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## Self-assessment competency tool for nurses working in critical care units: development and psychometric evaluation

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**Abstract:** Aim: this study aimed to develop a self-assessment competence tool for nurses working in critical care units, and to test its validity and reliability. Methods: Design: This operational action-oriented research for development of a tool include an experts group consisted of 41 senior registered senior nurses and physicians working in critical care units, jury group from academia and included 11 professors in field of specialty, a purposive sample of 50 nurses working in critical care units Data collection took place in June 2009 to September 2010 through a semi-structured questionnaire, a preliminary nursing competency list with initial 130 items pool through expert and jury opinionnaires, finally 79-item tool of clinical nurse competency was developed. Findings: Cronbach's  $\alpha$  rang from 0.81 to 0.96 for the new tool, with total internal consistency reaching  $\alpha = 0.98$ . Statistically significant positive moderate to strong correlations among the various components of the tool. Statistically significant positive correlations with almost all the new tool dimensions and the standardized tool ranging between 0.256 and 0.725. statistically significant positive correlation between the two scores, with a correlation coefficient  $r = 0.44$ . conclusion: a self-assessment competence tool for nurses working in critical care units was developed with a high level of reliability; its content validity was demonstrated, concurrent validity was borderline acceptable. Further improvement of the developed tool is recommended through including a variety of participants, both as experts and juries, and taking a more representative sample. Also, application of the tool in a variety of similar settings would offer data that can help in improving its psychometric characteristics. Moreover, periodic evaluation of nurses' competency can provide longitudinal data that may help in assessing the new tool's predictive validity.

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**Keywords:** self-assessment competence tool, critical care nursing, validity, reliability.

### 1. Introduction

The arena of critical care is currently receiving increasing scrutiny regarding developing advanced practice roles. This is challenging to critical care nurses who work in a wide variety of environments and specialties such as emergency departments and the intensive care units (Coombs and Chaboyer, 2007; Hurley *et al.*, 2008).

Critical care nursing is a dynamic specialty which positively and proactively responds to meet the ever changing, complex and challenging demands of the critically ill population and the overall health care system. This has resulted in the development of a diverse range of nursing roles, such as the bedside staff nurse, nurse manager, advanced practitioner, educator, researcher and consultant nurse (DOH, 2009). As well, critical care nurses are required to make a significant contribution in enhancing the quality and experience of patient care through involvement in professional activities such as the development of self and others, service improvement and research utilization (Bell, 2008).

Competency is crucial to reduce the risk of harming patients, and is of particular importance in

the critical care units (Hurley *et al.*, 2008). The literature that discusses the concept of competency in nursing often refers to the work of Benner (1982) who referred to competency as the "ability to perform a task with desirable outcomes under the varied circumstances in the real world" (P.304). The definitions of competency may be very simplistic, such as professional standards that nurses use to guide the practice (Kaiser and Rudolph, 2003), and individual's qualities and characteristics that lead to effective work performance (McMullan *et al.*, 2003). More complex definitions incorporate all aspects of nursing performance-how it is defined, acquired, developed and assessed (King, 2005). Nurses need personal attributes and characteristics to translate these skills and knowledge into effective action (Calman, 2006).

Competency statements are often grouped according to related facts of specialist practice known as domains. These domains include enabling, clinical problem solving, professional practice, reflective practice, teamwork and leadership (ACCCN, 2002). Meanwhile, Ramritu and Barnard (2001) described eight concepts of competence, which are safe

practice, limited independence, and utilization of resources, management of time and workload, ethical practice, performance of clinical skills, knowledge, and evolving.

Nurses should be assessed in some way to be deemed competent in their nursing practice. Assessing the competence of practicing nurses is essential in order to identify areas for professional development and educational needs (Meretoja *et al.*, 2004). However, measuring or assessing whether a nurse is competent is a challenging and problematic task. Many methods of assessing or measuring competency in nursing exist, including establishing and maintaining a professional portfolio, self-report measures, knowledge-based tests, assessment within a simulated nursing environment, and assessment through clinical bedside observation (Cashin *et al.*, 2008).

Nevertheless, the reliability and validity of assessment methods are still debatable (Watson *et al.*, 2002). For instance, rating nursing competence via assessment methods that stress the functional characteristics of practice may lead to inferring too much from performance and losing the ability to differentiate between nurses with functional skills and those with deeper personal qualities (Cowan *et al.*, 2007). On the other hand, qualitative competency measurement tools are criticized for being too task-oriented, while the concepts of caring, interpersonal interactions and decision-making are perceived as things that cannot easily be measured quantitatively (Bartlett *et al.*, 2000). Furthermore, the performance and competence observation tools may be subject to bias, may become outdated, and may be insensitive to low-visibility skills such as non-verbal communication. In addition, successful performance on one day is no guarantee of it on another (Redfern *et al.*, 2002).

In consideration of how to overcome these disadvantages of assessment tools, and given the available time and resources, the self-assessment competency tools would more practical (Cowan *et al.*, 2007). The methods of self-assessment are well supported, and are based on the concept that critical reflection is positively related to quality of care making it a powerful method for assessing clinical competence (Gopee, 2000; McCaughan and Parahoo, 2000; Way, 2002; Cowan *et al.*, 2005). Self-assessment tools are quick, efficient, and cost-effective (Cashin *et al.*, 2008). Moreover, they encourage practitioners to take an active part in the learning process and facilitate continuing education (Campbell and Mackay, 2001).

However, to ensure that they will provide accurate measurement, assessment tools must possess certain basic attributes. The most important of these

are to tool validity, reliability, and usability (Quinn, 2000). Validity refers to the extent to which a tool measures what it was designed to measure, and hence the tool should be relevant to the construct being assessed (Stuart, 2003). Face validity is the least time consuming method (Polit and Hungler, 2009), but the content, criterion-related, and concurrent validity methods are more important (Stuart, 2003). Concerning reliability, it refers to the tool ability to give similar results when used on separate occasions and with different assessors (Stuart, 2003). However, the issues of stability, equivalence, and homogeneity need to be considered when measuring reliability (Polit and Hungler, 2009)

### **Significance of the study**

Meeting the expectations of delivering safe, effective and timely health care services requires all health care professionals to identify their learning needs, refine and adapt their clinical roles through a process of ongoing self- assessment. Although the review of the literature identified several nursing competency instruments, few of them related to critical care nurses self assessment, but none of them was developed in the context of Egypt. Hence, a competency self-assessment tool would allow nurses to consider their practice within their own environment and assist them to provide high quality clinical practice. This is even more important in the critical care units. This study provides an instrument for self-assessment of competence for nurses working in critical care units.

### **Aim**

The aim of this study was to develop a self-assessment competency tool for nurses working in critical care units, and to test its validity and reliability.

## **2. SUBJECTS AND METHODS**

### **Research design**

This study is an operational action-oriented research for development of a tool.

### **Setting**

The study was conducted in the critical care units at Ain Shams University Hospital, Ain Shams Specialized Hospital, El Demerdash Hospital, and the Obstetrics Hospital.

### **Subjects**

The study subjects consisted of an expert group, a jury group, and a group of staff nurses for concurrent validity and reliability. The experts group consisted of 41 senior registered senior nurses and physicians working in critical care units, with the

only inclusion criterion of three years of experience in these units. The jury group was from academia and included 11 professors in medical-surgical nursing (4), critical and intensive care (4), and nursing administration (3) from the Faculties of Nursing and Medicine in Cairo, Ain-Shams, Tanta, and Alexandria universities. The second group consisted of a purposive sample of 50 nurses working in critical care units for at least one year.

### **Research procedures and tool development**

Data collection took place in June 2009 to September 2010. The first task in the process of developing the self-assessment competency tool was to review the current literature to find how the concept of competence had been used in instruments related to this concept. Then, the process of development of the tool and assessing its validity and reliability was started and involved the following six steps.

#### **Step I:**

In June 2009, a semi-structured questionnaire was developed and used to identify the concept and indicators or categories of nursing competency for nurses working in critical care. The questionnaire consisted of open and closed questions that reflect nurses' performance at the competence level, as well as the domains of nursing practice that represent the categories according to frequency of use (Shapiro 1998; Buchan 1999; Priest 1999; Lindberg, 2006; Toth, 2006). It included ten questions about functions, roles, skills, competencies, and care provided by nurses working in critical care. The experts group members were asked to complete the questionnaire. In a period of two months, they produced as many descriptions as they considered necessary for each category of competency, and created a total of 210 descriptions. The database of these descriptions was analyzed by deductive content analysis guided by Hanley and Higgins (2005), and Gill *et al.* (2006). This resulted in identification of 130 items for competent critical care nursing practice in nine domains: personal and professional development, professional and ethical practice, safety and injury prevention, comprehensive nursing care, interpersonal relationships, manage technical equipment, critical thinking and research utilization, teaching and coaching, and work management.

#### **Step II:**

Based on the forgoing, a preliminary nursing competency list was developed with the initial 130 items pool. These were classified into nine dimensions, and re-distributed to the same respondents for expert evaluation. An opinionnaire

form was designed for this purpose, and the respondent was asked to check each item on a 4-point rating scale (not relevant, somewhat relevant, quite relevant, and very relevant). Based on this assessment, the items judged to be not relevant or somewhat relevant by 50% or more of the experts were discarded. The process ended up with retention of 99 items in nine domains.

#### **Step III:**

At this step, the face validity and logical consistency of the competency domains and the appropriateness of the tool in terms of clarity, and comprehensiveness were evaluated by the jury group. They were asked to review the second list of 99 items agreed upon by the experts group. Based on their opinions, 20 overlapping items were deleted.

#### **Step IV:**

The 79-item tool of clinical nurse competence was translated into Arabic and back translated to ensure proper wording. The items were classified into the nine dimensions as follows: personal and professional development (8 items), professional and ethical practice (11 items), safety and injury prevention (6 items), comprehensive nursing care (21 items), interpersonal relationships (6 items), managing technical equipment (6 items), critical thinking and research utilization (5 items), teaching and coaching (7 items), work management (9 items). The scale of responses reflected the frequency the respondent is actually using the item in clinical practice: never, occasionally, usually, or always. The list was then re-distributed to the jury group to judge the clarity, comprehension, and accuracy of the Arabic version of the tool.

*Step V:* The list was distributed to ten nurses working in critical care units to evaluate its applicability as pilot testing. It was found easy to use. Cronbach  $\alpha$  coefficient of scale categories were estimated and proved to be acceptable and so no changes were made. The tool was then finalized for data collection with the purpose of psychometric evaluation.

#### **Step VI:**

Data collection was done using the developed self-assessment competence tool. The nursing competence scale (NCS) with known validity and reliability (Meretoja *et al.*, 2004) was used for assessment of the concurrent validity of the new tool. The two tools were distributed to 50 nurses in critical care units in the study settings.

### **Ethical considerations**

Before data collection, an approval from Ain Shams University Hospitals' authority and the Faculty of Nursing was obtained. All participants were informed about the purposes and procedures of the study. They were informed about their rights to refuse to participate or withdraw from the study at any time without being penalized. Moreover, the participants were reassured that their responses would be kept confidential and their identities would not be revealed on research tools or reports.

### Data analysis

Data entry and analysis were done using SPSS 16.0 statistical software package. Cronbach alpha coefficient was calculated to assess the reliability of the developed tools through their internal consistency. Pearson correlation analysis was used for assessment of the inter-relationships among the various dimensions and total scores of the two tools and to assess the convergent validity. Statistical significance was considered at p-value <0.05.

### 3. Results

The personal characteristics of nurses in the study sample, (Table 1) indicate that their mean age is 28.9±5.7 years; with half of them being singles. Slightly less than half of them (44.0%) have a bachelor degree, and their mean years of experience in the unit is 5.5±5.6. The majority (85.0%) had related training courses.

Table 2 summarizes the reliability of the total components of the new and the standardized competency assessment tools as ascertained by internal consistency. As evident from the table, Cronbach's  $\alpha$  rang from 0.81 to 0.96 for the new tool, with total internal consistency reaching  $\alpha=0.98$ . As for the standardized competency assessment tool, Cronbach's  $\alpha$  rang from 0.84 to 0.94, with total internal consistency reaching  $\alpha=0.97$ .

A correlation matrix of the scores of the nine dimensions of the newly developed competency assessment tool is presented in Table 3. It indicates statistically significant positive moderate to strong correlations among the various components of the tool. The correlation coefficients range from 0.467 between personal and professional development and managing technical equipment domains, to 0.794 between comprehensive nursing care and work management domains.

As for the standardized tool, Table 4 shows that the correlation coefficients of the dimensions matrix range from 0.006 to 0.838. The table indicates that the first dimension of the tool (helping role) has statistically significant correlations only with the second (teaching coaching) and the fifth (therapeutic interventions) dimensions.

Table 5 describes the correlations between the scores of various dimensions of the newly developed competency assessment and the standardized competency assessment tools. It shows statistically significant positive correlations among most dimensions, reaching as high as 0.765 between helping role (standardized tool) and teaching/coaching (new tool). It is also noticed that the first component of the standardized tool (helping role) has statistically significant correlations with all the dimensions of the new tool, whereas its third component (diagnostic role) has no statistically significant correlations with any of the dimensions of the new tool. On the other hand, the first two and the seventh dimensions of the new tool are significantly correlated only to the first dimension of the standardized tool.

**Table 1. Personal characteristics of nurses in the study sample (n=50)**

	Frequency	Percent
Age (years):		
<30	28	56.0
30+	22	44.0
Range	19-40	
mean±SD	28.9±5.7	
Marital status:		
Single	25	50.0
Married	22	44.0
Divorced /Widow	3	6.0
Nursing qualification:		
Secondary diploma	23	46.0
Technical institute diploma	3	6.0
Bachelor	22	44.0
Experience years (total):		
<10	32	64.0
10+	18	36.0
Range	1.0-30	
mean±SD	8.6±7.0	
Experience years (in dept):		
<5	29	58.0
5+	21	42.0
Range	<1-20	
mean±SD	5.5±5.6	
Attended training courses	42	84.0

Concerning the correlations between the standardized tool total score and the newly developed tool dimensions scores, Table 6 reveals statistically significant positive correlations with almost all the new tool dimensions ranging between 0.256 and 0.509. The only exceptions were with the dimensions of personal/ professional development, professional/ethical practice, and critical thinking/research utilization, which were not

statistically significant. The same table shows statistically significant positive correlations between the total score of the new tool and most of the dimensions of the standardized tool, ranging between 0.204 and 0.725. Only the dimensions of diagnostic role and managing situations of the standardized tool have no statistically significant correlations with the total score of the new tool.

To assess the concurrent validity of the newly developed tool, the correlation between its total score and the total score of the standardized tool was done. Figure 1 is a scatter plot indicating a statistically significant positive correlation between the two scores, with a correlation coefficient  $r = 0.44$ .

#### 4. Discussion

This study was carried out with the aim to develop and psychometrically evaluate a self-assessment tool to measure the competency of nurses working in critical care units. This tool was developed and proved to be a useful tool based on evidence of its reliability and validity as has been recommended by Polit and Hungler (2009).

**Table 2. Reliability of the newly developed and standard tools through internal consistency**

Tool components	No. of items	Cronbach Alpha Coefficient
Tool 1 (new tool) components:		
Personal / Professional development	8	.91
Professional / ethical practice	11	.90
Safety / injury prevention	6	.89
Comprehensive nursing care	21	.96
Interpersonal relationships	6	.91
Manage technical equipment	6	.92
Critical thinking / research utilization	5	.81
Teaching /Coaching	7	.94
Work management	9	.91
Total	79	.98
Tool 2 (standardized) components:		
Helping role	7	.87
Teaching-coaching	16	.92
Diagnostic role	7	.84
Managing situations	8	.88
Therapeutic interventions	10	.88
Ensuring quality	6	.85
Work role	19	.94
Total	73	.97

**Table 3. Correlation matrix of the scores of the components of the newly developed tool**

Tool 1 components	Pearson correlation coefficients (tool 1 components)								
	1	2	3	4	5	6	7	8	9
1. Personal / Professional development									
2. Professional / ethical practice	.500**								
3. Safety / injury prevention	.758**	.671**							
4. Comprehensive nursing care	.670**	.624**	.735**						
5. Interpersonal relationships	.590**	.705**	.710**	.576**					
6. Manage technical equipment	.467**	.557**	.607**	.608**	.574**				
7. Critical thinking / research utilization	.571**	.640**	.643**	.724**	.652**	.664**			
8. Teaching /Coaching	.717**	.572**	.737**	.787**	.695**	.736**	.666**		
9. Work management	.486**	.631**	.634**	.794**	.597**	.689**	.645**	.769**	

(\*\*) Statistically significant at  $p < 0.01$

**Table 4. Correlation matrix of the scores of the components of the standardized tools**

Tool 2 components	Pearson correlation coefficients (tool 2 components)						
	1	2	3	4	5	6	7
Helping role							
Teaching-coaching	.304*						
Diagnostic role	0.272	.713**					
Managing situations	0.159	.817**	.672**				
Therapeutic interventions	.296*	.751**	.684**	.710**			
Ensuring quality	0.006	.527**	.451**	.541**	.558**		
Work role	0.233	.838**	.680**	.768**	.783**	.570**	

(\*) Statistically significant at  $p < 0.05$

(\*\*) statistically significant at  $p < 0.01$

**Table 5. Correlation of the scores of the components of the newly developed and standard tools.**

Tool 1 components	Pearson correlation coefficients (tool 2 components)						
	Helping role	Teaching-coaching	Diagnostic role	Managing situations	Therapeutic interventions	Ensuring quality	Work role
Personal / Professional development	.559**	0.271	0.077	0.118	0.164	0.134	0.178
Professional / ethical practice	.464**	0.167	0.179	0.159	0.246	0.244	0.173
Safety / injury prevention	.592**	.330*	0.176	0.098	0.278	0.213	0.253
Comprehensive nursing care	.637**	.377**	0.198	0.213	.378**	0.231	.341*
Interpersonal relationships	.590**	0.27	0.212	0.165	.315*	0.249	0.271
Manage technical equipment	.564**	.308*	0.167	0.211	.391**	.320*	.406**
Critical thinking / research utilization	.558**	0.13	-0.016	0.081	0.198	0.248	0.125
Teaching /Coaching	.765**	.498**	0.235	.293*	.405**	0.257	.420**
Work management	.694**	.335*	0.224	0.242	.363**	.334*	.375**

(\*) Statistically significant at  $p < 0.05$

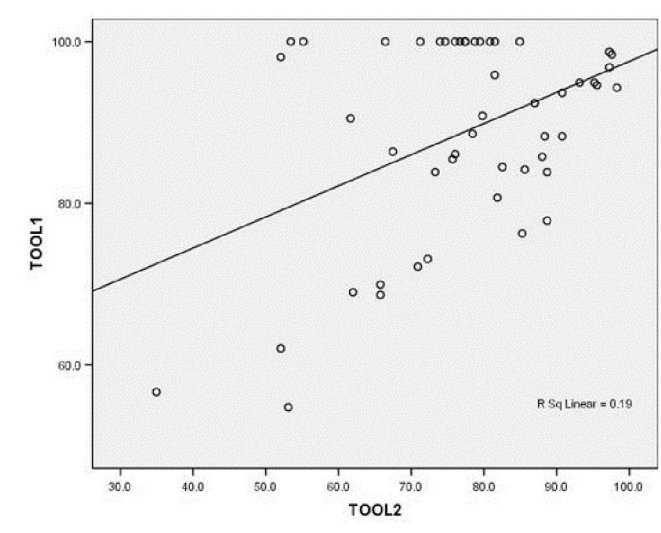
(\*\*) statistically significant at  $p < 0.01$

**Table 6. Correlation between the total score of each tool and the components of the other tool .**

	Pearson correlation coefficients	
	New	Standardized
Tool 1 (new) components		
Personal / Professional development		0.256
Professional / ethical practice		0.26
Safety / injury prevention		0.334*
Comprehensive nursing care		0.416**
Interpersonal relationships		0.348*
Manage technical equipment		0.419**
Critical thinking / research utilization		0.205
Teaching /Coaching		0.509**
Work management		0.438**
Tool 2 (standardized) components		
Helping role	0.725**	
Teaching-coaching	0.372**	
Diagnostic role	0.204	
Managing situations	0.221	
Therapeutic interventions	0.376**	
Ensuring quality	0.293*	
Work role	0.350*	

(\*) Statistically significant at  $p < 0.05$

(\*\*) statistically significant at  $p < 0.01$



**Figure 1. Correlation between the scores of newly developed and standard tools**  
Correlation coefficient:  $r = 0.44$  ( $p < 0.01$ )



The process of development of the new tool started with compilation of items based on literature review and the opinions of those involved in the job, followed by categorization of these items into related components or dimensions. These components or dimensions were based on different sources including recommendations and guidelines as well as similar assessment tools. The guidelines included the competency profile for critical care nurse duties and tasks (Coombs and Chaboyer, 2007), and the six-component competency framework for critical care nurses developed by the American Association of Critical-Care Nurses (AACN, 2005).

The newly developed tool has also added to the components of previous similar tools. For instance, the tool of the Australian College of Critical Care Nurses (ACCCN, 2002) included only five domains, namely enabling, problem solving, professional practice, reflective practice, teamwork and leadership. Similarly, the tool developed by Hanley and Higgins (2005) had five domains of competency: professional and ethical practice, interpersonal skills, practical and technical skills, critical thinking and clinical decision making, and organization and management of care. More recently, Liu *et al.* (2007) developed and tested a competency with 58 items and seven dimensions: leadership, clinical care, interpersonal relationships, legal/ethical practice, teaching-coaching, professional development, critical thinking and research aptitude. Therefore, our newly developed tool encompassed a wider scope of critical care nursing competencies.

The new tool content validity was assessed through judging the degree to which the items of the instrument adequately represent the universe of the content. This was tested by experts in the area, and then by a jury group. This type of validity evaluation is seen as the most important as it ensures a match between the target to be assessed and the tool (Burns & Grove, 2001). The information obtained from the experts group was used to delete or add items as recommended by Strickland (2000). The experts group had no problems in defining the scope of competency of nursing practice in critical care units, although in the early phases of indicators identification some overlap was detected between some of the categories. The deletion of overlapping items may have resulted in low correlations between certain items. Therefore, the factor analysis method was not very useful in the instrument development, and it was decided to resort to the concurrent validity method of assessment.

The tool selected for concurrent validation of our new tool is a self-assessment validated nurse competency scale composed of 73-item instrument distributed in seven competency categories (Meretoja

*et al.*, 2004). It was chosen because it measures the same construct using a similar method, i.e. self-assessment. Moreover, it is one of the most tested scales internationally (Dellai *et al.*, 2009). Cross-cultural validation of the instrument was also carried out in Australia, Italy, and Finland (Dellai *et al.*, 2009). Furthermore, the tool was tested by concurrent validity against a six-dimension scale developed by Schwirian (1978).

According to the current study findings, moderate to strong statistically significant intra-class correlation coefficients were revealed in the new tool. This adds to its content validity. Moreover, concurrent validity revealed ranges of total-to-items correlations that were between 0.2 and 0.7, which is close to those reported by Bekhet and Zauszniewski (2010) in their concurrent validation of the Arabic version of the Depressive Cognition Scale in first-year adolescent Egyptian nursing students; the corresponding range was 0.3-0.7. Additionally, the total score convergence correlation in the present study (0.44) was close to theirs (0.51). These figures are lower than previously reported in concurrent validity studies such as the study Cowan *et al.* (2008) who tested the convergent validity of two competency measures and reported a correlation of 0.75.

Meanwhile, Suleiman and Yates (2011) assessing the translation of the insomnia severity index into Arabic, found that the total tool score had a strong positive correlation with the Pittsburgh Sleep Quality Index global score ( $r= 0.76$ ). However, the same study reported a moderate correlation with the vitality tool ( $r= -0.38$ ), which is even lower than our figure.

The relatively low value of the convergent validity coefficient of the present study might be explained by the newly added dimensions, which were not covered by the standardized tool. This was evident from the total-to-dimensions correlations, which were insignificant for some dimensions of both tools. Nevertheless, the total correlation is close to the lower limit of acceptable coefficients as recommended by Carlson and Herdman (2010). However, these authors encouraged researchers to develop and report convergent validity data.

The second aspect of psychometric evaluation of the tool developed in the current study was the assessment of its reliability, which was ascertained through the internal consistency approach. The results indicated very high reliability coefficients of both the tool dimensions and its total score, mostly exceeding 0.8. The reliability measures of the new tool were similar or even better, compared to the standardized tool. The very high coefficients indicate a high level of reliability as indicated by Polit and

Hungler (2009). The total reliability coefficient of the present study (0.98) is higher than that reported in a similar study (Suleiman and Yates, 2011), which was 0.84.

### Conclusion and recommendations

To conclude, a self-assessment competence tool for nurses working in critical care units was developed with a high level of reliability; its content validity was demonstrated, but concurrent validity was borderline acceptable. The main study limitation is related to representativeness of the sample, which was purposive; therefore, the findings must be interpreted taking this limitation into consideration.

Further improvement of the developed tool is recommended through including a variety of participants, both as experts and novices, and taking a more representative sample. Also, application of the tool in a variety of similar settings would offer data that can help in improving its psychometric characteristics. Moreover, periodic evaluation of nurses' competency can provide longitudinal data that may help in assessing the new tool's predictive validity.

### Practical implications

The development of valid and reliable self-assessment tools to measure nurses' competence is the means for developing nurses' evaluation skills along with increasing their assertiveness and empowerment. These tools also are the way for evidence-based management to guarantee efficient clinical nursing practice as competency recognition helps to develop workforce planning and career opportunities of practicing nurses.

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## Molecular Detection and Predominance of Human Torque Teno Virus in Children's with acute hepatitis and Environmental Waters

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**Abstract:** Introduction: Epidemiological studies have shown that TTV is described worldwide in various populations. The prevalence of TTV viremia in healthy adults of developed countries is in the range of 1-34%. Prevalence reported from third world countries was found to be higher, typically 40-70%. In people who have received multiple blood transfusion; the virus is almost universally present with more than one subtype in each individual. Objectives: This study was carried out to determine the incidence of TTV infection among children's, wastewater, and drinking water, also genotyping of detected TTV strains circulating in the studied samples collected from Cairo. Subjects and Methods: stool samples were collected from 152 Children's with age range from 6 months to 12 years. Also, 45 wastewater samples and 45 drinking water samples were collected monthly from inlet and outlet from wastewater treatment plant (Zenin) and from El-Giza drinking water treatment plant. Collected samples were submitted to semi-nested PCR for amplification of 3' non translated region of TTV. The expected fragment sizes of PCR products were 390 bp and 271 bp for the first and second round of PCR, respectively. PCR products, of 2<sup>nd</sup> round of some positive samples, were purified for nucleotide sequence analysis in both directions. Fragments nucleotide sequences were compared to sequences derived from the corresponding TTV genome of the same regions deposited in the Gene Bank. Results and Conclusion: Obtained data showed that the incidence of TTV in children's stool samples was 59.2% (90/152), and in sewage samples was 64.4% (29/45), while it was 17.8% (8/45) in drinking water samples. Statistical analysis indicated that there no significant difference in TTV infection between male and female in infant and early childhood age groups. The phylogenic tree of positive samples confirmed that the isolated virus sequencing was 100% of nucleotide identity to TTV isolate isolated in Germany in 2001 and deposited in the GeneBank with accession no. AF435014, and the sequence of TTV isolated in the present study belong to TTV genotype 28.

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**Key words:** TTV, incidence, PCR, sequencing, genotyping, children, phylogenic tree.

### 1. Introduction

The Torque teno virus (TTV) was originally isolated from a Japanese patient who had contracted, following a blood transfusion, hepatitis caused by an unknown etiologic agent (Nishizawa *et al.*, 1997). Although TTV was discovered relatively recently in 1997, it seems to be a well-adapted virus of human that has been a persistent source of infection since the distant past. However, TTV differs from all other known viruses in its ability to sustain lifelong viremia, where it actively replicate and continuously produce virus in the blood for decades, even in healthy individuals. Due to its global distribution and persistent viremia in human population, there is no definitive causal association of TTV infection with the diseases that have been investigated. It may be possible that TTVs do not cause any disease and do not have any adverse effect whatever on human health (Griffiths 1999 and Simmonds *et al.*, 1999).

TTV is a small, non-enveloped virus measuring 30–32nm with a 3.4–3.9 kb single-stranded, circular DNA genome of negative polarity (Okamoto *et al.*, 2002). Hepatitis TT virus, is a parenterally transmitted DNA virus which has high prevalence among healthy population and chronic hepatitis patients. The virus can be transmitted both parenterally and orally; its presence in feces and its remarkable environmental stability suggested the possibility of using it as an indicator of fecal contamination in the environment (Verani *et al.*, 2006 and Griffin *et al.*, 2008).

The prevalence of TTV DNA in Egypt did not differ among patients with chronic hepatitis B (46%), chronic hepatitis C (31%), schistosomal liver disease (36%) and blood donors (29%) (Gad *et al.*, 2000). It seems that the infection neither contribute to the severity of liver disease nor to the causation of hepatocellular carcinoma ;where TTV was

determined in the serum of 60 samples obtained from hepatocellular carcinoma patients and 30 healthy individuals, and the most prevalence TTV genotype in Egypt was genotype1 (Hafez *et al.*, 2007).

## 2. Subjects and Methods

### A. Stool and environmental water samples:

#### 1. Stool samples:

During December 2007 to November 2008, 152 fecal specimens were obtained from Children's age range ; one week to 14 years old, from the Pediatric Hepatology Unit of three hospitals (Abo El-Rish, El-Safe and Embaba) located in Cairo and involving 86 males and 66 females. All of them showed the typical symptoms of acute hepatitis including nausea, vomiting, abdominal pains, diarrhea, fever, hepatomegaly, jaundice, and/or dark urine. Specimens were stored at 4°C, transported and processed within 6 hours. Fecal specimens were initially suspended in phosphate-buffered saline (15% wt/vol) using a glass rod to break up the solid particle, and were dissociated by vortex , then centrifuged at 5000 rpm at 4°C for 10 min. Supernatants were transferred to fresh sterile tubes and centrifuged for an additional 5 min at 5000 rpm at 4°C. Clarified supernatants were a liquated and stored at -80°C until virus detection.

#### 2. Wastewater samples:

During December 2007 to February 2009, 45 waste water samples were collected from Zenin wastewater treatment plant, where one sample was collected monthly, during a total study period of 15 months, from each wastewater type {15 sludge waste samples, 15 untreated wastewater samples (inlet) and 15 samples after treatment (outlet)}. TTV were concentrated from wastewater effluent samples by

filtration of 5 liters through negatively charged nitrocellulose membranes according to Rose *et al.*, (1984). Sample was then re-concentrated using an organic flocculation method according to Katzenelson *et al.*, (1976). Viruses were isolated from 100gm of sludge samples by using 10% beef extract and then the suspension was stirred at PH 9.5 for 30 min and centrifuged. The supernatant was re-concentrated using an organic flocculation method according to (EPA, 1984).

#### 3. Drinking water samples:

Drinking water samples were collected monthly in the same period as in the wastewater. 45 drinking water samples were collected from El-Giza drinking water treatment plant (15 drinking water sludge, 15 raw inlet water and 15 treated outlet water samples). TTV Virus was concentrated from drinking water by filtration of 100 liters by using ultra-filtration system (Amersham pharmacia Bioscience, USA) through hollow fiber cartridge of 50k dalton pore size and surface area of 8400 cm<sup>2</sup>. The one hundred drinking water samples were concentrated to one liter each and viruses were re-concentrated using 3% beef extract by organic flocculation method according to Katzenelson *et al.*, (1976).

### B. DNA extraction and Polymerase Chain Reaction (PCR):

TT virus DNA was extracted from stool, wastewater, and drinking water samples using viral DNA extraction kit (Axygen, USA), according manufacturer instructions.

#### 1. Oligonucleotide primers:

Two pairs of primers were used for first and second round semi nested PCR as shown in table (1).

**Table (1): Primers sets used for 3'NTR region amplification of TTV DNA.**

	Code of primer	Sequence of primer	polarity	Product size
1st round	TTVF1-P3B1	5'-GTGGGMSYTTCACTTGTCGGTGTC-3'	Sense	390bp
	TTVR1P3B3	5'-CMAATGGCRAGAAGATAAAGG-3'	Antisense	
2nd round	TTVF2-P3B2	5'-ARGTMRCYAAGCACTCCGAGCG-3'	Sense	271 bp
	TTVR1P3B3	5'-CMAATGGCRAGAAGATAAAGG-3'	Antisense	

Where Y represents C or T, R represent A or G, M represent A or C, H represent A, T or C and D represent G, A or T.

### 2. Detection of TTV DNA by conventional semi-nested PCR:

For amplification at 3'non-translated region of TTV genome, conventional semi nested PCR was used in a reaction volume of 50µl according to the method described by Biagini *et al.* (2001). Briefly, 10µl of the extracted DNA, was added to 5µl (10x) PCR Buffer, 4µl (25mM) MgCl<sub>2</sub>, 0.5µl (5U/µl) Go

Taq Flexi DNA Polymerase (Promega, Cat. No. M8305), 1µl (50 pmol/µl) of sense primer, 1µl (50 pmol/µl) of antisense primer, 4µl of dNTPs (10mM each, Promega, Cat. No. U122A) and 24.5µl of distilled water. For PCR amplification, an initial denaturation step of 5min at 95°C followed by 35 cycles at 94°C for 45sec., 52°C for 30 sec. and 72°C for 60 sec, and an additional final extension step at

72°C for 10 min. the second round PCR was carried out in a reaction volume of 50µl using 1µl of the first-round PCR product, with 1µl (50 pmol/µl) of sense primer and 1µl (50 pmol/µl) of antisense primer of the 2<sup>nd</sup> round primers for 30 cycles at 50°C annealing temperature and the same other temperature conditions as 1st round. Analysis of samples, for the presence of TTV DNA, were carried by mixing 10µl of 2<sup>nd</sup> round PCR products with 2µl of loading dye and analyzed by electrophoresis (General biosystem, Germany) using 2% agarose gel containing 10mg/ml ethidium bromide. The PCR products were analyzed by electrophoresis at 100V for 1hr, and bands were visualized under UV light compared by standard 100bp DNA ladder (SibEnzyme, Cat. No. M27, Russia), by using gel documentation system (Bio-doc analyzer, Biometra, Germany).

### C. Direct sequencing of DNA fragments:

#### 1. Sequencing reaction:

For sequencing, PCR products of random positive TTV DNA samples were sequenced to determine nucleotide identity among them and among TTV genomes deposited in the GeneBank. PCR products were cut and purified from agarose gel using Wizard® Sv Gel and PCR Clean-Up System (Promega, Cat. No. A1120) according the manufacturer instructions. Sequencing reactions on both strands of the obtained products were done as described by Biagini *et al.*, 2000, by using 20-75ng of the purified PCR products with 1µl (3.2 pmol/µl) PCR primers using ABI Prism® BigDye® Terminator Cycle Sequencing Ready Reaction kit version 1.1, according to the instructions of the manufacturer. The total reaction volume 20µl, of determinator products was purified by ethanol precipitation with 2µl (3M) sodium acetate, 3µl (5mg/ml) linear acrylamide, and cold absolute ethanol. The nucleotide sequences were determined directly with an ABI Prism® 310 Genetic Analyzer (Applied Biosystems).

#### 2. Computer-assisted sequence analysis:

Sequence data and changes analysis from both strands of the PCR products and extensive sequence information from the GeneBank database were aligned and compared by using the clustal X and blast programs (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed with neighbor-joining analysis using Tree View version 1.6.6 (Page, 1996).

#### D. Statistical analysis:

Data were analyzed by Chi-Square test .

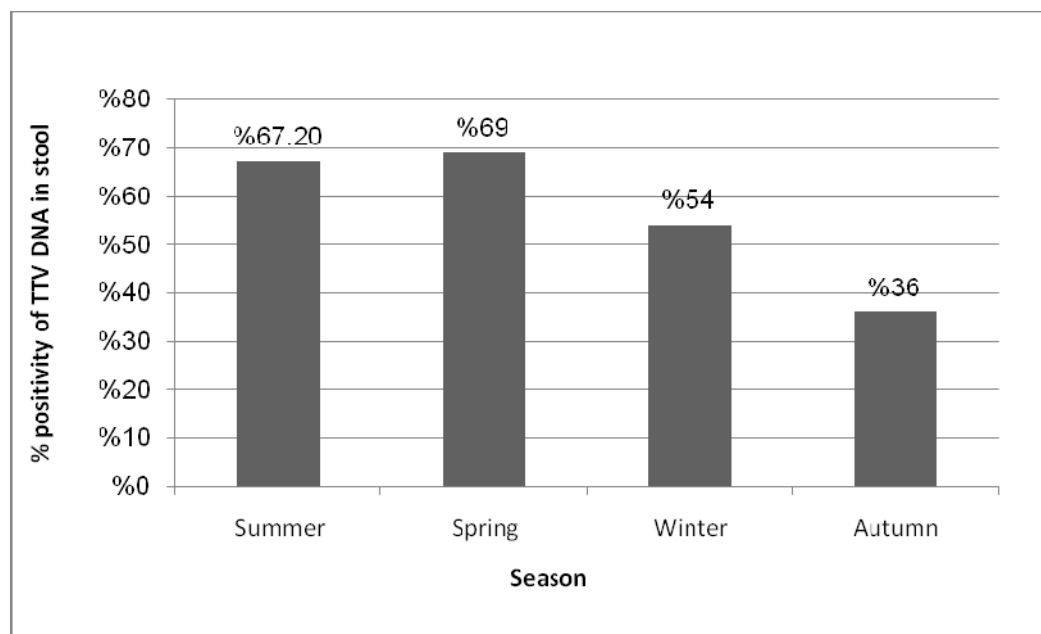
### 3. Results and Discussio

Data showed that the predominance of TTV DNA reach 59.2% (90/152) in stool samples of hospitalized children's with hepatitis, age range from 6 months to 12 years. Children's were grouped according to age into four groups. Data analysis showed that the percentage was 58% (11/19) in the age group 0.5-2 years; 62.9% (44/70), and 59.4% (19/32) in the age groups 3-5 years, and 9-12 years respectively. While the lowest prevalence rate of TTV was 51.6% (16/31) in the age group 6-8 years, Table (2). Prevalence of TTV DNA in children with hepatitis symptoms was considered as high incidence and it was slightly higher than previously reported in similar studies, where during a large outbreak in China it was 40% (45/112) Kangxian and Lian (2001), and in a similar study in Hawaii TTV was 22.4% (15/67) in stool samples of healthy children (Lin *et al.*, 2000). These results indicate that, TTV infection is common in children with symptoms of hepatitis in Cairo. The high prevalence of TTV DNA in infants and early childhood suggested that TTV may be transmitted in early life. The present study showed that the rate of transmission in female was higher than that in male in the age group from 0 to 2 years with no significant difference (Table 2), that may reflect that the hygienic care and behavior in male babies is much higher than that in female babies, or female babies are more susceptible to TTV infection than male babies in this age group. Prevalence of TTV in male and female was nearly equal within the same age group and also between all other studied age groups. Seasonal analysis of TTV in stool samples confirming that the TTV is endemic in the Egyptian children where the prevalence of virus was high and nearly equally distributed through the year, however summer season was higher than other seasons and the lowest prevalence of TTV was in autumn season (Fig. 1).

It was dependence between frequency of TTV infections and chronic diseases and mental-physical retardation of children ( $p=0.077$ ;  $p=0.01$ ). It was not confirming significant differences in depend on dwelling place, and children from worse social conditions were infected more often ( $p=0.05$ ). It was not significant dependence between frequency of TTV infections and breastfeeding. It was confirmed that in children of parents with chronic diseases and children with brothers and sisters with chronic diseases are infected more often (for mothers  $p=0.054$ ; fathers  $p=0.04$ , brothers or sisters  $p=0.062$ ). Disturbances in pregnancy or delivery were not connecting to frequency of TTV infections (Los-Rycharska, *et al.*, 2008).

**Table (2): Age and sex-specific Torque teno virus DNA in children's stool in Cairo**

Age (years)	Positive number of TTV		
	Male (%)	Female (%)	Total (%)
0–2	6/13 (46.1%)	5/6 (83%)	11/19 (58%)
3–5	21/31 (67.7%)	23/39 (59%)	44/70 (62.9%)
6–8	9/15 (60%)	7/16 (43.8%)	16/31 (51.6%)
9–12	16/27 (59.3%)	3/5 (60%)	19/32 (59.4%)
Total	52/86 (60.5%)	38/66 (57.6%)	90/152 (59.2%)

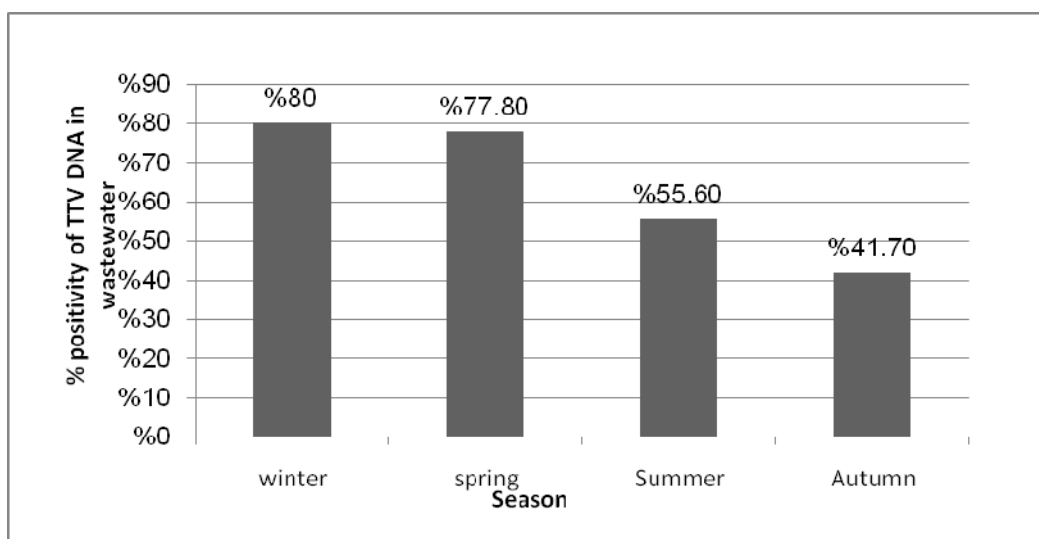
**Fig. (1): Seasonal variation of TTV DNA in children's stool samples**

In case of environmental water, generally the predominance of TTV DNA in Zenin wastewater treatment plant (WWTP) samples was 64.4% (29/45); where the percentage of TTV DNA in sludge samples was 26.7% (4/15) while it was 83% (25/30) in both treated and untreated wastewater effluent, represented as 93.3% (14/15) in raw untreated wastewater (inlet) and 73.3% (11/15) in treated wastewater (outlet) samples, Table (3). This result was slightly lower than a similar study of **Haramoto et al., (2005)** in Japan who detected TTV DNA in 97% (93/96), 24% (23/95), of influents and final effluents wastewater, collected from WWTPs in Japan, respectively. This difference may be due to the difference in technology used for TTV detection. On the other hand, our result was greatly higher than that of **Vaidya et al., (2002)** in India who found that, TTV was 12.7% (8/63) in

wastewater samples, and higher than that of **Carducci et al., (2009)** in Italy, who found that, TTV was 72% (21/29) and 62% (18/29) in inlet and outlet wastewater samples, respectively. These differences in incidence between our study and the studies of **Vaidya et al., (2002)** and **Carducci et al., (2009)**, may be due to lack of good hygienic knowledge and behavior in Egypt. However, all similar previously reported studies stated that TTV was distributed equally through the year (**Haramoto, et al., 2005** and **Hamza et al., 2011**). Seasonal variation of TTV DNA frequency in Zenin wastewater treatment plant showed that the presence of TTV virus was higher in winter and spring than in summer and autumn (Fig. 2), these results may reflect the sensitivity of TTV to high temperature and tropical weather.

**Table (3): Detection of Torque teno virus in Zenin WWTP by semi- nested PCR.**

No. of samples	Percentage of positive TTV semi RT-PCR			
	Inlet samples	Outlet samples	Total	Sludge samples
45	13.3% (2/15)	6.7% (1/15)	10% (3/30)	33.3% (5/15)



**Fig. (2): Seasonal variation of TTV DNA prevalence in Zenin wastewater treatment plant.**

Predominance of TTV in drinking water samples was 17.8% (8/45) where the percentage of TTV was 33.3% (5/15) in collected drinking sludge samples, while it was 10% (3/30) in drinking water

effluent samples represented as 13.3% (2/15), and 6.7% (1/15) in raw Nile river water (inlet) and in treated drinking water (outlet) samples, respectively, Table (4).

**Table (4): Detection of Torque teno virus (TTV) in EL-Giza DWTP by semi nested PCR.**

No. of samples	Predominance of TTV DNA			
	Raw Inlet samples	Treated outlet samples	Sludge samples	Total
45	93.3% (14/15)	73.3% (11/15)	26.7% (4/15)	64.4% (29/45)

TTV DNA recorded percentage in drinking water samples of the present study was lower than that of **Mendes *et al.*, (2008)** in Brazil who detected TTV in Brazilian Amazon using two molecular methods; conventional PCR, where TTV DNA was detected in a percentage of 37% (19/52) in the samples, and real-time PCR where TTV DNA could be detected in a percentage of 92% (48/52) in the samples. These results showed the role of difference in technology for the TT virus detection, where the sensitivity of viral genome detection differs from technique to other according to the technology used. Also **Haramoto *et al.*, (2010)** in Japan detected TTV in Tamagawa River water samples with a percentage of 5.6% (1/18). Japanese's author data is not far different than the present study data reflecting the wide distribution and diversity of TTV all over the world.

Result for the presence of TTV in drinking water showed also that the distribution of TTV in sludge was higher than that in raw Nile river water that was higher than treated drinking water, (Table 4).

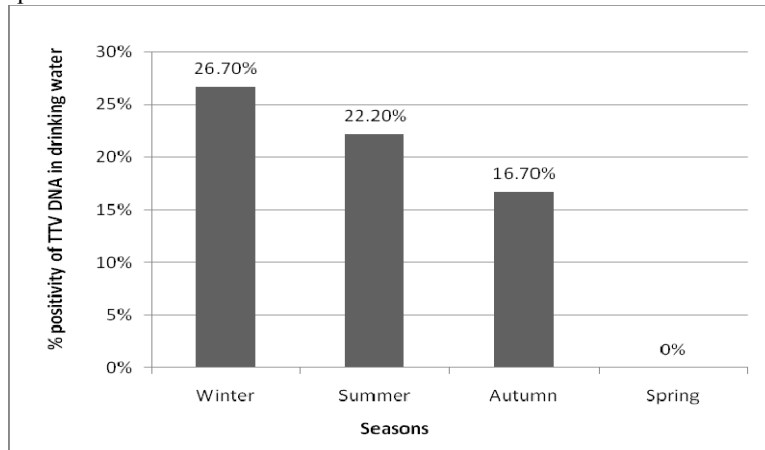
Seasonal variation of TTV DNA revealed that the viral incidence was 26.7% (4/15) during winter and 22.2% (2/9) during summer but it reached to 16.7% (2/12) in autumn season, while in spring season the virus was not detected (Fig. 3).

Generally the distribution of the TT virus in the present study was high in drinking water that should be free of virus contamination; however TTV is slightly less stable in the environment and less resistant to changes of environmental conditions. The high distribution of TT virus in raw drinking water reflecting that the probability of pollution of raw Nile river (the source of raw water in the studied drinking water treatment plant), with contaminated wastewater by TTV. The data showed also that the TTV is endemic in the Egyptian environment and the high prevalence of TTV in both stool and environmental water samples reflecting that the mode of transmission of virus in Egypt may be mainly by consumption of contaminated drinking water (fecal-oral route) rather than by blood transfusion specially because none of the children in the present study was



transfused blood in their live. The presence of TTV in treated drinking water showed also a defect in drinking water treatment technology because the treatment failed in complete removal of TTV from

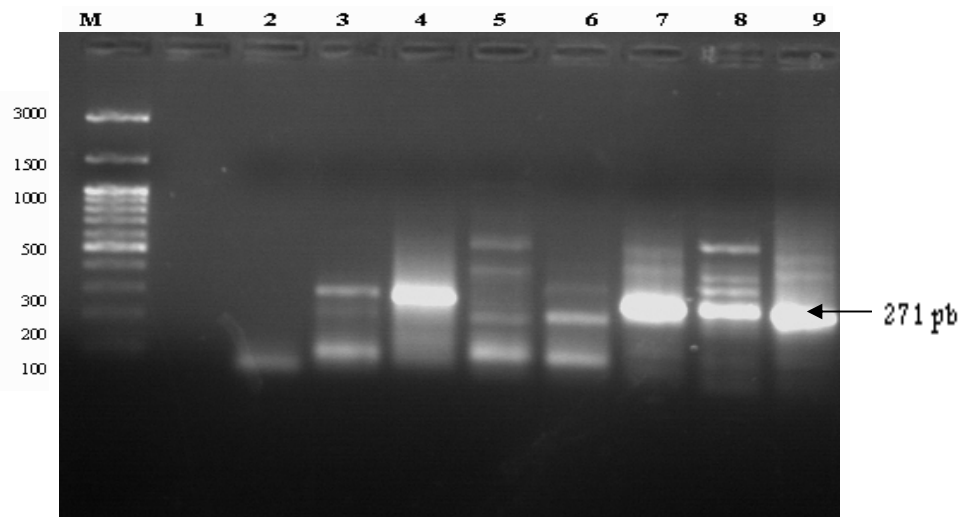
treated water and the percentage of removal was considered as low removal efficiency because it was 6.6% (Table 4).



**Fig. (3): Seasonal variation of TTV DNA prevalence in El-Giza drinking water treatment plant.**

The present study has checked the prevalence of TTV DNA in three different types of samples (stool, wastewater, and drinking water samples), using semi-nested PCR by two sets of primers from the 3'NTR to increase the specificity and sensitivity of TTV detection. TTV DNA was detected in collected positive samples where amplified PCR product of 2<sup>nd</sup>

round was 271 bp, the amplified PCR product appeared as a dense band in the most of sludge and wastewater samples than in the amplified drinking water samples as shown in Fig. (4), reflecting that the number of viral particles detected in these samples more than that in drinking water samples.



**Fig. (4).** Agarose gel electrophoresis of 2<sup>nd</sup> round semi-nested PCR for TTV DNA. Amplification product of 271 bp at 3'NTR. Lane M: 100 bp DNA ladder. Lane 1: Negative control, Lane 3, 4, 7, 8 and 9 were positive samples but 2, 5 and 6 were

Most of amplified PCR product fragments of positive samples were sequenced, compared to each other and to sequences derived from the corresponding TTV genome region deposited in GeneBank, (Fig. 5).

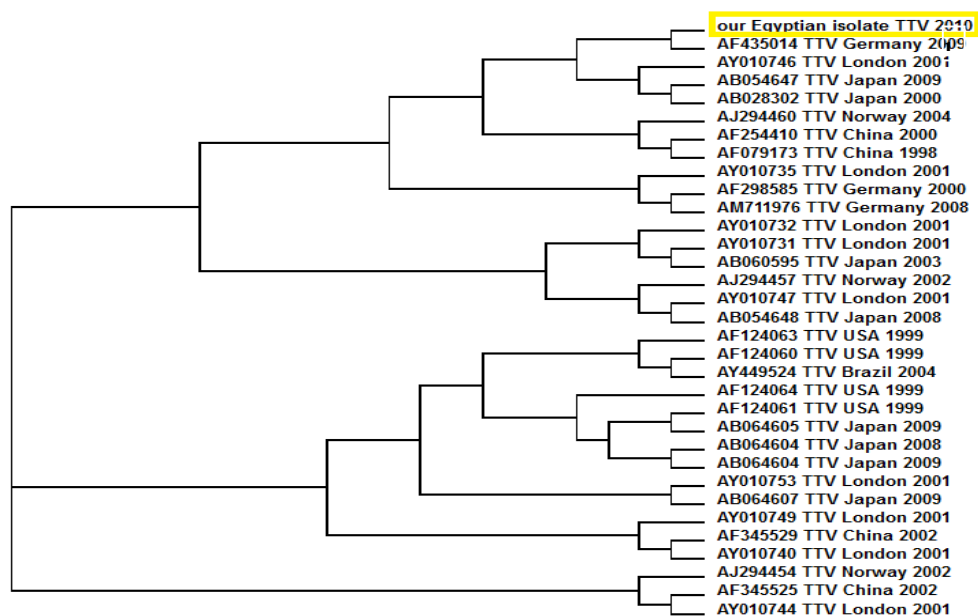
CTTCTTCGGAGCCGCGCTACGCCTTCGGCTGCGCGGGCACCTCAGACCCCGCTCGTGCTG  
ACACGCTTGC GCGTGT CAGACCACTTCGGGCTCCCGGGGGTTCGGGAAATTTGCTAAACAGACTC  
CAAGTTGCCATTGGACACTGGAGCTGTGAATCAGTAACA

**Fig. (5) Nucleotide sequence of amplified fragment at 3'NTR of TTV DNA.**

For further analysis of the obtained TTV partial genome detected from wastewater, drinking water and stool samples of hospitalized children, the complete sequence fragment was compared with the all highly similar partial TTV genome sequences deposited in the GeneBank to produce a phylogenetic tree. The phylogenetic tree was constructed by alignment of the obtained partial sequence with 32 highly similar TTV nucleotide sequences (**Fig. 6**). This analysis confirmed that the isolated virus

nucleotide sequence was most to the novel TTV KAV isolated that isolated from Germany in 2001.

Nucleotide sequence analysis showed that there is only one TTV genotype circulating in the studied Egyptian environment in the period from 2007 to 2009 and was closely related to the novel isolate named KAV that isolated from Germany in 2001 and deposited in the GenBank with accession no. AF435014, and considered as a new genotype of the TTV family and provisionally designated as genotype 28 (Heller, *et al.*, 2001).



**Fig. (6): A Neighbour-joining Phylogenetic Tree for highly similar sequences of TTV with the present study TTV sequence (Egyptian isolate 2010). Data showed that the obtained sequence is closely related to isolate KAV isolated from Germany in 2001 with Accession No. AF435014.**

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## Gender Differences in Financial Well-being, Financial Socialization and Financial Knowledge among College Students

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**Abstract:** This study aims to determine the gender differences in the financial well-being, financial socialization and financial knowledge among college students. The sample comprised 2,430 students in six public and five private universities across Malaysia, which were randomly selected for the study. A self-administered questionnaire was used as the data collection method. The perceived financial well-being was measured by asking students to rate their level of satisfaction on a scale of one (not satisfied at all) to ten (very satisfied) on six items including the amount of savings, financial situation, ability to purchase, savings for emergency expenses, skills to manage money, and ability to spend. The results revealed significant gender differences in financial well-being, financial socialization and financial knowledge. Female students perceived a higher level of financial well-being and a lower level of financial knowledge and later age financial socialization. [Leila Falahati, Laily Hj. Paim. **Gender Differences in Financial Well-being, Financial Socialization and Financial Knowledge among College Students**. Life Science Journal. 2011; 8(3):173-178] (ISSN:1097-8135). <http://www.lifesciencesite.com>.

