects of anisodamine on the expressions of tumor necrosis factor-α and cycloxygenase 2 in experimental infusion phlebitis

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Abstract: Objective This subject was designed to investigate effects of anisodamine on the expressions of tumor necrosis factor-α (TNF-α) and cycloxygenase 2 (COX-2) in a rabbit model of infusion phlebitis and to analyze the preventative and treatment mechanisms of anisodamine in experimental infusion phlebitis. **Method** The rabbits were randomly assigned to the control group, the model group, the magnesium sulfate group and the anisodamine group. Expressions of TNF-α and COX-2 were determined and contrasted with the control group treated with normal saline by histopathology, immunohistochemistry, reverse transcription polymerase chain reaction, and western blotting assay, respectively. **Results** Obvious pathohistological changes were observed and the model group showed the highest expressions of TNF-α and COX-2 in the four groups (P<0.01). On the contrary, anisodamine alleviated the inflammatory damage by significantly reducing the expressions of TNF-α and COX-2 compared with the model group (P<0.01). There was no difference in the expressions of TNF-α and COX-2 between the magnesium sulfate group and the anisodamine group (P>0.05). **Conclusion** Anisodamine alleviates the inflammatory damages by significantly reducing the expressions of TNF-α and COX-2, and shows significant protective effects in the animal model of infusion phlebitis.
[Zhang Zhenxiang, Zhang Qiushi, Wang Peng, Pan Xue, Zhao Qingxia, Wang Xiaokai. **Effects of anisodamine on the expressions of tumor necrosis factor-α and cycloxygenase 2 in experimental infusion phlebitis.** Life Sci J 2012;9(2):533-539]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 80

Keywords: anisodamine; infusion phlebitis; tumor necrosis factor-α; cycloxygenase 2

1. Introduction

Infusion phlebitis is the most common side effect of clinical intravenous drug therapy [1], and many drugs increase the risk for infusion phlebitis. However pathogenesis of infusion phlebitis has not been fully clarified and infusion phlebitis has not been effectively solved until now. Previously studies on the models of inflammation have implicated that tumor necrosis factor-α (TNF-α) and cycloxygenase 2 (COX-2) -induced prostanoids as the key factors in controlling the production of early inflammatory mediators [2].

Anisodamine is efficacious for the treatment of experimental and clinical bacterium shock [3-5]. Several clinical studies have demonstrated that anisodamine effectively prevents the occurrence of infusion phlebitis, but most of current studies of infusion phlebitis are clinical observations and influential factor analysis, lacking systematic mechanism study and animal-oriented model.

Based on these studies, this subject was designed to investigate the effects of anisodamine on the expressions of TNF-α and COX-2 in a rabbit model of infusion phlebitis and to analyze the prevention and treatment mechanisms of anisodamine for infusion phlebitis caused by intravenous administration.

2. Material and Methods

Rabbits

Specific pathogen-free male Japanese white rabbits (No. SCXK 2011-0001, age, 2 to 3 months; weight, 2.5 to 3.5 kg) were obtained from Experimental Animal Center of Henan Province, China. The rabbits were fed with standard diets in pathogen-free conditions and allowed free access to food and water. Animals were treated according to good laboratory practice (GLP).

Experimental infusion phlebitis was established as previously described [6]. Briefly, eighteen healthy rabbits were randomly assigned to three groups of the model group, the anisodamine group, and the magnesium sulfate group. All three groups continuously received intravenous injection of 2.5 ml/kg of 20% mannitol (Jiangsu Chia Tai Fenghai Pharmaceutical Co., Ltd.) 2.5 ml/kg via a peripheral ear vein twice a day for two days. The flow rate for each infusion was approximately 0.5 ml/min. After administration of mannitol, the ear vein was infused with 5 ml 0.9% NaCl solution (Ningbo Changfu Pharmaceutical Co., Ltd.) at the same flow rate, and then the infusion needle was removed. Moreover, the anisodamine group was smeared by 2% anisodamine (Beijing Double-Crane pharmaceutical Co., Ltd.) before intravenous injection, and the magnesium sulfate group was smeared by 25%

magnesium sulfate (Sanjing Pharmaceutical Co., Ltd.) after intravenous injection.

Meanwhile, the six normal rabbits of the control group received intravenous injection of pyrogen-free physiological saline via an ear vein in the same way as the control group.

Twenty-four hours after the fourth infusion, the appearances of vasculature and tissues were observed, and ears specimens around the vasculature (length 3 cm, width 1cm) were harvested and collected. All of the ears specimens were divided to two parts, one part of them was fixed in 10% neutrally buffered formalin, and the other was stored in liquid nitrogen.

Histopathological examination

Ears specimens were fixed in 10% buffered formalin and embedded in paraffin following routine methods. Deparaffinized thin sections from each paraffin block were stained with hematoxylin and eosin for histologic examination.

Immunohistochemistry

Immunohistochemistry was performed as previously described [7]. Briefly, after blocking for nonspecific sites, slides were incubated in a humid chamber overnight at 4°C with the primary antibodies, anti- TNF- α antibody or anti- COX-2 antibody, respectively. After washing with PBS, slides were incubated with horseradish peroxidase-conjugated secondary antibody at room temperature for two hours. Diaminobenzidine (DAB) was used as the chromogen, and the slides were counterstained with hematoxylin. Finally, the slides were analyzed with a spectral imaging system of Nikon NIS-Elements. Immunohistochemical expressions of TNF- α and COX-2 were represented by average optical density (OD) value of the staining positive cells.

Reverse transcription polymerase chain reaction (RT-PCR) assay

Grinded 100 mg frozen ears specimens in liquid nitrogen and the extractions of total RNA by Trizol were performed according to the manufacturer's instructions. RNA was extracted by using chloroform and precipitated by ethanol; the pellets were washed with 70% ethanol and dried. The dried RNA was suspended in 20 μl of sterile water and sent for RT-PCR analysis. 2 μg Total RNA was reverse-transcribed into cDNA in a 20 μl of reaction mixture containing: 0.5 μg Anchored Oligo (dT)18, 0.1 μg random Primers (N9), 25 U of ribonuclease inhibitor, 200 U of EasyScript RT reverse transcriptase and 4 μl of 5×RT Buffer, and 1 μl dNTPs (10 mmol/L). Samples were incubated at 25°C for 10 minutes, 42°C for 30 minutes and denatured at 85°C for 5 minutes in a S1000 PCR cycler. cDNA samples were subsequently amplified for the target sequences in a S1000 PCR cycler using a following reaction mixture contains: 5 μl cDNA, 5 μl 10×EasyTaq Buffer, 2.5 U EasyTaq DNA polymerase, 1 μl dNTPs (10 mmol/L) and 20 pmol of each primer in 50 μl final volume.

To prevent false positive results and contamination, RNA extraction, preparation of reaction mixtures and amplifications were carried out in separate rooms and each piece of RT-PCR experiment included a sample with no RNA as the negative control. The primers for GAPDH were added to each run as the positive control to assess RNA integrity and to confirm the absence of DNA polymerase inhibitors.

TNF- α primers were: 5′- GACAAGCCTCTAG CCCACG-3′, antisense 5′- GGCAAGGTCCAGGTA CTCA-3′, generating 405 bp. COX-2 primers were: sense 5′- TTGCTGAAGCCCTATGA -3′, antisense 5′- TGGGACGTTGAATGAAG-3′, yielding a 339 bp. GAPDH primers were: sense 5′-GGTCGGAGTG AACGGATTT-3′, antisense 5′-CTCGCTCCTGGAA GATGG-3′, generating 227 bp. The amplification cycling profile was as follows: initial denaturation 95°C for 2 minutes; cycling: 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute (35 cycles); final extension at 72°C for 10 minutes. After reaction completion, 5 μl of each PCR product was run on 1% agarose gel electrophoresis stained with ethidium bromide. Images were scanned by a Transilluminator JY04S Gel analysis system under UV light and analyzed by the Gel-PRO analyzer system. TNF- α mRNA and COX-2mRNA were assessed by comparing with GAPDH mRNA to investigate the mechanism of anisodamine on the expressions of TNF- α and COX-2.

Western blotting analysis

Expressions of TNF- α and COX-2 proteins were analyzed by Western blotting analysis. Briefly, the total proteins were extracted from frozen ears specimens and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to separate the proteins. At the end of the run, polypeptide bands in the gel were electrophoretically transferred to a PVDF membrane (Bio-Rad Laboratories, Inc., USA). The membrane was incubated for an hour at room temperature with anti- TNF- α antibody, anti- COX-2 antibody or anti-GAPDH antibody (Bioss Inc.), respectively. On the membrane, the binding of antibody to the specific protein band was detected with horseradish peroxidase-conjugated secondary antibody (Bioss Inc., China) and an analyzed by ECL Western blotting detection system (Beyotime Institute of Biotechnology, China).

Statistical analysis

Data were expressed as means± standard deviation (SD). All statistics were analyzed by SPSS

11.0 software (SPSS Inc., USA). The significance of differences in outcomes was determined by using oneway analysis of variance (ANOVA) followed by LSD t-test. Statistical significance was accepted at P <0.05.

3. Results

Histopathological examination

Pathohistological examination was performed by microscope. There were no abnormal changes in the control group (Figure 1A), but pathohistological changes of the model group were obviously observed such as loss of venous endothelial cells, inflammatory cell infiltration, edema and thrombus (Figure 1D). On the contrary, the magnesium sulfate group and the anisodamine group showed a significantly protective effect on vascular congestion, inflammatory cell infiltration, and proliferation, swelling of endothelium and perivascular hemorrhage (Figure 1B, 1C).

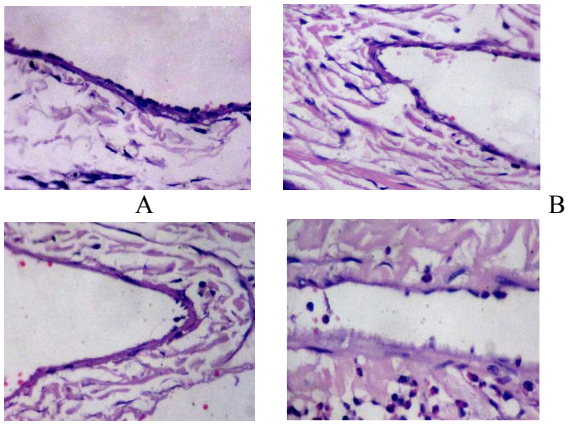

Figure 1. Histopathological examination of ears specimens in experimental infusion phlebitis (Hematoxylin and eosin staining, original magnification ×200). **A:** the control group. **B:** the magnesium sulfate group. **C:** the anisodamine group. **D:** the model group.

Immunohistochemistry

Expressions of TNF- α and COX-2 in ears specimens from the rabbit model were measured by immunohistochemistry. Specific staining of cells in the slides was obtained by using antibodies against TNF- α and COX-2 and lots of brown-yellow particles aggregated in the cytoplasm (Figure.2). Expressions of TNF- α and COX-2 were represented by OD values of the positive staining cells.

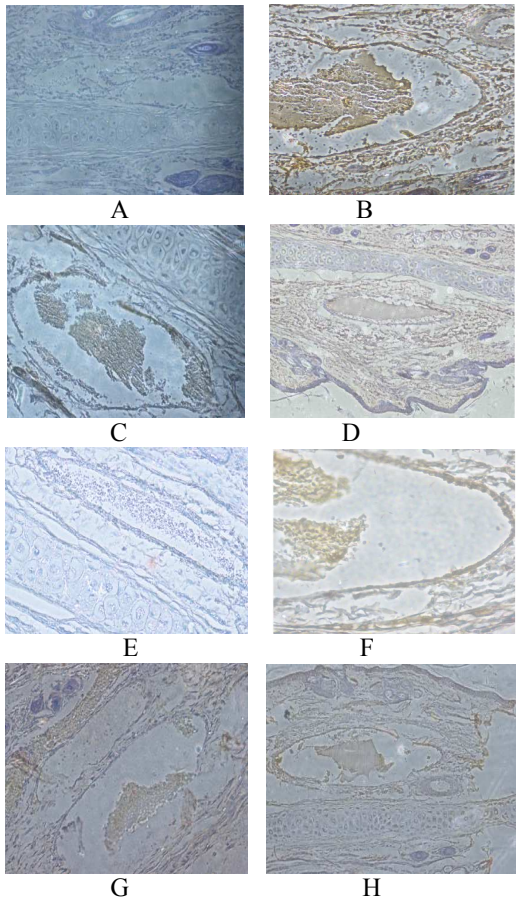

Figure 2. Immunohistochemistry examination of ears specimens in experimental infusion phlebitis (SP staining, original magnification ×100). **A:** the control group (anti-TNF- α antibody incubation). B: the magnesium sulfate group (anti-TNF- α antibody incubation). C: the anisodamine group (anti-TNF- α antibody incubation). D: the model group (anti-TNF- α antibody incubation). E: the control group (anti-COX-2 incubation). F: the magnesium sulfate group (anti-COX-2 incubation). G: the anisodamine group (anti-COX-2 incubation). H: the model group (anti-COX-2 incubation).

The results showed that the model group showed the highest expressions of TNF- α (0.6028±0.0515) of the four groups (P<0.01). The magnesium sulfate group and the anisodamine group displayed significantly higher levels of TNF- α protein expression (0.3690±0.0479 and 0.3925±0.0317) than the control group (0.1379±0.0309) (P<0.01), and there was no difference in the expressions of TNF- α between the magnesium sulfate group and the anisodamine group (P>0.05). Similarly, the model group

showed the strongest expressions of COX-2 (0.5268±0.0499) in the four groups (P<0.01), and the magnesium sulfate group and the anisodamine group displayed significantly higher levels (P<0.01) of COX-2 protein expression (0.4377±0.0609 and 0.4576±0.0394) than the control group (0.1239±0.0191). There was no difference in the expressions of COX-2 between the magnesium sulfate group and the anisodamine group (P>0.05). (Figure 3).

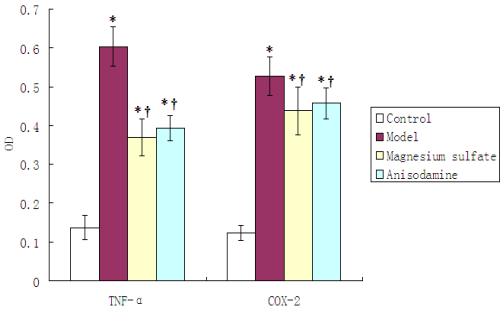

Figure 3. Semi-quantitative analysis of ***TNF- α*** and ***COX-2*** by immunohistochemistry. $^{*}P$<0.01 vs. the control group. $^{\dagger}P$<0.05 vs. the model group.

Semi-quantitative analysis of TNF- α and COX-2 by RT-PCR

The extracted total RNA had three ribosomal RNA bands, 5S, 18S and 28S. The densitometries at 260 and 280 nm were measured and all of the ratios of OD_{260}/OD_{280} were between 1.8 and 2.0. The results confirmed that extracted total RNA was stable and pure.

The expressions of TNF- α and COX-2 were positive in the four groups (Figure 4). The ratios of TNF- α mRNA/GAPDH mRNA in the model group (0.6789±0.0487) was significantly higher than those in the other groups (P <0.01), and the ratios of TNF- α mRNA/GAPDH mRNA in the magnesium sulfate group and the anisodamine group were 0.4970± 0.0513 and 0.5461±0.0501, significantly higher than those in the control group, 0.1388±0.0247, (P <0.01). There was no difference in the expressions of TNF- α mRNA between the magnesium sulfate group and the anisodamine group (P >0.05). Similarly, the ratio of COX-2 mRNA/GAPDH mRNA in the model group (0.6161±0.0504) was significantly higher than those in the other groups (P <0.05), and the ratios of COX-2 mRNA/GAPDH mRNA in the magnesium sulfate group and the anisodamine group, 0.4126± 0.0448 and 0.4219±0.0502, were significantly higher than those in the control group (0.1045±0.0183). There was no difference in the expressions of COX-2 mRNA between the magnesium sulfate group and the anisodamine group (P >0.05). (Figure 5).

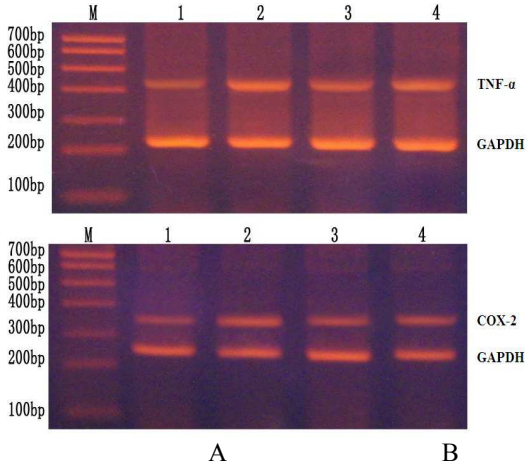

Figure 4. The expressions of TNF- α mRNA and COX-2 mRNA by RT-PCR. Lane 1: the control group; Lane 2: the magnesium sulfate group; Lane 3: the anisodamine group; Lane 4: the model group; M: Marker.

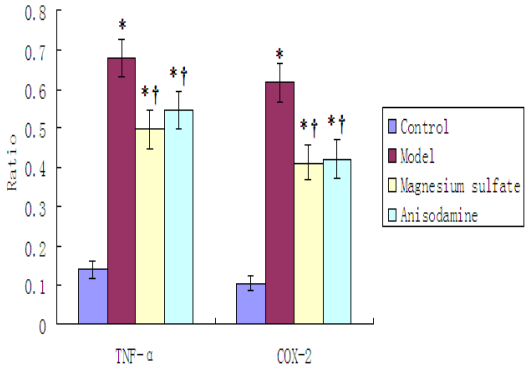

Figure 5. Semi-quantitative analysis of TNF- α mRNA and COX-2 mRNA by RT-PCR. $^{*}P$<0.01 vs. the control group. $^{\dagger}P$<0.01 vs the model group.

Western blotting analysis

Proteins extracted from frozen ears specimens were confirmed by Western blotting analysis. In the blots from extracts of specimens were probed with anti-GAPDH antibody, anti-TNF- α antibody, and anti- COX-2 antibodies, respectively (Figure 6). Blots were then scanned by a digital scanner, and the scans were examined by computerized densitometry. The expressions of TNF- α and COX-2 were assessed by comparing with GAPDH.

The ratio of TNF- α /GAPDH in the model group (0.8197±0.0647) was significantly higher than in the other groups (P <0.01), and the ratios of TNF- α /GAPDH in the magnesium sulfate group and the

anisodamine group (0.5425±0.0282 and 0.5865±0.0357) were significantly higher than in the control group (0.1688±0.0212) (P<0.01). There was no difference in the expressions of TNF-α between the magnesium sulfate group and the anisodamine group (P>0.05). Similarly, the ratios of COX-2/GAPDH in the model group (0.7925±0.0378) were significantly higher than in the other groups (P<0.05), and the ratios of COX-2/GAPDH in the magnesium sulfate group and the anisodamine group (0.4280±0.0484 and 0.4619±0.0474) were significantly higher than in the control group (0.1234±0.0186). There was no difference in the expressions of COX-2 between the magnesium sulfate group and the anisodamine group (P>0.05). (Figure 7).

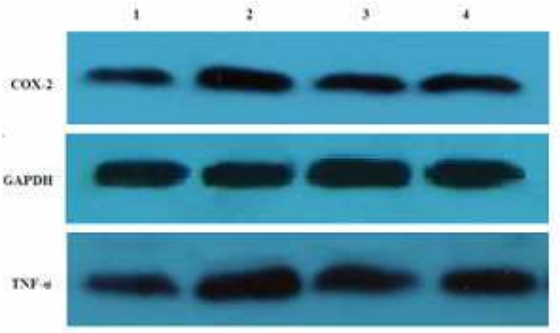

Figure 6. The expressions of TNF-α and COX-2 by Western blotting assay. Lane 1: the control group; Lane 2: the magnesium sulfate group; Lane 3: the anisodamine group; Lane 4: the model group.

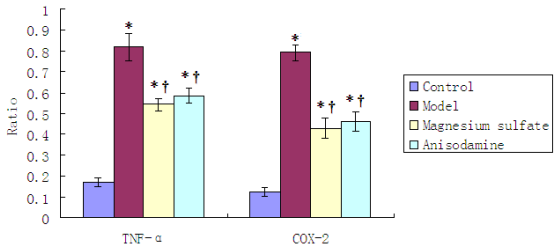

Figure 7. Semi-quantitative analysis of TNF-α and COX-2 by western blot assay. *P<0.01 vs. the control group. †P<0.01 vs. the model group.

4. Discussions

Chemical, physical, and microbial phlebitis have been described [8] and infusion phlebitis is the most common side effect of clinical intravenous drug therapy [1]. It is reported that infusion phlebitis occurs in 25% or more of hospitalized patients and its local symptoms are painful iatrogenic disorders with possible severe outcomes lasting from one week to several months; such as pain, swelling, and the appearance of tender cords. In most cases, infusion phlebitis induces to patients with more extra pain, additional medical cost, and greatly interferes with medication compliance and life qualities of patients [9].

Many drugs increase the risk for infusion phlebitis and antibiotics and cytotoxic drugs are the most common classes of drugs to increase risk [10]. It is reported that hyperosmolar solutions, drugs with high or low pH, and undissolved particles ranging from 1 to >25 μm in size are the most common classes of drugs to increase risk [11].

Frequent and intolerant phlebitis limited the usage of several effective agents, but pathogenesis of infusion phlebitis is not fully clarified and it has not been effectively solved until now. The most prevalent opinion is that the endothelium is intermittently exposed to high concentrations of the drug given intravenously, and chemical irritation of the endothelium leads to a sterile inflammation, endothelium sheds by toxicity, with granulocyte migration and subsequent induction of thrombosis[1, 12], in some cases followed by thrombosis[13].

It is generally accepted that acute inflammation starts a cascade of cytokines and chemokines that attract immune and nonimmune cells to infiltrate disrupted and damaged tissue [14]. TNF-α is a powerful and pleiotropic pro-inflammatory cytokine primarily produced by activated macrophages[15] and plays a critical role in inflammatory process and tissue destruction through the nuclear factor-κB (NF-κB) signaling pathway[16] and may initiate an inflammatory cascade consisting of other inflammatory cytokines[17]. Similarly, COX-2 induced production of prostanoids is often implicated in inflammatory diseases, characterized by edema, production of chemotactic factors, and infiltration of inflammatory cells [2]. Unlike COX-1, which is a constitutive enzyme expressed in most tissues and is essential for various physiological functions, COX-2 is an inducible enzyme that is induced in a variety of cell types by inflammatory stimuli, including lipopolysaccharide (LPS), phorbol ester, and cytokines such as interleukin (IL) -1β, and TNF-α [17].

Multiple cytokines, chemokines, and angiogenic factors have been identified as early participants and previous studies have shown that the models of inflammation have implicated TNF-α and COX-2-induced prostanoids as the key factors in controlling the production of early inflammatory mediators and expressions of both TNF-α and COX-2 are up-regulated during inflammation[2]. Those revelations intensify interests in controlling inflammation for disease prevention and treatment.

Anisodamine, a peripheral muscarinic receptor antagonist, is an alkaloid extracted from a Chinese herb. It is a naturally occurring atropine derivative

and a potent vasoactive drug and has been widely used for many years to improve microvascular perfusion and to treat acute disseminated intravascular coagulation in patients in bacteremic shock. It has been credited with contributing to improving the survival rate in the treatment of acute epidemic meningococcal meningitis and decreasing the mortality of meningococcemia from 67% to 12.4% [18]. Previous studies demonstrate that anisodamine has an anti shock effect contributing to prolong the survival of mice injected with Shiga toxin (Stx) and inhibit the production of TNF-α, IL-1β, and IL-8 from human peripheral blood monocytes stimulated with Stx[19]. Moreover, anisodamine is also an inhibitor of platelet aggregation, granulocyte aggregation, and thromboxane synthesis. Further study showed that anisodamine significantly inhibited the A-23187-induced release of PG and LT from mouse macrophages in a dose-dependent manner [20] and the TNF-alpha inhibitory effect was via a prostaglandin E2-dependent mechanism [19].

Several clinical studies have demonstrated that anisodamine effectively prevent the occurrence of infusion phlebitis, but little is known about its mechanism and most of current studies of infusion phlebitis are clinical observations and influential factor analysis, lacking systematic mechanism study and animal-oriented model.

Based on these reports, this study was designed to investigate the effects of anisodamine on the expressions of TNF-α and COX-2 in a rabbit model of infusion phlebitis comparing with magnesium sulfate as the positive control. The present study found obviously pathohistological changes, such as loss of venous endothelial cells, inflammatory cell infiltration, edema and thrombus. The model group showed the strongest expressions of TNF-α and COX-2 of the four groups (P<0.05). On the contrary, anisodamine alleviated the inflammatory damages by significantly reducing the expressions of TNF-α and COX-2 compared with the model group (P<0.05), and there was no difference in the expressions of TNF-α and COX-2 between the magnesium sulfate group and the anisodamine group (P>0.05). The results showed the significant protective effects of anisodamine on relieving vascular congestion, inflammatory cell infiltration, and proliferation, swelling of endothelium and perivascular hemorrhage in the animal model of infusion phlebitis with significantly reducing the expressions of TNF-α and COX-2.

In conclusion, the present study has established a method for preventing phlebitis caused by intravenous administration and analyzed the preventing mechanisms of anisodamine for infusion phlebitis. It has demonstrated that activations of TNF-α and COX-2 could be the important mechanisms of pathogenesis of infusion phlebitis and it gives us a theoretical basis of systematic mechanism study on the usage of anisodamine, which effectively prevents the occurrence of infusion phlebitis in clinical practice. This finding is a new strategy in the prevention and treatment of infusion phlebitis, and it should enrich the methods and theories of preventing and treating infusion phlebitis, and expand the use of anisodamine in clinical practices.

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References

1. Peter Lanbeck, Inga Odenholt, Kristian Riesbeck. Dicloxacillin and erythromycin at high concentrations increase ICAM-1 expression by endothelial cells: a possible factor in the pathogenesis of infusion phlebitis. J Antimicrob Chemother, 2004, 53(2):174 - 179.
2. Partha S. Biswas, Kaustuv Banerjee, Bumseok Kim, et al. Role of Inflammatory Cytokine-Induced Cycloxygenase 2 in the Ocular Immunopathologic Disease Herpetic Stromal Keratitis. J Virol, 2005; 79(16): 10589 - 10600.
3. Zhang HM, Ou ZL, Yamamoto T. Anisodamine inhibits shiga toxin type 2-mediated tumor necrosis factor-alpha production in vitro and in vivo. Exp Biol Med, 2001; 226: 597-604.
4. Li Q, Lei H, Liu A, et al. The antishock effect of anisodamine requires the upregulation of 7 nicotine acetylcholine receptors by IL-10. Life Sci, 2011; 89: 395-401.
5. Xu ZP, Wang H, Hou LN, et al. Modulatory effect of anisodamine on airway hyper-reactivity and eosinophilic inflammation in a murine model of allergic asthma. Int Immunopharmacol, 2011; 11: 260-265.

6. Kohno E, Murase S, Matsuyama K, et al. Effect of corticosteroids on phlebitis induced by intravenous infusion of antineoplastic agents in rabbits. Int J Med Sci. 2009, 6: 218-223.
7. Lee Jeong-Won, Han HD, Shahzad MMK.,et al. EphA2 Immunoconjugate as Molecularly Targeted Chemotherapy for Ovarian Carcinoma. J Natl Cancer Inst, 2009; 101: 1193 - 1205.
8. Yakir Rottenberg, Zvi G Fridlender. Recurrent Infusion Phlebitis Induced by Cyclosporine. Ann Pharmacother, 2004; 38: 2071-2073.
9. Uslusoy E, Mete S. Predisposing factors to phlebitis in patients with peripheral intravenous catheters: a descriptive study. J Am Acad Nurse Pract, 2008; 20: 172-180.
10. Dennis G Maki. Improving the safety of peripheral intravenous catheters. BMJ, 2008, 337: a630.
11. Maki DG. Improving the safety of peripheral intravenous catheters. BMJ, 2008, 337: a630.
12. Yoh K, Niho S, Goto K, et al. A randomized trial of 6-minutes drip infusion versus 1-minute bolus injection of vinorelbine (VNR) for the control of drug induced-phlebitis. ASCO Meeting Abstracts, 2004; 22: 8117.
13. Kilic B, Kruse M, Stahlmann R. The in vitro effects of quinupristin/dalfopristin, erythromycin and levofloxacin at low concentrations on the expression of different cell adhesion molecules on the surface of endothelial cells (Eahy926). Toxicology, 2006; 218: 30-38.
14. Babbar N, Casero RA Jr. Tumor Necrosis Factor-A Increases Reactive Oxygen Species by Inducing Spermine Oxidase in Human Lung Epithelial Cells: A Potential Mechanism for Inflammation-Induced Carcinogenesis. Cancer Res 2006; 66(23):11125-11130.
15. D'hulst AI, Bracke KR , Maes T,et al. Role of tumour necrosis factor-a receptor p75 in cigarette smoke-induced pulmonary inflammation and emphysema. Eur Respir J. 2006; 28(1): 102–112.
16. Masanobu Oshima, Hiroko Oshima, Akihiro Matsunaga, et al. Hyperplastic Gastric Tumors with Spasmolytic Polypeptide – Expressing Metaplasia Caused by Tumor Necrosis Factor-A-Dependent Inflammation in Cyclooxygenase-2/Microsomal Prostaglandin E Synthase-1 Transgenic Mice. Cancer Res 2005; 65(20): 9147-9151.
17. Fumitaka Kamachi, Hyun Seung Ban, Noriyasu Hirasawa, et al. Inhibition of Lipopolysaccharide-Induced Prostaglandin E2 Production and Inflammation by the Na+/H+ Exchanger Inhibitors. J Pharmacol Exp Ther, 2007; 321(1): 345 - 352.
18. Saori Nakagawa,1 Koji Kushiya,1 Ikue Taneike, et al. Specific Inhibitory Action of Anisodamine against a Staphylococcal Superantigenic Toxin, Toxic Shock Syndrome Toxin 1 (TSST-1), Leading to Down-Regulation of Cytokine Production and Blocking of TSST-1 Toxicity in Mice. Clin. Diagn. Lab. Immunol., 2005, 12(3): 399 - 408.
19. HM Zhang, ZL Ou, F Gondaira,et al. Protective effect of anisodamine against Shiga toxin-1: inhibition of cytokine production and increase in the survival of mice. J Lab Clin Med, 2001, 137(2): 93-100.
20. J Li, YY Jiang, TL Yue. Inhibition of release of prostaglandins and leukotrienes from calcimycin -induced mouse peritoneal macrophages and bovine aorta endothelial cells by anisodamine, Zhongguo Yao Li Xue Bao, 1989; 10(3): 274-278.

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Valeriana jatamansi : a phenotypically variable plant species of Kashmir Himalaya

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Abstract: Kashmir Himalaya harbours large number of plant and animal species and is credited all over the world as a treasure of medicinal and aromatic plants. *Valeriana jatamansi* inhabit wide variety of habitats and the species exhibit phenotypic variability across these habitats, the variability aids the species for its survival under different environmental conditions.

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Key words: Kashmir Himalaya; phenotypic variability; environmental conditions

1. Introduction

Many species occupy either a large geographical range or a variety of contrasting habitats within a limited area, or both. This raises the basic ecological question of how plants deal with these contrasting environmental conditions. Researchers have long known about genotypic and phenotypic variation in plant species and interaction of genotypes with environment, but these factors are often ignored in ecophysiological studies. In its simplest terms, genotypic variation deals with genetic differences among individuals or populations. If this variation can be related to habitat differences it may be considered ecotypic. Phenotypic variation involves non-genetic morphological or physiological plasticity or change to environmental variation such as seen in sun vs shade leaves (Abrams, 1993). Environmentally-induced phenotypic variation (phenotypic plasticity) in plants is often considered to be a functional response that maximizes fitness in variable environments. If there is relationship between environmental variation and phenotypic variation within species, it is reasonable to hypothesize that species which occupy a wide range of habitats will be more variable in phenotype than species that occupy a narrow range of habitats given by Van Valen (1965), Baker (1974), Sultan (2001).

Valeriana jatamansi inhabits an altitudinal gradient ranging from 1200-3000m asl, in addition the species also grows in wide variety of habitats which includes shady, moist, slopy and rocky habitats. Therefore, the present study was the first attempt to undertake phenotypic variability of the species in various habitats.

2. Materials and Methods

Plants were randomly selected in all the populations and the traits analyzed, include plant height, number of leaves per plant, internode length, leaf dimensions, number of flowers per inflorescence, flower dimensions, rhizome dimensions and number of roots per stock following methodology of Nath (1996), Kaufman *et al*.(1989).

3. Results

During the present investigation the species was found sporadically distributed in the mountain ranges of Kashmir Himalayas confined to sub-temperate and temperate regions, thriving in moist shady slopes, rocky slopes, land slide areas ranging in an altitude of 1200-3000m asl. The species is variable with respect to its quantitative traits (Figs.1-4). The phenotypic data gathered in different populations is described in detail below:

It was also observed that plants inhabiting shady, moist and fertile or humus rich soils attain vigorous growth, while plants growing under open sunny conditions and on rocky slopes were observed to be on the other extreme. The species produce two types of leaves- basal (radical) leaves which arise from rhizomatous portion and cauline (middle and apical) leaves which arise from the stem. Among this shade inhabiting plants showed maximum leaf dimensions. Root Stock, consisting of rhizome and roots, is characterized by underground thick horizontal rhizome with descending adventitious fibrous roots. Rhizome surface has nodes and internodes and terminates in a tuft consisting of leaf and flowering shoot bases.

Thus it is evident from the data that plants growing under dense canopy and humus rich moist soils as well as loose and fertile soil showed better plant development.

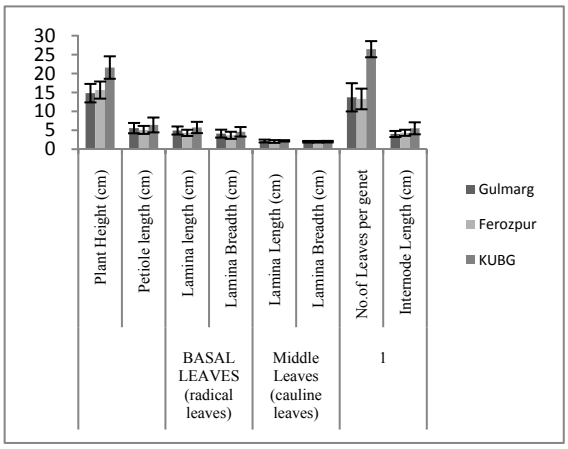

Fig. 1. Variability in morphological traits of hermaphrodite individual across different populations

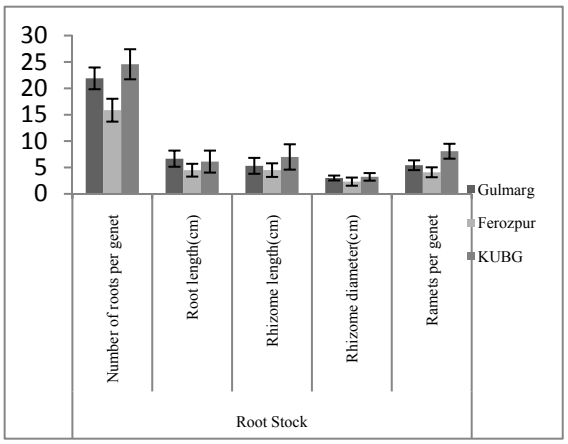

Fig. 2. Root stock dimensions of hermaphrodite individuals across different populations

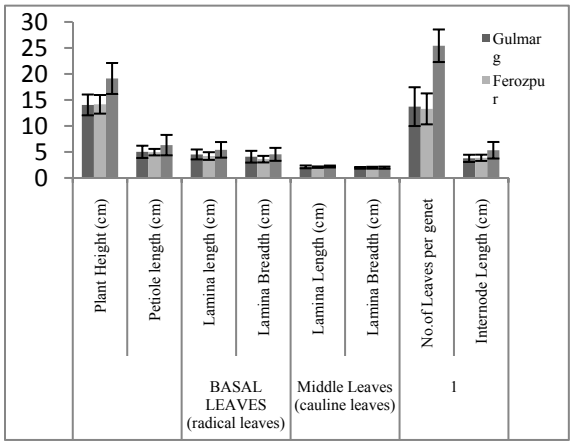

Fig. 3. Variability in morphological characters of female individual across different populations

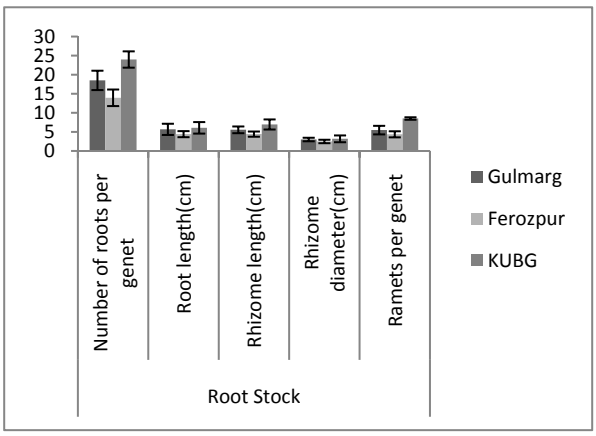

Fig. 4. Root stock dimensions of female individuals across different populations

4. Discussion

The valley of Kashmir, extending from Banihal to Baramulla is situated in the lap of the Himalaya. Himalaya is credited all over the world as a treasure of medicinal plants. Himalaya is ranked as one of the bio-diversity hot spots owing to its considerable abundance of medicinal plants. Among these medicinal gems *Valeriana jatamansi* (Valerianaceae) is a species with tremendous medicinal importance adding color to the crown of Himalayas. In Kashmir Himalayas *Valeriana jatamansi* inhabits sub-temperate and temperate habitats ranging from 1500 to 3000m asl. This species was found sporadically distributed over various sites of Kashmir Himalayas which include Shajnar, Dara, Harwan, Gulmarg, Yusmarg, Ferozpur, Sonamarg and Pahalgam by Naqashi and Dar (1982-86). These sites experience severe climatic conditions (low temperate, extreme variability in rainfall, fast winds, frequent clouds and high cosmic fallout etc.) and are too inaccessible. Within these specific natural habitats the individuals are sporadically distributed in a population, that too much less in number. This taxon has a greater endurance to extreme environments which are ecologically specific and unique in terms of habit, altitude, plant associations, edaphic conditions. These ecological preferences act as barriers preventing them from further spread. It needs to be borne in mind that species with highly stringent and specific habitat requirements have greater possibilities of extinction than species with a broad habit range (Samant *et al*.1996).

Valeriana jatamansi is displaying variability in various phenotypic traits at various stages in its populations. This wide distribution in trait values is the raw material for operative evolutionary forces and natural selection. This phenotypic variability speaks the language of a typical meiotic and breeding system,

which generate the variability in *Valeriana jatamansi*. The present study reveal that in response to their highly specific ecological environments this species have developed a spectacular diversity in their morphological characters viz., plant height, leaf number and dimensions, number of ramets, floral density, root length and number, rhizome dimensions etc. detailed morphological studies not only give specific botanical identity to a species but such studies reveal interesting features which are helpful in understanding the range of morphological variations present across different ecological zones (Anonymous,1976). The diversity across various ecological zones provides a strong edifice at which an ambitious plan for domestication and genetic improvement for commercial exploitation can be built. Plant height is highly plastic and varies among different populations. The plants growing in complete shady or dappled shade environments show maximum variability in this trait while the individuals growing in open or exposed conditions show least variation and are by and large uniform. Increase in plant height in shady environments seems advantageous for the species as the shady environments provide the conditions where plants have to compete for light. The species also exhibit enormous variability in leaf number per genet and leaf dimensions at inter-population level. However, it was also observed that plants growing under shade respond to shade by allocating more biomass to leaves and hence registers maximum leaf number and dimensions as well as plant height to compete for light, as holds true of many other plant species (Abrahmson and Gadgil, 1973). Stem-elongation responses in plants provide an example of adaptive plasticity that could involve an opportunity cost. Increased stem elongation is advantageous among plants growing in dense stands because taller plants overtop their neighbours and have higher lifetime light interception (Weinig, 2000a) and fitness (Schmitt et al. 1995 ; Dudley and Schmitt, 1996 ; Weinig 2000a). Elongation responses early in the life history may limit the duration of elongation or subsequent responsiveness because elongation lowers structural stability (Schmitt *et al*., 1995) and reduces resource acquisition by decreasing allocation to resource-harvesting organs such as leaves given by Morgan and Smith (1978), Robson et al. (1996), Cipollini and Schultz (1999). However, it was also observed that plants growing under shade respond to shade by allocating more biomass to leaves and hence registers maximum leaf number and dimensions as well as plant height to compete for light, as holds true of many other plant species (Abrahmson and Gadgil, 1973). Maximum amount of biomass is allocated to the organs of support i.e., rhizome and stem followed by leaves and inflorescences which is more in plants grown is shady habitat as holds true of other plants growing in shady environments of the forests (Abrahmson and Gadgil, 1973; Abrahmson, 1979).

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5. References

1. Abrahamson, W.G. (1979). Patterns of resource allocation in wild flower populations of fields and woods. Amer.J.Bot., 66: 71-79.
2. Abrahamson, W.G. and Gadgil, M. (1973). Growth form and reproductive effort in golden rods (*Solidego*, Compositae): Am. Nat., 107: 651-661.
3. Abrams, M. (1993).Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies.
4. Anonymous, (1976). The wealth of India, vol. x: Sp-D (Raw material), CSIR, publication, New Delhi, India, pp. 424-426.
5. Baker, H.G. (1974). The evolution of weeds. Ann. Rev. of Eco. and Syst., 5:1-24.
6. Cipollini, D.F. ; Shultz, C. (1999). Exploring cost constraints on stem elongation in plants using phenotypic manipulation. Am. Nat., 153(2):236–242.
7. Dudley, S. A. ; Schmitt, J. (1996). Testing the adaptive plasticity hypothesis: density dependent selection on manipulated stem length in *Impatiens capensis*. Am. Nat., 147:445–465.
8. Kaufman, P.B.; Carlson, T.F.; Dayanandan, P.; Evaus, M.L.; Fisher, J.B.; Parks, C. ; Wells, J.R. (1989). Plants their biology and importance, Hoppe and raw publishers, New York, PP. 714-730.
9. Morgan, D.C.; Smith, H. (1978). The relationship between phytochrome photoequilibrium and development in light grown *Chenopodium album* L. Planta, 142:187–193.

10. Naqashi ; Dar, G.H. (1982-1986). Kashmir University Herbarium Collection (KASH), Centre of Plant Taxonomy, University of Kashmir.
11. Nath, R. (1996). Comprehensive College Botany. Vol.11. Kalyami Publisher, New Delhi, PP 57-98.
12. Robson, P.R.; McCormac, A.C.; Irvine, A.S. and Smith, H. (1996). Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. Nat. Biotechnol. 14:995–998.
13. Samant, S.S.; Dhar, U.; Rawal, R.S. (1996).Natural Resource use by some natives within Nanda Devi biosphere Reserve in West Himalaya. Ethno Bot., 8: 40-50.
14. Schmitt, J.; McCormac, A.C. and Smith, H. (1995). A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. Am. Nat., 146:937–953.
15. Sultan, S.E. (2001). Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. Eco., 82:328-343.
16. Van Valen, L. (1965). Morphological variation and width of ecological niche. Am. Nat., 99:377-390.
17. Weinig, C. (2000a). Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. Evol., 54:124–136.

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Inhibition of EGFR signaling in prostate cancer treated with EGFR siRNA and Gefitinib

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Abstract: To validate the therapeutic effects and modification of EGFR-induced signaling proteins of gefitinib and a small interfering RNA targeting human EGFR (EGFR siRNA) on prostate cancer cell lines PC-3. MTT assay and tumor inhibitory rate were used to evaluate the antitumor activity of EGFR siRNA and/or gefitinib on PC-3 cells *in vitro* and *in vivo*. Real-time PCR was used to measure expression of EGFR mRNA; Western blot assay was applied to evaluate the level of EGFR and its downstream signalling proteins Akt (protein kinase B), MAPK (mitogen-activated protein kinase) and PKC (protein kinase C). Gefitinib inhibited PC-3 cells proliferation in a dose-dependent manner with significant decreased level of EGFR protein and phosphorylation of only Akt, but not either MAPK or PKC; on the other hand, knockdown of EGFR mRNA by siRNA led to lower proliferation of PC-3 cells with decreased phosphorylation of Akt and MAPK, but not PKC. Combination of both had more inhibitory effects on cells than gefitinib and EGFR alone with decreased level of Akt, MAPK and PKC phosphorylation ($P < 0.05$). In *in vivo* models, compared with control group, siRNA could significantly inhibited tumor growth at the rate of 34.83% ($P<0.05$) which is lower than 53.95% in gefitinb group and 59.28% in combined group ($P<0.05$), but no differences in the latter both groups ($P>0.05$). Gefitinib and EGFR siRNA could effectively inhibit the proliferation and tumor growth of prostate cancer, probably via inhibiting the activation of EGFR and the phosphorylation of Akt and MAPK.

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Keywords: Prostate cancer; epidermial growth factor receptor; small-interfering RNA; gefitinib; therapy

1. Introduction

Prostate adenocarcinoma is one of the most common cancers in aged men (Shteynshlyuger and Andriole, 2010). Most of the patients with prostate cancer died from the metastasis to the bone and lymph nodes of cancer cells that conventional androgen-deprivation therapy ultimately fails so that new therapeutic strategies are needed for its treatment and prevention. Prostate cancer commonly overexpresses several growth factors and their receptors, including epidermal growth factor (EGF) and its receptor (EGFR). EGFR plays a critical role in tumor growth and the prostate tissue becomes more susceptible to the growth-promoting action of EGF family growth factors during androgen withdrawal (Traish et al., 2009). Therefore, inhibiting the activation of EGFR and EGFR-induced tyrosine kinase signaling pathways provides therapeutic advantage especially against prostate cancer metastasis (Kim et al., 2006).

EGFR is a membrane glycoprotein composed of an extracellularbinding domain, a transmembrane domain containing a single hydrophobic anchor sequence and an intracellular domain containing tyrosine kinase activity. Activation of EGFR involved in the recruitment and activation of downstream intracellular-signaling cascades, mainly including the mitogen-activated protein kinase (MAPK) pathway, protein kinase C and phosphatidylinositol-3-kinase (PI-3K) /Akt pathways (Hynes and MacDonald, 2009). These signaling cascades can promote proliferation, angiogenesis and invasion and inhibit apoptosis, key mechanisms underlying tumor growth and progression (Iain et al., 2006).

Considering the overexpression of EGFR in prostate cancer, silencing of EGFR expression appears to be a rational strategy for targeting prostate caner. Small interfering RNA (siRNA) would specifically target cells expressing EGFR, providing significant cell specificity for this strategy. siRNA molecules can be efficiently introduced into cells in a permanent manner through expression systems such as lentiviruses that are capable of integration into the cellular genome. This approach for permanent delivery of siRNA has the potential of being translated into important clinical applications (Naldini, 1999). In this study, we have used siRNA-expression lentivirus targeting human EGFR to silence EGFR expression in prostate cancer.

Gefitinib (Iressa, ZD1839) is a quinazoline derivative and an orally active EGFR tyrosine kinase inhibitor (TKI) that competitively binds ATP of EGFR and blocks signal transduction processes implicated in the proliferation, metastasis and

angiogenesis of cancer cells. Gefitinib has been approved as a single drug therapy for lung cancer following very encouraging data obtained in clinical trials (Cohen et al., 2004). It has also shown antiproliferative and anti-invasive effects in other human cancers with amplified or transfected EGFR. Angelucci et al (2006) have shown that gefitinib was effectively inhibiting EGFR-dependent growth in prostate cancer primary cultures and cells lines, independently of their sensitivity to androgen.

In the present study, we undertook not only to investigate and compare the antitumor activity of gefitinib and/or EGFR siRNA against PC-3 cells *in vitro* and *in vivo*, but also to examine the inhibitory effect on EGFR expression and EGFR-induced downstream survival signaling proteins phosphorylation, such as Akt, MAPK and PKC.

2.Materials and Methods

Chemicals and antibodies: Gefitinib was purchased from AstraZeneca Pharmaceutical Co. (Macclesfied, UK). The primary antibodies included rabbit monoclonal antibody against phosphorylated (p)-Akt purchased from Cell Signaling (Beverly, MA, USA), and EGFR, p-PKC and p-MAPK purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibody were peroxidase-labeled antibodies against rabbit (Santa Cruz, CA, USA). Anti-β-actin antibody was purchased from Sigma-Aldrich Company Ltd. (Allentown, PA, USA). Drugs were dissolved in dimethyl sulfoxide (DMSO) for *in vitro* and stored at –20°C. The final concentration of DMSO in culture medium for all treatments was not greater than 0.1%.

Cell culture and transfection of siRNA: PC-3 cell was purchased from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China) and cultured in dulbecco's modified Eagle medium (DMEM, Gibco, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 mg/ml) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. A lentiviruse expressing siRNA targeting human EGFR (EGFR siRNA), marked with green fluorescent protein (GFP), was purchased from Shanghai Benefit Biothechnology Company (Shanghai, China). It's virus titer is 1.7×10^5 ifu/μl. The sequences of EGFR siRNA duplex are: sense strand, 5'-GCAGAGGAATTATGATCTT-3'; antisense strand, 5'-TAATCGTCGTAGACGGTTG-3'. It is homologous to 456–474 nt of human EGFR transcript. PC-3 cells were incubated with EGFR siRNA solution (final concentration of 150 nM) at 37°C. 72 h after initial transfection, cell infection rate was measured by counting green fluorescence and EGFR siRNA stably transfected PC-3 cells (si-PC-3 cells) were obtained. si-PC-3 cells were seeded into 96-well plates (for cell proliferation assays) or 6-well plates (for RT-PCR of immunoblot analysis).

MTT assay: The MTT ((3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide, Sigma, St. Louis, MO, USA) assay was performed to determine cell proliferation. Briefly, prostate cancer cells were plated in 96-well plates at a density of 3×10^3 per well in 100 μl of medium. After attachment overnight, cells were divided into different groups including PC-3 or si-PC-3 cells treated with DMSO (0.2%) as a control, PC-3 cells treated with gefitinib at varying final concentrations (0.5, 1, 2.5, 5 and 10 mg/mL) or si-PC-3 cells treated with gefitinib at a final concentrations of 5 mg/ml for various time periods (24 h, 48 h, 72h, 96h and 120h respectively). After treatment, 20 μL of MTT solution (5 mg/mL) was added to each well. After 4 h incubation, medium was removed and then dissolved by adding 150ul of DMSO to the plates. Color intensity of the solubilized formazan was measured at 570 nm and 630nm with an enzyme linked immunosorbent assay (ELISA) plate reader (Varioskan flash, American Thermo Instruments). This procedure was repeated four times. The growth inhibition was calculated according to the following formula: growth inhibition (%) = (mean OD of the control wells - mean OD of the treated wells)/mean OD of the control wells×100%. The 50% inhibitory concentration (IC50) was calculated from the linear equation, which was deduced using concentration versus growth inhibition regression curve.

Fluorescent realtime PCR: Following the provider's protocol, Trizol reagent kit (Promega, Shanghai, China) was used to isolated total RNA from PC-3 cells or si-PC-3 cells, treated or untreated with gefitinib (5mg/L) for 72 h. The purity of DNA was verified by the ratio A260/A280 = 1.80 – 1.93. The RNAs were frozen at -80℃ until analyzed. cDNA was synthesized from mRNA samples and subsequently used as template for fluorescent real-time PCR assays. Amplicons were visualized on a 1% agarose gel containing 0.2 μg / μL ethidium bromide. A 100-bp ladder (promega) was used as a size standard. A set of primers was designed for real-time PCR (sequences 5'→3') as follows: *EGFR* forward (F): GTGGGGCCGACAGCTATGAGAT, *EGFR* reverse (R): ACCGGCAGGATGTGGAGAT (190bp amplicon); β-actin (as an internal control) F: CCTGTACGCCAACACAGTGC, β-actin R: ATACTCCTGCTTGCTGATCC (211 bp amplicon). For fluorescent realtime PCR, the Qiagen Master Mix kit was used according to the vendor guidelines. A total reaction volume of 25 uL containing 12.5 uL Master Mix, 2 uL 25 mM MgCl2, and 0.25 uL 25x SyBr Green (BioWhittaker Molecular Applications,

Rockland, ME). For EGFR, 5 uL cDNA was used, and 3 uL was used for β-actin control. Samples were processed using the Cepheid Smart Cycler software (Cepheid Systems, Sunnyvale, CA) following 40 amplification cycles (15 seconds of denaturizing at 94℃; 20 seconds of annealing at 60℃; and 20 seconds of extension at 72℃). Melt curve analysis of each sample was supplemented with agarose gel electrophoresis of randomly selected samples to confirm the success of reactions. Fluorescence spectra were recorded during the annealing phase of the reaction. Second derivative analysis of the amplification curves was performed to determine the threshold cycles for each sample. The EGFR mRNA level for each sample was measured by using UVIDOC software (Topac, Cohasset, MA) in order to calculate the values of EGFR / β-actin.

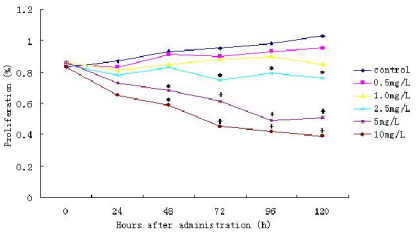
Western blotting: PC-3 or si-PC-3 cells were treated with DMSO (0.2%) as a control or gefitinib at a final concentration of 5 mg/mL for 72 hours. After treatment, cells were harvested and lysed on ice with lysis buffer. Protein concentrations of the supernatants were measured by using the Bradford assay (Bio Rad, Hercules, CA, USA). Protein (20 mg) was separated on 8% or 10% SDS-polyacrylamide gel by electrophoresis and transferred to polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA). Membranes were blocked overnight at 4°C in blocking buffer and then immunoblotted with primary antibodies (all at a 1:1000 dilution) overnight at 4°C. The blots were then incubated with the appropriate secondary antibodies (at a 1:2000 dilution) conjugated with horseradish peroxidase for 2 h at room temperature. The proteins were visualized with the Super Signal Chemiluminescent Substrate (Pierce, USA). The intensity of visualized bands was measured with Quantity One software (Ver 4.4.0, Bio-Rad, Hercules, CA).

Tumor xenograft model: Female nude mice (6 weeks old, weighing 20-25 g, Balb/c) were purchased from Suzhou University Animal Center (Suzhou, China). The certificate number is SYXK (Jiangsu) 2007-0035. Mice were introduced to establish xenograft tumor models of PC-3 cells and si-PC-3 cells respectively. All *in vivo* animal studies described here were carried out in compliance with the standards for use of laboratory animals. The axilla of the upper limb of nude mice were injected subcutaneously with exponentially growing 1×10^7 PC-3 cells or si-PC-3 cells suspended in 0.1 ml physiological saline respectively. When the diameter of the tumors reached over 0.5 cm in diameter after 2 weeks of implantation, selective 24 mice were randomized in average into 4 groups and then drug treatment was initiated. Four groups included: control group (treated with saline only, n = 6), gefitinib group (n = 6), si-PC-3 group (treated with saline only, n = 6) and si-PC-3 cells treated with Gefitinib group (combined treatment, n = 6). Gefitinib (50 $mg\cdot kg^{-1}\cdot d^{-1}$) were orally administrated for 6 days every week for 2 weeks. When all the mice were sacrificed 10 days after the last treatment, the tumors were removed and tumor volume (length × $width^2$ /2) was measured and weighed. The relative tumor volume at day n (RTVn) versus day 0 was expressed according to the following formula: RTVn = TVn/TV0. Tumor regression (T/C (%)) in treated versus control mice was calculated using: T/C (%) = (mean RTV of treated group)/(mean RTV of control group) × 100%.

Statistical analysis: Data are expressed as the mean ± standard deviation (SD). Pictures of the Western blot assay were analyzed using software Image J. Data of the representatives were analyzed for statistical significance using analysis of variance (ANOVA). All statistical analyses were performed with SPSS12.0. *P* values <0.05 was considered statistically significant.

3.Results

Inhibitory effect of gefitinib and/or EGFR siRNA on the proliferation of PC-3 cells: To determine the effects of gefitinib, EGFR siRNA and their combination on cellular proliferation, the MTT assay was performed on PC-3 cells at different time points. PC-3 cells were incubated with various concentrations of gefitinib for various time periods. Gefitinib inhibited cellular proliferation in a dose-dependent manner. The percentage of growth inhibition was lower from 30% to 80% at concentrations of 0.5, 1, 2.5, 5 and 10 mg/mL of gefitinib than control (Fig. 1A). The values of IC_{50} of gefitinib were (5.12 ± 0.41) mg / mL. GFP labeled EGFR siRNA (EGFR-GFP) was used to test the efficiency of EGFR siRNA expressing lentivirus transfecting PC-3 cells. When PC-3 cells were tranfected by EGFR-GFP, the intensity fluorescence from GFP was measured to calculate transfection efficiency under fluorescence microscope. A PC-3 cell line that stably expresses EGFR-GFP was generated 72 h after initial treatment. The transfection efficiency of lentivurus to PC-3 cells was about 85%. Compared with control, the viability of the PC-3 cells stably transfected with EGFR siRNA (si-PC-3 cells) maintained a lower rate at 40% - 50% ($P < 0.01$) and did not rebound (Fig. 1B and 1C). The combination treatment had a more potent inhibitory effect on cell viability than gefitinib or EGFR siRNA alone, with viability at 35%, 61% of gefitinib and 46% of EGFR siRNA at 72 h, respectively (P<0.05, Fig. 1B).



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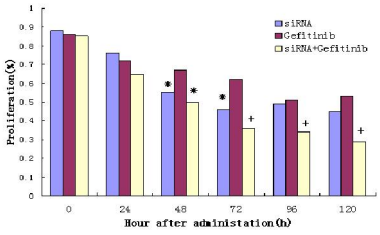

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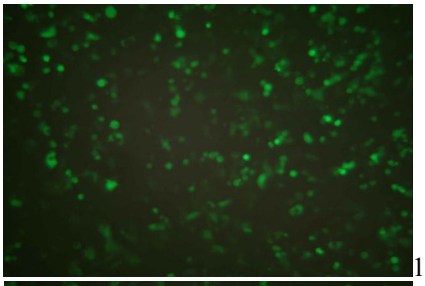

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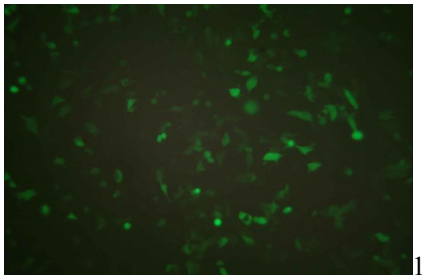

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Fig. 1 Inhibitory effect of gefitinib and/or EGFR siRNA on PC-3 cells measured by MTT. **1A.** Inhibitory effect of gefitinib on PC-3 cells at different doses for various periods. Gefitinib inhibited PC-3 cell proliferation in a dose-dependant manner. **P* < 0.05 versus control; +*P* < 0.05 versus gefitnib (2.5 mg/L). 1B. Combined effects of gefitinib (5 mg/L) and EGFR siRNA (150 nM) on PC-3 cell proliferation. Cell viability of PC-3 cells was measured by MTT assay after treatment with gefitinib, EGFR siRNA or a combination of both for various periods. **P* <0.05 compared with gefitinib; +*P* <0.05 compared with EGFR siRNA. 1C. PC-3 cells were transfected by EGFR siRNA expressing lentivirus labeled with GFR. The transfect efficiency of lentivirus for PC-3 cells was 85% (green, Fig. 1c1, × 100). After 72 h of initial tranfection, proliferation of PC-3 decreased dramatically (Fig. 1c2). The experiment was performed three times and each time triplicate cell cultures were examined. Data are shown as mean ±SD.

Gefitinib and/or EGFR siRNA decreased expression of EGFR: Overexpression or amplification of EGFR plays a key role in prostate cancer progression. To determine the effects of gefitinib (5 mg/mL), EGFR siRNA (150 nM) and their combination on cells, EGFR expression was measured on PC-3 cells at two selected time points (0 and 72 h) to determine the role of EGFR signaling in prostate cancer. Our study showed that the expression level of EGFR protein was high at 48% in PC-3 cells (Fig. 2B). Therefore, we measured EGFR expression by realtime PCR and Western blot respectively to determine the role of EGFR signaling in gefitinib-induced growth inhibition in PC-3 cells. Gefitinib decreased total EGFR protein by 78.3% after 72 h of exposure (Fig. 2B), but the level of EGFR mRNA unchanged compared with control (Fig. 2A). Such a discrepancy between the level of EGFR mRNA and protein strongly suggested that gefitinib, as a TKI would inhibit EGFR expression by regulating translation from EGFR, which would be elucidated by further study. Testing the efficiency and specificity of the individual EGFR siRNA in knocking down EGFR was necessary to confirm the results of the screen. Therefore, we examined EGFR siRNA effectiveness using real-time PCR and Western blot respectively. The result revealed that EGFR mRNA levels were reduced up to 92.5% after treatment with EGFR siRNA (Fig. 2A) consistent with the similar effect on EGFR protein expression (88.9%, Fig. 2B). Thus, EGFR siRNA, when added to PC-3 cells, demonstrated a near complete knockdown of EGFR expression. This experiment suggested that treatment of PC-3 cells with EGFR siRNA lead to an efficient depletion of EGFR mRNA and protein. As shown in Fig. 1B, proliferation of EGFR knockdown cells (si-PC-3 cells) was still inhibited by gefitinib despite that there was no big difference on EGFR expression between EGFR siRNA alone treatment and combination of both. It suggests that gefitinib may have other targets beside EGFR. It was reported that gefitinib induced apoptosis of cancer cells via VEGF and IL-8. However, Our above results showed downregulation of EGFR expression along with cell proliferation suppressed significantly, indicating that EGFR plays an important role in proliferation of PC-3 cells.

Gefinib and/or EGFR inhibited the phosphorylation of EGFR-induced downstream signaling proteins, such as p-Akt, p-MAPK and p-PKC: The PI3K/Akt, ras/MAPK and PKC pathway were recognized as playing critical regulatory roles in the cell survival/death decision.

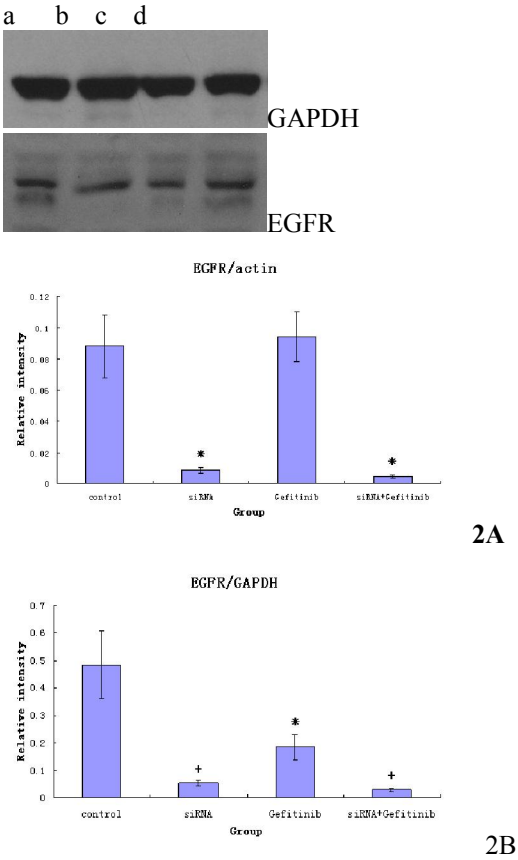

Fig. 2 Effects of gefitinib (5 mg/L), EGFR siRNA (150 nM) or combination of both on the EGFR expression after 72-hour exposure. 2A. Inhibition of EGFR mRNA expression in PC-3 cells treated by gefitinib, EGFR siRNA or combination of both. For evaluation of the EGFR mRNA, realtime RT-PCR was used. Actin was used as an equal loading control. Relative intensity of EGFR mRNA was measured by densitometry analysis. *P <0.05, compared with control and gefitnib. 2B. PC-3 cells were pretreated with compounds for 72 h. For evaluation of the EGFR protein, western blotting was used. GAPDH was used as an equal loading control. Relative intensity of EGFR was measured by densitometry analysis. *P <0.05 compared with control; +P <0.05 compared with gefitinib. a, control; b, gefitinib; c, EGFR siRNA; d, combination of gefitinib and EGFR siRNA.

To analyze those downstream signaling events of the EGFR pathway in our study, we next examined EGFR-induced phosphorylation of Akt, MAPK and PKCl in PC-3 cells treated with gefitinib, EGFR siRNA or their combination 72 h after initial treatment by using Western blot assay (Fig. 3). Our study showed that level of p-MAPK was higer than that of p-Akt and p-PKC in PC-3 cells. Gefitinib could efficiently inhibit level of p-Akt, but not levels of p-MAPK or p-PKC.

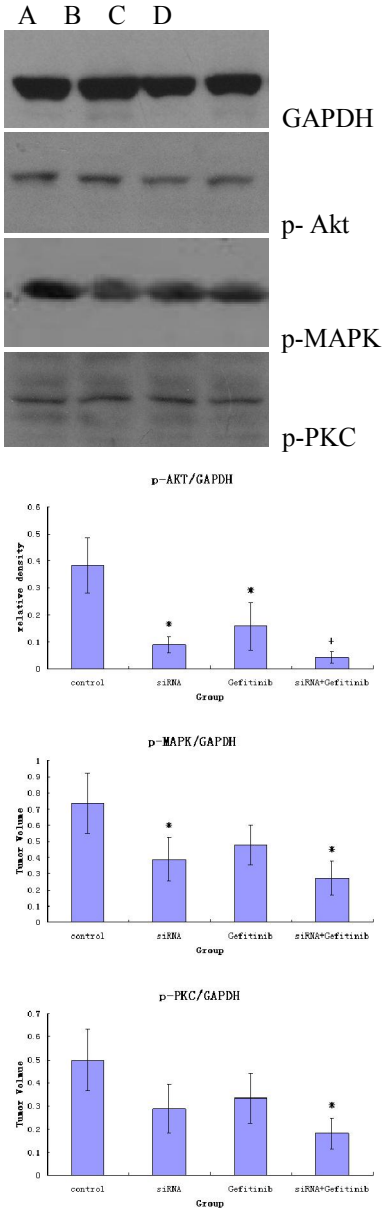

Fig. 3. Inhibition of EGFR-induced downstream signaling proteins phosphorylation in PC-3 cells by combination of gefitinib and EGFR siRNA. PC-3 cells were treated with compounds (gefitinib 5 mg/mL; EGFR siRNA 150 nM; or their combination) for 72 hours. Protein extracts were used for Western blot analysis of EGFR-induced downstream signaling proteins phosphorylation by using phospho-specific antibodies. Phosphorylation of Akt, MAPK or PKC was measured using phospho-specific antibodies by western blotting. Relative value of grey scale of p-Akt, p-MAPK, and p-PKC compared with GAPDH was calculated by software, respectively. *P<0.05 compared with control; +P<0.05 compared with gefitinib. A, control; B, gefitinib; C, EGFR siRNA; D, combination of gefitinib and EGFR siRNA.

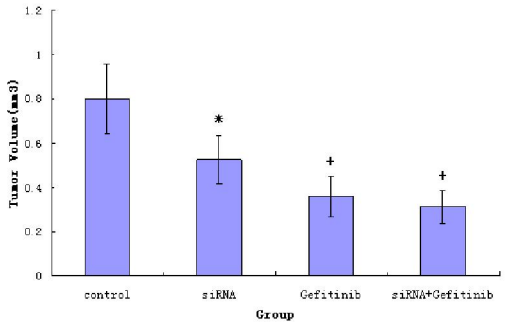

Fig. 4. Gefitinib and/or EGFR siRNA inhibited growth of PC-3 xenograft in nude mice. Luteolin inhibits growth of PC-3 xenograft in nude mice. Twenty-four mice were randomly divided into four groups (n=6 mice/each group). The mice of the treatment group were administered I.P. with gefitinib at 50 mg/kg. The mice of control group received solvent at equal volume. Treatment was begun 3 days after implantation and continued daily throughout the study. *P<0.05 compared with control; +P<0.05 compared with EGFR siRNA. For details, please see Materials and methods. After treatment, the tumors were harvested and measured to calculate volume. The data represent mean ± SD (n=6).

Densitometry analysis showed that p-Akt protein level was decreased by gefitinib by 59.01%, P<0.05; level of p-MAPK and p-PKC were decreased by 34.83% and 33.40%, respectively, P>0.05, as compared with control. However, EGFR siRNA decreased p-Akt and p-MAPK protein levels by 76.49% and 47.15%, respectively, P<0.05; and p-PKC protein level unchanged significantly by 42.2%, P>0.05, as compared with control. Importantly, the combination treatment of gefitinib and EGFR siRNA showed much more significant inhibition of the Akt, MAPK and PKC phosphorylation than the single treatment, especially with respect to the p-PKC protein. Densitometry analysis showed that p-PKC protein levels was inhibited by 33.4%, 44.2% and 63.8% when the cells were treated with gefitinib, EGFR siRNA and the combination, respectively. These data suggest that gefitinib, EGFR siRNA or their combination could selectively inhibit the phosphorylation of EGFR-mediated cell survival signals such as Akt, MAPK and PKC. Furthermore, the combination of gefitinib and EGFR siRNA demonstrated additive inhibitory effects on these critical cell survival signaling proteins and, importantly, in cell survival.

In vivo antitumor activity of gefitinib and/or EGFR siRNA on PC-3 tumor: Finally, the antitumor effect of gefitinib and/or EGFR siRNA *in vivo* was evaluated using the model of nude mice bearing PC-3 tumor xenografts. There was no evidence of systemic toxicity to the mice as evidenced by normal food intake and body weight (data not shown). The average size of tumors in the right flank (control group) reached 0.803±0.157 mm^3 at the end of experiment. Compared with the control group, EGFR siRNA showed a stronger tumor inhibitory effect with a percent tumor regression (T/C) of 34.83% (P <0.05). Gefitinib (50 mg /·kg) inhibited tumors growth more significantly than either EGFR siRNA or control treated with a T/C of 53.95% (P <0.05). But combination of gefitinib and EGFR siRNA did not show significant inhibitory effect against tumor growth than gefintinb alone with a T/C of 59.28% (Fig. 4), P > 0.05.

4.Discussion

Amplification or overexpression of EGFR was significantly associated with high grade, advanced stage, and high risk for prostate-specific antigen (PSA) recurrence in prostate cancer progression. EGFR-targeted drugs could be of therapeutic relevance in prostate cancer (Schlomm et al., 2007). There is increasing interest in the combined use of low doses of therapeutic agents with differing modes of action, rather than the administration of a single agent at a higher dose, as a means of obtaining increased efficacy and minimized toxicity. This approach is extremely important when a promising therapeutic agent demonstrates significant efficacy but may produce some untoward side effects at higher effective doses. Gefitinib is a molecular TKI that has showed significant inhibitory effects of many cancers. To date, small-molecule TKIs and siRNAs targeting EGFR are used frequently to treat prostate cancer (Festuccia, et al., 2009; Addepalli et al., 2010). It is interesting to evaluate the therapeutic effects of gefitinib, EGFR siRNA or combination of both on cells as well as their intracellular mechanisms useful to guide how to use these agents efficiently. In this study, we compared the inhibitory effects of gefitinib, EGFR siRNA and combined treatment on PC-3 cells along with investigating EGFR-induced downstream signaling proteins phosphorylation.

The overexpression of EGFR in prostate cancer has been implicated in the stimulation of EGFR signaling pathways that drive the progression of neoplastic events (de Muga, et al., 2010). In our *in vitro* study, overexpression of EGFR mRNA and protein on PC-3 cell was candidated again by PT-PCR and western blot assay. Gefitinib and EGFR siRNA could effectively inhibited PC-3 cells prolification with deceased expression of EGFR (Fig. 2). Gefitinib blocked the growth of PC-3 cells in a dose-dependent manner; however, PC-3 cells showed a lower growth

rate at 40%~50% for knockdown of EGFR 72 hours after stably trasfected by EGFR siRNA expressing lentivirus. siRNA molecules can be efficiently introduced into cells in a permanent manner through expression systems such as lentiviruses that are capable of integration into the cellular genome. Lentiviruses are considered as powerful integrative vector systems and are highly efficient for *in vivo* application (Naldini, 1999). The latest generation of lentiviral vectors is one of the safest and most efficient tools for stable gene transfer, even eliminating the risk of vector mobilization, due to infection with a wild-type HIV-1 (Montini, et al., 2006). The anti-EGFR lentivirus was efficiently capable of silencing EGFR, which resulted in a complete suppression at 120 h post-infection. Interestingly, in our study gefitnib still significantly inhibited the growth si-PC-3 cells with EGFR knockdown by EGFR siRNA. Therefore, combination of gefitinib and EGFR siRNA block PC-3 cells growths via other pathways, for example, directly blocking p-PKC that could not be effectively inhibited by gefitinib or EGFR siRNA alone. Protein kinase C activation can, in turn, result in MAPK and c-Jun NH2-terminal kinase activation (McClellan et al., 1999). One study investigated whether the antitumor effect of gefitinib was partly attributable to antiangiogenic activity that could be mediated directly by blocking EGF-induced neovascularization and also indirectly by inhibition of vascular endothelial growth factor (VEGF) or interleukin-8 production (Hirata et al., 2002). Our results was inconsistent with Bates et al (2005) who noted that successful therapy by such EGFR-targeting drugs could be expected for patients whose EGFR family members are amplified or overexpressed in cancer cells.

With regard to EGFR-induced downstream signaling proteins in PC-3 cells in our study, EGFR siRNA efficiently inhibited levels of p-Akt and p-MAPK, but not p-PKC; however, gefitinib significantly downregulated level of p-Akt and show no effects on the levels of p-MAPK and p-PKC. Activation of EGFR leads to the phosphorylation of key tyrosine residues within the COOH-terminal portion of EGFR and, as a result, provides specific docking sites for cytoplasmic proteins containing Src and phosphotyrosine-binding domains (Yarden et al., 2001). Akt, PKC and MAPK belong to cell-survival kinases. MAPK is a critically important protein in Ras/Raf pathway to regulate cell proliferation and survival. Activated MAPKs are imported into the nucleus where they phosphorylate specific transcription factors involved in cell proliferation (Liebmann et al., 2006). Both Ras-MAPK and PI3K-Akt signaling have been implicated in modulating androgen receptor (AR) activity in androgen-independent prostate cancer cells. Cross-talk between PI3K-Akt and Ras-MAPK pathways may also be important for androgen-independent prostate cancer cells to maintain their growth in an androgen-depleted state. Wu et al (2008) examined cross-talk between the Ras–MAPK and PI3K–Akt signaling pathways in androgen-independent C4-2 CaP cells. They found that PTEN expression in C4-2 cells made cells hypersensitive to EGF or serum stimulation as indicated by increased pERK levels. This hypersensitivity of MAPK signaling was due to the PTEN inhibition of PI3K–Akt pathway. PKC activation can result in MAPK and c-Jun NH2-terminal kinase activation. In PC-3 cells, combination of EGFR siRNA and gefitinib could more dramatically reduced level of p-PKC than either EGFR siRNA or gefitinib, but showed no significant reduction on the levels of p-Akt or p-MAPK compared with EGFR siRNA. Therefore, EGFR siRNA could inhibit PC-3 cells growth via efficiently downregulating expression of EGFR and the phosphorylation of EGFR-induced downstream signaling protein Akt and MAPK; however, gefitinib did so only selective inhibition of Akt activation, and its inhibitory effects on PC-3 cells could be enhanced by EGFR siRNA via further reducing p-PKC level.

Gefitinib is the first EGFR-targeting drug to be registered for advanced NSCLC, but might possesses slightly different pharmacologic characteristics on prostate cancer. In our study, PC-3 cells treated with gefitinib, p-Akt levels were downregulated by 59.01% lower than reduced levels of p-MAPK or p-PKC by 34.83% or 33.4%. Akt is another important regulator of cell survival and cell proliferation that significantly contributes to tumor growth and progression by promoting cell invasiveness and angiogenesis. Akt is phosphorylated on EGFR activation, transmitting signals for cell survival. It is reported that patients with phosphor-Akt positive tumours or EGFR-dependent Akt activation had a better response rate, disease control rate, and time to progression by gefitinib treatment (Cappuzzo et al., 2004; Ono et al., 2004). The Akt signaling pathway activated by EGFR harboring activating mutations or gene gain is rather more specifically involved in enhanced drug sensitivity and therapeutic efficacy than the ERK1/2 pathway, suggesting phosphorylated AKT as one of the molecular determinants of response to EGFR-targeting drugs. However, loss of PTEN gene in cancer cells such as PC-3 cells leads to constitutive activation of the PI3K/Akt signal transduction pathway. Gefitinib is unable to downregulate Akt activity in PTEN-negative cells while pharmacologic downregulation of constitutive PI3K/Akt pathway signaling using the PI3K inhibitor LY294002 restores EGFR-stimulated Akt signaling and sensitizes cells to

gefitinib. These results suggested that sensitivity to gefitinib requires intact growth factor receptor-stimulated Akt signaling activity. Reconstitution of PTEN in these cells re-established EGFR-driven Akt signaling and thereby restored gefitinib sensitivity. The factors that determine gefitinib sensitivity have long been an enigma (Uramoto and Mitsudomi, 2007). However, further study is required to determine how phosphorylated AKT expression can be applied to determination of the clinical therapeutic efficacy of gefitinib.

At last, we tested the effects of gefitinib and/or EGFR siRNA on prostate cancer growth by in vivo experiments. Gefitinib inhibited more significantly PC-3 xenografts growth in nude mice than EGFR siRNA. However, no significant difference in the tumor growth inhibition was observed between gefitinib group and combination group. This may be due to, (1) transfection of siRNA by lentivirus to PC-3 cells is insufficient, (2) the treatment time is not long enough or (3) the sample size is not big enough. This needs further investigation in our future study. In this study we have identified high levels of EGFR-induced p-Akt, p-MAPK and p-PKC as an essential mediator of growth factor-activated cell proliferation in PC-3 cells in vitro. Gefitinib and EGFR siRNA could inhibit the growth of PC-3 cells mainly via decreasing levels of p-Akt and p-MAPK. Considering great complexity and redundancy of EGFR pathway, it is natural to assume that one cannot expect a sole determinant of clinical benefit of EGFR-TKIs (Tsao et al., 2005). Combining EGFR-targeting drugs with anticancer agents could modify the characteristics of drug sensitivity in ways that might be unique for each drug type. Cooperative growth inhibition is often observed following a combination of EGFR-targeting drugs against various cancer cell types (Jimeno et al., 2005).

In summary, we have demonstrated that gefitinib in combination with EGFR siRNA mainly inhibits the activation of EGFR-induced downstream protein p-Akt and p-MAPK in PC-3 cells. EGFR/Akt or EGFR/MARP signaling pathways are critical for maintaining cell survival. These mechanisms may be exploited for the prevention and/or treatment of human prostate cancer. Our results clearly show that combination treatment of PC-3 cells with gefitinib and EGFR siRNA had a very potent inhibitory effect on the phosphorylation and activation of EGFR and Akt, and on the NF-kB pathway, when compared with single compound treatment. The concerted inhibitory effect of the combination on EGFR and its downscream proteins led to enhanced apoptotic cell death when compared with the effects of the individual compounds. The combination may thus offer therapeutic advantages in the treatment and prevention of human prostate cancer.

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References

1. Shteynshlyuger A, Andriole GL. Prostate cancer: to screen or not to screen? Urol Clin North Am. 2010; 37: 1-9.
2. Traish AM, Morgentaler A. Epidermal growth factor receptor expression escapes androgen regulation in prostate cancer: a potential molecular switch for tumour growth. Br J Cancer. 2009; 101:1949-56
3. Kim JH, Xu C, Keum YS, Reddy B, Conney A, Kong AN. Inhibition of EGFR signaling in human prostate cancer PC-3 cells by combination treatment with b-phenylethyl isothiocyanate and curcumin. Carcinogenesis, 2006, 27:475–482.
4. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol. 2009; 21:177-84.
5. Iain RH, Janice MK, Helen EJ, et al. Inductive mechanisms limiting response to anti-epidermal growth factor receptor therapy. Endocrine-Related Cancer, 2006,13:S89–S97.
6. Naldini L. In vivo gene delivery by lentiviral vectors. Thromb Haemost 1999; 82:552–554.
7. Cohen MH, Williams GA, Sridhara R, et al. United States Food and Drug Administration Drug Approval summary: gefitinib (ZD1839; Iressa) tablets. Clinical Cancer Research. 2004,10:1212-1218.
8. Angelucci A, Gravina GL, Rucci N, Millimaggi D, Festuccia C, Muzi P, Teti A, Vicentini C, Bologna M. Suppression of EGF-R signaling reduces the incidence of prostate cancer metastasis in nude mice. Endocrine-related Cancer, 2006,13:197-210.
9. Schlomm T, Kirstein P, Iwers L, Daniel B, Steuber T, Walz J, Clinical significance of epidermal growth factor receptor protein overexpression and gene copy number gains in prostate cancer. Clin Cancer Res. 2007; 13:6579-84.
10. Festuccia C, Gravina GL, Biordi L, et al. Effects of EGFR tyrosine kinase inhibitor erlotinib in prostate cancer cells in vitro. Prostate. 2009; 69:1529-37.
11. Addepalli MK, Ray KB, Kumar B, et al. RNAi-mediated knockdown of AURKB and EGFR shows enhanced therapeutic efficacy in prostate tumor regression. Gene Ther. 2010;

17:352-9.
12. de Muga S, Hernández S, Agell L, et al. Molecular alterations of EGFR and PTEN in prostate cancer: association with high-grade and advanced-stage carcinomas.Mod Pathol. 2010; 23:703-12.
13. Naldini L. In vivo gene delivery by lentiviral vectors. Thromb Haemost 1999; 82:552–4.
14. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, Sergi Sergi L, Benedicenti F, Ambrosi A, Di Serio C, Doglioni C, von Kalle C, Naldini L. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006; 24:687–96.
15. McClellan M, Kievit P, Auersperg N, Rodland K. Regulation of proliferation and apoptosis by epidermal growth factor and protein kinase C in human ovarian surface epithelial cells. Exp Cell Res 1999;246:471- 9.
16. Hirata A, Ogawa S, Kometani T, Kuwano T, Naito S, Kuwano M, Ono M. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. Cancer Res 2002; 62:2554-60.
17. Bates SE, FojoT. Epidermal growth factor receptor inhibitors: a moving target? Clin Cancer Res 2005; 11:7203 - 5.
18. Yarden Y, Sliwkowski M. Untangling the ErbB signaling network. Nat RevMol Cell Biol 2001; 2:127-37.
19. Liebmann C. Regulation of MAP kinase activity by peptide receptor signaling pathway: paradigms of multiplicity. Cell Signal 2001; 13:777- 85. GaestelM.MAPKAP kinasesIMKsItwo's company, three's a crowd. Nat Rev Mol Cell Biol 2006; 7:120 - 30.
20. Wu Z, Gioeli D, Conaway M, et al. Restoration of PTEN expression alters the sensitivity of prostate cancer cells to EGFR inhibitors. Prostate. 2008; 68:935-44.
21. Cappuzzo F, Magrini E, Ceresoli GL, et al. Akt phosphorylation and gefitinib efficacy in patients with advanced nonsmall-cell lung cancer. J Natl Cancer Inst, 2004,96: 1133– 1141.
22. Ono M, Hirata A, Kometani T, Miyagawa M, Ueda S, Kinoshita H, Fujii T, Kuwano M. Sensitivity to gefitinib (Iressa, ZD1839) in non-small cell lung cancer cell linesco rrelates with dependence on the epidermal growth factor (EGF) receptor/extracellular signal-regulated kinase 1/2 and EGF receptor/Akt pathway for proliferation. Mol Cancer Ther 2004; 3: 465-72.
23. Uramoto H and Mitsudomi T. Which biomarker predicts benefit from EGFR-TKI treatment for patients with lung cancer? Br J Cancer. 2007,96, 857 – 863.
24. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha Santos G, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J, Shepherd FA. Erlotinib in lung cancer-molecular and clinical predictors of outcome. N Engl J Med 2005; 353:133-44.
25. Jimeno A, Rubio-Viqueira B, Amador ML, Oppenheimer D, Bouraoud N, Kulesza P, Sebastiani V, Maitra A, Hidalgo M. Epidermal growth factor receptor dynamics influences response to epidermal growth factor receptor targeted agents. Cancer Res 2005; 65:3003-10.