**Keywords:** Gender, Financial Well-being, Financial Socialization, Financial Knowledge, College Students, Financial Education,

### 1. Introduction

Financial well-being and its impact on the quality of life have received considerable attention over the last decade. Satisfaction with personal financial affairs is an important factor that contributes to life satisfaction (Campbell et al. 1976). Several studies have indicated that efficient resource management is needed for a perceived higher level of financial satisfaction (Xiao et al. 2009). In other words, personal financial management has been identified as a major factor contributing to satisfaction or dissatisfaction with one's financial situation (Joo 2008).

Financial well-being is conceptualized as satisfaction with one's present financial situation, (Zimmerman 1995), however, Hayhoe and Wilhelm (1998) indicated that perceived economic well-being refers to the perception of one's economic situation in light of what is required and desired. Goldsmith (2005) described financial well-being as the degree of economic adequacy or security individuals and families have. A large number of studies have attempted to identify the dimensions of financial well-being among various groups towards the development of personal financial well-being. Young people, particularly college students, are one of the main consideration groups for financial studies. The assessment of college student's financial behaviour indicates that they are managing far more money, properties, and credit than their parents did at a

similar age. Students often begin their college careers without even having responsibility for their own personal finances (Cunningham 2001), however, most of them have cars, computers, credit cards, and cell phones. Therefore, to pursue more possessions they rely more heavily on credit (Davies and Lea 1995) resulting in a higher debt load as well as greater stress and decreased financial satisfaction (Norvilitis et al. 2006).

Diener (2004) pointed out that desirable outcomes, even economic ones, are often caused by well-being rather than others around, and that those people who are high in well-being later earn higher incomes and perform better at work than people who report low well-being. This statement is more true for college students for various reasons, including that they are potentially the future professional workers and high earners. However, their financial management during college life will have a significant effect on their future success or failure.

The studies concerning personal financial management among students reveal a noteworthy sex difference between males and females in financial matters (Barber and Odean, 2001; Goldsmith and Goldsmith 2006). Financial studies revealed that women and men perceived different levels of financial well-being as they perceived different levels of financial socialization (Hira and Mugenda 2000), financial knowledge and skills (Goldsmith and

Goldsmith 1997) and financial analysis (Webster and Ellis 1996).

While previous research has examined the relationship between a wide range of financial and economic variables with financial well-being, gender differences in financial well-being has received little attention except for some studies like Hira (1987), and Hayhoe and Wilhelm (1998). In reviewing financial studies (Joo and Grable 2004; Lyons 2004; Norvilitis et al. 2006) it revealed that most of them consider gender as a demographic factor and did not analyse from a gender perspective. The main aim of this study is to understand the gender differences in financial well-being among Malaysian college students. Identifying gender differences in financial well-being will give a clear view point about gender differences in financial behaviour and money management among college students.

## 2. Literature Review

The studies of financial well-being indicate that the single most powerful direct determinant of financial satisfaction is an individual's financial behaviour (Joo and Grable 2004). In other words, personal financial behaviour has been identified as a major factor contributing to the satisfaction or dissatisfaction with one's financial status (Joo 2008). Faber (1992), based on a review of several studies, concluded that financial behaviour is not necessarily influenced by economic factors only, but reflects social and psychological issues (Faber 1992), such as gender. Scholars have documented gender differences in financial matters, such as financial knowledge, attitudes and, particularly, financial behaviour, which has resulted in differences in financial well-being. In comparison to men, recent studies have also found that women are less knowledgeable financially (Goldsmith and Goldsmith 2006) late age financially socialized (Shim, et al. 2010) and have negative attitudes towards money (Dowling, 2009), which will likely result in significant differences in their behaviour and financial well-being.

Gender theories indicate that the behaviour of men and women are different, as they are socialized differently. According to the social learning theory, the socialization agents and other social structural variables are instrumental in shaping an individual's attitude and behaviour (Holmes 2009). Children learn financial management behaviour through observation and participation by socialization agents (Danes and Haberman 2007). Lime et al. (2003) pointed out through different socialization experiences that people come to understand money differently. The development stages from birth to adolescents contain socialization

and then entry to adulthood, which mostly happens by entering college life. Most students enter college immediately after high school without a sufficient level of financial knowledge and practice.

Garman and Fogue (2006) pointed out that financial knowledge involves concepts, principles, and technological tools that are essential for being smart about money. However, the research findings among college students indicate that students are burdened by the lack of knowledge concerning financial issues (Chen and Volpe 2002; Ibrahim et al. 2009; Lusardi et al. 2010; Mandell 2009). The research findings of financial knowledge among college students revealed significant differences between male and female students. The research findings indicate that females reported lower levels of knowledge concerning financial topics, such as money management (Hira and Mugenda 2000; Shim et al. 2009), financial analysis (Webster and Ellis 1996), and investing (Goldsmith and Goldsmith 1997) compared to male students. However, the research findings indicate that while financial studies show that woman are more concerned with investing issues than men are (Barber and Odean 2001), their lack of knowledge and confidence is likely to contribute to their dissatisfaction with their financial situation (Goldsmith and Goldsmith 2006; Hira and Mugenda 2000).

## 3. Materials and Methods

### 3.1 Instrumentation

#### 3.1.1. Financial well-being

In this study, financial well-being was measured by a six-item scale. The scale was adopted from Hira and Mugenda (1999), and Porter and Garman's (1993) financial well-being scale, and adapted to the Malaysian context. The scale comprised six components of financial well-being including satisfaction with: a) the amount of savings, b) amount of money owed, c) current financial situation, d) preparedness to meet emergency needs, e) financial management skills, and f) ability to spend money. The perceived financial well-being was measured by asking students to rate on a scale of one (not satisfied at all) to 10 (very satisfied) for six items. The alpha reliability for the financial well-being scale items was 0.898. A financial well-being score was computed by summing the average scores for all six statements.

#### 3.1.2. Financial Knowledge

Financial knowledge was measured by an instrument developed by Sabri et al. (2006) based on the Malaysian context. The instrument consisted of 25 true and false questions concerning financial goals, financial records, savings, investment,

retirement, banking system, time value of money, wills, insurance, education loan, and general knowledge on personal finance. However, finally, the components comprised six dimensions including: general knowledge, investment, savings, record, credit, insurance and risk management. The alpha reliability of the financial knowledge scale items was .747. A financial knowledge score was computed by summing the right answers to statements.

### 3.1.3. Financial socialization

To measure financial socialization a 10-item scale was adopted from Hira's (1997) study. Items were adapted to the Malaysian context and included statements about the age that financial practices start (e.g. own bank account, receive income from working, discuss with family about financial matters). The respondents were required to choose the age for starting to practice each statement on a score answer from never until 18 years old. The financial socialization score was computed by summing the score of the statement from one to six for 10 items. The alpha reliability of the financial socialization scale items was .544. The financial socialization score was computed by summing the score of statements with a higher score indicating earlier financial socialization.

### 3.2 Sample procedure and sample profile

Data were collected using a stratified sampling method. The study sample comprised students from public and private universities; a total of 11 universities were randomly selected for the study (six public and five private universities). For each university a total of 350 students were randomly selected using the list of names obtained from each student affairs office. The total number of questionnaires distributed to the 11 universities was 3,850.

A total of 2,500 completed and usable questionnaires were returned by the students, producing a 65% response rate. Of the 2,500 students who responded to the survey, 40.4% were male students and 59.6% were female students. The ethnic composition was Malay (71.4%), Chinese (21.7%), Indian (5.0%) and others (.8%). The age composition of students was lowest 20 years (39.3), 21-30 years (60.5%) and highest 31years (.2%). The mean age of the respondents was 20.9 years. Students from the public university were (60%) while the others (40%) study in private universities.

### 3.3 Data Analysis

As the main aim of this study was to determine gender differences in financial well-being, t-tests were conducted to determine the gender

differences among student's perceived financial well-being, financial knowledge and financial socialization.

## 4. Results and Discussions

The results of the mean comparisons are presented in Table 1, which indicates that there were statistically significant differences in the financial well-being score between male and female students. Female students (M= 32.2) have a higher level of financial well-being than male students (M= 31.17). The comparison of mean scores of financial well-being, ( $t = -2.24$ ,  $P \leq .00$ ) was found to be statistically significant between male and female students. The assessment of financial well-being components revealed that female students have a higher level of satisfaction with current financial status (M= 5.32), ability to meet wants (M=5.32), savings for emergency needs (M=5.31) and affordability to spend (M=5.74).

The results of the t-test presented in Table 1 indicate that the mean comparisons of current financial status, ( $t = -2.93$ ,  $P \leq .00$ ), ability to meet wants, ( $t = -3.07$ ,  $P \leq .00$ ), savings for emergency needs, ( $t = -2.68$ ,  $P \leq .00$ ) and affordability to spend, ( $t = -2.15$ ,  $P \leq .00$ ) were found to be statistically different between male and female students. The results reveal that female students have a higher level of financial well-being than male students. These findings concur with past research, such as Hayo and Seifert (2003), Di Tella et al. (2001), and Blanchflower and Oswald, (2000), who indicated that, overall, females are happier with the financial situation than males.

With respect to the financial well-being components, female students are significantly more satisfied than male students. Female students are satisfied with their ability to meet wants, savings for emergency needs, affordability to spend and overall current financial situation. Although the gender differences in financial management skills are not statistically significant, male students are more satisfied with their management skills than female students. This is consistent with the findings of Hira and Mugenda (2000), in that the majority of both male and female students are satisfied with their financial management skills. Likewise, although it was found by Hira and Mugenda (2000) that the majority of students are satisfied with management skills, satisfaction with the amount of savings is the lowest among all other components.

Furthermore, the results revealed that male students (M= 12.61) have a higher level of financial knowledge than female students (M= 12.21). These findings indicate that male students are more knowledgeable financially than female students,

which is consistent with previous findings (Chen & Volpe, 2002; Eitel & Martin, 2009; Shim, et al. 2010), and emphasizes that females have less financial knowledge than male students. In respect of financial socialization the results of the t-tests presented in Table 1 reveal that there were differences in the financial socialization mean score between male (M= 34.34) and female (M= 33.59) students. This comparison was found to be statistically significant,  $t= 3.28$ ,  $P \leq .00$ . This result indicates that male students have earlier financial socialization while female students have later financial socialization. These findings are consistent with the results of Shim et al. (2009) and Newcomb and Rabow (1999), which indicate that families use different strategies to socialize boys and girls in relation to financial issues.

### 5. Conclusions and Implications

One of the essential skills that people must acquire to function in our society is the ability to deal with money (Hira 1997). Most college students are at the age where they are developing the skills with which they will build their present and future economic well-being, and in which differences in financial issues may have a significant effect on their future success or failure. The overall findings of the present study indicated that female students are more satisfied with their financial situation; in other words, female students have a higher level of financial well-being than male students. The assessment of financial well-being components reveal that female students are more satisfied with their current financial status, ability to meet wants, savings for emergency needs, affordability to spend, while there are no differences

between males and females in satisfaction with the amount of savings and financial management skills. Although previous research indicated that females report a lower level of financial well-being (Barber and Odean 2001; Hira and Mugenda 2000) the results of this study reveal a higher level of financial well-being among female students. According to socialization studies (Moschis and Churchill 1978; Newcomb and Rabow 1999) males and females perform different behaviour as they experience different socialization and social-cultural expectations during childhood.

In the Malaysian culture, women are socialized in a way with lower levels of desire and expectations from life. Women report being more satisfied as they have a lower level of desire and expectation of life. The findings of the present research indicate late age financial socialization for female students, which may relate to the male preference culture in the Asian context, in that the economic role is male dominated and females are deprived from financial practices. The studies of financial socialization indicate that financial skills are most often learned by children and even adolescents from parents in the home rather than sources outside the home (Clarke et al. 2005). Therefore, parents with male preferences gender ideology may provide a different level of financial practice opportunities for sons and daughters at home. However, late financial socialization results in a lower level of financial skills in young adults, which may have a significant effect on financial behaviour during college studies and even later life.

**Table 1: The Results of t-Test for Financial Well-being, Financial knowledge and Financial socialization**

<i>Items</i>	<i>Male</i>	<i>Female</i>	<i>t</i>	<i>df</i>	<i>Sig</i>
Financial well-being	31.17	32.2	-2.24	2256	.00
Amount of saving	4.89	5	-1.17	2256	.234
Current financial status	5.04	5.32	-2.93	2256	.00
Ability to meet wants	5.07	5.32	-3.07	2256	.00
Saving for emergency needs	5.05	5.31	-2.68	2256	.00
Financial management skills	5.54	5.46	.970	2256	.332
Affordability to spend	5.55	5.74	-2.15	2256	.00
Financial Knowledge	12.61	12.21	2.58	2338	.000
Financial Socialization	34.34	33.59	3.28	2338	.000

The results of this study indicate the low level of financial knowledge among students with female students reporting a lower level. This result confirms previous findings (Borden et al. 2008; Markovich and DeVaney 1997) that male college students reported more financial knowledge than

female students. Overall, a low level of financial knowledge among college students indicates the educational needs during college. Garman and Forgue (2006) indicated that achieving success in financial matters required effective financial planning in all important areas, which is dependent on

sufficient financial education during school and college life.

Students after graduation will enter the labour force and, therefore, need to be equipped with basic financial knowledge and skills. Taken together, the findings of the present study indicate that gender differences in financial issues may relate to social and cultural norms and expectations during socialization. However, as eastern culture is imbued with higher advantages for males and a lower level of expectations of life among females, therefore, females are marginalized from financial practices and perceived lower financial practices and skills. Growing up with different socialization leads to different attitudes, beliefs and behaviour about money. It should be noted, based on the present study findings, that as male students possessed a higher level of financial knowledge and earlier financial experience they will have a stronger attraction to make money in future life.

## 6. Recommendations

These results indicate significant gender differences in financial matters between college students; hence, enhancement of opportunities in financial practices at home for females should receive more attention. The first financial experience happens within the family and, therefore, financial practices at home may affect their financial behaviour even in later life. Therefore, families might be aware of their financial style and financial behaviour and, more importantly, provide equal opportunity for males and females to practice financial matters.

In addressing efficient financial education, collaborative partnerships between families and schools are important. No single partner, such as schools, can be expected to meet all the demands for financial education. In line with the above development efforts, family economy educators should encourage a culture of life-long learning in order to expand financial issue practices from childhood in families until the retirement stage. Since the results of this study confirmed gender differences in financial matters, it is necessary to develop gender sensitive policies in financial issues. Supporting the mainstreaming of gender issues into all educational and related policies together with the collaboration of gender and family economy practitioners, to ensure the equal opportunities of financial affairs for males and females within the realms of the family and formal education is strongly recommended.

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## The Impact of Nurse Role Ambiguity and Role Conflict on Nursing Faculty Commitment in Saudi Arabia

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**Abstract:** Nursing education today is one of the merits of the Ministry of Higher Education. Higher education in the Kingdom of Saudi Arabia was established to enhance the nation's growth and the well-being of Saudis. There is movement toward advanced nursing education in order to keep pace with today's health demand. The Nursing Faculty in Saudi have a mission. Typically, this mission covers teaching, research, and community service. The interaction of these factors may provoke Faculty of Nursing role conflict and role ambiguity that has an impact on their commitments. Purpose: The purpose of this study is to examine the impact of academic nurse role ambiguity and role conflict on nursing faculty commitment in Saudi Arabia. Methods: A non-experimental descriptive cross-sectional correlational study was conducted. Three largest universities were selected. Full-time nurse faculty employees were recruited to participate in the study, with a total of 216 questionnaires returned over a three month period, giving a response rate of 88%. All respondents completed the Meyer and Allen organizational commitment instrument, the role conflict, and role ambiguity scale. Self-reported survey procedures were used to collect the study data. Descriptive procedures, Pearson's product-moment correlation coefficients were used in this study. Results: The majority of participants are expatriates with a mean age of  $36.61 \pm 10.47$ . The results revealed that the nursing faculty faced role ambiguity and role conflict. In addition, Nursing Faculty commitment was normative commitment followed by continuance commitment. Role ambiguity among faculty was negatively correlated to continuance and normative commitment while role conflict correlated to all commitment dimensions. There were significant mean differences between role ambiguity and role conflict and the nationality of nursing faculty. Recommendations: The results of this study reveal that many nursing faculty are experiencing role ambiguity, role conflict, which can lead to a decrease in organizational commitment. Nursing academic administrators ought to create a work environment that dilutes the role conflict and ambiguity in order to enhance excellence in academic scholarship. This will lead faculty to immerse in their own role of teaching, research, and community service and develop a sense of commitment to the organization.

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**Keywords:** Commitment, Role Ambiguity, Role Conflict, Saudi Arabia, Nursing Faculty

### 1. Introduction

Higher education in the Kingdom of Saudi Arabia was established to enhance the nation's growth and well-being for Saudis. For instance, the mission of the oldest university, King Saud University (KSU) is to provide students with a quality education conduct valuable research and serve the national and international societies. As well as to contribute to Saudi Arabia's knowledge economy through learning, creativity, the use of current and developing technologies and effective international partnership (KSU, 2009). The role of the nursing faculty in Saudi universities and colleges encompasses three folds: teaching, research, and community service. Nowadays, Saudi universities such as KSU have placed emphasis on reputation, image, and the pursuit for research level status. To sustain a significant link between faculty work and the discipline of nursing, nurse faculty are required to prioritize their work role to meet these changes.

Role has been defined as a prescribed or expected behavior associated with a particular position or status in a group or organization (Tarrant & Sabo 2010). However, these universities did not yet clearly define the faculty role. As result, role ambiguity and conflict may emerge frequently as problems and create an environment for misunderstanding and miscommunication. In this situation, nursing faculty will not have a clear understanding of what is expected of their performance or how they will be evaluated.

Classical organizational theory, Rizzo *et al.* (1970) studied how role conflict and ambiguity disrupt the function of an organization. In this theory, role ambiguity is defined as the predictability of outcomes based on one's behavior, and the existence of environmental guidelines indicating that one is behaving appropriately. However, Katz and Kahn (1966) described role conflict as "the simultaneous occurrence of two or more sets of pressure such that the compliance with one would make the other more

difficult" (p. 184). **Pearce (1981)** defined role ambiguity as the relative unpredictability of the outcomes of a person's behavior. According to **Rizzo et al. (1970)** role ambiguity consists of three types of unclear role expectations: (a) uncertain expectations, (b) disagreeing expectations about the position, and (c) incongruity between the role performer's own expectations and co-workers' expectations.

The stress of role conflict and ambiguity has been well studied in organizational research. **Miller and Anderson (2002)** conducted focus groups to examine how faculty feel about the work role. The findings indicated that mixed messages about expectations for work, needs for faculty development, idiosyncratic evaluation criteria, and lack of clarity of mission.

The major sources and the degree of role strain in nurse faculty were examined. The faculty experienced moderate to high levels of role strain to high job demands, pressure to conduct research and gain external funding, and poor preparation for their roles (**Mobily, 1991**). As it is well known, the variations in teaching, research, and grant productivity within research universities were strongly attributable to program area differences (**Caykoylu et al., 2007**). Furthermore, many factors can affect faculty commitment to the academic organization that can influence behavior and attitude in the academic workplace. Organizational commitment was defined as multidimensional psychological circumstances that characterize the person's connection with the organization (**Meyer and Allen, 1997**).

The components of commitment are affective, continuance, and normative. The employee's emotional connection and involvement in the organization refers to affective commitment, and continuance commitment refers to an awareness of the employee's costs associated with leaving the organization. As a result, the employee's sense of responsibility to the organization that reflect normative commitment (**Meyer et al., 2002**). **Meyer and Allen (2001)** study reveals a positive correlation between affective commitment and supervisory ratings and a negative correlation between continuance commitment and supervisory ratings.

**Gormley and Kennerly (2010)** explored how commitment is influenced by faculty work role in an educational setting. The study yielded a significant difference between work role, role ambiguity and role conflict. A negative relationship was discovered between affective continuance organizational commitments and between unclear role and role conflict. A study done by **Hinshaw (2001)** showed 45% of nurse faculties are dissatisfied with their work role. **Darwish (2002)** investigated the impact of role conflict and role ambiguity on

organizational commitment in the United Arab Emirates. The results revealed that role ambiguity directly and negatively influences both affective and normative commitments. It was also found that job satisfaction mediates the influences of role conflict and role ambiguity on various facets of organizational commitment, except continuance commitment-high personal sacrifice. Relationship was present between role ambiguity and role conflict, and affective and continuance organizational commitment.

According to **Zakari (2010)**, normative commitment received a high mean score of nursing faculty organizational commitment while organizational climate intimacy reflected the lowest mean score. A study was conducted in Saudi Arabia examine nursing faculty work role in academic setting. The results presented that the majority of nurse faculties never conducted or published research since they were employed in the current organization. In addition, 57% of faculty never participated in community services. The study concluded that around 85% of the faculty spent all of their time in teaching and their role is not well defined or unclear. However, the nurse faculties in Saudi Arabia are experiencing disengagement and production emphasis (**Zakari, 2010**).

The effect of role conflict and role ambiguity on organizational outcomes such as commitment has received less attention, and was typically conducted in non-academic settings. In Saudi Arabia, few research has explored the impact of role ambiguity and role conflict on commitment in academic setting. Organizational commitment in nursing academic settings in Saudi Arabia was not previously well considered and has not been well studied.

The purpose of this study is to examine how dimensions of organizational commitment are influenced by role ambiguity and role conflict in nursing academic settings in Saudi Arabia. Mainly this study will focus on the following objectives

1. To assess the demographical characteristics of nursing faculty in Saudi Arabia
2. To describe nursing faculty organizational commitment domains and role domains
3. To examine the relationships between organizational role ambiguity, role conflict and organizational commitment in nursing
4. To assess the differences between nursing faculty nationality and role domains

## 2. Material and Methods

### Design and Sampling

The design of this study is a non-experimental descriptive cross-sectional correlation.

The three oldest and largest universities under the Ministry of Higher Education responsibility were selected. Full-time nurses with professional ranks range from full Professor to clinical specialist and hold academic degrees ranging from the PhD degrees to baccalaureate degree in nursing were recruited to participate in the study. A total of 250 questionnaires were distributed over a three-month period. A total of 216 were returned, giving a response rate of 86%.

### Instrument

Three questionnaires sent to nursing faculty were: (1) **The Meyer and Allen Organizational Commitment Instrument (1993)** (2) **Role Ambiguity and Role Conflict Questionnaire (Rizzo et al., 1970)** and (3) a demographic questionnaire. Approximately 15 to 20 minutes were needed to complete the questionnaires.

Organizational commitment instrument consists of 18 items seven-point Likert scale. These statements (items) pertain to employees' awareness of their relationships with the organization and their reasons for staying. After faculty read each item, they showed the strength of their agreement by selecting a number from 1 (strongly disagree) to 7 (strongly agree). There are six statements for each of the three commitments scales: affective commitment scale (ACS), continuance commitment scale (CCS), and normative commitment scale (NCS) (Powell & Meyer, 2004). Reverse-keyed statements in the scale were recoded (i.e., 1 = 7, 2 = 6, .... 7 = 1) before scoring. The scores should range in value from 1 to 7 with higher scores indicating stronger commitment. In this study, Cronbach's alpha reliability coefficients for AC, CCS, and NCS subscale were 0.67, 0.73, and 0.77 respectively. Permission was granted by John Meyer to use the commitment scales for the purpose of this study.

Role Ambiguity and Role Conflict Questionnaire developed by **Rizzo et al. (1970)**. This questionnaire uses self-reporting to measure employees' perceptions of their jobs, work roles, and organizational features. **Rizzo et al. (1970)** reported construct validity for the two subscales labeled role conflict and role ambiguity. Internal reliability coefficients of .90 were reported for role ambiguity and .94 for role conflict. In the current study Cronbach's alpha reliability coefficients for role ambiguity and role conflict were .83 and .78 respectively. The questionnaire was comprised of 14-items divided into two subscales: six items for role ambiguity and eight items for role conflict. Subjects are asked to respond to each item according to their perceptions of how each statement applied to their role using a seven-point scale: 1 = Very False, 2 = Somewhat False, 3 = False, 4 = Neutral, 5 =

Somewhat True, 6 = True, and 7 = Very True. Scores for the two scales ranged from 6 to 42 for role ambiguity and 8 to 56 for role conflict. Items measuring role ambiguity were worded positively and were reverse scored, so that a high score indicates high role ambiguity.

The faculty demographic form was developed to measure characteristics of participants such as age, gender, nationality, highest degree earned, academic ranks, and years employed in baccalaureate nursing education.

### Procedures

Self-reported survey procedures were used to collect the study data. Faculty was contacted upon received of the college's agreement to participate. Study participants received data collection packets. Each packet contained (1) a cover letter that explained the study purpose, procedure, outlined participants' rights and confidentiality (2) a set of self-administered questionnaire. Data was collected on site. There was no identifying information on any of the data collection forms.

### Statistical analysis

Data were managed and analyzed with SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Descriptive analyses were used to examine demographic information and level of measured variables. Pearson's product-moment correlation coefficients were used in this study. The t-test was used to assess whether the means of nursing faculty nationality and role domains are statistically different from each other. An alpha level of .05 was the criterion level of significance for this study and .01 levels were reported as well.

### Ethical consideration

The study was approved by the ethical committee on deanship of research at King Saud University. Permission was obtained to conduct the study in selected universities. Throughout the study, protection of human rights was assured and adherence to ethical principles was secured. Thus, the researcher ensured that each individual's autonomy was supported. Participation was voluntary, and there was no penalty for withdrawal from or termination of the study. In addition, the research methodologies were non-invasive, and there were minimal or no anticipated risks to participants. A written consent form was obtained from all participants. Total confidentiality of information was also assured by de-identified all answers and data were coded. No written or computerized records were linked to the collected data with consent forms or the participants' identifying information.

### 3. Results

Table 1 presents demographic data of the participants, their ages ranged from 34 to 57 years ( $M = 36.61 \pm 10.47$ ). The majority are expatriates (135, 62.5%). Female nursing faculty dominate the workforce (192, 89%). The majority of faculty (114, 52.6%) have earned a Ph. D degree. From those who have earned doctoral degree, only 2.6% are Saudi nationals. However, faculty ranks are distributed across assistant professor (74, 34.2%) associate professor (37, 17%), and professor (3, 1.4%). Nursing faculty range from one to 24 years in experience ( $M = 5.97 \pm 5.61$ ).

The results in Table 2 revealed that the highest mean score of nursing faculty commitment was normative commitment ( $28.42 \pm 6.09$ ) followed by continuance commitment ( $26.75 \pm 6.6$ ) then affective commitment ( $23.73 \pm 5.7$ ). Thus, the nursing faculty are experiencing a sense of responsibility toward their academic organization. However, the nursing faculty had higher perceptions of role conflict ( $35.26 \pm 8.21$ ) and moderate of role ambiguity ( $29.88 \pm 6.60$ ).

**Table 1: Demographics Data of Faculty Members (N= 216)**

Demographics Data		Frequency	Percent
Age Group	23-32	93	43.1
	33-42	51	23.6
	43 +	72	33.3
Mean Age	36.61	Std. Deviation	10.475
Nationality	Saudi	81	37.5
	Expatriates	135	62.5
Gender	Female	192	88.9
	Male	24	11.1
Academic Degree	Ph.D	114	52.8
	Master	54	25
	Bsc	48	22.2
Professional Rank	Professor	3	1.4
	Assoc. Prof.	37	17.1
	Assist. Prof.	74	34.2
	Lecturer	54	25.1
	Clinical Specialist	48	22.2
Years of Experience	0-5	43	59.7
	6-10	16	22.2
	11+	13	18.1
Mean	5.97	Std. Deviation	5.61

Table 3 presents the matrix of statistically significant ( $P \geq .05$ ) correlation between organizational commitment domains and role domains. Role ambiguity among faculty was negatively correlated to continuance ( $r = -.24$ ) and significant negative relationship was observed between normative commitment and role ambiguity ( $r = -.48$ ). Role conflict negatively correlated to continuance ( $r = -.40$ ) and normative commitment ( $r = -.24$ ). A moderately positive relationship ( $P \geq .01$ ) was present between role conflict and affective commitment ( $r = .49$ ).

The findings in Table 4 shows expected differences between nationalities in a nursing faculty. A significant difference was found between nursing faculty expatriates and Saudi related to role domains. Nursing faculty expatriates experience higher role ambiguity ( $M = 32.17$ ) than Saudi nursing faculty ( $M = 26.07$ ). In addition, role conflict is higher among nursing faculty expatriates ( $M = 35.60$ ) than Saudi nursing faculty ( $M = 34.70$ ) in Saudi nursing universities

**Table 2. Descriptive Statistics of Commitment Domains and Role Domains**

Commitment Domains	Mean	SD(Standard deviation)
Affective Commitment	23.73	5.76
Continuance Commitment	26.75	6.68
Normative Commitment	28.41	6.09
Role Domains		
Role Ambiguity	29.88	6.60
Role Conflict	35.26	8.21

**Table 3. Correlations Matrix between Organizational Commitment Domains and Role Domain**

Correlations	Role		Organizational Commitment		
	Ambiguity	Conflict	Affective	Continuance	Normative
Role Ambiguity	—				
Role Conflict	.088	—			
Affective Commitment	.055	.494**	—		
Continuance Commitment	-.243**	-.400**	.412**	—	
Normative Commitment	-.482**	-.247**	.168*	.432**	—

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\*. Correlation is significant at the 0.05 level (2-tailed).

**Table 4. T-Test Differences between Nationality and Role Domains among Nursing Faculty**

Role Domains	Nationality	N	Mean	Std. Deviation	Levene's Test for Equality of Variances	
					F	Sig.
Role Ambiguity	Saudi	81	26.0741	7.64817	40.262	.000
	Expatriates	135	32.1778	4.58887		
Role Conflict	Saudi	81	34.7037	9.62347	11.015	.001
	Expatriates	135	35.6000	7.25464		

#### 4. Discussion

The new environment and the advanced movement in higher education in Saudi Arabia will make the faculty role more complex as it transforms from the job of instructor worker to the more fluid role of learning process manager. New paradigms and roles increase the probability of role conflict among faculty (Tarrant & Sabo, 2010). Clear task requirements help minimize confusion and increase

productivity. Faculty will experience role ambiguity when job-related expectations are in transition. Negative relationships existed between role ambiguity and role conflict, and affective, continuance, and normative organizational commitment in this study.

The findings from this study were similar to other studies that examined role ambiguity, role conflict, and organizational commitment domains.

**Gormley and Kennerly (2010)** found that role ambiguity and role conflict influenced all dimensions of organizational commitment negatively. When nursing faculty experienced high role ambiguity and role conflict, organizational commitment was diminished. **Wasti (2005), Karsh et al. (2005), and Piko (2006)** found that role ambiguity, role conflict, and organizational climate were antecedents to all dimensions of organizational commitment. When role ambiguity and role conflict were present, all dimensions of organizational commitment were lower. When nurse faculty experienced role ambiguity, and role conflict, the commitment to the organization was diminished. This result designated that the nursing faculty commitment is the obligation (normative commitment) base and they *have to* do so but their commitment is not desire (affective commitment) based and they *want to*. From culture perspectives obligation to the organization whether of necessity or of one's choosing is a bond of moral duty. Normative commitment is established through socialization, it is evident when an individual receives benefits and needs to reciprocate this social contract (**Herscovitch & Meyer 2002**). **Meyer and Allen (2001)** published work showing that individuals that received high scores in affective commitment tended to have higher performance levels. The discrepancy in the results can be attributed to difference in sample characteristics and demographics. Saudi citizens might place focus on desire to achieve and excel differently than other population due to cultural or environmental factors. In contrast, employees who experience a required need to maintain their employment and cannot afford to lose their jobs have little incentive to go beyond their job description. The academic field of nursing in Saudi Arabia is diverse; international expatriates dominate the workforce. Based on this composition, the development of affective continuance and normative commitment may be greatly affected. Affective commitment development needs to be altered to address the new desires and relevance of the international workforce (**Meyer & Herscovitch 2001**).

In addition, **Zakari et al. (2010)** studied conflict and professionalism perception among nurses. Their findings pointed to a low perception among the participating nurses regarding their professionalism, which includes the personal interest in the nursing profession. They conclude that due to the personal background of the nurses, which includes the personal interest in the nursing profession, as well as the family's, societies and the consumers' views of the profession may have an impact on their commitment. Suggestions for more systematic primary research concerning cultural

differences in professionalism and commitment is warranted (**Bhuian et al., 2001; Bentein et al., 2002**).

Furthermore, **Zakari's (2010)** and **Gormley and Kennerly's (2010)** studies results show that faculty members experience deliberation, disengagement, and lack of intimacy in the academic sitting. The reasons for this finding may be attributed to the imbalance in faculty role; these affected the deliberation relationship among faculty and increased the role conflict and role ambiguity. Therefore, this disengagement decreases work productivity, facilities welfare and increases ambiguity of role. Role ambiguity may increase as nurse faculty focus on one role rather than the other. It is important to implement balance between teachings, research, and community services as described in **Hinshaw's (2010)** and **Miller and Anderson's (2004)** studies. Furthermore, the creation of a bridge between official and unofficial roles of faculty members can aid in restoring this balance. Affective and normative commitment are likely to be required to ensure a willingness to work cooperatively with others and exert extra effort to achieve the objectives of the organization (**Chen, & Francesco, 2003; Wasti, 2003; Wasti, 2005**). The study shows that role ambiguity and role conflict is higher among expatriate nursing faculty. This study also highlights the need for improved guidance for new faculty in their roles as researcher and educator.

The findings of this investigation suggest several implications for both administrators and faculty. There is a need to improve the higher education climate in Saudi Arabia. Consequently, King Saud University (KSU) developed specific initiatives to promote academic climate to excel in all teaching and research fields. Furthermore, KSU established deanship of skills that aids in the development of faculty, lecturers, and teaching assistants skills to reach teaching excellence (**KSU, 2009**). Moreover, focusing on efficiency, flexibility and productivity is important to achieve this objective.

### Conclusion and Recommendations

Overall, role ambiguity, role conflict and organizational commitment are an important area of research; it provides evidence of the relationship between different organizational factors. Organizational commitment is a vital part of the organizational development of a college, and had been linked to motivation and behavior of faculty (**Christmas & Hart 2007**). It is important to understand how organizational commitment affect nursing faculty. The current national nursing faculty shortage in Saudi Arabia is evident as the number of colleges of nursing increase. This will create an

increase in the workload of full time nursing faculty that may inflame role conflict and role ambiguity. According to **Kaufman (2007)**, the average weekly workload of nurse educators has increased to 56-hours.

The results of this study have important implications in recruitment and retention of nurse faculty through the development of effective strategies that addresses both affective and normative commitment. For example, commitment can be improved through proper places socialization of faculty. The deans of the universities should be able to better match faculty goals and institutions. This can be done through interactive dialogue between both parties. Furthermore, other aspects of this study that yields questions can be answered by future research. Additional research can examine the difference in commitment components that interlink with higher education behavior over an extended time and how quality of work role is affected.

### Limitations

Findings from the current study offer an initial step to exploring organizational commitment, and nurse faculty role conflict and role ambiguity in Saudi academic settings. However, generalisability of the study's findings is limited because of the sampling method, which was based on responses from nurses working in three universities in Saudi Arabia.

To increase the power of generalisability of results, it is recommended that future investigations include nurses working in other governmental and private universities. Additional limitation in this study was the use of a cross-sectional design. A one-time measure does not permit testing causal effects relationship.

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## The Emergence of Gender Differences in Consumer Socialization among College Students

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**Abstract:** This study examines the gender differences in consumer socialization among college students. The sample comprised 2,430 students in six public and five private universities across Malaysia, which were randomly selected for the study. A self-administered questionnaire was used as the data collection method. The results revealed gender differences in consumer socialization, in which male students' perceived earlier socialization regarding financial matters. In addition, the results indicated that females are socialized earlier in receiving allowances and owning bank accounts while males are socialized earlier in financial activity and financial assets.

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**Keywords:** Gender, Consumer Socialization, Financial behavior, College Students, Financial Education

### 1. Introduction

Consumer socialization studies have developed extensively in recent decades, as the financial management of young adults is of concern and interest to businesses, educators and families. Prior research concerning consumer socialization has mostly focused on age-related differences in consumer socialization, while a study of gender differences is scarce. Gender matters have received considerable attention in statements concerning economics and finance, since differences have been observed in the male and female financial domains, such as behavior, investment and, most importantly, in financial skills.

Young adults, particularly college students, receive more attention from financial educators (Goldsmith and Goldsmith, 2006; Gutter et al, 2010; Hayhoe et al., 2000; Hira et al., 2000; Joo, 2008; Norvilitis et al., 2006), as they have easier access to financial resources such as credit, debt and educational loans than previous generations of students. However, a great body of research has documented significant gender differences in financial behavior and the level of perceived financial well-being among college students (Masuo et al., 2004; Shim et al., 2009).

The research findings indicated that in comparison to men, women are less knowledgeable financially (Goldsmith and Goldsmith, 2006), late age financially socialized (Shim et al., 2010) and have negative attitudes towards money (Dowling et al., 2009), which will likely result in significant differences in their behavior and, consequently, the different levels of perceived financial problems. Students like all other people learn financial behavior

early in life; however, it is widely held among researchers that family experiences, in particular, often provide the foundation for the student's financial behavior (Danes and Hira, 1986; Hira and Mugenda, 2000; Watchravesringkan, 2008).

Evidence has proven that different financial socialization will lead to differences in financial attitude (Hayhoe et al., 2000; Masuo et al., 2004), financial knowledge (Chen and Volpe, 2002), financial behavior (Xiao et al., 2009), which, in turn, results in different levels of financial independence that may affect their lives in a serious way. The main aim of this study is to investigate gender differences in consumer studies among college students; college students are the future labor force and financial users in the marketplace; hence, it is important to understand the male and female differences in consumer practices during socialization. Such knowledge about students' financial practices is important, as it provides an overview about students' financial needs. Most importantly, such knowledge is crucial for financial educators and practitioners in their efforts to educate male and female students about financial management.

### 2. Literature Review

The study of how and when children acquire the knowledge of conducting money has been a concern among financial researchers. Parents and the family have been identified as the most important source of influence concerning children's money beliefs and attitudes. Hira (1997), from a review of previous studies, suggested that at least some patterns of adult financial behavior are influenced by childhood and adolescent experiences, and the study

of these experiences should help us understand the development of financial behavior.

Consumer socialization was defined by Ward (1974) as the process by which individuals obtain the knowledge, skills, and attitudes relevant to their functioning as consumers in the marketplace (Danes and Haberman, 2007). Later Moschis (1987) indicated that socialization is a life spanning process, which begins in childhood and continues, to some extent, throughout life. Moschis (1987) and McNeal (1987) suggested that parents appear to play a central role in the consumer socialization of their children, and they are influential in teaching their children about appropriate consumer aspects (Hays, 2010; Newcomb and Rabow, 1999; Pinto et al., 2005; Shim et al., 2010).

Children learn financial management behavior through participation (incidental learning) and observation and through intentional instruction by socialization agents (Danes, 1994). Contained by the family, children learn how to handle their money and interact with the adult financial world; however, different financial expectations and rules govern thoughts and behavior that influence their social construction of gender and finance issues (Danes and Haberman, 2007). Consumer socialization studies reveal that gender issues are influenced by family strategies in the socialization process. Leach et al. (1999) pointed out that through different socialization experiences men and women come to understand money differently.

Many studies are consistent with the earlier financial socialization of the son, such as the ASEC (American Saving Education Council) (2001) report, which found that parents allow greater discretionary use of money for daughters than they do sons. Several studies (Rabow and Chames, 1991) indicated that families use a number of different strategies for the socialization of boys and girls regarding consumer matters, such as keeping their daughters dependent and uninformed regarding money. Although there are many consistencies across families relating to the gender socialization of financial roles, Clark et al. (2005) found substantial gender differences when assessing the modeling and teaching of adult financial roles to adolescents. Newcomb and Rabow (1999) findings revealed that boys and girls perceived different financial messages from parents, as daughters are protected from parental fiscal problems or do not perceive or evaluate them in the same way as sons.

They also found that sons, more than daughters, perceived and evaluated their parents as expecting them to know how to work and to save. In addition, sons were introduced to discussions of family finances at an earlier age than daughters,

reported that they currently work more than do women, and received less financial support from their families than did women (Newcomb and Rabow, 1999). Newcomb and Rabow (1999) concluded that women were expected to be more financially dependent than men, and that their participants' experiences regarding socialization about money reflected that expectation. Consumer socialization has been identified as a key concept in providing financial practices during childhood. Understanding gender differences in financial socialization provides knowledge about differences in financial knowledge, attitude and behavior, and, in turn, has an effect on future financial success or failure.

### 3. Hypotheses

For studying the gender differences in consumer socialization among college students four hypothesis was set including:

**H1:** There is a significant gender difference in consumer socialization between male and female students.

**H2:** There is a significant gender differences in receiving allowances between male and female students.

**H3:** There is a significant gender differences in financial practice between male and female students.

**H4:** There is a significant gender differences in financial assets between male and female students.

### 4. Material and Methods

#### 4.1. Instrument

Consumer socialization in the present study was measured using a 10-item instrument, including three main dimensions: receiving allowances and bank account, financial activity and financial assets. The first dimension was adopted from Hira's (1997) instrument; two items were used including "receiving allowance" and "having own saving account at the bank". The second dimension comprised financial activity; 6-items were considered to measure this dimension. The instrument for this dimension included items such as "receiving income from the current occupation", "discussing family's financial issues with parents", "borrowing money from colleagues for purchasing", "knowing financial condition of the family", "able to purchase alone at the store" and "budgeting by myself".

The third dimension was financial assets, which was measured by asking two items, including "owning personal cell phone" and "owning personal transportation". The instrument was measured by asking the respondents to choose the age they started to practice each statement on a score answer from "<7 years old", "7-12 years old", "13-15 years old",

“16-17 years old”, “>18 years old” and “Never”. The consumer socialization score was computed by summing the score of statements from one to six for 10 items; however, the earlier age (< 7years old) received the highest score (6), and never received the lowest score (1).

#### 4.2. Sample procedure and sample profile

Data were collected using the stratified sampling method at six public and five private universities across Malaysia. A self-administered questionnaire was used as the data collection methodology. Of the 2,500 students who responded to the survey, 40.4% were male and 59.6% were female students; 71.4% were Malay being the major ethnic group in Malaysia, 21.7 % were Chinese, 5 % were Indian and others (.8%). The mean age of the respondents was 21 years. Students from public universities were 60% while others, 40%, studied in private universities.

#### 4.3. Statistical Analysis

As the main aim of this study was to determine gender differences in consumer socialization, t-tests were conducted to determine gender differences in socialization regarding consumer and financial issues.

#### 5. The Results of Hypotheses Testing

In this section of the paper the analysis of research hypotheses were present. Since the main aim of the present study was to determine gender differences in consumer socialization, the analysis was provided for three dimensions and the total score of the variables.

##### 5.1. Results of Testing H1

The results of the t-tests presented in Table 1, revealed that there were differences in the

consumer socialization mean score between male (M= 34.34) and female (M= 33.59) students. This comparison was found to be statistically significant,  $t = 3.28$ ,  $P \leq .00$ . This result indicates that male students have earlier consumer socialization while female students have later socialization.

##### 5.2. Results of Testing H2

The findings showed (Table 1) that the mean comparisons for receiving allowance and bank account between males (M= 8.97) and females (M= 9.23), was statistically significant,  $t = -2.86$ ,  $P \leq .00$ . This result indicates that female students were socialized earlier in receiving allowances and own bank account. This finding indicates that families allocate bank accounts and allowances for the female child earlier than for the male.

##### 5.3. Results of Testing H3

Furthermore, the mean comparisons of financial activity and financial practice socialization between males (M= 4.49) and females (M= 4.01) showed significant differences as well. The findings showed that the mean comparisons for financial budgeting socialization was statistically significant,  $t = 8.09$ ,  $P \leq .00$ . This result indicates that male students socialized earlier in budgeting, and consumer socialization compared to female students.

##### 5.4. Results of Testing H4

The results of the mean comparisons in financial assets and ownership socialization revealed that male (M= 20.86) are socialized earlier than female (M= 20.34) students (Table1). The finding indicates the statistical significant differences  $t = 3.25$ ,  $P \leq .00$ , between male and female students.

**Table 1: The Results of t-test in Consumer Socialization by Gender**

Items	Male	Female	t	df	Sig
Financial socialization	34.34	33.59	3.28	2338	.00
Allowance/Bank account	8.97	9.23	-2.86	2338	.00
Financial Activity	4.49	4.01	8.09	2338	.00
Financial Assets/Ownership	20.86	20.34	3.25	2338	.00

#### 4. Discussion and conclusions

The results emphasize gender differences in consumer socialization, and that male students are socialized earlier in most of the items; these findings are consistent with the results of Shim et al. (2009), and Newcomb and Rabow (1999), who indicated that families use different strategies to socialize boys and girls in relation to financial issues. However, the findings show that females are socialized earlier in receiving allowances and owning bank accounts but are socialized later concerning financial activities, such as purchasing alone at the shop, and discussing with family about financial issues.

These findings indicate that although females receive money earlier, males are socialized earlier regarding money and are involved in financial issues and activities such as purchase alone at the shop, discussing with family, and budgeting on their own. These findings are consistent with previous research (Gutter et al., 2009; Hays, 2010; Newcomb and Rabow, 1999; Pinto et al., 2005; Shim et al., 2010), which indicates the gender differences in financial socialization between male and female students. However, the results indicate that males socialize earlier than females, which confirms the findings of Leach et al. (1999), ASEC (2001), and Newcomb and Rabow (1999).

Females receiving allowances and bank accounts earlier than males agreed with the findings of Allen et al. (2008); however, in financial activity males were socialized earlier. These findings indicate that although females receive allowances earlier, the family excluded them from financial practices. Males were involved in family financial issues and practices concerning money earlier than females, which is consistent with the findings of Edwards et al., (2007).

The findings highlight that the consumer socialization process is highly affected by gender issues in which families employ different strategies to socialize male and female children regarding financial and consumer matters. From this finding, the implications for family economics and gender educators seem even more apparent, given the important role of parents in socializing their children in financial issues. Regarding consumer socialization dimensions, the results indicate that most of the students, particularly females, are not involved in family financial issues and discussions.

By identifying the role of financial socialization in developing financial attitude and financial skills during childhood, parents may understand their key role in the financial life of their children. The awareness of families about the importance of providing equal financial practices for boys and girls may be enhanced.

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## Design and Synthesis of Acridine-4-Carboxamide and Acridine-4- Carboxylate Derivatives as Tyrosine Kinase Inhibitors

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**Abstract:** Acridine and quinazoline derivatives represent important classes for the treatment of cancer. Many derivatives of them found to be tyrosine kinase inhibitors. In this work novel eight acridine-4-carboxamide and acridine-4- carboxylate derivatives were synthesized from quinazoline and acridine scaffolds. Six of the newly synthesized compounds were chosen by NCI for screening as anticancer. The activity of six compounds (**8a-d**, **9a** and **9d**) was tested using the national cancer institute NCI disease oriented antitumor screen protocol. Compound **8c** was proved to be the most active member in this study. This acridine analog **8c** could be considered as useful template for further development to obtain more potent antitumor agents.

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**Key words:** Acridine-4-carboxamide, acridine-4- carboxylate, quinazoline, kinase inhibitors.

### 1. Introduction

Protein tyrosine kinases are enzymes involved in many cellular process such as proliferation, metabolism and apoptosis [1]. Several protein kinases are known to be activated in cancer cells [2]. Blocking tyrosine kinase activity therefore represents a rational approach to cancer therapy. Acridines and their analogs represent an important class for treatment of cancer via different mechanisms. They inhibit cancer cells by inhibition of different enzymes such as topoisomerase II [3], telomerase [4] or kinase [5]. They also intercalate with DNA [6]. Many acridine-4-carboxamide derivatives show activity on different cancer cell lines [6]. In recent years, combination chemotherapy with different mechanisms of action is one of the methods that are being adopted to treat cancer. Therefore a single molecule containing more than one pharmacophore, each with different mode of action could be beneficial for the treatment of cancer [7]. Enlightened by the reported activity of quinazoline derivatives as kinase inhibitors [8, 9] and as topoisomerase inhibitors [10], we combine both acridine and quinazoline moieties to produce more potent derivatives. In combining both moieties, different spacers were used. Such spacers were chosen to provide rigid structure and free rotation through phenyl ring in compounds **8a-d** and ethylene bridge in compounds **9a-d** respectively.

### 2. Experimental Chemistry

All melting points are uncorrected and determined by the open capillary method using

Gallenkamp melting point apparatus (MFB-595-010M; Weiss Gallenkamp, London, UK). IR spectra were recorded on a Shimadzu 435 Spectrometer (IR-435; Shimadzu, Japan) using KBr disks. <sup>1</sup>H NMR spectra were recorded on a Perkin-Elmer NMR FXQ-200 MHZ Spectrometer (Tokyo, Japan), using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX, Mass Spectrometer. Elemental analyses for C, H, and N were within ±0.4% of the theoretical values and were performed at the Microanalytical Center, Cairo University, and they were of the theoretical values. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F254 (Merck, Germany) and was visualized by UV lamp.

#### 9, 10-Dihydro-9-oxoacridine-4-carbonyl chloride **3**

It was prepared by the reported method [11] starting from 9, 10-dihydro-9-oxoacridine-4-carboxylic acid **2** [12] and thionyl chloride. It was used directly for preparation of compounds **8a-d**.

#### General method for preparation of 2,3-dihydro-3-substituted -2-thioquinazolin -4(1H)-one **5a-d**.

They were prepared according to the reported method from reaction of anthranilic acid **4** and different isothiocyanate in ethanol [13]

#### General method for preparation of 1-(4-aminophenyl)-2,3-dihydro-3-substituted -2-thioquinazolin-4(1H)-one **6a-d**.

A mixture of **5a-d** (0.01 mol) and *p*-bromoaniline (1.72g, 0.01 mol) was refluxed with sodium methoxide (2.16 g, 0.04 mol) in absolute ethanol (30 mL). The reaction was monitored by TLC. After the reaction was completed, it was filtered and the solvent removed under vacuum. The products were crystallized from ethanol/ ether mixture[14].

**General method for preparation of 2,3-dihydro-1-(2-hydroxyethyl)-3-substituted-2-thioquinazolin-4-(1H)-one 7a-d.**

A mixture of **5a-d** (0.01 mol), 2-chloroethanol (0.80 g, 0.01 mol) and potassium carbonate (6.90 g, 0.05 mol) was refluxed in dry acetone for 6-7 hours. The reaction mixture was then, filtered, left to cool and the crystals precipitated were filtered and recrystallized from ethanol [15].

**General method for preparation of N-[4-(3,4-dihydro-4-oxo-3-substituted-2-thioquinazolin-1(2H)-yl)phenyl]-9,10-dihydro-9-oxoacridine-4-carboxamide 8a-d.**

A mixture of **3** (2.57g, 0.01 mol) and the appropriate compound **6a-d** (0.01 mol) was refluxed in methylene chloride (75mL) and triethylamine (1mL) for 3-4 hours. The reaction was then filtered. The solvent was removed under vacuum. The precipitate which was formed was recrystallized from ethanol/ DMF.

**N-[4-(3-Butyl- 3,4- dihydro -4- oxo-2-thioquinazolin-1(2H)-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8a.**

Yield 60%; mp: 120°C. IR (cm<sup>-1</sup>):3300(2NH), 1650,1660 (3 CO), <sup>1</sup>H-NMR(DMSO-d6) δppm: 0.88 (t,3H,CH<sub>3</sub>), 1.31(m,2H,CH<sub>2</sub>-CH<sub>3</sub>), 1.62(m,2H,CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>),4.30 (t,2H,CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 7.29-7.93 (m,15H,Ar),12.88 (s,2H,2NH).Anal. Calcd. for C<sub>32</sub>H<sub>26</sub>N<sub>4</sub>O<sub>3</sub>S (546.64) : C 70.31, H 4.79 and N 10.25.Found: C 70.25,H 4.80 and N 9.80

**N-[4-(3-Benzyl- 3,4- dihydro -4- oxo-2-thioquinazolin-1(2H)-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8b.**

Yield 61%; mp: 148 °C. IR (cm<sup>-1</sup>) :3350 (2NH), 1720,1639 (3 CO), <sup>1</sup>H-NMR(DMSO-d6) δppm:5.53 (s,2H,CH<sub>2</sub>),7.18-7.83(m,20H, Ar),8.47 (s,1H,NH), 8.72(s,1H,NH NH-CO). MS: m/z 580 (M<sup>+</sup>, 0.09%) Anal. Calcd. for C<sub>35</sub>H<sub>24</sub>N<sub>4</sub>O<sub>3</sub>S(580.66): C 72.40, H 4.17 and N 9.65. Found: C 72.51, H 4.50 and N 9.27.

**N-[4-(3,4-Dihydro-3-methyl -4- oxo-2-thioquinazolin-1(2H)-yl) phenyl] 9,10- dihydro -9-oxoacridine-4-carboxamide 8c.**

Yield 55%; mp:225°C. IR (cm<sup>-1</sup>):3360(2NH), 1670 (3 CO), <sup>1</sup>H-NMR (DMSO-d6) δppm:2.51

(s,3H,CH<sub>3</sub>),7.18-7.99 (m, 15H, Ar),8.13 (s,1H, NH), 8.72 ( s, 1H,NH-CO). Anal. Calcd. for C<sub>29</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>S (504.56): C 69.03, H 4.00 and N 11.10. Found: C68.80, H4.10 and N10.9.

**N-[4-(3-(4-Fluorophenyl)-3,4- dihydro -4- oxo-2-thioquinazolin-1(2H)-yl) phenyl]-9,10-dihydro -9-oxoacridine-4-carboxamide 8d.**

Yield 64%; mp: 245 °C.IR (cm<sup>-1</sup>):3400(2NH), 1780 (3 CO), <sup>1</sup>H-NMR(DMSO-d6) δppm:6.92-7.79 (m ,19H Ar), 8.5 (s,2H, 2 NH). Anal. Calcd. for C<sub>34</sub>H<sub>21</sub>FN<sub>4</sub>O<sub>3</sub>S (584.62): C 69.85, H 3.62 and N 9.58. Found: C 69.89, H 4.10 and N 9.23.