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Government Expenditure and Economic Growth: Panel Evidence from Asian Countries

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Abstract: This paper attempted to examine the causality relationship between government expenditure and economic growth for two panels of 27 Asian countries over the 1970 to 2009 years. A Panel-VECM causality framework based on Wald's test employed to investigate short-run and long-run causality between government expenditure and economic growth and indicates bidirectional causality for Asian developing panel, while the results of long-run causality for advanced and newly industrialized countries does not support causality in any direction. These findings have the policy implication for policymakers and economists.
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1. Introduction

The relationship between government expenditure and economic growth is an important issue among the economist and policymakers for decades. Many empirical studies examined the relationship between government expenditure and economic growth for both developed and developing countries by using various econometric methods, but the results are mixed among the different studies. Some of these studies imply that government expenditure must decrease for achieving to more economic growth, and some of them indicate that government expenditure can stimulate growth. Further, some studies show that there is no relationship between government expenditure and economic growth in some countries.

The study of Gregoriou and Ghosh (2009) attempted to investigate the effect of government expenditure on economic growth for a panel of 15 developing countries over the 1972-1999 periods. The results of GMM method indicates that, for countries such as Brazil government expenditure plays a major role in long-run growth, whereas for countries like Sudan, government current expenditure have a minor role in economic growth. In other words impact of government expenditure is varying across the countries. Iyare and Lorde (2004) examined six versions of Wagner's law for nine Caribbean cointries, Empirical finding indicate the existence of long-run relationship between government expenditure and income for Grenada, Guyana and Jamaica for a specific version of Wagner's law. Results of Granger causality test indicate causality from income to government expenditure for Guyana and from government expenditure to income for Grenada and Jamaica. Results of short-run causality are mixed but causality from income to government expenditure is predominant causal relationship. Akitoby et al. (2006) studied the relationship between government spending and economic growth for 51 developing countries by employing error-correction model. The empirical results support existence of long-run relationship between government spending and GDP for 70% of countries. Wu et al. (2010) analyzed the Wagner's law hypothesis for 182 OECD and non-OECD countries by using panel data technique. Empirical results of this study indicate bi-directional causality for the full sample of countries. Also, the results of sub-sample countries support the bi-directional causality between government expenditure and economic growth. Huang (2006) investigates Wagner's law for China and Taiwan by employing Bound test approach proposed by Pesaran et al. (2001). The results of cointegration performed in this study cannot support existence of long-run relationship between government expenditure and GDP. In addition, the results of this study cannot support Wagner's law for China and Taiwan.

Wahab (2004) employed an ECM framework to analyze the nature of relationship between economic growth and government expenditure for OECD countries to investigate Wagner's law in these countries. The empirical finding reveals a limited support for Wagner's law. The study of Chang (2002) investigated five versions of Wagner's law for six emerging and industrialized countries over the 1951 to 1996 periods. The result

indicates unidirectional Granger Causality from income to government spending for South Korea, Taiwan, Japan, United Kingdom, and United States. Also, Chang et al. (2004) re-examined Wagner's law for ten emerging and industrialized countries and found same results as pervious work.

Landau (1983) tried to examine the relation between these variables for over 100 countries. The results of this paper indicate negative relationship between government consumption expenditure and the rate of growth of per capita GDP. Hsieh and Lai (1994) examined the relationship between government expenditure and economic growth for G7 countries by using Granger causality test and VAR technique. The results show that the relationship between government spending and growth can vary significantly across time as well as across the major industrialized countries. The empirical work of Dritsakis and Adamopoulos (2004) indicates bi-directional causality relationship between different category of expenditure and economic growth for Greek over the 1960 to 2001 years. Kolluri et al. (2000) showed short and long-run effects of economic growth on government expenditure for G7 countries by using annual time series data over the 1960 to 1993 years. Agell et al. (1997) examined relation between growth and the public sector for 23 OECD countries over the 1970 to 1990 years. The finding could not illustrate that relation is negative, positive or no relation exist between growth and public sector. Samudram et al. (2009) examined the relationship between different category of public expenditure and growth for Malaysia over the 1970 to 2004 years. The result indicates bi-directional causality between economic growth and spending on health and administration and for other kinds of spending causality run from economic growth to spending. The study of Loizides and Vamvoukas (2005) employed Granger causality framework to investigate the relationship between size of government and economic growth by examining a bi-variate model and two different tri-varibale models. The empirical results indicate causality from government size to economic growth in all countries in the short run and for Ireland and the UK in the long-run. In addition, causality from economic growth to government size in Greece and, when inflation included, in the UK.

As seen, there is no common consensus between the different studies. The difference between the findings could be due to different time periods or using different econometric methods. However, in this paper, we examine the causality relationship between government expenditure and economic growth for two panels of Asian countries by employing panel causality approach.

The rest of this paper is organized as follows: Section 2 discussed data and methodology. Section 3 present empirical results and finally conclusion presented in Section 4.

2. Data and Methodology

2.1 Data

The use of panel data has several benefits in contrast with time series data: controlling for individual heterogeneity and give more informative data, more variability, less colinearity among the variables, and more efficiency (Baltagi. 2005). Therefore, this paper applies panel data of government expenditure and real GDP of 27 Asian countries over the 1970 to 2009 years. Countries are categorized in two separate panels; one panel includes five Asian advanced economies: Hong Kong, Japan, Singapore, South Korea, Taiwan and five Asian newly industrialized countries: China, India, Malaysia, Philippines, and Thailand. Another's panel includes 17 Asian developing countries: Afghanistan, Bahrain, Bangladesh, Cambodia, Indonesia, Iran, Iraq, Jordan, Laos, Lebanon, Maldives, Nepal, Oman, Pakistan, Sri Lanka, Syrian Arab Republic, and Vietnam. Further, some of the countries excluded due to lack of data in the sample of 1970 to 2009 years.

The annual data of government expenditure and real GDP obtained from Penn Word Table 7.0. Government expenditure measured as the ratio of government expenditure to GDP, and real GDP measured in constant 2005 dollars, the natural logarithms of variables are denoted as LG and LGDP.

2.2 Methodology

2.2.1 Panel Unit Root Test

Several Panel unit root tests presented to investigate the stationary properties of panel data. This paper applied four tests proposed by Im et al. (IPS, 2003), Levin et al. (LLC, 2002), Breitung (2000) and Fisher-type test proposed by Maddala and Wu (1999) and Choi (2001) to test the null hypothesis of having unit root.

The test of Im, Pesaran and Shin (IPS, 2003) allow for a heterogeneous coefficient of y_{it-1} and propose an alternative testing procedure based on averaging individual unit root test statistics. IPS suggests an average of the ADF tests when u_{it} is serially correlated with different serial correlation properties across cross-sectional units.

The t-statistic of IPS can be expressed as follows:

$$t_{IPS} = \frac{\sqrt{N}(\bar{t} - \frac{1}{N}\sum_{i=1}^{N} E[t_{iT} \mid \rho_i = 0])}{\sqrt{\frac{1}{N}\sum_{i=1}^{N} \mathrm{var}[t_{iT} \mid \rho_i = 0]}} \Rightarrow N(0,1) \quad (1)$$

Values of $E[t_{iT} \mid \rho_i = 0]$ and $\mathrm{var}[t_{iT} \mid \rho_i = 0]$ obtained from the results of Monte Carlo simulations carried out by IPS.

Following Dickey and Fuller (1979, 1981), Levin and Lin (1993), and Levin, Lin and Chu (2002), consider a panel extension of the null hypothesis that each individual time series in the panel contains a unit root against the alternative hypothesis that all individual series are stationary.(Hsiao, 2003).

The adjusted t-statistic of LLC is:

$$t_{\rho}^{*} = \frac{t_{\rho} - N\tilde{T}\hat{S}_N \hat{\sigma}_{\varepsilon}^{2} \sigma(\hat{\rho}) \mu_{m\tilde{T}}^{*}}{\sigma_{m\tilde{T}}^{*}} \quad (2)$$

Where $\mu_{m\tilde{T}}^{*}$ and $\sigma_{m\tilde{T}}^{*}$ are the mean and standard deviation adjustments provided by table 2 of LLC. Levin, Lin and Chu show that t_{ρ}^{*} is asymptotically distributed as $N(0, 1)$.

As mentioned in Baltagi (2005), LLC and IPS tests may not keep nominal size well when either N is small or N is large relative to T. Breitung (2000) found that the LLC and IPS tests suffer from a dramatic loss of power if individual-specific trends are included. Breitung suggests a test statistic that does not employ a bias adjustment whose power is substantially higher than LLC or the IPS tests using Monte Carlo experiments.

Maddala and Wu (1999) and Choi (2001) proposed a Fisher-type test of unit root, which combines the p-values from unit root tests for each cross-section i to test for unit root in panel data. The Fisher test is nonparametric and distributed as chi-square with two degrees of freedom:

$$p\lambda = -2\sum log_e \pi_i \quad (3)$$

2.2.2 Panel Cointegration Test

Several test presented to examine the existence of cointegration in panel data. This paper applied panel cointegration test of Pedroni (1999, 2004) and Kao (1999).

Pedroni presented seven statistics for testing the null hypothesis of no cointegration in panel data. Four statistics called panel cointegration statistics and based on pooling along what is commonly referred to as the within-dimension. And other three statistics developed by Pedroni called group-mean panel cointegration statistics, are based on pooling along what is commonly referred to as the between-dimension.

Kao (1999) introduced parametric residual-based panel cointegration. He expanded four DF-types and one ADF-type tests for testing the null hypothesis of no cointegration. The tests are based on the spurious least squares dummy variable (LSDV) panel regression equation with one single regressor.

2.2.3 Granger Causality Test

To investigate the causality relationship between two variables in panel data we can use the following bi-variate vector autoregressive (VAR) model and employing Wald's test:

$$y_{it} = \lambda_i + \sum_{i=1}^{k} \alpha_{ik} y_{it-k} + \sum_{i=1}^{k} \beta_{ik} x_{it-k} + \varepsilon_{it} \quad (4)$$

$$x_{it} = \theta_i + \sum_{i=1}^{k} \gamma_{ik} y_{it-k} + \sum_{i=1}^{k} \delta_{ik} x_{it-k} + \upsilon_{it} \quad (5)$$

Where $i=1, \ldots, N$; $t=1, \ldots, T$; k refers to the lag, and ε_{it} and υ_{it} denote white-noise error terms.

3. Empirical Results

3.1. Panel Unit Root Test

The results of Im et al. (IPS, 2003), Levin et al. (LLC, 2002), Breitung (2000) and Fisher-type panel unit root test of Asian advanced economies and Asian newly industrialized countries presented in table 1 and results of Asian developing countries reported in table 2.

The results of different panel unit root tests indicates that LG is stationary in levels for developing panel while for advanced and newly industrialized countries become stationary after first difference. Furthermore, LGDP for both panels of countries is non-stationary in levels and become stationary after first difference, which means that LGDP is integrated of order one I (1).

3.2. Panel Cointegration Test

Table 3 presents the results of Pedroni panel cointegration tests for both panels of countries. Five statistics of pedroni test support the existence of cointegration between government expenditure and economic growth for advanced and newly industrialized countries. Further, all statistics of Pedroni tests reject the null hypothesis of no cointegration and indicate long-run relationship between LG and LGDP for developing countires.

Table 1. Panel Unit Root Tests – Advanced and Newly Industrialized Countries

Variable	*LG*		*LGDP*	
Test	Levels	1st differences	Levels	1st differences
IPS (2003)	0.512	-9.963 ***	1.002	-10.158 ***
LLC (2002)	1.108	-9.543 ***	-2.675 **	—
Breitung (2000)	-0.486	-3.608 ***	5.017	-4.211 ***
ADF-Fisher	17.433	138.114 ***	28.769	135.299 ***
PP-Fisher	21.291	184.035 ***	33.430 **	—

Note: *** and ** denote statistical significance at the 1 and 5% levels.

Table 2. Panel Unit Root Tests –Developing Countries

Variable	*LG*		*LGDP*	
Test	Levels	1st differences	Levels	1st differences
IPS (2003)	- 5.289 ***	—	3.294	-12.871 ***
LLC (2002)	-3.935 ***	—	3.686	-13.346 ***
Breitung (2000)	-2.711 ***	—	1.819	-5.144 ***
ADF-Fisher	99.170 ***	—	32.990	237.946 ***
PP-Fisher	73.804 ***	—	21.885	346.160 ***

Note: *** denote statistical significance at the 1% levels.

Table 3. Pedroni Panel Cointegration Test

Panel Group	Advanced and Newly Industrialized	Developing
Statistics		
Panel *v*-statistic	2.513 ***	3.200 ***
Panel ρ-statistic	-0.368	-4.684 ***
Panel non-parametric (PP) *t*-statistic	-1.322 *	-5.209 ***
Panel parametric (ADF) *t*-statistic	-3.161 ***	-6.934 ***
Group ρ-statistic	0.616	-2.539 ***
Group non-parametric *t*-statistic	-0.783 *	-4.024 ***
Group parametric *t*-statistic	-2.666 ***	-6.018 ***

Note: ***, ** and * denote statistical significance at the 1, 5 and 10% levels, respectively.

Table 4. Kao Panel Cointegration Test

Panel Group	Advanced and Newly Industrialized	Developing
Statistics		
DF_ρ	-0.739	-7.272 ***
DF_t	-0.807 *	-4.890 ***
DF_ρ^*	-5.282 ***	-17.137 ***
DF_t^*	-1.824 **	-4.904 ***
ADF	-1.899 **	-4.696 ***

Note: ***, ** and * denote statistical significance at the 1, 5 and 10% levels, respectively.

The results of Kao panel cointegration test reported in table 4. All statistics of Kao test except DF_ρ support the existence of cointegration between series for advanced and newly industrialized countries. Also, several statistics of Kao test reveal cointegration relationships between government expenditure and economic growth for developing panels. As seen, the results of Kao panel cointegration test adopt results of Pedroni panel cointegration test.

3.3. Panel Causality Test

As Granger (1969, 1988) points out, if there exists a cointegration between variables, there is causality among these variables at least in one direction. Therefore, to determine the direction of causality a panel-VECM causality which is based on Wald's test applied in this paper.

A bi-variate panel-VECM to examine the causal relationship between government expenditure and economic growth can be written as follows:

$$\Delta LG_{it} = c_{1i} + \sum_{i=1}^{k} \alpha_{1ik} \Delta LG_{it-k} + \sum_{i=1}^{k} \beta_{1ik} \Delta LGDP_{it-k} + \gamma_{1i} ECT_{t-1} + \varepsilon_{it} \quad (6)$$

$$\Delta LGDP_{it} = c_{2i} + \sum_{i=1}^{k} \alpha_{2ik} \Delta LG_{it-k} + \sum_{i=1}^{k} \beta_{2ik} \Delta LGDP_{it-k} + \gamma_{2i} ECT_{t-1} + \upsilon_{it} \quad (7)$$

Where Δ is the first difference operator and ECT_{t-1} is lagged values of the error correction term. The short-run causality from economic growth to government expenditure tested by H_0: $\beta_{1ik} = 0$ for all i and k in Eq. (6). Similarly, the null hypothesis for Eq. (7), is H_0: $\alpha_{2ik} = 0$ for all i and k, which test short-run causality from government expenditure to economic growth. Further, to investigate the long-run causality, the null hypothesis of no long-run causality in each Eq. (6)-(7), is tested by examining the significance of the coefficient of the respective error correction term.

Lag-length selection using Akaike's information criterion (AIC) indicated 3 lags for advanced and newly industrialized panel and four lag for developing panel. The results of panel causality reported in table 5 and 6.

Table 5. Panel Causality Test - Advanced and Newly Industrialized Countries

Dependent variable	Source of causation (independent variables)		
	Short-run		Long-run
	ΔLG	ΔLGDP	ECT
ΔLG	—	2.631 **	- 0.008
ΔLGDP	1.956	—	0.002

Note: ** denote statistical significance at the 5% levels.

The evidence of Panel–VECM causality framework in short-run reveals the unidirectional causality from LGDP to LG for advanced and newly industrialized countries and bidirectional causality between government expenditure and economic growth for developing countries. The results of causality in long-run indicate causality running in both directions for developing countries while for advanced and newly industrialized countries; we cannot find evidence of causality relationship between government expenditure and economic growth in any directions.

Table 6. Panel Causality Test - Developing Countries

Dependent variable	Source of causation (independent variables)		
	Short-run		Long-run
	ΔLG	ΔLGDP	ECT
ΔLG	—	4.316 ***	- 0.055 ***
ΔLGDP	2.423 **	—	-0.013 ***

Note: ***, ** and denote statistical significance at the 1 and 5% levels.

4. Conclusion

There are many empirical studies about the relationship between government expenditure and economic growth, but there are no common consequences between the different studies, so work on this issue is still debatable among economist. Therefore, this paper examined the causal relationship between government expenditure and economic growth in short-run and long-run for two panels of Asian countries; one panel consists of advanced and newly industrialized countries, and another's panel includes developing countries.

The empirical result of panel cointegration test indicates cointegration between government expenditure and economic growth for both panels. The panel-VECM causality framework based on Wald's test performed after investigates cointegration relationship and reveals bidirectional causality for developing countries in short-run and long-run. Furthermore, the empirical finding of panel causality test for advanced and newly industrialized countries indicates unidirectional causality running from economic growth to government expenditure in short-run, and no causality relationship in long-run. These findings can be important for policymakers, because they can conclude some policy implication for the size of government expenditure with respect to these results.

References

1. Agell J, Lindh T, Ohlsson H. Growth and the public sector: A critical review essay. European Journal of Political Economy 1997; 13: 33-52.
2. Akitoby B, Clements B, Gupta S, Inchauste G. Public spending, voracity, and Wagner's law in developing countries. European Journal of Political Economy 2006; 22: 908-924.
3. Baltaghi BH, Econometric Analaysis of Panel Data, Third Edition. John Wiley & Sons, Ltd 2005.
4. Breitung J. The local power of some unit root tests for panel data. Advances in Econometrics 2000; 15: 161–177.
5. Chang T. An econometric test of Wagner's law for six countries based on cointegration and error-correction modelling techniques. Applied Economics 2002; 34: 1157-1169.
6. Chang T, Liuy W, Caudill SB. A re-examination of Wagner's law for ten countries based on cointegration and errorcorrection modelling techniques. Applied Financial Economics 2004; 14: 577-589.
7. Choi I. Unit root tests for panel data. Journal of International Money and Finance 2001; 20: 249-272.
8. Dickey DA, Fuller WA. Distribution of the Estimators for Autoregressive Time Series with a Unit Root. Journal of the American Statistical Association 1979; 74: 427–431.
9. Dickey DA, Fuller WA. Likelihood Ratio Statistics for Autoregressive Time Series with a Unit Root. Econometrica 1981; 49: 1057–1072.
10. Dritsakis N, Adamopoulos A. A causal relationship between government spending and economic development: an empirical examination of the Greek economy. Applied Economics 2004; 36: 457-464.
11. Granger CWJ. Investigating causal relationships by econometric models and cross-spectral methods. Econometrica 1969; 37: 424-438.
12. Granger CWJ. Some recent development in a concept of causality. Journal of Econometrics 1988; 39: 199-211.
13. Gregoriou A, Ghosh S. The impact of government expenditure on growth: empirical evidence from a heterogeneous panel. Bulletin of Economic Research 2009; 61(1): 95-102.
14. Hsiao C. Analysis of Panel Data, Second Edition. Cambridge University Press: Cambridge 2003.
15. Hsieh E, Lai KS. Government spending and economic growth: the G-7 experience. Applied Economics 1994; 26: 535-542.
16. Huang CJ. Government expenditures in China and Taiwan: Do they follow Wagner's law? Journal of Economic Developments 2006; 31(2): 139-148.
17. Im KS, Pesaran MH, Shin Y. Testing for unit roots in heterogeneous panels. Journal of Econometrics 2003; 115: 53-74.
18. Kao C. Spurious regression and residual-based tests for cointegration in panel data. Journal of Econometrics 1999; 90: 1-44.
19. Kolluri B, Panik MJ, Wahab M. Government expenditure and economic growth: evidence from G7 countries. Applied Economics 2000; 32: 1059-1068.
20. Landau D. Government Expenditure and Economic Growth: A Cross-Country Study. Southern Economic Journal 1983; 49(3): 783-792.
21. Levin A, Lin CF. Unit Root Tests in Panel Data: Asymptotic and Finite Sample Properties. Working paper, 1993; University of California, San Diego.
22. Levin A, Lin CF, Chu CSJ. Unit-root test in panel data: asymptotic and finite sample properties. Journal of Econometrics 2002; 108: 1-24.
23. Loizides J, Vamvoukas G. Government expenditure and economic growth: evidence from trivariate causality testing. Journal of Applied Economics 2005; 8(1): 125-152.
24. Lyare SO, Lorde T. Co-integration, causality and Wagner's law: tests for selected Caribbean countries. Applied Economics Letters 2004; 11: 815-825.
25. Maddala GS, Wu S. A comparative study of unit root tests with panel data and a new simple test. Oxford Bulletin of Economics and Statistics 1999; 61: 631-652.
26. Pedroni P. Critical values for cointegration tests in heterogeneous panels with multiple regressors. Oxford Bulletin of Economics and Statistics 1999; 61: 653–678.
27. Pedroni P. Panel cointegration: asymptotic and finite sample properties of pooled time series tests with an application to the PPP hypothesis. Econometric Theory 2004; 20: 597–625
28. Pesaran H, Shian Y, Smith RJ. Testing Approaches to the Analysis of Level Relationships. Journal of Applied Econometrics 2001; 16: 289-326.
29. Samudram M, Nair M, Vaithilingam S. Keynes and Wagner on government expenditures and economic development: the case of a developing economy. Empirical Economics 2009; 36(3): 697-712.
30. Wahab M. Economic growth and government expenditure: evidence from a new test specification. Applied Economics 2004; 36: 2125-2135.
31. WU SY, Tang JH, Lin ES. The impact of government expenditure on economic growth: How sensitive to the level of development? Journal of Policy Modeling 2010; 32: 804-817.

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Study of Anxiety and Style Control Parenting

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Abstract: Strong, desirable and mutual relationship between parents and child is necessary so that nurses can achieve their duties and delivery of care to the child. This relationship should be identified in context of family (Style Control Parenting) in order for the nurses to maintain those parts of family relationship related to nursing duties. Anyhow, the actions should be planned in a manner that be well-adjusted with children' morale and help to decrease child's anxiety. The present descriptive correlation study aims to determine the relationship of behavioral models of parent with their children's anxiety (370 students in age 13 to 15) in 10 governmental girl guidance schools, Dist. 6 of Tehran city. The tools were questionnaires planned for students and parents consisting demographic characteristics, physiologic symptoms of anxiety, "Spiel Berger Anxiety Test" for parents and "Reynolds and Richmond Anxiety Tests" for students. The amount of anxiety among these groups was analyzed using Chi-square test and Fisher exact test in SPSS statistical software. Results showed that the amount of anxiety among students was 33%, with the most anxiety (92%) among adolescents who evaluated their parents' behavior as autocratic (P=0.001), and the least anxiety (7.6%) in adolescents who had democratic parents (P=0.003). These findings shows that there is statistically significant relationship between anxiety and style control parenting (P<0.05), ($df=2$). Style control parenting plays an important role in children's anxiety, therefore, health care providers can be effective in decreasing Children' anxiety by teaching style control parenting .

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Keywords: Style Control Parenting-Anxiety-Girls

Introduction:

Anxiety is considered as an inseparable part of human experience and nature, a mode which is created by stress. During the history, people have encountered many social, economical and technological changes resulted in reactions against stress situations (1). Anxiety is a vague annoyance feeling together with apprehension which has been developed in response to internal and external stimulations and can lead to cognitive, affective, physical and behavioral symptoms (2). Anxiety disorders is one of the most common psychological disorders, as annually, 23 millions persons in U.S.A. are affected and one of four involves in anxiety (3). Women experience anxiety disorders twice more than men (4).

Regarding etiology of anxiety, different causes has been addressed from psychological, biological, ethological, genetic and social-cultural theory points of view (5). Whereas parents deal with their children in different styles, the type of behavior with child can be influenced by cultural issues, their social class and economical sources (6). According to developmental theories of anxiety, acceptance of children by their parents, control style and their behaviors are related with appearing symptoms of anxiety in children (7). Although there are many behaviors in style control parenting, but, three autocratic, non-interventional and democratic models are mostly used (8).

Results of studies show an increase in various types of mental disorders such as anxiety and also demonstrate the effects of style control parenting in developing such disorders. The results of some studies about style control parenting show that the other methods should be found to control and decrease anxiety in children. Strong, desirable and mutual relationship between parents and child is necessary so that nurses can achieve their duties and delivery of care to the child. This relationship should be identified in context of family (Style Control Parenting) in order for the nurses to

maintain those parts of family relationship related to nursing duties. On the other hand, nurses of school can play an important role in decreasing anxiety arising from style control parenting and preventing mental and psychological diseases by acquaintance with morale of children and adolescents and informing parents of developmental needs of their children. Considering this issue, significance of the best kind of behavior of parents with student is distinguished. This study aims to determine relationship between students' anxiety with style control parenting.

Material and Methods:

This research is a descriptive correlation study performed to determine the relationship between students' anxiety with style control parenting in 2005 (1384). The sample consisted of 370 students in third grade of governmental guidance school in Dist. 6 of Tehran city by using 2-stage sampling method. At first, names of all schools in Dis. 6 of Tehran were prepared and ten schools were randomly selected, then, two classes from each school were randomly selected. Students were under guardianship of their main parents and did not have any history of known physical or mental diseases. As anxiety in students could be the result of morbid anxiety of their parents, parents of the sample students should have no physical and mental diseases and morbid anxiety so that the researchers can reach to anxiety resulting from style control parenting, therefore parents were screened by EspillBerger test and were omitted from research in case of any problem. After taking the permission from the school principle, we chose times which student had either no class or exercise classes. Our aim was to have all students in equal condition and without stress. The vital sign were calculated from all participants equally in sitting position. Those with abnormal signs were referred to health care centers for further evaluation. The questionnaires were given to student to fill and one for parents was taken home.

Tools for gathering data in this research consisted of two separate questionnaires relating to parents and students. The first questionnaire included demographic characteristics of parents (9 questions), Diana Brinder test for recognition of style control parenting (which consists of 20 questions related to control parenting and each question had 3 different answers. One was chosen and according to answer we could determine type of parenting), and eschpillberger evident and covert anxiety test (40 questions) based on Likert scale. Achievement of score (20-42) means light anxiety, (43-64) means middle anxiety, and (65-80) means intense anxiety. The second questionnaire consisted of demographic characteristics of students (4 questions), records of physiologic symptoms of students' anxiety (9 questions) (in this research physiologic symptoms means records of blood pressure, pulse, respiration, temperature, symptoms of abdominal pain, nausea, diarrhea, urine iteration, paleness, fever), and Revised Reynolds Richmond test of anxiety (37 questions) in which, achieving score of (0-19) is non-anxiety status and (20-27) shows anxiety. To determine validity and reliability of the style control parenting test, content validity method was used and reliability of which was reached from re-test method in the amount of 0.75. Assessment tools of parent and students' anxiety was standard and enjoyed high level of validity and reliability. Also, to ensure validity of physiologic symptoms, a fixed and same control tools were used and across reliability method was used to ensure reliability of the form for recording vital signs. Analysis of data was performed by using Fisher exact test and Chi-square test in SPSS statistical software.

Results:

In present study, most students (63.2%) were in 14-year age group and 57.3% had self-employed fathers, 60.5% had a desirable economical status and 76.8% were not in menstruation period at the time of research, regarding anxiety of students, results showed that 33% of students under study suffered from anxiety and 67% of them had no anxiety. There was no statistical significant relationship between anxiety and variables of age, birth rank, menstruation, father's age and parent's level of ($P>0.05$), but there was a statistically significant relationship between anxiety and its physiologic symptoms ($P=0.007$), parents' occupation (both mother and father) (P=0.017) and economical status of parents (P=0.016).

Findings of this research about father control style showed that 9.2% of them had non-interventional control style, 23.5% had autocratic control style, and 67.3% of them had democratic control style.(Table No. 1)

Regarding type of mothers' control style showed that 4.3% had non-interventional control style, 19.5%, had autocratic control style and 76.2% had democratic control style (Table No. 1), it means democratic control style is dominant in both groups.(Table No. 2)

Findings about student's anxiety status based on style control parenting shows that, 93.8% of mothers with non-interventional control style, 84.7% of mothers with autocratic control style and 16.3% of mothers with democratic control styles anxious children, it means that in democratic control style of mothers, students had the least anxiety. (Table No. 3)

Chi-square test showed that there is a significant relationship between students' anxiety status and style control parenting ($P<0.001$). ($df=2$)

Also, findings about relationship of students' anxiety status with fathers' control style shows that 92% of fathers with autocratic control style, 67.6% of fathers with non-interventional control style and 7.6% of fathers with democratic control style had anxious children, meaning that the least anxiety of students is related to fathers with democratic control style (Table No. 3). Chi-square test also showed that there is a significant relationship between students' anxiety status and fathers' control style. (P<0.001) (df=2)
(Table No. 3)

Discussion :

Findings shows that the most style control parenting of mothers under study (76.2%) and the most style control

parenting of fathers under study (67.3%) has been of democratic type which is consistent with the results of Izadpoor research showing that mothers and fathers in society prefer democratic education method and pattern to the other two methods(9). Also, findings showed that there was a significant relationship between style control parenting of mothers and students' anxiety status confirming the results of Hudson and Rapee research. In the methods that mothers have more intervention in duties of children and encourage them less, anxiety in their children is high (10).

Considering significance of relationship between students' anxiety status and style control parenting of fathers, results of Jerm and his colleagues' show that fathers have more involvements with their girls and amount of their involvement is effective in anxiety of their children (11).

According to the results, the researchers claim that style control parenting is related to the amount of anxiety of children' anxiety and is effective in its creation. Nurses in schools, considering significance of health of young society and its risks and consequences of anxiety can play an important role in on time preventing and decreasing anxiety resulting from style control parenting. Considering results of this research, it is suggested that the following topics in frame of future researches are emphasized.

According to our results which show that there is relationship between different control parenting and anxiety in students, there is usefulness of data in nursing fields. Nurses are health group which have close relation with families and their education could have great impact on family style. This shows that every school should have nurse present and all college nursing student should be aware and educated in this regard. This itself brings the inclusion of family mental health education as one of the subjects which should be taught in schools. The parents should also be able to contact the school nurse regarding the problems they are facing in their children education.

Conclusion :

Relation between parents' anxiety is assessed with anxiety of children. Also, style control parenting can be studied from viewpoint of anxious students; in addition, considering results of this research can not be generalized throughout of the country, it is suggested that the next researches to be performed with different cultures in different provinces.

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Table No. 1:
Absolute and relative frequency distribution of control style of fathers of Girl Students in Guidance School, 1384 (2005)

Style Control Parenting	Father		Mother	
	Number	Percentage	Number	Percentage
Non-Intervention	34	9.2	16	4.3
Despotic	87	23.5	72	19.5
Democratic	249	76.3	282	76.2
Total	370	100	370	100

Table No. 2:
Absolute and relative frequency distribution and anxiety status of girl students of guidance school based on control style of mothers, 1384 (2005)

Mother Control Style Frequency Students' Anxiety	Non-Intervention		Despotic		Democratic		Total		Test Result
	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	
Not exist	1	6.2	11	15.3	236	83.7	284	67	*x2=149.40 df=2 p<0.001 Meaningful*
Exist	15	93.8	61	84.7	46	16.3	122	33	
Total	16	100	72	100	282	100	370	100	

Table No. 3:
Absolute and relative frequency distribution and anxiety status of girl students of guidance school based on control style of fathers, 1384 (2005)

Father Control Style Frequency Students' Anxiety	Non-Intervention		Despotic		Democratic		Total		Test Result
	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	
Not exist	11	32.4	7	8	230	92.4	248	67	*x2=149.40 df=2 p<0.001 Meaningful*
Exist	23	67.6	80	92	19	7.6	122	33	
Total	34	100	87	100	349	100	370	100	

References:

1- Oton A. Psychiatric nursing Biological and Behavioral concepts,Philadelphia: Saunders Co. 1995 . 192-196

2- Boyd M. Psychiatric nursing contemporary practice. Philadelphia: Lippincoitt; 2002. 454 – 458

3- Verna B, Carson B. Mental health nursing. Philadelphia Saunders Co. 2000.301

4- Dikestin T.Gender differences in mood and anxiety disorders from bench to beside. American Journal of Psychiatry. 2000; 157 (18): 1186-87

5- Varcralis E. Psychiatricnursing Clinical Guide, assessment tools and Diagnosis. ST.Louis: Mosby; 2000.237 – 293

6- Wongs DL, Wally LF. Nursing care of infants and children. ST. Louis: Mosby; 2003

7- Wood J. Parenting and Childhood anxiety: theory, empirical finding and future direction. Journal of child psychology and psychiatry 2003; 44(1) : 134 – 151

8- Whaley LF, Wongs DL. Nursing care of Infantry and Children. Philadelphia: Mosby, 2001.pp.880-901

9- Izadpoor Ali, Study of Methods of Child Education of fathers and mothers whose children use Nursery. M.S. Thesis of Educational Sciences, Welfare & Rehabilitation University, 1380[Persian].

10- Hudson JL, Rapee PM. Parent – child interaction and anxiety disorders: Anobservational study. Behavioral research therapy. 2000; 39(1) 1411- 1427.

11- Jerm AF, Dear KBG, Rodgers B, et al. Interaction between mothers and fathers affection as a risk factor for anxiety and depression symptoms socpsychiatry psychiatry epidemiology. 2002; 38(2): 173-79

12- Mahram Ali. Test Norms of Schpil Berger Anxiety in Mashhad city, M.S. Thesis, Field of Study: Assessment & Measurement in Psychology – Faculty of Psychology, Tabatabaei University of Science, Mashhad, 1373[Persian].

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Application of Trinity Model on the First Aid in Community Residents

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Abstract: Purpose This study explores the effect of the first aid training model containing the Red Cross, universities and community which is used to train community residents. Method 426 residents from a selected community were extracted to be trained by the trinity first aid model. Respectively investigate their grasp of first aid knowledge before and after the training. Result Before training, the community residents' first aid knowledge score is 58.58±16.56 compare to 85.13±18.62 (P<0.01) after training, and the questionnaire score of the five latitude is also increased(P<0.01). About 63.2% of the residents considers that the first aid training is very good , 80.5% of residents consider the training time is appropriate, and 89.4% of residents says that learning first aid knowledge is very helpful to individuals and families.

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Key words: Trinity; First Aid; Community Residents

Introduction

In daily life, people will encounter a variety of emergencies, such as traffic accidents, anthracemia, food poisoning, and other events which can cause cardiac and respiratory arrest. When the accident happens, the family members or witnesses usually will take the injured rushed to hospital. This traditional approach to save the patients often makes the injured who is dying lost the rescued opportunity of 4~6 minutes' prime time. The correct treatment in time plays an important role on the recovery of the injured. However, a survey shows that about 65.7% of community residents in certain areas of China know nothing about CPR, and the level of understanding other first aid knowledge is low too[1-2] .In order to improve the level of the first aid knowledge for community residents, this study explored a new appropriate training model which was called "trinity" between July and August 2011 in Zhengzhou, a city of Central China's Henan province. The training model of "trinity" first aid knowledge combined the advantages of excellent educational resource in university with the Red Cross' cultural characteristics of the life-saving and universities. In this way, the first aid knowledge of community residents is improved effectively, and the research is as follows.

Materials and methods

Participants

There were about four hundred and twenty-six community residents participating in the first aid knowledge and skill training program in the City of Zhengzhou. Questionnaires were given to the residents before the training to appraise the level of the basement. After training the same kind of questionnaires were given to them who attending the training to survey the effect of the training. 426/426 questionnaires were distributed and 426/421 effective were retrieved at last. The effective rate was 100% / 98.8%. The basic informations of the residents were shown in Table 1.

Table 1. Participant (n=426/421)

	Pre-training	After training
Sex ratio	241females(56.6%) 185males(43.4%)	239females(56.8%) 182 males(43.2%)
Mean age (range)	35.12±10.336 (15-68)	34.07±10.327 (15-63)
Occupational	13workers(3.1%), 6 farmers (1.4%), 36 cadres (8.5%), 8 students(1.9%), 244 teachers(57.3%), 63 staff(14.8%), others 56 (13.1%).	13workers(3.1%), 6 farmers (1.4%), 36 cadres (8.6%), 8 students(1.9%), 241 teachers(57.2%), 62 staff (14.7%), others 55 (13.1%).

Methods

The training methods

The "trinity" first aid training model consists of the Red Cross, university and community. The training would last 2 hours each time. The training contents include the basic knowledge of the first aid, such as the principles of the first aid on site , cardiopulmonary resuscitation,the techniques of hemostasis, bandaging, fixation and transport the injured, and the handle knowledge of emergency which takes place commonly at home, such as fishbone card throat, bitten by dogs or cats, heart and brain disease and catastrophic events which need emergency relief immediately such as earthquakes, explosions and so on.

Assessment instruments

The current study presents a mixed method which is quantitative and qualitative way to assess the effect of the training. The quantitative questionnaire was made by researchers, and it includes two parts. The first part is the general demographic information for residents, including gender, age, occupation, etc. The second part is the first aid knowledge questionnaire which consists of 39 items, five dimensions. Each items is measured on a 4-point Likert scale ranging from 1"I know nothing", to 4 "I master it". The same questionnaire would be used after training to evaluate the residents' mastery degree of the first aid knowledge. Another questionnaire which was used to evaluate the training effectiveness was distributed meanwhile. All questionnaires were filled out in the anonymous and self-administered way. The residents who can't fill out the questionnaire by himself would be read and explained by the investigator. They would choose the right answers, according to the reaction of residents.

Semi-structured interviews were hold on with residents who participated in the training. After training, community workers and residents will receive on-site interviews. The semi-structure interview outline was developed by the Nursing College of Zhengzhou University. The interview Materials would be recorded and sorted by special man.

Quality Control

In this study, the model was demonstrated repeatedly before the training. Pretest was carried out twice to improve the training program and to evaluate the adaptation of the questionnaire. In order to ensure the consistency of the raining content , the assessors, teachers and investigators would get training in advance. On-site supervision was used to control the quality of training, and the questionnaires were recovered at the site.

Data collection and analysis

The Statistical software SPSS13.0 was used to analyse data. In order to reduce data entry errors, data was entered to the software by two persons, and the questionnaire which was unqualified would be eliminated in time. Count data was described by rate / percentage, and measurement data was described by mean / standard deviation. The differences between pre-training and after training were checked with Paired T test.

Results

The comparison of mastery degree for first aid knowledge before and after the training

After training the total score of the Residents' first aid knowledge had a significant increase. There was significant difference compared to pre-training.($P < 0.01$) (Table 2). There was significant difference for the score of every dimensions compared to pre-training ($P < 0.01$) (see Table 3).

Table 2. The Comparison of the total scores of the first aid knowledge to the residents in the city of Zhengzhou ($\overline{X} \pm S$)

	Score	t	P
Pre-training	56.58±14.56	23.09	<0.01
After raining	84.99±18.62		

Table 3. The Comparison of the scores of the different first aid knowledge to the residents in the city of Zhengzhou ($\overline{X} \pm S$)

Training Contents	Pre-training	After training	t	P
Basic Knowledge	8.35±2.10	12.42±3.37	13.89	<0.01
CPR Knowledge	5.74±1.98	8.29±2.08	10.67	<0.01
Wound management	9.87±2.96	14.23±3.51	15.76	<0.01
Home first-aid Knowledge	18.68±3.12	26.08±6.58	16.25	<0.01
Catastrophe management	13.46±4.99	19.20±5.03	15.98	<0.01

The residents' view of the training

The overall views of the community residents who participate in the training activities are as follows. 63.2% of the residents thought the training program was best, 19.7% of residents considered better, and 13.6% of residents thought it general; 3.8% of the residents thought that the training time is too long, 80.5% thought it was appropriate, while 15.1% residents believed that too short; In addition, 89.4% of residents considered that learn first aid knowledge was helpful to himself and families and 9.6% considered with a little help, only 0.9% considered no help.

Qualitative evaluation

After training, we had a conversation with the community workers. They were satisfied with the training. They thought that the form of training was standardized and innovative, the contents were very enriched and practical. The teacher could integrate theory with practice which made the contents were

more easy to understand. The teachers' attitude was serious and responsible and they had higher level for teaching. Many residents of the community said they had never took part in first aid training like this before, so they had higher enthusiasm for the training. Some training contents were very important to their daily life, such as cardiopulmonary resuscitation. So they look forward to the next deeper level training, for that when his family member or others get injured they could do something correct before the emergency staff reached. If they could do that the rate of the occurrence for undesirable consequences may be reduced.

Discussion

The feasibility of first aid training

The first aid is an important part for emergency defense function for every city, and it is also a sign of the degree of social civilization . There are some successful emergency trainings which are widely applied to help the community residents abroad, and the training content and depth are professional[3]. Judging from the situation of the training site, the knowledge of first aid training are very popular with community residents in our city, and this may be related to the development of our economy, society culture, and the knowledge of first residents. The general requirements of the knowledge for residents is increasing, so the requirements of safety is more and more important.

Training effect

The improvement of first aid knowledge for community is depended on the high quality training system. The level of the residents' knowledge for first aid is improved significantly after the training which is named "trinity" model to the community(Table 2). Compared to pre-training the mastery of first aid knowledge is increased significantly. All those who take part in the training program expressed (Table3). 68% of the residents consider that the difficulty of the training is moderate, and the content set is reasonable. In addition, the teachers who are involved in this training activities have abundant clinical and teaching experience, could master the degree of difficulty and take full advantage of the teaching skill.

The training model is reasonable

The Red Cross is the organizer of the first aid scene, the main force to carry out the general education. It rely mainly on social donations, state subsidies and other forms to raise funds to work, which have double difficult issues on implementation and funding. The community health care workers are rooted in the community, and the main force of the medical knowledge education, but studies have shown that 92% of community health care workers have not received first aid training [4]. They need first aid training for the reasons of lacking clinical experience, theoretical knowledge and knowledge aging. So they lack the ability of taking up first aid training for community residents. For this training program, which is organized by the Red Cross, based on community, the colleges and excellent teachers. The team had better basic training and scientific research funds which can support the emergency training work. For this model, we can make full use of the existing emergency network, and it can not only compensate for the shortage of community staff in first aid knowledge and skills, but also can carry forward the spirit of the Red Cross' life-saving. In this way the first aid knowledge could be transited to the community residents effectively.

Training should be strengthened

After training the mastery degree of the knowledge for community residents has greatly improved, but some of the contents still need to be improved, for example the cardiopulmonary resuscitation. Cardiopulmonary resuscitation is an important skill of first aid which every citizen should master it. Residents said that cardiopulmonary resuscitation played an important role on the emergency treatment for the man who was injured. They also look forward to the next training because they can't seize the core essentials of the technology only by one training. A great deal of specialized research had been carried out to find out the best training method and the effect of long-term application by foreign researchers[5-6]. Because of time constraints, this training program was carried out only once, and the survey of the master degree the training was completed only once too. We could not detect the long-term effects, however oblivion takes place immediately after learning [7]. The oblivion will become more prominent to community residents who had no medical knowledge background. Therefore, how to guarantee the long-term effects of first aid knowledge and skills will become the next research focus.

Summary

With the continued development of the economy, the rhythm of people's daily life becomes faster and faster, the requirements of safety is also increasing rapidly meanwhile. Because of the convenient and efficient transport in our daily life, it is not the responsibility of an individual or a hospital to protect the individual safely, your family safely, and improve the safety of whole society. It is a dynamic, continuing acts of individuals, families and society, and it is the responsibility of the whole society. Time is life, if the first witnesses of the scene can choose the effective first aid for the injured, the pain and

further damage of the injured will be reduced, even the further diagnosis and treatment chance will be created. Therefore, exploring effective training methods and content for community residents is a strong guarantee for improving the efficiency of first aid , adopting effective on-site first aid treatment, and improving the success rate. It lays a certain foundation for exploring a long-term oriented and systematic training by this first aid training.

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References

1. Zhang Yan, Zhang Zhengxiang, Liu Lamei. Surveying the knowledge of first aid on field of 592 of citizens in Zhengzhou[J].Chinese Health Service Management, 2011,(2):158-159.
2. Zou Xiaoping, Qin Hong. Report on residents' attitude,knowledge and action towards emergency treatment on sport in community of Wuxi city [J]. Journal of Preventive Medicine , 2006,33 (12) :2404-2405.
3. Okudera H, Wakasugi M. Immediate Cardiac Life Support(ICLS) course developed by Japanese Association for Acute Medicine[J]. NihonRinsho, 2011,69(4):684-690.
4. Liao Quanquan,Ye Liangyu, Zhang Youhui,et al. A survey of first aid training require ment of medical and nursing staffs in community health service centers of Bao'an district of Shenzhen city [J].Nursing Research, 2008, 22(11A) : 2904-2906.
5. Okudera H, Wakasugi M.Immediate Cardiac Life Support(ICLS)cours developede by Japanese Association for Acute Medicine[J]. NihonRinsho, 2011, 69(4):684-690.
6. Wik L, Myklebust H, Auestad B H, et a1.Twelve-month Retention of CPR Skills with Automatic Correcting Verbal Feedback[J]. Resuscitation, 2005, 66(1):27-30.
7. Zhang Guozhong.The exploration and application of the forgotten law[J]. Continuing Education, 2002, (6) :86-88.

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The Relationship between Perfectionism and Depression with Academic Achievement among the Students of Ilam University of Medical Sciences

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Abstract: Introduction- Perfectionism is an irrational or illogic belief that people have about themselves and their surroundings. Depression is one of the results of extreme perfectionism. Perfectionism and depression are of the factors influencing on academic progress. The purpose of this study was to determine the relationship between perfectionism and depression with academic achievement in the students of Ilam University of Medical Sciences. Methodology-200 students of Ilam University of Medical Sciences were enrolled in this study by classification sampling method. Data collection was performed using Frost Multi-Dimensional Perfectionism Scale (FMPS) and Beck Depression Questionnaire. The questionnaire reliability was confirmed by Alpha coefficient of 87%. The FMPS questionnaire has six sub-scales. The collected data were analyzed using the SPSS16 software and Mann-Whitney and Kruskal-Wallis tests. Findings- There was no significant relationship between gender, educational field, semester and faculty with depression, while a significant relationship was seen between the sex and some of the sub-scales of perfectionism ($P < 0.01$). There was a significant and negative correlation between the depression ($P = 0.04$, $r = -0.193$) and the parental criticism ($P = 0.000$, $r = 0.346$). Conclusion-Depression and parental criticism were the effective factors on academic achievement.

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Keywords: Perfectionism, Depression, Academic Achievement, Students, Ilam

Introduction

Perfectionism in fact is an irrational or illogic belief that people have towards themselves and their surroundings. The perfectionist people believe that they and their surroundings have to be perfect and any attempt in life must be without error and mistake. The perfectionism dimensions should be added to the research components, the dimensions that represent the inner-personal, inter-personal and social multi- aspects nature of perfectionism (1). Hewitt and Flett have distinguished three dimensions, namely, self-oriented perfectionism, other-oriented perfectionism and community-oriented perfectionism (2). The self-oriented perfectionism is associated with a tendency to establish unrealistic measures for itself and focusing on imperfections and failures in performance and is specified with precise self-controlling. The other-oriented perfectionism tend to have extreme expectations from others and critical evaluation of them. The community-oriented perfectionism is applied to feel the necessity to observe the criteria and realization of expectations prescribed by the important people in order to obtaining approval. The characteristics of perfectionist individuals can be mentioned as ambition, fear of making mistake, fear of disapproval by others, all or nothing thinking, frustration and finally depression. Depression occurs in these people since they consider themselves as an incompetent person, and when they fail to achieve their goals, they become depressed and disappointed. Depression is a serious and common disease that is specified with obvious disorder in the mood. The prevalence rate of depression has also been reported up to 25% (3). Depression can be chronic and recurrent and leads to the loss of social, personal and career functionality (4), (5), (6) (7) . Since the perfectionism and depression can be of influencing factors on academic achievement, thus, the aim of this research is to study the relationship of perfectionism and depression with academic achievement in the students of Ilam University of Medical Sciences.

Materials & methods

This study is carried out based on evaluation of the students of Ilam University of Medical Sciences employing 200 students of both genders using the clustering sampling method. The research tool was a three-part questionnaire, including the demographic questions as the first part, the Frost multidimensional perfectionism questionnaire (EMPS) as the second part and the Beck standard questionnaire as the third part. The demographic questions include questions about gender, educational field, faculty of education, semester, previous semester grade and the total average of the previous semesters. The Frost multidimensional perfectionism scale has been developed in order to assess different dimensions of perfectionism. This test consists of 35 statements and 6 sub-scales, including concerning about mistakes or errors, hesitation than to actions, parental expectations, parental criticism, individual standards and discipline. The overall perfectionism score is obtained by summing the scores of all the 35 statements of the test. To obtain the score of each sub-scale, the scores of all statements related to the studied sub-scale should be summed; a high score indicates the excessive perfectionism of the individuals in the studied area. High scores on this test indicate a high perfectionism of the person in the studied area. The method of grading the options in this test is as follows: Completely Disagree = 1; Disagree = 2; 3 = No Comment; Agree = 4; Completely Agree = 5. (8) have been reported the internal consistency coefficient of the test sub-scales between 73% to 93% and the total internal consistency coefficient of the test as 90% (9), (10). The reliability coefficient in the present study was as 87%. The Beck-2 depression questionnaire (BDI-||) includes 21 questions that measures physical, behavioral and cognitive symptoms of depression. Each question has four options that are respectively graded from 0 to 3 scores and determines different degrees of depression from mild to severe. The maximum score on this questionnaire is equal to 63 and its minimum is zero. The validity and reliability of this test has been proven in various studies (10). A high score indicates more severe depression.

The questionnaires were provided for research samples by the researcher, and after giving them the necessary explanations about the objectives of the study, if they were

announcing their consent to participate in the study, they would be asked to return the questionnaires to the researcher after completion. From a total of 200 questionnaires that was distributed at the first stage, 176 questionnaires were returned to the researcher. Thus, the responding percentage was 88%. 14 questionnaires were also excluded due not-completing the first, second or third section. Finally, 48 other questionnaires were prepared and made available for the students who have not attended in the first stage. After completion of these questionnaires and end of inquiry, the necessary data was extracted from the questionnaires and entered into SPSS software database.

Data analysis was performed using SPSS19 software. Data normalizing was investigated using Kolmogorov – Smirnov test. Normal data was analyzed using parametric tests and non-normal data was analyzed using non-parametric tests. Based on this test, the scores of the following were not normally distributed: the depression score ($P < 0.007$), the sub-scales of concern about errors ($P < 0.01$), hesitation than to actions ($P < 0.01$) and discipline ($P < 0.002$).

Research findings

Of a total of 200 studied students, 98 subjects (49%) were female and 32 subjects (16%) were medical students. 53 students (5/26%) were at third semester and 87 subjects (5/43%) were paramedical college students. The Grade Point Average (GPA) of the previous semester and the overall GPA of last semesters of the studied students were 16.2± 1.4 and 16.0± 1.3, respectively. Demographic characteristics of the studied students are given in Table 1.

The mean depression score was 14.65 ± 12.41and the depression distribution based on severity were as follows: more than half of the samples (54%) with minor depression, 15.5% with mild depression, 16.5% with moderate depression and 14.5% with severe depression. The average of perfectionism overall score was 18.2 ± 2.9 and the scores about its sub-scales were as the following: concerning about the mistakes (2.6 ± 0.6), hesitation than to actions (2.9 ± 0.6), parental expectations (3.1 ± 0.7), parental criticism (2.5 ± 0.7), individual standards (3.3 ± 0.6) and discipline (3.6 ± 0.8). There was no significant relationship between depression score and sex, educational field and educational year. There was a significant relationship between sex and perfectionism overall score (CI 95%, 0.19-1.8, P =0.01). There were also significant relationships between sub-scales of perfectionism, including parents expectations (CI 95%, 0.12-0.54, P =0.002), individual standard (CI 95%, 0.04-0.38, P =0.01) and discipline (P =0.001) with sex. No significant relationship was observed between the educational filed and academic year with perfectionism subscales.

A negative and significant correlation was observed between depression score and the GPA of the last semester (r =-0.193, P =0.04) and the overall GPA of the previous semesters (r =-0.197, P =0.04) of the studied students. There was a negative correlation between the total score of perfectionism and the GPA of the last semester (r =-0.150, P =0.12) and the total GPA of the previous semesters (r =-0.028, P =0.78) of

the studied students, but it was not significant. There was a negative and significant correlation between the parental criticism subscale and the GPA of the last semester (r =-0.346, P =0.000) and the total GPA of the previous semesters (r =-0.255, P =0.01) of the studied students. There was a positive correlation between the sub-scales of hesitation than to actions and discipline and academic achievement, but this correlation was weak and was not statistically significant. There was a positive but weak correlation between depression and perfectionism and also it was not statistically significant. There was a positive, strong and significant correlation between the depression and concerning about the mistakes (P =0.004, r =0.201). Except for the individual standards and discipline, there was a positive correlation between the depression and other sub-scales of perfectionism. There was a significant relationship between different degrees of depression and concerning about the mistakes based on Kruskal – Wallis test, so that the students who were suffering from severe depression had a higher average scale score in the sub-scale of concerning about the mistakes (P =0.05). There was a significant and inverse relationship between parental criticism and depression with academic achievement and also a significant difference between sex and perfectionism. The mean depression score was 14.65± 12.41, and 14.5% of the students were suffering from severe depression based on Beck depression questionnaire (11), (12). There was a significant relationship between the depression and previous semester GPA and the total GPA of last semesters, so that, with increasing the depression score, the students' GPA was declined.

Discussion and Conclusion

A relationship between each of the two aspects of perfectionism (positive and negative perfectionism) with depression and stress was also shown by Afshari et al. that, so that the positive perfectionism is considered as a protective factor to prevent depression and stress in a person (13) but the negative or extreme perfectionism, itself, causes depression and stress in a person. There was a negative correlation between perfectionism score with the last semester GPA and the total GPA of previous semesters of studied students, but not significant. There was a negative and significant correlation between the parental criticism subscale with the last semester GPA and the total GPA of previous semesters of studied students. Parents' over-criticism of children not only causes loss of their academic achievement, but also can cause depression and loss of academic in them (13), (14).

Hamacheck in a research to review the issue that personality traits are related to perfectionism have noted that the positive perfectionism is associated with the sense of duty. People who expect to be in full from themselves, tend to perfectionism due to individual differences that one of its reason includes family factors, including non-critical parents or sometimes critical, some times strict (15). Given that individual characters began to form in the family, the stresses and depressions due to extreme perfectionism and unrealistic expectations can be reduced by giving awareness to the parents in children's educational areas as well as notifying them in the areas of perfectionism and its positive and negative aspects in social life of the person. Reducing the stress of extreme perfectionism and the resulted depression, its negative effects on individual' academic achievement can be prevented.

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References:

1. Sherry SB, Vriend JL, Hewitt PL, Sherry DL, Flett GL, Wardrop AA. Perfectionism dimensions, appearance schemas, and body image disturbance in community members and university students. Body Image. 2009 Mar;6(2):83-9.
2. Hewitt PL, Flett GL. Perfectionism in the self and social contexts: conceptualization, assessment, and association with psychopathology. J Pers Soc Psychol. 1991 Mar;60(3):456-70.
3. Sadock BJ, Sadock VA. Kaplan and Sadock's synopsis of psychiatry : behavioral sciences/clinical psychiatry. 9 ed. Philadelphia PA , ... Lippincott Williams & Wilkins; 2003.
4. Castro JR, Rice KG. Perfectionism and ethnicity: implications for depressive symptoms and self-reported academic achievement. Cultur Divers Ethnic Minor Psychol. 2003 Feb;9(1):64-78.
5. Kenney-Benson GA, Pomerantz EM. The role of mothers' use of control in children's perfectionism: implications for the development of children's depressive symptoms. J Pers. 2005 Feb;73(1):23-46.
6. Davis B, Sheeber L, Hops H, Tildesley E. Adolescent responses to depressive parental behaviors in problem-solving interactions: implications for depressive symptoms. J Abnorm Child Psychol. 2000 Oct;28(5):451-65.
7. Sadock BJ, Sadock VJ. Kaplan and Sadock's Pocket Handbook of Clinical Psychiatry. 4 ed: Lippincott Williams & Wilkins; Fourth edition; 2005.

8. Frost RO, Heimberg RG, Holt CS, Mattia JI, Neubauer AL. A comparison of two measures of perfectionism. Personality & Individual Differences. 1993;14 119-26.
9. Purdon C, Antony MM, Swinson RP. Psychometric properties of the frost multidimensional perfectionism scale in a clinical anxiety disorders sample. J Clin Psychol. 1999 Oct;55(10):1271-86.
10. Gelabert E, Garcia-Esteve L, Martin-Santos R, Gutierrez F, Torres A, Subira S. Psychometric properties of the Spanish version of the Frost Multidimensional Perfectionism Scale in women. Psicothema. 2011 Feb;23(1):133-9.
11. Joe S, Woolley ME, Brown GK, Ghahramanlou-Holloway M, Beck AT. Psychometric properties of the Beck Depression Inventory-II in low-income, African American suicide attempters. J Pers Assess. 2008 Sep;90(5):521-3.
12. Winter LB, Steer RA, Jones-Hicks L, Beck AT. Screening for major depression disorders in adolescent medical outpatients with the Beck Depression Inventory for Primary Care. J Adolesc Health. 1999 Jun;24(6):389-94.
13. Afshar H, Roohafza H, Sadeghi M, Saadaty A, Salehi M, Motamedi M, et al. Positive and negative perfectionism and their relationship with anxiety and depression in Iranian school students. J Res Med Sci. 2011;16(1):79-86.
14. Ferrari JR, Mautz WT. Predicting perfectionism: applying tests of rigidity. J Clin Psychol. 1997 Jan;53(1):1-6.
15. Davis C. Normal and neurotic perfectionism in eating disorders: an interactive model. Int J Eat Disord. 1997 Dec;22(4):421-6.

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The importance of lifelong education

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Abstract: Lifelong learning is the continuous building of skills and knowledge throughout the life of an individual. It occurs through experiences encountered in the course of a lifetime. These experiences could be formal (training, counseling, tutoring, mentorship, apprenticeship, higher education, etc.) or informal (experiences, situations, etc.) Lifelong learning, also known as LLL, is the "lifelong, voluntary, and self-motivated" pursuit of knowledge for either personal or professional reasons. As such, it not only enhances social inclusion, active citizenship and personal development, but also competitiveness and employability. Adult learning is any 'post-compulsory' learning. Adult learning is not just about formal qualifications but includes all forms of skill development activities including formal college education, job related and workplace training, informal and community education, skills updating and refreshing, or general self improvement. In the context of demographic and technological change and globalisation, an educated and skilled population represents one of the strongest foundations for ensuring a strong and healthy economy and a socially inclusive society.adult who is able to recognize their needs.
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Keywords: lifelong education, learning

Introduction:

The most serious challenge will be to motivate low-skilled, under-educated adults within the working age population to seek further education. Simply expanding the number of providers and programs will not necessarily increase demand from the populations and communities where the needs are greatest. Deepseated social, economic and cultural barriers—many dating back generations—lead people to undervalue education. In addition, in many counties it is difficult for people to see a direct relationship between better education and better-paying jobs. Either there are no jobs available or many existing employers do little to emphasize the connection between better education and the possibilities for getting a job, keeping a job, or earning a higher wage. For many, getting more education and earning a high school diploma or a college degree has little positive meaning.

Only the negative consequences are obvious: getting more education often means leaving one's family and community for jobs and opportunities for advancement somewhere else. The future of Kentucky depends on uplifting the quality of life and economy of all of Kentucky. The social and economic costs of neglect of large parts of the state will drag down the rest of the state and seriously hinder its capacity to compete in the global economy.

Much like strategies to curb epidemic, strategies to reduce illiteracy and raise the educational attainment of Kentucky's population must include both short-term efforts to face the immediate crises as well as long-term strategies to get at the underlying causes. Short-term crises include the imperative to keep helping welfare clients make the transition from welfare to work within the constraints of federal and state mandates and the need to train workers for immediate employer demands. Long-term prevention must address the underlying, persistent problems of the state's economic structure as well as the low awareness--if not appreciation--among segments of the population of the vital connection among education, employment, and improved standards of living.

Stages of Lifelong Learning

Lifelong education means education resulting from integration of formal, non-formal, and informal education so as to create ability for continuous lifelong development of quality of life. Learning is therefore part of life which takes place at all times and in all places. It is a continuous lifelong process, going on from birth to the end of our life, beginning with learning from families, communities, schools, religious institutions, workplaces, etc. The African traditional society envisioned lifelong learning by the roles one was expected to play in society from child, youth (boy or girl), young adult, junior elder to senior elder. Today with less defined changes in life roles there is need for new strategies to motivate lifelong learning.

Age 0-5 years

In this age group, a lot of learning takes place, providing a foundation for future learning habits and resourcefulness. This is probably the age group where the greatest amount of informal learning occurs, as children imitate almost everything from parents, peers and their environment. Psychologists such as Sigmund Freud and other behavioral psychologists emphasize the importance of childhood learning. Learning in this stage

affects the development of all other learning abilities later in life.

Learning in the 6-24 age group

Learning in the 6 – 24 age group primarily takes place in educational institutions, from primary and secondary to tertiary levels. Family life, social organizations, religious institutions, and mass media can also play a role in non-formal and informal learning during this time. The objective of learning in this period is the holistic development of learners in four aspects, namely: physical, intellectual, social capacity, emotional and mental development

Learning in the 25-60 age group

Individuals in the 25 – 60 age group can learn informally during their working lives through the use of instructional media, mostly from their occupations, work-places, colleagues, touring, mass media, information technologies, environment and nature. Adults learn from experiences and problem solving. They therefore need continuous development of intellect, capability and integrity.

Learning in the 60+ age group

In their senior years people may seek new knowledge for their own sake. This could be termed autoandragogy, from pedagogy, an adult who tutors him/herself. The challenge of seeking new knowledge and teaching themselves may result in a sense of pride of accomplishment and help maintain self-esteem. This may be further enhanced by offering their new knowledge in service to their local community, thus continuing to make valued contributions to society.

Concept of adult education:

Several definitions of adult education has been done Community

- Adult Education is a⎫in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age⎫ limits as formal and informal, voluntary and at different levels of time, place
- Adult Education is a process in which people who⎫and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after⎫ school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

Issues Beyond the Department of Adult Education and Literacy

Beyond the issues relating directly to DAEL (Department of Adult Education and Literacy), the task force heard a number of concerns about the Commonwealth's overall approach to adult literacy.

- Lack of coherent statewide leadership and coordination among multiple complementary initiatives aimed at the same problem.
- Lack of continuity in state leadership. Cited in particular was the difficulty sustaining a high level commitment to the issue long enough to make a difference because of changes in priorities of the state's political leaders. A high level of turnover in the leadership of the Department of Adult Education and Literacy has also contributed to the instability.
- Tendency to think of adult education as a separate categorical program rather than a strategy that cuts across the mission and responsibility of multiple Commonwealth programs and initiatives (e.g., early childhood education, welfare reform, economic development, and corrections).
- Multiple uncoordinated categorical federal initiatives that tend to drive (and fragment) policy for an overall state effort that is largely funded by Kentucky.
- A tendency to commingle and confuse different functions. The most important distinction is between functions focused on the needs of clients (adult learners, employers, communities, regions, and the Commonwealth as a whole) and functions associated with the operations and performance of providers. It is important that each of these functions receive attention, yet the tendency is for one (e.g., overseeing a network of providers) to drive out attention to overall system strategy.
- Inadequate coordination of services to meet the needs of individual adults, communities, employers, and regions is hindered by:

- Vertical financing and regulatory relationships between separate federal and state programs and local providers and administrative units. These vertical relationships can hinder the horizontal coordination of services for individual adult learners, communities, and employers.
- Turf wars among providers, local politics, and long-standing conflicts among neighboring counties.

- Inadequate links with and leverage of other public and private initiatives and investments to reach the target population. Major sources of

help include employers, postsecondary education, and workforce development.

- Lack of a state financing policy and strategy for provider performance incentives and collaboration, and tax and other employer incentives for leverage of non-state resources.
- Lack of programmatic and administrative flexibility to meet the rapidly changing needs of adult learners, employers, regional economies, and communities.

Conclusion:

Material often set different types of materials and educational content in books and pamphlets, books, training guides, trainers, equipment auxiliary audio, visual and material are included such that during actual teaching sessions, are used in the transmission and content but also to achieve the goals of making education programs are important.

Additional material for the next stage of learning often means to be expected when developing your learning skills Learners to increase awareness and enjoyment of reading and studying to operate.

To improve the quality of life, learning materials should reinforce the skills they acquired previous. This material should have access to information and provide new technology. should also have to make learning more fun. Additional materials should provide opportunities for literacy skills to read and to strengthen their cognitive awareness.

Track materials (continued) which increased literacy skills and knowledge gained is also effective in enriching learning environment for learners are important. Participatory materials to ensure the participation of learners in the learning process and codification are included out of class activities, dialogue, role playing, etc.

In traditional programs that the principles of psychology and curriculum planning, less attention is the form of content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided Affect the selection of pictures and images related to the concepts and content produced by including them.

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

1- Preparation for adults to learn how much he depends on previous learning. Knowledge that has accumulated because of an ability to absorb new information more person is. Past educational experience features a diverse group of adult learners, the starting point of any activity on the diversity training is emphasized.

2- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable

3- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.

4- To maximize learning, information must be provided an organized manner. Entries can be simple or complex can be arranged around related concepts are organized. Starting point for organizing content knowledge for adults and adults is linked to past experiences

Reference:

1. Cranton, P. (1992). Working with Adult Learners. Toronto: Wall & Emerson.
2. Cranton, P. (1996). Professional Development as Transformative Learning. San Francisco: Jossey- Bass.
3. Creighton S. (2000). Participation trends and patterns in adult education: 1991-1999. United States: National Center for Education Statistics.
4. Egan, K. (1992). Imagination in Teaching and Learning. Chicago: University of Chicago Press.
5. Fabry, D. L.,&Higgs, J. R. (1997). Barriers to the effective use of technology in education: Current status. Journal of Educational Computing Research, 17(4), 385-395.
6. Frye, N. (1993). The Educated Imagination. Toronto: Canadian Broadcasting Corporation.
7. Ginsburg, L. (1998). Integrating technology into adult learning. In C. Hopey (Ed.), Technology, basic skills, and adult education: Getting ready and moving forward (Information Series No. 372, pp. 37- 45). Columbus, OH: Center on Education and Training for Employment. (ERIC Document Reproduction Service No. ED 423 420).
8. Ginsburg, L.,&Elmore, J. (2000). Captured wisdom: Integrating technology into adult literacy instruction. Naperville, IL: North Central Regional Education Laboratory. (ERIC Document Reproduction Service No. ED 454 408).
9. Glenn, A. D. (1997). Technology and the continuing education of classroom teachers. Peabody Journal of Education, 72(1), 122-128.

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The Effect Of Cognitive-Behavioral Couneseling On The Level Of Anxiety In Woman With Sexual Dysfunction

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Abstract: Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral components. It is the displeasing feeling of fear and concern. The root meaning of the word anxiety is 'to vex or trouble'; in either presence or absence of psychological stress, anxiety can create feelings of fear, worry, uneasiness, and dread. The prevalence of female sexual dysfunction is high and it may significantly affect self-esteem and quality of life. Even sexual dysfunction of short duration can create frustration and anguish. When chronic, it may lead to anxiety and depression, harm relationships, and cause problems in other aspects of life. The goal of the present research is to study the effect of cognitive-behavioral counseling on the level of anxiety in women with sexual dysfunction. Method and materials for this research study are Cognitive behavior therapy (CBT) that focused on cognitive restructuring, modification of cognitive distortions and training of behavioral techniques. Data analysis showed that the cognitive behavior therapy has significantly effect on reduction of anxiety. Cognitive counseling as a therapeutic method can have a significant role in improvement of women suffering from anxiety which is resulted from sexual dysfunction.
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Keywords: Sexual Dysfunction, Anxiety, Cognitive Behavior Counseling, Women

1. Introduction

Anxiety is a generalized mood condition that can occur without an identifiable triggering stimulus. As such, it is distinguished from fear, which is an appropriate cognitive and emotional response to a perceived threat. Additionally, fear is related to the specific behaviors of escape and avoidance, whereas anxiety is related to situations perceived as uncontrollable or unavoidable. (Ohman2000) Another view defines anxiety as "a future-oriented mood state in which one is ready or prepared to attempt to cope with upcoming negative events" (Barlow, 2002), suggesting that it is a distinction between future and present dangers which divides anxiety and fear. In a 2011 review of the literature, (Sylvers, 20011) fear and anxiety were said to be differentiated in four domains: (1) duration of emotional experience, (2) temporal focus, (3) specificity of the threat, and (4) motivated direction. Fear was defined as short lived, present focused, geared towards a specific threat, and facilitating escape from threat; while anxiety was defined as long acting, future focused, broadly focused towards a diffuse threat, and promoting caution while approaching a potential threat.

The **physical effects** of anxiety may include heart palpitations, tachycardia, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, stomach aches, or headaches. As the body prepares to deal with a threat, blood pressure, heart rate, perspiration, blood flow to the major muscle groups are increased, while immune and digestive functions are inhibited (the *fight or flight* response). External signs of anxiety may include pallor, sweating, trembling, and papillary dilation. Someone who has anxiety might also experience it subjectively as a sense of dread or panic. Although panic attacks are not experienced by every person who has anxiety, they are a common symptom. Panic attacks usually come without warning and although the fear is generally irrational, the subjective perception of danger is very real. A person experiencing a panic attack will often feel as if he or she is about to die or lose consciousness.

The **emotional effects** of anxiety may include "feelings of apprehension or dread, trouble concentrating, feeling tense or jumpy, anticipating the worst, irritability, restlessness, watching (and waiting) for signs (and occurrences) of danger, and, feeling like your mind's gone blank" as well as "nightmares/bad dreams, obsessions about sensations, deja vu, a trapped in your mind feeling, and feeling like everything is scary." (Smith , 2008)

The behavioral effects of anxiety may include withdrawal from situations which have provoked anxiety in the past. Anxiety can also be experienced

in ways which include changes in sleeping patterns, nervous habits, and increased motor tension like foot tapping. (Barker, 2007)

Female sexual dysfunction (FSD) is defined as persistent or recurring decrease in sexual desire , persistent or recurring decrease in sexual arousal , dyspareunia and a difficulty in or inability to achieve an orgasm (Basson et al, 2000). The prevalence of female sexual dysfunction is high, ranging from 43% to 88% .(Dennerstein , 2002) It may significantly affect self-esteem and quality of life. Even sexual dysfunction of short duration can create frustration and anguish. When chronic, it may lead to anxiety and depression, harm relationships, and cause problems in other aspects of life.(Dennerstein, 2005) Several factors, including interpersonal ,psychological, physiological, medical, social and cultural variables, have been shown to correlate with sexual dysfunctions (Basson, 2005) Anxiety disorders can preclude women's ability to attend to sexual stimuli and to be lost in the moment (Meston, 2004). The anxiety resulting from sexual functioning put people in trouble psychologically. Instead of focusing on sexual arousal stimuli, one involves in a sense of anxiety concerning sexual functioning (Adams, 1985).The results of a comprehensive research by Holvorsen & Metz on various methods of treating sexual disorders showed that the most prevalent methods for psychopathic treatment of sexual malfunctioning that have been in practice from 1996 onward include sensory focus, CBT (Cognitive Behavior Therapy), relaxation practice, hypnosis, and group therapy; the results also showed the above-mentioned treatments have achieved considerable results in improvement of different sexual disorders like sexual idiosyncrasy in women (Kabakci, 2003). Cognitive behavior therapy focuses on decreasing anxiety and promoting changes in attitudes and sexual thoughts, which increase the ability to achieve orgasm and to gain satisfaction from orgasm (Soykan, 2005).The goal of the present research is to study the effect of cognitive-behavioral counseling on the level of anxiety in women with sexual dysfunction. The assumption was based on the fact that the method can alleviate the anxiety which is one of the co- morbidities of sexual dysfunction.

2. Material and Methods

The subjects included 20 women aged 25-45 years old with sexual dysfunction who had referred to TALEGHANI Hospital in Tehran-the capital of IRAN. First the demographic questionnaire, together with Spilburger's Anxiety questionnaire, were filled by the subjects in order to measure their level of anxiety. This questionnaire was presented by Spilburger et al. in 1970, and was renewed in 1983. The questionnaire measures the anxiety in two scales of situation and trait. The Chronbach's alpha coefficient in the scale of situation was reported 0.92% , and the corresponding coefficient for trait was 0.90 %. The questionnaire includes 40 questions, and questions 1-20 assess the anxiety of situation. Each question is followed by four options- ***never, sometimes, often, very often***. Questions 21-40 deal with anxiety of trait consisting of four options : ***almost never, sometimes, most often, and almost always***. The scores of 20-30 signify low level of anxiety, and scores 31-45 denote medium level of anxiety, and eventually the scores above 46 indicate high level of anxiety.

After conducting the test, subjects group underwent cognitive-behavioral treatment (CBT), which consisted of 4 groups and 8 individual sessions. The sessions were decided to be twice a week, and each session lasted one-and –a half hours. Throughout the session the focus was mainly on cognitive restructuring, modification of cognitive distortions, and training of behavioral techniques such as relaxation education. Following the counseling sessions, they sat a post-test, and SPSS software, version 18, and Chi-Square test together with T-test were used to analyze the data.

Protocol of implementation of cognitive-behavioral therapy: First session of group counseling: the aim of this session was introduction, and assessing the level of the subjects' awareness of sexual behavior.

Second session of group counseling: this session aimed at teaching sexual behavior and giving information, and focused on teaching the relaxation skill in order to reduce their anxiety in intercourse.

Third session of group counseling: this session focused on analyzing the wrong images as well as suppositions of the subjects by themselves, and learning some skills and doing some assignments.

Fourth session of group counseling: in this session all the subjects' questions were answered, and all the previous subjects were reviewed.

Following the group counseling sessions, since they did not feel free to put forward some of their problems, 8 individual counseling sessions were organized with the following goals:

The first session focused on individual interviews, assessment of their manner of intercourse, and determining the problem. In the second session, false negative views and thoughts that often lead to the expression of negative feelings towards sexual issues were discussed. The purpose of the third session was further cognitive reconstruction in the subjects. In the fourth session, the main objective was sensual focus type II, as well as training the Kegel exercises.

During the fifth session, penetration without orgasm, and self-stimulation was practiced, and in the sixth session, reaching orgasm was practiced in the presence of their spouse, and some other assignments. The aim of the seventh session was individual counseling, intercourse, and orgasm; and eventually, in the last session, all the material covered during the previous sessions were reviewed and conclusions were drawn. The subjects were categorized and assigned to each level of the counseling process depending on the nature of their problems.

3. Results

Considering the results gained from demographic questionnaire, the average age for the subjects was determined 32years. 60% of the subjects group, had middle school education; 25% of them had high school diploma, and 10% of them had bachelor degree. 5% in subjects group had primary level of education. Also, 60% of subjects group were housewives, while 35% in subjects groups were office employees, and finally, 5% of them were retired. Regarding their economic status, 60% in subject group had an average economic situation; 30% of them had bad economic situation, and 10% of them , had a decent economic state. The results can be seen in the following tables 1and 2.

Table1: Distribution frequency scores demographic data in subjects group

	Variables	Frequency	Percent
Education	Primitive	1	%5
	High school	12	%60
	Diploma	5	%25
	Bachelor	2	%10
Occupation	Housewife	12	%60
	Employee	7	%35
	Retired	1	%5
Economy	Bad	6	%30
	Moderate	12	%60
	Good	2	%10

Table2:distribution frequency and compare mean scores situational anxiety and trait anxiety before and after CBT in subjects group

Groups	No.	Mean	STD	T	DF	Sig
Situational - pre	20	62.9	7.226	0.09	38	0.93
Situational -post	20	35.11	6.189	13.12	38	0.000
Trait-pre	20	62.60	7.598	0.243	38	0.81
Trait-post	20	35	8.349	11.07	37	0.000

As it can be seen from the table2, the average Pre-test score for situation anxiety for subjects group was 62.9, and it was high; however, the average score for the Post-test concerning the situation anxiety was 35.11 , which means there has been a considerable difference between the pre-test and the Post-test ($P< 0.05$) in subjects group. The difference in figures, in fact, denotes a decrease in anxiety in subjects group and effectiveness of the interference. Also, it was concluded that the average Pre-test score for Trait Anxiety was 62.60 and they had a high level of Trait anxiety. In contrast, the average Post-test scores for Trait Anxiety were quite different: 35 in subjects group. This implies a significant difference between the pre-test and the Post-test ($P< 0.05$) and a reduction of anxiety as well as effectiveness of interference.

4. Discussions

Results of the research concerning the women who referred to Taleghani Hospital in Tehran showed in subject groups, the level of anxiety was high and acute; also the group and individual counseling sessions offered to them had significant effect on reduction of anxiety for both Situation and Trait. Planning the details of the method of intercourse, and also discussion around fears, anxieties and concerns, coming over the sense of guilt, existing misunderstandings, as well as correcting the misconceptions about sexual behavior, and finally the radical alteration of women's view to sex and sexual act are among the many issues that justify the effectiveness of this therapeutic method.

The findings of the present research corresponds with the results of another study implying that those who enjoyed this type of counseling experienced a significant drop in their level of anxiety.(Kaiser, 2008) It also corresponded with the results of another research concluding the effectiveness of cognitive-behavioral treatment for sexual disorders in Vaginistic women and specific phobia of female diseases, and anxiety(Crespo, 2004). In another research, cognitive-behavioral counseling was conducted to promote the sexual intercourse, and reduce the anxiety and fear of sex act, the results of which corresponds with the present study . (Turkuile, 2007)

In the studies conducted by Mehrabi, Jaberi and Mehryar on assessing the level of effectiveness of cognitive-behavioral treatments concerning the women inflicted with the sex-phobia disorder, as well as studying the sex intercourse that was conducted, the results showed that as a result of cognitive-behavioral treatment, the level of anxiety in the subjects reduced considerably, and their efforts to have more intercourse was successful. The results of the research also corresponded with the present study.(Mehrabi, 2002) Therefore, it is recommended that longer similar therapeutic methods and more number of sessions be organized and conducted, and in order to monitor the consistency of the treatment

effects, follow-up tests be performed at various intervals, following the termination of the therapeutic interference. Since the subject who referred to Taleghani Hospital were limited, there any kind of generalization must be cautioned.

As it was mentioned, sexual disorder has had high prevalence among women and caused several problems in their personal life including anxiety and depression, as well as in their inter-personal relations, and as it was noticed, individual cognitive counseling as a therapeutic method can have a significant role in improvement of people suffering from anxiety which is resulted from sexual dysfunction.

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References

1. Chris Scarre, Chronicle of the Roman Emperors, Thames & Hudson, 1995. pp.168-169.
2. Seligman, M.E.P., Walker, E.F. & Rosenhan, D.L..*Abnormal psychology*, (4th ed.) New York: W.W. Norton & Company, Inc.
3. Davison, Gerald C. (2008). *Abnormal Psychology*. Toronto: Veronica Visentin. pp. 154. ISBN 978-0-470-84072-6.
4. Bouras, n. and Holt, G. (2007). *Psychiatric and Behavioural Disorders in Intellectual and Developmental Disabilities* 2nd ed. Cambridge University Press: UK.
5. National Institute of Mental Health Retrieved September 3, 2008.
6. Ohman, A. (2000). Fear and anxiety: Evolutionary, cognitive, and clinical perspectives. In M. Lewis & J. M. Haviland-Jones (Eds.). *Handbook of emotions*. (pp.573-593). New York: The Guilford Press.
7. Barlow, David H. (2002). "Unraveling the mysteries of anxiety and its disorders from the perspective of emotion theory". *American Psychologist* **55** (11): 1247–63. PMID 11280938. http://psycnet.apa.org/journals/amp/55/11/1247.pdf.
8. Sylvers, Patrick; Jamie Laprarie and Scott Lilienfeld (2011). "Differences between trait fear and trait anxiety: Implications for psychopathology". *Clinical Psychology Review* **31** (1): 122–137. doi:10.1016/j.cpr.2010.08.004.
9. Smith, Melinda (2008). Anxiety attacks and disorders: Guide to the signs, symptoms, and treatment options. Retrieved March 3, 2009, from Helpguide Web site: http://www.helpguide.org/mental/anxiety_types_symptoms_treatment.htm
10. Adams, A.E, & Haynse, S.N. (1985). Cognitive distraction in female sexual arousal .Journal of pychophysiology. 22,689-696.
11. Basson R, Berman J, Burnett A, Derogatis L, Ferguson D, Fourcroy J et al. Report of the international onsensusdevelopment conference on female sexual dysfunction: definitionsand classifications. J Urol 2000; 163: 888–893.
12. Basson R (2005). Women's sexual dysfunction: revised andexpanded definitions. Canadian Medical Association Journal172, 1327–1333.
13. Crespo ,E,& Fermandez, F. (2004), Cognitive behavioral treatment of a case of vaginism and phobia about pelvic examination. Journal of psychology,106-121.
14. Dennerstein L, Randolph J, Taffe J, Dudley E, Burger H. Hormones, mood, sexuality, and the menopausal transition. Fertil Steril. 2002;77:S42-8.
15. Kabakci, E, & Batur, S. (2003). Who benefit from cognitive behavioral therapy for Vaginism? Journal of Sex Marital Therapy, 29(4), 277-88.
16. Kaiser FE. Sexual function and the older woman. Clin Geriatr Med. 2003;19:463-72.
17. Mehrabi, & Fereidun,& Jaberi, & Parivash, & Mehriar, & Amirhushang. (1999). The manners of cognitive behavioral therapy in therapy on women with sexual phobia.The Journal of Thinking and Behavior.79-91.
18. Meston CM, Hull E, Levin RJ, Sipski M. Disorders oforgasm in women. *J Sex Med*. 2004;1(1):66-68.
19. Soykan A, Boztas H, Kutlay S, Ince E, Nergizoglu G, Dileko¨z AYet al. Do sexual dysfunctions get better during dialysis?Results of a six-month prospective follow-up study from Turkey. Int J Impot Res 2005; 17: 359–363.
20. Turkuile, M.M., Vanlankveld, J.J, Groot, E.D, Melles, R, Neffs, J, Zandbergen, M. (2007). Cognitive behavioral therapy for women with lifelong vaginismus:process and prognosis factors.Behavioral Research and therapy, 45(2):359-73.