**General method for preparation of 2-(3,4-dihydro-4-oxo-3-substituted-2-thioquinazolin-1 (2H)-yl) ethyl-9,10- dihydro-9- oxoacridine -4- carboxylate 9a-d.**

Acridone-4-carboxylic acid **2** (2.39g,0.01 mol) was dissolved in dichloromethane/ THF (1:1 mix.)(75mL) and drops of DMF (till complete dissolution). DCC (2.06g, 0.01 mol) is added and stirred for 5 minutes, followed by addition of the appropriate compounds **7a-d** (0.01 mol). The reaction is kept stirring at room temperature over night. The reaction was then filtered. The filtrate was evaporated to dryness under reduced pressure and the precipitate was crystallized from ethanol/DMF.

**2-(3-Butyl-3,4-dihydro-4-oxo-2-thioquinazolin-1(2H)-yl)ethyl-9,10-dihydro-9- oxoacridine -4- carboxylate 9a.**

Yield 50%; mp:226 °C.IR (cm<sup>-1</sup>):3350(NH), 1712,1670 (3CO) <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δppm: 0.92 (t,3H,CH<sub>3</sub>), 1.32 (m, 2H, CH<sub>2</sub>-CH<sub>3</sub>), 1.54 (m, 2H,CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>), 2.91(t,2H,CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub>) , 3.43 (t,2H, N-CH<sub>2</sub>) , 4.23 (t,2H,CH<sub>2</sub>-O) , 7.21-7.90 (m,11 H, Ar).Ms:m/z 499.58( M<sup>+</sup>,25% ). Anal. Calcd. for C<sub>28</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>S (499.58): C 67.32, H 5.04 and N 8.41. Found: C 67.60, H 5.60 and N 8.59.

**2-(3-Benzyl-3,4-dihydro-4-oxo-2-thioquinazolin-1(2H)-yl)ethyl-9,10-dihydro-9- oxoacridine -4- carboxylate 9b.**

Yield 45%; mp: 195°C.IR (cm<sup>-1</sup>):3400(NH), 1756,1690 (3 CO), <sup>1</sup>H-NMR(DMSO-d6) δppm:2.49 (t, 2H, N-CH<sub>2</sub>) , 3.03 (t,2H,CH<sub>2</sub>-O) , 3.73 (s,2H,CH<sub>2</sub>-Ph) ,7.21-7.93 (m, 16H,Ar), 8.56 (s,1H,NH). Anal. Calcd. for C<sub>31</sub>H<sub>23</sub>N<sub>3</sub>O<sub>4</sub>S (533.60): C 69.78, H 4.34 and N 7.87. Found: C 69.80, H 4.41 and N 8.30.

**2-(3,4-Dihydro-3-methyl-4-oxo-2-thioquinazolin-1(2H)-yl)ethyl-9,10-dihydro -9- oxoacridine -4- carboxylate 9c.**

Yield 69%; mp:233°C .IR (cm<sup>-1</sup>):3350(NH), 1694,1650 (3 CO), <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δppm: 2.88(



s,3H,CH<sub>3</sub>), 3.62(t,2H,CH<sub>2</sub>-N), 3.67(t,2H,CH<sub>2</sub>-O),7.21-7.93 (m,11H,Ar). Anal. Calcd. for C<sub>25</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S (457.50): C 65.63, H 4.19 and N 9.18. Found: C 65.80, H 4.41 and N 8.60.

**2-(3-(4-Fluorophenyl)-3,4-dihydro-4-oxo-2-thioxoquinazolin-1(2H)-yl)ethyl-9,10-dihydro-9-oxoacridine-4-carboxylate 9d.**

Yield 40%; mp:194<sup>o</sup>C.IR (cm<sup>-1</sup>):3320(NH), 1670,1630 (3 CO), <sup>1</sup>H-NMR(CDCl<sub>3</sub>) δppm: 2.91 (t,2H,CH<sub>2</sub>-N), 3.43(t,2H,CH<sub>2</sub>-O),7.22-7.97 (m,15H,Ar). Anal. Calcd. for C<sub>30</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>4</sub>S (537.56): C 67.03, H 3.75 and N 7.82. Found: C 67.29, H 3.81 and N 8.30.

**Antitumor screening**

Under a sterile condition, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Biocell, CA, USA); 5×10<sup>5</sup> cell/ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilized in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 ml) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 ml) containing a cell population of 6×10<sup>4</sup> cells/ml was pipetted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 5% CO<sub>2</sub> atmosphere. After 48 hrs, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution.

**3. Results and Discussion**

**Chemistry**

The title compounds were prepared firstly synthesized the tricyclic nucleus acridone-4-carboxylic acid **2** then functionalizing it with quinazoline derivatives. Acridone-4-carboxylic acid **2** was prepared from condensation of anthranilic acid and 2-chlorobenzoic acid to give **1**, followed by cyclization according to the reported method [12]. This one was used to prepare intermediate **3** after reacting with thionyl chloride as shown in scheme 1 [11]. Scheme 2 shows preparation of compounds **5a-d**. They were obtained when a mixture of anthranilic acid and different isothiocyanate derivatives were refluxed in ethanol [13]. These compounds later react

with p-bromoaniline in presence of sodium methoxide and ethanol and with 2-chloroethanol in presence of potassium carbonate and acetone to produce compounds **6a-d** and **7a-d**, respectively [14, 15]. Acridone-4-carboxamide derivatives were obtained when intermediate **3** was reacted directly with compounds **6a-d** in methylene chloride in presence of triethylamine. Esterification of acridone-4-carboxylic acid **2** with compounds **7a-d** in presence of N,N-dicyclohexyl carbodiimide (DCC) leads to formation of acridone-4-carboxylate derivatives **9a-d** as shown in scheme 3.

**Biological activity**

The synthesized compounds **8a-d**, **9a** and **9d** were subjected to the NCI in vitro one dose primary anticancer assay using a 3 cell line panel consisting of MCF-7(breast), NCI-H460 (lung) and SF-268 (CNS) cancers. Compounds which reduce the growth of any one of the cell lines to 32% or less passed for evaluation in the full panel of 60 cell lines over a 5-log dose range [16]. Growth percent was shown for each cell line using the known drug 5-fluorouracil (5FU) as positive control.

**Conclusion**

All compounds show specificity in their action especially on UO-31 cell line (renal cancer) with percent growth range from 85%-63%. The most active one was compound **8c**, while the least active one was compound **9a**. Compound **8c** also show slight activity against SNB-75(CNC) with growth percent equal to 68%. In general compounds **8a-d** were more active than **9a-d**. This may be attributed to the rigidity of structure in compounds **8a-d** exhibited by the presence of phenyl ring as spacer between acridone and quinazoline moieties rather than the ethylene bridge in compounds **9a-d**. This could be considered as useful model for further improvement of activity.

**Acknowledgments**

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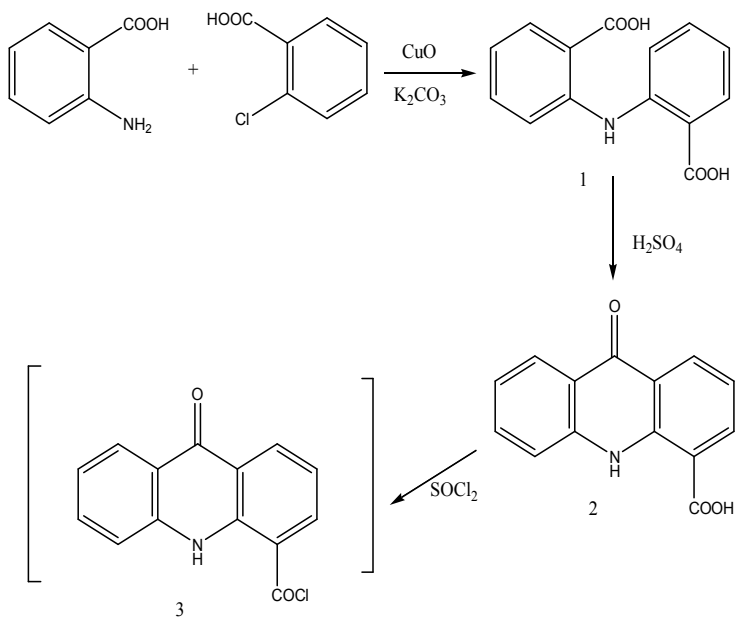
**Corresponding author**

Gehan H.Hegazy

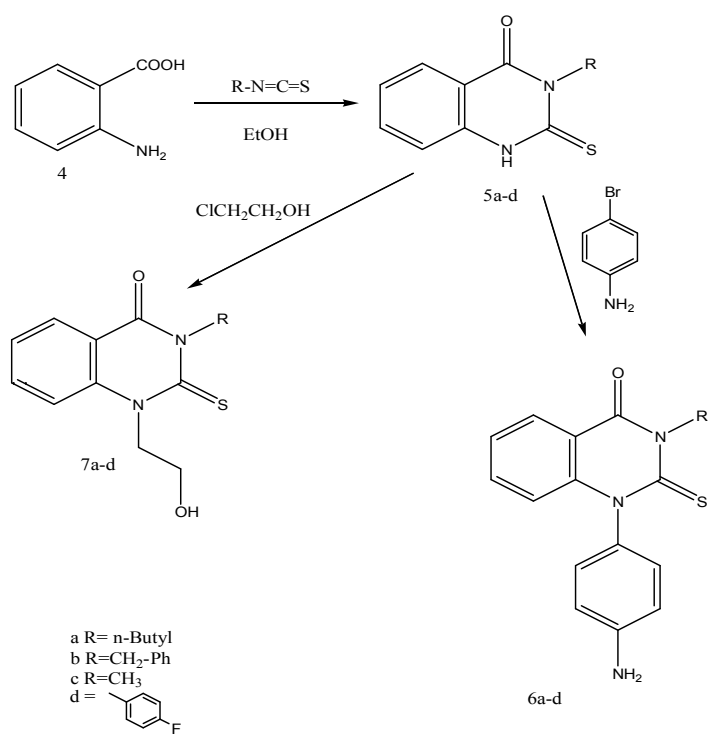
Department of Pharmaceutical Chemistry, Faculty of Pharmacy Cairo University, Cairo, Egypt

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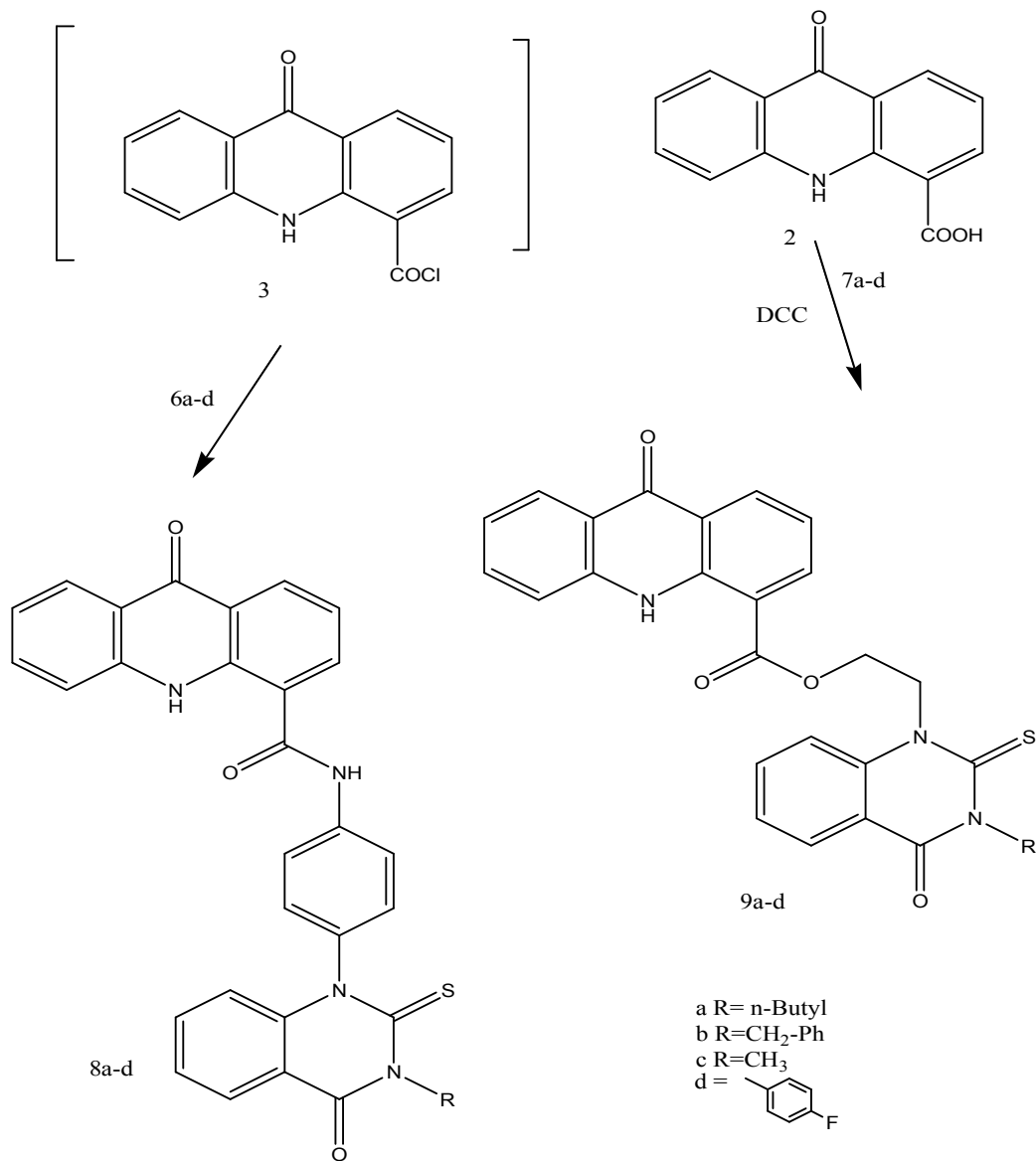
Scheme 1



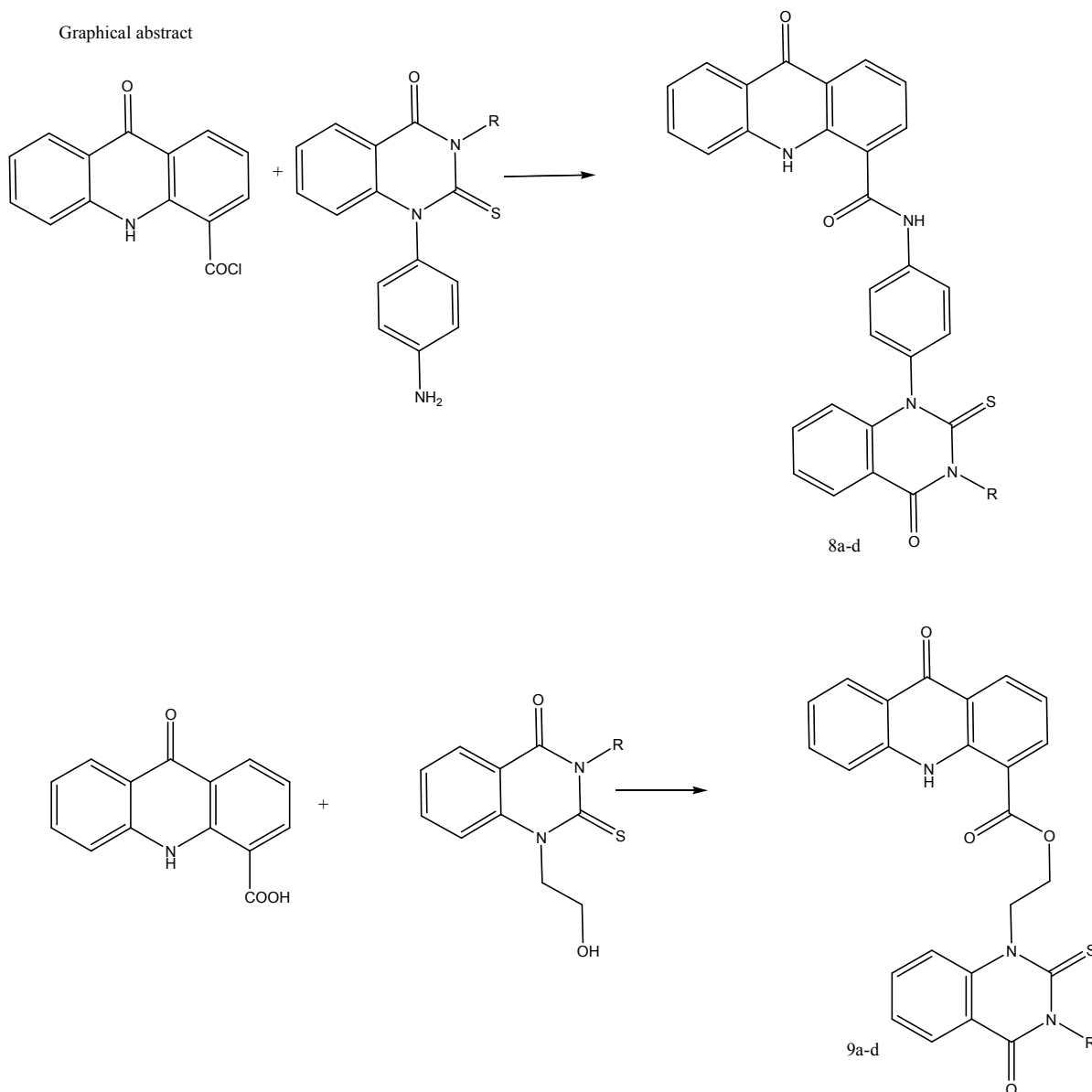
Scheme 2



Scheme 3



## Graphical abstract



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## Effect of Therapeutic Guidelines for Bronchial Asthma on Adult Patients' Knowledge, Practice, Compliance, and Disease Severity

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**Abstract:** Asthma places a large burden on affected patients and their families. Although asthma is a major cause of patient disability and in rare cases causes premature death, asthma morbidity and mortality are largely preventable when patients and their families are adequately educated about the disease and have access to high quality health care. That is, poor outcomes for patient asthma, such as hospitalizations and deaths, are at least partially sensitive to the quality of ambulatory health care. Aim of the study: to assess asthmatic adult needs, designing therapeutic and nutritional guidelines, and evaluating their effectiveness on improvement of the knowledge, practices, and severity of bronchial asthma as well as patient's therapeutic compliance. The research hypotheses were that asthmatic adult patients receiving the designed guidelines will have significant improvements in their knowledge, practices, severity of asthma, and compliance to therapeutic regimen. Design: a quasi experimental research design was used with pre-post assessment of outcome. It involved four steps; pre-guidelines, guidelines implementation, post-guided and follow up. Setting: The study was conducted in the outpatient clinic of bronchial asthma at Zagazig University Hospitals and Helwan University Hospitals. Subjects: The study was carried out on (60) a convenience sample of asthmatic adults consecutively recruited from the study setting, uses of inhaler. Patients with other chronic diseases were excluded. Tools: Four tools were used for data collection, 1- An Interview form, 2- An Observation check list, 3- A Compliance assessment form 4- An Asthma severity assessment scale. Results: There were statistically significant effect of bronchial asthma on patient's daily life activities, work, psychology, self-image, diet and sleep. There was Improvement in knowledge, practice, decreased level of severity of asthma and therapeutic compliance after guidelines implementation. There was a positive coefficients change in knowledge score and guidelines intervention. Also there were negative coefficients change in the severity score of bronchial asthma and Educational level and Knowledge score. The study concludes that the developed guidelines have a significant positive impact on asthma patients' knowledge, practices, therapeutic compliance, and disease severity. This success is attributed to that the guidelines are based on needs assessment and integrate updated technology. Therefore, these guidelines should be adopted as an essential component of the care provided to asthma patients. Continuous follow-up together with selecting the optimal treatment options for each individual patient are recommended. The long-term effects of following the guidelines need to be further studied.

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**Keywords:** Adult asthma, asthmatic needs, guidelines, compliance, nebulizer, deep breathing, coughing exercise and Peak expiratory flow (PEF).

### 1. Introduction

Bronchial Asthma (BA) is one of the most frequent chronic and recurrent diseases. In the last few years the incidence of the disease has been on the rise virtually everywhere. It is estimated that an average of 8% of the world population suffer from bronchial asthma. Although exact epidemiological data are lacking, its prevalence is estimated to be approximately 5.5% of the general population. Most people develop it before the age of 30 years (Payne *et al.*, 2003; Payne *et al.*, 2004). In Cairo, Egypt, the prevalence of bronchial asthma was reported to be 9.4% (Georgy *et al.*, 2006).

According to the Global Initiative for Asthma guidelines final update (*Global Initiative for Asthma*

[GINA], 2009) BA is clearly defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. This inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment (Paul and O'Byrne, 2009). Common risk factors for asthma symptoms include exposure to allergens (such as those from house dust mites, animals with fur, cockroaches, pollens, and molds), occupational irritants, tobacco smoke,

respiratory infections, exercise, strong emotional expressions, chemical irritants, some foods, and drugs such as aspirin and beta blockers (*Chakir et al., 2003; Tarek, 2007*).

Guidelines are written strategies or protocols for health care delivery that are developed to facilitate clinical decision-making and to provide patients with critical information concerning the different treatment options available (*Expert Panel Report 3, 2007*). The BA guideline is intended for health care professionals, including family physicians, nurses and social workers providing primary health care to patients with bronchial asthma. It includes practical and evidence-based information about diagnosis, outpatient management and prevention of the disease in adults (*Rabe et al., 2004*).

Asthma can often be diagnosed on the basis of a patient's symptoms and medical history. Measurements of lung functions by spirometry or Peak Expiratory Flow (PEF) meters provide an assessment of the severity, reversibility, and variability of airflow limitation, and help confirm the diagnosis of asthma (*Global Initiative for Asthma [GINA], 2009*). Traditionally, BA is classified according to severity into intermittent, mild persistent, moderate persistent or severe persistent depending on the grade of symptoms, airflow limitation, and lung function variability. However, it is important to recognize that asthma severity involves both the severity of the underlying disease and its responsiveness to treatment. In addition, severity is not an unvarying feature of an individual patient's asthma, but may change over months or years (*El-shafey, 2006*).

The goal of BA care is to achieve and maintain control of the clinical manifestations of the disease for prolonged periods. When asthma is controlled, patients can prevent most attacks, avoid troublesome symptoms day and night, and keep physically active. To reach this goal, the asthmatic patient can learn how to avoid risk factors, take medications correctly, understand the difference between "controller" and "reliever" medications, monitor their status using symptoms and, if relevant, PEF. They should also be able to recognize dangerous signs indicating that their asthma is worsening and take action or seek medical help as appropriate (*Tarek, 2007*).

To improve the control of BA and reduce the needs for medication, patients should follow certain instructions. Although physical activity is a common cause of asthma symptoms, patients should not avoid exercise. Common strategies for avoiding allergens and pollutants include staying away from tobacco smoke, avoiding drugs, foods, and additives if they are known to cause symptoms, and reduce or

preferably avoid exposure to occupational sensitizers (*Ali et al., 2010*).

Treatment reliever medication should be prescribed for quick relief of symptoms as needed. Patients also require one or more regular controller medications, which keep symptoms and attacks from starting. According to *Global Initiative for Asthma [GINA], (2009)* guidelines, inhaled glucocorticosteroids are the cornerstone treatment in BA. They demonstrate high efficiency in reducing asthma symptoms, frequency and severity of exacerbations, decreasing airway hyperresponsiveness, controlling airway inflammation, as well as improving lung functions and the quality of life (QOL) of patients. They were also associated with lower mortality. Long-acting Beta2 agonists showed great asthma control outcomes together with a marked reduction in the need of rescue medications and an overall improvement in asthmatics QOL (*Nuhoglu et al., 2005*).

Compliance or adherence refers to patient's accurate abiding to a prescribed regimen of treatment in terms of taking medication, following diet, exercising, or undergoing other lifestyle changes. It is an observable behavior that can be measured (*Bastable, 2003*). Inadequate compliance to the recommended plan remains a significant problem facing health care professionals. Many reports emphasize the impact of lack of compliance on the morbidity and mortality associated with BA. It has also been associated with increasing costs of care (*Gorman et al., 2002*).

Ongoing monitoring is essential to maintain control and establish the lowest step and dose of treatment to minimize costs and maximize safety. Typically, patients should be seen one to three months after the initial visit, and every three months thereafter. As severe asthma attacks may be life threatening, patients and health care providers should not underestimate the severity of an attack. Treatment requires close supervision. Fortunately asthma can be effectively treated and most patients can achieve good control of their disease (*Tarek, 2007*).

Achieving asthma control is the focus of asthma management (*Rodrigo, 2005*). Achieving day-to-day asthma control is indicated by the absence of symptoms, minimal use of reliever medication, normal activity levels, and lung function values close to normal. A second objective is to minimize future risks to the patient by ensuring the absence of asthma exacerbations, the prevention of accelerated decline in lung function over time, and no side effects from medications (*Rabe et al., 2004*).

Nonetheless, many patients lack the knowledge and self-care abilities that they need to



achieve their health goals. The nurse who is a skilled educator should take the lead in improving patients' compliance. The responsibilities of the nurse include ensuring that the patient understands the regimen and arranging needed follow-up (Taylor et al., 2001).

### **Rationale and aim of the study**

Despite all advances in the management of asthma, the morbidity and mortality rates are increasing. Both the nurse as well as patients play a pivotal role for the under treatment and mismanagement of the disease. This causes concern in the field of asthma care. Unless the patient possesses basic knowledge about the ailment and its management, there is no likelihood to make the best use of the available facilities. This study was planned with the aim to assess asthmatic adult needs, designing therapeutic and nutritional guidelines, and evaluating their effectiveness on improvement of the knowledge, practices, and severity of bronchial asthma as well as patient's therapeutic compliance. The research hypotheses were that asthmatic adult patients receiving the designed guidelines will have significant improvements in their knowledge, practices, severity of asthma, and compliance to therapeutic regimen.

## **2. Subjects and Methods**

### **Research design, setting, and sample:**

The study was conducted in the outpatient clinic of BA at Zagazig and Helwan University Hospitals. A quasi experimental research design was used with pre-post assessment of outcomes. The study was carried out on a convenience sample of 60 asthmatic adults consecutively recruited from the study settings. Patients using inhalers and those with other chronic diseases were excluded.

### **Data collection tools:**

Four tools were used for data collection, namely an interview form, an observation checklist, the asthma severity scale, and a patient compliance form. The interview form was constructed by the researchers and consisted of four parts. The first part covered patient's personal characteristics as well as the duration of the disease, past and present complaints, and therapy and diet followed. The second part included questions about patient's knowledge of asthma definition, diagnostic measures, symptoms and signs, follow-up schedule, and prevention. The third part involved patients knowledge about the therapies used in BA, as the types, routes, side effects, drugs that aggravate an asthma attack, how to use inhaler, and how to practice deep breathing and coughing exercise. The fourth part was concerned with patient's dietary

knowledge and the types of food that can precipitate the attack. The form included a total of 55 closed and multiple choice questions. Patients' responses to knowledge questionnaire were checked with model answered and given 1 point if correct and 0 if incorrect. The points were summed up and converted into a percent score. A total score of 50% or higher was considered as satisfactory knowledge.

The second tool was an observation checklist: designed to assess actual asthmatic patient's practice regarding deep breathing, coughing exercise, use of inhaler and incentive spirometry. The form was developed based on Hashem (2000), Elshamy (2002), Kamal (2004), Temple (2006), and Elkin (2007). The actual practice was compared with standardized procedures. Accordingly, subjects were given 1 point if the step was correctly done and zero if incorrect. The points were summed up and converted into a percent score. A total score of 60% or higher was considered adequate practice.

The compliance assessment form was adopted from Lenfant and Khaltoev (1995) to assess asthmatic patient's therapeutic compliance. Factors associated with non-compliance were classified into medication related and non medication related. The last tool was the asthma severity assessment scale. It was adopted from Emery et al. (1996) to assess asthma severity according to the criteria set by the National Asthma Educational Program guidelines. Both daytime and nocturnal symptoms were scored as follows: once per week or less (score 0), 2-6 time per-week (score 1), daily (score 2). Oral corticosteroids use was scored as follows: no corticosteroid use (0), sometimes during acute attacks (1), usually during acute attacks (2), and daily (3) even without shortness of breath. Spirometry was scored as follows: forced expiratory volume (FEV1) >80% of predicted value (0), 60-80% of predicted value (1), <60% of predicted value (2). These three scores were summed up to give on overall score of asthma severity levels. These scores were categorized into mild (0-2), moderate (3-5), and severe score (6-7).

### **Pilot study:**

A pilot study was conducted on ten asthmatic adult patients selected from the same study settings to check and ensure the clarity, applicability, and relevance of the tools, to identify any difficulties with their application, and to estimate the time needed to completion of the tools. Modifications of the tools were done according to pilot results to reach to the finalized form. Subjects who shared in the pilot study were not included in the main study sample.

### **Administrative design and ethical considerations:**

The necessary official approvals were obtained from the Heads of the outpatient Departments, and from the General Directors of Zagazig and Helwan University Hospitals. Letters of request were issued to them from the Faculties of Nursing at Zagazig and Helwan Universities explaining the aim of the study and its procedures.

Before the initial interview, an oral consent was secured from each subject after being informed about the nature, purpose, and benefits of the study, as well as any potential side effects. Patients were also informed that participation is voluntary and about their right to withdraw at any time without giving reasons. Confidentiality of any obtained information was ensured through coding of all data. The researcher reassured patients that the data will be used only to improve their health and for the purpose of the study.

#### **Study maneuver:**

After securing official permissions to carry out the study, the researchers met with the potential participants in the outpatient clinics. The aim of the study was explained to them and their informed consent was secured before collecting data. The fieldwork was carried out along a period of twelve months (January 2010 till January 2011), three days weekly. Every patient was interviewed for about 30-45 minutes. Every researcher interviewed five patients per day.

Based on actual educational needs assessment of the patients and guided by relevant literature, the researchers developed the guidelines. They included theoretical background about asthma definition, types, triggers, signs and symptoms, diagnoses, prevention, medical, nursing and self-care management, nutritional guidelines, as well as compliance and follow-up schedule. The theoretical part was presented in two sessions using different teaching strategies as lectures, discussions, and media as posters and videos. The program had five practical sessions that involved demonstration and re-demonstration on diaphragmatic deep breathing and coughing exercises, steps for using the inhaler, nebulizer, spirometry and positioning by using real objects. Moreover, colored booklets were designed by the researchers and distributed to each patient or accompanying relative. Teaching was carried out individually by the researchers for each patient with one of his/her family member to help in following the program guidelines at home. During the five practical sessions, each patient was assessed in performing the prescribed guideline.

The evaluation of the guidelines' effect was carried out using the aforementioned tools. Each patient was evaluated three times during the study:

immediately upon presenting to the outpatient asthma clinic (pre-test), immediately after implementation of the guidelines (post-test), and two months after implementation of the guidelines (follow-up).

#### **Statistical analysis:**

Data entry and statistical analysis was done using SPSS 16.0 statistical software package. Qualitative categorical variables were compared using chi-square or Fisher exact tests as suitable. To identify the independent predictors of knowledge and asthma severity scores, logistic regression analysis was used. Statistical significance was considered at p-value <0.05.

### **3. Results**

Table shows that the age of the patients ranged between 22 and 80 years, with mean $\pm$ SD 48.7 $\pm$ 3.7 years. Two thirds (66.7%) of the patients were females and from rural areas, and more than three quarters (76.2%) were married and illiterate. Only less than one third (31%) of the patients were employed, with mostly sufficient income (59.5%). Only 14.3% of them were smokers. The duration of asthma ranged between 5 and 35 years, with a mean of 17.0 $\pm$ 1.4 years.

As seen in Table 2, about two thirds (64.3%) of the patients' total knowledge was unsatisfactory before implementation of the guidelines. The worse knowledge was regarding exercises, which was unsatisfactory among all the studied patients. The post guidelines phase showed a statistically significant improvement in patient's knowledge ( $p < 0.001$ ), with all patients having satisfactory knowledge in almost all areas and in total knowledge. This improvement persisted during the follow-up period with no decline in any of the areas of knowledge ( $p < 0.001$ ). Similarly, the table indicates that all the patients had total inadequate practices before the guidelines. The post guidelines phase showed statistically significant improvement in patients' practices ( $p < 0.001$ ), with all the patients except one (97.6%) having adequate practice. As in knowledge, the improvement in practice persisted throughout the follow-up. Only the area of use of nebulizer did not demonstrate a significant improvement after guidelines implementation ( $p = 0.27$ ).

Concerning the effect of BA on patients' life, Table 3 shows statistically significant decreasing effects on patients' daily life activities, work, psychology, self-image, and diet ( $p < 0.001$ ). The decline even continued during the follow-up phase. The table also demonstrates statistically significant improvements in patients' sleep and resting positions during asthma at the post and follow-up phases

( $p < 0.001$ ). It is evident that for more than half of the patients the sleep and resting positions were the semi-sitting in the pre guidelines phase. At the post phase, about two thirds assumed the lying down position; and this rose to almost all patients at the follow-up phase.

Patients in the study sample have also demonstrated statistically significant improvements regarding the severity of their asthma. As Table 4 shows, there were improvements in the frequency of the attacks, the use of oral cortisone, and the pulmonary function tests both after the implementation of the guidelines and at the follow-up phases. Overall, 88.1% of the patients had moderate to severe asthma at the pre-guidelines phase. This decreased to 38.1% at the post phase, and down to 35.7% at the follow-up phase ( $p < 0.001$ ).

Table 5 displays the best fitting multiple linear regression model for the change in patients' knowledge score throughout the study phases. The only statistically significant independent predictor of the knowledge score was the program implementation, while patient's age, sex, education, residence, and duration of illness had no influence. The model r-square indicates that the program explains 92% of the improvement in knowledge score. As regards the change in asthma severity, the same table shows that the educational level and the knowledge score were the statistically significant independent negative predictors of the severity score. Meanwhile, patient's age, sex, residence, and duration of illness had no significant influence on the severity. The model explains 49% of the improvement in asthma severity as indicated by the value of r-square.

Regarding the factors underlying patients' non-compliance with medications, Table 6 shows statistically significant decreases in all medical and non-medical reasons at both at the post and follow-up phases, compared to the pre-program phase ( $p < 0.001$ ). Some of the factors even disappeared at the follow-up phase such as difficult use of atomizer, concern for side effects as addiction, and cost and dislike of drugs.

#### 4. Discussion

This study aimed at evaluating the effectiveness of therapeutic and nutritional guidelines for bronchial asthma developed by the researchers based on assessment of adult asthmatic needs, on patients' knowledge, practices and therapeutic compliance, as well as on the severity of their asthma. The findings revealed significant improvements in these outcomes, which lead to acceptance of the research hypotheses regarding the effectiveness of the guidelines.

**Table 1. Socio-demographic characteristics and duration of bronchial asthma among patients in the study sample (n=42)**

	Frequency	Percent
Age (years)		
<50	22	52.4
50+	20	47.6
Range	22.0-80.0	
Mean±SD	48.7±13.7	
Sex:		
Male	14	33.3
Female	28	66.7
Marital status:		
Unmarried	10	23.8
Married	32	76.2
Education:		
Illiterate	32	76.2
Educated	10	23.8
Job status:		
Unemployed/house wife	29	69.0
Employed	13	31.0
Living:		
With family	38	90.5
Not with family	4	9.5
Residence:		
Rural	28	66.7
Urban	14	33.3
Income:		
Insufficient	17	40.5
Sufficient	25	59.5
Smoking		
No	36	85.7
Yes	6	14.3
Duration of asthma (years)		
<10	15	35.7
10+	27	64.3
Range	5.0-35.0	
Mean±SD	17.0±1.4	

The study sample involved 60 adult asthmatic patients. Those who pursued all the intervention phases were 42, i.e. a dropout rate of 30%. This is often encountered in follow-up studies and is known as cohort attrition, which is on the limitations of longitudinal studies (*Booker et al., 2011*). However, in the present study, the dropouts were not due to reasons related to the intervention or the disease outcomes, as most of the dropouts were just reluctant to come for follow-up and were not different from those who continued.

The study sample was representative of the population of adult asthmatic patients attending

outpatient clinics. It covered a wide range of duration of the disease, between 5 and 35 years. The representativeness was also evident from the preponderance of female patients in the sample, which is an often cited finding in asthma research. In this regard, *Niitsuma et al. (2004)* reported that bronchial asthma was slightly more frequent among females compared to males.

According to the present study, patient's knowledge about asthma was deficient before introducing the guidelines, with only about one third of them having satisfactory knowledge. This was particularly evident in relation to knowledge about exercise, which has a lot of misconceptions among asthmatic patients (*Martínez-Gimeno, 2009; Williams et al., 2010*). This lack of knowledge can be attributed to the lack of educational programs and unavailability of information resources about the disease and its effect. It reflects a deficiency in providers' educational role.

After implementation of the guidelines, patients' knowledge demonstrated significant improvement, which was confirmed through multivariate analysis, which indicated that the implementation of intervention was the only statistically significant independent predictor of the knowledge score. This asserts the assumption that the lack of knowledge was due to lack of educational activities provided to these patients, and indicates that the meeting of patients' information needs would fill this gap of knowledge. In agreement with this, *Rai et al. (2007)* mentioned that the asthmatic patient needs knowledge about the disease process, precipitating factors, treatment, preventive measures, and guidelines for self-care and use of ventilator aids. This further highlights the importance of developing and implementing guidelines for increasing patient's knowledge about management of asthma attack in order to maintain health and prevent complications.

On the same line, *Temple (2006)* stated that asthma self-management education is essential to provide patients with the skills necessary to control asthma and improve outcomes. Therefore, health care providers should reinforce and expand key messages such as the patient's level of asthma control, inhaler techniques, and self-monitoring. The author also emphasized the importance of use of a written asthma action plan by all members of the health care team. This can help effective treatment so that most patients can achieve good control of their disease (*Paul and O'Byrne, 2009*).

Patients' practices and compliance related to asthma have also improved after implementation of the present study guidelines. The improvement has even been sustained after a two-month follow-up. This improvement involved performance of exercises

and compliance to follow-up. Although the use of nebulizer has also improved, it did not reach statistical significance probably because it was already high at the pre-intervention phase. This success of the guidelines may be attributed to the fact that the procedures were practiced under supervision and guidance of the researchers, with demonstration and re-demonstration, using real objects. This is in agreement with *Milani et al. (2004)* and the *Expert Panel 3 (2007)* who emphasized the importance of training and reinforcing correct techniques before patients are discharged home, with provision of written asthma action plans, peak flow meters, spacer devices, deep inspiration, slow expiration and coughing technique as has been done in the current study.

The positive effects of the present study guidelines were not only limited to patients' knowledge and practices, but also extended to their compliance, and various aspects of their life. For instance, asthma may have deleterious effects on patients' daily life activities, work, self-image, psychology, and diet. It may also prevent patients from lying down during rest or sleep at the time of the attacks. All these aspects have shown improvements among patients in the current study secondary to implementation of the guidelines. The findings are in agreement with *Rai et al. (2007)* who clarified that the application of treatment guidelines for bronchial asthma can achieve minimal or nil day time and night time symptoms, prevent acute exacerbations, and attain normal or near normal lung function, thus improving the overall quality of life.

The present study assessed the effect of implementation of the guidelines on the severity of asthma using objective measures such as the pulmonary function tests, in addition to patients' reporting of the frequency of the attacks and the oral use of cortisone. The result of the study indicated significant improvements in all these criteria of disease severity, which extended through the follow-up phase. Furthermore, the multivariate analysis confirmed the effect of the guidelines on the improvement of the disease severity, but through improvement in patient's knowledge. This confirms the importance of improving knowledge, which will be reflected on compliance, practices, and consequently on disease severity. Patient's education had also a significant positive impact on the severity of asthma, which is in line with *Brooten et al. (2003)* who showed that higher education would diminish the severity of the disease, and also encourage better therapeutic compliance.

**Table 2. Knowledge, practice, and compliance related to bronchial asthma among patients in the study sample throughout study phases.**

	Time						X <sup>2</sup> Test (p-value) Pre-post	X <sup>2</sup> Test (p-value) Pre-FU
	Pre		Post		FU			
	No.	%	No.	%	No.	%		
Satisfactory knowledge (60%+) about:								
Definition/types	13	31.0	42	100.0	42	100.0	44.29 ( $<0.001^*$ )	44.29 ( $<0.001^*$ )
Time/season	21	50.0	42	100.0	42	100.0	28.00 ( $<0.001^*$ )	44.29 ( $<0.001^*$ )
Precipitating factors	13	31.0	42	100.0	42	100.0	44.29 ( $<0.001^*$ )	44.29 ( $<0.001^*$ )
Symptoms/signs	19	45.2	42	100.0	42	100.0	31.67 ( $<0.001^*$ )	31.67 ( $<0.001^*$ )
Complications	13	31.0	42	100.0	42	100.0	44.29 ( $<0.001^*$ )	44.29 ( $<0.001^*$ )
Management	19	45.2	42	100.0	42	100.0	31.67 ( $<0.001^*$ )	31.67 ( $<0.001^*$ )
Exercises	0	0.0	41	97.6	41	97.6	80.09 ( $<0.001^*$ )	80.09 ( $<0.001^*$ )
Total knowledge:								
Satisfactory	15	35.7	42	100.0	42	100.0	39.79 ( $<0.001^*$ )	39.79 ( $<0.001^*$ )
Unsatisfactory	27	64.3	0	0.0	0	0.0		
Adequate practice (60%+) and compliance:								
Use nebulizer	32	76.2	36	85.7	36	85.7	1.24 (0.27)	1.24 (0.27)
Comply to exercise	7	16.7	42	100.0	42	100.0	60.00 ( $<0.001^*$ )	60.00 ( $<0.001^*$ )
Exercise performed:								
Deep breathing	2	28.6	34	81.0	34	81.0	Fisher (0.01*)	Fisher (0.01*)
Coughing	5	71.4	8	19.0	8	19.0		
Comply to FU	2	4.8	40	95.2	41	97.6	68.76 ( $<0.001^*$ )	72.47 ( $<0.001^*$ )
Total practice:								
Adequate	0	0.0	41	97.6	41	97.6	80.09 ( $<0.001^*$ )	80.09 ( $<0.001^*$ )
Inadequate	42	100.0	1	2.4	1	2.4		

(\*) Statistically significant at  $p < 0.05$

**Table 3. Effect of bronchial asthma on patients throughout study phases**

	Time						X <sup>2</sup> Test (p-value) Pre-post	X <sup>2</sup> Test (p-value) Pre-FU
	Pre		Post		FU			
	No.	%	No.	%	No.	%		
Effect of asthma on:								
Daily life activities	36	85.7	20	47.6	7	16.7	13.71 ( $<0.001^*$ )	40.07 ( $<0.001^*$ )
Work	38	90.5	16	38.1	8	19.0	25.10 ( $<0.001^*$ )	43.25 ( $<0.001^*$ )
Psychology	30	71.4	12	28.6	8	19.0	15.43 ( $<0.001^*$ )	23.26 ( $<0.001^*$ )
Self-image	27	64.3	9	21.4	9	21.4	15.75 ( $<0.001^*$ )	15.75 ( $<0.001^*$ )
Diet	38	90.5	21	50.0	6	14.3	16.46 ( $<0.001^*$ )	48.87 ( $<0.001^*$ )
Sleep position:								
Lying down	3	7.1	27	64.3	40	95.2	35.70 ( $<0.001^*$ )	65.43 ( $<0.001^*$ )
Semi-sitting	25	59.5	15	35.7	2	4.8		
Stooping	14	33.3	0	0.0	0	0.0		
Resting position:								
Lying down	10	23.8	28	66.7	41	97.6	20.93 ( $<0.001^*$ )	48.02 ( $<0.001^*$ )
Semi-sitting	21	50.0	14	33.3	1	2.4		
Stooping	11	26.2	0	0.0	0	0.0		

(\*) Statistically significant at  $p < 0.05$

**Table 4. Changes in bronchial asthma severity among patients in the study sample throughout study phases**

	Time						X <sup>2</sup> Test (p-value) Pre-post	X <sup>2</sup> Test (p-value) Pre-FU
	Pre		Post		FU			
	No.	%	No.	%	No.	%		
Frequency of attacks/week:								
1	0	0.0	23	54.8	24	57.1	31.67	33.60
2+	42	100.0	19	45.2	18	42.9	<0.001*	<0.001*
Oral cortisone use:								
None	8	19.0	18	42.9	18	42.9	5.57	5.57
Sometimes with attacks	34	71.0	24	57.1	24	57.1	0.02*	0.02*
Pulmonary function tests (% of predicted):								
>80%	3	7.1	20	47.6	23	54.8	24.23	27.47
60-80	20	47.6	19	45.2	16	38.1	<0.001*	<0.001*
<60	19	45.2	3	7.1	3	7.1		
Severity index:								
Mild	5	11.9	26	61.9	27	64.3		
Moderate	27	64.3	16	38.1	15	35.7	22.55	24.43
Severe	10	23.8	0	0.0	0	0.0	<0.001*	<0.001*

(\*) Statistically significant at  $p < 0.05$

**Table 5. Best fitting multiple linear regression model for the change in knowledge and severity scores throughout study .**

	Un standardized Coefficients		Standardized Coefficients	t-test	p-value
	Beta	Std. Error			
<b>Knowledge score:</b>					
Constant	45.65	1.05		43.471	<0.001*
Intervention	49.90	1.29	.961	38.796	<0.001*
<i>r-square=0.92</i>					
<i>Model ANOVA: F=1505.12, p&lt;0.001</i>					
<i>Variables excluded from model: age, sex, education, residence, duration of illness</i>					
<b>Severity score:</b>					
Constant	6.622	.407		16.268	<0.001*
Educational level	-.172	.056	-.199	-3.083	0.003*
Knowledge score	-.050	.005	-.667	-10.340	<0.001*
<i>r-square=0.49</i>					
<i>Model ANOVA: F=58.52, p&lt;0.001</i>					
<i>Variables excluded from model: age, sex, residence, duration of illness</i>					

**Table 6. Reasons for non-compliance as reported by patients in the study sample throughout study phases.**

	Time						X <sup>2</sup> Test (p-value) Pre-post	X <sup>2</sup> Test (p-value) Pre-FU
	Pre		Post		FU			
	No.	%	No.	%	No.	%		
<b>Medical reasons:</b>								
Not realizing importance of medication	26	61.9	6	14.3	12	28.6	20.19 (<0.001*)	9.42 (0.002*)
Non-practical diet regimen	27	64.3	9	21.4	5	11.9	15.75 (<0.001*)	24.43 (<0.001*)
Difficult use of atomizer	26	61.9	4	9.5	0	0	25.10 (<0.001*)	37.66 (<0.001*)
Concern for side effects as addiction	29	69.0	1	2.4	0	0	40.65 (<0.001*)	44.29 (<0.001*)
Cost of drugs	40	95.2	2	4.8	0	0	68.76 (<0.001*)	76.36 (<0.001*)
Dislike drugs	31	73.8	2	4.8	0	0	41.98 (<0.001*)	49.13 (<0.001*)
<b>Non-medical reasons:</b>								
Denial	33	78.5	6	14.3	7	16.7	34.89 (<0.001*)	32.26 (<0.001*)
Unclear treatment plan	38	90.5	10	23.8	7	16.7	38.11 (<0.001*)	46.00 (<0.001*)
Unrealistic expectations	35	83.3	4	9.5	2	4.8	46.00 (<0.001*)	52.60 (<0.001*)
Lack of guidance for self management	36	85.7	3	7.1	3	7.1	52.12 (<0.001*)	52.12 (<0.001*)
Dissatisfaction with health care	37	88.1	5	11.9	2	4.8	48.76 (<0.001*)	58.63 (<0.001*)
Poor training or follow up	40	95.2	3	7.1	3	7.1	65.23 (<0.001*)	65.23 (<0.001*)
traditional, beliefs about asthma and treatment	33	78.5	5	11.9	4	9.5	37.68 (<0.001*)	40.62 (<0.001*)
Family issues (smokers, pets)	38	90.5	7	16.7	5	11.9	46.00 (<0.001*)	51.89 (<0.001*)

(\*) Statistically significant at  $p < 0.05$

The improvement in asthma severity revealed in the present study is quite plausible given the associated improvements in patients' knowledge, practices, and therapeutic compliance. Similar improvements in asthma severity have been reported secondary to interventions involving asthma guidelines *Ali et al. (2010)*, or naturopathy treatment (*Manjunath and Shirley, 2006*). Furthermore, patients' therapeutic compliance has been shown to improve asthma severity (*Horvath and Wanner, 2006; Slats et al., 2006*). The improvement in the different aspects of patients' life, including the psychological state could also have contributed to the decrease in disease severity. In congruence with this, *Bateman et al. (2008)* highlighted the importance of the psychological factors in asthma, which can play an important role in precipitating exacerbations and possibly act as a risk factor for an increase in disease severity.

### Conclusion and recommendations

The study concludes that the developed guidelines have a significant positive impact on asthma patients' knowledge, practices, therapeutic compliance, and disease severity. This success is attributed to that the guidelines are based on needs assessment and integrate updated technology. Therefore, these guidelines should be adopted as an essential component of the care provided to asthma patients. Continuous follow-up together with selecting the optimal treatment options for each individual patient are recommended. The long-term effects of following the guidelines need to be further studied.

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## Geographical analyses of rural well-being of Iran (case study sistan region)

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**Abstract:** In framework of recognize logic, reason of searching the geographical phenomena in rural well being arena and due to recognize of making clearly is from scientific special necessities in this connection. Conforming to making clear study and analyses and existence pattern socio welfare field problem, and it is meaning rate vulnerability condition and sistan rural area discussed as a principle of the study. Proportional with the subject, the hypothesis that illustrative relationship among vulnerability in socioeconomic general dimensions and all of the performances was formed. In regard of operational and also providing recognize aspects in relation to scientific theories and explanatory introduces, used by several resources, that it's result was practicable; intelligible framework for field work achievement, human, , physical, finance social capitals for reach to rural well being, also, should not be neglect from poverty, bereavement, retirement and rural vulnerability reagents. However, modification in theoretic attitudes from physical and material planning direction toward social and human planning is in result of intervention in capacities, abilities, and organize process and have also opportunity and security with regard to above mentioned, in rural; in relationship to different rural well being aspects and vulnerability in framework of training options, information, increasing intervention in making decision, participation increasing, using of local knowledge, reduction of discrimination, diversity livelihood has evaluated. The assessment in sistan region with selection 40 rural used by rural Islamic council. Results shown lack of follow actions pattern of the rural well-being in sistan region, with have rural vulnerability rate. In addition, created changes in depending variable never explained with created chance in independent variables.

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**Keywords:** Vulnerability, Rural well being; Sistan region; Iran.

### 1. Introduction

Providing the well-being is related to the human rights and national governance and it contributes to the peace, stability, justice and security, especially when it is enhanced by a set of values and ideologies. Regarding the inefficiencies of the planning systems based on the simply concentrated economical views with some characteristics such as having no regard for the social and cultural dimensions of the development, centralism, increased regional and district differences, lack of people's participation, spatial, sexual and social inequalities, geographical isolation and deprivation as well as poverty resulted in the unity of thoughts concerned the well being. In 1980s, after providing a conceptual framework and a generic and specific perception, there was a right direction towards the evolution for the trend of discussions concerned the development. Also, there have been some various changes in the concept and content of the well being during the recent decades. In 1970s, it was being used for a set of rules, plans and organized services with the aim of satisfying the least urgent needs of the all people of a country and in 1980s, for a set of various social actions and services, and for the time being, it refers to the conditions that gives satisfaction to the people in their lives. Also, concerning the schools, target group

and ideologies, there are different viewpoints that form a basis of thoughts and reflections for the well being. In the meantime, villages as the biological residential places and the villagers as their residents are particularly concerned the well being in terms of having no merit and no language, being poor, less healthy, more vulnerable and being far from the decision making centers and facilities as well as geographical isolation. Therefore, the rural well being is also particularly important as a basic subclass of the national well being, because it is believed that the main reason for the failures in managerial and planning system is the having no regard for the villages. In this direction, with knowledge that there has been paid little attention to the villages and their spatial conditions in the concentrated systems and especially in the developing countries, it should be initially mentioned that in a rural community every one has a right to benefit from an honorable life and the government as the administrator and the main agent of planning and resource management is responsible for providing the well being for the villagers. Hence, concerning the principal deficiencies in rural structures, especially more illiteracy, lack of confirmed ownership, less accessibility, more susceptible economy, and two

basic approach should contain the bases for the social rural life:

- First, decreasing the (social, physical, economical, and environmental) vulnerability rate;
- Second, increasing the opportunities and equal accessibility to the facilities, resources and so on.

Wellbeing is a positive physical, social and mental state; it is not just the absence of pain, discomfort and incapacity. It requires that basic needs are met, that individuals have a sense of purpose, that they feel able to achieve important personal goals and participate in society.

It is enhanced by conditions that include supportive personal relationships, strong and inclusive communities, good health, financial and personal security, rewarding employment, and a healthy and attractive environment.

Government's role is to enable people to have a fair access now and in the future to the social, economic and environmental resources needed to achieve wellbeing. An understanding of the effect of policies on the way people experience their lives is important for designing and prioritizing them."

## 2. Theoretical basis of the research:

Regarding the above-mentioned cases as well as various spatial, environmental, social and economical conditions, the historical breadth, growth rate, and the development of the countries, there have been raised some different theories and viewpoints concerned the rural well being that the most important of them are referred here; from a morphological and philosophical viewpoint, of course, rural well being is as important as justice, equality, freedom, and citizen rights, that in some cases, they are considered as the complements of it and sometimes they are regarded as the bases for it or even as an aim or a tool for achieving it. From an economical point of view, as we know, the western countries economy has been influenced by the classical schools of thought derived from some thinkers' theories such as Adam Smith, David Ricardo, and Malthus, during two hundreds years ago. In this kind of the schools, the social rural welfare, in a wide sense, is influenced by the individual values and satisfaction. At the same time, from the viewpoint of the macroeconomics thinkers, unemployment is defined as the main problem in the economical system of the community and the government can provide the well being by balancing the economy through providing the social helps, social security, education, and health. In contrast to this thought and in the direction of the social analyses, Marxists introduce the radical alternative. And from their viewpoint, the ownership of

production tools can be changed through the understanding the historical nature of the relationship between human and material and also by using the work sharing and the social classes can be changed based on it (Alwang Jeffry and et al. 2005).