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Serum Soluble Interleukins-2 Receptors in Bronchial Asthmatic Children

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Abstract: Background: Bronchial asthma is an inflammatory airway disease characterized by infiltration of inflammatory cells into bronchial tree and increased airway hyper-reactivity to various physical and chemical stimuli. The aim of this study was to detect soluble interleukin-2 receptors (sIL-2) serum levels, as marker of T lymphocyte activation in *vivo,* among bronchial asthmatic children in relation to infection, atopy status and disease severity. **Methods:** Sixty bronchial asthmatic children (30 with acute and 30 with stable asthma); and 17 apparently healthy children as controls were recruited. History taking and clinical examinations were performed among all studied groups. Venous blood sample was withdrawn for measuring of sIL-2R using ELISA technique. Pharyngeal swabs were taken for detecting organism causes the disease.**Results:** The predominant infection was viral with total 40% of examined cases; *respiratory syncitial virus* and *Adenovirus* were prevalent virus pathogens in asthmatic children. While *Haemophilus influenza* and *Candida albicans* were most common causes of bacterial infections. sIL-2R serum level was significantly elevated in acute and chronic asthmatic children versus controls and in acute versus chronic patients. Meanwhile, in acute asthmatic children, insignificant differences were recorded between different degrees disease severity or allergic status. **Conclusion:** sIL-2R is an important interleukin associated with bronchial asthma in children; this interleukin can indicate disease activity. In addition it can't be used as indicator for severity or atopy of the disease.
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Keywords: Bacteria, Bronchial asthma; Children; Soluble interleukin-2 receptors; Viruses.

1. Introduction

Asthma is a chronic inflammatory lung disease that leads to significant morbidity, mortality, and financial burden (1) Bronchial asthma is characterized by episodic reversible narrowing of the airway, with associated bronchial hyper reactivity. Inflammation is responsible for airway obstruction and hyper responsiveness (2). Persistent inflammation of the respiratory mucosa, characterized by an eosinophilic infiltrate, and also involving other cell types (lymphocytes, mast cells, basophils and neutrophils) is thought to be important in the pathogenesis of asthma and is associated with airway hyper-responsiveness, a hallmark of the disease (3).

T-cells may orchestrate inflammatory responses to inhaled antigen and other stimuli in asthma by producing several cytokines (4).

Activation of T lymphocytes results in expression of interleukin-2 receptor (IL-2R) on the cell surface and releases of soluble interleukin 2 receptor (sIL-2R), a subunit of the IL-2 cell surface receptor molecules into the circulation (5). Various studies have confirmed the strong association between serum sIL-2R levels and the activation of T lymphocytes *in vitro* and have indicated that sIL-2R production is directly proportional to cellular IL-2R expression (6).

There is a worldwide trend of increasing asthma prevalence, with large international variations. A number of studies have been undertaken to try to explain the variations and to detect risk factors for the development of childhood asthma. The risk factors are several; including allergy, eczema, antibiotic use, reactions to food, early respiratory syncytial virus infection and parental asthma. Viruses are the most common causative agents of lower respiratory tract infections in young infants' worldwide (7). Among them, *respiratory syncytial virus* (RSV) and *adenovirus* (ADV) are responsible for 30 and 5% of hospitalized cases, respectively (8; 9).

To our knowledge, there are no studies that have examined the activation status of T cells in stable and acute asthma in children in relation to causative organisms. It is very important to measure peripheral blood markers (minimally invasive method) to assess airway inflammation in children with asthma. So, this cross sectional study aimed to evaluate the serum levels of soluble marker of T-cell activation in *vivo* sIL-2R level in the serum in acute and stable asthmatic children in relation to causative organisms, allergic status and disease severity.

2. Material and Methods:

This cross sectional study was conducted on 60 children suffering from bronchial asthma with duration of at least 6 months before attending the Pediatric Clinic at King Abdulaziz University Hospital from January 2011 to December 2011 and 17 apparently healthy non-asthmatic children as control group. All patients were diagnosed by a respiratory medicine specialist and diagnosis was confirmed by lung function tests on older children. The patients were subdivided into acute asthmatic group which consisted of 30 children suffering from acute attacks of bronchial asthma (16

boys, 14 girls; mean ±SD of age 5.33±1.70 years, range 2–8 years); chronic stable asthmatic group consisted of 30 children (17 boys, 13 girls; mean ±SD of age 5.46±1.30 years, range 2–9 years); control group consists of 17 children (9 boys, 8 girls; mean ±SD of age 4.75±2.20 years, range 2–9 years).

Written informed consent was obtained by all patients' caregivers for interviewing and blood sampling. The study was conducted according to Declaration of Helsinki and approved by the local ethics committee.

All recruited children were subjected to complete history intake, physical examination**,** complete blood picture, X-rays chest. After clinical examination, the children with acute exacerbation were assigned as mild, moderate and severe acute asthma according to established guidelines (10). Patients with acute asthma were classified into atopic and non-atopic groups. Atopy was defined as a positive skin prick test (wheal diameter >4mm at 15 minutes) to extracts of three common aeroallergens (mixed grass pollen, cat dander, house dust mite: Soluprick; ALK, Horsholm, Denmark) and/or a serum IgE concentration > 150 IU/ml (PRIST; Pharmacia, Uppsala, Sweden) (11). The atopic subjects in acute asthmatics included 16 subjects who had also a history of seasonal nasal symptoms. Non-atopic asthmatic subjects had a history of post-infectious onset of asthma, negative reaction to skin prick tests, and negative specific IgE against house dust mite. All normal volunteers were non-atopic. All acute patients were treated with nebulized β2-agonists, oral or intravenous corticosteroids, and oxygen. Stable asthma was required no more than intermittent inhalation of β2-agonists and regular inhaled steroids.

All recruited children were investigated for bacterial and viral infections by taking pharyngeal swabs which immersed in thioglycolate broth. The pharyngeal specimens were Gram stained, streaked on nutrient, blood, chocolate, MacConkeys and Sabarauds agar plates. The bacteria were recognized by their colony morphology and Gram smears. Blood culture was done for each patient. The result of blood culture was reported as negative if there was no growth in the blood culture up to 10 days. any sample showed visualized colonies in solid phase it was subculture into blood, chocolate, MacConkey and Sabaurauds and samples were diluted with virus transport medium (0.5% gelatin hanks balance salt solution with penicillin, streptomycin) and supernatant were obtained and treated in the same way three times .

Venous blood samples were taken (5ml) from all patients before any medications, serum was obtained from each sample and kept frozen at -20ºC for viral and soluble interleukin-2 receptors detection. EIA rapid diagnosis of *Respiratory syncytial virus* was done for all samples by using Abbott Test Pack RSV enzyme immunoassay (EIA), and (Virotech) system diagnostic GmbH) for and *Parainfluenza. Adenovirus* detection was done using Adenocolone EIA diagnostic kit (Cambridge bioscience) (12). Soluble interleukin-2 receptors was measured by enzyme-linked immunosorbent assay using (Genzyme; Cambridge, MA, USA) (13). All methods were used according to instruction manufacturers.

Statistical analysis

The obtained data were expressed as mean+/- standard deviation (SD) and range or number (%) as appropriate. Two-sided unpaired Student's t-tests and one way ANOVA tests were performed for comparison for parametric and Chi square test for non-parametric parameters. Results were considered significant at P <0.05. All statistical analyses were performed using the SPSS statistical software package version 16.

3. Results

Table (1) showed the demographic and clinical characteristic of all the studied groups. sIL-2R showed significant increase in acute and chronic asthmatic patients versus controls (P <0.0001) and in acute versus chronic patients (P <0.0001) (Table 1).

Table (2) showed that viral and bacterial infections were (n=14 and n= 16) in acute asthmatics and (n=10 and n= 6) in chronic asthmatics. In both acute and chronic patients, viral infections were mostly due to *Respiratory syncytial virus* (23.33%, 13.30%) followed by *Adenovirus* (10.00%, 6.70%), then *Influenza virus* (6.70%, 6.70%), *Parainfluenza virus* (6.70%, 6.70%); meanwhile bacterial infections were mostly due to *Haemophilus influenza* (23.30%, 6.70%) followed by *Candida albicans* (16.70%, 13.30%). In acute asthmatics only bacterial infections were also due to by *Streptococcus pneumonia* (2.7%) *and Mixed infections* (2.7%).

Table (3) showed that the serum levels of sIL-2R in different viral and bacterial infections were significantly higher in acute versus stable asthmatic patients.

Tables (4a and 4b) showed that in acute asthmatic patients, there were no significant differences in the serum sIL-2R levels according to severity or atopic state of the disease.

4. Discussion

Athma is a chronic inflammatory disease of the airways characterized by fibrosis of the airways, hyperplasia and hypertrophy of smooth muscle cells and mucous secreting cells due to infiltration of activated eosinophils and activation of resident mast cells and lymphocytes. These chronic inflammatory changes are mediated by secretion of cytokines from inflammatory cells such as IL-2 , antigen activate T cells to express genes encoding IL-2 and its receptor; therefore, the rate of release of the soluble form of IL-2R appears to reflect T cell activation *in vivo* (14).

Table (1). Demographic Characteristics and soluble interleukin-2 receptors (sIL-2) of different studied groups

Item	**Control** (n=17)	**Patient**	
		Acute (n=30)	**Stable** (n=30)
Age (years)			
mean	4.75±2.20	5.33±1.70	5.46±1.30
range	2.00-9.00	2.00-8.00	2.00-9.00
P value		*P* >0.05	*P* >0.05
			**P* >0.05
Sex			
Number of male	9 (52.94%)	16 (53.33%)	17(56.66%)
Number of Female	8 (47.06%)	14 (46.67%)	13 (43.34%)
P-value		*P >0.05*	*P >0.05*
Height (cm)			
Mean	103.00±10.24	100±11.24	103.00±9.26
Range	90.00-130.00	87.00±120.00	97.00-118.00
P-value		*P* >0.05	*P* >0.05
			**P* >0.05
Weight (kg)			
Mean	3.70±1.70	3.20±1.70	4.60±18.9.0
Range	14.00-2.50	13.00-2.60	15.00- 3.00
P-value		*P* >0.05	*P* >0.05
			**P* >0.05
Temperature (ºC)			
Mean	37.00±0.25	37.00±0.45	37.00±0.12
range	36.70-37.50	36.70-37.30	37.00-37.20
P-value		*P* >0.05	*P* >0.05
			**P* >0.05
sIL-2R (U/ml)			
mean	240.34 ±355.67	705.00±1550.00	308.00±570.24
range	154.00-1252.00	450.00-2854.00	155.00-1255.00
P-value			
		***P* <0.001**	***P* <0.001**
			**P* <0.001

P: significance versus controls; **P*: significant acute versus chronic asthmatic patients

Table (2). Type of respiratory tract infections among different studied groups

Type of infection	**Patients**		**Significance**
	Acute (n=30)	**Stable** (n=30)	
Viral infections	(n= 14, 46.67%)	(n= 10, 33.33%)	
Respiratory syncytial virus	7 (23.33%)	4 (13.30%)	***P* <0.05**
Adenovirus	3 (10.00%)	2 (6.70%)	*P* >0.05
Influenza virus	2 (6.70%)	2 (6.70%)	*P* >0.05
Parainfluenza virus	2 (6.70%)	2 (6.70%)	*P* >0.05
Bacterial infections	(n= 16, 53.33%)	(n= 6, 20.00%)	
Haemophilus influenza	7 (23.30%)	2 (6.70%)	***P* <0.05**
Candida albicans	5 (16.70%)	4 (13.30%)	*P* >0.05
Streptococcus pneumonia	2 (6.70%)	-	*P* >0.05
Mixed infections	2 (6.70%)	-	*P* >0.05

Data are expressed as number (%), *P*: significant between acute and chronic groups.

Table (3): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among different studied asthmatic groups according to type of infections.

Items	Acute asthma	Stable asthma	Significance
Viral infections			
Respiratory syncytial virus (n=11)			
Mean	1790.00±250.00	550.00 ±230.6.00	***P* <0.001**
range	635.00-2555.00	154.00-955.00	
Adenovirus (n=5)		590.00±220.10	***P* <0.001**
Mean	1680.00±550.00	254.00 -1254.00	
range	452.00-2854.00		
Influenza virus (n=4)		620.00 ± 310.50	***P* <0.001**
Mean	1850.00±250.00	254.00-852.00	
range	605.00 -2452.00		
Parainfluenza virus (n=4)			***P* <0.001**
Mean	1690.00±320.00	599.00±300.80	
range	566.00-2225.00	152.00-955.00	
Bacterial infections			
Haemophilus influenza (n=9)			***P* <0.001**
Mean	1855.00±55.00	640.00 ±230.00	
Range	1425.0-2585.000	254.00- 850.00	
Candida albicans (n=9)			***P* <0.001**
Mean	1880.00±60.00	670.00±210.00	
Range	1251.00-2452.00	451.00-1252.00	
Streptococcus pneumonia (n=2)	0.00	0.00	-
Mixed infections (n=2)	0.00	0.00	-

Data are expressed as mean ±SD and range, P: significant between acute and chronic groups.

Table (4a): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among acute asthmatic patients' subgroups according to severity of the disease.

Items **Severity**	**Acute asthma** (n= 30)	**Significance**
Mild (n=12)	1574.40±714.00 455.00-2585.00	
Moderate (n=10)	1391.00±747.00 452.00-2854.00	**P* >0.05
Severe (n=8)	1594.00±699.00 455.00-2555.00	**P* >0.05 ***P* >0.05

Data are expressed as mean ±SD and range. **P*: significance versus mild asthmatic, ***P*: significance versus moderate asthmatic.

Table (4b): Serum levels of soluble interleukin-2 receptors (sIL-2R) U/ml among atopic and non-atopic group in acute asthmatic patients.

Items	Atopic (n= 17)	Non-atopic (n= 13)
Mean	1546.60±794.00	1455.20±596.70
range	452.00-2854.00	455.00-2555.00
P-value		*P* >0.05

Data are expressed as mean ±SD and range. *P*: Significant between atopic and non-atopic groups.

Our study revealed in both acute and stable asthmatic patients that virus infections was predominant infection in both acute (46.67%) and stable asthma (33.33%) with total (24 children) 40.00% of examined cases. Viral infections were mostly due to *Respiratory syncytial virus* (23.33%, 13.30%) followed by *Adenovirus* (10.00%, 6.70%), then *Influenza virus* (6.70%, 6.70%), *Parainfluenza virus* (6.70%, 6.70%). These results agreement with others (15-18). reported that viral respiratory tract infections, predominantly those caused by *human rhinoviruses*, are associated with asthma exacerbations. Also, Malek-shahi *et al.* (19) reported that wheezing episodes early in life due to *human rhinoviruses* are a major risk factor for the later diagnosis of asthma at age 6 years. Recently mechanisms for virus-induced exacerbations of childhood asthma are beginning to be focused on and defined. Viruses cause systemic immune activation and also produce local inflammation. These factors are likely to affect airway pathogenesis leading to airway narrowing, an increase in mucus production, and eventually bronchospasm, and airway obstruction (20). These new insights related to the pathogenesis and disease activity are likely to provide new targets for the therapy and prevention of early asthma in childhood.

Our data declared bacterial infection in both acute and stable asthma with (53.33% and 20.00%) with total (22 children) 36.67%% of examined cases. In acute and stable asthmatic patients, *Haemophilus influenza* was the prevalent bacteria (23.30%, 6.70%) then *Candida albicans* (16.70%, 13.30%). In only acute asthmatics, bacterial infections were also due to by *Streptococcus pneumonia* (2.70%) *and Mixed infections* (2.70%). Our result were in partial agreement with others (21) who found that over 50% of patients have direct and/or indirect evidence of infection, most commonly bacterial, bacteria as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis,* or atypical organisms as *Chlamydia pneumoniae*, *Mycoplasma pneumonia*. Also, Hare *et al.* (22) reported that *Streptococcus pneumoniae, nontypable Haemophilus influenzae (NTHi), and Moraxella catarrhalis* were prevalent causes of lower airway infection.

In this study, serum sIL-2R levels in acute and stable asthmatics were found to be significantly elevated versus healthy controls and in acute versus chronic asthmatics. Also, in acute asthmatic children, serum sIL-2R levels were significantly elevated in different types of viral and bacterial infections than stable cases. Our data are in agreement with previous study (23) which indicate that the serum concentration of sIL-2R in asthmatic children remained significantly higher than in controls subjects. Although other study (12) have reported that no changes in serum sIL-2R concentration between patients with acute severe asthma and patients with stable asthma. Other study (24) indicate that serum sIL-2R concentration was elevated in asthmatic patients especially in patients during acute exacerbation. Apparently, from the above, elevated serum sIL-2R levels should be carefully interpreted in the presence of clinical, acute and stable asthma.

Whether the serum sIL-2R level can reflect the severity of asthma is a subject of considerable debate, and for paediatric asthma, there is a scarcity of data. In this study, in acute asthmatics, serum sIL-2R levels were not different according to severity of the cases. On contrary, Park *et al.* (25) reported that circulating sIL-2R was the reflection of local inflammatory activity within lung involved, suggesting that sIL-2R test might clinically be useful in the evaluation of patients with bronchial asthma with respect to the severity. Tang and Chen (23) reported that the serum level of sIL-2R in asthmatic children correlated positively with the severity of exacerbation, significantly higher than at clinical remission and could be a potential index of asthma severity.

It is well established that CD4+ Th2 lymphocytes and Th2-associated cytokines (IL-4, IL-5, IL-13) play a crucial role in orchestrating the chronic inflammatory response in atopic asthma (26). However; The immunological mechanisms and the role of T-cell activation occurring in patients with non-atopic asthma are less well characterized, particularly in children. In

this study, we examined T-cell activation in vivo by measuring sIL-2R concentration in serum of acute atopic asthma and non-atopic asthma subjects. We found that serum sIL-2R levels were not different in non-atopic and atopic acute asthmatic patients. However, the data of elevated concentrations of IL-2 and IL-5, and elevated numbers of IL-2R on CD3, CD4, and CD8+ lymphocytes in the bronchial tree, as well as sIL-2R and interferon gamma elevations in serum of the status asthmaticus of the non-atopic patients with bronchial asthma, indicate strong evidence of T-cell activation even in non-atopic bronchial asthma (27,28).

In conclusion, this study emphasize the role of T cells in asthma and suggest that regulation of their function may be important in the treatment of acute and stable asthmatic children as evident by elevated serum levels of sIL-2R. In addition, serum levels of sIL-2R can't be used as indication of acute asthmatic disease severity or allergic state. This study suggests that the therapeutic strategy for asthma should be targeted at inflammatory phenomena rather than at symptoms alone.

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References

1. Warrier MR, Hershey GK. Asthma genetics: personalizing medicine. J Asthma. 2008; 45:257-64
2. Hanania NA. Targeting airway inflammation in asthma: current and future therapies. Chest. 2008; 133:989-98.
3. Akdis CA, Blaser K, Akdis M. Apoptosis in tissue inflammation and allergic disease. Curr Opin Immunol .,2004; 16:717–23.
4. Lee NA, Gelfand EW, Lee JJ. Pulmonary T cells and eosinophils: coconspirators or independent triggers of allergic respiratory pathology? J Allergy Clin Immunol., 2001; 107:945–57.
5. Rubin LA, Kurman CC, Fritz ME. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J Immunol., 1985; 135: 3172-7
6. Lai KN, Leung JCK, Chow CC, Cockram CS. T lymphocyte activation in euthyroid Graves' ophthalmopathy: soluble interleukin 2 receptor release, cellular interleukin 2 receptor expression and interleukin 2 production. Acta Endocrinol (Copenh), 1989; 120:602-9.
7. .Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. JAMA. 1999; 282: 1440-6.
8. .Avendano LF, Palomino MA, Larrañaga C. Surveillance for respiratory syncytial virus in infants hospitalized for acute lower respiratory infection in Chile (1989 to 2000). J Clin Microbiol., 2003; 41:4879-82.
9. .Palomino MA, Larranaga C, Villagra E, Camacho J, Avendano LF. Adenovirus and respiratory syncytial virus adenovirus mixed acute lower respiratory infections in Chilean infants. Pediatr Infect Dis J., 2004; 23:337-41.
10. Williams SG, Schmidt DK, Redd SC, Storms W; National Asthma Education and Prevention Program. Key clinical activities for quality asthma care. Recommendations of the National Asthma Education and Prevention Program. MMWR Recomm Rep., 2003 ; 52:1-8.
11. Stelmach I, Jerzynska J and Kuna P. A randomized, double-blind trial of the effect of treatment with montelukast on bronchial hyperresponsiveness and serum eosinophilic cationic protein (ECP), soluble interleukin 2 receptor (sIL-2R), IL-4, and soluble intercellular adhesion molecule 1 (sICAM-1) in children with asthma. J Allergy Clin Immunol., 2002; 109:257-63.
12. 12.Hite SA, Huang YT. Microwave-accelerated direct immunofluorescent staining for respiratory syncytial virus and influenza A virus. J Clin Microbiol., 1996; 34:1819-20.
13. Limas CJ, Goldenberg IF, Limas C. Soluble Interleukin-2 Receptor Levels in Patients with Dilated Cardiomyopathy: Correlation with Disease Severity and Cardiac Autoantibodies. Circulation, 1995; 91:631-4..
14. Bousquet J, Corrigan CJ, Venge P. Peripheral blood markers: evaluation and inflammation in asthma. Eur Respir J., 1998; 26:42S-8S
15. Cabalka AK. Physiologic risk factors for respiratory viral infections and immunoprophylaxis for respiratory syncytial virus in young children with congenital heart disease. Pediatr Infect Dis J. 2004; 23:S41-5.
16. Oh JW. Respiratory viral infections and early asthma in childhood. Allergol Int. 2006; 55(4):369-72.
17. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal

carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. BMC Infect Dis., 2010; 23; 10:304
18. Noyola DE, Rodríguez-Moreno G, Sánchez-Alvarado J, Martínez-Wagner R, Ochoa-Zavala JR. Viral aetiology of lower respiratory tract infections in hospitalized children in Mexico. Pediatr Infect Dis J., 2004; 23:118-23.
19. Malek-shahi SS, Azad TM, Yavarian J, Shahmahmoodi S, Naseri M, Rezaei F. Molecular detection of respiratory viruses in clinical specimens from children with acute respiratory disease in Iran. Pediatr Infect Dis J., 2010; 29:931-3
20. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet, 2010; 376:826-34.
21. Aebi T, Weisser M, Bucher E, Hirsch HH, Marsch S, Siegemund M. Co-infection of Influenza B and Streptococci causing severe pneumonia and septic shock in healthy women. BMC Infect Dis., 2010; 27:308.
22. Hare KM, Grimwood K, Leach AJ, Smith-Vaughan H, Torzillo PJ, Morris PS et.al. Respiratory bacterial pathogens in the nasopharynx and lower airways of Australian indigenous children with bronchiectasis. J Pediatr., 2010; 157:1001-5.
23. Tang RB, Chen SJ. Soluble interleukin-2 receptor and interleukin-4 in sera of asthmatic children before and after a prednisolone course. Ann Allergy Asthma Immunol. 2001; 86(3):314-7.
24. Lai CK, Chan CH, Leung JC, Lai KN. Serum concentration of soluble interleukin 2 receptors in asthma. Correlation with disease activity. Chest, 1993; 103:782-6.
25. Park CS, Lee SM, Chung SW, Uh S, Kim HT, Kim YH. lnterleukin-2 and Soluble Interleukin-2 Receptor in Bronchoalveolar Lavage Fluid From Patients With Bronchial Asthma. Chest, 1994; 106:400-6.
26. Romagnani S. The role of lymphocytes in allergic disease. J Allergy Clin Immunol., 2000; 105:399–408.
27. .Heath H, Qin S, Rao P, et.al. Chemokine receptor usage by human eosinophils. The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. J Clin Invest. 1997; 99:178-84.
28. Bettiol J, Bartsch P, Louis R, *et al.* Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. Allergy, 2000; 55:1134-41.

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A Literature Review of Factors Influencing Breast Cancer Screening in Asian Countries

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Abstract: Breast cancer is a major public health concern among Asian women. As breast cancer is often diagnosed in advanced stages in younger women, mortality rates are frequently higher compared with rates in developed nations. Due to the influence of various psychological, social, and cultural factors on breast cancer, women are reluctant to screen their breast cancer symptoms at the early stages when treatment is most expected to be successful. Screening options for Asian women are also limited because of demographic constrains and their knowledge of preventive health measures. This paper proceeds to review the existing literature on factors influencing breast cancer screening among Asian women. For the most part, health care professionals, medical doctors, gynecologists, and breast cancer advocates should find actual ways to overcome psychological barriers such as beliefs about pain, fear, embarrassment, and modesty of women through public awareness campaigns. Considerable attention should be also devoted to lower socioeconomic status women. In the same way, health care providers should explain to the women about the importance of breast cancer as a common disease and the existence of breast cancer screening programs in a small scale approach, as well as the benefits that participation in these programs can offer. This initiative is about enhancing health status among women and it is part of community development endeavor.

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Keywords: Asian women, Breast cancer screening, Socio-cultural factors, Community development.

Introduction

The burden of breast cancer continues to increase in Asian countries, although some strategies for breast cancer prevention and treatment are in place. Two systematic literature reviews on cancer prevention and breast cancer screening barriers (Parsa et al., 2006; Yoo, 2010), have shown wide disparity among breast cancer incidences in Asian countries. Breast cancer incidence rates increased significantly until the end of the 1990's in Asian women (Yoo et al., 2006). It was reported that, the percent of increase in breast cancer mortality for middle-aged women from the mid-80s to the mid-90s was the highest in Korea, followed by China, and Japan (Bray et al., 2004). In Asian countries, breast cancer is also the most frequently diagnosed cancer among women. According to the National Cancer Registries for Asian countries, the crude incidence rate of breast cancer varied from 21.3 per 100,000 population in Jordan, 21.4 in Iran, 24.1 in Turkey, 34.86 in Malaysia, 48 in Japan to 54 per 100,000 population in Singapore (Ferlay, 2001; Petro-Nustus, 2002; Harirchi, 2004; Secginli, 2006; Hisham, 2004).

Breast cancer arises in the younger age group of Asian women, 40 to 49 years-old compared to the other Western counterparts, where the peak prevalence is realized between 50 to 59 years. As has been described, in Singapore (Yip and Ng, 1996), Malaysia (Hisham and Yip, 2004), Iran (Harirchi et al., 2004), Thailand (Thongsuksal, 2000), Pakistan (Usmani et al., 1996) and Arab women in Palestine (Nissan et al., 2004), more than half of new cases of breast cancer were diagnosed in women below the age of 50 years and in advanced stages III or IV.

There are many factors related to breast cancer prevention such as psychological, social, cultural, and structural and policy factors of breast cancer screening. For example, a study from Iran showed that the breast cancer incidence will grow up considerably in the future as a result of the national shortage of breast cancer screening programs which originates from incomplete registration and diagnosis of cancer patients. The westernized life style will also help this up-growing trend (Mousavi et al., 2009).

What is more important in breast cancer issue in Asian countries is that the breast cancer screening behaviours is characterisized largely by the women's psychosocial attributes and demographic factors. It appears that many barriers to breast cancer screening are also related to culture, income, education, immigration status, and language barriers. As a result, breast cancer screening practices are few among Asian women.

Similarly, Asian immigrant women in America, especially those who have immigrated within the last 10 years, tend to have less cancer screening activity than native women. For instance, only 41% of Filipino and 25% of Korean immigrant women in Los Angeles

reported receipt of a mammogram in the previous two years. Arab Americans represent the largest immigrant group but only 58.1 percent of them reported to have mammogram every 1–2 years. But Iranian American showed a higher rate of mammography screening. A total of 81.1% of the women aged 40 and above reported having a mammogram less than two years ago, and 9.4% of the women more than two years ago (Shirazi, 2006).

On the whole, breast cancer screening behaviours among Asian women residing in their native country are low. To illustrate, mammography screening in Middle Eastern countries are low (Schwartz et al., 2008). Reviews of the literature have shown that only 25% of Turkish people reported having at least one mammogram. Fewer women about 10.3% in the United Arab Emirates had mammography, which was attributed to poor knowledge of breast cancer screening and infrequent offering of screening by health care workers (Schwartz et al., 2008).

Parsa (2006) also noted that previous studies have illustrated that only 3.8% in Malaysia (Hisham and Yip, 2004), 6% in Iran (Jarvandi et al., 2002), 7% in Jordan (Petro- Nustus, 2002), 12% in South Asia (Choudhry et al., 1998), and 16% in China (Fung et al., 1998) women reported performing breast self exam regularly, compared to Sweden where 70% of women aged 25-80 years examined their breasts on a regular basis (Persson et al., 1997). Furthermore, mammography was carried out only in 3.8% of Malaysian women; and there was a significant difference in screening rates between urban and rural areas (50.6% versus 42.3% respectively, $P<0.05$) (Narimah, 1997).

Diversity of Asian population in terms of psychological, social, cultural, and religious ideas also cause challenges in the breast cancer screening rates. While randomized trials comparing mammography with no mammography screening found that women might benefit a 15% relative risk reduction in mortality from mammography (Gotzsche, 2006). It is believed that breast cancer screening options for women in Asian countries are required to adapt tailored strategies and factors influencing breast cancer screening methods should be considered in long-term care on reducing in the number of advanced stage tumors. To some extent, the cost-effectiveness of breast cancer screening activities is not successful without women's participation within those programs. Therefore, this paper presents the previous literature to look into the factors influencing breast cancer screening among Asian women.

Methodology

More than 100 articles published from the year of 1990 through 2011 were found and reviewed by four databases namely, Pub Med, Medline, Science Direct, Google Scholar. But we summarized the findings from relevant published studies to identify factors influencing breast cancer screening in Asian countries. The inclusion criteria were “psychosocial factors”, “early detection” or “breast cancer screening” in combination with “mammography practices”, “clinical breast exam”, “breast self exam”, and “Asian women”. The factors influencing breast cancer screening were regarded as three main subjects such as psychosocial issues, socio demographic or individual factors and knowledge. On the other hand, the potential obstacles for breast cancer screening among Asian women are related to the attitudinal, logistic, and demographic determinants.

Psychosocial Factors

There are many psychosocial factors that explain the different aspects of health-related behaviors. Recently, the concentrate on the factors such as self efficacy, beliefs, social influence, and barriers, have been studied by a number of researchers to understand breast cancer screening behavior. Here are some illustrations of the factors which we have drawn recently on breast cancer early detection among Asian women.

Beliefs toward Breast Cancer and Early Detection

Several researchers have reported that there is a strong relationship between beliefs and health behaviors, such as mammography (Sheeran, 2002; Ajzen, 2004). In line with breast cancer screening via mammography, beliefs include knowing the time and place of doing mammogram and other information such as arranging for work leave and transportation are subjects that will increase the mammography usage among women (Gollwitzer, 1993; Rutter, 2006). Similarly, belief components such as touching of breasts by the technician, living longer, and X-ray exposure are effective in doing mammography (Motano, 1997).

The belief in the benefit of early detection among Asian such as Korean (Han et al., 2000) and Turkish women (Secginli et al., 2006) are positively associated with screening behaviors. Poss (2001) also stated that significant beliefs allow a better understanding of the cultural perspective affecting the people’s behaviour. For instance, with regard to clinical breast exam in Tehran, although, more than half of the women preferred to be examined by a female physician, forty seven percent said that clinical breast exam by a male physician was not against their Islamic beliefs. Likewise, the results showed the vast majority of the women believed that breast self-exam was not against their religious beliefs (Montazeri et al., 2003). It is therefore, religious belief among Asian Muslim women is an important contributor to the breast cancer early detection. In contrast, a survey by

Ahmadian (2011) in Iran showed that adherent women to mammography had more positive beliefs in doing screening than non-adherent group. Focus groups revealed that most Iranian women are not interested in those practices that require their bodies be touched by physicians, so breast cancer screening practices would be neglected by women.

Sometimes beliefs can be altered by other factors. For example, in a study of Vietnamese American women, mostly from first generation immigrants, socio-demographic and acculturation factors showed a high correlation with breast cancer screening rather than attitudes and beliefs (McPhee, 1997). Up to point, Asian women always occupy a lower position which it can itself result in ignorance of their own needs including health care needs (Im, 2004; Benner, 2002; Nissian, 2004; Hisham; 2004).

Attitude and Practice of Breast Cancer Screening

Previous researchers showed that attitudes toward mammography is an important factor for low participation rate among Asian women (Im et al., 2002; Hisham and Yip, 2003; Rashidi et al., 2000). For instance, modesty was reported to be an inhibiting issue that influenced women's participation in mammography (Im et al., 2002). One reason for low participation rate in mammography among Asian women was their inability in perceiving the importance of breast cancer screening test (Parsa, 2006).

Within Iranian context, cultural and social characteristics are very important factors for participation in mammography. A focus group by Ahmadian (2011) in Iran showed that religious boundary and modesty prohibit the performance of breast cancer screening among women. Moreover, from their view of point, destiny is a strong reason for any disease such as breast cancer. Death is viewed as God's will by most Iranian women, especially among the traditional ones and this negatively affects their attitude. Attitudes toward mammography are also developed by women's beliefs about the expected outcomes resulting from the screening performance. The antecedent of attitude is about personal belief concerning the perception of what they should do regarding breast cancer which eventually prescribed their action to seek treatment or early detection. Consequently, this is portrayed in the difference between adherent women to mammography and non-adherent one might contribute to a sense of fatalism.

Previous studies revealed that women in Korea (Lee et al., 2000; Im et al., 2004), Malaysia (Hisham and Yip, 2003), Iran (Jarvandi et al., 2002) , and Singapore (Straughn and Seow, 2000), did not notice the importance of early detection which influences their attitude toward breast cancer screening.

Self-efficacy *to* Perform *Breast Cancer* Detection *Practices*

In terms of prevention of disease, self-efficacy and barriers are the strongest predictors to explain people's behaviour to prevent diseases (Wallace, 2002). Bandura (1977) observed that low self-efficacy shows avoidance behaviour among people and in reverse, high self-efficacy tends to result in initiating behaviours and high efforts to overcome personal obstacles like fear. Self-efficacy is positively correlated with attendance at the breast screening exercise (Straughan and Seow, 2000).

Along with early detection, there must be adequate self-efficacy to challenge the psychosocial obstacles. It seems women who are aware of cancer detection are more likely to take part in the screening programs. A significant positive relationship has been found between breast self-exam and self-efficacy (Edgar et al., 1984; Brailey, 1986). However, according to Shirazi (2006) although most women seem to believe in the efficacy of breast self-exam, they are not so easy to do. In addition, women seem to find that it is embarrassing to perform breast self-exam and to look at their own body in the mirror. The majority also lacked confidence in performing breast self examination.

Self-efficacy was also investigated to be a significant variable for mammography screening (Savage, 1996; Lechner, 1997; Allen et al., 1998; Wallace, 2002). A survey by Ahmadian (2011) in Iran revealed that adherent group to mammography had more self-efficacy towards mammography uptake than the non-adherent group. On the contrary, self-efficacy had no significant relationship with participation in mammography in Kerman, Iran (Abbaszadeh, 2007). Beyond socio-demographic characteristics, self-confidence may influence the health status of women because they may believe in changing of their health behavior prior to every decision making. If women do not tend to undergo breast cancer screening, any attempt to recruit them for their participation in early cancer detection such as health care providers or doctors' advice will be fruitless.

A study by Kim et al., (2009) showed that Korean American women, who are not thinking about having a mammogram, had significantly lower self-efficacy for having a mammogram. Thus increasing women's self efficacy towards cancer detection is an important step to overcome psychological barriers to screening and may lead to changes in breast cancer prevention behaviors.

Social Influence on Breast Cancer Screening

Social influence is a significant contributor of behavioral intention in health issues (Bosompra, 2001; Smith, & Biddle, 1999). Regarding breast cancer

screening, Allen, Sorensen, Stoddard, Colditz, and Peterson (1998) have reported that social network influence was significantly associated with mammography intention in women.

Previous literature showed, if the social support network, including the employers, colleagues in the workplace, family, and friends, is being improved through appropriate health education campaign, then it is likely that more positive attitude toward preventive health behavior will be observed (Straughan and Seow, 2000; Abdullah and Leung, 2001; Juon et al., 2004). Likewise, supportive social influences along with self-efficacy were found to be strongly linked to mammography intention adjusting for prior mammography use (Allen et al., 1998). Instead, a study in Iran demonstrated that women adherent with mammography had lower social influence in comparison to non-adherent ones. The result was not anticipated because non-compliant women also admitted that they were influenced by their social networks such as friends, families and doctors regarding participation in mammography. Indeed, social influence in Iran is not the same as the other parts of the world (Ahmadian, 2011).

In some Asian countries, culturally norms inhibit the discussion of particular issues, such as cancer behavior. Similarly, Iranian women do not tend to talk about cancer disease, as they believe breast cancer affect their body and attractiveness. So, participating women could not benefit information which has been received in their social network. On the other hand, most women who have participated in mammography in the past two years have indicated that their mammography was diagnostic. It can be concluded that there is resistance against family or friends' advice regarding mammography use and as a result they were less influenced by social factors.

With regard to Muslim women, Rajaram and Rashidi (1999) pointed out that Muslim men inappropriately use Islam to justify their authority and dominance over their spouses which creates another barrier for breast cancer screening. Usually, an expectation of obedience to spouse who exerts control over family health decisions is in conflict with the expectation of remaining healthy in order to serve the needs of the family.

Further many studies have reported the positive influence of social support on women's psychological well-being through every stage of breast cancer (Hoskins et al., 1996; Lugton, 1997). Emotional support is offered by family members in the form of trust, concern, and listening and examples of instrumental support such as money, time, labour, and transportation. Peers provide appraisal support that increases the individual's self-esteem. Information support includes advice, suggestions, information, and directives (Gotay and Wilson, 1998).

Previous literature also showed relationship of higher social support levels with higher income and higher education. Women who did not adhere to screening guidelines (for breast self exam or clinical breast exam) reported less social support (Katapodi et al., 2002). Influence of family, friends or someone with breast cancer is significant for participation in screening (McCance, 1996). Other researchers reported that lack of encouragement by family members and physicians leads to low participation in breast cancer screening (Han et al., 2000). Social support network, including employers, colleagues in the workplace, family, and friends, can be improved through appropriate health education campaign, then it is likely that a more positive attitude toward preventive health care will be provided (Straughan and Seow, 2000; Abdullah and Leung, 2000; Juon et al., 2004).

Barriers *of* Breast Cancer Screening

The most significant construct of the health belief model is the perceived barrier that determines behavior change (Janz & Becker, 1984). It involves individual's own estimation of the obstacles in his or her way in adopting a new behavior. Some of the barriers include difficulty with starting a new behavior or a new habit, fear of not being able to perform a desired behavior and embarrassment (Umeh & Rogen-Gibson, 2001).

Perceived barrier as a salient factor also affects on breast cancer screening. Taking no care of oneself, lack of information, and fear are the three most commonly cited barriers (Garbers et al., 2003). Barriers in the case of mammography could include fear of cancer, pain, cost, travel and time (Champion and Menon, 1997).

The socio-cultural barriers such as patient-physician communication difficulties, beliefs about cancer, and cancer prevention impact women's involvement in breast cancer screening programs. Previous studies revealed that physicians are less likely to allocate information with individuals differ from them by social class, ethnicity, gender, and age (Meleis et al., 1995; O' Malley et al., 1997). Health care professionals also have stereotypical ideas about Muslim women as being powerless, uneducated and subservient (Meleis et al., 1995). Rashidi and Rajaram (2000) argued that the unique complexities in the socio-cultural backgrounds of Asian Muslim immigrant women could also delay access to healthcare services. Physician communication problems exist due to religious, cultural and linguistic differences between older Asian Muslim women and their physicians.

Likewise, findings of many studies showed that women were fearful about cancer and death which make them reluctant to participate in breast cancer screening (Benner et al., 2002; Juon et al., 2004; Nissan et al., 2004). Researchers have proved that increased benefits and decreased barriers are linked to increased screening (Slenker and Grant, 1989; Champion 1992; Rakowski et al., 1992). Previous studies highlighted barriers to screening behavior including fear of results, fear of treatment and fear of the test itself. These studies include countries such as, Iran (Jarvandi et al., 2002), Malaysia (Hisham and Yip, 2003), United Arab Emirates (Bener et al., 2002) and Jordan (Petro-Nustas and Mikhail, 2002). Smith et al., (2006) also investigated that fatalism, fear, language barriers, and preference for traditional healers are barriers. Lack of time and costs also were the most frequently reported reasons for Chinese women from Hong Kong reluctance to participate in clinical breast examinations or mammography screenings (Chua, 2005).

Physical examination of body parts is a barrier to screening for Asian women. This barrier includes a woman's concern for maintaining her own expectations of modesty and attitudes of her male sexual partner. Although there is little information about the cancer screening behaviour of Muslim women, modesty has also been concerned in these communities (Rashidi and Rajaram, 2000). In Asian traditional culture, women embarrassment prevents them to show their breasts to others, including health care providers (Smith et al., 2006; Im et al., 2004; Juon et al., 2004). Asian women are unwilling to show their breasts to others, including to health care providers (Smith et al., 2006; Im et al., 2004; Juon et al., 2004). Sometimes unpleasant previous experiences stresses the modesty issues of the Korean, Chinese, and Iranian women further (Im et al., 2004; Juon et al., 2004; Abdulah, 2001). Male physicians also do the clinical exams in Asian countries which needs women expose their breasts to them. Thus, they feel ashamed and as a result they do not tend to undergo a stressful screening. A study by Ahmadian et al., (2011) identified barriers that may have an impact on women's adherence to mammography in Iran. The 400 women admitted embarrassment, lack of doctor or health care provider's advice regarding mammography, and worry about mammogram devices as the most selective barriers.

Besides, Asian immigrants are more disadvantaged and faced with numerous barriers in accessing health care than non-immigrant minority women. Cancer screening barriers include: cost, particularly for undocumented immigrants, lack of female physicians, women's lower status and men's gate keeping, transportation and language (Crane et al., 1996). Latina, Chinese, and Vietnamese American women who were born outside the United States were significantly less likely to have mammography compared to white women (Hiatt, 1996). It seems to me that the lack of knowledge is a barrier to regular cancer screening for minority women related to Asian communities.

Researchers have demonstrated that increased benefits and decreased barriers are linked to increased screening (Champion, 1992; Rakowski et al., 1992; Slenker and Grant, 1989). However, previous literature on increasing breast self-examination practices in women revealed that the fear of breast cancer would encourage women to accept early detection, but there are some barriers with more effect on breast self-exam performance (Champion, 1993; Champion& Menon, 1997; Umeh &Rogan- Gibson, 2001). Similarly, a survey in Iran by Ahmadian (2011) showed that non-adherence with mammography was associated with high levels of distress among Iranian women which ends in being unable to overcome their problems on taking mammography. Although, the study highlighted that participating women are advantaged by socio-demographic characteristics. It is believed that respondents in the lower socioeconomic classes had more barriers to screening.

Some studies suggest that having a gynaecologist, as a regular physician, and physician referral are important predictors in mammography (Jarvandi et al., 2002; Im et al., 2004; Juon et al., 2004; Secginli et al., 2006). Also, the rate of referral by a physician was substantially higher among participating women in mammography. In some Asian countries such as Iran, Turkey, and Korea insurance for having mammography requires doctor's reference to ensure payments (Jarvandi et al., 2002; Juon et al., 2004; Secginli et al., 2006; Parsa, 2006).

For the most part, women who had been screened before cited fear, pain, or other attitudinal barriers more often, but women who had never had been screened cited cost or other logistical barriers. As shown in this review, women's socio-psychological aspects influence participation behaviour.

Demographic Determinants

Available information is limited about the health status and health practices within diverse cultural groups and socio-demographic factors, and there is poor understanding about the amount of these factors affecting health education (Hoare et al., 1994). It appears that many barriers to breast cancer screening are related to culture, income, education, immigration status, and language barriers.

Esterada, Trevino, & Ray, (1990) also noted that culture, education, income, and age, contribute to underuse of cancer screening methods among women population. For example, factors such as high

education, married status, employment were predictors of performance of breast self-exam (Madan et al., 2000). Previous studies showed that education and socioeconomic conditions are major factors for women delay in this trial exam and response to treatment in several cancers, including breast cancer (Blanchard, 2004). For example, less educated or recently immigrated women aged forty and older were less likely to have mammograms in the past two years (McPhee, 1997).

Besides, higher levels of education, income, health insurance, and access to health care reduce the feelings of powerlessness, denial, and turmoil (Saint-Germain and Longman, 1993). Several studies have also proposed that income and education level may be important variables associated with mammography use (Straughan and Seow, 2000; Juon et al., 2002; Finney et al., 2003). Blanchard (2004) argued that that education and socioeconomic conditions are major factors for women delay in the screening and response to treatment in several cancers, including breast cancer. Instead, Chua et al., (2005) reported that education level had no effect on the awareness. According to this study, full-time housewives were more likely to have heard of mammographic screening compared to non-housewives.

Other studies also hinted that age was associated with mammography (Abdulah and Leung, 2001; Petro-Nustas and Mikhail, 2002; Katapodi et al., 2004; Juon et al., 2004; Wu et al., 2006). To illustrate, 66% of Filipino American women with the average age of 65 years had never had a screening mammogram, and 42% had once in the past 12 months. Instead, age was not shown to be a significant reason in mammography practices upon a cross-sectional study which carried out among female teachers in Malaysia on breast cancer screening in 2006 (Parsa et al., 2008).

A baseline survey of participation in mammography showed that 21.9% of Korean-American women aged 40 and older had a mammogram in past 12 month (Sadler, 2001). On the contrary, age was not shown to be a significant reason in mammography practices upon a cross-sectional study which carried out among female teachers in Selangor in Malaysia on breast cancer screening in 2006 (Parsa et al., 2008). At the same time, Navon (1999) stresses the inadequacy of attributing each and every difference to cultural factors which are interrelated to the economic or educational disparities. Undoubtedly, socio-demographic factors make women modify their cultural beliefs.

A study by Yu et al., (2003) investigated the factors influencing breast cancer screening utilization among Chinese and Korean women, living in the United States, and examined similarities and differences between the two sub-populations. The results showed that breast cancer screening among Asian women was significantly associated with their ability to speak English, and availability of health insurance. Even for immigrant communities, social discrimination is believed to account for much of the differences in cancer screening behavior (O'Malley et al., 1997).In addition, women with health insurance were more likely than other women to do screening (Juon et al., 2004; Secginli, 2006). Tessaro and Smith (1994) also found that family history as a significant risk factor.

Therefore, establishing a breast cancer screening program, particularly mammography among Asian women of low socio-economic status should be inaugurated. In a way, combining of other but less accurate screening modalities such as breast self-examination or physical examination would be important to seek earlier detection.

Knowledge of Breast Cancer and its Early Detection

Many Asian women may not know that they should obtain an intervallic breast cancer screening (Im et al., 2004). Misconception on breast cancer reasons and expectations from screening program is also a big issue among them. A study showed that misconception on breast cancer was highest among Pakistani women (Gilani et al., 2010). Several studies demonstrated that lower screening rate among Asian women is associated with their knowledge of preventive health measures (Benner et al., 2001, Petro- Nustus, 2002; Juon et al., 2004; Nissan, 2004; Chua, 2005; Parsa, 2006). Suarez et al., (1997) have also found that older women have less knowledge about the importance of mammography.

Studies in Korea (Joun et al., 2004) and Turkey (Secginli et al., 2006) showed knowledge of breast cancer screening guidelines was a major predictor of regular screening. Women who had knowledge of mammography guidelines were ten times more likely of having regular mammograms (Secginli et al., 2006). The results of some studies carried out in Korea (Lee et al., 2000; Im et al., 2004), Singapore (Straughn and Seow, 2000), Malaysia (Hisham and Yip, 2003), Iran (Jarvandi et al., 2002) showed that women had poor information on breast cancer and screening test.

Knowledge is one important influencing factor in mammography use and breasts self-exam (Jarvandi et al., 2002; Secginli et al., 2006; Han et al., 2000; Miller and Champion, 1996). Alternatively, Schulter (1982) has found that there is no correlation between breast cancer knowledge and screening behavior. As argued by Reddy and Alagna (1986), the relationship between knowledge and participation in mammography as a trial exam is not simple.

Electronic media and TV were noted as the most important sources of getting information on breast cancer in Iran, while health care providers were ranked last (Montazeri et al., 2008). Relatives and friends also were the most common sources for getting information among less educated women (Hatefnia et al., 2010). Regardless of low knowledge of breast cancer in Asian countries, only a few studies evaluated on the methods for increasing awareness by health care providers and local awareness campaigns (Ali and Baig, 2006; Dow et al., 2007; Adib et al., 2009; Tavafian et al., 2009; Moshfeghi and Mohammadbeigi, 2010; Keshtgar and Baum, 2010; Noroozi et al., 2010; Garg et al., 2010). This literature was already cited in the research by Asadzadeh et al., (2011). Definitely, women's knowledge regarding the breast cancer symptoms and screening behaviors is a significant factor to detection of less advanced stage and intensify women's participation in cancer preventive behaviors.

Conclusion

Previous studies conducted in Asian countries have proved that women's individual characteristics, psychosocial factors, and knowledge are imperative to breast cancer health seeking behavior. This literature review is an account of what has been published on the factors influencing breast cancer early detection by scholars and researchers. Even though, fundamental studies in breast cancer prevention and control within the above mentioned factors are still limited.

In order to improve women's participation in breast cancer prevention programs/ screenings, especially among the at-risk subgroup, the intervention strategies should tailor to their knowledge and socio-demographic factors. The strategies adopted should also take into account the women psychological and cultural matters in order to encourage lifelong mammography screening practice for Asian population which is based on theoretical interventions. Based on the socio-psychological theories and models, the interventions are able to change individuals and communities' attitudes towards health. On the same note, healthcare professionals working with Asian women should carefully address the misconceptions such as worry about mammogram devices and fatalism.

Active recruitment strategies and educational materials also have an important effect on women's adherence to breast cancer screening behavior. Women's awareness concerning breast self-examination and physical examination must be taken into account in screening protocols in younger women to further promote breast cancer screening. As breast cancer is a widespread disease in Asian countries, recommendations on breast cancer screening, and its intervals must be made clear to women, and this is a primer role among healthcare professionals.

The Ministry of Health, health care organizations, national cancer councils, cancer programming and research institutes, and advocates in Asian countries would allocate funds to carry out cancer research in identified priority areas to help reduce the breast cancer disease. Future researchers should also focus on designing qualitative studies on barriers to screening like embarrassment, pain, and fear with asymptomatic women from different socioeconomic backgrounds to discover ways to overcome psychosocial, individual and structural barriers to screening.

Diversity of Asian population in terms of cultural and social limits the applicability of an exclusive breast cancer prevention program. Consequently, small scale approaches for breast cancer detection such as compulsory and free mammogram for low socioeconomic status women seem to be practical initiative to identify breast cancer and decrease in the number of late stage tumors among Asian women.

In sum, the aim of this paper is to highlight factors influencing breast cancer screening among Asian women. The above mentioned factors showed how psychosocial and individual determinants can be used to explain and predict individual health-promoting behavior. Many of the examples covered in this paper concern personal and community development strategies. It is through understanding the psychological and demographic barriers to screening, a more affirmative action through appropriate strategies can be developed to change the human's (women) attitudes, broaden their knowledge, and enhance their awareness about the disease. All this effort is about promoting health and well-being, which is parcel of community development endeavor.

References

1. Asadzadeh Vostakolaei F, Broeders MJM, Kiemeney LALM, Verbeek ALM. (2011). Asian Pacific Journal of Cancer Prevention, 12, 2467-2475
2. Abbaszadeh, A., Haghdoost, A., Taebi, M., & Kohan, S. (2007). The relationship between women's health beliefs and their participation in screening mammography. Asian Pacific Journal of Cancer Prevention, 8, 471-475.
3. Abdullah A, Leung T (2000). Factors associated with the use of breast and cervical cancer screening services among Chinese women in Hong Kong. Public Health Journal, 115, 212-7.
4. Adib SM, Sabbah MA, Hlais S, et al (2009). Research in action: mammography utilization following breast cancer awareness campaigns in Lebanon 2002-05. East Mediterr Health J, 15, 6-18.
5. Ahmadian , M (2011). Factors Influencing Women's Participation in Breast Cancer Prevention Program in Tehran, Iran. Doctoral research, Universiti Putra Malaysia.

6. Ahmadian M, Samah A A, Emby Z, Redzuan M (2011). Barriers to Mammography among Women Attending Gynecologic Outpatient Clinics in Tehran, Iran, Journal of Scientific Research and Essays Vol. 6(27), pp. 5803-5811.
7. Ajzen, I., Brown, T. C., & Carvajal, F. (2004). Explaining the discrepancy between intentions and actions: The case of hypothetical bias in contingent valuation. Personality and Social Psychology Bulletin, 30, 1108.
8. Ali TS, Baig S (2006). Evaluation of a cancer awareness campaign: experience with a selected population in Karachi. Asian Pacific J Cancer Prev, 7, 391-5.
9. Allen, J. D., Sorensen, G., Stoddard, A. M., Coldits, G., & Peterson, K. (1998). Intention to have a mammogram in the future among women who have underused mammography in the past. Health Education and Behavior, 25, 474-488.
10. Bandura, A., & Adams, N. E. (1977). Analysis of self-efficacy theory of behavioral change. Cognitive Therapy and Research, 1(4), 287-310.
11. Bener A, Honein G, Carter A, Da'ar Z. (2002). The determinants of breast cancer screening behavior: A focus group study of women in the United Arab Emirates. Oncology Nursing Forum, 29, 91-8.
12. Blanchard, K. Colbert, J. A., & Puri, D. (2004). Mammographic screening: Patterns of use and estimated impact on breast carcinoma survival. Cancer 101, 3, 495–507.
13. Bosompra, K. (2001). Determinants of condom use intentions of university students in Ghana: An application of the theory of reasoned action. Social Science and Medicine, 52 (7), 1057-1069.
14. Brailey, L. J. (1986). Effects of health teaching in the workplace on women's knowledge, beliefs, and practices regarding breast self-examination. Research in Nursing and Health, 9, 223-231.
15. Bray, M. (1996). Decentralization of Education: Community Financing. Washington, DC: World Bank.
16. Champion, V. (1993). Instrument refinement for breast cancer screening behaviors. Nursing Research, 42, 139-143.
17. Champion, V. L. (1992). Compliance with guidelines for mammography screening. Cancer Detection and Prevention, 16, 253-258.
18. Champion, V., & Menon, U. (1997). Reliability and validity of breast cancer screening scales in African American women. Nursing Research, 46, 331-337.
19. Choudhry, U. K., Srivastava, R., & Fitch, M. I. (1998). Breast cancer detection practices of South Asia women: Knowledge, beliefs, and beliefs. Oncology Nursing Forum Journal, 25, 1693-701.
20. Chua M, Franzcr M, Mok T, et al (2005). Knowledge, perceptions and attitudes of Hong Kong Chinese women on screening mammography and early breast cancer management. Breast Journal ,11, 52–6.
21. Crane, L. A, Kaplan, C. P, & Bastani, R. (1996). Determinants of adherence among health department patients referred for a mammogram. Women and Health, 24(2), 43-6.
22. Dow Meneses K, Yarbro CH (2007). Cultural perspectives of international breast health and breast cancer education. J Nurs Scholarsh, 39, 105-12.
23. Edgar, L., Shamian, J., & Patterson, D. (1984). Factors affecting the nurse as a teacher and practice of breast self-examination. International Journal of Nursing Studies, 21, 255-265.
24. Estrada, A., Trevino, F., & Ray, L. (1990). Health care utilization barriers among Mexican-Americans: Evidence from 1982-1984. American Journal of Public Health, 80(1), 27-31.
25. Ferlay J, Bray F, Pisani P (2001). Globocan 2000: Cancer Incidence,Mortality and Prevalence Worldwide, Lyon: IARC Press, version 1.0 IARC Cancer Base. No 5.
26. Finney, R. L., & Iannotti, R. (2003). Health beliefs, salience of breast cancer family history, and involvement with breast cancer issues: Adherence to annual mammography screening recommendations. Cancer Detection and Prevention Journal, 27, 353-59.
27. Fung, S. Y. (1998). Factors associated with breast self-examination behavior among Chinese women in Hong Kong. Patient Education Counseling, 33, 233-43.
28. Garbers, S., Jessop, D. J., Foti, H., Uribelarrea, M., & Chiasson, M.A. (2003). Barriers to breast cancer screening for low-income Mexican and Dominican women in New York City. Journal of Urban Health, 80(1), 81-91.
29. Garg P, Bansal M, Garg M, Arora B (2010). Creating awareness about the painless nature of early breast cancer lump is important in low-income countries. Breast J, 16, 101-2.
30. Gilani S.I., Khurram M., Mazhar T., Mir S.T., Ali S., Tariq S. & Malik A.Z. (2010) Knowledge, attitude and practice of a Pakistani female cohort towards breast cancer. Journal of Pakistan Medicine Association 60, 205–208.
31. Gollwitzer, P. M. (1993). Goal achievement: The role of intentions. European Review of Social Psychology, 4, 141.
32. Gotay, C., & Wilson, M. E. (1998). Social support and breast cancer screening in African American, Hispanic, and Native American women. Cancer Practice, 6, 31-37.
33. Gotzsche PC, Nielsen M: (2006). Screening for breast cancer with mammography. Cochrane Database System Review Art No.: CD001877.
34. Han, Y, Williams, R. D., & Harrison, R. A. (2000). Breast cancer screening knowledge, attitudes, and practices among Korean American women. Oncology Nursing Forum, 27, 1585-1589.
35. Harirchi I, Karbakhsh M, Kashefi A, Momtahen AJ (2004). Breast cancer in Iran: results of multi-center study. Asia Pacific Journal Cancer Prevention, 5, 24-27.
36. Hatefnia E, Niknami S, Bazargan M, et al (2010). Correlates of mammography utilization among working Muslim Iranian women. Health Care Women Int, 31, 499-514.
37. Hiatt, R. A. (1996). Pathways to early cancer detection in the multiethnic population of San Francisco Bay Area. Health Education Quarterly, 23, 10-27.
38. Hisham A N, Yip C H (2003). Spectrum of breast cancer in Malaysian women: An overview. World Journal of Surgery, 27, 921-3.
39. Hisham AN, Yip CH (2004). Overview of breast cancer in Malaysian women: a problem with late diagnosis. Asian Journal of Surgery, 27, 130-133.
40. Hoare, T., Thomas, C, Biggs, A., Booth, M., Bradley, S., Friedman, E. (1994). Can the uptake of screening behavior by Asian women be increased? A randomized controlled trial of link worker intervention. Journal of Public Health Medicine, 16(2), 179-185.
41. Hoskins, C. N., Baker, S., Sherman, D., Bohlander, J., & Bookbinder, M. (1996). Social support and patterns of adjustment to breast cancer. Scholarly Inquiry for Nursing Practice, 10, 99-123.

42. Im E. O, Park Y S, Lee EO (2004). Korean women's attitudes toward breast cancer screening tests. International Journal of Nursing Studies, 41, 583-9.
43. Janz, N. K., & Becker, M. H. (1984). The Health Belief Model: A decade later. Health Education Quarterly, II, 1-47.
44. Jarvandi S, Montazeri A, Harirchi I , Kazemnejad A (2002). Beliefs and behaviors of Iranian teachers toward early detection of breast cancer and breast self-examination. Public Health, 116, 245-249.
45. Juon H S, Kim M, Shankar S (2004). Predictors of adherence to screening mammography among Korean American women. Preventive Medicine, 39, 474-81. .
46. Katapodi, M. C., Facione, N. C., & Miaskowski, C. (2002). The influence of social support on breast cancer screening in a multicultural community sample. Oncology Nursing Forum, 29(5), 845-52.
47. Keshtgar M and Baum M (2010). A new approach to treating breast cancer combining tumor removal and intraoperative radiotherapy: is it viable? Womens Health, 6, 9-12.
48. Kim, R (2002). Use of a theoretical framework to understand factors that influence participation in mammography screening among Korean women. Doctoral research, University of Texas.
49. Lechner, L., de Vries, H., & Offermans, N. (1997). Participation in a breast cancer screening program: Influence of past behavior and determinants on future screening participation. Preventive Medicine, 26(4), 473-482.
50. Lee C Y, Kim H S, Ham O. (2000). Knowledge, practice, and risk of breast cancer among rural women in Korea. Nursing and Health Science, 2(4), 225-230.
51. Lugton, J. (1997). The nature of social support as experienced by women treated for breast cancer. Journal of Advanced Nursing, 25(6), 1184.
52. Madan, A., Barden, C., & Beech, B. (2000). Socioeconomic factors, not ethnicity, predict breast self examination. Breast Journal, 6, 263-6.
53. McCance, K. L., Mooney, K. H., & Field, R. (1996). The influence of others in motivating women to obtain breast cancer screening. Cancer Practice, 4(3), 141-6.
54. McPhee, S. J. (1997). Barriers to breast and cervical cancer screening among Vietnamese-American women. American Journal of Preventive Medicine, 13, 205-213.
55. Meleis, A. & Hatter-Pollard, M. (1995). Arab Middle Eastern American women. Stereotyped, invisible, but powerful. In D. L. Adams (Ed.), Health Issues for Women of Color: A cultural diversity perspective (pp. 133-163). Sage Publications.
56. Miller, A. M., & Champion, V. L. (1997). Attitudes about breast cancer and mammography: Racial, income, and educational differences. Women and Health, 26, 41-63.
57. Montaño, D. E., Thompson, B., Taylor, V. M., & Mahloch, J. (1997). Understanding mammography intention and utilization among women in an inner city public hospital clinic. Preventive Medicine, 26(6), 817-824.
58. Montazeri A, Haji-Mahmoodi M, Jarvandi S. (2003). Breast self-examination: do religious beliefs matter? A descriptive study. Journal of Public Health Medicine, 25 (2): 154-5.
59. Montazeri, A., Vahdaninia, M., Harirchi, I., Harirchi, A. M., Sajadian, A., & Khaleghi, F. (2008). Breast cancer in Iran: The need for greater women awareness of warning signs and effective screening methods. Asia Pacific Family Medicine, 7(1), 6.
60. Moshfeghi K, Mohammadbeigi A (2010). Comparison the effects of two educational methods on knowledge attitude and practices of Arak physicians about breast cancer. Pakistan J Biological Sci, 13, 901-5.
61. Mousavi, S., Gouya, M., Ramazani, R., Davanlou, M., Hajsadeghi, N., & Seddighi, Z. (2009). Cancer incidence and mortality in Iran. Annuals of Oncology, 20(3), 556.
62. Narimah, A. (1997). Breast examination. Report of second national health and morbidity by Public Health Institute, Ministry of Health Malaysia, 145-148.
63. Navon, L. (1999). Voices from the world. Cancer Nursing, 22, 39-45.
64. Nissan A, Spira M, Hamburger T (2004). Clinical profile of breast cancer in Arab and Jewish women in the Jerusalem area. American Journal of Surgery, 188, 62-7.
65. Noroozi A, Jomand T, Tahmasebi R (2010). Determinants of breast self-examination performance among Iranian women: An application of the health belief model. J Cancer Educ, 26, 365-74.
66. Okobia MN, Bunker CH, Okonofua FE, Osime U. (2006) Knowledge, attitude and practice of Nigerian women towards breast cancer: a cross-sectional study. World Journal of Surgical Oncology. Feb 21; 4:11.
67. O'Malley, A. S., Earp, J. A., & Harris, R. P. (1997). Race and mammography use in two North Carolina counties. American Journal of Public health, 87(5), 782-786.
68. Parsa, P., & Kandiah, M. (2005). Breast cancer knowledge, perception and breast self-examination practices among Iranian women. Internal Medicine Journal. 4, 17Y24.
69. Parsa, P., Kandiah, M., Abdul Rahman, H., & Zulkefli, N. (2006). Barriers for breast cancer screening among Asian women: A mini literature review. Asian Pacific Journal of Cancer Prevention, 7(4), 509.
70. Parsa, P., Kandiah, M., Mohd Zulkefli, N., & Rahman, H. (2008). Knowledge and behavior regarding breast cancer screening among female teachers in Selangor, Malaysia. Asian Pacific Journal of Cancer Prevention, 9(2), 221-227.
71. Persson, K., Svensson, P., & Ek, A. (1997). Breast self-examination: An analysis of self-reported practice. Journal of Advanced Nursing, 25, 886-92.
72. Petro-Nustus W, Mikhail B (2002). Factors associated with breast self examination among Jordanian women. Public Health Nursing, 19, 263-71.
73. Poss, J. E. (2001). Developing a new model for cross-cultural research: Synthesizing the Health Belief Model and the Theory of Reasoned Action. Advances in Nursing Science, 23(4), 1-15.
74. Rajaram, S., & Rashidi, A. (1999). Asian-Islamic women and breast cancer screening: A socio-cultural Analysis. Women and Health, 28(3), 45-58.
75. Rakowski W, Dube C E, Marcus B H (1992). Assessing elements of women's decisions about mammography. Health Psychology Journal, 11, 111.
76. Rashidi, A., & Rajaram, S. (2000). Middle Eastern Islamic Women and Breast Self-Examination. Cancer Nursing, 23, 64-71.
77. Reddy and Alagna, 1986. D. Reddy and S.A. Alagna, Psychological aspects of cancer prevention and early detection among women. In: B.L. Andersen, Editor, Women with cancer: Psychological perspectives, Springer-Verlag, New York (1986), pp. 93–137.
78. Rutter, D. R., Steadman, L., & Quine, L. (2006). An implementation intentions intervention to increase uptake of mammography. Annals of Behavioral Medicine 32, 127.

79. Sadler GR, Ryujin LT, Ko CM, Nguyen E.(2001) Korean women: breast cancer knowledge, attitudes and behaviors. BMC Public Health. 1:7.
80. Saint-Germain, M. A., Longman, A. J. (1993). Breast Cancer Screening among older Hispanic Women: Knowledge, attitudes, and practices. Health Education Quarterly, 20(4), 539-553.
81. Savage, S. A., & Clarke, V. A. (1996). Factors associated with screening mammography and breast self-examination intentions. Health Education Research, 11(4), 409.
82. Schulter, L.A. (1982). Knowledge and beliefs about breast cancer and breast self-examination among athletic and non-athletic women. Nursing Research, 31, 348-353.
83. Schwartz, K., Fakhouri, M., Bartoces, M., Monsur, J., & Younis, A. (2008). Mammography screening among Arab American women in metropolitan Detroit. Journal of Immigrant and Minority Health, 10(6), 541-549.
84. Secginli S, Nahcivan NO (2006). Factors associated with breast cancer screening behaviors in a sample of Turkish women: A questionnaire survey. International Journal of Nursing Studies, 43, 161-71.
85. Sheeran, P. (2002) Intention-behavior relations: A conceptual and empirical review. European Review of Social Psychology, 12, 1
86. Shirazi, M., Champeau, D., & Talebi, A. (2006). Predictors of breast cancer screening among immigrant Iranian women in California. Journal Women's Health, 15(5), 485-506.
87. Slenker, S. E., & Grant, M. C. (1989). Attitudes, beliefs, and knowledge about mammography among women forty years of age. Journal of Cancer Education, 4, 61-65.
88. Smith R, Maira C, Ute S, et al (2006). Breast cancer in limited resource countries: early detection and access to care. BreastJ, 12, 16-26.
89. Smith, R. A., & Biddle, S. J. (1999). Attitudes and Exercise Adherence: Test of the Theories of Reasoned Action and Planned Behavior. Journal of Sports Science, 17(4), 269-81.
90. Straughan, P., & Seow, A. (2000). Attitude as barriers in breast screening: A prospective study among Singapore women. Social Science and Medicine, 51, 1695-703
91. Suarez, L., Roche, R. A., Nichols, D., & Simpson, D. M. (1997). Knowledge, behavior, and fears concerning breast and cervical cancer among older low-income Mexican-American women. American Journal of Preventive Medicine, 13, 137–142.
92. Taleghani F, Yekta ZP, Nasrabadi AN. (2006).Coping with breast cancer in newly diagnosed Iranian women. Journal of Advanced Nursing. May; 54(3):265-272
93. Tavafian SS, Hasani L, Aghamolaei T, et al (2009). Prediction of breast self-examination in a sample of Iranian women: an application of the health belief model. BMC Womens Health, 9, 37-40.
94. Tessaro, I., & Smith, J. (1994). Breast cancer screening in older African-American women: Qualitative research findings. American Journal of Health Promotion, 8(4), 286-92.
95. Thongsuksai P, Sripung H (2000). Delay in breast cancer care: a study in Thai women. Medical Care. 38,108-14.
96. Umeh, K. & Rogan-Gibson, J. (2001). British Journal of Health Psychology, 6(4), 361-372.
97. Usmani K, Khanum A, Afzal H, Ahmad N (1996). Breast cancer in Pakistani women. Journal of Environmental Pathology, Toxicology and Oncology, 15, 251-3.
98. Wallace, L. S. (2002). Osteoporosis prevention in college women: Application of the expanded health belief model. American Journal of Health Behavior, 26(3), 163-172.
99. Wu, T. Y., West, M. A., Chen, Y. W., & Hergert, C. (2006). Health beliefs and practices related to breast cancer screening in Filipino, Chinese and Asian-Indian women. Cancer Detection and Prevention Journal, 30, 58-66.
100. Yip CH, Ng EH (1996). Breast cancer- a comparative study between Malaysian and Singaporean women. Singapore Medical Journal J, 37, 264-7.
101. Yoo KY (2010). Cancer prevention in the Asia pacific region, Asian Pacific Journal of Cancer Prevention. 11.839-44
102. Yoo KY, Kim Y, Park SK, et al (2006). Lifestyle, genetic susceptibility and future trends of breast cancer in Korea. Asian Pac J Cancer Prev, 7, 679-82.
103. Yu, M. Y., Hong, O. S., & Seetoo, A. D. (2003). Uncovering factors contributing to under utilization of breast cancer screening by Chinese and Korean women living in the United States. Ethnicity and Disease, 13(2), 213-9.

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Factors Influencing Households' Income Shock Exposure and Coping Options in Nigeria

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Abstract: Income shock is the driving force of poverty in Nigeria. This study analyzed the different forms of shocks that households experienced with some welfare losses. The Core Welfare Indicator Questionnaire (CWIQ) data that comprise of 75329 households were used. The data were analyzed with simple descriptive methods and Probit regression. The results show that probability of shock exposure decreases significantly ($p<0.01$) with access to improved drinking water, improved toilet, health facility well/borehole, agricultural inputs, agricultural produce buyers, consumer goods, employment opportunities, assets and credit facilities. It was recommended that ensuring that development projects target the poor will assist in reducing their exposure to shocks.

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1. Introduction

Conventional approaches to analyze poverty typically focus on understanding the levels and distribution of welfare in a specific context. Such efforts are essentially crude, and are rarely channeled towards informing policy makers about the underlying processes that contribute to poverty dynamics through poverty modeling that takes cognizance of households' exposure to income or consumption shocks. Recently, a natural complement to the traditional poverty analysis is the introduction of shocks variables that makes it possible to assess households' vulnerability. This adds some values to the processes of policy dialogue by introducing a comprehensive framework to properly understand poverty dynamics or vulnerability and the reasons behind those that are chronically poor (Hoogeveen *et al*., no date).

Conceptually, vulnerability describes occurrence of events that have undesired effects on individuals, households, communities, countries and enterprises (Cuna, 2004). It is the outcome of risk exposure and coping capacity of the households (Chambers, 1989). Cuna (2004) defined "vulnerability as the inability of a household to secure its living standards in the face of a certain negative event". This definition portrays vulnerability as a combination of exposure to negative events and the capacity of the households to cope with it (Chambers, 1989). Therefore, it is important to have a clear understanding of the nature of the shock (Sinha and Lipton, 1999), transmission mechanism and households' or communities' coping options for better policy information (Shaffer, 2001; Dercon, 2001).

In Nigeria, the growing problem of poverty had been described as suffering in the midst of plenty (World Bank, 1996). Precisely, 65.6 percent of the population - (about 67.5 million) - was poor in 1996. The proportion reduced to 54.4 percent in 2004 (about 72 million) (FGN, 2005) before increasing to 69 percent in 2010 {National Bureau of Statistics (NBS), 2010}. These scenarios clearly reveal that poverty as a problem in Nigeria should be addressed with some notions of emergencies. The Nigerian government has focused on the National Poverty Eradication Programme (NAPEP) that was introduced early in 2001 as one of the foremost poverty alleviation programs. Also, given the multidisciplinary approach that is required for poverty alleviation, some government parastatals have been saddled with the responsibilities of implementing some development programs that are meant for reaching the poor. Thus, achieving the MDG of halving poverty level by 2015, which is a prerequisite for achieving the other seven profoundly attractive goals, is a daunting challenge that Nigerian policy makers must address.

It should be emphasized that previous poverty reduction programs in Nigeria did not fully achieve their objectives. It is therefore not sure whether the country lacks sufficient capacity to mitigate social risks faced by households and communities, and/or whether the country has not paid sufficient attention to the issue of shocks and uncertainty that are important for understanding the processes of poverty dynamics (Alayande and Alayande, 2004). Therefore, to fully address poverty, Nigerian policy makers need a more comprehensive understanding of the different forms of shocks that subject households to perpetual poverty. This is important because of the implications of different policy reforms that the economy had undergone in the recent time.

Furthermore, given the importance of welfare shocks, policy makers are beginning to implement programmes to cater for the needs of vulnerable households. Essentially, Christiansen and Subbarao (2001) submitted that the need for addressing vulnerability in any human development strategy in conjunction with poverty is two fold. First, not being vulnerable has some intrinsic value. This is because for a person to be considered non-poor, he must not only have enough to live a comfortable life today, but he must also possess some good prospect today that he will have enough to live a comfortable life tomorrow. Second, addressing vulnerability has instrumental value. Because of the many shocks household face, they often experience wide variability in their incomes. In absence of sufficient assets or insurance to smooth consumption, such shocks may lead to irreversible welfare losses, such as distress sale of productive assets, reduced nutrient intake, or interruption of education that permanently reduces human capital, thereby locking their victims in perpetual poverty.

It should also be noted that while many studies have addressed the impact of shocks on households' welfare, not much emphasis had been placed on determining those factors that expose them to shocks. For instance, we may ask ourselves, is it because of their lack of education, assets, residence in certain part of the country or some other reasons that make households to experience income shock? Therefore, this paper seeks to answer two questions: First, what are the socio-economic characteristics of those that are affected by income shocks? Second, what are the different coping options available to shock affected households? Provision of answers to these questions will form some bases for policy formulation in order to reverse the upward trend of poverty in Nigeria. In the remaining parts of the paper, materials and methods, results and discussions and recommendations are presented.

2. Materials and Methods

The Data

We used the 2006 Core Welfare Indicator Questionnaire (CWIQ) survey data. The survey was conducted by the National Bureau of Statistics (NBS). A two-stage cluster sample design was adopted in each LGA. The first stage involves the Enumeration Areas (EAs), while Housing Units (HUs) constitute the 2nd stage. The National Population Commission (NPopC) EAs as demarcated during the 1991 Population Census served as the sampling frame for the selection of 1st stage sample units. In each LGA, a systematic selection of 10 EAs was made. Prior to the second stage selection, complete listing of Housing Units (and of Households within Housing Units) was carried out in each of the selected 1st stage units. These lists provided the frames for the second stage selection. Ten (10) HUs were then systematically selected per EA and all households in the selected HUs were interviewed. The projected sample size was 100 HUs at the LGA level. The sample size using other defined reporting domains (FC, senatorial, state and geo-political zone) varied, depending on the number of the LGAs that made the reporting domain. Overall, 77,400 HUs were drawn at the national level out of which 59567 were from the rural areas and 17833 from urban areas. However, only 75329 were properly completed, and these were used for this study.

Probit Regression

The Probit regression method was used to determine the factors that predispose farm households to shock. We are interested in estimating the probability that the respondents are vulnerable to welfare shocks given some implemented development projects and their socio-economic characteristics (X_i). The estimated equation can be expressed as:

$$Y_i = \propto + \beta_i \sum_{i=1}^{n} X_{i+} e_i \qquad 1$$

$\propto$ is the constant, β_i is slope of coefficient e_i is the error term. Also, X_i are the explanatory variables where i = 1, 2, 3, ,….., n. The explanatory variables are Food problem (yes = 1, 0 otherwise), school fees problem (yes = 1, 0 otherwise), house rent problem (yes = 1, 0 otherwise), utility problem (yes = 1, 0 otherwise), health bill problem (yes = 1, 0 otherwise), materials of the roof (improved = 1, 0 otherwise), materials of the wall (improved = 1, 0 otherwise), materials of the floor (improved = 1, 0 otherwise), type of housing unit (flat/duplex/whole building = 1, 0 otherwise), improved drinking water (yes =1, 0 otherwise), problem with drinking water (yes = 1, 0 otherwise), improved toilet (yes = 1, 0 otherwise), electricity (yes = 1, 0 otherwise), modern cooking fuel (yes = 1, 0 otherwise), safe type of refuse collection (yes = 1, 0 otherwise), building of school project (yes = 1, 0 otherwise), rehabilitation of school (yes = 1, 0 otherwise), building of health facility (yes = 1, 0 otherwise), rehabilitation of health facility (yes = 1, 0 otherwise), sanitation project (yes = 1, 0 otherwise), building of new roads (yes = 1, 0 otherwise), tarring/grading of roads (yes = 1, 0 otherwise), transport services (yes = 1, 0 otherwise), sinking of well/borehole (yes = 1, 0 otherwise), piping of water (yes = 1, 0 otherwise), rehabilitation of pipe water (yes = 1, 0 otherwise), agriculture input on credit (yes = 1, 0 otherwise), agricultural inputs readily available (yes = 1, 0 otherwise), buyer of

agriculture produce (yes = 1, 0 otherwise), availability of extension services (yes = 1, 0 otherwise), veterinary services (yes = 1, 0 otherwise), consumer goods now available (yes = 1, 0 otherwise), employment opportunities available (yes = 1, 0 otherwise), (yes = 1, 0 otherwise), more people owning houses (yes = 1, 0 otherwise), police services now available (yes = 1, 0 otherwise), credit facility now being provided (yes = 1, 0 otherwise), electrification (yes = 1, 0 otherwise), rehabilitation of electric facility (yes = 1, 0 otherwise), reforestation (yes = 1, 0 otherwise), rural area (yes = 1, 0 otherwise), household size, age (years), North East (yes = 1, 0 otherwise), North West (yes = 1, 0 otherwise), North Central (yes = 1, 0 otherwise), South East (yes = 1, 0 otherwise), South West (yes = 1, 0 otherwise), Monogamy (yes = 1, 0 otherwise), Polygamy (yes = 1, 0 otherwise), Divorced/separated (yes = 1, 0 otherwise), Asset index, Gender (male =1, 0 otherwise) and tertiary education (yes = 1, 0 otherwise).

3. Results

Income shocks experienced by households

The different form of income shocks experienced by the households, presented against their socio-economic characteristics are contained in tables 1. It shows the distribution of income shocks experienced by the households across the six geopolitical zones (GPZ) and economic sectors in Nigeria. It shows that 50.75 percent of the respondents in North East did not experience any shock, which is also the highest in all the zones. South West zone has the next highest value of 41.10 percent. In the rural sector, 35.45 percent of the respondents did not experience any shock, which can be compared with 42.99 percent for urban sector. In the combined data, 37.17 percent of the respondents did not experience any shock.

The table further shows that across the GPZs and sectors, the most commonly experienced shocks include not able to afford agricultural input prices, agricultural inputs not available, hard economic times/economic decline, lack of capital to start or expand agricultural production, low agricultural production, lack of employment/job opportunities and prices of commodities too high. Also, the least experienced shocks include delayed payment of gratuities, cultural/religious shocks, irregular payment of pension, too much competition and retrenchment/redundancy.

Table 1: Percentage distribution of respondents' shocks across geopolitical zones and sectors in Nigeria

Shocks experienced	NW	NE	NC	SE	SW	SS	Rural	Urban	All
No shock	25.06	50.75	38.45	24.58	41.10	31.80	35.45	42.99	37.17
Cannot afford agricultural input prices	23.76	18.15	17.82	20.74	15.28	29.58	21.80	16.35	20.56
Agricultural inputs not available	15.00	8.76	10.53	4.96	3.41	2.15	8.55	3.77	7.46
Low agricultural production	5.60	3.27	4.39	3.44	2.43	2.12	3.99	1.67	3.46
Drought	0.72	0.78	0.63	0.33	0.34	0.04	0.54	0.34	0.50
Lack of adequate land	1.02	0.95	0.29	3.05	0.23	1.09	1.14	0.59	1.02
Low prices for agricultural produce	1.89	0.65	2.24	1.69	2.94	1.49	1.98	0.90	1.74
Lack of market/buyers	0.74	0.46	0.86	1.93	1.70	1.32	1.09	1.12	1.10
Lack/loss of cattle/oxen due to disease	0.62	0.30	0.21	0.40	0.11	0.11	0.33	0.10	0.28
Lack of capital to start or expand agricultural production	4.55	3.12	4.25	7.09	2.68	3.88	4.40	2.78	4.03
Lack of capital to start or expand own business	1.62	1.28	2.78	5.08	4.05	2.92	2.38	4.14	2.78
Lack of credit to start or expand agricultural production	0.70	0.97	0.79	0.84	1.20	0.68	0.88	0.90	0.88
Lack of credit facilities to start or expand own business	0.71	0.54	0.85	1.42	1.32	1.19	0.92	1.11	0.96
Lack of employment/job opportunities	2.30	1.74	2.75	7.54	2.91	7.65	3.72	4.26	3.84
Salaries/wage too low	2.17	0.59	2.48	1.65	1.66	1.96	1.29	2.83	1.64
Retrenchment/redundancy	0.07	0.03	0.20	0.26	0.20	0.08	0.09	0.27	0.13
Prices of commodities too high	3.08	2.11	2.29	2.72	3.92	1.77	2.46	3.17	2.62
Hard economic times/economic decline	7.96	4.09	5.74	8.61	9.34	7.03	6.36	8.57	6.86
Business not doing well	0.62	0.45	0.92	1.74	2.15	1.72	1.02	1.85	1.21
Low profit	0.88	0.54	0.58	0.94	1.99	0.71	0.81	1.34	0.93
Too much competition	0.21	0.12	0.29	0.12	0.33	0.07	0.17	0.26	0.19
Cultural/religious reasons	0.05	0.04	0.04	0.01	0.03	0.00	0.03	0.04	0.03
Irregular payment of pension	0.04	0.02	0.19	0.45	0.28	0.20	0.13	0.33	0.18
Delayed payment of gratuities	0.06	0.03	0.05	0.14	0.06	0.08	0.06	0.07	0.06
Others	0.57	0.26	0.39	0.28	0.34	0.34	0.39	0.24	0.36

Source: Author's computations from the Core Welfare Indicator Questionnaire (CWIQ) Survey Data 2006

Specifically, 29.58 percent and 23.76 percent of the respondents from South South and North West GPZs indicated inability to afford prices of agricultural inputs as a major shock that had promoted poverty. In addition, 15.00 percent of the respondents in North West, 10.53 percent in the North Central and 8.76 percent in the North East indicated non-availability of agricultural inputs as the major shock experienced. These values constitute the highest proportions across the zones. In the rural sector, 21.80 percent and 8.55 percent could not afford agricultural input prices and unable to get the needed agricultural inputs, respectively.