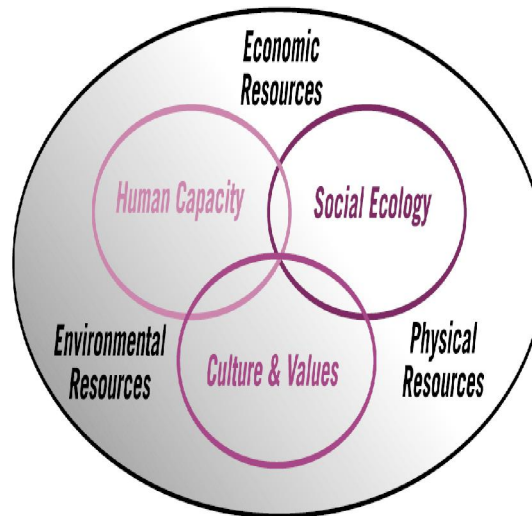


Figure 1. Basic conceptual framework

At the same time, the interaction between a flow derived from all the presented functions by the parts of a social system in a static and dynamic state and recognizing its functional inefficiency and using the religion alternative as a social feature is also the result of the functionalists' thoughts.

Unlike the above-mentioned cases, in the discussion of collectivism, grassroots and the regard for the social groups that are in the majority and lead to the forming of pluralist governments (White, S. 2008) are particularly important. The theory of the interaction perspective, balance rate, and the interaction between surfaces and the components of the social phenomena are of a great importance and the relations in the spatial (rural, urban) systems, rate of the relations and its feedbacks determine the survival of the system (Gasper, D. 2007).

One of other most basic and key discussions are the social justice theory. It has been considered in a main part of the ancient Greek philosophy to the modern thoughts. Also, it has been raised in a framework of dialectic thoughts and it has been considered to be related to appealing for the rank and proportion, that is, to be in a natural rank and it has been considered to have a natural right. At the same time, to present a definition for justice is subject to the presentation of a definition for social justice, and for this reason, justice is a subordinate confirmation of its definition. In addition, there are two problems concerned justice, that are considered as the

distributive justice and submissive justice; there are some special conditions concerned each of them. Also, there has been considered two forms for the social justice: exclusion and inclusion; its exclusive form is defined according to the just manners and its inclusive form makes the government to give some special and certain rights such as employment and housing to the people.

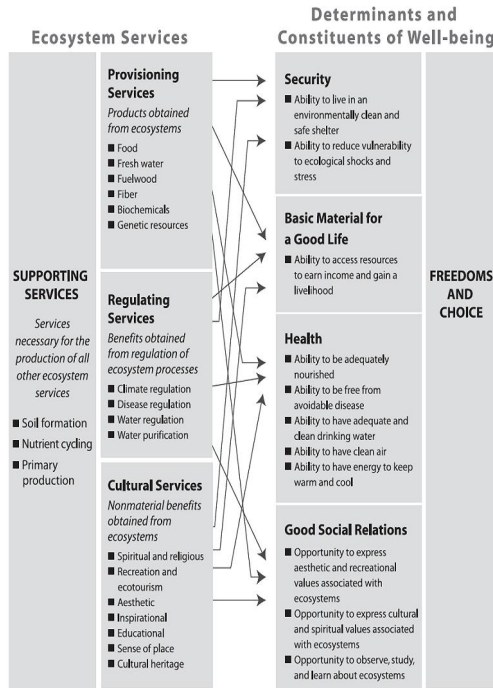


Figure 2: Ecosystems and wellbeing

Poverty is considered as a basic problem in a rural society that in some third world countries, it is accompanied by having no power, no land, no sanitation, as well as hunger, illiteracy, deprivation and familial and social isolation. At the same time, all parts interact with each other and result in the poorest of the poor (Jones Howard, 1990). Fifty percent of the poor in the world live in the rural regions. There are a lot of definitions for poverty and there are a number of different viewpoints on this issue. There are a lot of factors, reasons, effects, and consequences concerned poverty; and there have been written a great deal of material concerned them. In contrast to this theory, there is the theory of the social conscience and humanitarian stimuli that based on it, the government pay attention to the well being especially in the villages because of their own more undertaking towards the humanitarian values (WHOQOL-SRPB Group ;2006).

According to the citizen theory, a rich society can be defined as a society in which citizen rights have been given and all people have

opportunity to show their maximum capabilities. According to this theory, and concerned village and villagers, having the structural functions for improving the relations, potential, self-sufficiency among spaces, people and activities, and the ethnic, environmental, spatial, social and economical varieties in the villages as well as analysis of an explanatory range of the rules for the spatial differences and their regard range for the consequences, limitations and capabilities are definable and explicable (UNICEF; 2007).

The theory of the urgent and physical needs is one of the most basic thoughts and reflections related to the well being. According to this theory, the regard for the sanitation, nutrition, education, employment conditions, income and savings, transportation, housing, clothing, free time and insurance should be paid systematically. In this school, providing the urgent needs of the people as the necessary minima for their growth and exaltation with no regard for their living place is of great importance. According to the theory of spatial distribution, to consider the phenomena flow, to recognize the flows, the way, rate and the quality of their spread that may contains social services and the conditions of providing well being is definable and explicable. Determination of the distribution points in limitative and attractive environmental and spatial conditions of the distributive elements is considered in the conceptual area of this theory (Sointu, E.; 2005).

In addition, in this direction, the theory if the spatial right explains the basic and natural rights for human beings concerned the maximizing the social interests of human, improving the interests, minimizing the costs and increasing the rate of exploitation of human beings on the basis of their settlements. It is very important to serve the people in the place where they live and work and the distribution and justice should be considered on a spatial basis.

One of the other theories that is concerned the differences is the theory of industrialization; this theory that is based on the basic hypothesis of the urbanization, has intensified the spatial inequality so that the process of a rural-urban migration is considered as a natural incident. Instead of putting a positive effect on the growth and development, this theory resulted in leaving the villages empty, increasing the migration rate, leaving the farmlands and the spread of suburbanization and some other issues (Rojas, M.; 2007).

Everybody has a right to benefit from development, especially the villagers who are the most vulnerable and the minimum costs are allocated to them and the maximum costs are allocated to the

interests of the rich. With growing the colonization and a narrow view of development pivots, the theory of the development rights raised (New Economics Foundation; 2004).

Of course, the most basic and introductory discussion in this theory are to get rid of the discrimination, to stop tortures, to respect children's rights, women's ownership right and so on. At the same time, economical inference from the word "development", resulted in raising the theory of human development. It is true that access to the income may help people to achieve their wishes, but well being depends on how people use this income rather than other issues. Human development is not only an aim, but also it is tool, because it is a multipurpose activity for human beings to develop and perfect. Presupposition for this theory is the no regard for human beings in the human development process (Gasper, D. 2007).

In the direction of well being and with regard for the emptiness in environmental preservation and ignoring the biological cycles and nature, the theory of sustainable development raised. This theory is based on this fact that all creatures have an indisputable right to share in nature and world and so, nature and the world should not be damaged or destroyed because of the human arrogance.

There are some common points regarding the above-mentioned theories that include:

1. Emphasis on the vulnerability areas in general;
2. Emphasis on the mechanisms for decreasing the vulnerability rate;
3. Having a limited attitude in each area (having no systematic attitude);
4. Regard for the tools for decreasing the vulnerabilities
5. Regard for the palpable and defined dimensions.

Also, there are some main differences concerned these schools that include:

1. Having the different attitudes towards setting (individual, social, functional, and structural and ...) goals;
2. Having the cliché attitudes of ideologies and thoughts;
3. Effect of the social context and bed on them
4. Effect of the progress and development of the society on them;
5. Using the mechanisms related to the macro – policies;
6. Using the wisdom in presenting the policies and basic approaches.



Figure 3. Map of sistan

### 3. Methodology and introducing the study area:

Concerning the theoretical basis and the subject nature in the direction of the spatial organization, to be logical, having a scientific spirit, realism, this research method follows a particular pattern that is:

- Width-based in terms of the activity width;
- Depth-based in terms of different elements of the vulnerability in four dimensions: natural, physical, social and economical.

The Sistan area is located at the tail end of a large closed inland (endorheic) basin, in one of the driest regions of the world. It is comprised of three geographical sub-units: (i) the upper plain of the inland delta of the Helmand (Hirmand) river, which is mostly drained and used for agriculture; (ii) the wetlands (Hamoons) covering the lower delta plain and (iii) a hypersaline lake (Gowd-e-Zareh) in the lowest part of the basin, which collects the overspill from the wetlands and – in case of extreme floods – from the Helmand River. There is no outflow from this terminal lake; water is lost from Gowd-e-Zareh only by evaporation. The Sistan basin has been continuously inhabited by complex cultures for more than 5,000 years. One of the key archaeological sites on the Iranian side is the Burnt City, founded next to a presently dried-up branch of the Helmand River in 3100 B.C. Livelihoods in this region are strongly interlinked with and dependent on the wetland products and services. The reed beds provide fodder for livestock, fuel for cooking and heating, and raw materials for handicraft and constructions.

Statistical society containing all the villages in Sistan Region (310 villages with more than 20 families). In Iran that based on a sample survey, 40

villages have been chosen as the sample volume. The analysis unit has been chosen in two different levels containing sample villages where there are Islamic Councils and three persons have been questioned.

The research tools have been the questionnaire that its done according to the test has compiled at a very good level. The chosen region is Sistan Region is a frontier area of that is located east of Iran. Its population is 350000 people that 64% of it lives in rural area. 38 % of its population is under 15 years old and its annual population growth is 3/2 %.

#### 4. Results

In this research all the accomplished stages were in the direction of refusal or approval the research hypothesis. In recognizing and the presenting the research, the following stages have been considered:

- providing the presuppositions: with respect to the researcher's mentality, differences in well being actions were the cause for raising the different question and hypotheses concerned spatial changes of these kinds of actions.
- analyzing the presuppositions: the presented presuppositions in exploratory research and through the observation and analysis of the presuppositions. Finally the relations between accomplished actions and the rate of vulnerability of the village were considered as the standard of action.
- purifying the hypotheses: through a meaning distinction and using the honorable professors' consultations, all the indicators between rural well being were extracted and then the relationship between different factors were presented in a testable framework.

In order to access to the research hypotheses and their refusal or approval, through the theoretical analogical conclusive approach (the studied theory) accompanied by the observation and experience and even last experiences were used.

So that in a first part, the theories concerned the well being and vulnerability were considered and in the second part, the research hypotheses were refused or approved with the field studies.

At the same time, concerning the non-directional nature of the hypotheses, the current conditions were considered and this matter was considered in the interpretation of the results.

With an introduction that was presented, the research hypotheses were evaluated using the special statistical techniques that here we explain their refusal or approval. In addition, the focus has been on the explaining the spatial pattern of the rural well being, because the spatial effective factors and

welfare have been implicitly considered in the original research and inside the indicators.

There is a meaningful relationship among the accomplished actions concerning the provision of the rural well-being and the decrease of the rural vulnerability rate in Sistan Region.

According to this theory, the correlation coefficient test was used that its results can be presented as follows:

meaningfulness	$0.437 > 0.05$
correlation	-0.079

So it can be said undoubtedly that there is not a meaningful relationship between the accomplished actions concerning the rural well being and the rate of vulnerability in the villages in Sistan Region, because the accomplished actions neither decrease the vulnerability rate nor increase its rate. Therefore, the rate of the changes in the set of the independent variables (the accomplished actions in all dimensions) do not explain at all the changes of the dependent variables of the vulnerability. So, the spatial pattern of the actions concerned welfare, don't follow the rural vulnerability rate and it has not been accompanied by the setting goals for decreasing the vulnerability rate.

There is a natural-environmental meaningful relationship between the accomplished actions concerned the rural well-being and the decrease of the vulnerability rate of the villages in Sistan Region. Regarding the approval and refusal of this hypothesis and according to the presented results:

meaningfulness	$0.033 < 0.05$
correlation	-0.288

Firstly, it can be said this is a negative relationship, that is , the accomplished actions have not concerned the decrease of the natural and environmental vulnerability, and they have been in the villages with the lower rate of the natural vulnerability, secondly, the obtained correlation rate have been acceptable and is approved with 99% certainty. So, accomplished actions do not follow a special environmental pattern for decreasing the rate of the vulnerability in the villages and it has not increases simultaneously with the increase of the environmental vulnerability, on the contrary, it has partly decreased. In this case, the spatial pattern of the accomplished actions concerned rural welfare, is not homogenous with the pattern of the natural and environmental vulnerability rate.

There is an environmentally meaningful relationship between accomplished actions concerned rural well being and the vulnerability rate in the villages of the Sistan Region. The following results

came from the explanatory tests for eh approval and refusal the hypothesis:

meaningfulness	0.200 > 0.05
correlation	0.231

The obtained correlation is not meaningful and the hypothesis of the independence between two variable set is acceptable. So, these accomplished actions is neither concerned the decreasing the economical vulnerability in the villages of Sistan Region nor concerned their economical vulnerability. Therefore, the changes in the dependent variable (economical vulnerability) are not explained at all by the independent variable (Accomplished actions). For interpreting these results, it can be said that accomplished actions do not follow a spatial pattern for decreasing or increasing the vulnerability rate and have been aimless.

There is a meaningful relationship between the accomplished actions concerned the rural well being and decrease of the social vulnerability rate in Sistan Region. Concerned the nature of the hypothesis, non-parametrical tests of correlation have been used that the obtained results are:

meaningfulness	0.204 > 0.05
correlation	0.131

The obtained meaningfulness shows that the hypothesis of the independence between two sets of variables is acceptable. So, it can be said that the changes in independent variable (actions) is not related at all to the changes in dependent variable (social vulnerability) and the accomplished actions in the villages of Sistan Region do not follow the rate of social vulnerability in the villages, so, it does not decrease or increase the vulnerability rate.

There is a physical meaningful relationship between the accomplished actions concerned the provision of the rural social security and the decrease of the vulnerability rate in the villages of Sistan Region. After carrying out the tests of hypotheses, the rate of the obtained correlation is as follows:

meaningfulness	0.489 > 0.05
correlation	-0.042

Therefore, there is no meaningful correlation between two sets of the variables and the rate of the change in the variables is not correlated to each other. So, we can conclude that the spatial pattern of the accomplished actions concerned the physical affairs, does not follow the rate of the vulnerability of the villages in Sistan Region. So, as usual, the

accomplished actions have not been concerned the decrease of the vulnerability rate in the villages.

There are some results concerned the analysis of the meaningful difference rate between distribution of facilities and resources in administrative organizations of rural well being in the villages of Sistan Region and rate of the vulnerability in the villages that obtained from a correlation analysis:

meaningfulness	0.489 > 0.05
correlation	-0.044

Regarding the correlation level and acceptable correlation, it can be said undoubtedly with 99 % certainty that there is a positive relationship between two sets of the variables. So that as the number of the organizational facilities and resources increase in the villages, the rate of the vulnerability in these villages increases acceptably. So, the changes in the dependent variable (vulnerability in all dimensions) can be explained with the rate of the changes in independent variable (organizational facilities and resources).

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## A Study on Efficacy of Empowerment Training among Diabetes Patients

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**ABSTRACT:** OBJECTIVES — The main purpose of this research is to evaluate the effectiveness of the treatment for diabetic patients by empowerment intervention<sup>[1]</sup>. RESEARCH DESIGN AND METHODS — The research sample enrolls 30 patients with type 2 diabetes from one family medicine outpatient department of regional teaching hospitals in south Taiwan. The subjects are randomly assigned into experimental group and control group. Data is collected from July 2010 to December 2010 (7~12) and the effectiveness is re-evaluated three months later<sup>[2]</sup>. The intervention model is based on empowerment theory and application of diabetes self-management. The intervention of empowerment program which contains major six components: 1. self-management support 2. clinical information systems 3. delivery system design 4. decision support 5. health care organization 6. to make use of community resources. Data collection includes patient's demographic information, physical examination, serum relevant biochemical parameters, and life quality<sup>[3]</sup>. RESULTS — After completing such a program, experimental group shows significant improvement than that of the controlled group in the score of life quality (The 36-Item Short Form Health Survey, SF-36), and serum relevant biochemical parameters including glycated hemoglobin, cholesterol, and uric acid ( $p < 0.05$ )<sup>[4]</sup>. CONCLUSIONS — Application of empowerment, intervention of empowerment by medical professional team and program would improve the treatment outcome of diabetes mellitus, life quality in diabetic patient and significantly improving the ability of self-management<sup>[5]</sup>.

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**Keywords:** Empowerment, Diabetes Mellitus, Life quality, Self-management

### 1. Introduction

Diabetes is a chronic disease that changes the way that the body used glucose for energy and leads to lower health-related quality of life. It is associated with an impaired glucose cycle, unstable blood glucose concentration, altering metabolism, and glucose metabolic cycle. Treatment goals for type 2 diabetic patients are related to effective control of blood glucose, blood pressure, body weight, uric acid level<sup>[6]</sup> and lipids to minimize the risk of long-term consequences and complications associated with diabetes. Management of this disease may include carefully managing diet, exercising, body weight control, taking oral diabetes medication, using some form of insulin, maintaining proper circulation in extremities and may be further complicated by other external factors such as stress, trauma, poor healing wound, and other physiological factor unique to individual patients.

Well serum glucose level control and keeping health is particularly important for anyone with diabetes. In spite of the great strides that have been made in the treatment of diabetes in recent years, many patients do not achieve optimal outcomes and still experience unresolved complications that result in a decreased length and quality of life. The medical professional team wants to give the best recommended level of diabetes care within the present status. Because

our health care system is designed to deliver acute, symptom-driven care, it is poorly configured to effectively treat chronic diseases such as diabetes that require the development of a collaborative daily self-management plan. Traditionally, the success of patients to manage their diabetes has been judged by their ability to adhere to a prescribed therapeutic regimen<sup>[7]</sup>.

A great deal of effort has been spent in developing methods for measuring compliance and techniques and strategies to promote adherence. The multiple daily self-care decisions that diabetes requires mean that being adherent to a predetermined care program is generally not adequate over the course of a person's life with diabetes and let patient having the self-care ability to create tailored by empowerment may be a good idea. Patients would be able to set goals and make frequent daily decisions. The self-management style is effective and fit their philosophy, values and lifestyles, while taking into account multiple physiological and personal psychosocial factors. To manage diabetes successfully, intervention strategies that enable patients to make decisions about goals, therapeutic options, and self-care behaviors and to assume responsibility for daily diabetes care are effective in helping patients care for themselves.<sup>[8]</sup>

This is particularly true when the self-management plan has been designed to fit patients' clinical diabetic condition, but has not been tailored to fit their priorities, control goals, wealth, staple food, resources, racial culture, and lifestyle<sup>[9]</sup>. The control goals are suggested in clinical practice guidelines released by various national and international diabetes agencies. The targets are:

- Hb<sub>A1c</sub> of 6% to 7.0%
- Preprandial blood glucose: 4.0 to 6.0 mmol/L (72 to 108 mg/dl)
- 2-hour postprandial blood glucose: 5.0 to 8.0 mmol/L (90 to 144 mg/dl)<sup>[10]</sup>.

## 2. Materials and methods

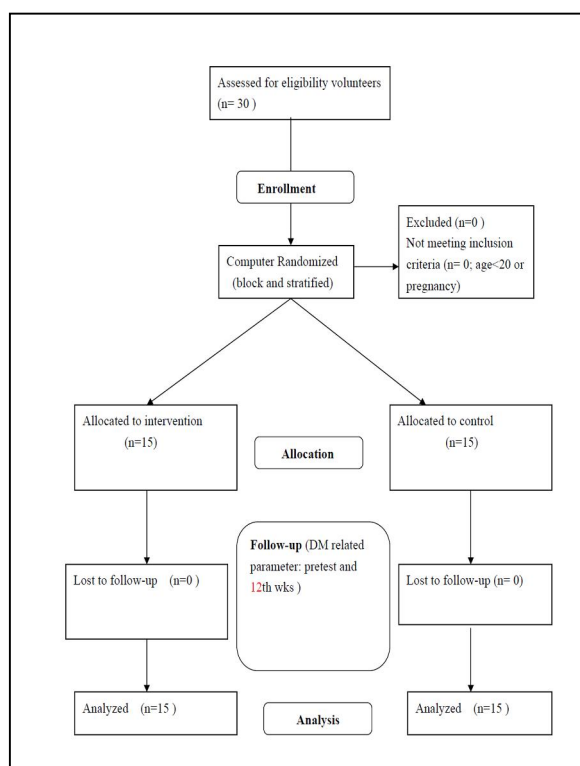


Figure 1: Flow diagram. The study team collected 30 patients with type II Diabetes by Quasi-Experimental design in Family Medicine clinic of a Regional Teaching Hospital. Random assignment is used for assigning subjects to different treatments and no treatment.

In older patients, clinical practice guidelines by the American Geriatrics Society states for frail older adults, persons with life expectancy of less than 5 years, and others in whom the risks of intensive glycemic control appear to outweigh the benefits, a less stringent target such as Hb<sub>A1c</sub> of 8% is appropriate. The HbA<sub>1c</sub> data is the ratio of glycosylated hemoglobin in relation to the total hemoglobin. Persistent raised plasma glucose

levels cause the proportion of glycosylated hemoglobin to go up. The diabetic control of glycosylated hemoglobin over a period originally thought to be about 3 months (the average red blood cell lifetime). In the non-diabetic, the HbA<sub>1c</sub> level ranges from 4.0-6.0%; patients with diabetes mellitus who manage to keep their HbA<sub>1c</sub> level below 6.5% are considered to have good glycemic control.<sup>[11]</sup>

The study team collected 30 patients with type II Diabetes by Quasi-Experimental design in Family Medicine clinic of a Regional Teaching Hospital. Random assignment is used for assigning subjects to different treatments and no treatment. The thinking behind random assignment is that by randomizing treatment assignment, then the group attributes for the different treatments will be roughly equivalent and therefore any effect observed between treat groups can be linked to the treatment effect and is not a characteristic of the individuals in the group.(figure1)

Participants are randomly assigned in additional treatment of empowerment of 30 minutes every 4 weeks for totally 12 weeks in experimental group by medical staff. The subjects in control group received usual care of clinical treatment program at the outpatient department for 12 weeks<sup>[12]</sup>.

The empowerment intervention includes six major parts: 1. self-management support. 2. clinical information systems. 3 delivery system design. 4. decision support. 5. health care organization. 6. community resources.

The research members collect all references and guideline to design including six fundamental aspects of chronic illness care: self-management support \ clinical information systems \ decision support \ health care organization \ and community resources. Every two weeks, we give the patients of experimental groups a topic of empowerment intervention and recheck the life quality, glucose level, and etc

There seems to be no significant difference in age, sex, height, body weight, BMI (body mass index), education level, rate of hypertension, rate of smoking, rate of drinking, history and frequency of exercise, serum relevant biochemical parameters, liver function , and etc (table. 1).

The personal characteristic questionnaire includes information about persons participated in the study as follows: age, gender, height, weight, frequency of taking exercise habit, education level, medical and illness history , coping strategies, attitudes in diabetes, and knowledge about diabetes<sup>[13]</sup>. The 36-Item Short Form Health Survey (SF-36)<sup>[14]</sup> is used to elevate the life quality of diabetic patients. The SF-36 is shown to have good internal consistency, stability, and concurrent validity. The research team consults the specialists of the 36-Item Short Form



Health Survey (SF-36) in order to get the using permit [15].

The 36-Item Short Form Health Survey Questionnaires [16] are sent to patients family members in the first week and the end of empowerment course. SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) is used to analyze the data. (table2).

### 3. Results

Post empowerment, the experimental group have improve data with significance including: HbA1c, cholesterol, uric acid level, life quality ( $p < 0.05$ ). The control group has elevated glucose level with significant change post 12 weeks follow.

Paired- Samples t test was took to examine the pre-test and post-test serum biochemical parameters and it showed improvement with significance on post-test for the intervention group [17]. In within-group comparisons, the subjects in the experimental group, statistically significant improvement was observed in the glycated hemoglobin, cholesterol, and uric acid , and the SF36 scores ( $p < 0.05$ ).

There was no significant difference in other items included preprandial glucose 、renal function (Creatinine) 、liver function(GPT) 、Triglyceride [18].(table3).

Table 1: The major basic characteristics of the recruits the study team use

Basic data	Experimental group	Control group	p-value
age	65.13(14.56)	57.07(12.80)	0.12(NS)
Sex (female)	40%	40%	1.00(NS)
Body weight	73.69(12.87)	72.35(12.68)	0.78(NS)
BMI	27.07(3.68)	27.55(3.78)	0.73(NS)
Glucose(preprandil)	162.60(34.58)	169.93(57.59)	0.68(NS)
HbA1c	7.41(1.11)	7.70(1.84)	0.60(NS)
cholesterol	194.33(50.87)	191.07(38.40)	0.84(NS)
TG	158.20(77.87)	217.40(156.86)	0.20(NS)
Uric acid	5.52(1.19)	6.52(1.20)	0.03*
Creatinine	0.89(0.23)	0.84(0.20)	0.51(NS)
GPT	30.4(17.44)	29.2(19.91)	0.86(NS)
Hypertension	13.3%	13.3%	1.00(NS)
Hyperlipidemia	26.7%	26.7%	1.00(NS)
Without habit of smoking	93.3%	80.0%	0.60(NS)
Without habit of drinking	86.7%	86.7%	1.00
Without habit of taking Semen Arecae	93.3%	100%	1.00
Without habit of taking fruits and vegetable	20%	26.7%	1.00
Without regular exercise	6.7%	6.7%	1.00
36-Item Short Form Health Survey (SF-36)	56.4(7.03)	58.0(7.21)	0.543

Table 2: Basic data and analysis of serum relevant

Basic data and analysis of serum relevant biochemical parameters	Average	Standard Deviation
Age	61.10 y/o	14.08
Body weight	73.02 kg	12.58
BMI	27.31	3.67
Glucose level(before)	166.27 mg/dl	46.82 mg/dl
Glucose level(after)	172.27 mg/dl	65.59 mg/dl
HbA1c(before)	7.55%	1.50%
HbA1c(after)	7.08%	1.37%
Uric acid(before)	6.02 mg/dl	1.28 mg/dl
Uric acid(after)	5.67 mg/dl	1.37 mg/dl
cholesterol(before)	192.7 mg/dl	44.32 mg/dl
cholesterol(after)	182.8 mg/dl	51.59 mg/dl
Triglycerides (before)	187.8 mg/dl	125.35 mg/dl
Triglycerides (after)	218.33 mg/dl	225.79 mg/dl
GPT(before)	29.8IU/L	18.4 IU/L
GPT(after)	29.07 IU/L	14.85 IU/L
Creatinine (before)	0.87 mg/dl	0.22 mg/dl
Creatinine (after)	1.16 mg/dl	1.68 mg/dl
Life quality	57.2 mg/dl	7.04 mg/dl
Life quality(after)	78.2 mg/dl	18.0 mg/dl

HbA1C=glycosylated hemoglobin ,GPT= Glutamic Oxaloacetic Transaminase

Table 3: Serum biochemical parameters of treatment conditions compared to non-intervention conditions

	Before intervention	Post intervention	p-value
	Average (Standard Deviation)	Average (Standard Deviation)	
	N1=15,N2=15	N1=15,N2=15	
Glucose 1	162.60(34.58)	151.73(72.13)	0.570
Glucose 2	169.93(57.59)	192.8(52.96)	0.012*
HAb1C 1	7.41(1.11)	6.53(0.89)	0.014*
HAb1C 2	7.70(1.84)	7.63(1.56)	0.836
Cholesterol 1	194.33(50.87)	166.80(38.63)	0.004**
Cholesterol 2	191.07(38.40)	198.80(59.50)	0.487
TG1	158.20(77.87)	160.67(71.42)	0.908
TG2	217.40(156.86)	276.00(305.59)	0.301
Uric acid 1	5.52(1.19)	4.90(1.10)	0.030*
Uric acid 2	6.52(1.20)	6.43(1.20)	0.608
Cr1	0.89(0.23)	1.44(2.37)	0.365
Cr2	0.84(0.20)	0.89(0.24)	0.131
GPT1	30.4(17.44)	27(14.40)	0.222
GPT2	29.2(19.91)	31.1(15.50)	0.699
Live Quality (SF-36 score)1	58.0(7.21)	91.13(15.55)	0.000**
Live Quality (SF-36 score)2	56.4(7.03)	65.27(8.45)	0.001**

Note: 1 = experimental group , 2 = control group. HbA1C=glycosylated hemoglobin ,TG=Triglyceride ,Cr=Creatinine ,GPT= Glutamic Oxaloacetic Transaminase. \* $p < 0.05$ ; \*\*  $p < 0.05$

## 5. Discussion

There are many limitations in our study. First, total number of patients is only 30 persons, there is a recommendation for future researches to get more patients joining the related research. The second limitation relates to the focus on empowerment models and there is a lack of principles and guidelines to carry out the method procedure<sup>[19]</sup>.

In the past, patient education was generally prescriptive and therapeutic goals were set by medical professionals without discrimination. Most health professional training is based on a medical model designed to treat acute health care problems and relatively ignores chronic diseases management and health management ability for long time.

In empowerment model, the patients are the authority responsible for the treatment programs, self-health management, and the treatment outcomes. As chronic illnesses become more prevalent, and lead in high medical costs. Perhaps this health care model of empowerment model can be extended to most chronic disease patients as well. This approach is based on the belief that patients have an obligation to follow the direction of themselves and that the benefits of compliance outweigh the impact of these recommendations on patient quality of life<sup>[20]</sup>.

Education is designed to promote compliance or adherence using motivational and behavioral strategies in an effort to get patients to have the ability of creating health style. The model was effective in diabetes care about patient compliance cause of diabetes related management program. A new approach of think outside the box was needed that recognized that patients are in control of and responsible for the daily self-management of diabetes and that, to succeed, a self-management plan had to fit patients' goals, priorities, and lifestyle as well as their diabetes.

This approach is based on six fundamental aspects of chronic illness care six major: self-management、support、self-management support、clinical information systems、delivery system design、decision support、health care organization、community resources.

The choices that patients make each day as they care for diabetes have a greater impact on their outcomes than those made by their doctors. In addition, patients are in charge of their self health-management behaviors and implement because the care programs are made by themselves.

Finally, because the implications, complications, and consequences for these decisions accrue directly to patients, they have both the right and the responsibility to manage diabetes in the way that is best suited to the context and culture of their lives<sup>[21]</sup>.

## 6. Conclusion

The study puts efforts and concern on the research and techniques in empowerment interventions which help diabetic patients deal with self health care. Post empowerment intervention, the related data including life quality and serum biomarkers of experimental patients is significant improved ( $p < 0.05$ ). The clinical physicians would take empowerment into consideration about the diabetic treatment program cause the research result. Application of empowerment, intervention of empowerment by medical professional team would improve life quality in diabetic patient and management<sup>[22]</sup>. The Diabetes empowerment Process has satisfactory treatment outcome for diabetic patients. Further studies are needed to test applicability of the empowerment to other populations<sup>[23]</sup>.

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## Effect of Gamma Irradiation on Enhancement of Some Economic Traits and Molecular Changes in *Hibiscus Sabdariffa* L.

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**Abstract:** Seeds of *Hibiscus sabdariffa* were irradiated with gamma rays (100, 200, 300, 400, 500, 600, 700 and 800 Gy) for determining the effectiveness of different doses of irradiation on growth behaviour, yield and evaluate of roselle calyx extract and quality. Gamma irradiation at 600 Gy was superior in growth criteria enhancement. Maximum mean values for fresh and dry weight of leaves, stems and roots/plant were recorded at 600 Gy in the first season and 500 or 600 in the second one. The application of 600 Gy gave the highest effect on increasing number of fruit per plant and the most significantly effective treatment for increasing anthocyanin. The significantly higher calyx yield per plant recorded by the application of 700 Gy. The variation in DNA of the irradiated seeds in comparison to the control were successfully assessed using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR).

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**Key words:** *Hibiscus sabdariffa*, gamma rays, anthocyanin, calyx yield, RAPD- PCR.

### 1. Introduction

*Hibiscus sabdariffa* L., popularly known as roselle, is a member of the family Malvaceae and one of the most important and popular medicinal and industrial plants, the calyx is widely used for producing drinks or tea because of its high content of anthocyanins and organic acids (Hong and Wrostrlad, 1990; Gomez-Leyva *et al.*, 2008; Cissé *et al.*, 2009) as well as flavour and colour additives in the manufacture of jam, liquor, and jellies (Akindahunsi and Olaleye, 2003). In ethno medicine, *H. sabdariffa* is traditionally used to deal with several health problems, including hypertension, pyrexia and liver disorders, microorganism growth; it is also used as a diuretic, sedative, or digestive (Faraji and Tarkhani, 1999; Chen *et al.*, 2003; Akindahunsi and Olaleye, 2003). The positive physiological effect of this plant extract could be related to the presence of anthocyanins with potent antioxidant activity. Anthocyanins in addition to their colorful characteristics possess antioxidant properties (Francis, 2000).

The study of the effects of radiation on plants is a broad and complex field. Gamma irradiation was found to increase plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues depending on the

irradiation level (Gunckel and Sparrow, 1961). It is one of the important physical agents used to improve the characters and productivity of many plants (Jaywardena and Peiris, 1988, Sharma and Rana, 2007). The gamma ray had adverse effect on traits of plants and this depended on plant species or varieties and the dose of irradiation (Artk and Peksen 2006). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kim *et al.*, 2004, Wi *et al.*, 2005). Mokobia and Anomohanran 2005) found that gamma irradiation were very useful not only for sterilization of medicine but also for the preservation of food and cereals in nutrition and agriculture.

Irradiation also been successfully used for mutation in breeding of various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003; Yoon *et al.*, 1990). Many of the complications of a phenotypic or biochemical based assay can be mitigated through direct identification of genotypes with DNA based assays (Mengoni *et al.*, 2000) One such method is RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) which amplifies random

genomic DNA sequences using single, short arbitrary primers, and these can be effectively used as genetic markers. The RAPD technique therefore surveys (scans) numerous loci in the genome, which makes this method particularly attractive for analysis of genetic distance and similarity between closely related species (Persson and Gustavsson 2001 and Crockett *et al.*, 2002).

The present work aimed to investigate the effect of different doses of gamma irradiation (0.0, 100, 200, 300, 400, 500, 600, 700 and 800 Gys) on growth, yield, calyx extract and quality as well as molecular changes of roselle plants.

## 2. Materials and methods

Roselle (*Hibiscus sabdariffa* L.) variety "Sabahia 17" used in this investigation. Seeds of roselle were obtained from Medicinal and Aromatic plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Dry seeds were divided into nine groups. The first group was kept without irradiation as control, while the rest were exposed to 100, 200, 300, 400, 500, 600, 700 and 800 Gray gamma-irradiation doses using Egypt's Mega Gamma-1 type J 6600 cobalt-60 irradiation at Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Egypt.

A field experiment was conducted during the two successive seasons of 2009 and 2010 at the Faculty of Agriculture Experimental Station, Suez Canal University, Ismailia, Egypt. Seeds were sown in sandy soil on the 4th May for both seasons. Each treatment was planted in 6 rows, 4 m long and 0.6 m wide, making an area of 14.4 m<sup>2</sup>. Hills were 50 cm apart; 5 seeds per hill then thinned, three weeks later to one plant/ hill. Other agricultural practices such as irrigation and weeding were carried out as recommended.

### Measurements were taken on

#### Vegetative growth characters

After 132 days from sowing before harvesting, the following growth criteria were recorded, using eight random plants from each treatment, plant height (cm), number of branches/ plant, main root length/ plant, number of roots/ plant, fresh and dry weight of leaves, stems and roots/ plant (g).

#### Yield components

In both seasons at harvest (180 days seed sowing), number of fruits/ plant and fresh and dry weight of calyx/ plant (g) were taken.

### Chemical analysis in leaves and calyces

#### Photosynthetic pigments:

The fourth leaf from top was picked after 132 days from sowing. Chlorophylls a and b in leaves were determined calorimetrically according to A.O.A.C. (1980).

Total anthocyanin in dried air harvested roselle calyx determined according to the method described by Fuleki and Francis (1968) and developed by Du and Francis (1973).

### Genomic DNA extraction

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was adopted for obtaining good quality total DNA. 100 mg of 40-days-old young fresh leaves of roselle was collected and quickly frozen in liquid nitrogen then ground using mortar and pestle. Five ml of CTAB extraction buffer (60°C), 50 mg PVP (polyvinyl pyrrolidone) and 15 µl β-Mercaptoethanol (0.3%) were added. The tubes were mixed by inversion and incubated at 65°C for one hour. Then, 6 ml of chloroform: isoamyl alcohol (24:1) was added and contents were mixed by inversion to form an emulsion. The tubes were centrifuged at 5000 rpm for 20 min at room temperature. The top aqueous layer was further centrifuged at 5000 rpm after addition of 6 ml of chloroform: isoamyl (24:1). Half-volume of 5 M NaCl and two volumes of absolute cold ethanol were added to the supernatant and were mixed well. The tubes were incubated at -20°C overnight, and then centrifuged at 8000 rpm for 15 min. The supernatant was discarded, the pellet was washed with 70% cold ethanol, and dried in Speed Vac (Savant, USA) for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4° C. Then, it was transferred to 1.5 ml centrifuge tube. To remove RNA contamination, 4 µl (10 mg/ml) RNase (Sigma, USA) were added to the DNA solution and the mixture was incubated at 37°C for 2 hours. The extracted DNA was deproteinized by adding 4 µl (1mg/ml) proteinase K (Sigma, USA) and was incubated at 37°C for 2 hours. Three hundred µl of Tris-saturated phenol-chloroform were added and mixed well by inversion. Tubes were centrifuged at 14000 rpm for 15 min in a centrifuge (Eppendorf, USA). The upper layer was transferred to new tube and 150 µl of TE buffer was added to the phenol phase, mixed, spun for 10 min, then the upper layer containing the DNA was removed and was added to the sample. DNA was precipitated overnight at -20°C using 0.1 volume 3 M sodium acetate (pH 8.0) and two volumes of chilled absolute ethanol. Samples were centrifuged at 14000 rpm at 4°C for 15 min. The DNA was washed with 70 % ethanol, briefly air-dried and re-dissolved in TE buffer.

**Estimation of DNA Concentration:**

DNA concentration was determined using NanoDrop 3300 (Thermo Scientific, Wilmington, USA).

**Randomly Amplified Polymorphic DNA (RAPD) RAPD-PCR Reactions**

A set of twenty-eight random 10-mer primers (Table 1) was used in the detection of polymorphism among the irradiated seeds and

control. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) RAPD-PCR was carried out according to the procedure given by Williams *et al.* (1990) with minor modifications. The amplification reaction was carried out in 25  $\mu$ l reaction volume containing 1X PCR buffer, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 1  $\mu$ M primer, 1 U Go *Taq* DNA polymerase (Promega, USA) and 25 ng templates DNA.

**Table (1): Sequence of the twenty-eight decamer arbitrary primers assayed in RAPD-PCR.**

Name	Sequence (5'-3')	Name	Sequence (5'-3')
OPA-05	ACGGGTCTTG	OPD-02	GGACCCAACC
OPA-07	GAAACGGGTG	OPD-07	TTGGCACGGG
OPA-14	TCTGTGCTGG	OPD-07	TTGGCACGGG
OPA-16	AGCCAGCGAA	OPG-10	AGGGCCGTCT
OPB-02	TGATCCCTGG	OPH-05	AGTCGTCCCC
OPB-06	TGCTCTGCCC	OPO-15	TGGCGTCCTT
OPB-08	GTCCACACGG	OPO-20	ACACACGCTG
OPB-12	CCTTGACGCA	OPZ-12	TCAACGGGAC
OPB-13	TTCCCCGCT	OPZ-13	GACTAAGCCC
OPk-01	CATTCGAGCC	OPZ-14	TCGGAGGTTC
OPk-02	GTCTCCGCAA	OPZ-15	CAGGGCTTTC
OPk-03	CCAGCTTAGG	OPZ-18	AGGGTCTGTG
OPk-12	TGGCCCTCAC	OPZ-19	GTGCGAGCAA
OPk-16	GAGCGTCGAA	OPZ-20	ACTTTGGCGG

**Thermocycling profile and detection of the PCR products**

PCR amplification was performed in a C1000- Thermocycler (*BIO RAD, USA*) programmed to fulfill 40 cycles after an initial denaturation cycle for 2 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 $\mu$ g/ml) in 1X TAE buffer at 95 volts. PCR products were visualized on UV light and photographed using a GelDoc 2000 (BIORAD, USA). Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

**Statistical analysis**

Experiment was set up in randomized complete block design with eight replicates per treatment. Data were statistically analyzed using ANOVA/MANOVA of Statistica 6 software (Statsoft, 2001), the significance of differences among means was carried out using the Least Significant Test (L.S.D) at  $p = 0.05$ .

**3. Results and Discussions****Plant growth and yields**

It should be pointed out that, seed germination started simultaneously six days after sowing, and the germination rate was 100% in the control as well as in the treated seeds of roselle plant during both seasons (data not shown).

In general, gamma irradiation significantly increased plant height, number of branches, number of root and root length of roselle plant as compared with control as shown in Table (2). At harvest time (132 days after sowing) 600 Gy gave the highest plant height (154.7 cm and 127.0 cm), highest number of branches (14.7 and 14.0 branches/ plant) and longest root (34.3 cm and 31.3 cm) in 2009 and 2010 seasons respectively. It can be noticed that the higher dose of gamma rays (800 Gy) was less effective than the other lower doses, seeds exposed to (800 Gy) produced the shortest plants with reduction root length compared to the other doses (Table 2). This reduction could be attributed to reduced mitotic activity in meristematic tissues and reduced moisture content in seeds as reported by Khalil *et al.* (1986). Similarly Norfadzrin *et al.* (2007) noticed that higher gamma ray doses (600 and 800 Gy) had

negative effect on the morphological characteristics of tomato and okra seedlings derived from irradiated seeds. A reduction in plant height and number of branches for many crops that exposed to higher gamma ray doses had already been reported by Thimmaiah *et al.* (1998), Muhammad and Afsari (2001), Al-Salhi *et al.* (2004), Yaqoob and Ahmad (2003), Token *et al.* (2005) and Kon *et al.* (2007).

#### Fresh and dry weights of plant

The fresh and dry weight of leaves stems and roots of roselle plant were significantly increased as result of gamma ray compared with control in the two seasons as shown in Table (3). The maximum values of fresh and dry weight were obtained by 600 Gy in the first season and by 500 or 600Gy in the second one. Similer trend have been reported by Veeresh *et al.*, (1995) and Kon *et al.*, (2007) on beans. Abo-El-Seoud *et al.* (1994) assumed the stimulation of gamma radiation to its impact on the auxins balance within the plant tissues. The reduction in fresh and dry weight of plant may be due to reduce moisture content due to radiation stress, when exposed to high gamma radiation doses.

#### The calyx yield

The application of 600 Gy gave the highest effect on increasing number of fruit per plant (Table4) compared with the other radiation doses and the control. The significantly higher calyx fresh weight per plant recorded by the application of 500 and 400 Gy (171.8 and 151.4 g per plant) in 2009 and 2010 respectively at harvest stage 180 days after sowing (Table 4). Increased growth of plants (plant height, number of branches, root length, fresh and dry weight of leave, stems and roots) as result of exposed to 600 Gy hence higher yields (fruit yield) of roselle plant (Tables 2and3). The stimulatory effect of 600 Gy dose is due to the fact that mutagens stimulate the role of enzyme and growth hormone responsible for growth and yield. Increased number of fruits per plant as a result of gamma irradiation was recorded by (Dubey *et al.*, 2007; Mishra *et al.*, 2007; Sharma and Mishra, 2007 Sujaya-Das *et al.*, 2007 and Sundaravadivelu *et al.*, 2006).

#### Photosynthetic pigment

Data presented in Table (5) indicated that all photosynthetic pigment contents were significantly increased as a result of gamma irradiation increased except 300and 400 Gy. In the two seasons, best results were obtained by using 700 Gy. Higher doses of mutagens were most effective to produce chlorophyll mutations in roselle which consequently increased all yield-related traits. These results are in agreement with those obtained by (Rasico *et al.*,

2001; Osama, 2002 and Rejili *et al.*, 2008), who reported that the improvement of yield components and chlorophyll parameters in plants was induced after various mutagenic treatments such as E.M.S, sodium azide and gamma rays.

#### Anthocyanine content

The concentrations of anthocyanin are given in Fig. (1). Results indicated that anthocyanin content of roselle calyxes were increased by increasing the dose of gamma irradiation treatments when compared with control treatment. Moreover, at harvest (180 days after sowing), the 600 Gy was the most effective treatment for increasing anthocyanin which gave 3.63% and 3.68% in 2009 and 2010 respectively. These results agree with those reported by Abo-El-Seoud, *et al.* (1994) who found that the 40 Gy had the capacity to enhance anthocyanin concentrations. Gamma rays belong to ionizing radiation and interact on atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level (Kim *et al.*, 2004, Wi *et al.*, 2005).

#### RAPD-PCR of genomic DNA

RAPD-PCR was used for detection of DNA profile changes due to treatments (0.0, 100,200,300,400,500,600,700 and 800 Gys). Six primers out of twenty-eight random 10-mer primers (OPA-16, OPB-02, OPD-02, OPD-07, OPG-10 and OPH-05) successfully amplified DNA fragments from *Hibiscus sabdariffa* L DNA samples (Table1 and Fig2). The results indicated occurrence of structural changes in treatment with six primers (Table 6, Fig2). A total of 54 fragments were visualized across the six primers (Table 6). Genomic DNA polymorphisms due to treatments are presented in Figure (2). The percentage of polymorphism was (38.4, 70, 50, 77.3, 54.5 and 38.4%) (Table 6). The result of RAPD analysis indicted the disappearance of DNA polymorphic bands in response to treatments with doses of all gamma irradiation. In primer OPD-07 bands with molecular size 1651 bp disappeared under the effect of gamma irradiation. Bands with molecular size 1207, 322, 506 and 370 bp appeared under the effect of gamma irradiation, the first appeared only at high doses (200, 300 and 500 Gy). Bands with molecular size 370 bp appeared only at high doses (800 Gy). In primer OPA-16, band with molecular size 1234bp appeared under irradiation by 700 and 800 Gy. The bands with molecular sizes 442bp appeared only under irradiation by 300, 500, 700 and 800 Gy in primer OPD-02 (Fig2).

The results agreed with Wendt *et al.* (2001) who used the RAPD markers to study the effect of gamma radiation on potato. Ganapathi *et al.* (2008) studied the effect of gamma irradiation on banana using RAPD-DNA analysis. They observed changes in the DNA bands, where the main changes in the RAPD profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages. Appearance of new bands is usually result from different DNA structural changes (Breaks, transpositions, deletion etc) (Danylchenko and Sorochinsky, 2005).

### Conclusion

The results of the experiment indicated that increasing doses of gamma irradiation caused severe effects on the plant development. In general, according to the results of the present work, the best treatment was the application of 600 Gy irradiation which stimulate roselle plant growth and, intern, to increase its active substances productivity. The ultimate aim of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait and selection of economically important mutants. For breeding purposes mutagenic treatments with low physiological effects and strong genetic effects are desirable.

**Table (2) Effect of gamma irradiation on growth criteria of *roselle* plants during 2009 and 2010 seasons.**

Treatments	Plant height (cm)	Number of branches/ plant	Number of roots/ plant	Length of the Longest root (cm)
<b>1st season</b>				
Control	78.3d*	6.3c	3.7e	19.0d
$\gamma$ 100Gy	106.3c	10.6abc	12.0a	22.0bcd
$\gamma$ 200 Gy	107.3c	12.7ab	5.7cde	26.0bc
$\gamma$ 300 Gy	131.3b	6.7c	5.3de	21.3cd
$\gamma$ 400 Gy	123.3b	13.7a	10.0ab	21.1cd
$\gamma$ 500 Gy	121.5b	14.3a	6.3cd	27.3b
$\gamma$ 600 Gy	154.7a	14.7a	9.0b	34.3a
$\gamma$ 700 Gy	130.0b	10.3abc	8.0bc	22.3bcd
$\gamma$ 800 Gy	87.0d	8.3bc	3.8e	21.0cd
<b>2nd season</b>				
Control	85.7cd	6.0d	5.3d	15.0d
$\gamma$ 100Gy	108.0b	6.7cd	13.5a	24.0bc
$\gamma$ 200 Gy	108.3b	13.7ab	6.3bcd	22.3bc
$\gamma$ 300 Gy	126.3a	10.7abc	7.3bc	25.0bc
$\gamma$ 400 Gy	126.7a	9.3bcd	6.7bcd	25.3abc
$\gamma$ 500 Gy	109.7b	10.0abcd	6.5bcd	26.7ab
$\gamma$ 600 Gy	127.0a	14.0a	7.3bc	31.3a
$\gamma$ 700 Gy	99.0bc	12.7ab	6.1cd	24.3bc
$\gamma$ 800 Gy	78.3d	12.3ab	6.0cd	19.7cd

\* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test



**Table (3) Effect of gamma radiation on fresh and dry weight g / of leaves, stem and root of roselle plants during 2009 and 2010 seasons.**

Treatments	Leaves fresh weight/ plant (g)	Leaves dry weight/ plant (g)	Stem fresh weight/ plant (g)	Stem dry weight/ plant (g)	Roots fresh weight/ plant (g)	Roots dry weight/ plant (g)
<b>1st season</b>						
Control	75.7de*	12.6d	64.9f	20.3d	8.1e	3.1d
γ 100Gy	87.8de	13.6cd	94.8e	36.1c	9.6de	4.1cd
γ 200 Gy	92.8de	16.8cd	75.4f	22.9d	18.0ab	5.5abc
γ 300 Gy	101.9d	24.9b	112.2d	34.3c	12.5cde	4.1cd
γ 400 Gy	91.8de	18.9c	113.9d	34.6c	13.6bcd	5.2bc
γ 500 Gy	148.5c	27.0b	212.1d	52.3b	14.2abc	5.1bc
γ 600 Gy	214.2a	36.4a	218.3a	70.6a	18.8a	6.9a
γ 700 Gy	174.1b	36.2a	200.7b	50.6b	14.7abc	6.1ab
γ 800 Gy	83.6de	12.7d	141.5c	25.3d	8.4e	4.8bc
<b>2nd season</b>						
Control	63.6c	11.6f	59.0g	17.8d	8.6c	2.5e
γ 100Gy	88.5c	11.8f	107.2e	31.1c	8.8c	4.2bcd
γ 200 Gy	92.4bc	17.5de	76.4f	20.5d	9.4c	3.7cde
γ 300 Gy	98.2bc	20.6cd	128.0cd	37.5b	14.5b	5.1bc
γ 400 Gy	86.1c	15.0ef	124.8d	31.3c	15.9b	5.3b
γ 500 Gy	166.6a	34.6a	164.3b	42.0a	9.4c	4.0bcde
γ 600 Gy	153.5a	30.3b	198.8a	42.2a	26.0a	8.2a
γ 700 Gy	131.1ab	22.9c	141.5c	41.7a	23.5a	7.8a
γ 800 Gy	90.4c	17.6cd	130.2cd	40.4ab	8.7c	2.6de

\* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

**Table (4) Effect of gamma irradiation on some yield components in roselle plants during 2009 and 2010 seasons.**

Treatments	No. of fruits / plant	Calyx weight	
		Fresh (g/plant)	Dry (g/plant)
<b>1st season</b>			
Control	31.7e*	70.7b	8.8e
γ 100Gy	43.0de	68.8b	8.5e
γ 200 Gy	40.0de	60.4b	8.0e
γ 300 Gy	42.0de	130.5ab	12.0cde
γ 400 Gy	86.0b	151.4ab	13.9bcd
γ 500 Gy	53.0cd	171.8a	22.4a
γ 600 Gy	113.5a	103.7ab	16.4b
γ 700 Gy	66.3c	90.7ab	15.7bc
γ 800 Gy	46.7d	73.8b	10.4de
<b>2nd season</b>			
Control	33.0d	80.3ab	12.4abcd
γ 100Gy	36.0d	68.7b	9.9bcd
γ 200 Gy	43.3cd	89.7ab	13.4abcd
γ 300 Gy	49.3bc	90.3ab	14.4abcd
γ 400 Gy	58.7ab	151.4a	17.2a
γ 500 Gy	50.0bc	108.5ab	16.0ab
γ 600 Gy	61.0a	84.5ab	9.9bcd
γ 700 Gy	56.7ab	109.9ab	8.6cd
γ 800 Gy	38.3d	53.9ab	7.2d

\* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

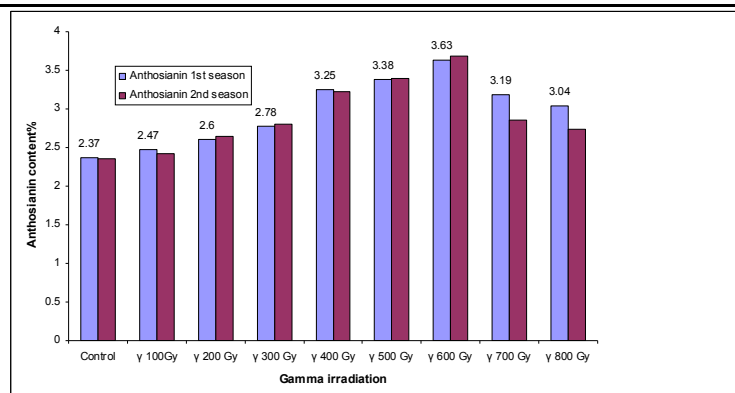
**Table (5) Effect of gamma irradiation on chlorophyll (mg/100 g F.W) of roselle plants during 2009 and 2010 seasons.**

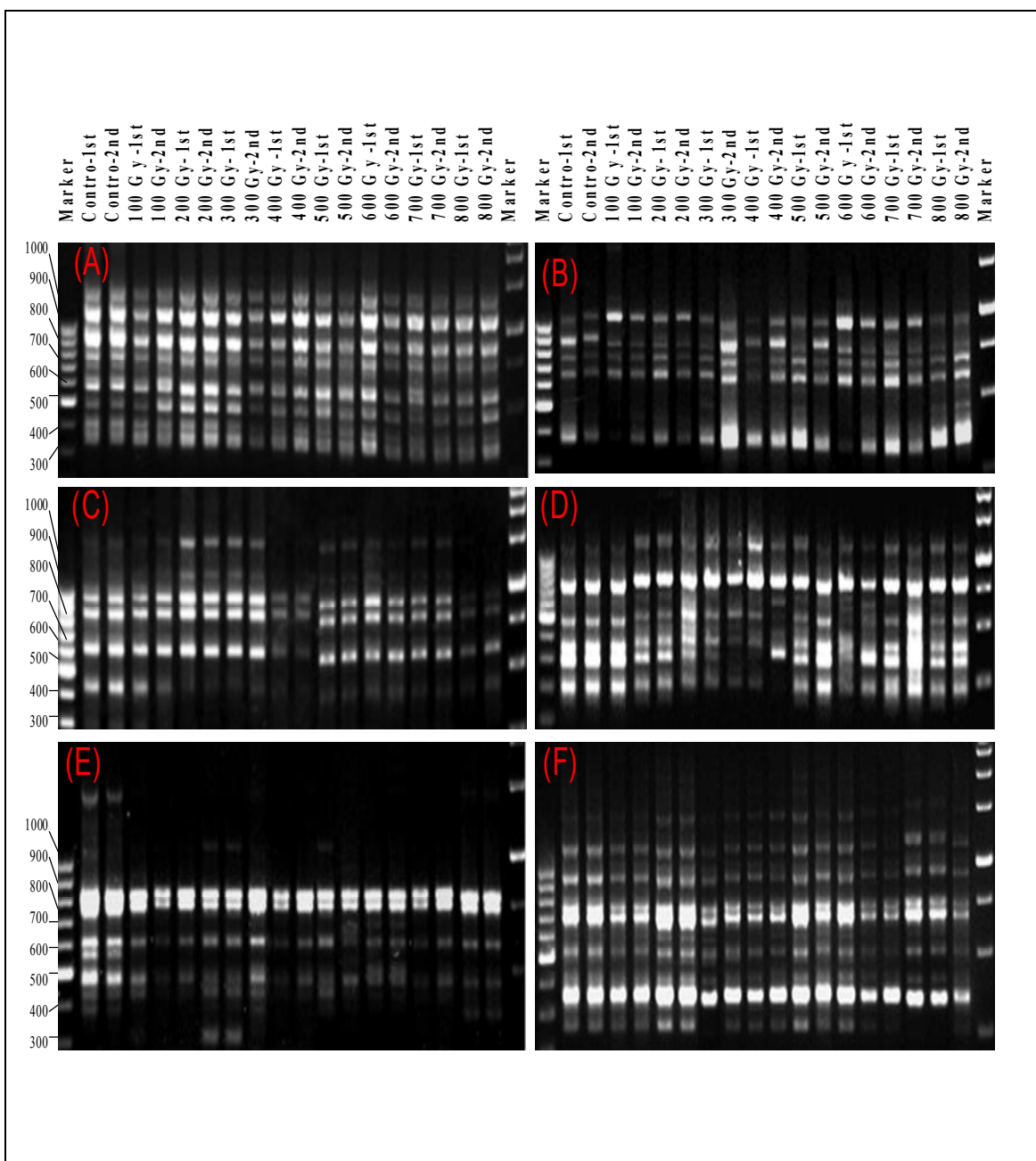
Treatments	Chlorophyll "a"	Chlorophyll "b"	Chlorophyll "a+b"
<b>1st season</b>			
Control	47.7b	42.9ab	90.6b
$\gamma$ 100Gy	47.8b	52.6ab	100.4ab
$\gamma$ 200 Gy	68.1a	51.5ab	119.6ab
$\gamma$ 300 Gy	70.2a	25.2b	95.8ab
$\gamma$ 400 Gy	62.8a	48.3ab	110.9ab
$\gamma$ 500 Gy	65.2a	46.4ab	111.6ab
$\gamma$ 600 Gy	68.5a	57.6ab	126.1ab
$\gamma$ 700 Gy	70.7a	69.4a	139.6a
$\gamma$ 800 Gy	68.6a	71.8a	140.3a
<b>2nd season</b>			
Control	70.2a	23.5b	94.0c
$\gamma$ 100Gy	64.4ab	44.5ab	108.9abc
$\gamma$ 200 Gy	59.4ab	40.9ab	100.4bc
$\gamma$ 300 Gy	65.5ab	40.8ab	106.3abc
$\gamma$ 400 Gy	54.8b	39.2ab	94.0c
$\gamma$ 500 Gy	61.7ab	52.1ab	113.8abc
$\gamma$ 600 Gy	67.2ab	60.4a	127.6ab
$\gamma$ 700 Gy	70.5a	62.1a	132.3a
$\gamma$ 800 Gy	57.2ab	35.4ab	92.6c

\* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

**Table (6) DNA polymorphism using randomly amplifying DNA (RAPD) for roselle plants during 2009 and 2010 seasons.**

Primer	Total # of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
OPA-16	13	8	5	38.4
OPB-02	10	3	7	70
OPD-02	10	5	5	50
OPD-07	15	4	11	77.3
OPG-10	11	5	6	54.5
OPH-05	13	8	5	38.4
Total	72	33	39	54
Average	12	5.5	6.5	

**Fig (1) Effect of gamma irradiation on total anthocyanin content (%) of roselle plants during seasons 2009 and 2010.**



**Fig. (2) DNA polymorphism using randomly amplifying DNA (RAPD) for *Hibiscus sabdariffa* treated with different doses of gamma irradiation during seasons 2009 and 2010. (A), (B), (C), (D), (E) and (F) refer to primers OPH-05, OPD-02, OPG-10, OPB-02, OPD-07 and OPA-16 respectively.**

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## Attitude of Academic Ambulatory Nurses toward Patient Safety Culture in Saudi Arabia

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**Abstract:** Patient safety issues in Saudi Arabia have received increasing attention. Pressures to improve patient safety within academic healthcare settings are gaining momentum daily. Health care organization providers and policy makers continually strive to promote patient safety culture. The purpose of this study was to examine the nurses' attitude toward patient safety culture in academic ambulatory healthcare settings in Saudi Arabia. Methods: A cross-sectional correlational descriptive design, using the Safety Attitude Questionnaire Ambulatory version (SAQ-A), was carried out in year 2010. The survey was distributed to four ambulatory academic departments, which included medical, surgical, obstetrics/gynecological, and pediatric in Riyadh. All 250 available (not on vacation or deployed out of the area) ambulatory care staff nurses and nurse managers were recruited yielding 221 responses with a response rate of 88%. Results: The highest positive attitudes were toward job satisfaction and the work experience. Working conditions and the quality of the work environment and its logistical support received the second highest positive attitudes. However, the quality of collaboration between personnel and the proactive organizational commitment to safety had the lowest positive attitudes among nurses. Strong significant relationship was indicated between job satisfaction among nurses and work conditions. Statistically significant differences in mean scores were observed for registered nurse toward stress recognition and acknowledgement of the effects of stress on patient safety than nurse managers. Recommendations: Enhancing the quality of collaboration between personnel and the proactive organizational commitment to safety may promote safety culture in academic care settings. Assessment of workplace safety culture is the first step in identifying barriers that nurses face to provide safe patient care. Improving safety culture attitudes in academic settings can take a considerable amount of effort and resources.