The results further indicate that while low agricultural production was mostly reported in the northern zones, lack of market/buyers, lack of capital to start or expand agricultural production, lack of capital to start or expand own business, lack of credit facilities to start or expand own business, lack of employment/job opportunities, hard economic times/economic decline and business not doing well were reported most in the zones from the south. Across the sectors of the economy,

Determinants of shock exposure

Table 2 shows the results of the Probit regression to determine the factors influencing shock exposure. The Likelihood Ratio Chi-Square value is statistically significant ($p<0.01$). This shows that the model produced a good fit for the data. Many of the included parameters are statistically significant ($p<0.05$). Specifically, households that sometimes have problems meeting their food needs have significantly lower probability of experiencing shocks ($p<0.01$). This is because food is basic need for everybody. Also, those households that indicated having problems with payment of children's school fees have significantly lower probability of experiencing shocks. The households that have problem with payment of school fees have significantly higher probability of experiencing shocks ($p<0.01$). Those that indicated problem with payment of utility bill have significantly lower probability of experiencing shocks ($P<0.01$).

The households that used improved materials for the floor of their houses have significantly lower probability of experiencing shocks ($p<0.01$). Also, the households that are resident in flats, duplex or a whole house and those with access to improved drinking water sources have significantly lower probability experiencing shocks ($P<0.01$). Similarly, access to improved cooking fuel and toilet have significantly lower probability of experiencing shocks ($p<0.01$).

Table 2: Probit Regression Results of the Determinants of Shock Exposure

Variables	Coefficients	t-	Mean
Food problem	-.3371284***	-21.30	.8253614
School fees problem	-.1003013***	-5.99	.8337515
House rent problem	.1941164***	10.44	.8738567
Utility problem	-.151428***	-9.46	.8108514
Health bill problem	-.005453	-0.39	.7834375
Materials of the roof	-.00116	-0.08	.6454923
Materials of the wall	-.0088784	-0.62	.4278944
Materials of the floor	-.0571622***	-4.35	.5555644
Type of housing unit	-.0585466***	-5.23	.3411127
Improved drinking water	-.0383306***	-7.98	.8022356
Problem with drinking water	.0202815	1.53	.8342294
Toilet	-.0922786***	-9.06	.5299693
Electricity	.1258705***	9.38	.4335364
Cooking fuel	-.1860918***	-11.12	.1653192
Type of refuse collection	-.0189912	-0.68	.0342905
Building of school	-.1286696***	-9.85	.2163368
Rehabilitation of school	.0574952***	4.51	.2699563
Building of health facility	-.0691322***	-4.22	.123767
Rehabilitation of health	-.0691322	-1.31	.1402021
Sanitation	-.0029482	-0.18	.1146601
Building of new roads	.0134982	0.70	.0779535
Tarring/Grading of roads	-.0072887	-0.46	.1280948
Transport services	-.0007769	-0.04	.0969374
Sinking of well/borehole	-.0455199***	-3.62	.2015346
Piping of water	.0034884	0.13	.0388307
Rehabilitation of pipe water	.0337656	1.10	.029737
Agriculture input on credit	-.1350295***	-3.82	.0225284
Agricultural inputs readily	-.1404852***	-5.33	.045256
Buyer of agriculture produce	-.0734263***	-4.40	.140056
Availability of extension	-.0389538	-1.16	.0266438
Veterinary services	.0687996***	2.59	.0399724
Consumer goods now	-.0411177***	-2.71	.1723419
Employment opportunities	-.0826801***	-2.71	.0294317
More people owning houses	-.0229253	-1.62	.1760059
Police services now available	.0107068	0.63	.1180055
Credit facility now being	-.1016799***	-2.54	.0164881
Electrification	.0006792	0.04	.1088853
Rehabilitation of electric	.0440585**	2.17	.0734796
Reforestation	.0528063	1.41	.0175103
Household size	.0008664	0.48	4.953377
Age	-.000259	-0.78	47.46374
North East	-.0162383	-0.79	.1469725
North West	-.6030676***	-31.99	.244255
North Central	-.1900136 ***	-10.27	.1492559
South East	.2689677 ***	13.03	.1225723
South West	-.173503 ***	-9.63	.1778645
Monogamy	.0205809	0.92	.069736
Polygamy	-.0135351	-1.04	.5995725
Divorced/separated	.0535271**	2.30	.1421668
Asset index	-.2990758***	-38.67	9.64e-07
Gender	.0038449	0.18	.8644311
Tertiary education	.0262002	1.62	.1090446
Constant	1.078803***	25.80	
LR chi2(53) =	Pseudo R^2 =		

Note: *** statistically significant at 1 percent, ** statistically significant at 5 percent

Source: Author's computations from the Core Welfare Indicator Questionnaire (CWIQ) Survey Data 2006

However, connection of house to electricity increases the probability of experiencing shocks ($p<0.01$). This is a reflection of the erratic nature of electricity supply, that can even generate some other forms of shocks to the households.

On development projects, the results show that those that benefited from building of schools have significantly lower probability of experiencing shocks ($p<0.01$).However, those that benefited from rehabilitation of schools have significantly higher probability of experiencing shocks ($p<0.01$). Also those that benefited from building of health facilities and sinking of borehole have significantly lower probability of experiencing shocks ($p<0.01$). Our results also show that those households that benefited from agricultural inputs on supply, agricultural input on credit and buyers of agricultural produce significantly reduce probability of experiencing shocks ($p<0.01$). Similarly, households that benefited from available consumer goods, employment opportunities and credit facilities have significantly lower probability of experiencing shocks ($p<0.01$). However, those that benefited from veterinary services and electricity service rehabilitation have significantly higher probability of experiencing shocks ($p<0.01$).

Furthermore, the estimated parameters for North-East, North-West, North-Central and South West show that residing in those zone significantly reduces the probability of experiencing shocks ($p<0.01$). However, residence in South-East increases the probability of experiencing shocks. Those that were divorced or separated have higher probability of experiencing shocks ($P<0.01$). Also, as the asset index increases, the probability of experiencing shocks decreases significantly ($p<0.01$).

Shock coping methods by the households

The coping methods of households are presented in table 3. It shows that the highest proportion of households in North East (22.45 percent) and South East (17.30 percent) depended on piece work on farms belonging to other households. Engagement in other piece works was used as a coping method by 17.17 percent of the households in the North East, 15.59 percent in the North West and 13.96 percent in the South South. Substitution of ordinary meals with fruits, reduction in the number of meals and informal borrowing are largely used by households from southern zones. Specifically, 20.01 percent, 20.84 percent and 18.78% of households in the South East, South South and South West zones respectively depended on reducing the number of meals to cope with income shocks. These values can be compared with 10.49%, 8.23% and 16.40% for North West, North East and North Central respectively. It should also be noted that sale of asset was largely used by zone from the north. Precisely, 13.68 percent of the households in North West and 7.63 percent in North East used this method.

Table 3: Percentage distribution of respondents' shock coping methods across geopolitical zones and sectors in Nigeria

Coping methods	NW	NE	NC	SE	SW	SS	Rural	Urban	All
None	5.65	7.62	13.47	9.55	5.84	4.49	7.12	9.35	7.63
Piece work on farms belonging to other households	15.59	22.45	11.54	17.30	4.90	15.93	17.02	8.25	15.02
Other piece works	15.59	17.17	11.22	4.88	10.18	13.96	13.31	10.94	12.77
Working on food-for-work programme	1.08	2.93	1.37	1.44	1.03	3.29	2.08	1.57	1.96
Relieve food, free food from government/other bodies	1.14	1.09	0.53	0.75	0.92	0.33	0.82	0.81	0.82
Eating wild food only	1.50	1.94	2.64	2.85	5.44	0.86	2.55	2.52	2.54
Substituting ordinary meals with fruits	5.73	2.93	6.65	9.76	6.91	6.74	5.90	6.60	6.06
Reducing number of meals	10.49	8.23	16.40	20.01	18.78	20.84	14.14	18.47	15.13
Reducing other household items	5.56	3.89	8.95	4.50	6.61	5.14	5.45	6.31	5.65
Informal borrowing	8.94	6.75	7.49	9.19	12.96	9.49	8.68	10.18	9.02
Formal borrowing in cash or kind	1.09	1.53	1.86	0.99	1.77	1.06	1.15	2.31	1.42
Church charity	0.10	0.16	1.11	0.56	0.80	0.56	0.44	0.80	0.52
Withdrawing children out of school	0.23	0.46	0.41	1.44	0.24	0.65	0.48	0.73	0.53
Sale of assets	13.68	7.63	1.37	1.49	0.58	0.87	5.04	2.59	4.48
Petty trading	3.66	6.57	5.74	8.39	9.30	6.01	6.39	7.48	6.64
Asking from friends, neighbors, relatives,	9.01	7.22	7.78	6.22	12.38	7.63	8.11	9.47	8.42
Begging from the street	0.46	0.37	0.17	0.13	0.14	0.11	0.24	0.22	0.24
Others	0.50	1.08	1.30	0.56	1.22	2.03	1.07	1.39	1.14

Source: Author's computations from the Core Welfare Indicator Questionnaire (CWIQ) Survey Data 2006

In the rural sector, 17.02 percent of the households depended on piece work on other people's farms. This can be compared with 8.25 percent for the urban sector. Similarly, 13.31 percent of rural dwellers depended on other piece works, as against 10.94 percent for urban. Reduction of the number of meals was used by 18.41 percent of urban households, as against 14.14 percent for rural. Informal borrowing was also used by 10.18 percent of urban households, which can be compared with 8.68 percent for rural. In the rural sector, 5.04 percent of the respondents sold other assets in order to cope with income shocks, while only 2.59 percent used this in urban. Petty trading was used by 7.48 percent and 6.39 percent of the respondents from urban and rural sectors, respectively. Asking from friends, neighbors and relatives was used by 9.47 percent of the households in urban sector and 8.11 percent of those from the rural areas.

4. Recommendations

Understanding the correlates of shock exposure is vital for dealing with rising poverty in Nigeria. This is very paramount because of the different hardships that recent economic reforms have brought upon the people. The paper probed into different forms of shocks that households have faced with the goal of determining the characteristics the shock-exposed and their coping methods. The findings have shown that majority of Nigerian households have experienced one form of shock or the other. This further confirms that issue of shocks should be taken seriously because affected households have linked them to severe welfare losses. Majority of the households were affected by sudden rise in the prices of agricultural input and their scarcity. Government therefore needs to put in place appropriate mechanism for ensuring timely provision of agricultural inputs. The issue of diversion and allocation of agricultural inputs that are meant to be used by farmers to unintended beneficiaries should be addressed.

Also, there is the need for government to implement development programmes that can be of tremendous benefits to the people. The results show that provision of improved drinking water, improved toilet, building of health facility, sinking of well/borehole, agricultural inputs on credit, agricultural inputs readily available, buyer of agriculture produce, consumer goods, employment opportunities and credit facilities significantly reduced the probability of experiencing shocks. This implies that development efforts that can be channeled more into those areas will go a very long way in assisting households to be less susceptible to shock exposure.

Also, government should ensure provision of adequate environment for shock reduction in the South Eastern part of the country. It should be noted that at the time of collecting the data, the problems of Niger Delta militants and oil pipe vandalization were prominent. Although the militants have been given amnesty by the government, Boko Haram Islamic Sect is presently troubling the northern part of the country. This implies that if recent data were available, the picture might be quite different. It was also found that those that divorced/separated have higher probability of experiencing shocks. Ensuring workability of marriages by religious or cultural norms is therefore vital for reducing exposure to shocks and its impacts.

It was found that the poorest among the people have higher probability of being exposed to shock. Also, a good number of the people were selling their assets in order to reduce the impact of shocks. There is the need for government to provide adequate social protection to cater for the vulnerable poor in the event of shocks. This becomes so pertinent because out of the coping options that were reported, very few of the affected people were able to receive food aids.

References

1. Hoogeveen J, Tesliuc E, Vakis R Dercon S. A guide to the analysis of risk, vulnerability and vulnerable groups. (nd) Unpublished Paper.
2. Cuna L. Assessing household vulnerability to employment shocks: a simulation methodology applied to Bosnia and Herzegovina 2004. Internet file retrieved
3. Chambers R. Vulnerability, coping and policy, Editorial Introduction to IDS Bulletin, Special Issue on "Vulnerability: how the poor cope", *IDS Bulletin* 1989: Vol. 20, No 2; p.1-7.
4. Sinha S, Lipton M. Damaging fluctuations, risk and poverty: a review", 1999: Poverty Research Unit at Sussex (PRUS), University of Sussex, Brighton.
5. Shaffer P. New thinking on poverty: implications for poverty reduction strategies. 1999: CIS – University of Toronto, UNDESA.
6. Dercon S. Assessing vulnerability to poverty. Paper prepared for the Department for International Development (DfID), 2001, London.
7. World Bank 1996. Poverty in the midst of plenty: the challenge of growth with inclusion in Nigeria. A World Bank Poverty Assessment Paper, 1996, May 31, World Bank, Washington, D.C.
8. FGN (Federal Government of Nigeria). Poverty profile for Nigeria. National Bureau of Statistics (NBS) 2005, FGN.

9. National Bureau of Statistics. The Nigeria Poverty Profile 2010 Report. Press briefing BY The Statistician-General of the Federation/Chief Executive Officer,13th February, 2012.
10. Alayande B, Alayande O. A quantitative and qualitative assessment of vulnerability to poverty in Nigeria. Being a paper submitted for presentation of CSAE Conference on Poverty reduction, Growth and Human Development in Africa, March, 2004.
11. Christiaensen L, Subbarao K. Towards and understanding of vulnerability in rural Kenya. 2001: Mimeo.

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Cytological, Histological Uni- and Multi-Immunohistochemical Marrow Examinations in Detecting Early Disseminated Tumor Cells in De Novo Breast Cancer Patients.

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Abstract: Many studies have demonstrated the independent prognostic value of detecting bone marrow (BM) disseminated tumor cells (DTCs) at initial diagnosis of 1^{ry} breast cancer (BC) patients. Therefore, an accurate detection of these DTCs in the BM is very critical and must be obtained by using the most reliable and sensitive detection methodologies. In this respect, our study aimed to evaluate the detection capacities of the cytological, histological, uni- and multi-immunohistochemical (IHC) marrow examinations for early DTCs in the BM of newly diagnosed patients with non-stage IV 1^{ry} BC. This study included 80 of these patients that were subjected to CBC, BM aspiration/biopsy and IHC staining by a panel of monoclonal antibodies (McAbs) including Cytokeratin (CK), Mammaglobin and cancer antigen 15-3 (CA15-3). The detection rate of the histological BM examinations (11.3%) was significantly higher than that of the cytological one (2.5%), *p*-value =0.04. Our individual interpretation of the uni-IHC marrow examinations, using the above mentioned 3 McAbs, revealed that their detection rates (21.3%, 26.3% and 35%) were considerably variable but were significantly higher than that of the routine histological ones, *p*-values= 0.049, 0.035 and 0.02, for CK, Mammaglobin and CA15-3, respectively. The results obtained from the uni-IHC marrow examinations, using the same 3 McAbs, also showed variable degrees of agreement between each other. Therefore, a total interpretation of multi-IHC marrow examinations for these 3 McAbs was established. From the quantitative point of view, our multi-IHC total interpretation revealed a detection rate (47.5%) significantly higher than that of our histological interpretation (11.3%), *p-value* = 0.01; also, from the qualitative point of view, our results of both histological and multi-IHC total interpretations showed a highly significant statistical difference, *p*-value = 0.001. We concluded that for optimal increase in the detection capacity for early DTCs in the BM of de novo patients with non-stage IV 1^{ry} breast cancer, a total interpretation for combined histological/multi-IHC marrow examinations must be performed.

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Key words: Histological - immunohistochemical - detection capacity - disseminated tumor cells - marrow - breast cancer.

1. Introduction

Breast cancer (BC) is considered a systemic disease in which a hematogeneous dissemination of tumor cells, essentially to bone marrow (BM), may occur at very early stages of primary tumor development and form an occult isolated tumor cells, called disseminated tumor cells (DTCs) or micro-metastases that subsequently lead to an overt metastases **[1]**. Over the last two decades many studies have demonstrated the independent prognostic value of detecting BM micro-metastases or DTCs at initial diagnosis of 1^{ry} BC patients **[2 - 7]**. Other studies described a significant correlation between the presence of DTCs in BM and an unfavorable clinical outcome **[8-10]**.Therefore, accurate detection of BC micro-metastases in BM is very critical and must be based on standardized detecting methodologies. Nevertheless, among the currently used procedures, the reported incidence of BM micro-metastatic cell detection fluctuates considerably. This might be due to variations in patient series, stage distribution, expression of targeted antigen, sensitivity and/or specificity of the used monoclonal antibody (McAb) and in sensitivity of the procedure itself **[11]**. The detecting procedure for DTCs in BM is still investigational according to the American Society of Clinical Oncology, 2007 update of recommendations for the use of tumor markers in breast cancer **[12]**. At initial diagnosis of any 1^{ry} BC, especially in non-stage IV patients, the continuous real challenge for hematopathologists is the uppermost increase of detection capacity for any DTCs hidden in the BM or peripheral blood. In this respect, many different procedures have been used **[11, 13 - 15]**, but some limiting factors were always experienced with some of these detection methods. Therefore a combination of techniques and markers might help to overcome limitations experienced with these detection procedures **[16]**. In this study, we aimed to evaluate the detection capacities of cytological, histological, uni- and multi-immunohistochemical (IHC) marrow examinations for early DTCs in the BM of de novo patients diagnosed with non-stage IV 1^{ry} BC.

2. Material and Methods

This study included 80 newly diagnosed female patients with apparently non-metastatic 1ry BC staged [I-III] and received neither chemotherapy nor radiotherapy. These patients were selected from the surgical out-patient clinics, in National Cancer Institute – Cairo University, between October 2008 and July 2011. All patients' medical records were reviewed for data concerned with clinical examinations, pathological reports and radiological findings that confirm their clinical staging. Patients were subjected to CBC, BMA, BMB and IHC staining of BM sections using a panel of monoclonal antibodies (McAbs) including CK with (AE1/AE3) clone, Mammaglobin and CA15-3.

Bone marrow sampling, preparation, routine staining and interpretation:

Bone marrow biopsy cores (≥ 2cm long) and aspirates were consequently obtained from each patient under local infiltration anesthesia. BM smears were stained with routine leishman stain. BMB cores were fixed, decalcified, processed, paraffin embedded, sectioned and H&E-stained according to the well known routine techniques **[17].** Their morphological interpretation was accomplished according to the following precise criteria **[18, 19]**:-

Morphologically positive BM:

The BM was considered morphologically positive based on: **(1)** BMA smears showed large pleomorphic neoplastic cells with hyperchromatic coarsely reticular nuclear chromatin and moderately abundant variably basophilic cytoplasm with or without vaculation. These cells were arranged in tight small clusters either in syncytial formation or in cell columns pattern named "Indian Files". Solitary individually dispersed cells were also taken in consideration. **(2)** BMB sections showed these neoplastic cells occurred in randomly scattered small aggregates [2-4 cells] forming "micro-metastases" and associated with stromal reactions like [a-fibrous reaction among the involved areas b-interstitial increase in marrow eosinophils, histiocytes, plasma cells and/or fibroblasts c- active angiogenesis and/or d- increased osteoblastic/ osteoclastic activities with occurrence of trabecular bone erosion and/or widening].

Morphologically suspicious BM:

The BM was considered morphologically suspicious based on: **(1)** Absence of frank non-haemopoietic cells in BM smears and rare or occasional presence of their single or clustered "bare" nuclei associated with increased marrow osteoblasts and/or osteoclasts, **(2)** Absence of frank micro-metastatic colonies in BM sections and presence of one or more of the above-mentioned marrow stromal reactions and **(3)** The morphologic expectation of hidden metastatic cells that might be entangled among a prominent fibrotic reaction.

Morphologically negative BM:

The BM was considered morphologically negative based on: **(1)** Morphological absence of frank or suspicious individual cells, micro-metastatic colonies or sheets in all examined BM smears and sections **(2)** Absence of any suspicious marrow stromal reactions in marrow sections.

Immunohistochemical staining and interpretation for BM sections:

The BM sections were subjected to IHC staining using (DAKO Envision™ + System, peroxidase (HRP)/DAB, Mouse, K4006) as universal visualization system. BM sections were first de-paraffinized, in 2 changes of xylene, and re-hydrated in descending ethyl alcohol concentrations till distilled water. The concentrated primary antibodies [Mammaglobin, DAKO, code M3625, Cytokeratin (AE1/AE3), BioGenex, code MU071-UC and CA15.3, BioGenex, code MU323-UC] were diluted by antibody diluent in ratios of 1:100, 1:50-100 and 1:15-30, respectively. Sections were then pretreated by heat-induced epitope retrieval (DAKO 10x citrate buffer solution, Ph 6.1, S1699) for 15 minutes in microwave. After blocking the endogenous peroxidase activity by incubating the sections 10 min in a blocking solution, the diluted primary Abs were applied for 1 h and after washing in 3 changes of phosphate buffer saline (PBS) the HRP-labeled Polymer was applied for 30 min. After washing, the staining was completed by applying freshly prepared DAB + substrate-chromogen solution (20 µl DAB+Chromogen to 1 ml buffered substrate) to the sections and leaving them in dark for 8 minutes to produce a brown-colored precipitate at the antigen-sites. After washing, the sections were counter stained by light green stain 5% for 20 minutes, washed by distilled water, dehydrated in ascending grades of ethyl alcohol, cleared by xylene and finally mounted by DPX to be ready for microscopic examination. Unless test sections showed specific surface and/or cytoplasmic brown coloration in non-haemopoietic cells as positive control sections, they were considered negative. Also the positive staining intensity in test sections was assessed within the context of any non-specific background staining appeared in the negative control sections.

Individual interpretation of uni-IHC-staining [using a single McAb]:

To avoid false positive staining and to increase specificity of each individual McAb, its interpretation was accomplished in view of the standardized objective morphological criteria established by **Borgen *et al.* [20]** for the evaluation of immuno-stained DTCs in the BM. These criteria include: the occurrence of DTCs in clusters (e.g. in doubles, triples or more) and/or the presence of large-sized cells showing high N/C ratio, strong cytoplasmic staining with or without

large nucleoli. Sections showed cells with these criteria were considered positive. Sections showed absence of any immuno-stained cells or showed immuno-stained cells but without these criteria were considered negative.

Total interpretation of multi-IHC-staining [using a panel of McAbs]:

The total interpretation of multi-IHC staining (using 3 McAbs) was obtained depending on (1) the individual interpretation of each McAb as mentioned above (2) the detection rate of each McAb in the used panel and (3) the degrees of agreement between the used McAbs i.e. Firstly, out of the 3 used McAbs, the two with highest detection rates and substantial degree of agreement were selected for the total interpretation. Secondly, among these selected two McAbs, all cases that showed at least one McAb positive were totally interpreted as positive, while, all cases that showed the two selected McAbs negative were totally interpreted as negative.

Statistical Analysis

Data was analyzed using [SPSSwin] statistical package version 15.0.1 (Echo Soft Corporation, Chicago, IL). Quantitative (numerical) data, for non-parametric results, were expressed as median (50^{th} percentile) and interquartile range (IQR: 25^{th} – 75^{th} percentile). Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. Sign test was used to examine the relation between 2 related qualitative variables. Kappa (interrater reliability) was used to examine the agreement between two tests on the assignment of categories of a categorical variable. The probability of being by chance (p-value) was calculated for all parameters and was evaluated as follows: p-value ≥ 0.05 was considered non-significant and p-value < 0.05 was considered significant.

3. Results

This study included 80 females with de novo apparently non-metastatic 1ry BC; staged I to III and received neither chemotherapy nor radiotherapy. Their ages ranged from 27 to 77 years with a median of 53 years. Table (1) shows the relation between the detection rates of cytological versus histological marrow examinations in detecting early DTCs in these 80 patients. We noted that the histological detection rate (11.3%) was significantly higher than that of the cytological one (2.5%), (*p*-value = 0.04, Figs. 1, 2 and 3).

Cytokeratin (AE1/AE3), Mammaglobin and CA15-3 McAbs were used to highlight early DTCs in the IHC-stained marrow sections of the 80 patients included in this study. Individual interpretation of the uni-IHC marrow examinations revealed different detection rates of these McAbs. Cytokeratin (AE1&AE3) McAb showed the least detection rate (17/80, 21.3%), Mammaglobin showed a higher detection rate (21/80, 26.3%) and CA15-3 McAb showed the highest detection rate (28/80, 35%). Table (2) shows the relation between the detection rates of the histological marrow examination and that of each uni-IHC marrow examinations; using the above mentioned McAbs. The obtained results revealed that the detection rates of all uni-IHC marrow examinations, using CK (AE1/AE3), Mammaglobin and CA15-3 McAbs were significantly higher than that of the histological marrow examination, (*p*-values= 0.049, 0.035 and 0.02, respectively, Figs. 4, 5 and 6).

In table (3) the degrees of agreement between results of the marrow uni-IHC staining of the three McAbs were highlighted. The results of CA15-3 McAb showed a substantial agreement with that of Mammaglobin McAb (Kappa = 0.644) and a moderate agreement with that of Cytokeratin (AE1&AE3) McAb (Kappa = 0.515). Mean-while, the results of Mammaglobin McAb showed a weak agreement with that of Cytokeratin (AE1&AE3) McAb (Kappa = 0.275).

Our individual interpretation of the uni-IHC marrow examinations for CK (AE1/AE3), Mammaglobin and CA15-3 McAbs showed a considerable variation in their detection rates as well as their degrees of agreement; therefore a total interpretation of multi-IHC marrow examinations for these three McAbs was established based on recruiting only two of them (CA 15-3 and Mammaglobin McAbs) that showed the highest detection rates as well as a substantial degree of agreement. In this respect, all cases that showed positive staining of at least one of these two McAbs were multi-IHC totally interpreted as positive, while, cases that showed negative staining of these two McAbs were multi-IHC totally interpreted as negative. Accordingly, from the quantitative point of view, our total interpretation of the multi-IHC marrow examinations revealed a detection rate of (47.5%) which is higher than those of the individually interpreted uni-IHC marrow examinations (mentioned above) and, as shown in table (4), is significantly higher than that of the histological marrow examination (11.3%), *p*-value = 0.01. However, from the qualitative point of view, the relation between results of the histological interpretation of BM sections and that of the total interpretation of their multi-IHC staining for the 80 patients is studied and revealed, as shown in table (5), that all the 9 cases that histologically interpreted as positive were also multi-IHC totally interpreted as positive. Mean-while, among the 45 cases that were histologically interpreted as negative, 14 were multi-IHC totally interpreted as positive.

Also, among the 26 cases that were histologically interpreted as suspicious, only 15 were multi-IHC totally interpreted as positive and 11 cases were interpreted as negative. Thus, the results of both histological and multi-IHC total interpretations showed a highly significant statistical difference (*p*- value = 0.001).

Table (1): The relation between the detection rates of cytological and histological marrow examinations for detecting early DTCs in patients with de novo 1ry breast cancer

		BM examinations for 80 patients		*p*-value
		Cytological (BMA)	**Histological (BMB)**	
Detection rates	Positive marrow	2 (2.5%)	9 (11.3%)	**0.04**
	Negative marrow	73 (91.2%)	45 (56.2%)	
	Suspicious marrow	5 (6.3%)	26 (32.5%)	

BM= bone marrow, **DTCs=** disseminated tumor cells, **BMA=** BM aspiration, **BMB=** BM biopsy.

Table (2): The relation between the detection rates of the histological and each of the uni-IHC marrow examinations for detecting early DTCs in patients with de novo 1ry breast cancer; using CK, Mammaglobin and CA 15-3 McAbs.

		BM examinations for 80 patients		*p*-value
		Histological (BMB)	**Uni-IHC by CK (AE1/AE3) McAb**	
Detection rates	Positive marrow	9 (11.3%)	17 (21.3%)	**0.049**
	Negative marrow	45 (56.2%)	63 (78.7%)	
	Suspicious marrow	26 (32.5%)	-	

		Histological (BMB)	**Uni-IHC by Mammaglobin McAb**	
Detection rates	Positive marrow	9 (11.3%)	21 (26.3%)	**0.035**
	Negative marrow	45 (56.2%)	59 (73.7%)	
	Suspicious marrow	26 (32.5%)	-	

		Histological (BMB)	**Uni-IHC by CA 15-3McAb**	
Detection rates	Positive marrow	9 (11.3%)	28 (35%)	**0.02**
	Negative marrow	45 (56.2%)	52 (65%)	
	Suspicious marrow	26 (32.5%)	-	

IHC= immunohistochemical, **BM=** bone marrow, **DTCs=** disseminated tumor cells, **BMA=** BM aspiration, **BMB=** BM biopsy, **CK**= Cytokeratin, **McAb**= monoclonal antibody.

Table (3): Degrees of agreement between different McAbs used in the IHC-staining for detecting early DTCs in BM of patients with de novo 1ry breast cancer **[a]** agreement between CA15-3 and Mammaglobin McAbs, **[b]** agreement between CA15-3 and CK (AE1/AE3) McAbs and **[c]** agreement between Mammaglobin and CK (AE1/AE3) McAbs.

	Patients number=80		**CA15-3 McAb**		**Kappa**
			28 + ve	**52 - ve**	
[a]	**Mammaglobin McAb**	**21 +ve**	20	1	**0.644**
		59 - ve	8	51	

	Patients number=80		**CA15-3 McAb**		**Kappa**
			28 + ve	**52 - ve**	
[b]	**CK (AE1/AE3) McAb**	**17 +ve**	15	2	**0.515**
		63 - ve	13	50	

	Patients number=80		**Mammaglobin McAb**		**Kappa**
			21 +ve	**59 - ve**	
[c]	**CK (AE1/AE3) McAb**	**17 +ve**	5	12	**0.275**
		63 - ve	16	47	

McAbs= monoclonal antibodies, **IHC=** immunohistochemical, **DTCs=** disseminated tumor cells, **BM=** bone marrow, **CA15-3=** cancer antigen 15-3, **CK=** cytokeratin

Table (4): The relation between the detection rates of histological and multi-IHC marrow examinations for detecting early DTCs in patients with de novo 1ry breast cancer

		BM examinations for 80 patients		*p*-value
		Histological (BMB)	Multi-IHC staining	
Detection rates	Positive marrow	9 (11.3%)	38 (47.5%)	**0.01**
	Negative marrow	45 (56.2%)	42 (52.5%)	
	Suspicious marrow	26 (32.5%)	-	

BM= bone marrow, **BMB=** BM biopsy, **IHC=** Immunohistochemical.

Table (5): The relation between the outcome of histological interpretation of BMB sections and that of the total interpretation of their multi-IHC staining for patients with de novo 1ry breast cancer

Patients number=80		Histological interpretation of BMB sections			*p*-value
		Positive BM (n=9)	Negative BM (n=45)	Suspicious BM (n=26)	
Total interpretation of multi-IHC staining	Positive BM (n=38)	9	14	15	**0.001**
	Negative BM (n=42)	0	31	11	

BM= bone marrow, **BMB=** BM biopsy, **IHC=** Immunohistochemical.

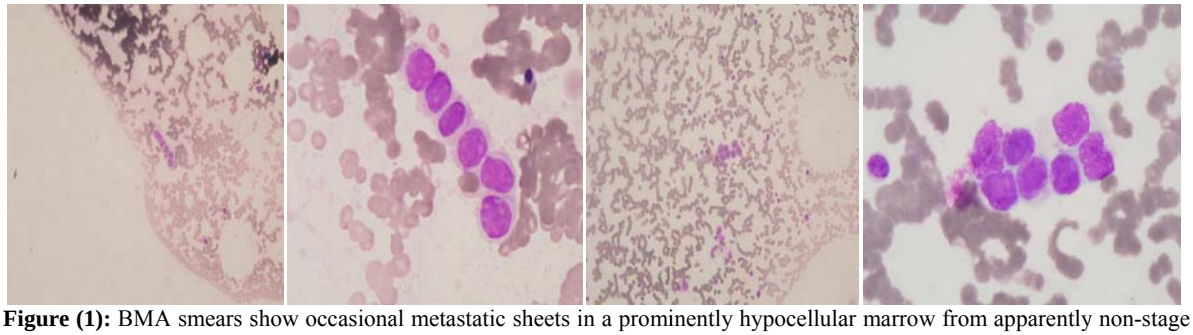

Figure (1): BMA smears show occasional metastatic sheets in a prominently hypocellular marrow from apparently non-stage IV 1^{ry} breast cancer patients, Leishman's stain, 20x and 100x.

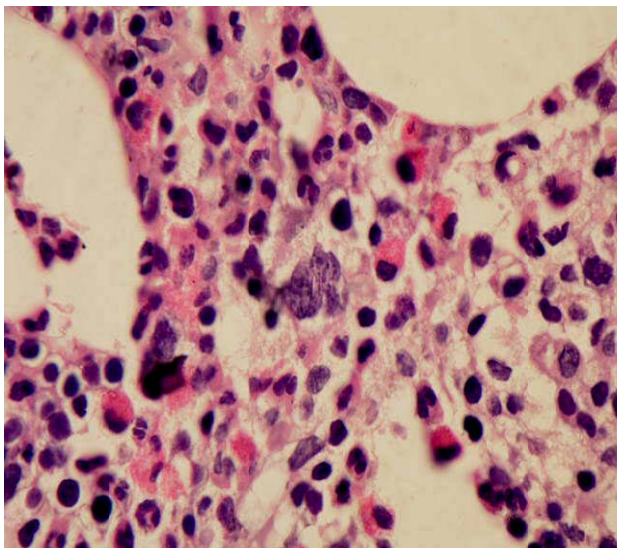

Figure (2): BMB section, from apparently non-stage IV 1ry cancer breast patient, shows a collection of 2 to 3 disseminated tumor cells (in center); associated with reactive interstitial increase in marrow eosinophils, H&E, 100x.

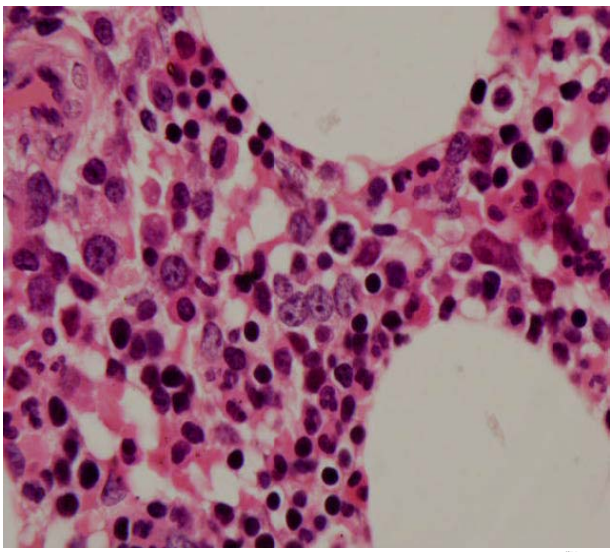

Figure (3): BMB section, from apparently non-stage IV 1^{ry} breast cancer patient, shows 3 to 4 morphologically suspicious (malignant-looking) cells (in center) arranged in an Indian file pattern, H&E, 100x.

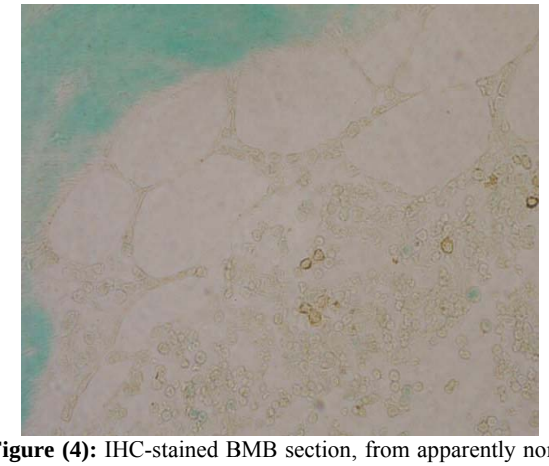

Figure (4): IHC-stained BMB section, from apparently non-stage IV 1ry breast cancer patient, shows few DTCs positive for Cytokeratin (AE1/AE3) McAb and arranged in singles, doubles and triples, 40x

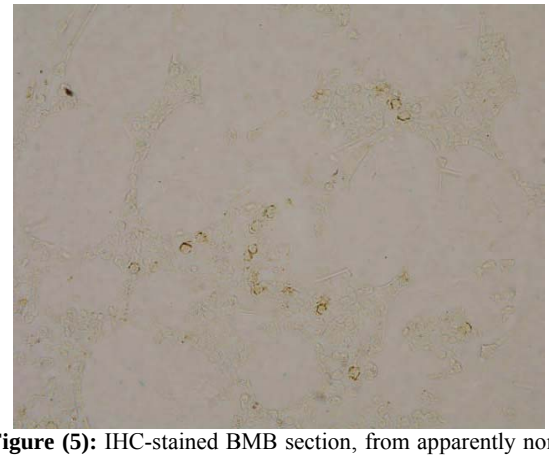

Figure (5): IHC-stained BMB section, from apparently non-stage IV 1ry breast cancer patient, shows some DTCs positive for Mammaglobin McAb and arranged in singles, and doubles, 40x

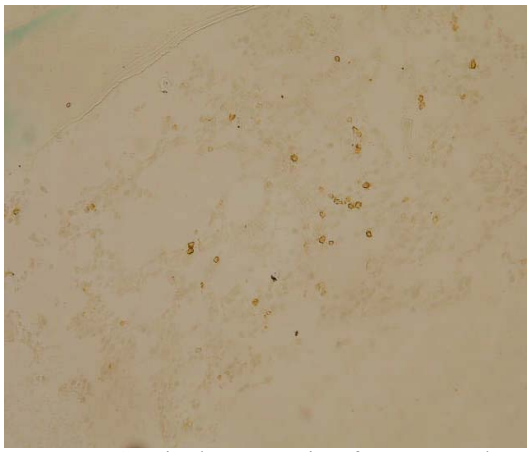

Figure (6): IHC-stained BMB section, from apparently non-stage IV 1ry breast cancer patient, shows multiple scattered DTCs positive for CA 15-3 McAb and arranged either individually, in doubles or in a collection showed an Indian file pattern, 20x

4. Discussion

In the haematopathology practice the cytological, histological and immuno-histochemical marrow examinations are known to be frequently used methodologies to detect metastatic lesions in BM of BC patients. In this respect, our study aimed firstly to evaluate the detection capacities of these different marrow examinations in detecting early DTCs in de novo cases of 1ry BC patients that clinically appear healthy and considered non-stage IV. Secondly, in view of the priority of cost/benefit relationship in our low socioeconomic communities, we tried to verify the impact of the step-wise combining of cytological, histological and immuno-histochemical marrow examinations on their detection capacities for early DTCs in the BM and eventually whether an individual interpretation of a uni-IHC staining will be satisfactory and reliable or a total interpretation of a multi-IHC staining should be performed.

In our results, the cytological BM examinations for early DTCs showed the weakest detection capacity with the lowest detection rate (2.5%). When the histological BM examinations are combined to the cytological one, the detection capacity became stronger and the detection rate was significantly increased to (11.3%), *p*-value =0.04. However, because of our histological interpretation of BM sections was restricted to precise morphological criteria, 32.5% of the histologically examined cases were still morphologically considered as suspicious and 56.2% were considered as negative for DTCs. Therefore, further BM examinations for early DTCs were required by an immunohistochemical staining.

As cancer breast is an epithelial cell tumor and the BM is normally free of any epithelial cells, the detection of DTCs in BM by immuno-staining procedures was based on using different McAbs raised against epithelial markers on the breast carcinoma cells **[11, 16]**. The specificity of some antibodies, used to characterize epithelial cells, remains controversial as they have been shown to cross-react with some hematopoietic cells **[21]**. In this study the IHC staining were performed using three properly selected McAbs; including CK [AE1/AE3], Mammaglobin and CA15-3. Our morphological interpretation to their immunohistochemical staining was preliminary accomplished on individual basis i.e. we performed an individual interpretation of the uni-IHC marrow examination for each McAb alone. These individual interpretations were done in view of the standardized objective morphological criteria established by Borgen et al. for the evaluation of immuno-stained DTCs **[20]**.

Cytokeratins are proteins that constitute a part of the cytoskeleton of epithelial cells, hence are regularly expressed by these epithelial cells and their malignant descendents **[22]**. They belong to a large multi-gene family thus are expressed at various levels and

compositions in all epithelial tumors **[23]**. We used CK McAb with the clone [AE1/AE3] that reacts with basic and acidic keratins covering a large spectrum of cytokeratins (e.g. CK1-8, CK10, CK14-16 and CK19). Among our 80 cases, its uni-IHC marrow examination for early DTCs showed a detection rate of 21.3% which is the lowest rate among the 3 selected McAbs. However, this detection rate is still significantly higher than that of the histological BM examinations (11.3%), *p-value*= 0.049. In comparison to previous studies, **Landys *et al.* [24]** used CK McAb with the same AE1/AE3 clone in uni-IHC BM examinations for 128 BC patients. They obtained a 19% detection rate which is a bit close to ours. **Salvadori *et al.* [25]** also performed uni-IHC marrow examinations on biopsies from 121 BC patients by using MBr1 McAb. They reported a detection rate of 17% which is obviously lower than ours. This is may be due to their use of a different McAb. Also their larger number of cases may partially explain their lower detection rate.