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**Keywords:** patient safety culture, nursing, academic ambulatory, Saudi Arabia, attitude, healthcare organization

### 1. Introduction

Patient safety issues in Saudi Arabia have received increasing attention. Pressures to improve patient safety culture within academic healthcare settings are gaining momentum daily. Many safety-oriented organizations develop and foster a patient safety culture that is defined as the set of shared values, attitudes, perceptions, beliefs and behaviors that support safe practices among individuals in healthcare organizations (**World Alliance for Patient Safety, 2008**). Safety climate, teamwork climate, working conditions and acknowledged mutual dependency are some components of a patient safety culture (**Pronovost & Sexton, 2005**).

Academic healthcare organizations have grown increasingly complex with many component parts that are expected to optimize patient safety. In addition, they provide the most advanced clinical education, significant amounts of research, and substantial innovations in both technology and delivery systems. Moreover, a quality multidisciplinary endeavor emerges essentially through the process of quality improvement (**Hall et al., 2006**). The care is often delivered in a fast-

moving environment, involving a multitude of technologies and many individual decisions by healthcare providers. Such circumstances provide fertile ground for erroneous events that may lead to serious patient injuries and sometimes death. Health experts believe organization safety culture is a fundamental factor that influences patient safety (**Nieva & Sorra, 2003**).

Thorough research and improvement efforts have primarily focused on inpatient care, while substantial patient safety risks exist in ambulatory care. Improving patient safety in the outpatient setting poses unique challenges for healthcare leaders. Even though ambulatory care may be less technologically complex than inpatient care it is often more complex logistically (**Schauberger & Larson, 2006**). Like all healthcare institutions, academic healthcare have been challenged by considerable data indicating large numbers of medical errors and quality defects in patient care (**Aspden et al., 2007**). In addition, The World Health Organization estimated an average of 10% of all inpatient visits result in some form of unintended harm done, and suggested that developing countries account for

around 77% of all reported cases of counterfeit and substandard drugs (**World Alliance for Patient Safety, 2008**). Most care is provided in ambulatory care settings rather than acute care inpatient hospital settings. Recent research finds that over 77% of medical procedures are now performed in ambulatory care settings (**Day & Smith, 2007**).

In addition the ambulatory care setting present some challenges to patient safety. Factors as waiting and delays, poor communication, environmental issues, parking, security, service quality deficiencies, and inadequate staffing was associated with unsafe care (**Weingart et al., 2007**). Furthermore, several studies examined diagnostic errors in the ambulatory care setting which were due to multiple process breakdowns, including failure to order an appropriate diagnostic test and inadequate follow-up planning (**Wachter, 2006; Woods et al., 2007**).

Few studies regarding patient safety culture were conducted in Saudi Arabia. Nevertheless, socioeconomic and organizational/system factors affecting patient safety and quality perceptions were examined from nurses in five Riyadh hospitals in Saudi Arabia. The results revealed that system factors significantly affect patient safety improvements, including functional feedback, suggestions, and error reporting (**Mwachofi et al., 2011**). **Al-Ahmadi (2009)** compared between Riyadh's public and private hospitals in Saudi Arabia to explore the perceptions of health care providers on patient safety and error reporting using Hospital Survey on Patient Safety Culture (HSPSC). The findings presented that safety culture dimension of organizational learning received the highest positive response whereas the non-punitive response to error received the lowest positive response. All types of mistakes were reported more frequently in private hospitals than in public hospitals. Teamwork across units, supervisor/managers expectations and actions promoting patients safety are factors that influence event reporting. The study concluded that staffing and non-punitive response to error need improvement in both hospital types. The Hospital Survey on Patient Safety Culture questionnaire was used to assess patient safety culture in Saudi Arabian hospitals. Overall Patient Safety perception was rated as excellent or very good by 60% of the respondents, acceptable by 33% and failing or poor by 7%. Under reporting of events, non-punitive response to error, staffing, teamwork across hospital units needed improvement for most hospitals (**Al Ahmadi, 2010**).

In most healthcare systems, academic healthcare institutes with their multiple mandates of care provision, teaching, training and research add to the complexity of this environment that becomes

potentially more vulnerable to errors and adverse events (**Day & Smith, 2007; Schnall et al., 2008**). Moreover, a study was conducted to evaluate the perception of nursing staff on the quality of patient care pre and post accreditation process in the multicultural and multi-language academic health care organization. The results indicated that despite all the barriers created by the multicultural and multi-language, the accreditation process has generated a positive impact on the quality of patient care and patient safety as perceived by nursing staff (**Al Awa et al., 2011**). However, promoting safety culture among nurses present some challenges. A study compared attitudes toward patient safety among health providers of outpatient care with a modified version of the safety attitudes questionnaire (SAQ) and adapted it for use in this group of providers. The results revealed that physicians had the least favorable attitudes about perceptions of management, while managers had the most favorable attitudes. Nurses had the most positive stress recognition scores. All providers had similar attitudes toward teamwork climate, safety climate, job satisfaction, and working condition. They concluded that attitudes are associated with medical error and may differ among health providers (**Modak et al., 2007**). According to **Holden et al. (2009)**, the technicians scored less on the stress recognition subscale than other health providers did. The youngest health care providers scored less on teamwork climate, safety climate, and perception of management, job satisfaction than elders in primary care did.

Nursing work environment has been characterized by serious threats to patient safety. These threats are related to organizational management practices, workforce deployment practices, and organizational culture (**Page, 2004**). A study revealed that nurse managers reported significantly more positive safety culture perceptions compared to staff nurses. Additionally, staff nurses employed in government facilities had significantly less positive safety culture perceptions (**Wagner et al., 2009**). The results of a comparison study between teaching and community hospitals showed that the mean scores for work quality, job satisfaction, nursing leadership, quality of care, and job stress were higher for nurses in teaching hospitals than for nurses in community hospitals. Nurses in teaching hospitals reported higher perceptions of the quality of the work and work environment than in community hospitals (**Hall et al., 2006**). The greatest possible gains in patient safety result from management of information, communication, and coordination of patient care (**Hammons et al., 2003**).

While there has been a clear rise in the level of awareness of the issue of patient safety. Wide-

scale patient safety activities in Saudi Arabia have been initiated at many hospitals, but little is known about the safety culture at these hospitals. The academic setting in Saudi Arabia has been developing a range of policies and procedures to promote and improve patient safety culture. In addition, the patient safety movement was launched to establish the patient safety concept and to raise awareness among all health staff working in the academic setting. Such movements require ensuring and promoting patient safety culture (**College of Medicine, 2010**).

### **Purpose of the study**

Nurses' attitude toward ambulatory patient safety culture in academic settings in Saudi Arabia has not been extensively examined. This study was designed to examine nursing attitudes toward patient safety culture in an academic ambulatory healthcare organization. The study addressed the following research questions:

1. What are the nurses' attitudes toward patient safety culture in an academic ambulatory healthcare organization?
2. What is the relationship among the subscales of patient safety culture in an academic ambulatory healthcare organization?
3. Is there any difference between patient safety culture subscales and some demographic data?

## **2. Material and Methods**

### **Design and Sampling**

This was a descriptive cross-sectional correlational study. The current study was conducted in 800-bed academic facility with a unique multicultural, multi-language environment. It provides general and subspecialty medical services. In 2008, the total patients who sought ambulatory health care services in this academic facility were 664932 patients (**Ministry of Health, 2009**). This academic facility provides primary and secondary care services for its patients. It also provides tertiary care services on referral bases. It contains a special outpatient building with eight departments. Four ambulatory departments were selected randomly. These four ambulatory departments are medical, surgical, Obstetrics and Gynecology, and pediatric. All 250 available (not on vacation or deployed out of the area) ambulatory care staff nurses and nurse managers were recruited yielding 221 responses with response rate of 88%.

### **Instrument**

The full version of the Safety Attitude Questionnaire Ambulatory version (SAQ-A) is 62 items, of which only 30 were scaled (**Nieva & Sorra,**

**2003**). The SAQ-A has been successfully used in inpatient and ambulatory clinics (**Sexton et al., 2006; Modak et al., 2007**). It was developed to assess the attitude of health providers in an ambulatory setting. Furthermore, SAQ-A has been demonstrated to have good psychometric properties (**Modak et al., 2007**). The questionnaire was comprised of seven subscales. Stress Recognition (SR) is four items that acknowledges how the performance is influenced by stressors. Job Satisfaction (JS) is five items, which is related to positivity about the work experience. Perception of Management (PM) is four items related to approval of managerial action. Working Conditions (WC) is four items that perceives quality of the work environment and logistical support such as staffing, training, etc. Safety Climate (SC) is seven items related to perceptions of a strong and proactive organizational commitment to safety. Teamwork Climate (TC) is six items that perceives quality of collaboration between personnel. In the current study, scale reliabilities of the six safety attitudes were assessed using Cronbach's alpha. For each subscale, the reliability was SR (0.85), JS (0.80), PM (0.76), WC (0.74), SC (0.69), and TC (0.67). The questionnaire used a 5-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = agree strongly). Negative statements were reversed so that the higher the score, the more positive the attitudes. Extra items were added to identify respondents' demographic information such as age, gender, management role, and working experience in the outpatient areas.

### **Procedures**

The questionnaire was distributed through the nurse manager as the point of contact at each of the four outpatient departments over two-month period. All questionnaires included a letter that explained the purpose of the study and explicitly stated that the survey was entirely voluntary. The investigator passed through each department to explain the research, answer questions, and collect the completed surveys in order to reduce the inconvenience to the clinic point of contact.

### **Statistical Analysis**

The Statistical Package for Social Sciences software (SPSS 17.01 for Windows; SPSS, Chicago, IL, USA) was used for data analysis. Cronbach's alpha was calculated and determined the reliability of the subscales of the Safety Attitude Questionnaire Ambulatory version (SAQ-A). Descriptive statistics were computed to describe the participants' characteristics. The correlation coefficients were calculated to indicate the strength and direction of the relationships between the variables. To calculate



safety attitudes for each of the six safety dimensions (stress recognition, job satisfaction, perception of management, working conditions, safety climate, and teamwork climate), converting results from categorical to continuous variables as follows: strongly disagree = 0; disagree = 25; neutral = 50; agree = 75; and strongly agree = 100 was performed. Some items were reverse scored so that a higher score always represented a more positive attitude. For each respondent, a mean score of  $\geq 75$  for the items in a particular dimension indicated a positive safety attitude for that dimension.

### Ethical consideration

The study's protocol was approved by the deanship of research at King Saud University and ethical approval was obtained from the academic health organization administrator. Permission was obtained to conduct the study in selected departments. Throughout the study, protection of human rights was assured and adherence to ethical principles was secured. Thus, the researcher ensured that each individual's autonomy was supported. Participation was voluntary, and there was no penalty for withdrawal from or termination of the study. In addition, the research methodologies were non-invasive, and there were minimal or no anticipated risks to participants. A written consent form was obtained from all participants. Total confidentiality of information was also assured by de-identified all answers and data were coded. No written or computerized records were linked to the collected

data with consent forms or the participants' identifying information.

### 3. Results

For the present study, the mean age of the total sample ( $n=221$ ) was  $38.5 \pm 8.9$  years and 84% of the nurses were married. As expected, the majority of nurses are international expatriates (98.3%). The most numerically predominant cultures were the Filipino (48%), the Indian (39%), and the Middle East (11.3%). The dominant gender in nursing field as usual was female (96.7%). In the ambulatory setting, around 92% are staff nurses and the rest work as nurse managers.

Table 1, show percentage of nurses with positive safety attitudes and mean scale scores. Nurses in academic health organization had the highest percent (88%) of positive attitudes toward their job and their work experience compared to their teamwork climate that reflected the quality of collaboration between personnel had the lowest positive attitudes (6%). Although the second highest positive attitudes present (77%) is working conditions and the quality of the work environment and logistical support, only (9%) they see how strong and proactive organizational commitment to safety is. However, 29 percent of nurses in the academic health organization acknowledged how stressors influence their performance and only (20%) had positive attitudes toward perception of management and items related to approval of managerial action.

Table 1. Percentage of nurses with positive safety attitudes and mean scale scores

% and mean scores	Stress recognition	Job satisfaction	Perception of management	Working conditions	Safety climate	Teamwork climate
% with positive attitude*	29	88	20	77	9	6
Mean score (SD)	60 (11.9±3.7)	84 (21.6±2.5)	63 (12.7±2.1)	77 (15.4±1.9)	59 (20.5±4.5)	65 (19.5±2.3)

\*Positive attitudes were defined as having scale scores  $>75$ , the equivalent of agree or strongly agree on the Likert scale used for the response options

Table 2. Correlation matrix between six of safety culture attitudes dimensions

	1	2	3	4	5	6
(1) Stress Recognition	—					
(2) Job satisfaction	-.032	—				
(3) Perception of Management	-.260**	.531**	—			
(4) Working Condition	.091	.660**	.321**	—		
(5) Safety Climate	.102	-.412**	-.251**	-.423**	—	
(6) Teamwork Climate	.325**	-.128	-.287**	.034	.199**	—

\*\* . Correlation is significant at the 0.01 level (2-tailed).

Table 2, presents correlation matrix between six of safety culture attitudes dimensions. The highest positive significant correlations existed between job satisfaction and work condition and perception of management (0.66, 0.53,  $P < 0.01$ , respectively). Furthermore, the highest negative significant correlations (-0.423,  $P < 0.01$ ) existed between safety climate and working condition and job satisfaction (-0.41,  $P < 0.01$ ).

The current study results revealed that there were statistically significant differences in mean scores between registered nurse and nurse managers

in all patient safety culture dimensions presented in Table 3. The statistically significant differences in mean scores ( $P < 0.05$ ) for registered nurse were observed. The registered nurses were more positive attitudes toward stress recognition and acknowledgement of the effects of stress on patient safety than nurse managers. In addition, perception of management dimension attitudes was higher among nurse managers than registered nurses. The significant mean difference was observed for the job satisfaction dimension. The nurse managers had more higher positive attitude than registered nurses.

**Table 3. Mean difference between registered nurse and nurse manager related to patient safety culture dimension**

Position	safety culture dimensions	t	df	Sig. (2-tailed)	Mean Difference
Registered nurse	Safety Climate	55.106	168	.000	20.625
	Job satisfaction	108.437	168	.000	20.873
	Perception of Management	82.866	168	.000	12.672
	Working Condition	103.724	168	.000	15.210
	Teamwork Climate	120.078	168	.000	19.441
	Stress Recognition	43.965	168	.000	11.855
Nurse manager	Safety Climate	43.125	51	.000	20.123
	Job satisfaction	57.074	51	.000	21.796
	Perception of Management	43.788	51	.000	12.854
	Working Condition	63.164	51	.000	15.988
	Teamwork Climate	79.374	51	.000	19.604
	Stress Recognition	23.131	51	.000	12.200

#### 4. Discussion

Improvement of patient safety culture has become an important aspect by aiming for high quality and safe healthcare. Healthcare leaders should maintain an organizational culture that supports and promotes patient safety. The systems within an organization are highly influenced by its culture or are an expression of its culture (Frankel *et al.*, 2003). The nursing profession has prided itself on being the patient's advocate and the keeper of quality and safety. Evidence shows that nurses are key factors in providing quality of care, which leads to improve patients' outcomes (Page, 2004).

The nurses who participated in the present study recognized six different cultural backgrounds. In congruence with Al Awa *et al.* (2011) result, this current study reflects the unique multicultural, multi-language environment. The predominant cultures were Filipino, then Indian, and then Middle Eastern. This might represent considerable effects on the findings of the study as being rooted in the society and consequently might have dominant cultural

effects. The remaining cultural minorities (1.7%) represent Saudi nationals.

Examination of the attitudes of nurses toward patient safety culture at academic health organizations in Saudi Arabia found that the highest positive attitudes were toward their jobs and their work experience. This finding was supported by Carayon *et al.* (2005) and Abdou and Saber (2011) they indicated that staff nurses were more satisfied with their job. The teamwork climate which reflects the quality of collaboration between personnel had the lowest positive attitudes among nurses. This might support the evidence that the unique multicultural and multi-language environment might represent considerable effects on the concept of collaboration between personnel and attitudes toward safety climate. According to the Al Awa *et al.* (2011) attitude varied considerably among unique multicultural, multi-language environments and sites that help identify strengths and weaknesses and highlights the potential identified weaknesses.

Interestingly, the second highest positive attitude among nurses was working conditions while their attitude toward the academic health organization and proactive commitment to safety climate was low. However, hospital safety culture in Taiwan was assessed and the results revealed that safety climate received lower scores related to safety culture (Lee *et al.*, 2010). This contrasts the findings that presented that nurses have relatively low perceptions of working conditions and high perception of safety climate. Underlying reasons for the observed dissimilarity in safety attitudes are not well understood. Safety culture attitudes are reflection of complex culture that resulting from the complex interactions among unique individuals and unique circumstances over time (Modak *et al.*, 2007).

A novel finding of the present study showed that the stress recognition attitude and perception of management among nurses received lower mean scores. In addition, statistically significant differences in mean scores between registered nurses and nurse managers related to these safety culture dimensions. Recent research has identified the same results. Perceptions among pharmacists toward stress recognition showed the least (Nordén-Hägg *et al.*, 2010). Unexpectedly Modak *et al.* (2007) presented that nurses had the highest stress recognition score. Higher stress recognition scores may indicate more recognition of the effects of stress on the ability of a nurse to perform optimally in delivering safe care. The prominent grounds for the noticeable dissimilarity may relate to factors such as size, location, and residency program affiliation in academic health organization.

In the current study, the highest positive significant correlations existed between job satisfaction and work condition and perception of management. Furthermore, the highest negative significant correlations existed between safety climate and working condition and job satisfaction. The perception of teamwork climate based on professional role was studied among labor and delivery staff nurses. The nurse perceived difficulties with the conflict resolution, ease in asking questions, and heeding nurse input (Sexton *et al.*, 2006). These factors are associated with breakdown in communication and teamwork, which has a deleterious impact on the patient safety. Job satisfaction and work conditions among nurses displayed relatively high values across nurses.

However, results obtained from the current study indicate that the importance of the managers' role is creating an organizational climate that promotes the emotional stability of nurses. Patient safety can be improved by promoting safety culture

in academic care settings where nurses are free of negative emotions (Judge & Bono, 2001).

There were some notable differences in scores between the two types of nursing positions. Registered nurses show statistically significant mean differences in perception of management dimension, in contrast to the mean difference, nurse managers have higher positive attitudes toward themselves. The other statistically significant finding was that nurse managers had the highest stress recognition scores than registered nurse. Higher stress recognition scores indicate more recognition of the effects of stress on the ability of a provider to perform optimally in delivering safe care. This is consistent with other research that reports different attitudes from leaders. While it can be helpful for leaders to have positive attitudes, it may be a problem if their attitudes reflect an unrealistic view of the practice or if their attitudes are markedly different from staff nurses (Modak *et al.*, 2007; Teng *et al.*, 2009). Moreover, the management in academic culture may be relatively non-hierarchical and democratic, by international comparison. It is also considered to be practiced with a collective orientation and reduced hierarchies. Consequently, the results of this current study may reflect a reality of better collaboration whereby good relationships are a fundamental and well-established part of the academic working life (El-Jardali *et al.*, 2010).

There is a clear consensus that transforming patient safety will require a substantial change in the culture of future clinicians. The institutions that educate and train future healthcare providers have a unique opportunity to establish a culture of patient safety. Setting such a culture will require a commitment from senior leadership, along with faculty and staff with the requisite knowledge and skills in patient safety and quality improvement (Griner, 2007; Sachdeva *et al.*, 2007).

### Conclusion and Recommendations

Enhancing safety culture in academic care settings will facilitate improvements in patient safety. Assessment of the workplace safety culture is the first step in identifying barriers that nurses face to provide safe patient care. Improving safety culture perceptions in academic settings can take considerable effort and resources. Leaders in academic facilities can use safety culture assessments to uphold and evaluate their patients and identify where critical needs exist. Moreover, to improve safety culture, nurse leaders must include interventions aimed at breaking down barriers between managers and nursing staff. They have the ability to cause change and are therefore in a unique position to help create a safety culture in an academic

setting where safety is valued. The present study offers academic setting managers an alternative means of improving safety culture through stabilizing nurse emotions. The results provide managers with insights into the potential benefits of improving support to nurses in terms of job content and context redesign.

Saudi Arabia will continue to experience diversity in its nursing workforce for the near future. Therefore, further qualitative research is recommended to focus on to what extent attitudes and values are sources of promoting safety culture. Replication of this study should be carried out with a health team in different regions and settings. Additionally, further exploration of this area could generate useful information about multiple disciplines, including health care, service management and social psychology.

Nurse Managers play a critical role in supporting safety culture, and effective leadership has shown to be important in creating a positive safety environment. Additionally, the concept of teamwork is a key in patient safety culture and is enhanced by clear leadership expectations between nurse managers and nursing staff (Hall *et al.*, 2006; Wagner *et al.*, 2009). Facilitating teamwork between staff members and managers is imperative. Interventions designed to improve safety culture in academic settings should be focused on the concerns of multinational staff nurses and the improvement of teamwork. Nurse leaders have the ability to foster an environment in which nursing staff feel comfortable with identifying safety issues.

### Limitations

The present study has limitations that must be acknowledged. Firstly, self-report questionnaires were used for data collection with research assumption of trustworthiness of the respondents. Lastly, the data are from one academic practice and may not be generalizable to other settings.

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## Aerobic Degradation of Paraffin and Olefin Synthetic Based Drilling Mud Base Fluids by Gulf of Guinea Sediments under Natural Environmental Conditions

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**Abstract:** Aerobic biodegradation of synthetic Paraffins and Olefins in the Gulf of Guinea sediments were monitored over a 120 day period in an indoor benthic chamber basin tests measuring 18 x 30 inches. At each 30 day interval, residual hydrocarbons were measured with gas chromatograph while microbial populations were quantified with the most probable plate number method (MPN). At the end of the 120 day monitoring period, the following % degradation rates were recorded for different hydrocarbon substrates; Linear Olefin (90%), Synthetic Paraffin (82%), and Internal Olefin (86%). The overall degradation sequence showed that the Olefins degraded faster than the Paraffins but both hydrocarbon substrates were readily biodegradable by the indigenous microbial flora of the Gulf of Guinea sediments. This study demonstrated that over 85% of the degradation of Synthetic Paraffins and Olefins on the surface of sediments were carried out by aerobic microorganisms.

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**Keywords:** Aerobic biodegradation, Synthetic Paraffins, Linear Olefins, Internal Olefins, Gulf of Guinea Sediments.

### Introduction:

Synthetic based fluids (SBF) are new class of fluids that are currently in use for drilling oil and gas wells. They include Linear Alpha Olefins, Internal Olefins, Synthetic Paraffins and Esters. These fluids are environment friendly and they provide lubricity, stability at high temperature and well bore stability (American Chemistry Council, 2006). In the present study, we dealt only with synthetic Paraffins and Olefins. Ester based synthetic fluids have already been dealt with in our previous studies.

Paraffins consists of a broad class of compounds that have the general formula  $C_nH_{2n+2}$  where "n" is the number of carbon atoms which are joined by single bonds. Paraffins can be categorised as normal meaning that they are linear, iso meaning that they are branched and cyclo meaning that they consists of ring structures (American chemical council, 2006). Olefins are similar to Paraffins but contain at least two fewer hydrogen atoms providing at least one double bond between adjacent carbon atoms. Olefins with one double bond have the general formula  $C_nH_{2n}$  (American Chemistry Council, 2006).

Aerobic biodegradation of Paraffin and Olefin synthetic base fluids (SBF) in the sediment is a major criterion in selecting appropriate base fluids that are suitable for drilling purposes (Gardline 1988, Neff *et al*, 2000). Ester based fluids are known to have higher biodegradation potential than the Paraffins and Olefins but their major disadvantage is that they are susceptible to calcium and acidic gas contamination as well as thermal limitations and as a result of this, some drillers do prefer synthetic paraffins and olefins

(West *et al*, 2009). Toxicity tests results carried out by the American chemistry Council (2006) showed that the Olefin and Paraffin SBF are not toxic to the water and sediment dwelling organisms and on the biodegradation potential of the synthetic Paraffins and Olefins, the council advanced that they are readily biodegradable both in aerobic and anaerobic environment. Other investigators have also advanced that the synthetic based fluids in oily cuttings are biodegradable under aerobic conditions (Kjeilen, 1997, Robert and Nguyen 2006, Okoro 2011). In addition to this, Cobby (2002) advanced that biodegradation rates of SBFs in the sediment can be influenced by factors like seabed temperature, fluid concentrations and loadings, the surface area of the cuttings and the sediment particle size.

A wide variety of aerobic hydrocarbon degrading microorganisms such as *Flavobacterium* sp., *Micrococcus* sp., *Alkaligenes* sp., *Corynebacterium* sp., *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium* sp. have been isolated from the Gulf of Guinea sediments (Okoro 2010<sub>a</sub>). In a related development, another investigation carried out by Okoro (2010<sub>b</sub>) revealed that aerobic microorganisms are very active in the Gulf of Guinea sediments up to a depth of 2-5cm with a total heterotrophic bacterial counts of  $3.20 \times 10^6$  cfu/g and  $2.20 \times 10^4$  cfu/g respectively. Other aerobic microorganisms implicated in organic carbon degradation in marine sediments by other researchers include; *Pseudomonas* sp. (Tagger *et al*, 1990), *Flavobacterium* sp. (Okpokwasili *et al*, 1984) and *Vibrio* sp. (West *et al*, 1984). Naturally,

biodegradation occurs more rapidly under aerobic conditions than in anaerobic conditions. It is also likely that aerobic biodegradation of SBF may deplete the oxygen in sediments making the sediment anoxic if the loading of the sediment with biodegradable organic matter from SBF cuttings is high and aeration of sediment is low (CSA, 2004), at this stage microaerophilic and anaerobic microorganisms might become relevant in the degradation of the residual SBF in the sediment.

In the present study, aerobic biodegradation potential of 3 synthetic Paraffin and Olefin based drilling fluid namely; SBF-LO (Linear olefins), SBF-SP (Synthetic paraffin) and SBF-IO (Internal olefins) were tested on Gulf of Guinea sediments under natural environmental conditions over a period of time.

## 2. Material and Methods:

### Experimental Design:

3 rectangular shaped glass containers measuring 18x30 inches (about 18 inches deep) were used for the experiment. Each of the glass container was filled with the wet sediment up to 12 inches depth followed by the introduction of 150mls of each of the representative SBF to the respective containers. The sediment/fluid mixture was mixed thoroughly by manual means using a metallic mixer. The experimental set up was allowed to settle for about 6hrs before the collection of the first sediment sample at day 0. The experiment was monitored for a 120 day period and at each 30-day interval, sediment samples were collected and analysed for residual hydrocarbon (SBF) and hydrocarbon utilizing bacteria.

### Description of the Synthetic-based fluids (SBF) used for the study.

The SBF samples which were collected from the Nigerian Department of Petroleum Resources (DPR) were coded and have the following descriptions. 1. SBF-LO (Linear Olefins), 2. SBF-SP (Synthetic Paraffin) and 3. SBF-IO (Internal Olefins).

### Microbiological and Physicochemical Analysis of the Sediment samples.

#### Enumeration of Total Heterotrophic Bacterial and Fungal Counts.

Heterotrophic bacteria and Fungi were enumerated by adopting the standard plate count technique using spread plate method. Appropriate dilution of samples were plated out on nutrient agar plates for Bacteria and potato dextrose agar plates (PDA) for Fungi. The plates for Bacteria were made in duplicates and incubated aerobically at 29°C for 24hrs while that of Fungi were incubated aerobically

for 3-4 days. 2µg/L of chloramphenicol was added to PDA plates to inhibit bacterial growth as described in Eaton *et al*, 1995.

#### Enumeration of Hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacterial counts were obtained by plating out at low dilutions  $10^{-1} - 10^{-3}$  of samples on mineral salt medium of Mills *et al* (1978). The composition of the medium in (g/L) is as follows NaCl (10), MgSO<sub>4</sub>.7H<sub>2</sub>O (0.42), KCl (0.29), KH<sub>2</sub>PO<sub>4</sub> (0.83), Na<sub>2</sub>HPO<sub>4</sub> (1.25), NaNO<sub>3</sub> (0.42), Agar bacteriological (15), distilled water (1000 ml), and pH (7.2). The medium was autoclaved at 1.1 kg/cm<sup>2</sup> for 15 mins. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light) and held in the lid of the petri dishes. The dishes were wrapped round with a masking tape so as to increase the vapour pressure within the petri dishes while the plates were incubated at 29°C for 6 days after which the growth of hydrocarbon degrading bacteria were observed and counted. For Fungal plates, 0.1g of Penicillin was added to 250 ml mineral salt medium to inhibit bacterial growth.

#### pH, Temperature measurement and Salinity

The pH of the sediment was measured with a portable water proof pH meter (Jenway, 3150, USA), Temperature was measured using portable thermometer (Hanana , H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described in (Eaton *et al*, 1995).

#### Estimation of Background Nutrient Concentration of the sediment

Interstitial water samples were withdrawn with a simple apparatus as described in McKee *et al*, 1998. The collected interstitial water was filtered and inorganic nutrients such as Phosphorus and Potassium were analysed with ICP (Inductively coupled argon plasma emission spectrometer) as described in Eaton *et al*, 1995). Amonium-Nitrogen was analysed with auto analyser as described in Eaton *et al*, 1995.

#### Detection of heavy metals:

Heavy metals were detected using the Atomic absorption Spectrophotometer (Perkin Elmer 5100PC, England) after sample preparation and digestion as previously described (Eaton *et al*, 1995).

#### Moisture content:

The moisture content of the sediment samples were measured by simple gravimetric analysis. 10grams of the sediment sample containing water was dried in the oven at a temperature of 200°C after

which, the sample was measured again and the difference in weight is the moisture content as previously described (Eaton *et al*, 1995).

#### **Solvent extraction of Residual Hydrocarbon**

One gram of the sample was introduced into a separating funnel containing 50mls of Methylene chloride, this was followed by vigorous shaking for 10mins and filtration using Watman no.1 filter paper as previously described (Eaton *et al*, 1995) and the filtrate was collected in a clean conical flask.

#### **Gas Chromatography of Hydrocarbon**

Degraded oil were analyzed by Gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1micron film thickness were used. A temperature program of 50-305<sup>o</sup>C increasing at 3.5<sup>o</sup>C per minute for 27.15 min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was 325<sup>o</sup>C while the injection port temperature was 305<sup>o</sup>C. 1 ml of the residual oil extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of 0.2  $\mu$ l was injected into the GC.

#### **Identification Microorganisms capable of utilizing SBF**

The growth and morphology of bacterial isolates in minimal salts medium and on nutrient agar plates were noted with regards to the following characteristics; form, pigmentation, texture, colour and elevation. Fungal cultures were stained with Methylene blue and observed under a microscope (x40) and each fungal culture was identified based on its morphological characteristics with the aid of an identification manual. Bacterial cultures were stained using grams staining procedure and proper identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

### **3. Results.**

#### **Microbiological and Physicochemical properties of the Gulf of Guinea Sediments**

The three sediment samples tested showed a total heterotrophic bacterial counts which ranged between 1.30 – 1.80 x 10<sup>6</sup> cfu/g. The hydrocarbon

utilizing bacterial population among the heterotrophs ranged between 0.031- 0.066 x 10<sup>6</sup> cfu/g. Heterotrophic Fungal and Yeast counts in the sediment ranged between 0.001 - 0.011 x 10<sup>6</sup> cfu/g while the hydrocarbon utilizing Fungal and Yeast species ranged between 0.00013 – 0.00080 x 10<sup>6</sup> cfu/g.

The concentration of total petroleum hydrocarbons in all the sediments were less than 10ppm on the average, this is an indication that the sediments used in the experiment were all pristine and have not undergone any form of petroleum hydrocarbon pollution in the past. The endogenous concentrations of nitrogen, potassium and phosphorus in the sediment was sufficient enough to sustain microbial growth and proliferation. The detailed results are shown in table 1.

#### **Aerobic degradation of synthetic Paraffins and Olefins in the sediment.**

Varieties of aerobic microorganisms present on the sediment surface degraded considerably the synthetic Paraffins and Olefins that were used to spike the sediment within the 120 day period the experiment lasted. After the 120 -day experimental period, Sample SBF-LO which is a linear olefin recorded 90% degradation, sample SBF-SP (a synthetic paraffin) recorded 82% degradation while sample SBF-IO (an internal olefin) recorded about 86% degradation. The detailed results are shown in Table 2.

#### **Population dynamics of hydrocarbon utilizing bacteria during biodegradation of SBFs in the sediment**

The population dynamics of hydrocarbon utilizing bacteria during biodegradation of the SBFs in the sediment showed relatively low concentrations of indigenous heterotrophs with the capability to utilize the SBFs as their sole carbon source at the beginning of the experiment at day 0 when the sediments were just spiked with the SBFs. Thereafter when the microorganisms started to utilize the SBFs, their population density gradually increased and peaked at day 60. However when considerable levels of the SBFs had been degraded after day 60, the population density started to decline and the downward trend was sustained till the termination of the experiment at day 120. The trend in population dynamics were similar in all the sediment samples tested as shown in table 3.



**Table 1. Microbiological and Physicochemical properties of Gulf of Guinea sediments**

		SBF-LO-SD	SBF-SP-SD	SBF-IO-SD
1	Total Heterotrophic Bacterial Counts (Cfu/g x 10 <sup>6</sup> )	1.30	1.80	1.40
2	Hydrocarbon utilizing bacterial counts (Cfu/g x 10 <sup>6</sup> )	0.031	0.066	0.051
3	Total Heterotrophic Fungi/Yeast Counts. (Cfu/g x 10 <sup>6</sup> )	0.001	0.011	0.0064
4	Hydrocarbon Utilizing Fungi/Yeasts. (Cfu/g x 10 <sup>6</sup> )	0.00013	0.00022	0.00086
5	Total Petroleum Hydrocarbon (TPH) (ppm)	9.20	8.60	8.40
6	pH	6.60	6.40	6.80
7	Temperature (°C)	23	25	24
8	Salinity (mg/g)	5260	5320	5540
9	Moisture content (%)	56	57	60
10	Phosphorus (mg/g)	116	121	105
11	Potassium (mg/g)	92	43	46
12	Ammonia-N (mg/g)	3.10	4.20	2.30
13	Heavy Metals detected (ppm)	Zn(0.62), Cr (0.001), Pb(0.020)	Cd(0.02), Pb(0.018)	Fe(0.022), Zn (0.004)

**SEDIMENT TYPES; 1. SBF-LO-SD, 2. SBF-SP-SD, 3. SBF-IO-SD****Table 2. Aerobic Degradation of Synthetic Paraffin and Olefin based drilling fluids in the Sediment (in ppm) after a time period of 120 days**

	SBF-LO	SBF-SP	SBF-IO
<b>DAY 0</b>	22,400	23,240	22,280
<b>DAY 30</b>	19,200	20,600	19,800
<b>DAY 60</b>	8,850	15,260	10,250
<b>DAY 90</b>	4,800	8,950	5,200
<b>DAY 120</b>	2,200	4,250	3,100
<b>% Degradation after 120 days</b>	<b>90</b>	<b>82</b>	<b>86</b>

**SBF-LO (Linear Olefin), SBF-SP (Synthetic Paraffin), SBF-IO (Internal Olefin)****Table 3. Population dynamics of Hydrocarbon utilizing bacteria during biodegradation of Synthetic based fluids in the sediments. (Bacterial Population x 10<sup>6</sup> cfu/g)**

SEDIMENT	DAY 0	DAY 30	DAY 60	DAY 90	DAY 120
SBF-LO-SD	0.026	0.420	1.180	0.620	0.056
SBF-SP-SD	0.010	0.230	1.210	0.640	0.260
SBF-IO-SD	0.053	0.380	1.680	1.060	0.610

**Aerobic microorganisms isolated from the Gulf of Guinea sediments with the capability to utilize the SBFs as their sole carbon source.**

Varieties of aerobic microorganisms that showed capability to utilize the SBFs in the sediment were isolated and identified from 3 different sediment

samples tested. In sediment sample SBF-LO-SD which was spiked with linear olefin, the predominant microbial flora were *Pseudomonas* sp., *Achromobacter* sp., *Aspergillus niger* and *Penicillium* sp., and the total percentage of indigenous heterotrophs that utilized the SBFs as their sole

carbon source was 2.38%. Sediment sample SBF-SP-SD which was spiked with synthetic paraffin was dominated by the *Alkaligenes* sp., *Pseudomonas* sp., *Flavobacterium* sp and *Aspergillus niger* with about 3.66% of the indigenous heterotrophic microbial population having the capability to utilize the SBFs as their sole carbon source. Sediment sample SBF-IO-SD which was spiked with internal olefin showed its predominant microbial flora as *Micrococcus* sp., *Pseudomonas* sp., *Penicillium* sp., and *Aspergillus niger*. 3.64% of the indigenous heterotrophic population possessed the capability to utilize the SBFs as their sole carbon source. The detailed results are shown in Table 4.

#### 4. Discussion:

Aerobic degradation of synthetic Linear Olefins, Paraffins and Internal Olefins by the indigenous microbial flora of Gulf of Guinea sediments were monitored over a period of 120 days. Incubation at hydrostatic pressure was not necessary

because previous research have shown that incubation at such deep offshore pressure had no effect on the hydrocarbon substrate degradation (Benka-Coker and Olumagin, 1995, Alan *et al*, 2006, Robert and Nguyen, 2006). The analytical data derived from the present study on the Gulf of Guinea sediments showed that they are populated with wide varieties of indigenous microbial populations that have the capability to utilise the SBF as their sole carbon and energy sources. The background nutrient composition of Gulf of Guinea sediments used in the present study also showed that the sediments have fairly good nutrient composition that can sustain microbial growth and proliferation.

Previous investigations have also shown that the Gulf of Guinea sediments which is mostly sandy in nature have considerable background nutrient composition and is populated with a wide variety of indigenous microbial flora with the capability to utilise the organic carbons in the sediment as their sole carbon and energy sources (Okoro, 2010<sub>a</sub>).

**Table 4. Microorganisms isolated from Gulf of Guinea Sediments with the capability to utilize the SBF**

	SBF-LO-SD	SBF-SP-SD	SBF-IO-SD
<b>DAY 0</b>	<i>Pseudomonas</i> sp., <i>Achromobacter</i> sp., <i>Acinetobacter</i> sp., <i>Alkaligenes</i> sp., <i>Flavobacterium</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i> sp., <i>Penicillium crysogenum</i>	<i>Acinetobacter lwoffii</i> , <i>Pseudomonas</i> sp., <i>Vibrio</i> sp., <i>Micrococcus</i> sp., <i>Alkaligenes</i> sp., <i>Flavobacterium</i> sp. <i>Bacillus</i> sp., <i>Aspergillus niger</i> <i>Aspergillus Fumigatus</i> , <i>Penicillium</i> sp.	<i>Micrococcus</i> sp., <i>Pseudomonas</i> sp., <i>Alkaligenes</i> sp. <i>Pseudomonas mallei</i> . <i>Penicillium</i> sp <i>Aspergillus fumigatus</i> <i>Rhizopus</i> sp. <i>Candida</i> sp.,
<b>DAY 30</b>	<i>Pseudomonas</i> sp. <i>Acinetobacter</i> sp., <i>Achromobacter</i> sp., <i>Flavobacterium</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i> sp.,	<i>Pseudomonas</i> sp, <i>Alkaligenes</i> sp., <i>Flavobacterium</i> sp., <i>Aspergillus niger</i> <i>Penicillium</i> sp	<i>Pseudomonas</i> sp. <i>Alkaligenes</i> sp., <i>Pseudomonas mallei</i> , <i>Micrococcus</i> sp. <i>Penicillium</i> sp., <i>Candida</i> sp. <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i>
<b>DAY 60</b>	<i>Pseudomonas</i> sp., <i>Achromobacter</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i> sp.,	<i>Pseudomonas</i> sp, <i>Alkaligenes</i> sp., <i>Flavobacterium</i> sp. <i>Aspergillus niger</i>	<i>Pseudomonas</i> sp. <i>Alkaligenes</i> sp., <i>Micrococcus</i> sp., <i>Penicillium</i> sp., <i>Aspergillus niger</i> <i>Aspergillus fumigatus</i>
<b>DAY 90</b>	<i>Pseudomonas</i> sp <i>Achromobacter</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i> sp.,	<i>Alkaligenes</i> sp., <i>Pseudomonas</i> sp., <i>Flavobacterium</i> sp <i>Aspergillus niger</i>	<i>Pseudomonas</i> sp. <i>Alkaligenes</i> sp., <i>Penicillium</i> sp., <i>Micrococcus</i> sp <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i>
<b>DAY 120</b>	<i>Pseudomonas</i> sp. <i>Achromobacter</i> sp., <i>Aspergillus niger</i> , <i>Penicillium</i> sp.,	<i>Alkaligenes</i> sp., <i>Pseudomonas</i> sp., <i>Flavobacterium</i> sp. <i>Aspergillus niger</i>	<i>Micrococcus</i> sp., <i>Pseudomonas</i> sp sp., <i>Penicillium</i> sp., <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i>
<b>% of Indigenous heterotrophs utilizing SBF</b>	<b>2.38%</b>	<b>3.66%</b>	<b>3.64%</b>

The American Chemistry Council (2006) have equally observed that sediment dwelling microorganisms are able to utilise Paraffin and Olefin

SBF as a source of nutrition and aerobic biodegradation of SBF in the sediment results in decrease in sediment oxygen concentration.

The sediments used in the present study had a total heterotrophic microbial population that ranged between  $1.30 - 1.80 \times 10^6$  cfu/g but only about 2.38-3.64% of the indigenous heterotrophic microbial population in the sediment had the capability to utilise the spiked SBFs as their sole carbon and energy sources. Robert and Nguyen demonstrated that the indigenous aerobic microbial populations of the Gulf of Mexico sediments ranged between  $1.0 \times 10^8 - 1.4 \times 10^9$  cfu/g but less than 10% of this had the capability to utilise the SBF as their sole carbon and energy source. In a similar study conducted by Okoro (2011) using Ester based fluids and the Gulf of Guinea sediments, about 0.92-3.30% of the indigenous heterotrophic microbial population in the sediments were able to utilise the Ester based SBF as their sole carbon and energy source. All these investigations points to the fact that in marine sediments, the heterotrophic microbial populations that have the capability to utilise hydrocarbons as their sole carbon and energy source are naturally present in small numbers but their numbers increases astronomically as the microorganisms starts acting on the exogenous hydrocarbons as their input increases.

We observed in the present study that 90% of the original Linear Olefin concentration that was used to spike the sediment degraded after 120 days. Synthetic paraffins recorded 82% degradation while Internal Olefins recorded 86% degradation within the same period. An evaluation of the overall degradation sequence showed that Linear Olefins and Internal Olefins degraded faster than Synthetic Paraffins. American Chemistry Council (2006) have also observed that Synthetic Paraffins degrade slowly and may persist in the environment for longer periods than the Olefin based SBF. A similar investigation by Okoro (2011) showed that Ester based fluids degraded faster than the Paraffin and Olefin based SBFs under the same experimental conditions. The assertion that the Ester based SBF degrade faster than the Paraffins and the Olefins have also been confirmed by other investigators such as Robert and Nguyen (2006) and OGD (2003).

Another important observation in the present study is the unique roles played by mixed microbial populations in the degradation of the SBFs. The predominant aerobic microbial flora that were associated with the degradation of the SBFs used in the present study in the Gulf of Guinea sediments were *Pseudomonas* sp., *Achromobacter* sp., *Alkaligenes* sp., *Flavobacterium* sp., and *Micrococcus* sp. among the bacterial species and *Penicillium* sp., *Aspergillus niger* and *Aspergillus fumigatus* among the fungal species. These microbial flora were very active in the utilisation of the SBFs from the initial period they were used to spike the

sediment up to the time the experiment was terminated. In a similar study conducted by Okoro (2011) using Ester based SBF and the Gulf of Guinea sediments, the predominant microbial flora that utilised the SBF in the sediment were found to be *Pseudomonas* sp., *Alkaligenes* sp., *Micrococcus* sp., and *Achromobacter* sp. among the bacterial species and *Aspergillus niger* and *Penicillium crysogenum* among the fungal species but in another related study conducted by Ben-Coker and Olumagin (1995) in the Gulf of Guinea sediments, the predominant microbial flora with the capability to utilise the SBF in the sediment were found to be *Alkaligenes* sp. and *Micrococcus* sp. among the bacterial species and *Penicillium* and *Cladosporium* species. among the fungal species. This study also demonstrated that about 6% of the total heterotrophic population possessed the ability to utilise the SBF in the sediment as their sole carbon and energy source. Khodja (2008) and Khoja *et al.*, (2010) have also demonstrated the roles of mixed microbial culture comprising of *Enterobacter* sp., *Citrobacter freundii*, *Erogenous pseudomonas*, *Staphylococcus guricularis*, *Bacillus thuringensis* and *Micrococcus varians* in the degradation of drilling fluids deposited on the sediment.

#### Conclusion:

The present study have clearly demonstrated that the Synthetic Paraffins and Olefins used in the present study are readily biodegradable by the aerobic indigenous microbial flora of the Gulf of Guinea sediments and considerable concentrations of the original SBF used to spike the sediments were removed from the sediments after a period of 120 days. The study also showed that the Olefins degraded relatively faster than the Paraffins. We can safely conclude from this study that over 85% of degradation of SBF in the sediments were carried out by aerobic microorganisms.

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## Impact of *Nigella Sativa* Supplementation on the Outcome of Systemic Inflammatory Response /Multiple Organ Dysfunction Syndromes in Aged Rats

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**Abstract:** Systemic inflammatory response syndrome (SIRS) is a poorly understood condition that may proceed to multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF) and death. Aged people are more susceptible to SIRS/MODS than adults with more morbidity, mortality and increased cost burden on health care systems. The present study was planned to investigate the effects of pretreatment with *Nigella Sativa* seeds on the prognosis of systemic inflammatory response/multiple organ dysfunction syndromes in aged rats. The present study was carried out on 42 aged male Wistar albino rats (23-25 months). Rats were allocated into three groups; Sham-operated group (C, n=10); Colonic ligation and puncture group (CLP, n=17) comprised of rats given distilled water (10 ml/Kg b.w./day) by gavage for 4 weeks and *Nigella Sativa*/Colonic ligation and puncture group (NS/CLP, n=15) comprised of rats given ground seeds of *Nigella Sativa* (1 g/Kg b.w./day) by gavage. for 4 weeks. Thereafter, CLP and NS/CLP rats underwent colonic ligation and puncture operation (CLP) and after 2 days, rats were examined for body weight, rectal temperature and ECG recording. Blood samples were withdrawn to estimate arterial blood gases, complete blood picture and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and adiponectin. Livers, kidneys and lungs were excised for histopathological study. Obtained results revealed that two days after colonic ligation and puncture operation, CLP and NS/CLP groups showed high death rate (35% and 20%, respectively), significant decrease in body weight, hyperthermia, hypoxemia, significant increase in serum levels of ALT and creatinine as well as significant decrease in serum adiponectin compared to C group. CLP group exhibited significantly higher *in vivo* heart rate, deeper Q wave, lymphopenia and elevated serum levels of AST compared to C group, while NS/CLP group exhibited significant decrease in hemoglobin content, packed cell volume and red blood cell count compared to C group. However, when compared to CLP group, NS/CLP rats showed significant decrease of Q wave voltage as well as leukocytosis. Histopathological examination of livers, kidneys and lungs from CLP group revealed leukocytic infiltration, cytoplasmic vacuolation and vascular congestion compared to C group. These changes were less extensive in NS/CLP group. In conclusion pretreatment with ground seeds of *Nigella sativa* in diet improved survival rate as well as some features of SIRS/MODS in aged rats.

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**Key words:** Multiple organ dysfunction syndrome (MODS), Systemic inflammatory response syndrome (SIRS), Colonic ligation and puncture (CLP), *Nigella Sativa* (NS). Intensive care unit (ICU).

### 1. Introduction

The concept of multiple organ failure and related abnormalities was first developed in the 1970s, when several reports appeared describing remote organ failure as a complication of severe sepsis (Baue, 1997). In the UK and USA, mortality rates due to multiple organ dysfunction syndrome (MODS) are currently comparable with and expected to exceed those from single-organ failure or unexpected cardiac arrest as a cause of death in the ICU (Brown *et al.*, 2006 and Mayr *et al.*, 2007).

Systemic inflammatory response syndrome (SIRS) and eventually MODS are characterized by

increased oxygen consumption, hyperglycemia, accelerated protein catabolism and unrecognized perfusion deficits (Beal and Cerra, 1994). It has been reported that five of six patients who develop SIRS for more than 30 days proceed to severe MODS, and three of them die and it has been suggested that early recovery from SIRS might arrest the progression of organ dysfunction (Yoshio *et al.*, 1997).

Health benefits of *Nigella Sativa* seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) have been known for centuries in folk medicine for treatment of hypertension, bronchial

asthma and asthmatic bronchitis (**Randhawa and Ghamdi, 2002**). Scientific research reported anti-inflammatory, analgesic, antipyretic (**Abd-El-Fattah et al., 2000**), antineoplastic effect of these seeds (**Badary and Gamal El-Din, 2001**), antimicrobial (**Morsi, 2000**) as well as hypolipidemic and hypoglycemic effects (**Benhaddou-Andalousi et al., 2010**). Also the seed has cytoprotective and antioxidant actions (**Kruk et al., 2000**), in addition to their effect on some mediators of inflammation (**Alkharfy et al., 2011**)

The present study was designed to investigate the effect of pretreatment of aged rats with *Nigella Sativa* ground seeds on the development of SIRS and progression to MODS.

## 2. Materials and Methods

### Experimental animals

This study was carried out on 42 aged male Wistar albino rats (23- 25 months) weighing 370-410 g at the start of the experiment. Rats were purchased from Ophthalmic Diseases Research Institute (Giza) and housed 3/cage in plastic cages with food and water available *ad libitum* and were maintained in Physiology Department Animal House, Faculty of Medicine, Ain Shams University under standard conditions of boarding at room temperature.

Rats were randomly allocated into the following three groups:-

**Sham operated group C** (n=10); Comprised of rats fed ordinary rat chow and after 4 weeks were subjected to all the steps of colonic ligation and puncture operation but the cecum was neither ligated nor punctured.

**Colonic ligation and puncture group CLP** (n=17); Comprised of rats that were given distilled water (10ml/kg b.w./day ) by gavage for 4 weeks. Then subjected to colonic ligation and puncture operation.

***Nigella sativa*/ colonic ligation and puncture group NS/CLP** (n=15); Comprised of rats given *Nigella sativa* ground seeds in oral suspension in a dose of (10 ml / Kg b.w./day) for 4 weeks by gavage ,then subjected to colonic ligation and puncture operation.

*Nigella sativa* oral suspension was prepared by grinding 10 gm of *Nigella Sativa* seeds and freshly adding 100 ml distilled water to get a final concentration of 100 mg of *Nigella Sativa* ground seeds / ml distilled water. The suspension was mixed thoroughly and given by gavage (10 ml/Kg b.w) to achieve daily intake of *Nigella sativa* (1 g/Kg b.w.) modified from **Al-Hariri et al. (2009)**.

### Experimental procedure

After 4 weeks, CLP and NS/CLP rats were subjected to induction of sepsis by cecal ligation and puncture operation. C rats were subjected to all the

steps of colonic ligation and puncture operation except for ligation and puncture of the colon.

### Colonic ligation and puncture operation (CLP):

Rats were weighed to estimate their preoperative body weight then anesthetized using diethyl ether (ADWIC). The rat was fixed on the dissecting table. A midline abdominal incision was made; the cecum was exteriorized and ligated by 3.0 silk ligature at its base without obstructing intestinal continuity. The cecum was punctured twice with an 18 gauge needle, squeezed gently to extrude a small amount of feces from the perforate site. The cecum was returned to the peritoneal cavity and the abdominal incision was closed with 3.0 running silk suture (**Zingarelli et al., 2003**). Fluid resuscitation of the animal was performed by flushing 0.5 ml sterile saline solution into the peritoneal cavity before closure of the abdominal wall (**Zantl et al., 1998**).

After 2 days, all rats were fasted overnight; weighed to estimate postoperative body weight and rectal temperature was measured using medical thermometer. Rats were anesthetized by thiopental sodium (40mg/kg .i.p). ECG was recorded, then a midline abdominal incision was made to expose the abdominal aorta and blood samples were collected as follows

1. 0.5 ml blood in heparinized plastic syringe for determination of blood gases
2. 2 ml blood in plastic whole blood with spray- coated K<sub>2</sub> EDTA tube for complete blood picture (CBC).
3. 1 ml blood in clean plastic tubes, centrifuged at 4000 r.p.m. for 15 minutes to separate serum then stored at - 80° C for later biochemical study.

### 1-ECG recording:

Needle electrodes were placed beneath the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph (Cardimax FX 121, Fukuda Denshi Co, LTD). The electrocardiographic tracing was recorded from lead II at paper speed of 25 mm/sec, heart rate, P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following equation:

$$HR = \frac{7500}{\text{Distance in mm between 6 successive peaks of R waves}}$$

### 2-Biochemical measurements:

Biochemical measurement of blood gases, CBC and serum levels of ALT, AST and creatinine were done in Alfa Laboratories, Cairo, Egypt. Blood gases were analyzed by ABL 5 blood gas analyzer (Diamond Diagnostics). CBC was analysed by Cell Dyn 1700

hematology analyzer (Abott Diagnostics). Serum creatinine was measured using Creatinine reagent OSR6178 kinetic color test (Jaffé method) for the quantitative determination of creatinine on Olympus analyzers according to **Mazzachi et al. (2000)**. Serum alanine aminotransferase (ALT) was measured using OSR6007 kinetic UV test for the quantitative determination of alanine aminotransferase on Olympus analyzers according to **Thomas (1998)**. Serum aspartate aminotransferase (AST) was measured using OSR6009 kinetic UV test for the quantitative determination of alanine aminotransferase on Olympus analyzers according to **Thomas (1998)**. Serum adiponectin was estimated using Alpco ELISA kit for rat adiponectin (ALPCO Diagnostics) according to the method described by **Shimada et al. (2004)**.

Lungs, kidneys and livers were excised and kept in 10% formaline for histopathological examinations, dehydrated, cleared in zylol and embedded in parablaxt. Paraffin sections were cut serially at 6  $\mu\text{m}$  thickness and stained by Hematoxylin and Eosin (Hx & E) as described by **Drury and Wallington, (1980)**.

#### Statistical Analysis:

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1 according to **Armitage and Berry (1987)**. Statistical significance was determined by one-way ANOVA for differences between means of different groups; further analysis was made by LSD (least significance difference) to find intergroupal differences. A probability of  $P < 0.05$  was considered significant. Correlations and Lines of Regression were calculated by linear regression analysis (ranking data directly or indirectly) using the Least Square Method. A probability of  $P < 0.05$  (2-tailed). All data were expressed as mean  $\pm$ SEM.

### 3. Results

Postoperatively, CLP and NS/CLP groups exhibited death rates 35% and 20% respectively.

Preoperative body weights were comparable between the three studied groups. However postoperative body weights were significantly ( $P < 0.05$ ) decreased in CLP and NS/CLP groups compared to C group. Body temperature was significantly ( $P < 0.05$ ) increased in CLP and NS/CLP groups compared to C group (Table 1).

ECG recording revealed significant ( $P < 0.05$ ) increase of *in vivo* heart rate in CLP group compared to C group and. Q wave was significantly ( $P < 0.05$ ) deeper in CLP group compared to C group but showed significantly ( $P < 0.05$ ) decreased depth in NS/CLP group compared to CLP group (Table 2; Fig. 1).

Blood picture revealed that CLP group had significant ( $P < 0.05$ ) lymphopenia compared to C group. NS/CLP group showed significant ( $P < 0.05$ ) decrease in blood hemoglobin content, packed cell volume and red blood cell count compared to C group and showed significant leukocytosis compared to CLP group (Table 3).

Arterial blood gas analysis revealed significant ( $P < 0.05$ ) hypoxemia in both CLP and NS/CLP groups compared to C group. Serum levels of ALT and AST increased significantly ( $P < 0.05$ ) in CLP group compared to C group, while in NS/CLP, only ALT increased significantly ( $P < 0.05$ ) compared to C group. Serum creatinine increased significantly ( $P < 0.05$ ) in CLP and NS/CLP compared to C group. Serum adiponectin showed significant ( $P < 0.05$ ) decrease in CLP and NS/CLP groups compared to C group (Table 4).

Correlation study of serum adiponectin versus other parameters in CLP and NS/CLP groups showed that in CLP group, serum adiponectin correlated negatively and significantly with body temperature, *in vivo* heart rate, Q wave voltage and serum levels of ALT and creatinine and correlated positively and significantly with arterial  $\text{Po}_2$  and blood hemoglobin content. In NS/CLP group, the significant negative correlations between serum adiponectin and body temperature and serum levels of ALT and creatinine persisted while its correlations with *in vivo* heart rate, Q wave voltage and arterial  $\text{Po}_2$  became insignificant (table 5).

#### Histopathological examination:

Microscopic examination of the livers of sham operated rats showed normal architecture of hepatic lobules with each lobule formed of radially arranged cords of hepatic cells extending from the central vein towards the periphery of the lobule separated by the blood sinusoids. The central vein was lined by flat endothelial cells, blood sinusoids were lined by flat endothelial and Von Kupffer cells. The hepatocytes were polygonal in shape with acidophilic cytoplasm and rounded vesicular nuclei with prominent nucleoli. Portal tracts were seen around the periphery of the lobule, each tract contained branches of hepatic artery, bile duct and portal vein and surrounded by connective tissue. On the other hand, microscopic examination of livers of CLP group revealed loss of hepatic architecture in the form of marked disruption of the cords of hepatocytes and poorly defined portal tracts. Hepatocytes especially at the periphery of the hepatic lobules exhibited signs of degeneration in the form of marked cytoplasmic vacuolation which was extensive in some areas giving a ballooning appearance of the hepatocytes. Prominent cellular infiltration was also observed between degenerated hepatocytes as well as marked congestion of the portal vein branches. Fibrous deposition increased around central veins and

portal tracts. Livers of NS/CLP rats retained almost normal hepatic architecture with less vacuolation of the cytoplasm of the hepatocytes and scanty fibrous deposition around central veins. The central vein and the branches of portal vein appeared normal. No signs of hepatocyte degeneration were observed (Figs.2-a,b,c).

Microscopic examination of the respiratory portion of the lungs of sham operated group showed thin alveolar septa lined with continuous simple squamous epithelium with dark flat nuclei and thin cytoplasm. The bronchioles were lined with normal simple ciliated columnar epithelium and its lumen appeared clear from any cellular debris. On the other hand, lungs from CLP group showed increased thickness of the alveolar septa with marked cellular infiltration, narrowing of alveolar spaces, desquamated cells with vacuolated cytoplasm in the lumen of some bronchioles, congested blood capillaries as well as aggregates of cellular infiltration and pink hyaline material in some areas. Lungs from NS/CLP group exhibited almost normal microscopic picture of the lung with thin alveolar septa and less

cellular infiltration, wide alveolar spaces, less congested blood vessels and less desquamated cells in the bronchiolar lumen (Figs.3-a,b,c).

Microscopic examination of the kidneys from sham operated group showed normal appearance of renal corpuscles, proximal and distal convoluted tubules surrounded by sparse interstitial tissue. Each renal corpuscle was formed of a central tuft of anastomosing glomerular capillaries surrounded by Bowman's capsule with no cellular debris in Bowman's space. The proximal convoluted tubules were packed with large cuboidal cells with large spherical basal nuclei and acidophilic cytoplasm with no obvious demarcation between the cells. On the other hand, kidneys from CLP group showed cellular infiltration in some renal corpuscles, narrowed Bowman's spaces and dilated renal tubules as well as tubular cell degeneration in the form of vacuolated cytoplasm. In the NS/CLP group, renal corpuscles appeared almost normal with distinct glomerular capillaries and Bowman's capsule with no degenerative changes in tubular cells (Figs.4-a,b,c).

Table (1): Changes in preoperative body weight (BW<sub>1</sub>, g), postoperative body weight (BW<sub>2</sub>, g) and rectal temperature (Temp., ° C) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/colonic ligation and puncture (NS/CLP) groups.

Groups	BW <sub>1</sub> (g)	BW <sub>2</sub> (g)	Temp.( ° C)
C(n=10)	390±3.9	390±3.9	37.6±0.6
CLP(n=10)	389±4.9	372±5 <sup>a</sup>	38.3±0.1 <sup>a</sup>
NS/CLP(n=10)	381±3.8	361±4.7 <sup>a</sup>	38.5±0.2 <sup>a</sup>
<i>P</i>	<i>NS</i>	<0.001	<0.001

*a*:significance by LSD at significance level  $P < 0.05$  from C group.

*b*:significance by LSD at significance level  $P < 0.05$  from CLP group.

*P*: significance by one way ANOVA among the three studied groups.

*NS* not significant

In parenthesis is the number of rats.

Table (2): Changes in Heart rate (HR, beats/min.), PR interval (msec.), QRS duration (msec.), QT interval (msec.), Q wave (μv), R wave (μv), and ST segment elevation (μv) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/colonic ligation and puncture (NS/CLP) groups.

Groups	HR (beats/ min.)	PR (msec.)	QRS (msec.)	QT (msec.)	Q (μv)	R (μv)	ST (μv)
C (n=10)	426±11.4	52±3.2	46±3.7	92±6.1	55±5	625±61.5	130±25.7
CLP(n=10)	490±24 <sup>a</sup>	51±2.7	51±3.1	96±4.9	95±8.9 <sup>a</sup>	495±70.4	95±11.6
NS/CLP(n=10)	474±19.1	52±4.4	45±3.7	90±6.8	65±7.6 <sup>b</sup>	645±66.8	105±11.0
<i>P</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<i>NS</i>	<0.01	<i>NS</i>	<i>NS</i>

*a*:significance by LSD at significance level  $P < 0.05$  from C group.

*b*:significance by LSD at significance level  $P < 0.05$  from CLP group.

*P*: significance by one way ANOVA among the three studied groups.

*NS* not significant

In parenthesis is the number of rats.



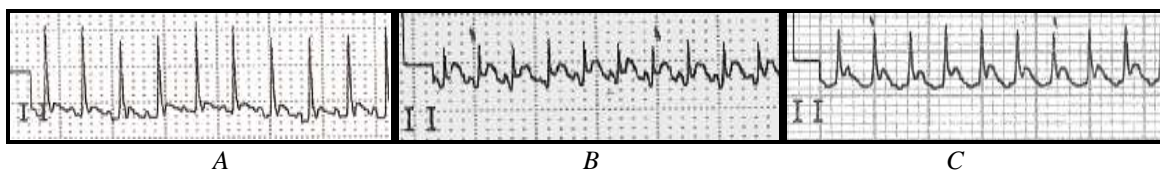


Figure (1): ECG tracing of Sham operated control group (C), panel (A); Colonic ligation and puncture group (CLP), panel (B) and *Nigella Sativa*/ colonic ligation and puncture group (NS/CLP), panel (C).

Table (3): changes in hemoglobin content (Hb, g/dl), Packed cell value (PCV, %), red blood cell count (RBC,  $10^6/\text{mm}^3$ ), white blood cells (WBC,  $10^3/\text{mm}^3$ ), Neutrophil (N,%) and lymphocyte (L, %) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

Groups	Hb (g/dl)	PCV (%)	RBC ( $10^6/\text{mm}^3$ )	WBC ( $10^3/\text{mm}^3$ )	N (%)	L (%)
C(n=10)	13.6±0.1	39.9±0.4	7.1±0.1	9.1±1.2	30.1±6.2	62.7±6.7
CLP(n=10)	12.4±0.1	36.7±0.6	6.8±0.1	6.8 ±1.2	42.8±4.9	45.6±4.4 <sup>a</sup>
NS/CLP(n=10)	11.8±0.9 <sup>a</sup>	34.9±2.6 <sup>a</sup>	6.23±0.4 <sup>a</sup>	11.1±1.4 <sup>b</sup>	43.4±4.5	50±4.6
<i>P</i>	NS	NS	NS	NS	NS	NS

*a*:significance by LSD at significance level  $P < 0.05$  from C group.

*b*:significance by LSD at significance level  $P < 0.05$  from CLP group.

*P*: significance by one way ANOVA among the three studied groups.

NS not significant

In parenthesis is the number of rats.

Table(4):Changes in arterial blood gases ( $\text{Po}_2$ ,  $\text{PCo}_2$ , mmHg). Serum levels of alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST,U/L), creatinine (Cr., mg/dl) and adiponectin (ng/ml) in Sham operated control (C), Colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

Groups	$\text{Po}_2$ , (mmHg)	$\text{PCo}_2$ (mmHg)	ALT (U/L)	AST (U/L)	Cr. (mg/dl)	Adp. (ng/ml)
C(n=10)	110±8.3	33.2±4.6	44.3±1.5	223.3±13.9	0.5±0.01	1.06±0.05
CLP(n=10)	82.7±6.6 <sup>a</sup>	34.4±1.9	84.5±6.1 <sup>a</sup>	291.4±24 <sup>a</sup>	0.6±0.0 <sup>a</sup>	0.2±0.04 <sup>a</sup>
NS/CLP(n=10)	82.7±9.9 <sup>a</sup>	33.6±2.1	71.6±5.4 <sup>a</sup>	282.4±25	0.6±0.1 <sup>a</sup>	0.4±0.02 <sup>a</sup>
<i>P</i>	<0.05	NS	<0.001	NS	<0.001	<0.001

*a*:significance by LSD at significance level  $P < 0.05$  from C group.

*b*:significance by LSD at significance level  $P < 0.05$  from CLP group.

*P*: significance by one way ANOVA among the three studied groups.

NS not significant

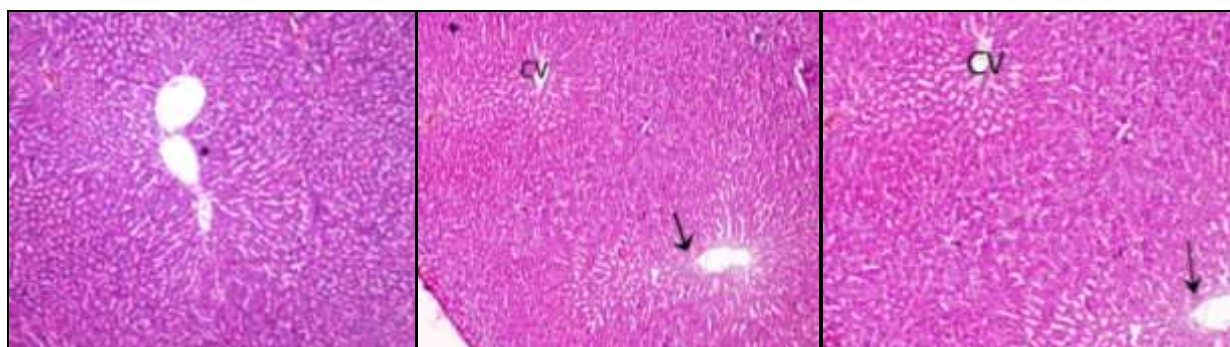
In parenthesis is the number of rats.

Table(5):Correlations of serum adiponectin with rectal temperature, heart rate, Q wave voltage,  $\text{Po}_2$ , Hemoglobin content (Hb), white blood cells (WBC), alanine aminotransferase (ALT) and creatinine (Cr.) in colonic ligation and puncture (CLP) and *Nigella Sativa*/ colonic ligation and puncture (NS/CLP) groups.

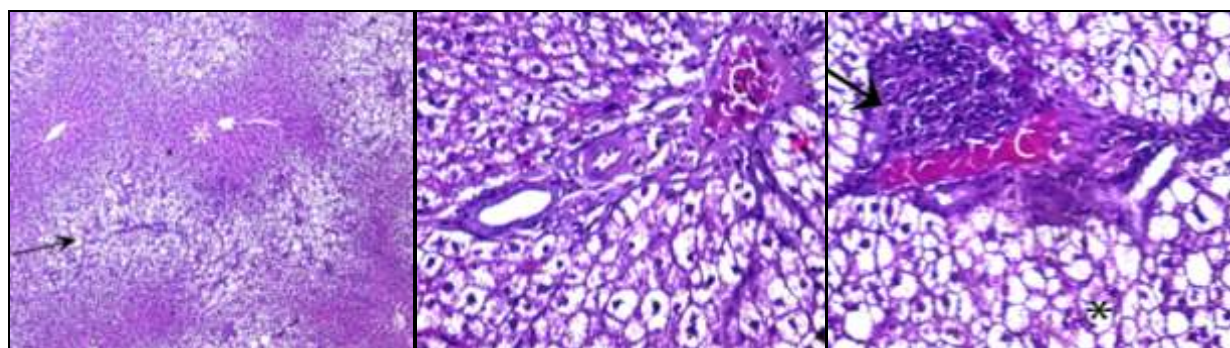
Groups	Temp.	HR	Q	$\text{Po}_2$	Hb	WBC	ALT	Cr	
CLP	<i>r</i>	-0.73	-0.48	-0.69	0.44	0.66	0.19	-0.82	-0.57
	<i>p</i>	<0.001	<0.05	<0.01	<0.05	<0.01	NS	0.001	<0.05
NS/CLP	<i>r</i>	-0.7	-0.28	0.32	0.39	0.447	-0.36	-0.7	-0.58
	<i>p</i>	<0.01	NS	NS	NS	<0.05	NS	<0.01	<0.01

*r*: correlation coefficient

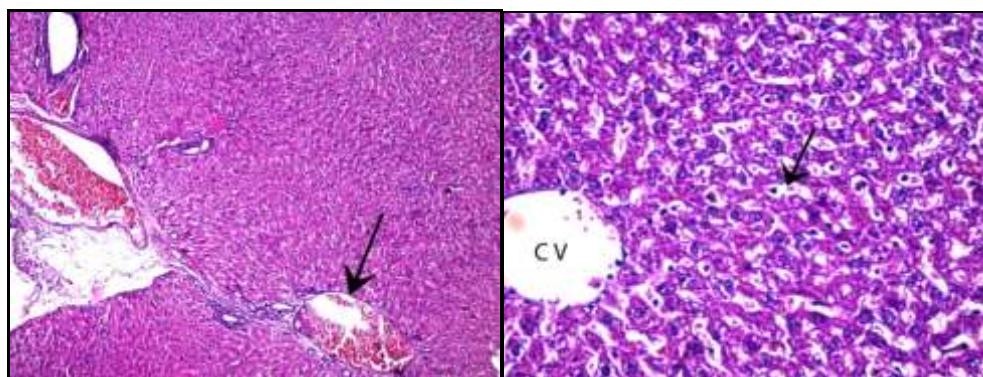
*P*: significance at the 0.05 level



**A** (Hx &E . x200, x100, x 200)  
CV: central vein, Arrow: portal tract.

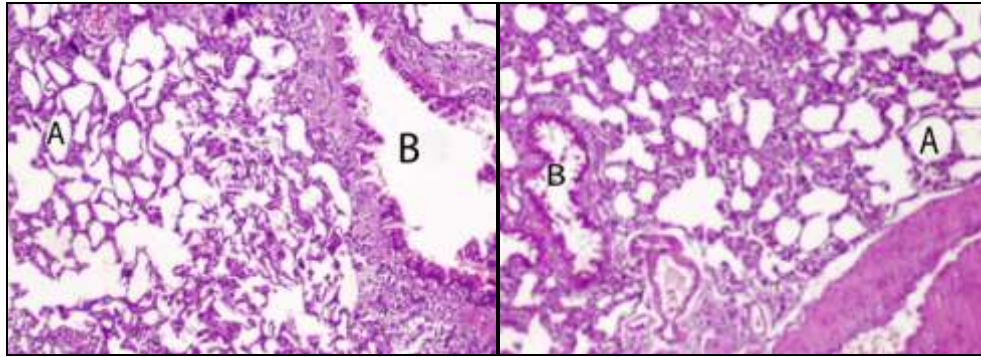


**B** (Hx &E . x100, x400 ,x 400)  
C: congested portal vein, \*: areas of vacuolations., Arrow: mononuclear infiltration.

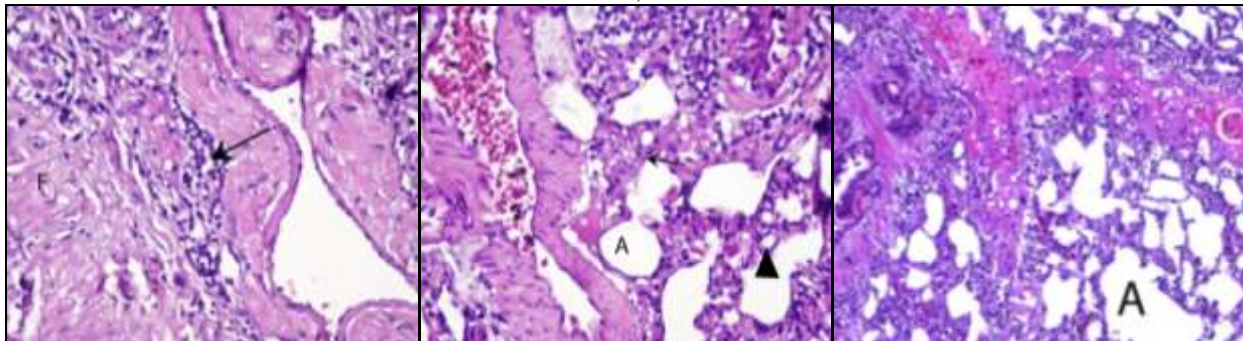


**C** (Hx &E . x100, x 400)  
Arrow: portal tract, CV: central vein.

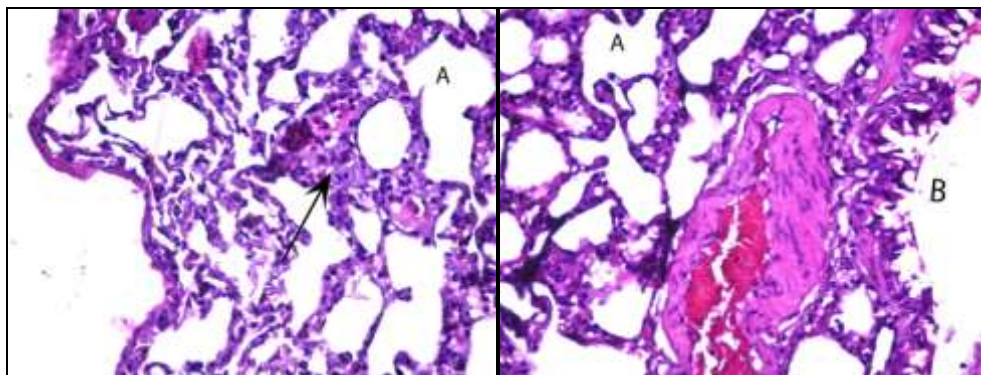
Figure .(2) (A) sham operated group(C) showing normal liver architecture; (B) colonic puncture and ligation group (CLP) showing disrupted liver architecture, poorly defined portal tracts, degenerated hepatocytes, cellular infiltration and congested blood vessels; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal hepatic architecture.



**A** (Hx &E . x100 x 100)  
**B:** bronchiole, **A:** alveoli



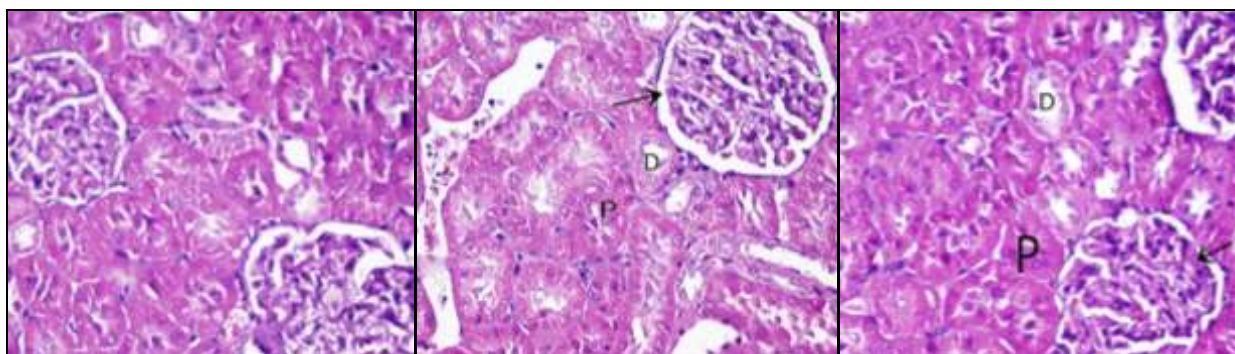
**B** (Hx &E . x400, x400, x 100)  
 Arrow: mononuclear infiltration, Arrow Head: vacuolations, **A:** alveoli.



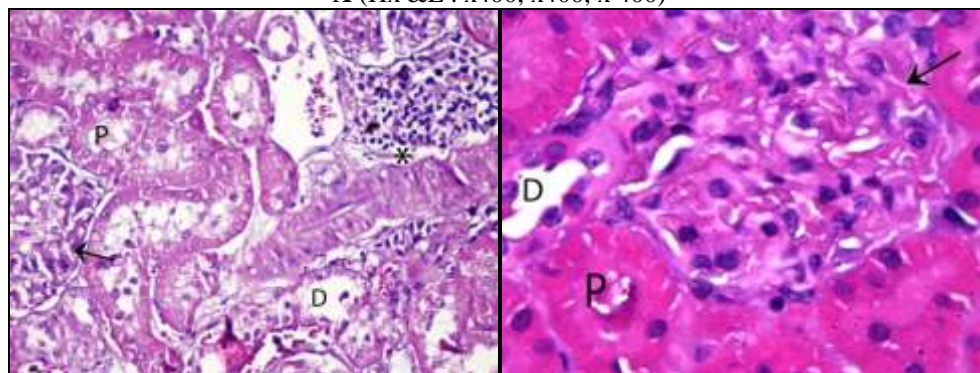
**C** (Hx &E . x400, x400)

Arrow: mononuclear infiltration, **A:** alveoli, **B:** bronchiole.

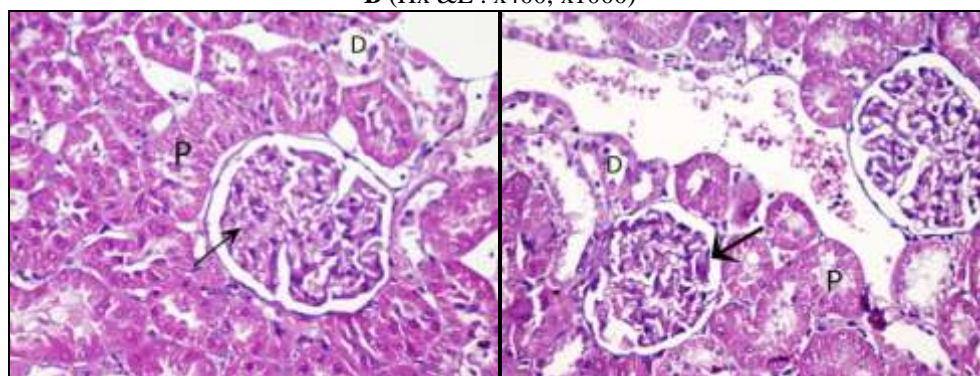
Figure (3) (A) sham operated group(C) showing normal appearance of the lung; (B) colonic puncture and ligation group(CLP) showing thick alveolar septa with cellular infiltration, narrowing of alveolar spaces, desquamated cells with vacuolated cytoplasm in the bronchioles and congested capillaries; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal appearance of lung tissue.



A (Hx &amp;E . x400, x400, x 400)



B (Hx &amp;E . x400, x1000)



C (Hx &amp;E . x400, x400)

Arrow: Glomeruli, P: proximal convoluted tubules, D: distal convoluted tubules, \*: mononuclear infiltration.

Figure.(4) (A) sham operated group (C) showing normal appearance of renal corpuscles and tubules;(B) colonic ligation and puncture group(CLP) showing cellular infiltration of renal corpuscles, widening of Bowman's spaces, dilated renal tubules with degenerative changes in tubular cells; (C) *Nigella Sativa*/colonic ligation and puncture group (NS/CLP) showing almost normal appearance of renal corpuscles and tubules.

#### 4. Discussion

The concept of systemic inflammatory response syndrome (SIRS) was established in 1992, it describes a hyperinflammatory state represented by elevated levels of proinflammatory mediators with development of multiple organ dysfunction (MOD), multiple organ failure (MOF) and finally death (**Bone et al., 1992**). Various therapeutic approaches were tried in experimental models of SIRS/MODS with

variable success (**Zingarelli et al. 2003; Barichello et al., 2007 and Park et al., 2011**). However, when these approaches were tried in human cases of SIRS/MODS, the results were disappointing partly due to the poorly understood pathology of this syndrome and partly due to the mismatching between animal models and human patients. One aspect of this mismatching is that most humans with sepsis and SIRS are above 50 years (**Martin et al., 2003 and Derek et al., 2001**), while animals used in

experimental studies were of young age. It had been reported that SIRS is a preventable condition and that early intervention in septic patients with supportive non specific measures could be beneficial (**Rivers et al., 2001**).

In the present study, we chose CLP model of sepsis in aged rats as a surrogate of human sepsis, SIRS and MODS as previously described by **Overhaus et al.(2004)** and **Rittirsch et al.(2007)**. Forty eight hours after the CLP operation, all rats showed wound sepsis and adhesions between the perforated colon and intestinal loops. Survival rate was 65 % for CLP group and 80 % for NS/CLP group which was consistent with the findings of **Zingarelli et al. (2003)**. CLP and NS/CLP rats showed hyperthermia compared to sham operated rats which agree with the findings of **Zingarelli et al. (2003)** and **Rittirsch et al. (2007)** as well as leukocytic infiltration of remote organs which denotes a systemic inflammatory reaction rather than discrete organ affection. Manifestations of multiple organ dysfunctions were also observed in the form of tachycardia, low arterial  $PO_2$ , elevated serum levels of ALT and creatinine. The significant increase in body core temperature with sepsis can be explained by release of the endogenous pyrogen (IL-1) from blood leukocytes, tissue macrophages and large granular killer lymphocytes (**Guyton and Hall, 2006**). CLP rats showed insignificant change in their total leukocytic count from control values with observable decrease in lymphocytes. However, their vital organs (livers, kidneys and lungs) were heavily infiltrated with leukocytes which indicate exaggerated extravasation of white blood cells from unhealthy blood capillaries possibly due to accumulation of chemotactic mediators in these organs (**Neumann et al., 1999**). On the other hand, the significantly higher leucocytic count in NS/CLP rats compared to CLP rats with less organ infiltration denotes potent immune response with less inflammatory reaction in the vital organs which might reflect restoration of the normal balance between immunity and inflammation, the disruption of which had been claimed to be the underlying pathology of this syndrome (**Butt and Shrestha, 2008**). The significant decrease of hemoglobin content in NS/CLP group compared to normal control rats could be the result of excessive nutrient utilization by stimulated leukopoietic cells in the bone marrow, thus limiting the rate of erythropoiesis. Although both CLP and NS/CLP rats showed evidence of multiple organ dysfunctions, yet cardiac dysfunction was more evident in CLP rats. This can be deduced from the observation that although CLP and NS/CLP rats exhibited significant comparable body temperature, yet *in vivo* heart rate increased significantly only in CLP group compared

to C group which was consistent with the findings of **Zingarelli et al. (2003)**. Moreover, Q wave was significantly deeper in CLP rats compared to C rats which might indicate myocarditis (**Goldberger, 2006**). The significant drop in arterial  $PO_2$  in both CLP and NS/CLP groups with no significant change in arterial  $P_{CO_2}$  indicates decreased diffusing capacity of alveolocapillary membrane, thus interfering with  $O_2$  diffusion rather than  $CO_2$  (**Barrett et al., 2009**). Lack of significant difference in liver and kidney functions between NS/CLP and CLP rats despite regression of most of the inflammatory signs by microscopic examination of these organs in NS/CLP rats might be due to the short time interval between induction of sepsis and sacrifice of rats.

Up till now, pathogenesis of SIRS/MODS is not well understood but several clinical observations reported that a wide variety of systemic insults including surgery, trauma, burns or severe infection prime the host immune system with subsequent overwhelming reaction of the innate immune system (**Butt and Shrestha, 2008**). Primed polymorphnuclear neutrophils, macrpahages and monocytes exhibit generation of reactive oxygen and nitrogen species (ROS and RNS), degranulation of enzymes, expression of cytokine and delayed apoptosis (**Schaeffer et al., 2007**). Also, proinflammatory cascades (.e.g. the complement cascade) become activated with appearance of various mediators like  $TNF-\alpha$ , IL-1, IL-6, C5a, (**Cavillon et al., 2003**). This inflammatory milieu in different tissues will recruit more leukocytes with release of too much proinflammatory mediators and uncontrolled inflammatory response resulting in intavascular coagulation, tissue hypoxia, cellular damage, consumptive depletion of the clotting system, excessive release of anti-inflammatory mediators (**Bone, 1996**), immunosuppression, anergy and SIRS/MODS (**Rittirsch et al., 2007**). Nevertheless, a question remains to be answered about the triggering event that shifts the normal immune response into widespread uncontrolled inflammation of vital organs with subsequent failure and death. **Butt and Shrestha, (2008)** proposed the two hit hypothesis to explain the SIRS/ MODS which postulated that a severe insult like trauma or infection prime the host immune system so that a subsequent trivial insult produces a markedly exaggerated immune response of the host leading to MODS and death. Signs of disturbed metabolism in the form of increased levels of gluconeogenic hormones, diversion of skeletal muscle protein to splanchnic area, altered transport of glucose, and insulin resistance are serious sequelae of uncontrolled release of inflammatory mediators which would add more

risk to those patients with sepsis making them more susceptible to mortality (**Michie, 1996**).

Insight into this interwoven pathogenesis of SIRS / MODS requires that the effective therapy should have the potency of interrupting all these vicious circuits to restore the normal balance between inflammation and immunity. *Nigella Sativa* oil was reported to inhibit 5-lipoxygenase products in polymorphonuclear leukocytes of rats (**El-Dakhakhny et al., 2002**) as well as endothelin -1 production and oxidative stress induced by sepsis in rats (**Alici et al., 2011**). Thymoquinone- a constituent of *Nigella Sativa* was also reported to decrease inflammatory markers in septic rats like IL-1, IL-10, TNF and IL-2 (**Alkharfy et al., 2011**), to normalize liver GSH and to decrease liver MDA and caspase-3 activity and serum levels of TNF-alpha and total bilirubin (**Helal, 2010**). The observation that *Nigella sativa* -pretreated rats could retain almost normal microscopic picture of their lungs, livers and kidneys during sepsis was consistent with the findings of **Helal (2010)** and further confirmed the previous reports of the antiinflammatory, antioxidant protective properties of *Nigella Sativa* seeds. Our study demonstrated that all rats with sepsis (CLP and NS/CLP groups) had significant decrease in serum adiponectin compared to sham operated rats which was consistent with the findings of **Lago et al. (2007)** and although serum adiponectin showed substantial increase in NS/CLP group, yet it was not significantly different from CLP group. Hepatic, cardiac and renal protection conferred by adiponectin can be deduced from the significant inverse relationship between it and body temperature, liver enzymes and creatinine in both CLP and NS/CLP groups and came in accordance with the results of **Kondo et al. (2010; Wang et al. (2010) ; Hamed et al. (2011) and Latif et al. (2011)**. Whether or not using a higher dose of *Nigella Sativa* for a longer duration would elevate serum adiponectin level towards normal values thus adding more protection during sepsis is a matter of debate that needs further investigation to be clarified.

Results of the present study demonstrated that dietary supplementation of aged rats with ground *Nigella Sativa* seeds improved their survival on exposure to SIRS/MODS that complicated a septic insult. *Nigella sativa* -pretreated rats showed amelioration of inflammatory changes in vital organs (liver, kidney and lungs) although organ dysfunctions were not evidently improved. *Nigella sativa* seeds can be included as dietary supplement for elderly people to improve their prognosis on exposure to sepsis. Clinical studies should be encouraged to extrapolate these findings to human patients.

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**Molecular Modeling Based, Design Synthesis and Cytotoxic Activity of Substituted Arylidene Piperazinoquinoline, a Hybrid Pharmacophore, Targeting Epidermal Growth Factor Receptor (EGFR), Tyrosine Kinase**

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**Abstract:** A series of 1-(2-(4-substitutedarylidene) hydrazinyl-4-(7-chloro-quinolin-4-yl)piperazine-2,5-dione VI was designed by molecular hybridization approach and synthesized for biological evaluation. Virtual screening was carried out through docking the designed compounds into the ATP binding site of epidermal growth factor receptor (EGFR) to predict if these compounds have similar binding mode as the EGFR inhibitors. Subsequently, the compounds were examined for their cytotoxic effect on human breast cell-line (MCF-7) in which the EGFR is highly expressed. Although most of the compounds were quite effective on the breast cancer cell line examined, the compounds II, III, IV a, IVc, VIa, VIc emerged as the most active among the prepared series. Thus 7-chloro-4-(2,5-dioxo 4-substitutedarylidene piperazinoquinoline can serve as the prototype molecule for further development of a new class of EGFR Tyrosine Kinase inhibitors and anti-breast cancer agents.

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**Keywords:** Anti-breast cancer activity . 4-Arylidine piperazinoquinoline. EGFR inhibitors. Hybrid pharmacophore approach

### 1. Introduction:

The growing incidence of drug resistance to cancer chemotherapeutic agents represents a serious medical problem<sup>(1)</sup>. Therefore there is an urgent need to develop new classes of chemotherapeutic agents with different mechanism of action to treat cancer<sup>(2)</sup>. It has been reported that designing a single molecule with more than one pharmacophore each with different mode of action could be beneficial for the treatment of cancer<sup>(3-6)</sup>, as well as to reduce unwanted side effects<sup>(3,4,6,7)</sup>.

Many quinolines were found to possess antineoplastic activity. Although the antineoplastic activity of these quinolines was attributed to intercalating binding to DNA, there were additional advantages in quinolines that interact with DNA, with low association constant. The corresponding significant increase in the amount of free drug at equilibrium may have an important effect upon distribution and hence the spectrum of activity and accessibility of these molecules to solid tumor<sup>(8,9)</sup>. In addition a number of quinoline derivatives were reported to reverse tumor cell multidrug resistance<sup>(10,11)</sup>.

Recently it has been demonstrated that 10  $\mu$ M chloroquine significantly increases cancer cell killing effects<sup>(3,4,12)</sup>. Several CQ analogues (Fig 1, 1-3) have

### 2. Chemistry

Compounds (II-VI) described in this study were synthesized as outlined in scheme 1. 4,7-Dichloroquinoline I was reacted with glycine ethyl

been synthesized and examined as cytotoxic agents against (MCF-7) breast cancer cell line. Some of these compounds were very effective<sup>(13)</sup>. It has been reported that CQ and its analogs has a unique property in being accumulated in the lysosomes, raising intra-lysosomal pH, and results in enhancement of cell killing by cancer therapeutic agents in a variety of different tumors<sup>(14)</sup>. Published data showed that the piperazinoquinoline pharmacophore, is one of the most effective newly emerging class of heterocyclic molecules that possessed antitumor activity<sup>(15,16)</sup> (Fig 2, 4&5). In addition many researches proved the importance of azomethine link or Schiff's base in anticancer drug<sup>(17,18)</sup>.

Moreover there are accumulating line of evidence that hybridization of two or more bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often render synergistic effects<sup>(19-24)</sup>. Encouraged by these previous reports and in an effort towards developing effective anticancer agents by a hybrid pharmacophore approach, herein (7-chloroquinolines, azomethine, piperazinedione) we designed different set of compounds (scheme 1) in the aim of prospecting their anticancer potentiality.

ester to afford compound II which upon reaction with hydrazine hydrate produced the key compound III. The target compounds VIa-e were obtained through two different pathways. The first includes the reaction of

compound III with different aromatic aldehydes to yield Schiff's base IV a-f which upon cyclization with chloroacetylchloride afforded the desired compounds VIa-e. Alternatively the second pathway involves

## 2.2. In vitro cytotoxicity

Since breast cells are known to over express EGFR, which leads to continuous activation of the EGFR pathway involved in cell proliferation, therefore all the compounds synthesized were evaluated for their cytotoxicity on breast cancer cell line MCF-7 using the sulfo-Rho-damine-B stain (SRB) assay using the method of Skehan<sup>(25)</sup>.

Cells were plated in 96 – multiwell microtiter plate ( $10^4$  cells / well) for 24h before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volumes. Different concentration of the compound under test (0.1, 2.5, 5 and 10  $\mu\text{g}$  / ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48h at 37°C and in atmosphere of 5%  $\text{CO}_2$ . After 48h, cells were fixed, washed and stained for 30 minutes with 0.4% (wt / vol) with SRB dissolved in 1% acetic acid unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve. Most of the tested compounds exhibited potent inhibitory activity against MCF-7 cell line (Table 1, Fig. 3-6).

### 2.2.1. Results and Discussion

The biological screening results (table 1) revealed that cytotoxic activities increase following the conversion of the ester II to the corresponding acid hydrazide III with  $\text{IC}_{50}$  values equal to 3.09 and 2.84

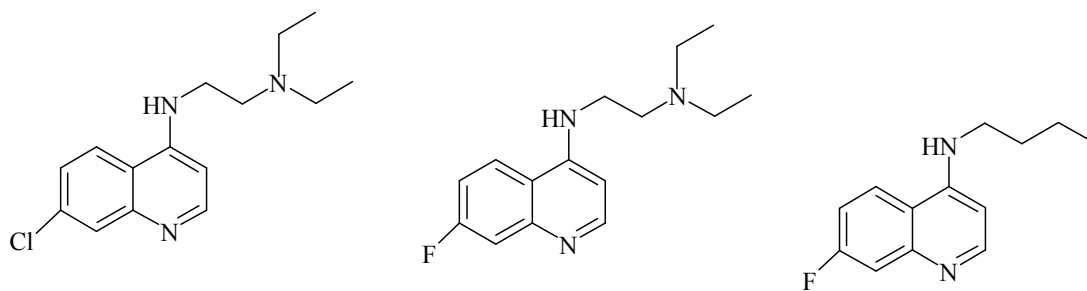
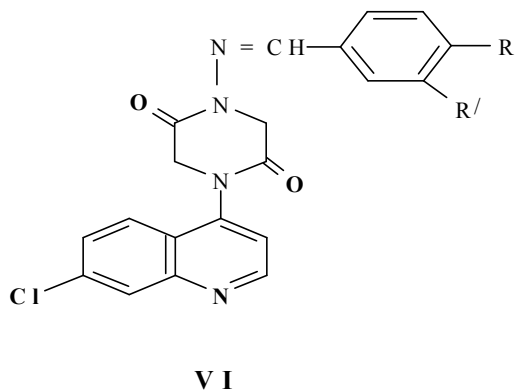
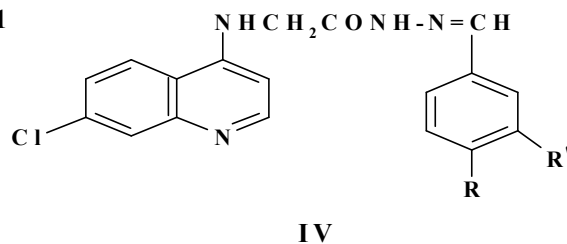
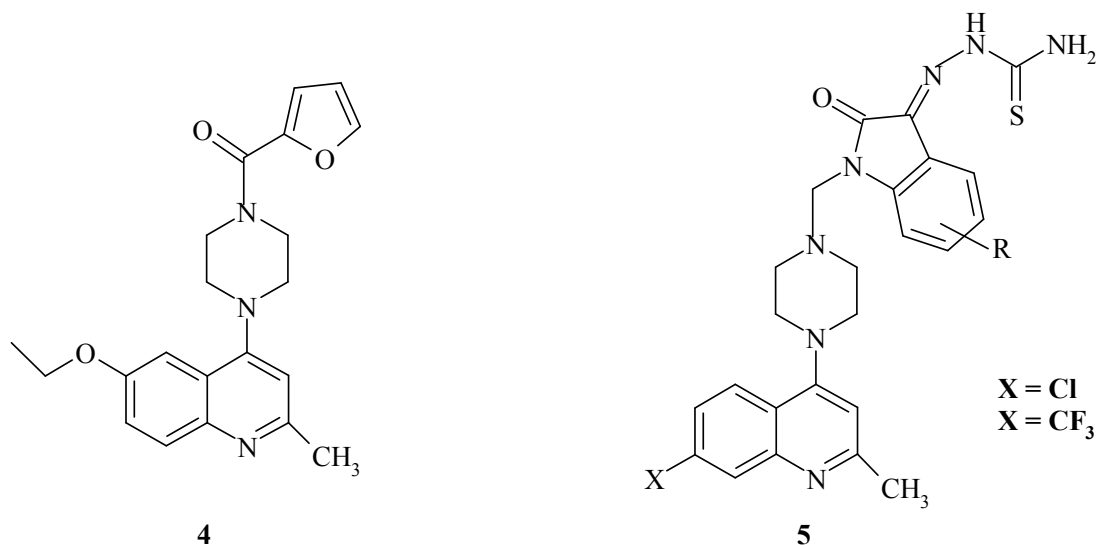
M respectively. Converting the acid hydrazide III to the corresponding arylidene derivatives reduces the cytotoxic activities as seen from compounds IVa and IVc where  $\text{IC}_{50}$  were 5.37 and 8.83 M respectively. Unfortunately cyclization of compound III to produce compound V abolishes the activity, which was restored again as seen from compound VIa and VIc with  $\text{IC}_{50}$  1.41 and 2.75 M respectively; a result that supports our hypothesis of designing a hybrid compound bearing three cytotoxic pharmacophore; herein: 7-chloroquinoline, a piperazine moiety and an azomethine group. It is also clear from the obtained data that electronic effect of the substituent in the para position of the arylidene group in the series comprising compounds VI(a-e), seems to be an important determinant of activity since the bromo derivative VIa

primarily cyclization of the key compound III via reaction with chloroacetylchloride to produce compound V which through interaction with different aromatic aldehydes ends into our target compounds. VIc was more active than its methoxy congenere VIc ( $\text{IC}_{50}$  equal to 1.41 and 2.75 M respectively).

## 3. Molecular modeling study:

Docking study was carried out for the target compounds into EGFR using SYBYL version 7.3. Tripos Inc with malegro virtual docker program version 2007. The crystal structure of the enzyme and lapatinib (1XKK) was obtained from protein data bank PDB<sup>(26)</sup> since it was found that lapatinib mimic ATP and bind to the ATP binding region of the kinase active site. Therefore our compounds were modeled by positioning them in the lapatinib binding site in accordance with the published crystal structures of quinazoline derivatives bound to kinase<sup>(27)</sup>. The entire complex was then subjected to alternate cycles of minimization and dynamics the intent was to get a satisfactory structure for the complex that was consistent with the published crystal structure<sup>(28,29)</sup>.

From the comparative docking study of our compounds with many structurally related lead compounds, such as lapatinib and gefitinib we could observe how our compounds might bind to the kinase binding site. Based on a knowledge of the structure of similar active sites, we docked Lapatinib into the active site of the enzyme (Fig. 7). Docking studies have revealed that Lapatinib ring binds to a narrow hydrophobic pocket in the EGFR TK domain with three hydrogen bond interaction with amino acids in vicinity while the aniline moiety lies in a deep and hydrophobic pocket. The bulky sulfamoyl group at C-4 of aniline moiety lies at the same position of the 3' chloro-4'-(3-fluorobenzyloxy) moiety of Lapatinib with total interaction energy equal to - 71 k cal/mol and RMSD equal 0.004 indicating that the ligand chosen interact with the enzyme at the same sites as do the main ligand. Compound IVc was then docked in the ATP binding site of EGFR TK with total interaction energy equal to -65.3 kcal/mol and showing hydrogen bond with D 855 (Fig. 8). Compound VIc was also docked in the ATP binding site of EGFR TK with total interaction energy equal to - 66.3 k cal/mol and showing two hydrogen bond with R 841&C 797 (Fig. 9). We can observe that the quinoline ring lies in a deep and hydrophobic pocket in the EGFR as in case of the chosen lead compounds with total interaction energy which nearly approaches that of the lead compound Lapatinib.

**Graphical Structure: 1****1 Fig. 1. Chemical structure of 4-2minoquinolines reported for an<sub>3</sub>ancer activity****Fig. 2. The design of hybrid compounds**

## Scheme 1

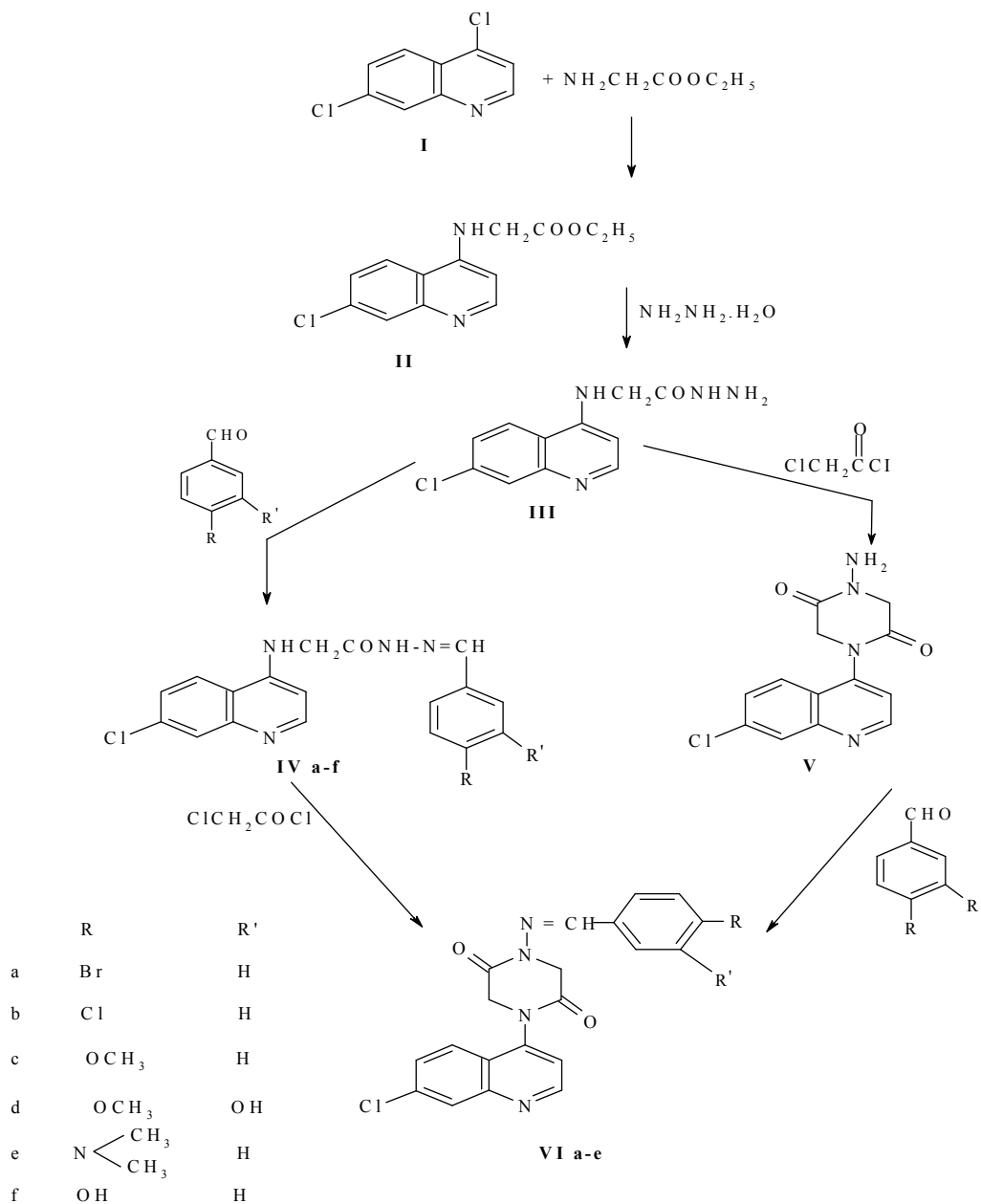


Table 1: In vitro cytotoxic activities of some synthesized compounds against human breast cancer cell (MCF-7)

Compounds	Cytotoxicity ( $\text{IC}_{50}$ ) <sup>a,b</sup>
II	3.097
III	2.84
IVa	5.37
IVc	8.83
V	n.a.
VIa	.41
VIb	1.5
VIc	2.75

<sup>a</sup> $\text{IC}_{50}$ . Compounds concentration required to inhibit tumor cell proliferation by 50%.