Up to our knowledge, we considered Mammaglobin and CA15-3 McAbs as novel markers; being used as McAbs in IHC marrow examinations for detecting early DTCs in patients with non-stage IV 1ry breast cancer. Mammaglobin is a member of the Uteroglobin/Clara cell protein [secretoglobin] superfamily **[26]**. It has been discussed as a promising diagnostic marker in breast cancer for almost ten years **[27 - 29]**.It is almost exclusively expressed in breast epithelial cells and is also over expressed in 61–93% of 1^{ry} and metastatic breast cancer tissues **[30, 31]**. Furthermore, **Ferrucci *et al.* [32]** included Mammaglobin among a new comprehensive gene expression panel to study tumor micro-metastases in patients with high-risk breast cancer. **Li *et al.* [33]** specified the detection of Mammaglobin m-RNA, by reverse transcriptase PCR, and considered it a superior biomarker for circulating tumor cells in BC patients. We could not find any previous studies that used Mammaglobin as McAb in immunohistochemical detection of early DTCs in the BM. Our individual interpretation of uni-IHC marrow examinations, for early DTCs, using Mammaglobin McAb revealed a detection rate of 26.3% which is higher than that of CK [AE1/AE3]McAb and is significantly higher than that of the histological BM examinations (11.3%), *p-value*= 0.035. Recently, **Liu *et al.* [34]** studied the expression of human Mammaglobin m-RNA, in the BM of 102 patients with stage I-III breast cancer, by RT-PCR. They reported a positive expression rate of 38.2% which is much higher than ours. This may be attributed to the higher detection capacity (sensitivity) of RT-PCR technique for detecting Mammaglobin m-RNA than that of uni-IHC staining using single anti-Mammaglobin McAb.

CA15-3 is a common well-known breast tumor marker. It is considered one of the markers that showed evidence of clinical utility and was recommended for use in practice **[12]**. **Velaiutham *et al.* [35]** reported that CA15-3 has an independent prognostic impact in both uni- and multi-variate analysis. In our study, CA15-3 is the third McAb selected in our immunohistochemical staining. Its clone [BGX323A] is considered very specific to react with CA15-3 antigen in mammary cancer cells and, as stated by its manufacturer, it has no cross reactivity with human CEA or CA125 and has no binding with non-specific tissues or cells. We could not find any previous studies that used CA15-3 as McAb in an immunohistochemical detection of early DTCs in the BM. In our individual interpretation of uni-IHC marrow examinations for early DTCs, CA15-3 McAb showed a detection rate of 35% which is higher than that of CK [AE1/AE3] and Mammaglobin McAbs (21.3% and 26.3%, respectively) and is significantly higher than that of the histological BM examinations (11.3%), *p-value*=0.02. Thus, our individual interpretation of the uni-IHC marrow examinations, using the above mentioned 3 McAbs, revealed a considerable variation in their detection capacities for early DTCs in the BM. This variation could be attributed to the heterogeneity of expression of these markers in breast carcinoma cells. Also, the results obtained from the uni-IHC marrow examinations, using the same 3 McAbs showed variable degrees of agreement between each other (Table 3). Therefore, a total interpretation of multi-IHC marrow examinations for these 3 McAbs was established on the basis of recruiting only two of them (CA15-3 and Mammaglobin) that showed the highest detection rates as well as a substantial degree of agreement. Accordingly, from the quantitative point of view, our total interpretation of the multi-IHC marrow examinations revealed an obvious increase in the detection rate (47.5%) which is higher than those of the individually interpreted uni-IHC marrow examinations (Table 2) and, also, is significantly higher than that of the histological BM examinations (11.3%), *p-value* = 0.01 (Table 4). On the contrary of our results, **Mathieu *et al.* [36]** performed first multi-IHC staining on 12 histologically positive BM biopsies; using a panel of McAbs including anti-CK with different clones (KL1, AE1/AE3 and CAM-5) and anti-EMA. Secondly, they selected out of these McAbs the most sensitive one to be used in the IHC detection of any occult metastases among series of 93 BM biopsies negative by conventional histological examinations. They found only one case stained positive with CK (KL1) demonstrating isolated tumor cells. Therefore, they stated that "Single BM biopsy techniques whether stained by conventional or IHC methods do not appear to be useful tests to detect occult BM metastases, especially at initial diagnosis of clinically M0 breast carcinoma patients".

Also, in comparison to our study, **Vannucchi *et al.* [37]** evaluated the presence of BM micrometastases in bilateral BM biopsies obtained at diagnosis of 33 patients with stage II/IIIA breast cancer using RT-PCR assay for CK19 m-RNA, histology and multi-IHC marrow examinations with a panel of three McAbs. They detected CK19 transcripts in one or both BM samples in 48% of patients, with an overall 85% concordance with the results of their multi-IHC analysis. On the other hand, 56% of PCR- and IHC-positive BM samples were diagnosed as 'normal' on histological analysis. Previously, **Lyda *et al.* [38]** used a combination of different CK clones; including AE1/AE3, CAM5-2 and 35ßH11, in performing multi-IHC marrow examinations for 65 BM biopsies from 54 patients with lobular breast carcinoma. They obtained a detection rate (30.8%) higher than that of their routine histological marrow examinations. The detection rate of their multi-IHC marrow examinations is lower than ours (47.5%) because in our multi-IHC marrow examinations we used 3 totally different McAbs (CK, Mammaglobin and CA15-3) rather than 3 different clones of the same McAb.

From the quantitative point of view, our multi-IHC total interpretation revealed a detection rate (47.5%) significantly higher than that of our histological interpretation (11.3%), *p-value* = 0.01; also, from the qualitative point of view, our results of both histological and multi-IHC total interpretations showed a highly significant statistical difference, *p*-value = 0.001. Therefore both interpretations are complementary to each other and neither of them can substitute the other.

In this study, we confirmed the popular finding that ensured the superiority of the histological detection capacity over the cytological one for DTCs in the BM. We found that the combined histological/uni-IHC interpretations significantly increase the detection capacity but to a different rates according to the individually used McAb and with variable degrees of agreement between the used McAbs. Eventually, we concluded that for optimal increase in the detection capacity for early DTCs in the BM of de novo patients diagnosed clinically with non-stage IV 1ry breast cancer, a total interpretation for combined histological/multi-IHC marrow examinations must be performed.

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References

1. Benoy IH, Eiast H, Philip M, Wuyts H, VanDam P, Scharpe S, *et al.* (2006): Detection of disseminated tumor cells in bone marrow has superior prognostic significance in comparison with circulating tumor cells in patients with breast cancer. Br J Cancer, 94: 672-680.
2. Benoy IH, Elst H, Philips M, Wuyts H, VanDam P, Scharpe S, *et al.* (2006): Prognostic significance of disseminated tumor cells as detected by quantitative real-time reverse-transcriptase polymerase chain reaction in patients with breast cancer. Clin Breast Cancer; **7**(2): 146–152.
3. Naume B, Wiedswang G, Borgen E, Kvalheim G, Karesen R, Qvist H, *et al.* (2004): The prognostic value of isolated tumor cells in bone marrow in breast cancer patients: evaluation of morphological categories and the number of clinically significant cells. Clin Cancer Res.; 10: 3091–3097.
4. Sola M, Margeli M, Castella E, Julian J, Rull M, Gubern J, *et al.* (2011): Prognostic value of hematogeneous dissemination and biological profile of the tumor in early breast cancer patients . A prospective observational study. BMC Cancer, 11: 252. [Available from: http://www.biomedcentral.com/1471-2407/11/252].
5. Alexandrova E, Sergieva S, Nikolova V, Danon S. (2003): Bone marrow micro-metastases as prognostic factor in early breast cancer patients. J BUON, 8(2): 133-137.
6. Wiedswang G, Borgen E, Karesen R, Kvalheim G, Nesland J, Qvist H, *et al.* (2003): Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. J Clin Oncol., 21(18): 3469-3473.
7. Braun S and Naume B. (2005): Circulating and disseminated tumor cells. J Clin Oncol. , 23(8): 1623-1626.
8. Braun S and Pantel K. (2001): Clinical significance of occult metastatic cells in bone marrow of breast cancer patients. The Oncologist.; 6 (2): 125-132.
9. Gebauer G, Fehm T, Merkle E, Berck E, Lang N, Nager W. (2001):Epithelial cells in bone marrow of breast cancer patients at time of primary surgery: clinical outcome during long term follow-up. J Clin Oncol., 19(6): 3669-3674.
10. De Boer M, van Deurzen C, van Dijck J, Borm G, van Diest P, Adang E, *et al.* (2009): Micrometastases or isolated tumor cells and the outcome of breast cancer. N Eng J Med., 361(7): 653-663.
11. Choesmel V, Pierga J, Nos C, Vincent-Salmon A, Sigal-Zafrani B, Thierry J, *et al.* (2004): Enrichment methods to detect bone marrow micro-metastases in breast carcinoma patients: clinical relevance. Br Cancer Res. , 6(5): 556-570.
12. Harris L, Fritsche H, Mennel R, Norton L, Ravidin P, Taube S, *et al.* (2007): American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. , 25(33): 5287-5312.
13. Forus A, Hoifodt HK, Overli GE, Myklebost O, Fodstad O. (1999): Sensitive fluorescent in situ hybridization method for the characterization of breast cancer cells in bone marrow aspirates. Mol Pathol., 52: 68-74.
14. Zhong XY, Kaul S, Lin YS, Eichler A, Bastert G. (2000): Sensitive detection of micro-metastases in bone marrow from patients with breast cancer using immuno-

magnetic isolation of tumor cells in combination with reverse transcriptase polymerase chain reaction for Cytokeratin-19. J Cancer Res Clin Oncol. , 126:212-218.
15. Aerts J, Wynendaele W, Paridaens R, Christiaens R, Vanden-Bogaert W, van Oosterom AT, *et al.* (2001): A real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to detect breast carcinoma cells in peripheral blood. Ann Oncol., 12: 39-46.
16. Vincent-Salmon A, Bidard F, Pierga J. (2008): Bone marrow micro-metastases in breast cancer: review of detection methods, prognostic impact and biological issues. J Clin Pathol. , 61:570-576.
17. Bain BJ, Clark DM, Wilkins BS. (2010): Technical methods applicable to trephine biopsy specimens, in Appendix, in Bone Marrow Pathology. Fourth Edition, Wiley-Blackwell, a John Wiley & Sons, Ltd., Publication; pp. 601-10.
18. Frisch B and Bartl R. (1999): Metastatic bone disease, in Biopsy interpretation of bone and bone marrow: Histology and immunohistology in paraffin and plastic. Second Edition, Arnold Publication – a member of the Hodder Headline Group, London NW1-3BH,; pp. 121-143.
19. Bain BJ, Clark DM, Wilkins BS. (2010): Metastatic tumours, in Bone Marrow Pathology. Fourth Edition, Wiley-Blackwell, a John Wiley & Sons, Ltd., Publication; pp. 549-586.
20. Borgen E, Naume B, Nesland J, Kvalheim G, Beiske K, Fodstad O, *et al.* (1999): Standardization of the immunocytochemical detection of cancer cells in BM and blood: Establishment of objective criteria for the evaluation of immuno-stained cells. The European International Society of Hemato-therapy and Graft Engineering (ISHAGE) Working Group for standardization of tumor cell detection, Cytotherapy, 1: 377-388.
21. Borgen E, Beiske K, Trachsel S, Nesland J, Kvalheim G, Herstad TK, *et al.* (1998): Immunohistochemical detection of isolated epithelial cells in bone marrow: non-specific staining and contribution by plasma cells directly reactive to alkaline phosphatase. J Pathol.; 185: 427-434.
22. Janni W, Rack B, Lindmann K, Harbeck N. (2005): Detection of micro-metastatic disease in bone marrow: Is it ready for prime time? The Oncologist.; 10 (7): 480-492.
23. Lacroix M. (2006): Significance, detection and markers of disseminated breast cancer cells. Endocrine Related Cancer; 13(4): 1033-1067.
24. Landys K, Persson S, Kovarik J, Hultborn R, Holmberg E. (1998): Prognostic value of bone marrow biopsy in operable breast cancer patients at the time of initial diagnosis: results of 20 years follow-up. Can Res Treat.; 49(1): 27-33.
25. Salvadori B, Squicciarini P, Rovini D, Orefice S, Andreola S, Rilke F, *et al.* (1990): Use of monoclonal antibody MBr1 to detect micrometastases in bone marrow specimens of breast cancer patients. Eur J Cancer; 26: 865-867.
26. Klug J, Beier H, Bernard A, Chilton B, Fleming T, Lehrer R, *et al.* (2000): Uteroglobin/Clara cell 10-kDa family of proteins: Nomenclature committee report. Ann New York Acad Sci.; 923: 348-354.
27. O'Brien N, Magiure T, O'Donovan N, Lynch N, Hill A, McDermott E, *et al.* (2002): Mammaglobin A: a promising marker for breast cancer. Clin Chem., 48(8): 1362-1364.
28. Zehentner BK and Carter D. (2004): Mammaglobin: a candidate diagnostic marker for breast cancer. Clin Biochem. , 37(4): 249-257.
29. Zatch O and Lutz D. (2005): Mammaglobin remains a useful marker for the detection of breast cancer cells in peripheral blood. J Clin Oncol., 23(13): 3160.
30. Han J, Kang Y, Shin H, Kim H, Kim Y, Oh Sl. (2003): Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. Archives Path Lab Med.; 127: 1330-1334.
31. Span P, Wanders E, Manders P, Heuvel J, Foekens J, Watson M, *et al.* (2004): Mammaglobin is associated with low grade steroid receptor positive breast tumors from postmenopausal patients and has independent prognostic value for relapse free survival time. J Clin Oncol.; 22: 691-698.
32. Ferrucci PF, Rabascio C, Gigli F, Corsini C, Glordano G, Bertolini F, *et al.* (2007): A new comprehensive gene expression panel to study tumor micrometastases in patients with high-risk breast cancer. Int J Oncol. , 30(4): 955-962.
33. Li G, Zhang J, Jin K, He K, Wang H, Lu H, Teng L. (2011): Human Mammaglobin: a superior marker for reverse-transcriptase PCR in detecting circulating tumor cells in breast cancer patients. Biomark Med. , 5(2): 249-260.
34. Liu Y, Ma L, Liu X, Wang L. (2011): Expression of Mammaglobin as marker of bone marrow micrometastases in breast cancer. Expr Therp Med.; 2 (12): 550 – 554.
35. Velaiutham S, Taib N, Ng K, Young B, Yip C. (2008): Does the pre-operative value of serum CA15-3 correlates with survival in breast cancer? Asian Pacific J Cancer Prev.; 9: 445-448.
36. Mathieu MC, Friedman S, Bosq J, Caillo B, Spielmamm M, Travalgi J-P, *et al.* (1990): Immunohistochemical staining of bone marrow biopsies for detection of occult metastases in breast cancer. Breast Cancer Res Treat. , 15(1): 21-26.
37. Vannucchi AM, Bosi A, Glinz S, Pacini P, Linari S, Saccardi R, *et al.* (1998): Evaluation of breast tumour cell contamination in the bone marrow and leukapheresis collections by RT-PCR for cytokeratin-19 m-RNA. Br J Haematol.;103(3): 610-617.
38. Lyda MH, Tetef M, Carter NH, Ikle D, Weiss LM, Arder DA. (2000): Keratin immunohistochemistry detects clinically significant metastases in bone marrow biopsy specimens in women with lobular breast carcinoma. Am J Surg Pothol. , 24(12):1593-1599.

Characterization of Fennel Fruits: Types and Quality (I)

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Abstract: Four samples of different fennel fruit cultivars (F **1**-F **4**), obtained from El-Fayoum, Egypt (F **1**), El-Menia, Egypt (F **2**), Sudan, El-Khartoum (F **3**), and Germany (F **4**) were cultivated in MEPACO`s Farm (Arab Co. for Pharm. and Med. Plants, Cairo, Egypt) and the obtained fruits were subjected to macro- and micro-morphological stereomicroscopic examination as well as GC-MS analysis of their volatile oils. **The aim** of the study is to determine the differences in the macro- and micro- characters of different fruit cultivars as well as their oil constitutes. **The results** show different exomorphic parameters *viz*. shape, color, dimensions and surface sculpture. Also the stereomicroscopic examination showed differences in the epicarp, mesocarp; vitti and endosperm. GC-MS analysis of volatile oils of (F **1**-F **4**) showed on comparing three parameters; fenchone, estragole and *trans*-anethole that F **4** has the highest percentage of *trans*-anethole (78.98%), while F **1** and F **2** have close values (1.05 and 1.02%, respectively) followed by F **3** (3.02%). F **4** has the lowest percentage of estragole (3.97%); while (F **1**-F **3**) have higher values (78.58, 64.81 and 25.79%, respectively). Also F **4** has doubled the percentage of fenchone (6.73%) of F **1** and F **2** (2.54 and 2.57%, respectively), while F **3** has 0.69%. Thus results show that the two cultivars growing in Egypt (F **1** and F **2)** have almost the same ratios of the compared parameters while, the Sudan cultivar F **3** is closer to F **1** and F **2** than it is to F **4**. Also the three cultivars (F **1**-F **3**) are far from specification of sweet fennel oil but close to bitter fennel oil. The German cultivar (F **4)** has the best oil quality as a sweet fennel. Investigation of the powdered samples (F **1**-F **4**) showed that only F **4** is different in having higher abundant fragments of reticulate parenchyma cells with ratio of 1:3 {F **4**:(F **1**-F **3**)}. **In conclusion:** These findings are of pharmaceutical-industrial value helping in the production of herbal pharmaceutical products of fennel fruit and/or oil of known higher quality.
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Key Words: Foeniculum vulgare, Fenchone, Estragole, Anethole, Stereomicroscopic examination.

1. Introduction

Fruit of Foeniculum vulgare Mill. (Fam. Umbellifereae) is cultivated in Europe and much is imported from India, China and Egypt (**Trease and Evan, 1976**). Fennel oil has long been used as an aromatic stomachic (**Gunn, 1920**). Fennel oil is a natural antispasmodic agent (**Plant and Miller, 1926**), it also has anti-foaming and carminative effect (**Harries *et al.,* 1978**). It is used in treatment of irritable bowel syndrome as it exerts gastric relief due to its local anesthetic effect on the gastrointestinal tract (**Holt, 1990**). Also it is used in management of bronchial asthma due to the strong diaphoretic effect of anethole (**Haggag *et al.,* 2003**). Fennel oil emulsion showed to reduce intestinal spasms and increase motility of small intestine in infantile colic (**Alexandrovich *et al.,* 2010**). Fennel oil have been investigated for its constituents as sweet and bitter oil (**Wichtl and Bissett, 1994; Leung and Foster, 1996**). The therapeutic value of fennel fruit as well as its presence in several commercial cultivars, varying considerably in size and appearance (**Barthlott, 1981 & 1990; Barthlott *et al.,* 1998 & 2003; Javadi and Yamaguchi, 2004; Salimpour *et al.,* 2007**), encouraged the authors to make close investigation of macro- and micro- characters of different fruit cultivars as well as their oil constitutes.

2. Materials and Methods

Plant materials:

Four samples of different fennel fruit cultivars (F **1**-F **4**), obtained from El-Fayoum, Egypt (F **1**), El-Menia, Egypt (F **2**), Sudan, El-Khartoum (F **3**), and Germany (F **4**) were cultivated in MEPACO`s Farm (Arab Company for Pharmaceutical and Medicinal Plants, Cairo, Egypt) and the obtained fruits were subjected to this study.

Macromorphological Investigation:

The investigated fruits were dried, cleaned and examined by Stereomicroscope and photographed by Digital Camera 7.2 mega pixels to show the different exomorphic parameters viz. shape, dimensions and color. For stereo-electro-microscopic (SEM)

examination, the fruits were dried, fixed in 70% alcohol and were mounted on brass stubs and coated with a thin layer of gold using Edwards Sputter coater and examined using different magnifications by JEOL- T100 scanning electron microscope at the SEM unit, Faculty of Science, Ain Shams University. The terminology used for the description of leaves as examined by SEM is that of **Stearn (1966); Barthlott (1981 & 1990); Barthlott *et al.* (1998 & 2003); Javadi and Yamaguchi (2004) and Salimpour *et al.* (2007).**

Micromorphological Investigation:

Mature fruits were softened in warm water for 12 – 72 hrs and then dehydrated using a tertiary butyl alcohol series and sectioned at a thickness of 15 - 20 µm; sections were double stained with safranin and light green according to the traditional methods of **Johanson (1940).** The fruit sections were described as LM, and photographed using Digital Camera (Sony cyber-shot DSC.W55, 7.2 mega pixels). The anatomical descriptive terms of fruit coat in the present study based on the terms of **Corner (1976).**

Preparation of the essential oils:

The fresh fruits (500g) were separately hydro-distilled for 6 hours in a Clevenger type apparatus. The resulting oils were collected, dried over anhydrous sodium sulphate and stored in refrigerator until analyzed. Percentage yields were determined according to the Egyptian Pharmacopoeia, 1984.

GC/MS Analysis:

GC/CMS analysis was performed on a GC/MS system (Shimadzu GC/MS- QP2010) with software (Class 5000). Gas chromatograph equipped with a TR-5MS (5% Phenyl Polysil Phenylene Siloxane), column (DB 30m × 0.25 mm i.d × 0.25 um film thickness). The analyses were carried out under the following conditions: Carrier gas: He with flow rate 1 ml/min; 235°C; Detector temp. FID: 250°C; Injector temp.: 235°C; split ratio; 1:10; Oven temp. Program: initial temp.; 40°C (0.5 min) increasing to 150°C (at 7.5°C/min), 150°C (1min) then increasing to 250°C (at 5°C/min)- 250°C (2min). The capillary column was directly coupled with mass spectrometer HP 5973 (Agilent). EI-MS were recorded at 70 ev. The analysis has been done at the Quality Control Department, Chemistry Section of Arab Co. for Pharm. and Med. Plants (MEPACO), Cairo, Egypt. Identification of the components were performed by aid of the computer library search (Class 5000 lab software package) comparison of mass spectra with literature data and by comparison of their retention times and mass fragmentation patterns with those of the library data base (**Massada, 1967; Egyptian Pharmacopoeia, 1984; Adams, 1995; Guido *et al.*, 2005**).

3. Results

Macromorphological characters of the different cultivars (F **1**-F **4**) whole mount are shown in Figure 1, microphotographs of the fruit surface sculpture of the different cultivars (F **1**-F **4**) are shown in Figure 2 and microphotographs of fruit anatomy of the different cultivars (F **1**-F **4**). The results of macromorphological and micromorphological characters of the four cultivars (F **1**-F **4**) are summarized in Table **1** and GC-MS analysis of volatile oils of the four cultivars (F **1**-F **4**) is summarized in Table **2**.

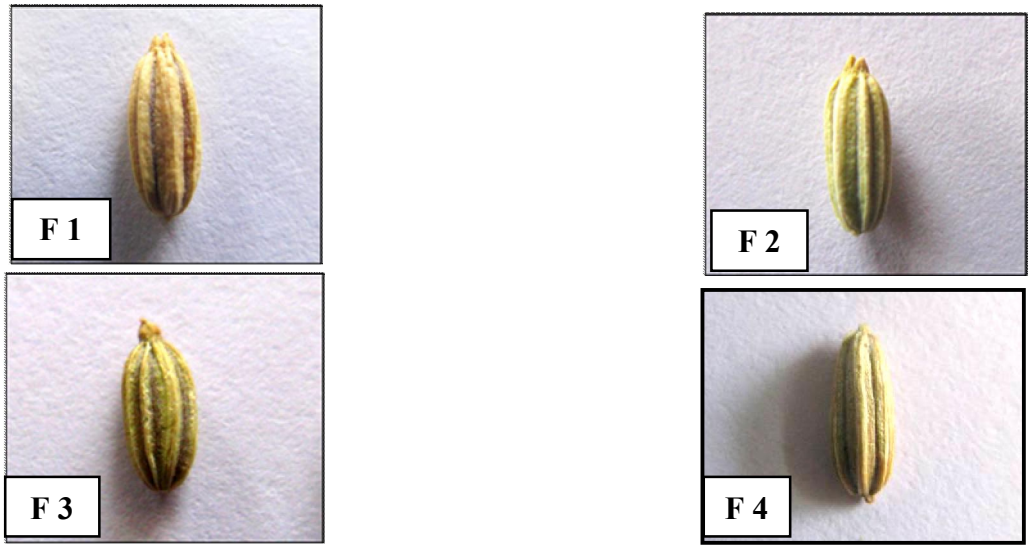


Fig. (1): Macrophotographs of fennel different cultivars fruits (whole mount) X=10

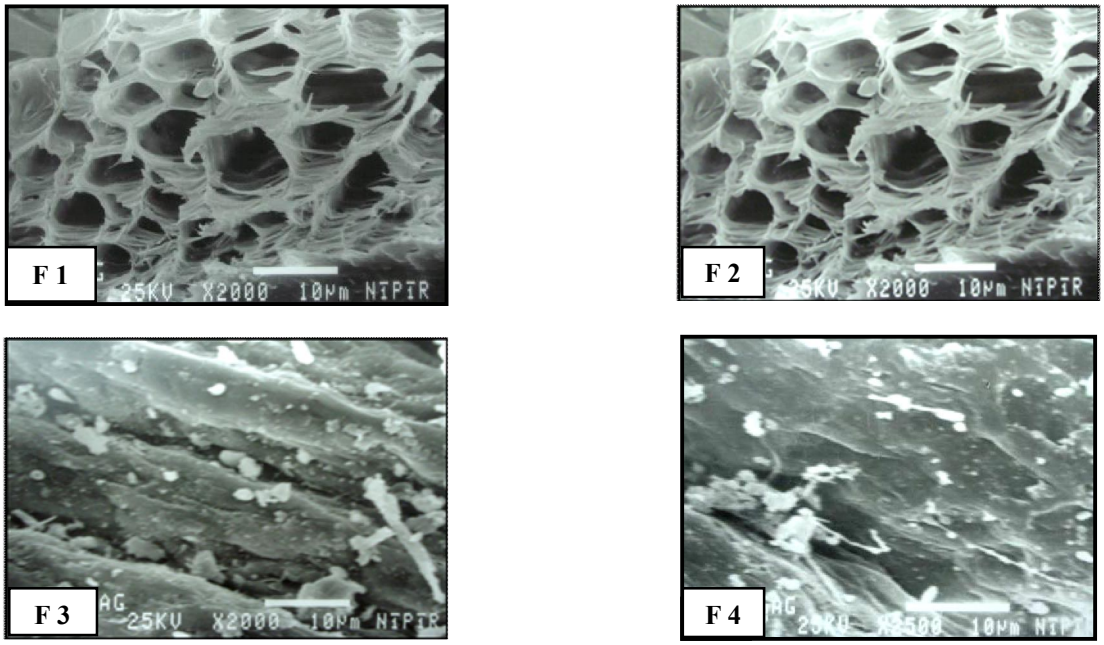


Fig. (2): Microphotographs of fruit surface sculpture of fennel different cultivars X=400

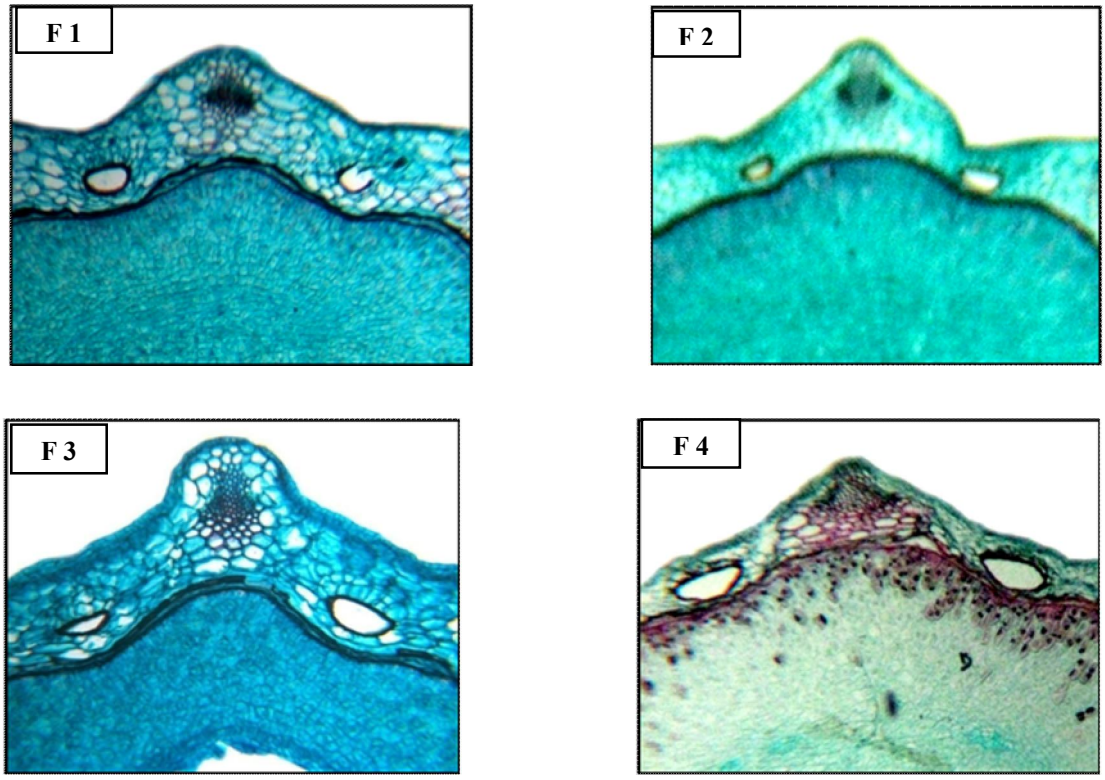


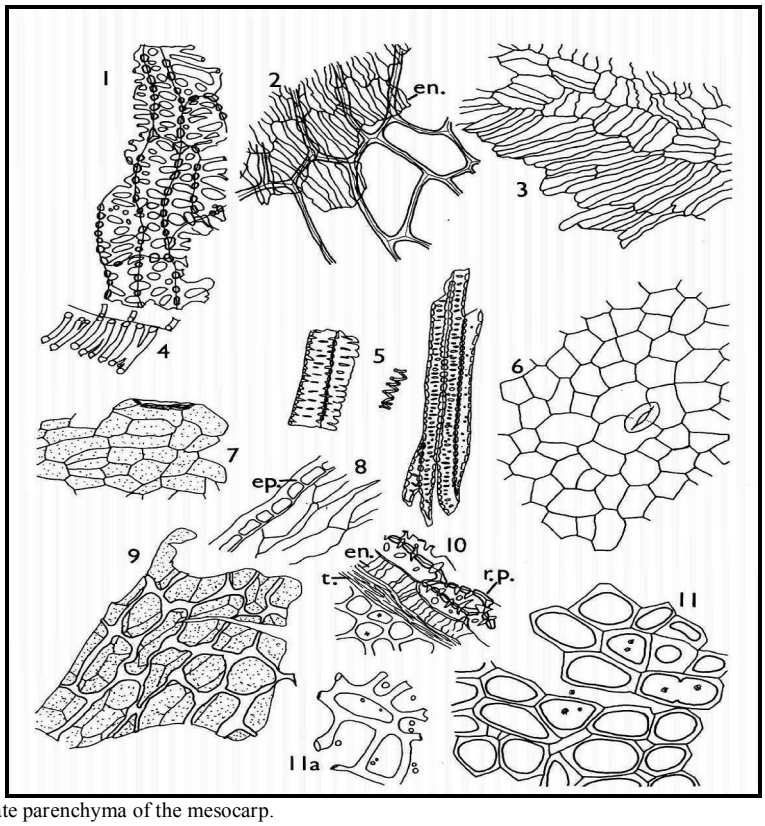
Fig. (3): Microphotographs of fruit anatomy of fennel different cultivars X=400

Table (1): Macro- and micro-morphological characters of the four fennel cultivars

Description			F 1	F 2	F 3	F 4
Macromorphological Characters	Shape		Elliptical	Elliptical	Oval	Oval
	Color		Brownish green	Greenish yellow	Brownish green	Light brown
	Dimensions		9 (±1)x 2 (±1) mm	9 (±2)x 3 (±1) mm	5 (±2)x 2 (±1) mm	6 (±1)x 2 (±1) mm
	Fruit surface sculpture		Rugose	Verrucate	Striated	Reticulate-Foveate
	Anticlinal wall	Level	Elevated	Elevated	Elevated	Elevated
		Surface sculpture	Smooth	Wrinkled	Wrinkled	Smooth
		Margin	Straight	Straight	Straight	Straight
	Periclinal wall	Level	Slightly Depressed	Slightly Depressed	Slightly Depressed	Slightly Depressed
		Surface sculpture	Smooth	Wrinkled	Wrinkled	Wrinkled
	Wax Shape		Granulate / Rodlets	Granulate / Rodlets	Granulate	Granulate
Micromorphological Characters	Epicarp	Cuticle	Thick	Thick	Thin	Thin
		No. of Layer	Two	Two	One	One
		Shape	Tangential	Tangential	Tangential/ Radial	Tangential
	Mesocarp	Reticulate Parenchyma	Above the V.B	Above the V.B	Above the V.B	Above and below the V.B
		No. of Layer	6 -1 0	8 - 12	6 - 10	4 - 6
		Type of cells	Polyhedral parenchyma / Collenchyma	Polyhedral parenchyma / Collenchyma	Polyhedral parenchyma / Collenchyma	Polyhedral parenchyma / Collenchyma
		No. of Vascular Bundle	5	5	5	5
		Size of V.B.	5 small bundles	5 small bundles	3 small + 2 medium	3 small + 2 large
		No. of Vitti	6	6	6	6
		Shape	Terete	Terete	Compressed oval	Oval
	Endocarp	No. of Layer	One	One	One	One
		Shape	Polyhedral	Compressed	Polyhedral	Compressed
	Seed Coat	No. of Layer	One	One	One	One
		Shape	Compressed	Compressed	Tangential	Compressed
	Endosperm	No. of Layer	18 - 20	13 - 18	15 - 18	18 - 22
		Shape	Elliptic - Square	Elliptic - Square	Globoid	Globoid

Table (2): GC-MS analysis of volatile oils of the four fennel cultivars

Active principle	R_t (min)	Relative percentage			
		F 1 (El-Fayoum)	F 2 (El-Menia)	F 3 (El-Khartoom)	F 4 (Germany)
Fenchone	11.66	2.54	2.57	0.69	6.73
Estragole	15.02	78.58	64.81	25.79	3.97
Trans-Anethole	17.54	1.05	1.02	3.02	78.98



1 Reticulate parenchyma of the mesocarp.
2 Endocarp (en.) with overlying cells of the innermost layer of the mesocarp, in surface view.
3 Endocarp in surface view.
4 Fragment of a reticulately thickened vessel.
5 Elements from the fibro-vascular tissue.
6 Epicarp in surface view showing a stoma.
7 Fragment of a vitta.
8 Epicarp (ep.) and parenchyma of the mesocarp in sectional view.
9 Fragment of a vitta with overlying thick-walled cells of the inner- cells of the most layer of the mesocarp, in surface view.
10 Part of the pericarp and seed in sectional view showing the reticulate parenchyma (r.p.), endocarp (en.), testa (t.) and endosperm.
11 Endosperm containing microrosette crystals of calcium oxalate.
11a Thick-walled cells of the endosperm

Fig. (4): Items of powdered fennel sample X=80

4. Discussion

The four samples of the different fennel fruit cultivars (F 1-F 4), showed macro-morphological differences in parameters *viz*. shape, color, dimensions and surface sculpture. Also the micro-morphological stereomicroscopic examination showed differences in the epicarp, mesocarp; vitti and endosperm. GC-MS analysis of volatile oils of (F 1-F 4) showed on comparing three parameters; fenchone, estragole and *trans*-anethole that F 4 has the highest percentage of *trans*-anethole (78.98%), while F 1 and F 2 have close values (1.05 and 1.02%, respectively) followed by F 3 (3.02%). F 4 has the lowest percentage of estragole (3.97%); while (F 1-F 3) have higher values (78.58, 64.81 and 25.79%, respectively). Also F 4 has doubled the percentage of fenchone (6.73%) of F 1 and F 2 (2.54 and 2.57%, respectively), while F 3 has 0.69%. Thus results showed that the two cultivars growing in Egypt (F 1 and F 2) have almost the same ratios of the compared parameters while, the Sudan cultivar F 3 is closer to F 1 and F 2 than it is to F 4. Also the three cultivars (F 1-F 3) are far from specification of sweet fennel oil but close to bitter fennel oil and the German cultivar (F 4) has the best oil quality as a sweet fennel when all compared with reported data *(*Wichtl and Bissett, 1994; Leung and Foster, 1996*)*. Investigation of the powdered samples (F 1-F 4) showed that only F 4 is different in having higher abundant fragments of reticulate parenchyma cells with ratio of 1:3 {F 4:(F 1-F 3)} (Figure 4).

Conclusion

GC-MS analysis of volatile oils of (F 1 - F 4) showed on comparing the three parameter; fenchone, estragole and *trans*-anethole that the two cultivars

growing in Egypt (F 1 and F 2) have almost the same ratios of the compared parameters while, the Sudan cultivar F 3 is closer to F 1 and F 2 than it is to F 4. Also the three cultivars (F 1 - F 3) are far from specification of sweet fennel oil but close to bitter fennel oil. The German cultivar (F 4) has the best oil quality as a sweet fennel. Thus these findings are of pharmaceutical-industrial value helping in the production of herbal pharmaceutical products of fennel fruit and/or oil of known higher quality.

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References

1. *Adams, R. P. (1995):* Identification of Essential Oil Components by Gas Chromatography Mass spectroscopy. Allured Publ. Corp., Carol Stream.
2. *Alexandrovich, I.; Rakovitskaya, O.; Kolmo, E.; Sidorova, T. and Shushunov, S.* (2003): The effect of fennel (*Foeniculum vulgare*) seed oil emulsion in infantile colic: a randomized, placebo-controlled study. Altern. Ther. Health. Med.; 9:58-61.
3. *Barthlott, W. (1981):*.Epidermal and seed surface characters of plants: Systemic applicability and some evolutionary aspects. Nord. J. Bot.; 1: 345-355.
4. *Barthlott, W. (1990): Scanning Electron Microscopy of the Epidermal Surface in Plants*. In: Scanning Electron Microscopy in Taxonomy and Functional Morphology, Systematic Association, Special Clarendon Press, Oxford.
5. *Barthlott, W.; Neinhuis, C.; Cutler, D.; Ditsch, F.; Muesel, I.; Theisen, I. and Wilhelm, H. (1998)*: Classification and terminology of plant epicuticular waxes. Bot. J. Linn. Soc.; 126: 237-260.
6. *Barthlott, W.; Theisen, T.; Borsch, T. and Neinhuis, C. (2003):* Epicuticular Waxes and Vascular Plant Systematics: Integrating Micromorphological and Chemical Data. In: Deep Morphology: Toward a Renaissance of Morphology in Plant Systematic, Regnum Vegetabile Ganter Verlag, Rugell, Liechtenstein.
7. *Corner, E. J. H. (1976):* The Seeds of Dicotyledons. Vol. II Cambridge University Press, Cambridge.
8. *Egyptian Pharmacopoeia (1984):* General Organization for Governmental Printing Affairs, Cairo.
9. *Evans, W.C. (2002):* Trease and Evans Pharmacognosy, 15th ed. WB Saunders, Edinburg, UK.
10. *Flamini, G.; Cioni, P.L. and Morelli, I. (2005):* Composition of the essential oils and *in vivo* emission of volatiles of four *Lamium* species from Italy: *L. purpureum, L. hybridum, L.bifidum and L. amplexicaule*. Food Chemistry, 91: 63-68.
11. *Gunn, J.W.C. (1920)*: The carminative action of volatile oils. J. Pharmacol. Exp. Ther.; 16: 39-43.
12. *Haggag, E.G.; Abou-Moustafa, M.A.; Boucher, W. and Theoharides, T.C. (2003):* The effect of a herbal water-extract on histamine release from mast cells and on allergic asthma. J. Herbal Pharmacotherapy; 3: 41-54.
13. *Harries, N.; James, K.C. and Pugh, W.K. (1978):* Antifoaming and carminative action of volatile oils. Br. J. Surg.; 2: 171-177.
14. *Holt, S. (1990):* Observations on the relationship between nonulcer dyspepsia and gastric motor function. Gastroenterol J. Club; 2: 9-12.
15. *Javadi, F. and Yamaguchi, H. (2004):* A note on seed coat and plumule morphological variation in the genus *Cicer* L. (Fabaceae). Sci. Rep. Grad. Sch. Agric. and Biol. Sci.; 56:7-16.
16. *Johansen, D. A. (1940):* Plant Microtechnique. New York Book Company.
17. *Leung, A.Y. and Foster, S., (1996): Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics,* 2nd ed. New York, John Wiley & Sons, Inc.
18. *Massada, Y. (1967):* Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. Wiley, New York.
19. *Plant, O.H. and Miller, C.H. (1926):* Effect of carminative volatile oils on the muscular activity of the stomach and colon. J. Pharmacol. Exp. Ther.; 27: 149-156.
20. *Salimpour, F.; Mostafavi, G. and Sharifnia, F. (2007):* Micromorphologic study of the seed of the genus *Trifolium*, section Lotoidea, in Iran. Pak. J. Biol. Sci.; 10: 378-382.
21. *Stearn, W. T. (1966):* Botanical Latin. Thomas Nelson & Sons London.
22. *Wichtl, M. and Bisset, N.G. (1994):* Herbal Drugs and Phytopharmaceuticals. Stuttgart, Medpharm Scientific Publishers.

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