<sup>b</sup>Values are averages of three experiments n.a. = No activity

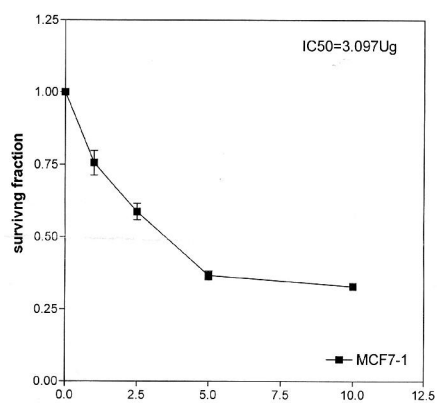


Fig. 3. Concentration of II in g/ml

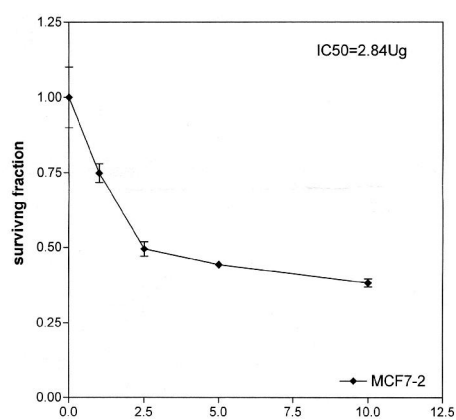


Fig. 4. Concentration of III in g/ml

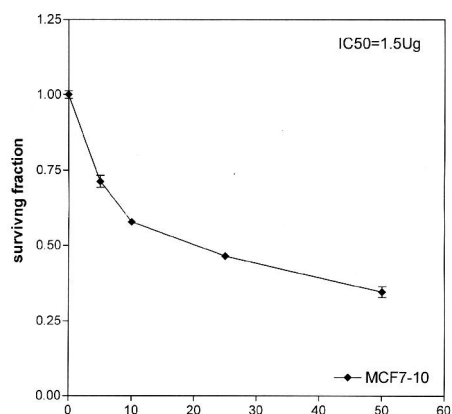


Fig.5. Concentration of VIa in g/m

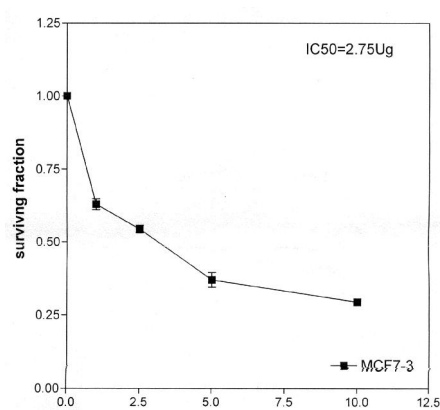


Fig. 6. Concentration of VIc in g/m

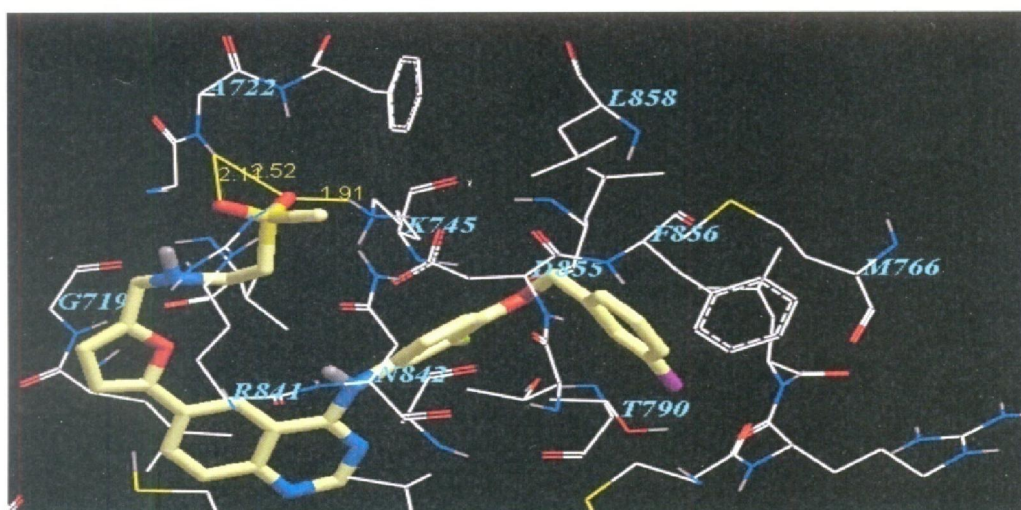


Fig. 7. Lapatinib in the ATP binding site of EGFR – TK. With  $\Sigma E$  equal to -71, RMSD = 0.004 with 3 HB. This picture was created with SYBYL version 7.3.

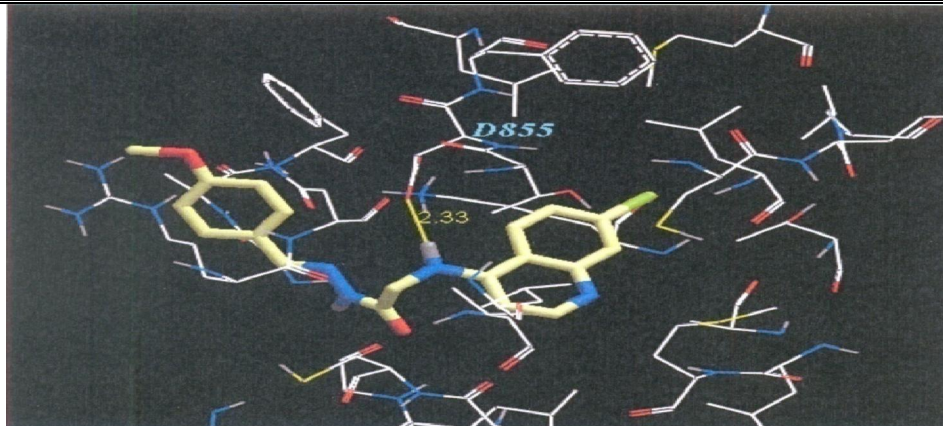


Fig. 8. Compound IVc docked at the ATP binding of EGFR – TK with  $\Sigma E$  equal to – 65.3 the fig shows HB with D 855.

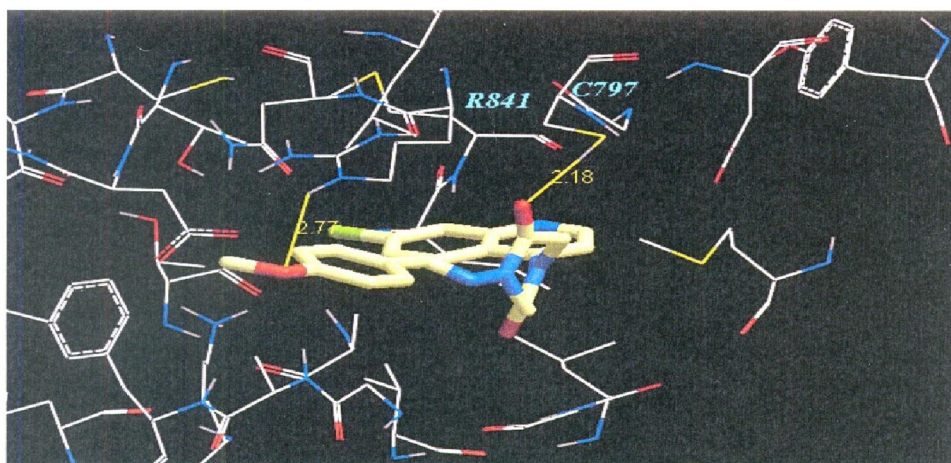


Fig. 9. Binding mode of compound VIc in the ATP binding site of EGFR – TK with  $\Sigma E$  equal to – 66.3 and showing 2HB with R841 & C 797.

#### 4. Material and methods

##### 4.1. Experimental

Melting points were determined with a Gallenkamp (London, U.K.) melting point apparatus and are uncorrected. IR spectra ( $\text{KBr}, \text{cm}^{-1}$ ) were recorded on Bruker Vector, 22 FT-IR (Fourier Transform Infrared (FTIR)) (Germany) spectrometer.  $^1\text{H}$ NMR spectra were recorded on a Varian Gemini-200 (200-MHz, Foster City Calif, USA) and Varian Mercury-300 (300-MHz, City: Palo Alto, State: Calif, USA) spectrometers using dimethylsulphoxide ( $\text{DMSO}-d_6$ ) as a solvent and tetramethylsilane ( $TMS$ ) as an internal standard (Chemical shift in  $\delta$ , ppm). Mass spectra were determined using Mass spectrometers GC/MS Shimadzu QP 1000 EX (Shimadzu Corporation, Tokyo, Japan) with ionization energy 70 eV. Elemental analyses were determined using Automatic Elemental Analyzer CHN Model 2400 Perkin Elmer (USA) at Microanalytical Center, Faculty of Science, Cairo University, Egypt. All the results of elemental analyses corresponded to the calculated values within experimental error. Progress of the reaction was monitored by thin-layer chromatography

(TLC) using precoated TLC sheets with Ultraviolet (UV) fluorescent silica gel (Merck 60F254) and spots were visualized by iodine vapors or irradiation with UV light (254 nm). All the chemicals were purchased from *Sigma-Aldrich*.

##### 4.1.1 Ethyl -2-(7-chloroquinolin-4-ylamino) acetate II

A mixture of glycine ethyl ester (0.01 mole) and 4,7-dichloro-quinoline I (0.01 mol) in absolute ethanol (10 ml) containing few drops of HCl was refluxed for 2 h. to give a creamy white precipitate which was filtered, washed with cold aqueous alcohol and recrystallized from absolute alcohol yield 80%; MP: 185-87°C; IR ( $\text{KBr}, \text{cm}^{-1}$ ) 3300, 1610;  $^1\text{H}$  NMR: 1.2 (t, 4H,  $\text{CH}_3$ ) 2.4 (s, 2H,  $\text{CH}_2$ ) 4.2 (q, 2H,  $\text{CH}_2$ ) 6.8-8.5 (m, 5H, aromatic) 9.5 (s, 1H, NH) MS m/z 265. Anal. Calcd for  $\text{C}_{13}\text{H}_{13}\text{N}_2\text{ClO}_2$ : C, 58.9; H, 4.9; N, 10.5. Found C, 58.72; H, 4.62; N, 10.5.

##### 4.1.2. 7-Chloro-N-(2-hydrazinyloxy)-2-oxoethyl quinoline-4-amine III

A mixture of (0.01 mol) of II and hydrazine hydrate (0.03 mol) was refluxed in absolute ethanol (10

ml) for 2h. The solvent was evaporated under vacuum; the resulting oil was triturated with ice. The buff precipitate formed, was filtered, washed with alcohol/water and crystallized from ethanol. Yield 85% MP: 195-97°C; IR (KBr,  $\text{cm}^{-1}$ ) 3150-3300, 1650;  $^1\text{H}$  NMR: 2.4(s, 2H,  $\text{CH}_2$ ); 6.8-7.8 (m, 5H, aromatic) 8 (s, 2H,  $\text{NH}_2$ ) 9-9.4 (2s, 2H, 2 NH) exchangeable with  $\text{D}_2\text{O}$  MS m/z 250. Anal. Calcd. For  $\text{C}_{11}\text{H}_{11}\text{N}_4\text{ClO}$ : C, 52.8; H, 4.3; N, 22.11. Found: C, 52.6; H, 4.58; N, 22.32.

#### 4.2. General procedure for the preparation of N-(2-(2-(4-substituted arylidene)hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV a-f

A mixture of III (0.01 mol) and the appropriate aldehyde (0.01 mol) in absolute ethanol and drops of glacial acetic acid was refluxed for 3 h. The precipitate formed was filtered off, washed with alcohol dried and recrystallized from DMF/water.

##### 4.2.1 N-(2-(2-(4-Bromobenzylidene) hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV a

Yield: 70%; mp: 198-200°C, IR (KBr,  $\text{cm}^{-1}$ ) 3300-3400, 1650;  $^1\text{H}$  NMR 2.4 (s, 2H,  $\text{CH}_2$ ), 7.2-8.6 (m, 9H aromatic) 9.4 (s, 1H, ald), 11.6-11.8 (2s, 2H, 2 NH) exchangeable with  $\text{D}_2\text{O}$ , MS/Z 416. Anal Calcd for  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{Br ClO}$ : C, 51.7; H, 3.3; N, 13.4. Found: C, 51.8; H, 3.16; N, 13.4.

##### 4.2.2. N-(2-(2-(4-Chlorobenzylidene) hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV b

Yield: 65% mp: 205-7°C, IR (KBr,  $\text{cm}^{-1}$ ) 3200-3400, 1680;  $^1\text{H}$  NMR 2.4 (s, 2H,  $\text{CH}_2$ ) 7.2-8.6 (m, 9H, aromatic) 9.4 (s, 1H, ald), 9.8, 10.2 (2s, 2H, 2 NH) exchangeable with  $\text{D}_2\text{O}$  Anal. Calcd. For  $\text{C}_{18}\text{H}_{14}\text{N}_4\text{Cl}_2\text{O}$ : C, 58; H, 3.7; N, 16.5; Found: C, 58.1; H, 3.8; N, 17.

##### 4.2.3 N-(2-(2-(4-Methoxybenzylidene) hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV c

Yield: 68% mp: 118-20°C; IR (KBr,  $\text{cm}^{-1}$ ) 3200-3400, 1600;  $^1\text{H}$  NMR 2.4 (s, 2H,  $\text{CH}_2$ ) 4 (s, 3H,  $\text{OCH}_3$ ) 7-8.4 (m, 9H, aromatic + 1H ald) 11.8 (broad, 2H, 2 NH) exchangeable with  $\text{D}_2\text{O}$ . Anal. Calcd. For  $\text{C}_{19}\text{H}_{17}\text{N}_4\text{ClO}_2$ : C, 61.8; H, 4.6; N, 15.1; Found: C, 61.83; H, 4.59; N, 14.91.

##### 4.2.4. 5-(2-(2-(7-Chloro quinoline-4-ylamino) acetoxy) hydrazino) methyl)-2-methoxy phenol IV d

Yield. 80%; mp: 190-2°C; IR (KBr,  $\text{cm}^{-1}$ ) 3200-3400-3500, 1650;  $^1\text{H}$  NMR 2.4 (s, 2H,  $\text{CH}_2$ ), 4 (s, 3H,  $\text{OCH}_3$ ), 6.8-8.2 (m, 9H, aromatic), 8.8 (s, 1H, ald), 9.9 (s, 1H, OH), 11.8 (br. 2H, 2NH) exchangeable with  $\text{D}_2\text{O}$ . Anal. Calcd. For  $\text{C}_{19}\text{H}_{17}\text{N}_4\text{ClO}_3$ : C, 59.2; H, 4.3; N, 14.5; Found C, 59.12; H, 4.7; N, 14.9.

##### 4.2.5. N-(2-(2-(4-Dimethylaminobenzylidene)hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV e

Yield 80%; mp. 245-7°C; IR (KBr,  $\text{cm}^{-1}$ ) 3200-3400, 1650;  $^1\text{H}$  NMR 2.4 (s, 2H,  $\text{CH}_2$ ), 3.4 (s,

6H,  $2\text{CH}_3$ ) 6.8-8.6 (m, 9H, aromatic + 1H, ald) 9.8, 12 (2s, 2H, 2 NH) exchangeable with  $\text{D}_2\text{O}$  Anal. Calcd. for  $\text{C}_{20}\text{H}_{20}\text{N}_5\text{ClO}$ : C, 62.9; H, 5.2; N, 18.3 Found: C, 63; H, 5.2; N, 18.69.

##### 4.2.6. N-(2-(2-(4-hydroxybenzylidene) hydrazinyloxy)-2-oxoethyl)-7-chloroquinoline-4-amine IV f

Yield 65%; mp. 173-5°C; IR (KBr,  $\text{cm}^{-1}$ ) 3300-3400, 1600;  $^1\text{H}$  NMR. 2.4 (s, 2H,  $\text{CH}_2$ ) 4 (s, 3H,  $\text{OCH}_3$ ), 7-8.6 (m, 10H, aromatic + CH ald.) 10 (s, 1H, OH), 11.8 (broad, 2H, 2NH) exchangeable with  $\text{D}_2\text{O}$ . Anal. calcd for  $\text{C}_{18}\text{H}_{15}\text{N}_4\text{ClO}_2$ : C, 60.9; H, 4.2; N, 15.7; Found: C, 60.88; H, 4.3; N, 15.41.

#### 4.3. General procedure for the preparation of 1-(2-(4-substituted arylidene) hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VI a-f

##### Method A

Compounds IV (a-f) (0.1 mole) were refluxed with chloroacetyl chloride (10 ml) in absolute ethanol (10 ml) for 4 h. The refluxed mixture was poured into ice/ water to produce compounds VI a-f.

##### Method B

A mixture of compound V (0.1 mole) and the appropriate aromatic aldehyde (0.1 mole) in absolute ethanol (10 ml) containing few drops of glacial acetic acid was refluxed for 4h. The solid product was filtered off and recrystallized from DMF/water.

##### 4.3.1. 1-(2-(4-Bromobenzylidene) hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VI a

Yield 75%; mp. 240-2°C; IR (KBr,  $\text{cm}^{-1}$ ) 1610-1620;  $^1\text{H}$  NMR: 2.4 (s, 4H,  $2\text{CH}_2$ ) 6.6-8.8 (m, 10H, aromatic + CH ald) Anal. Calcd for  $\text{C}_{20}\text{H}_{14}\text{N}_4\text{Br ClO}_2$ : C, 52.4; H, 3.0; N, 12.2; Found C, 52.5; H, 2.8; N, 12.25.

##### 4.3.2. 1-(2-(4-Chlorobenzylidene) hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VI b

Yield 60%; mp: 295-7°C; IR (KBr,  $\text{cm}^{-1}$ ) 1608-1670;  $^1\text{H}$  NMR 2.4 (s, 4H,  $2\text{CH}_2$ ), 7.5-8.8 m (10H, arom + CH ald). Anal. Calcd. For  $\text{C}_{20}\text{H}_{14}\text{N}_4\text{Cl}_2\text{O}_2$ : C, 58.25; H, 3.39; N, 13.59; Found: C, 58.4; H, 3.3; N, 13.93.

##### 4.3.3. 1-(2-(4-Methoxybenzylidene) hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VI c

Yield 65%; mp: 275-7°C, IR (KBr,  $\text{cm}^{-1}$ ) 1610-1620;  $^1\text{H}$  NMR 2.4 (s, 4H,  $2\text{CH}_2$ ), 3.8 (s, 3H,  $\text{OCH}_3$ ) 7 - 8.8 (m, 10H, arom. + CH ald) MS/Z 409. Anal. Calcd for  $\text{C}_{21}\text{H}_{17}\text{N}_4\text{ClO}_3$ : C, 61.6; H, 4.16; N, 13.7; Found: C, 61.29, H, 3.79; N, 13.8.

##### 4.3.4. 1-(2-(3-Hydroxy-4-methoxybenzylidene) hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VI d

Yield: 65%; mp: 246-8°C; IR (KBr, cm<sup>-1</sup>) 1600-1650; <sup>1</sup>H NMR. 2.4 (s, 4H, 2CH<sub>2</sub>) 3.8 (s, 3H, OCH<sub>3</sub>) 6.9-8.7 (m, 10H, arom. + CH ald.) 9.8 (s, 1H, OH) exchangeable with D<sub>2</sub>O. MS/Z 423.5. Anal. Calcd for C<sub>21</sub>H<sub>17</sub>N<sub>4</sub>ClO<sub>4</sub>: C, 59.36; H, 4.0; N, 13.1; Found: C, 59.22; H, 4.11; N, 13.5.

#### 4.3.5. 1-(2-(4-Dimethylaminobenzylidene)hydrazinyl-4-(7-chloroquinolin-4-yl)piperazine-2,5-dione VIe

Yield: 70%; mp: 228-30°C; IR (KBr, cm<sup>-1</sup>) 1600-1610; <sup>1</sup>H NMR. 2.4 (s, 4H, 2CH<sub>2</sub>) 3.4 (s, 6H, 2CH<sub>3</sub>) 6.7-8.5 (m, 10H, arom. + CH ald.). Anal. Calcd for C<sub>22</sub>H<sub>20</sub>N<sub>5</sub>ClO<sub>2</sub>: C, 62.6; H, 4.7; N, 16.6; Found: C, 62.33; H, 5.33; N, 17.

### 5. Conclusion

We have synthesized and tested a series of 1-(2-(4-substitutedarylidene)hydrazinyl-4-(7-chloroquinoline-4-yl)piperazine 2,5-dione VI variously substituted at the 3 and 4 phenyl moiety. Cytotoxic activity against human breast cell line (MCF-7) was evaluated as well as molecular modeling study was carried out through docking the designed compounds into the ATP binding site of EGFR. Biological screening results revealed that compounds VIa and VIc were the most active as shown in Table 1. It is crucial and advantageous that the electronic effect and the lipophilicity of the substituent in the para position of the arylidene moiety seems to be an important determinant of activity since the bromosubstituted derivative VIa was more active than the methoxy substituted derivative VIc. A parallel correlation was observed concerning molecular modeling study and cytotoxic activity. We can conclude that the designed hybrid pharmacophore VI might present good antitumor lead targeting EGFR-TK.

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## Study of the predictive factors of body mass index in a group of Iranian undergraduate students

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**Abstract:** As part of research, predictive factors of body mass index (BMI) such as nutritional behavior (NB), nutritional knowledge (NK), attitude toward nutrition (ATN), physical exercise (PE) and demographic variables of age and gender among undergraduate students (US) of hormozgan university, Iran were studied, in a correlation trial. Two hundred seventy five US (137 boys and 138 girls) were randomly selected, responded to the study questionnaire and then weighed as the height was measured using a standard protocol in order to calculate BMI. Predictor factors such as NB, NK, ATN and PE were significantly positive for the normal BMI while age and gender were not positively predicted the normal BMI. Among the predicted variables, NB and PE had more important roles for explain the normal body mass index variance. It is concluded that, nutritional behavior and physical exercise could be an applicable approach for predict the health status of students.

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**Keywords:** undergraduate students; BMI; nutritional behavior and knowledge; attitude towards nutrition; physical exercise

### 1. Introduction

Wrong nutritional trends and the related disorders in advanced countries are increasing considerably (Mokdad et al., 2000), even some of scientific communities called it as epidemiology (WHO, 2006). Until most recently the importance of obesity and overweight among children had not been emphasized enough. It is estimated that, there are a total of 155 million overweight children and around 30-45 million are classified as obese (Azizi et al., 2001a). Much concern is being expressed both about the early consequences and lifetime effects of obesity in both children and adolescents. In Iran, obesity and overweight are under investigations. Overweight and obesity are caused by an imbalance between physical activity and nutritional intake. According to the obtained results, 18% of teenagers in Tehran were suffered from overweight and obesity (Azizi et al., 2001a). Another study reported that 12-18% of teenagers (11-19 years old) were suffered from cardiovascular risks like high bad cholesterol or LDL-C (low density lipoprotein cholesterol) and those aged >6-12 years had high triglycerides and low good cholesterol or HDL-C (high density lipoprotein cholesterol) (Azizi et al., 2001b).

The researchers were focused on behavioral and cognitive subjects linked to psychological - physical pathologies. Cox et al. (2006) referred to some problems as emotional failures, alcoholism, smoking, illegal drugs and eating disorders and reported that it seems that relationship between behaviors and nutrition are more complex than

smoking and drinking alcohol, so that quantitative indices can used easily to assess alcohol and Psychoactive drugs but nutritional behaviors are more complex and multidimensional in terms of evaluation. Some indices as rate of the food intake, food calorie, feelings of satiety and anorexia and overweight are various factors that evaluate some aspects of nutritional behaviors.

One of the most acceptable and common indices in most of the researches is body mass index (BMI) that is calculated via weight and height of the person and it is a rather reliable index of body fat in most of the people. It is worth to mention that BMI doesn't measure body fat directly, but the researches showed that BMI has linear correlation with direct evaluation of body fat such as weighting under water and dual energy x-ray absorptiometry (DXA) (Mei et al., 2002). There are various standards about this index, but one of the most common standards is classification of the degrees of this index by Centers for Disease Control and Prevention (CDC).

According to the classification of this center, the range of BMI for teenagers and adults is such as BMI range 18.5 - 24.9 is defined as normal BMI and the upper and lower limit of this range is defined and abnormal BMI. In the current research the classification of this center is used as BMI of this range that was considered as normal and lower and also upper amounts of this range was considered as abnormal. BMI in most of the researches is related to a wide range of health issue (increasing risk of cardiovascular diseases, diabetic, cancer and arthritis)

(Callee et al., 1999). So BMI is considered as an important variable in health researches. Hence, one of the important scopes of experiments is the examination of the factors related to BMI. In some of the studies, cognitive variables (memory, attention range and etc) are investigated as BMI predictive (Pothos et al., 2008). In other studies, some indices as food external index, emotional factors and abstain eating are supported as BMI predictive (Braet and van Strien, 1997; Van Strien et al., 2009; Aboserea et al. 2011). Some of the researches also referred to some factors as stress, anxiety and depression as probable predictor of BMI and moderator effects of some variables such as gender and physical activity (Pothos et al., 2008). In recent researches, some variables such as knowledge, attitude and performance of guidance and high school students about healthy nutrition were investigated (Azadbakht et al., 2001) so that results did showed a small percentage of teenagers that have good nutritional performance and in most of the cases their attitude and performance is not according to their nutritional knowledge (Mohammadi et al., 2000, in adults).

Nutritional knowledge is not the only effective factor on nutritional behaviors (Kline, 1993) and there are other variables that were more effective in this regard. So, in the current study, according to the past researches results and the importance of BMI predictive factors in programming and intervention on nutritional behaviors of students, some predictive variables were investigated. Therefore, general objective of the current research is the examination of BMI predictive variables among the undergraduate students of hormozgan university, Iran. So the partial aims of the research were the investigations of nutritional knowledge, nutritional behavior, attitude toward nutrition, physical exercise variables and also demographic variables such as age and gender as BMI probable predictor among undergraduate students.

According to the research aims, the research hypotheses are presented as the followings:

- 1- Nutritional knowledge variable predicts normal BMI positively and significantly.
- 2- Attitude toward nutrition variable predicts normal BMI positively and significantly.
- 3- Nutritional behavior variable predicts normal BMI positively and significantly.
- 4- Physical exercise variable predicts normal BMI positively and significantly.
- 5- Age variable predicts normal BMI positively and significantly.
- 6- Gender variable predicts normal BMI positively and significantly.

## 2. Material and methods

This experiment is correlative research so that normal BMI is considered as criterion variable and gender, age, nutritional knowledge or behavior, attitude toward nutrition and physical exercises are considered as predictive variables. To analyze data, descriptive statistics such as mean, standard deviation and frequency tables were used and in the prediction of criterion variable by predictive variables, logistic regression, enter method is used. The research statistic population was undergraduate students in Hormozgan University who were studied in 2009 and 2010. A total number of three hundred volunteers (150 girls and 150 boys) of undergraduate students were selected from university statistics center randomly then research questionnaires were given to the selected students. Considering that, 275 students could the completed the questionnaire and returned it. Questionnaire and method of collecting data were used according to work of Azadbakht et al. (2001). Questionnaire was contained 34 items including, nutritional knowledge or behavior, attitude toward nutrition and their age and gender.

The providers of the questionnaire reported the test – retest reliability coefficient equal to 0.75 and examined the validity of the questionnaire by factor analysis method and evaluated it at a good level. BMI also was calculated from measured height and weight of participants. Data collected from experiment were analyzed by SPSS statistical software (version 16).

## 3. Results

Descriptive statistics of the research variables were shown in Tables 1 and 2. To assess hypothesis of the current study, Correlative matrices of predictive and criterion variables was calculated. As a consequence, nutritional behavior had the highest correlation with BMI variable while gender variable showed the lowest correlation (Table 3).

In addition, when logistic regression analysis carried out, applying goodness of fit tests of model is necessary. The results of omnibus tests for the coefficients of the model showed significant chi-square value ( $X^2(6, N = 275) = 261.207, p < 0.001$ ) and indicates that model variables are suitable to predict criterion variable.

The results obtained by McCreary (2002) showed were not significant so that P level equals 0.73 in this regard that is considered another index of model fit and was agreement with the observed results in the presented study. Generally, the results of the analysis showed that, the criterion variable was properly predicted by the model in 94.2 % of cases (Table 4).

Table 1. Descriptive statistics of research continuous variables

Variables	M	SD	Min	Max
Age	20.6	5.33	18	24
Nutritional knowledge	16.44	3.09	11	22
Nutritional attitude	18.28	1.16	16	20
Nutritional behavior	5.10	1.60	3	7

Table 2. Descriptive statistics of research categorical variables

Variables	Gender			Body Mass Index		
	Girl	Boy	Total	Normal	Abnormal	Total
Frequency	138	137	275	171	104	275
Percent	50.2	49.8	100	62.2	37.8	100

Table 3. Correlative matrices of predictive and criterion variables

Variable	BMI	Exercise	Age	Knowledge	Attitude	Gender	Behavior
BMI	-----						
Exercise	0.64**						
Age	-0.48**	-0.48**					
Knowledge	0.53**	0.45**	-0.52**				
Attitude	0.21**	0.19**	-0.11	0.051			
Gender	0.07	0.004	0.038	0.039	0.30**		
Behavior	0.69**	0.40**	-0.42**	0.46**	0.15*	-0.004	-----

Note: P<0.05\*, P<0.01\*\*

Table 4. The predictive model of criterion variable classification and the prediction amount

Observed	Predicted		
	Abnormal BMI	Normal BMI	% of Correct predicted
Abnormal BMI	93	11	89.4
Normal BMI	5	166	97.1
Total			94.2

Table 5. The existing variables in predictive model, Wald statistics and significance level

Variables	B	Wald	Df	Significant
Nutritional behavior	1.74	38.68	1	P<0.001
Exercise	4.03	35.45	1	P<0.001
Nutritional knowledge	0.31	6.22	1	P<0.01
Nutritional attitude	0.90	9.14	1	P<0.05
Age	0.00	0.00	1	-----
Gender	1.02	3.05	1	-----

The above analyzed model explained the range 61 to 83% of normal BMI variance and correct prediction for normal and abnormal BMI was 97.1% and 89.4%, respectively. The following table presents Wald statistics and the related freedom degree and significance level for each predictive variable. As a consequence, it is shown that nutritional behavior variables, physical exercise, nutritional knowledge and attitude toward nutrition significantly predicted

normal BMI, while demographic variables (age and gender) were not significant predictors of normal BMI.

#### 4. Discussion

In the current study because of increasing the overwhelming obesity and overweight in developing countries and the increasing trend of this phenomenon in Iran, BMI predictive factors were investigated as one of the common indices of obesity and overweight. According to the results of previous researches, some factors such as nutritional behavior (NB), nutritional knowledge (NK), attitude toward nutrition (ATN), physical exercise (PE) and demographic variables of age and gender were investigated as predictive variables of body mass index. As it was said in the results section, some factors such as NB, NK, ATN and PE predict normal BMI significantly and four hypotheses of the research were supported. On the other hand, demographic variables (age and gender) don't predict normal BMI significantly and fifth and sixth hypotheses were not supported.

The current research findings obtained similar results in compatible with the studies inside and outside of Iran. Study of the results obtained from Sadrzadeh Yeganeh et al. (2005) showed relationship of the obesity with some the nutritional behaviors among high school girls. In another study (Azadbakht et al., 2001), it was shown that despite having good nutritional knowledge, NB of high school students was at low level. This was important because it shown the strong influence of nutritional behavior on being in a good weight range and this

result is supported in the current study and predictive variable of nutritional behavior is referred as the most important BMI predictive variable. The various studies showed that NB, NK and ATN are good variables to investigate the nutritional trend of people and their weights (Mohammadi et al., 2000; Buttriss, 1997). Another variable that in the current research is significant to prediction of normal BMI is physical exercise. The results observed in the current study is similar to the findings of the same researches carried out in this field (Lahti-Koski et al., 2002; Henningsson and Ekelund, 2007; Metallinos-Katsaras et al., 2007). In all of these researches, PE did showed significantly positive relationship with normal BMI.

Although the above results supported the hypotheses 1-4, two other results of the current study related to hypotheses of demographic variables of age and gender resulted unlike and not supported as significant predictors of normal BMI. In different researches, obesity and overweight were reported more among men than women (McCreary, 2002) and by increasing age, the weight of the person was increased and their BMI is far from the normal status. For example, in study conducted by Najafi et al. (2006), obesity was increased by increasing age. One of the probable reasons of non-significance of gender predictive variable could related to the difference of the results in different studies and the lack of constant gender differences in BMI status of people. For example, gender in study carried out by Najafi et al. (2006) was not reported as significant variable. In terms of non-significance of age predictive variable, one of the probable reasons of the study carried out on students with the mean age of 16.2 is due to the rather wide age range among selected people (age range of 15-17), age variable is not significant predictor for normal BMI. It is worth to mention that in the current study, the simple correlation between age and normal BMI is calculated -0.47 that is significant and it indicates that by increasing age, BMI will be far from normal status (Najafi et al., 2006).

Generally, the current research findings indicate that the examination of the effective factors on obesity, overweight and BMI status of people requires a systematic and multidimensional view that considers important factors such as NB, NK and ATN beside some factors as physical exercise, age and gender. So it is appropriate to consider them together in intervention programs about student's health status.

Although it is attempted to investigate the predictive factors of normal BMI but research limitations restricts the generality of the findings. One of the most important limitations of the current

research is using the self report questionnaire that reduces the reliability of the research data. It is recommended in the future studies to assess the predictor variables used objective indices such as calculating the daily calorie and observing people in real condition of nutrition. Other limitation of the research was implementation the research among undergraduate students that makes the generalization of the results to other educational environments more difficult. It is recommended to carry out it in the other educational courses and compare the results with the existing study findings. Finally all of the investigated factors in this research were not effective factors on normal BMI and other factors were more effective in the prediction of normal BMI. Considering other variables instead of the investigated variables in this study such as the social economics status, education level, full breakfast, the amount of daily calorie and etc can be important in nutrition of today's society and further researches is needed to help address the problem of obesity in this group of undergraduate students.

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## Serum Amyloid A an Early Diagnostic Marker for Neonatal Sepsis

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**Abstract:** 100 term neonates were included in the study. 50 neonates with clinically suspected sepsis, in which blood culture, SAA measurement, CRP, CBC, were done for all neonates when sepsis was first suspected (Reading A) and 48 hours post sepsis evaluation (Reading B). The other 50 matched neonates served as a control group and the same investigations were done once for all of them, during routine follow-up or bilirubin evaluation. **Patients and Methods:** Of 50 neonates with clinically suspected sepsis, 41 had positive blood culture (septic group) and 9 had negative blood culture (non-septic group). The SAA levels of septic group were significantly higher than those of the control and non-septic group ( $p < 0.001$ ) at Reading A. in comparison with CRP, SAA levels rose earlier and in a sharper manner, had the higher levels and returned faster to normal. **Results:** When sepsis was first suspected (Reading A), SAA had an overall diagnostic accuracy for early diagnosis of neonatal sepsis (97.6%), compared to CRP (36.6%). Statistical evaluation of SAA testing showed a sensitivity of 97.6%, specificity of 88.9%, positive predictive value of 97.6%, negative predictive value of 88.9%, and test efficiency of 96% in diagnosing of neonatal sepsis.

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### 1. Introduction

Neonatal sepsis is one of the leading causes of morbidity and mortality among the newborns. As many as 2% of fetuses are infected in utero and up to 10% of infants are infected during delivery or the first month of life (Gonzalez et al., 2004).

Serum Amyloid A (SAA) proteins comprise a family of apolipoproteins synthesized in response to cytokines released by activated monocytes, macrophages in a number of different inflammatory process (Malle and DeBeer, 1996).

Serum Amyloid A (SAA) was found to be increased in bacterial and fungal infections, invasive malignant disease, tissue injury in the acute myocardial infarction and autoimmune diseases such as rheumatoid arthritis and vasculitis (Jovanovic, 2004). The aim of the study was to evaluate the diagnostic value of serum Amyloid A as an early diagnostic marker for neonatal sepsis.

### 2. Subjects and Methods:

#### Subjects:

The study included 100 term neonates, 50 in the study group and 50 healthy neonates as control group. The study group (suspected group) included 50 neonates with maternal and neonatal criteria suggestive of sepsis. This study is a case control study conducted on a group of neonates admitted into NICU of El-Galaa Teaching Hospital in Cairo. The maternal criteria are intra partum fever, premature rupture of membrane

(PROM) more than 18 hours and foul smelling amniotic fluid. The neonatal criteria are temperature instability ( $< 36.5^{\circ}\text{C}$  or  $> 37.5^{\circ}\text{C}$ ), poor skin perfusion (capillary refill  $> 3$  seconds), poor activity and crying, poor suckling reflex, poor Moro's reflex, apnea or respiratory distress, pallor, lethargy or irritability, bradycardia and abdominal distention and vomiting. We excluded any case with traumatic tissue injury, congenital anomalies, metabolic liver disease and history of perinatal, natal and postnatal asphyxia.

According to blood culture results, the suspected group (50 neonates) was subdivided into two subgroups, septic subgroup (41 neonates) they are infants with clinical and biochemical evidence of infection and positive blood culture.

Non-septic subgroup (9 neonates) they are infants who suspected of having sepsis initially, but with negative blood culture and no evidence of localized infection such as pneumonia or NEC.

The control group includes 50 healthy neonates, age and sex-matched in whom serum Amyloid A (SAA) and C-reactive protein (CRP) were measured during routine blood sampling for biochemical or hematological tests.

#### Methods:

All cases were subjected to the following:

- Careful history taking including, natal history.
- Full clinical examination for early detection of manifestations of neonatal sepsis.

- Assessment of gestational age by using new Ballard Score (**Ballard, 1991**).
- Anthropometric measurements including bodyweight, length and skull circumference.
- Laboratory investigations:

Two consecutive blood samples were collected from the suspected cases and one sample were taken from the control cases. The first sample (Reading A) when the sepsis was first suspected. The second sample (Reading B) after 48 hours from the first sample. The blood samples were investigated for complete blood picture (CBC), quantitative C-reactive protein (CRP), blood culture and serum Amyloid A (SAA) level measured by an immunoenzymometric assay using human SAA Elisa Kit (**Biosource Europe S.A., Belgium**).

#### Statistical Analysis:

Results are expressed as mean  $\pm$  standard

deviation (SD) or number (%). Comparison between the mean values of different variables in Reading A and Reading B in septic group was performed using paired student t-test. Comparison between the mean values of the different variables in the two groups was performed using unpaired student t-test SPSS computer program (version 12 windows) was used for data analysis.

#### 3. Results:

The septic subgroup, the frequency of clinical manifestations were increased gradually with progress of illness from Reading A (when the sepsis was first suspected) to Reading B (after 48 hours from Reading A). There was a significant increase of the frequency of manifestation as regard poor Moro, poor suckling, lethargy, respiratory distress, and feeding intolerance as shown in table (1).

**Table (1): Comparison between clinical findings among septic subgroup when the sepsis was first suspected (Reading A) and after 48 hours (Reading B).**

Characteristics	Reading A	Reading B	P.value	Sig.
Poor Moro	20 (48.8%)	39 (95.1%)	0.000	HS
Poor suckling	22 (53.7%)	37 (90.2%)	0.000	HS
Hypotonia	7 (17.1%)	11 (26.8%)	0.286	NS
Lethargy	14 (34.1%)	35 (85.4%)	0.000	HS
Convulsions	1 (2.4%)	3 (7.3%)	0.305	NS
Apnea	9 (22%)	9 (22%)	1.0	NS
Respiratory distress	17 (41.5%)	30 (73.2%)	0.004	S
Bradycardia	7 (17.1%)	7 (17.1%)	1.0	NS
Hypothermia	9 (22%)	8 (19.5%)	0.785	NS
Poor skin perfusion	12 (29.3%)	14 (34.1%)	0.635	NS
Jaundice	6 (14.6%)	6 (14.6%)	1.0	NS
Pallor	4 (9.8%)	3 (7.3%)	0.693	NS
Abdominal distension	12 (29.3%)	13 (31.7%)	0.810	NS
Feeding intolerance	13 (31.7%)	23 (56.1%)	0.26	S

Value are expressed as number

NS = Non-significant    HS = Highly significant    S = Significant

Blood culture was done for all cases of the suspected group. According to the blood culture results, they were subdivided to non-septic group with negative blood cultures (9 cases) and septic group (41 cases) with positive cultures.

Gram negative organisms were predominant in 28 cases (68.3%), they were klebsiella 16 cases (39%),

pseudomonas 5 cases (12.2%), Escherichia coli 4 cases (9.8%) and enterobacter 3 cases (7.3%). On the other hand gram positive organism were cultured in 13 cases (31.7%) of the septic group, mainly staphylococcus aureus 6 cases (14.6%), staphylococcus coagulase negative 5 cases (12.2%), and streptococcus pneumoniae 2 cases (4.9%), as shown in table (2).



**Table (2): Distribution of the blood cultured organisms among the septic group (n=41).**

Organism	No. of cases	%
• <b>Gram negative organism:</b>	28	68.3%
- <i>Klebisella</i>	16	39%
- <i>Pseudomonas</i>	5	12.2%
- <i>E.coli</i>	4	9.8%
- <i>Enterobacter</i>	3	7.3%
• <b>Gram positive organism:</b>	13	31.7%
- <i>Staph. aureis</i>	6	14.6%
- <i>Staph. coagulase negative</i>	5	12.2%
- <i>Srept. pneumonie</i>	2	4.9%

Different laboratory parameters were studied in the three groups, when sepsis was first suspected (Reading A), as shown in table (3).

**Table (3): Laboratory data of the studied groups (when sepsis was first suspected = Reading A).**

Variable		Control (n=50)	Suspected group (n=50)		P. value	Sig.
			Septic subgroup (n=41)	Non-septic subgroup (n=9)		
<b>Hb (gm/dL)</b>	Range	11.5 – 20.7	8.2 – 17.8	12.8 – 18.6	0.000	HS
	Mean $\pm$ SD	15.8 $\pm$ 1.99	13.49 $\pm$ 2.42	16.04 $\pm$ 1.86		
<b>Platelets (x10<sup>3</sup>/cm)</b>	Range	112 – 268	38 – 237	168 – 213	0.000	HS
	Mean $\pm$ SD	192.74 $\pm$ 28.4	155.94 $\pm$ 44.92	191.33 $\pm$ 14.72		
<b>TLC (x10<sup>3</sup>/cm)</b>	Range	8.9 – 18.3	5.3 – 22	11.7– 19.2	0.000	NS
	Mean $\pm$ SD	13.20 $\pm$ 2.23	14.25 $\pm$ 04.72	15.27 $\pm$ 2.42		
<b>Immature neutroph. (x10<sup>3</sup>/cm)</b>	Range	1 – 7	3 – 20	3 – 9	0.000	HS
	Mean $\pm$ SD	2.9 $\pm$ 1.47	8.37 $\pm$ 03.94	5.89 $\pm$ 2.03		
<b>Total neutroph. (x10<sup>3</sup>/cm)</b>	Range	24– 45	32 – 69	29 – 40	0.000	HS
	Mean $\pm$ SD	34.56 $\pm$ 5.65	42.56 $\pm$ 9.25	34.44 $\pm$ 4.1		
<b>I/T ratio</b>	Range	0.3 – 0.18	0.08 – 0.31	0.1 – 0.23	0.000	HS
	Mean $\pm$ SD	0.08 $\pm$ 0.03	0.19 $\pm$ 0.06	1.17 $\pm$ 0.04		
<b>I/M ratio</b>	Range	0.3 – 0.22	0.08 – 0.45	0.11 – 0.29	0.000	HS
	Mean $\pm$ SD	0.09 $\pm$ 0.04	0.24 $\pm$ 0.09	0.2 $\pm$ 0.06		
<b>Degenerative changes</b>	No. (%)	0	3(7.3%)	0	0.000	NS
<b>HSS</b>	Range	0 – 2	0 – 5	0 – 3	0.000	HS
	Mean $\pm$ SD	0.42 $\pm$ 0.58	2.46 $\pm$ 1.36	1.22 $\pm$ 0.83		
<b>CRP (mg/L)</b>	Range	0 – 6	0 – 48	0 – 6	0.000	HS
	Mean $\pm$ SD	0.12 $\pm$ 0.85	7.17 $\pm$ 10.58	2.67 $\pm$ 3.16		
<b>SAA (<math>\mu</math>g/m)</b>	Range	0 – 9	8-245 – 6	4 – 17	0.000	HS
	Mean $\pm$ SD	3.16 $\pm$ 2.97	116.8 $\pm$ 54.04	7.78 $\pm$ 03.77		

HS = Highly significant    NS = Non significant

- The CRP level ranged from 0-6 mg/L with a mean of 0.12 $\pm$ 0.85 in control group, increased in septic group and ranged from 0-48 mg/L with a mean of

7.17 $\pm$ 10.58, and finally it ranged from 0-6 mg/L in non-septic group with a mean of 2.67 $\pm$ 3.16 (table 3).

When comparing the CRP level between septic and control group, there was a highly significant difference ( $p=0.000$ ), but no significant difference between septic and non-septic groups ( $p=0.079$ ), and between control and non-septic groups ( $p=0.309$ ) (table 3)

- As regard **Hematological scoring system of sepsis (HSS)**, there was statistically significant difference between septic and control group, and septic and non-septic group ( $p=0.000$ ) (table 3).
- Newborns of the septic group had a higher levels of **Serum Amyloid A (SAA)** ranged from 8-245  $\mu\text{g/ml}$ . with a mean of  $116.8 \pm 54/04$ , while in the control

group, it ranged from 0-9  $\mu\text{g/ml}$ , with a mean of  $3.16 \pm 2.97$ . In the non-septic group, it ranged from 4-17  $\mu\text{g/ml}$ , with a mean of  $7.78 \pm 3.77$  (table 3).

As regard SAA levels, there was a highly significant statistical difference between septic group and both non-septic and control groups ( $p=0.000$ ), while there was no statistically significant difference between control and non-septic groups ( $p=0.715$ ) (table 3).

A comparative study was done in between the mean values of different hematological parameters among the septic neonates with progress of illness.

**Table (4): Comparison of the laboratory data of the septic group when sepsis was first suspected (Reading A) and after 48 hours (Reading B).**

Variable		Suspected group (n=41)		P. value	Sig.
		Reading A	Reading B		
Hb (gm/dL)	Range	8.2 – 17.8	8 – 16	0.001	HS
	Mean $\pm$ SD	13.49 $\pm$ 2.42	12.5 $\pm$ 2.08		
Platelets ( $\times 10^3/\text{cm}$ )	Range	38 – 237	48 – 211	0.001	HS
	Mean $\pm$ SD	155.94 $\pm$ 44.92	138.24 $\pm$ 41.73		
TLC ( $\times 10^3/\text{cm}$ )	Range	5.3 – 22	4.2– 23	0.001	HS
	Mean $\pm$ SD	14.25 $\pm$ 04.72	15.45 $\pm$ 5.33		
Immature neutroph. ( $\times 10^3/\text{cm}$ )	Range	3 – 20	7 – 23	0.001	HS
	Mean $\pm$ SD	8.37 $\pm$ 03.94	11.88 $\pm$ 3.97		
Total neutroph. ( $\times 10^3/\text{cm}$ )	Range	32 – 69	29 – 72	0.001	HS
	Mean $\pm$ SD	42.56 $\pm$ 9.25	45.17 $\pm$ 8.56		
I/T ratio	Range	0.08 – 0.31	0.17 – 0.40	0.001	HS
	Mean $\pm$ SD	0.19 $\pm$ 0.06	0.26 $\pm$ 0.06		
I/M ratio	Range	0.08 – 0.45	0.2 – 0.67	0.001	HS
	Mean $\pm$ SD	0.24 $\pm$ 0.09	0.36 $\pm$ 0.11		
Degenerative changes	No. (%)	3(7.3%)	12(29.3%)	0.001	S
HSS	Range	0 – 5	2 – 6	0.10	HS
	Mean $\pm$ SD	2.46 $\pm$ 1.36	4.07 $\pm$ 0.99		
CRP (mg/l)	Range	0 – 48	12 – 192	0.001	HS
	Mean $\pm$ SD	7.17 $\pm$ 10.58	47.41 $\pm$ 44.98		
SAA ( $\mu\text{g/m}$ )	Range	8-245	0 – 73	0.001	HS
	Mean $\pm$ SD	116.8 $\pm$ 54.04	20.39 $\pm$ 17.40		

HS = Highly significant S = Significant

From table (4), there was a highly statistically significant different ( $p<0.001$ ) in all hematological parameters if we compare the results of Reading A and Reading B.

Among the septic neonates (n=41), a comparative study was done between the sensitivity of the results of SAA, CRP, and HSS (using cross tab) in diagnosing neonatal sepsis.

**Table (5): Comparison between laboratory results of SAA, CRP and HSS for early diagnosis of neonatal sepsis.**

	Reading A	Reading B
CRP (mg/L)	15/41 (36/6%)	41/41 (100%)
SAA ( $\mu\text{g/ml}$ )	40/41 (97.6%)	25/41 (61%)
Heamtological sepsis scoring	11/41 (26.8%)	32/41 (78%)

Statistical evaluation of SAA testing as an early diagnostic parameter of neonatal sepsis showed a sensitivity of 97.6%, specificity of 88.9%, positive

predictive value (PPV) of 97.6%, negative predictive value (NPV) of 99.9%, and test accuracy of 96%.

**Table (6): Accuracy of SAA in diagnosis of neonatal sepsis in suspected group (after using blood cultures as standard test).**

	Blood culture +ve	Blood culture -ve
SAA +ve	40 TP	1 FP
SAA -ve	1 FN	8 TN

#### 4. Discussion

In this study, analysis of maternal and obstetrical data revealed that the most important risk factors for sepsis was premature rupture of membrane (PROM) and maternal fever. Maternal fever was found in 14.6% of septic subgroup and PROM was found in 12.2% of them. This agrees with **Ahmed et al (2002)** who found PROM in 11.9% of cases' and maternal fever in 6.8% cases. In a study done by **Veskari et al. (2000)**, PROM was found in 19% of cases.

On clinical evaluation of the septic group, poor suckling, poor Moro, respiratory distress, and lethargy were the commonest clinical presentations at early suspicion of sepsis (Reading A) (54%, 49%, 42%, and 34% respectively), **Fathy et al. (2009)** found RD, poor Moro and suckling, and lethargy in 65%, 60%, and 55/0 respectively, that is slightly higher than our results.

Respiratory symptoms in this study was RD in 41.5% and apnea in 22% of cases. **Gotoll (2000)**, stated that, RD in the form of apnea, mild tachypnea, increase in oxygen requirement, and severe RD requiring mechanical ventilation (MV) occurs in 90% of infants with sepsis.

In the present study; gastrointestinal manifestations of sepsis, mainly in the form of abdominal distension were found in 29.3% of cases, while feeding intolerance and jaundice occurred in 31.7% and 14.6% of cases, respectively. **Cloherly et al. (2004)**, found abdominal distension in 45% of cases of neonatal sepsis. It might be due to NEC or toxic ileus which was reported to be frequently associated with sepsis (**Robertson, 2002**).

It was also found that the clinical manifestations of neonatal sepsis were increased gradually with progress of illness. In reading A, poor suckling, poor Moro, RD, and lethargy were found in 54%, 41%, 42%, and 34% of cases respectively. While in reading B (after 48 hours) the same manifestations were found in 90%, 95%, 73%, and 85% respectively. This agrees with **Weisman et al. (1992)** who stated that

the early symptoms and signs of neonatal sepsis are usually delayed and non-specific.

Blood culture is the gold standard method for isolation of the organisms, and it should be obtained before the initiation of antibiotics. There is no laboratory test having 100% sensitivity and specificity for diagnosis of neonatal sepsis with the exception of blood culture (**Buttery, 2002**)

In the present study, *Klebsiella* was the dominant organism isolated from the blood of infected group (39%), followed by *Staph. aureus* (14.6%), *Pseudomonas* (12.2%), Coagulase-negative *Staph.* (12.2%), *E.coli* (9.8%), *Enterobacter* (7.3%), and *Strept: pneumoniae* (4.9%).

These results were in agreement with other several studies done by **Fathy et al., (2009)**; **Badrawi et al., (2005)**; **Abou Hussein et al., (2005 and Hashim et al. (2004)**, who reported that *Klebsiella* is the commonest isolated organism in septic newborns, with a ratio ranging from 35-56% of all isolated organisms.

In our study, the findings of total leucocytic count (TLC) were not informative for the diagnosis of neonatal sepsis. There was non-significant difference in TLC between septic and control groups. This was in concordance with **Thurlbeck and Meintoch (2002)**, who stated that, TLC is the least useful index for sepsis because the normal range is so wide, varies with gestational and postnatal age.

In the present study, HSS was positive (score above 3) in only 26.85% of septic neonates when sepsis was first suspected, and became positive in 78% of them after 48 hours, not to be considered as an early marker for sepsis diagnosis.

This was in agreement with **Awad et al. (2002)**, who found a positive HSS in 13.3% of septic neonates when the sepsis was suspected~ and 73.3 % of the same cases after 48 hours.

In our study, there was a highly significant increase of CRP level between septic and control groups, and this agrees with the results of **Fathy et al. (2009)**, **Abou Hussien et al., (2005)**.

CRP was positive ( $>6$  mg/L) in 36.6% of septic neonates in reading A and 100% in reading B. This was in concordance with **Awad et al., (2002)**, who found positive CRP in 26.7% in early readings and became 100% after 48 hours.

In 2007, **Arnon et al.** found that SAA, is an early and accurate marker of neonatal early-onset sepsis (EOS).

In the present study, we tested the diagnostic accuracy and the dynamics of SAA during neonatal sepsis in term neonates.

The findings of our study revealed that serum concentrations of SAA were significantly elevated in septic group, compared to non-septic and control groups.

The mean value of SAA in septic group was ( $116.8 \pm 54$   $\mu\text{g/ml}$ ), compared to control group ( $3.16 \pm 2.97$   $\mu\text{g/ml}$ ), and this was proved highly statistically significant ( $p < 0.001$ ). This was in agreement with **Arnon et al. (2002)** who found the mean value of SAA among septic group ( $187.6 \pm 78.3$   $\mu\text{g/ml}$ ) compared to ( $10.2 \pm 8.3$   $\mu\text{g/ml}$ ) of the non-septic group. Also, **Arnon et al. (2005)** found that the median value of SAA among septic group was 122  $\mu\text{g/ml}$  which was significantly higher than the level of the control group 6  $\mu\text{g/ml}$ .

If we are comparing the results of SAA among the septic group when the sepsis was suspected (Reading A) and after 48 hours (Reading B), there was a highly statistical significant difference in between both readings. The mean value of SAA in reading A was  $116.8 \pm 54$   $\mu\text{g/ml}$ , while the mean value of SAA in reading B was  $20.4 \pm 17$   $\mu\text{g/ml}$ . This was in agreement with **Litmanovitz et al. (2007)**, who stated that SAA is more sensitive at onset of sepsis, rose earlier and in a sharper manner, had higher levels and returned faster to normal values in infants who recovered. Same results were found by, **Arnon et al., (2005)**, who found that the medians of SAA at sepsis onset was 122 (79-185  $\mu\text{g/ml}$ ) compared to 16 (4-29  $\mu\text{g/ml}$ ) 48 hours after sepsis onset.

Compared with CRP, SAA was positive ( $\geq 10$   $\mu\text{g/ml}$ ) in 97.6% of septic group in reading A and 61 % of cases in reading B, while CRP was positive ( $>6$  mg/l) in only 36.6% of septic group in reading A and 100% of cases in reading B.

This results was in agreement with the findings of **Litmanovitz et al. (2007)**, who stated that SAA was more sensitive than CRP at onset of sepsis. In a study done by **Arnon et al. (2007)**, SAA was sensitive in 96% of septic group at onset of sepsis, compared to 30% sensitivity of CRP at the same time.

In our study, at a cut-off value of SAA  $\geq 10$   $\mu\text{g/ml}$ , the sensitivity of measuring SAA level was 97.6%, specificity was 88.9%, positive predictive value

(PPV) was 97.6%, negative predicative (NPV) was 88.9%, and test accuracy was 96% in diagnosis of neonatal infection when the sepsis was suspected.

On the same cut-off value of SAA, **Arnon et al., (2007)** found a sensitivity 96%, specificity 95%, PPV 85%, and NPV 99% at onset of sepsis.

From the results, we can concluded that SAA seems to be an early, highly sensitive and specific marker for the diagnosis of neonatal sepsis at the first suspicion of infection, especially if compared to diagnostic accuracy of CRP and HSS at this time. The Quick and reliable use of SAA in early diagnosis of neonatal sepsis can be useful in; early initiation of antibiotic treatment, duration, response, and outcome after therapy.

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## Damage Assessment of Buildings Due to Different Parameters of Pipeline Deterioration

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**Abstract:** Due the high interaction between sewer pipelines deterioration and existing structures in urban areas, the operation of pipeline failure in urban areas draws much attention. In this study a thorough analysis of the pipeline failure influence in different soils on adjacent buildings was investigated. Numerical simulations were performed by means of the finite element program ANSYS/CivilFEM. The purpose of the coupled analyses (soil, pipeline and building in the same model) was to investigate the general mechanisms of soil structure interaction that occur in this type of problem. Each of these analyses produced a large amount of output data. This study highlights how the ground surface and building foundation displacements are used to estimate the damage category of buildings due to failure in pipeline. The variable parameters used to simulate the pipeline failure are pipeline settlement, position of settlement, burial depth, soil stiffness, infiltration of sewage and groundwater. For each case, results are presented as vertical and horizontal displacements of ground beneath building and estimated category of damage is calculated.

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**Keywords:** Soil structure interaction, Sewer, Pipeline, Deterioration, building damage.

### 1. Introduction

This paper investigates the interaction between the pipeline failure and adjacent structure. It also develops the coupling effect models of pipeline failure, soil, foundations and upper structure. A full three-dimensional finite element analysis, "ANSYS+CivilFEM", which takes into consideration the elasto-plastic behavior of the soil, the pipeline failure mechanisms and the presence of the structure, is employed to perform the study (Swanson 2007). The results of the models include the vertical settlement and horizontal displacement of the foundation of the adjacent structure.

Analysis of the pipeline-structure interaction problem is performed in two steps (steady state and pipeline failure state). The pipeline failure operation is modeled by either the settlement of pipes or reducing the stiffness of soil around the pipeline.

Settlement of structures, whether from nearby pipeline failure or other causes, can result in noticeable damage. Such damage can be significant and costly. Usually, the most settlement sensitive buildings are those with frames with masonry in-fill walls or masonry load bearing walls. Simplified criteria including "angular distortion", "deflection ratio" and "horizontal strain" have been used to assess such damage. By combining ground deformation patterns, well-known damage category criteria, strain superposition and critical strain concepts, the potential effects of building deformations can be estimated with a great accuracy. The report by Aye (2007) was used as a basic reference in ground

deformation prediction and building damage assessment. For cut-and-cover excavation zone, the work of Ruwanpura (2007), Clough (1990) was used whereas published papers of Burland (1977), Boscardin and Cording (1989) were applied for bored tunnels. The damage categories are based directly on the descriptions of damage provided in Table 1.

The cumulative tensile and principal crack widths were calculated from the output settlement and run within spreadsheets. The simple cumulative deformation was used directly considering that the buildings may have exhibited some initial cracking due to construction defects, thermal cracking, or from ageing. In addition, calculation of tensile cracks were calculated at the first bay of building (from 5.0 to 10.0 m), because it is the nearest place to pipe failure.

The numerical model result was used to estimate the effect of each of the main parameters that induce pipeline failure on the category of damage of adjacent buildings. These parameters include the pipeline settlement, position of settlement, burial depth, soil stiffness, infiltration of sewage and groundwater. The numerical modeling was previously adopted to analyze such problem for a practical case study by Metwally (2009) and fairly accurate results were achieved (A&A 2008).

### 2. Numerical Modeling

Figure 1 depicts the problem under consideration which is used to quantify the interaction between sewer pipeline and the reinforced concrete building. The pipeline is characterized by its

depth  $H$ , diameter  $D$ , pipe thickness  $e$ , while the building is modeled by a spatial reinforced concrete

framed structure with floor height 3.0 m and column's spacing 5.0 m in two directions.

**Table 1: Building damage classification after Burland (1977) and Boscarding and Cording (1989)**

Risk Category	Degree of Damage	Description of Typical Damage	Approximate Crack Width (mm)
0	Negligible	Hairline cracks	Null
1	Very Slight	Fine cracks easily treated during normal decoration	0.1 to 1
2	Slight	Cracks easily filled. Several slight fractures inside building. Exterior cracks visible	1 to 5
3	Moderate	Cracks may require cutting out and patching. Door and windows sticking	5 to 15 or a number of cracks > 3
4	Severe	Extensive repair involving removal and replacement of walls, especially over doors and windows. Windows and door frames distorted. Floor slopes noticeably.	15 to 25 but also depends on number of cracks
5	Very Severe	Major repair required involving partial or complete reconstruction. Danger of instability.	> 25 but depends on number of cracks

The behavior of the building is assumed to be linear-elastic. The soil behavior is assumed to be governed by an elastic perfectly-plastic constitutive relation based on the Mohr–Coulomb criterion with a non-associative flow rule.

Numerical simulations were performed by means of the finite element program ANSYS/CivilFEM. Analysis of the pipeline–structure interaction problem is performed with two steps (steady state and pipeline failure state). The first step (steady state) is concerned with the determination of initial stresses in the soil mass prior to the pipeline failure. It is performed using a finite element calculation considering the self-weight of both the soil and the structure. Displacements are reset to zero at the end of this stage; consequently, results referred to hereafter are due to the pipeline failure. The second step (pipeline failure state) deals with the numerical simulation for the failure of the pipeline in presence of the structure (Metwally 2004). The pipeline failure operation is modeled by the settlement of pipes or by reducing the stiffness of soil around the pipeline.

### 2.1. Full Three-Dimensional couple analysis

The full three-dimensional coupled approach is adopted in this research to study the influence of the pipeline failure on the building. The longitudinal

section of the pipeline is assumed to coincide with that of the building. The pipeline and structure characteristics are given by: pipeline diameter  $D=2.0$  m, pipe thickness  $e=0.2$  m, pipeline depth  $H=5.0$  m, the column's spacing in two directions  $=5.0$  m, and height of each level  $h=3.0$  m. Material properties for soil, lining and structure are listed in Tables 2 and 3.

Finite element analysis for the coupled model is carried out using the mesh presented in Figures 2. The finite element mesh was 30 m long, 12 m high and 30 m wide. Eight-noded brick elements were used to model the soil and the concrete pipe. The structure is modeled using eight-noded brick elements and four-noded shell. In the model, the number of pipes is 15, where the connections between the pipes are contact element. The contact element of pipes connection was taken no separation element. In this element (no separation contact), the two contact surfaces (target and contact surfaces) are tied, although sliding is permitted, elements for the frames and slabs respectively. The pipeline encased in a homogeneous soil mass. The contact element between the foundation of the building and the soil was taken rough element. In this element (rough contact), the two contact surfaces (target and contact surfaces) are not slipping, although separation is permitted.

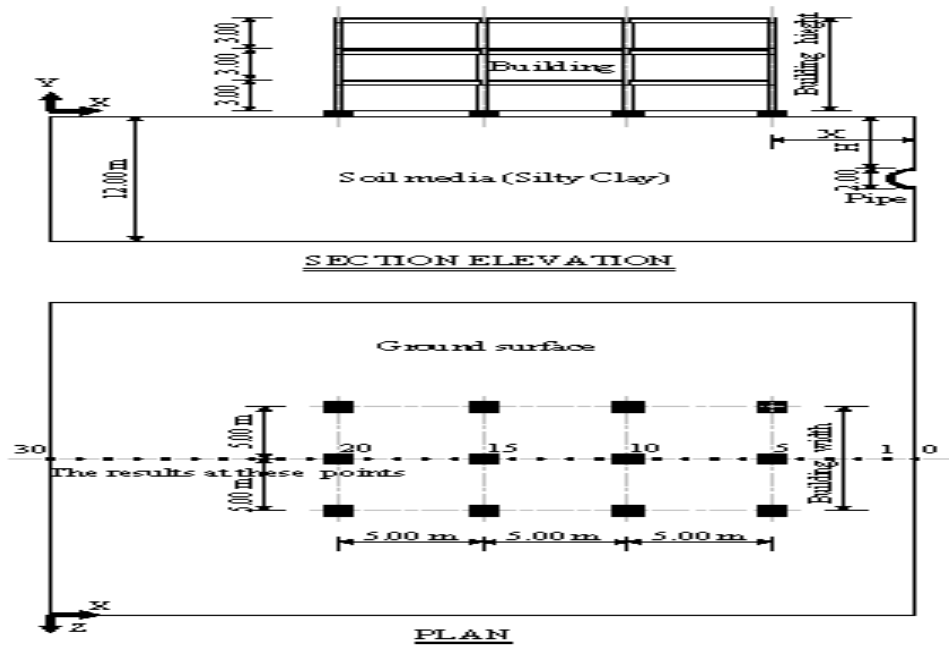
**Table 2: Soil and pipeline properties after Metwally (2009)**

Soil properties		Pipeline properties	
Soil elastic modulus $E_s$	2000 t/m <sup>2</sup>	Pipe diameter $D$ (interior)	2.00 m
Soil Poisson's ratio $\nu$	0.35	Wall thickness of concrete $e$	0.20 m
Soil cohesion $C$	2.00 t/m <sup>2</sup>	Pipe length $L_p$	2.00 m
Angle of internal friction $\phi$	30°	Number of pipes in pipeline	20 pipes
Density of soil over pipe $\gamma$	1.85 t/m <sup>3</sup>	Concrete elastic modulus $E_c$	3.5E6 t/m <sup>2</sup>
Soil height above crown $H_t$	5.0 m	Concrete Poisson's ratio $\nu_c$	0.20
$\mu$ (Between soil & pipes)	0.32	$\mu$ (Between pipes segments)	0.60

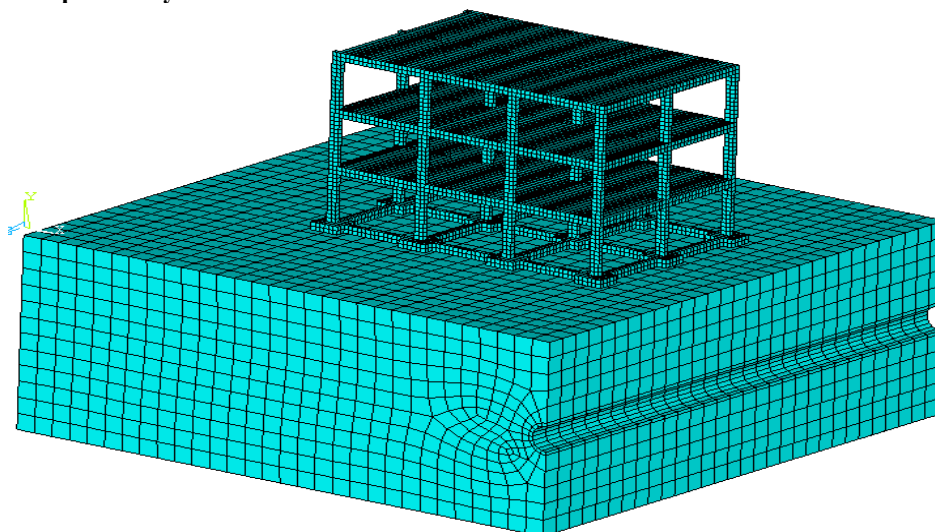
**Table 3: Structural material data after Metwally (2009).**

Properties	Notation & Unit	Building elements
Density	$\gamma$ (t/m <sup>3</sup> )	2.5
Compressive stress*	$f_c$ (kg/cm <sup>2</sup> )	90
Tensile stress*	$F_t$ (kg/cm <sup>2</sup> )	10.8
Shear stress*	$q$ (kg/cm <sup>2</sup> )	19
Young's modulus	$E$ (t/m <sup>2</sup> )	2.1E06
Poisson's ratio	$\nu$	0.20
compressive strain*	$\epsilon_c$	0.003
tensile strain*	$\epsilon_t$	0.003
Shear strain*	$\epsilon_s$	0.003

\*Allowable stress or strain.



**Figure 1 Geometry; (a) pipe-building-soil interaction geometry in the coupled analysis, (b) building geometry in the coupled analysis.**



**Figure 2 Finite element mesh adopted in the coupled analysis (R.C. building)**



**3. Parametric Study and Results:**

Many variables affect the behavior of the system. Some of them are:

- Values of pipeline settlement.
- Position of settlement in pipeline relative to the building.
- Burial depth and pipeline settlement.
- Soil stiffness changing above pipeline.
- Sewage infiltration.
- Groundwater saturation.

The purpose of the coupled analyses (soil, pipeline and building in the same model) was to investigate the general mechanisms of soil structure interaction that occur in this type of problem. Each of these analyses produced a large amount of output data. This section highlights how the ground surface and building foundation displacement changes due to failure in pipeline. Figure 3 illustrates the place

(ground surface) of results (vertical settlements and horizontal displacements). The cumulative tensile crack width and cumulative principle crack width were calculated (Table 4) from the output displacement to assess the building condition. The calculations of tensile cracks were worked within spreadsheets. Damage categories are based directly on the descriptions of damage provided in the Table 1. In all data analyses, critical cracking strain was not included as a criterion (i.e.  $\epsilon_c = 0\%$ ). The simple cumulative deformation was used directly considering that the buildings may have exhibited some initial cracking due to construction defects, thermal cracking, or from age. In addition, the calculation of tensile cracks were calculated at the first bay (from 5.0 to 10.0 m), where the first bay is the nearest place to the pipe failure.

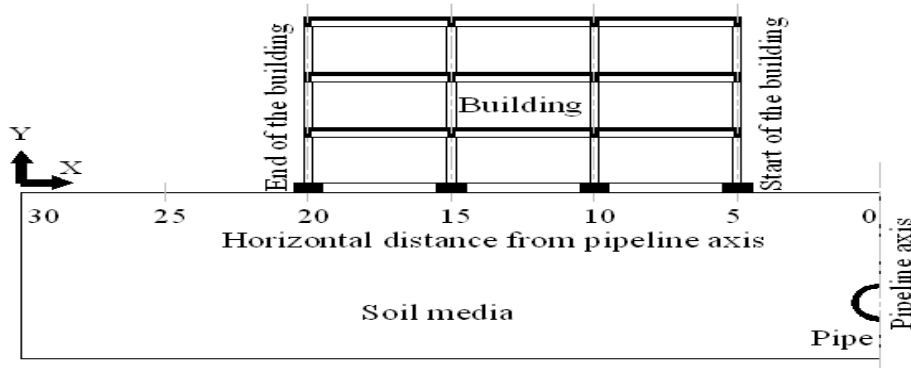


Figure 3 Schematic view of the model at place of results (displacements).

**4. Effect of Pipe Settlement on Buildings:**

**4.1 Effect of value of pipeline settlement:**

The influence of settlement in the pipelines is explained by considering three values of vertical settlement in the middle five pipes; 1% D, 3% D, and 5% D, where D is the pipe diameter. Tables 2 and 3 give the criteria of silty clay soil, pipe, and building

criteria respectively. Figures 4 and 5 show respectively the relations between the vertical and horizontal settlement of both ground surface and building (the building lies at distances from 5 to 20m from pipeline axis), and the pipeline settlement; we can find out that the minimum results are for minimum value of pipeline settlement.

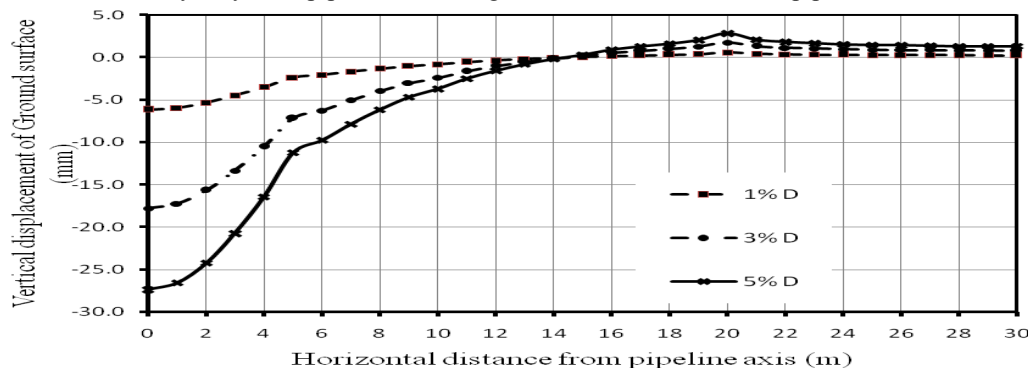
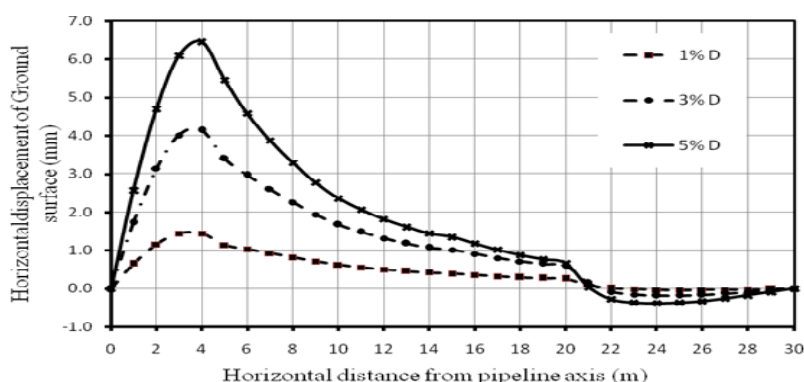


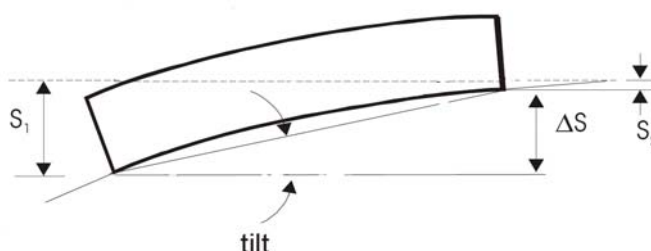
Figure 4 Effect of pipe settlement on vertical settlement of ground surface.



**Figure 5 Effect of pipe settlement on horizontal displacement of ground surface**

Table 4 illustrates the results for evaluating the potential damage category for in-fill walls and beams within frames due to different values of

pipeline settlement. The table shows the values of maximum and minimum vertical displacement, tilting angle  $\alpha$  (Figure 6) for the base of building.



**Figure 6 Definition of Tilting Angle  $\alpha$**

**Table 4: Evaluation of potential damage of building due to pipeline settlement**

Properties	Case			Unit
	1% D	3% D	5% D	
Max. Settlement (S1)	-2.4	-7.2	-11.2	mm
Min. Settlement (S2)	0.6	1.8	2.8	mm
Differential Sett. ( $\Delta S$ )	2.9	8.9	14.0	mm
Angle of Tilt $\alpha$	0.011	0.034	0.054	deg.
Cumulative Tensile crack width ( $C_t$ )	0.74	2.39	4.11	mm
Cumulative Principle crack width ( $C_p$ )	0.77	2.37	3.84	mm
Damage Category	Very Slight(1)	Slight(2)	Moderate(3)	

The results presented in previous table show the effect of pipeline settlement on the value of  $\alpha$  and the crack width, it is clear that the value of pipeline settlement plays an important role in building deformation and damage.

From the above, the major effect of the vertical settlement of pipeline on the increase of the deformations of adjacent buildings is within about 6 times the pipe diameter or two times of burial depth and slightly varying after this distance.

#### 4.2. Effect of settlement location relative to the building

The influence of settlement location in the pipelines is explained by considering three locations from vertical axis of symmetry for vertical settlement in the five pipes ;  $B=0$ ,  $B=1$  and  $B=2$ , where  $B$  is the horizontal shift in the symmetric axis of pipes settlement and equals 3 times the pipe diameter. The

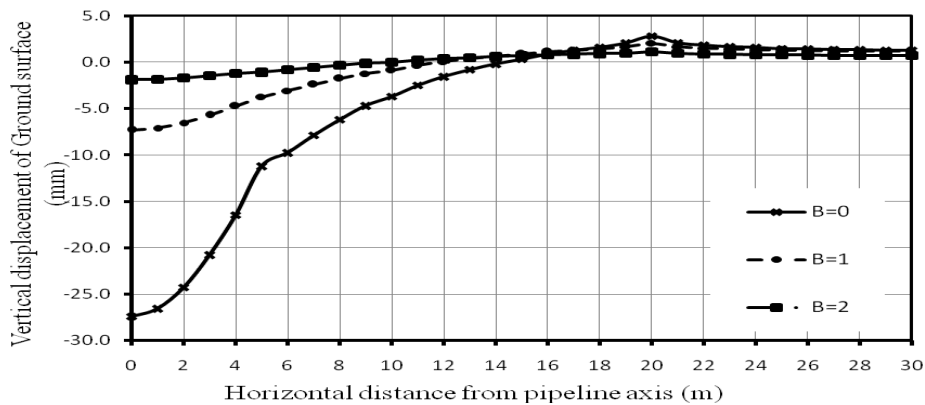
settlement value was taken 5% D (D is pipe diameter).

Figures 7 and 8 show respectively the relations between the vertical and horizontal

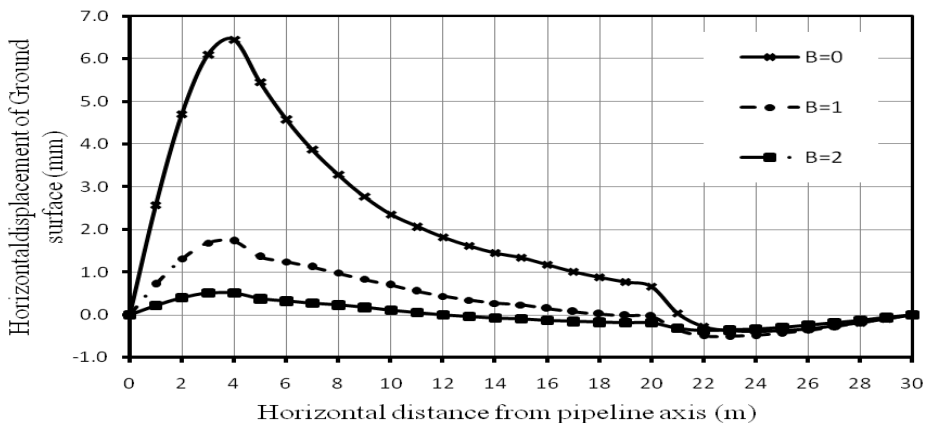
settlement of building and the horizontal location of pipeline settlement. From Table 5, we can find out that; the maximum results are for nearest location (B=0) of pipeline settlement.

**Table 5: Evaluation of potential damage of building due to the settlement location.**

Properties	Case			Unit
	B=2	B=1	B=0	
Max. Settlement (S1)	-1.1	-3.7	-11.2	mm
Min. Settlement (S2)	1.2	2.0	2.8	mm
Differential Sett. ( $\Delta S$ )	2.2	5.8	14.0	mm
Angle of Tilt $\alpha$	0.008	0.022	0.054	deg.
Cumulative Tensile crack width ( $C_t$ )	0.5	1.1	4.1	mm
Cumulative Principle crack width ( $C_p$ )	0.5	1.3	3.8	mm
Damage Category	Very Slight (1)	Slight (2)	Moderate (3)	



**Figure 7 Effect of settlement location on vertical settlement of ground surface.**



**Figure 8. Effect of settlement location on horizontal displacement of ground surface.**

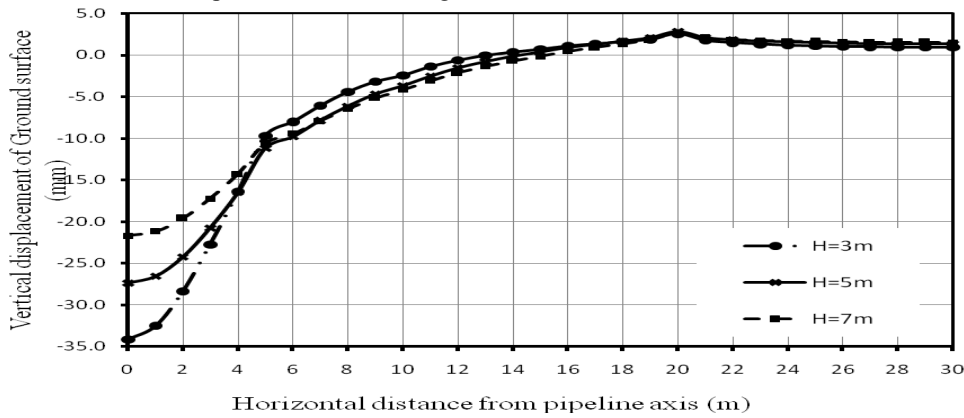
**4.3. Effect of burial depth**

The effect of burial depth is demonstrated by considering three heights of soil above the pipe; 3,

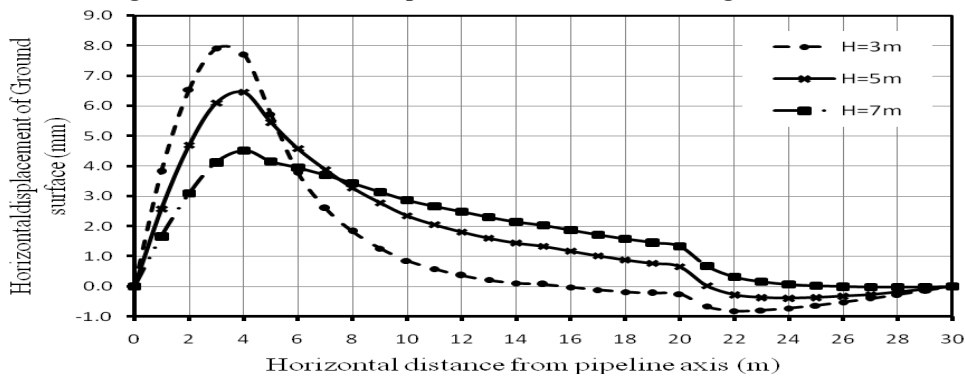
5, and 7 m of silty clay soil. The settlement value was taken 5% D (D is pipe diameter).

Figures 9 and 10 illustrate the effect of burial depth and pipeline settlement on the vertical settlement and horizontal displacement of building;

we can notice that; increasing the height of soil above the pipe causes slight decrease in the building differential settlement.



**Figure 9 Effect of burial depth on vertical settlement of ground surface.**



**Figure 10 Effect of burial depth on horizontal displacement of ground surface.**

From Table 6, we can find out that; the maximum results of building deformation and

damage are for smallest burial depth of pipeline settlement.

**Table 6: Evaluation of potential damage of building due to burial depth of pipes**

Properties	Case			Unit
	H=3m	H=5m	H=7m	
Max. Settlement (S1)	-9.7	-11.2	-10.6	mm
Min. Settlement (S2)	2.6	2.8	2.5	mm
Differential Sett.( ΔS)	12.3	14.0	13.1	mm
Angle of Tilt α	0.047	0.054	0.050	deg.
Cumulative Tensile crack width (C <sub>t</sub> )	5.6	4.1	2.1	mm
Cumulative Principle crack width (C <sub>p</sub> )	4.4	3.8	2.7	mm
Damage Category	Moderate (3)	Moderate (3)	Slight (2)	

### 5. Effect of the Soil Stiffness Changing Above Pipeline on Building

The case of soil stiffness changing above pipeline may takes place from the deteriorated pipeline. This case of deterioration may be failure in pipeline or separation of joints between pipes, which leads to soil infiltration to the pipes.

The influence of the soil stiffness changing above pipeline is explained by considering three values of soil stiffness for the part of soil, which it is above the pipeline. These values are relative to the

value of the existing soil stiffness; 0.25E, 0.50E, and 0.75E.

Figures 11 and 12 shows the relation between the vertical settlement and the horizontal displacement of building, and the soil stiffness changing above pipeline. From Table 7, we can find out that the building deformation and damage decrease with increasing the soil stiffness above the pipeline. In addition, the maximum results are from soil with relative stiffness 0.25E relative to the original soil.

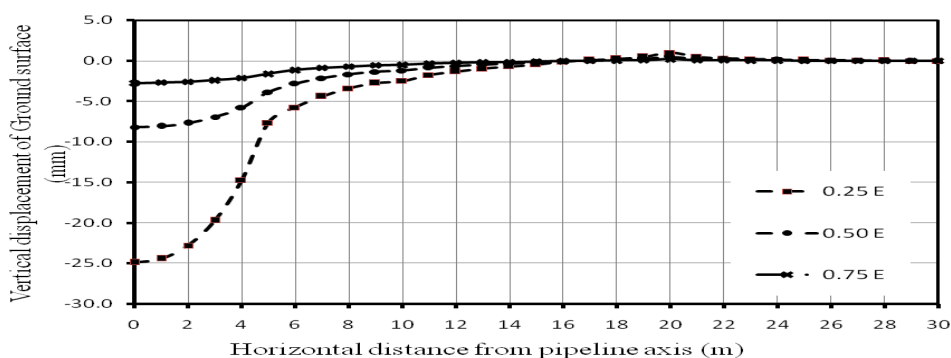


Figure 11 Effect of soil stiffness on vertical settlement of ground surface.

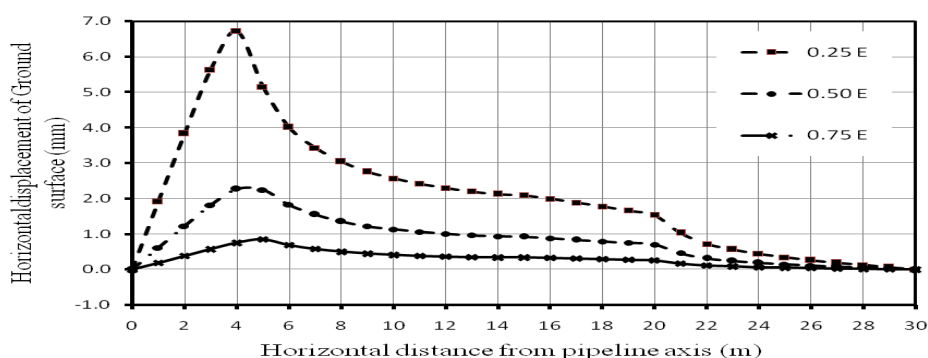


Figure 12 Effect of soil stiffness on horizontal displacement of surface.

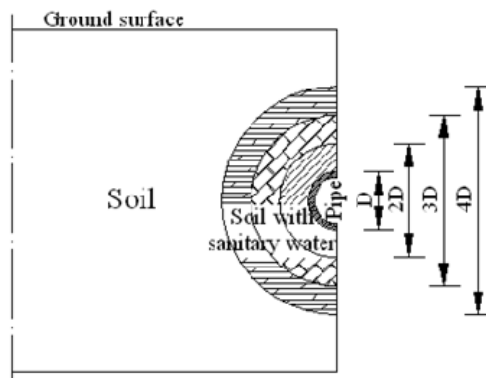
Table 7 Evaluation of potential damage of building due to change of soil stiffness.

Properties	Case			Unit
	0.75 E	0.50 E	0.25 E	
Max. Settlement (S1)	-1.6	-3.9	-7.6	mm
Min. Settlement (S2)	-0.1	-0.2	-0.4	mm
Differential Sett.( ΔS)	1.5	3.7	7.2	mm
Angle of Tilt α	0.006	0.014	0.027	deg.
Cumulative Tensile crack width (C <sub>t</sub> )	0.5	1.3	3.1	mm
Cumulative Principle crack width (C <sub>p</sub> )	0.6	1.5	3.0	mm
Damage Category	Very Slight (1)	Slight (2)	Moderate (3)	

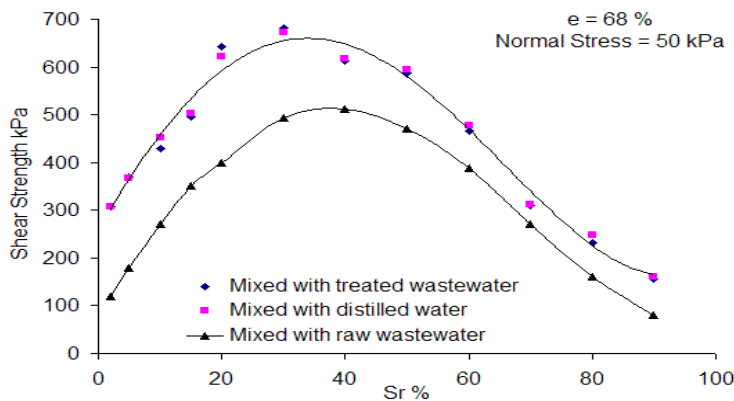
**6. Effect of Sewage Water on Building**

The influence of the sewage water around pipeline is explained by considering three areas with soil saturated with sewage water around the pipe. These areas are 3 circular rings around the pipe with thicknesses  $D/2$ ,  $D$  and  $3D/2$  respectively (figure 13), where  $D$  is the pipe diameter. Maaitah et al. (2005) have been concluded in their research that the raw

wastewater has a negative effect on the shear strength, compaction process and soil swelling. They reported the relationship between the degree of saturation and shear strength for soils that mixed with raw wastewater, treated wastewater and distilled water as show in Figure 14 where the effect of sanitary water on soil properties was illustrated (Ana, 2007).



**Figure 13 Schematic View of the Problem.**



**Figure 14 Unsaturated shear strength versus degree of saturation (Maaitah 2005).**

Figures 15 and 16 shows the relation between the vertical settlement and the horizontal displacement of building and the saturated soil with wastewater around the pipeline. From Table 8, we

can find out that the building deformation and damage increases with increasing the area of sewage water around pipeline.

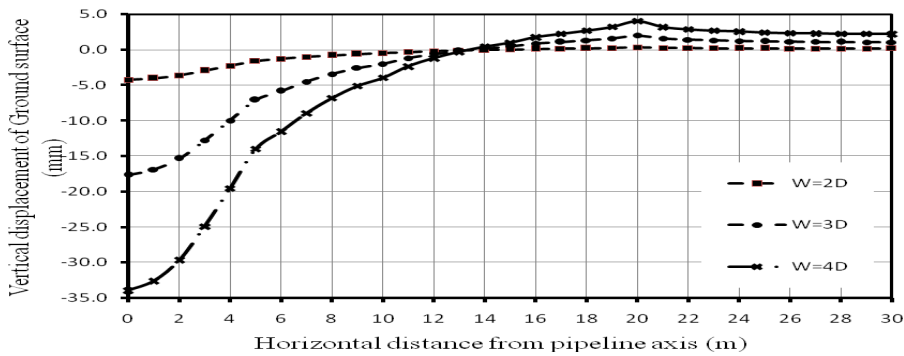


Figure 15 Effect of sewage water on vertical settlement of ground surface.

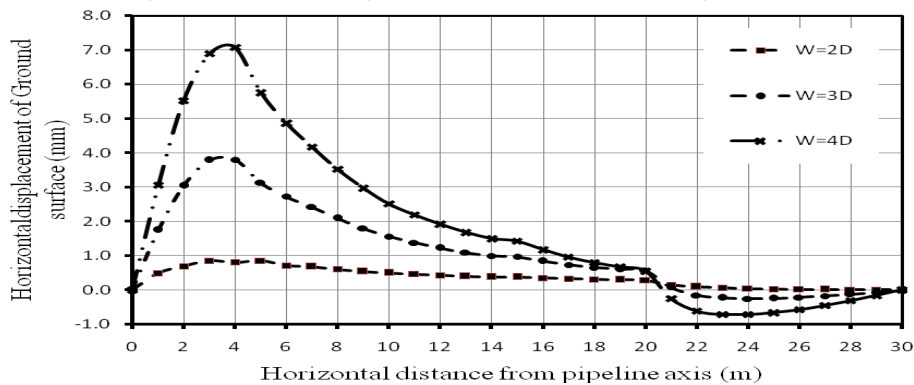


Figure 16 Effect of sewage water on horizontal displacement of ground surface.

Table 8: Evaluation of potential damage of building due to sewage water.

Properties	Case			Unit
	W=2D	W=3D	W=4D	
Max. Settlement (S1)	-1.6	-7.1	-14.0	mm
Min. Settlement (S2)	0.0	0.5	1.0	mm
Differential Sett. ( $\Delta S$ )	1.6	7.6	15.0	mm
Angle of Tilt $\alpha$	0.006	0.029	0.057	deg.
Cumulative Tensile crack width (C <sub>t</sub> )	0.5	2.2	4.3	mm
Cumulative Principle crack width (C <sub>p</sub> )	0.6	2.7	5.3	mm
Damage Category	Very Slight (1)	Slight (2)	Moderate (3)	

**7. Effect of Groundwater on Building**

The influence of groundwater is explained by considering three types of silty clay soil with different degrees of saturation; 50%, 70%, and 90%. The More-Coulomb parameters (cohesion) of the three soils are: 1.8, 1.0, and 0.5 t/m<sup>2</sup>, respectively (AL-Shayea, 2001). In addition, the angles of internal friction for the three soils are 27, 16, and 8 degree (C-CORE, 2003). Figures 17 and 18 show the relation between the vertical settlement and horizontal

displacement of building and the degree of saturation in soil; we can find out that the minimum results are for 50% saturated soil relative to the saturated soil. In addition, due to large values of settlement the number of cracks is high which increases the category of damage even for very low differential settlement. From Table 9, we can find that the building deformation and damage increase as the groundwater saturation degree increases due to decrease in cohesion value.

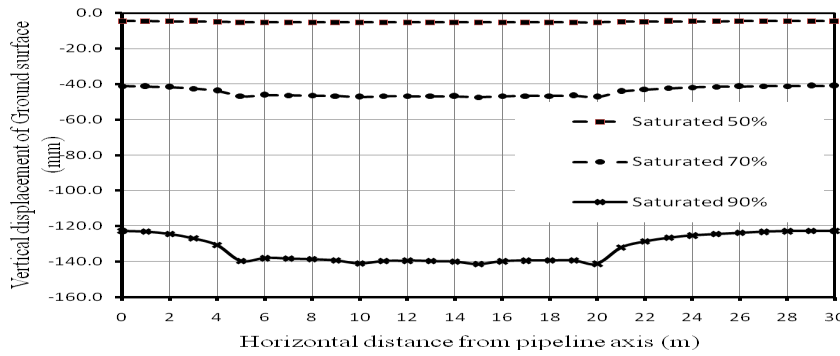


Figure 17 Effect of groundwater on vertical settlement of ground surface.

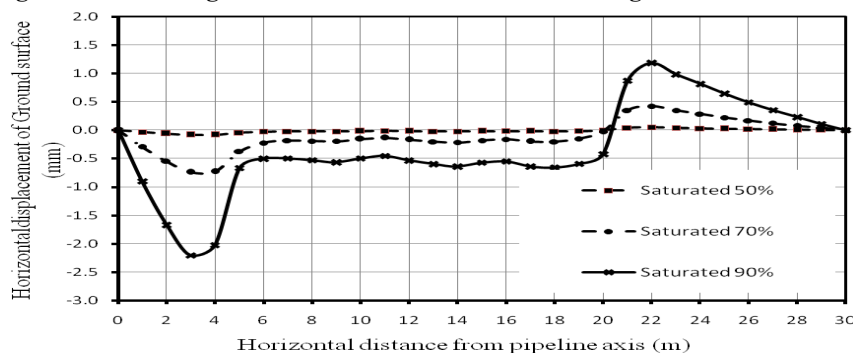


Figure 18 Effect of groundwater on horizontal displacement of ground surface.

Table 9. Evaluation of potential damage of building due to groundwater.

Properties	Case			Unit
	Saturated 50%	Saturated 70%	Saturated 90%	
Max. Settlement (S1)	-5.2	-46.8	-139.7	mm
Min. Settlement (S2)	-5.3	-47.3	-141.2	mm
Differential Sett. ( $\Delta S$ )	-0.1	-0.5	-1.5	mm
Angle of Tilt $\alpha$	0.000	-0.002	-0.006	deg.
Cumulative Tensile crack width ( $C_t$ )	0.0	0.0	0.0	mm
Cumulative Principle crack width ( $C_p$ )	0.0	0.3	0.6	mm
Damage Category	Slight (2)	Moderate (3)	Severe (4)	

**8. Conclusion**

We can conclude from results that the damage of adjacent buildings due to pipeline failure is increased by:

1. The increase of pipelines settlement and how near is its location.
2. The decrease of the position of settlement with respect to the building.
3. The decrease of soil stiffness above pipeline.

4. The increase of area of exfiltration of sewage water around pipelines.

5. The increase of the degree of soil saturation due to groundwater.

6. The decrease of burial depth of pipes.

The major effect of all above factors occurs within soil width equal about six times the pipe diameter or two times the burial depth from vertical axis of pipelines.



Therefore, this part of soil should be monitored on a regular basis for early prevention of buildings damage and pipeline deterioration.

The presence of building increases the soil stiffness in contact to the footings. That is why the rate of variation of displacement is decreased below the building.

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**Effects of knocking-down Nucleostemin gene on apoptosis of HL-60 cells *in vitro***

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**Abstract:** *Objective.* To explore whether the apoptosis of leukemia cells could be induced by knocking-down Nucleostemin (NS) gene *in vitro*. *Methods.* HL-60 cells were taken as a model, and was directly transfected with Nucleostemin specific short hairpin RNA (NS-shRNA). Sequences that unrelated with NS gene were taken as a control. The blocking effect of NS-shRNA was detected by Reverse Ttranscription PCR (RT-PCR), the morphology changes in culture state were observed under inverted microscope, and the changes of cell shape and nucleus were detected by Wright-Giemsa staining. The amount of apoptotic cells were assayed by flow cytometer (FCM) and Tunel technique, and the positive rate of apoptosis was determined in the meanwhile. *Results.* Two NS-shRNA were synthesized *in vitro*, and the more effective one was selected to be transfected into HL-60 cells. The blocking rate of NS-mRNA reached up to 74.94%. 48 hours after transfection, nuclear fragmentations and “apoptosis bodies” showed in HL-60 cells, observed by Wright-Giemsa staining. The apoptosis rates in transfected groups were (25.32±3.06)% and (27.3±3.21)% respectively, but were only (3.12±0.38)% and (3.30±1.52)% in control group, detected by FCM and Tunel technique. The difference between the treated group and the control group was significant ( $P<0.01$ ). *Conclusion.* The apoptosis of HL-60 leukemia cells can be induced by the silencing of NS gene *in vitro*, which means NS could be a candidate gene for the theoretical therapy principle of leukemia.

[YUE Bao-hong, WANG Yuan-yuan, YU Li-na, FU Shu-zhen, ZHANG Qin-xian, KAN Quan-cheng. **Effects of knocking-down Nucleostemin gene on apoptosis of HL-60 cells *in vitro***. Life Science Journal. 2011;8(3):290-294] (ISSN:1097-8135). <http://www.lifesciencesite.com>.

**Keywords:** Nucleostemin; apoptosis; short hairpin RNA; leukemia; HL-60 cell line

## 1. Introduction

Nucleostemin(NS) was originally identified by Tsai and McKay in 2002, and abundantly expressed in both embryonic and adult CNS stem cells in culture. It is also present in embryonic stem cells, primitive cells in bone marrow, and cancer cells. Later, it is found that NS also exists in some human solid tumor cells, but not in the differentiated cells. NS protein weights 61kD, it contains a highly basic region in the N terminus, two GTP-binding motifs, a coiled-coil domain and an acidic domain in the carboxyl end. NS locates in the nucleolus, and its expression is abruptly down-regulated during differentiation prior to terminal cell division<sup>[1,2]</sup>. NS plays a significant role in the self-renewal and infinite proliferation state of stem cells and malignant tumor cells, also, it probably participate in the G2/M transition as a key regulation factor<sup>[1,8,9]</sup>. Previous studies of our research group had certified the high expression of NS gene and NS protein in leukemia cells<sup>[3]</sup>. RNA interference(RNAi) technique<sup>[4,5]</sup> is used in our present study. In order to

inhibit the expression of NS gene, we synthesized Nucleostemin specific short hairpin RNA (NS-shRNA) *in vitro*, and directly transfected NS-shRNA into HL-60 cells, then we observed the changes of the apoptosis induced in HL-60 cells, and approached the apoptotic state after targetingly knocked down NS gene in leukemia cells. This research will be helpful for us to understand further the function of NS, and also support a new strategy to treat leukemia by using RNAi technique.

## 2. Materials and Methods

### 2.1 Cell culturing

The leukemia cell line of HL-60 was kindly provided by Shanghai Institutes for Cell, Chinese Academy of Medical Science, and was conserved by the Department of Histology and Embryology of Zhengzhou University. Cells were cultured in RPMI1640 medium, supplemented with 10% fetal bovine serum (containing 100 U/ml penicillin and 100µg/ml streptomycin) at 37 °C with 5% CO<sub>2</sub>. The culture medium was changed on alternate days.

## 2.2 Designing and synthesizing of NS-shRNA

Three variants (variant, NM014366, NM206825, NM206826) of complete cDNA in human were retrieved from NCBI GenBank Web site, they have a common sequence of 1833bp. Other homologous coding sequences and expressed sequence tag (EST) homologous sequences were discarded according to the principals of siRNA designing. Finally, two of 21 bp sequences were determined as targeting cDNA sequences (below):

cDNA-NS-1: 5' -AAGCTGAGCTAAGGAAACAGA-3'

cDNA-NS-2: 5' -AAGCTAGGAAAGACCCAGGA-3'

The corresponding RNA sequences are shown as follows:

RNA-NS-1: 5' -UCUGUUUCCUUAGCUCAGCUU-3'

RNA-NS-2: 5' -UCCUGGGUCUUUCCUAGGCUU-3'

The shRNA contains 19 fully paired bases. These bases constitutes the sense strand and the antisense strand of interfering RNA, and the two strands are connected by a 9 bp loop [aaguucucu] and ended in a 2-uridine 3'-overhang. The total length of the designed NS-shRNA was 49 bp. The sequences of the two NS-shRNA are listed as follows:

NS-shRNA- 1:

5'-GCUGAGCUAAGGAAACAGA ucucuugaa  
UCUGUUUCCUUAGCUCAGCUU-3'

NS-shRNA- 2:

5'-GCCUAGGAAAGACCCAGGA aaguucucu  
UCCUGGGUCUUUCCUAGGCUU-3'

To synthesize NS-shRNA, first of all, the complementary single DNA templates for NS-shRNA, which contained T7 promoter sequence, were constructed *in vitro*, and then they were annealed to generate double DNA template. 1  $\mu$ l of NS-shRNA products were fractioned in 25g/L of agarose gel electrophoresis while 1  $\mu$ l and 0.5  $\mu$ l of the annealed DNA template were taken as the control, so as to detect the synthesizing effect of NS-shRNA.

## 2.3 Transfection of NS-shRNA into HL-60 leukemia cells

Logarithmic growth phase cells were adjusted to the density of  $4 \times 10^5/\mu$ l with whole medium, then aliquoted into 6-well plates for 2.5 ml per well. Cells were randomly grouped into the transfected groups (R<sub>1</sub> group were treated by NS-shRNA-1, and R<sub>2</sub> group were treated by NS-shRNA-2) and the control groups (C<sub>1</sub> were only treated by transfection reagent as blank control, and C<sub>2</sub> were treated by unrelated siRNA sequences as negative control). Preparing for the transfection, 7.5  $\mu$ l Code Breaker siRNA transfection agent (Promega Corporation, Madison in Wisconsin, USA. <http://www.promega.com>) was added into 625  $\mu$ l non-serum medium, the transfected group was added NS-shRNA to the final concentration of 10 nmol/, according to preliminary experiment, while the control

group was added non-related shRNA.

## 2.4 Blocking effect on NS-mRNA in HL-60 cells after transfection

48 hours after transfection, total RNA was extracted from each group for Reverse Transcription PCR(RT-PCR), and then the image analysis was carried out after electrophoresis, the NS-shRNA blocking effect was calculated by the decrease of NS-mRNA expression. The forward primer of NS gene was 5'-AAAGCCATTCGGGTTGGAGT-3', and the reverse primer was 5'-ACCACAGCAGTTTGGCAGCAC-3', with the expected size of 418 bp.  $\beta$ -actin gene was taken as internal control with the expected size of 315 bp, its forward primer was 5'-TCCTGTGGCATCCACGAAACT-3', and the reverse primer was 5'-GAAGCATTGCGGTGGACGAT-3'.

## 2.5 Observation of apoptosis in culture state by inverted microscope

After routinely cultured for 24 hours, cell morphology was observed under the inverted microscope in culture state both in the transfected groups and control groups.

## 2.6 Detection of apoptosis by Wright-Giemsa staining

Cells were harvested 48 hours after transfection, then centrifuged to condense and smeared. The morphology and karyomorphism of HL-60 cells were observed by Wright-Giemsa staining and micrography.

## 2.7 Observation of apoptosis by flow cytometry (FCM)

After routinely cultured for 24 hours, cells both in transfected groups and control groups were harvested and centrifuged, then treated according to the operating instruction of Annexin V-FITC Kit, and washed twice by PBS which precooled to 4°C, then 250  $\mu$ l 1:4 diluted binding buffer was added to reconstitute the cells, and the cells were adjusted to the density of  $1 \times 10^6/\text{ml}$ . 100  $\mu$ l suspension was added into the 5 ml flow tube, then 5  $\mu$ l Annexin V/FITC and 10  $\mu$ l 20  $\mu$ g/L propidium iodide (PI) were added and mixed, the solution was incubated in dark area for 15 minutes, 400  $\mu$ l PBS was added into the reaction tube, then detected by flow cytometry (FCM) and analyzed by Cell quest 1.2 software.

## 2.8 Detection of apoptosis by dUTP nick end labeling (Tunel) technology

48 hours after transfection, cells in each group were harvested and smeared on the anti-run slides that enveloped by APES, and washed three times with PBS,

then incubated with endogenous peroxidase blocking solution (0.3% H<sub>2</sub>O<sub>2</sub> methanol solution) for 30 minutes, and again washed 3 times with PBS, then incubated with penetration solution in ice bath for 2 minutes, and then treated according to the instruction manual of Tuel Kit.

### 2.9 Statistical analysis

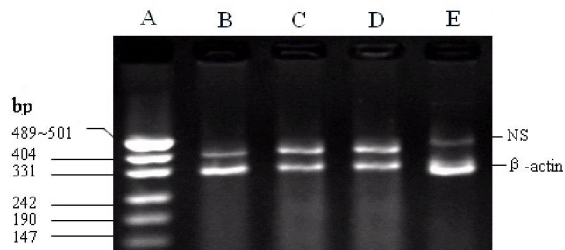
All experimental data were processed by SPSS 13.0 software. The experimental variables were determined using ANOVA or *t*-test. Data was reported as  $\bar{x} \pm SD$ , statistical significance was set *P* less than 0.05.

## 3. Results

### 3.1 NS-mRNA and NS protein changes after treated 48 hours with NS-shRNA

#### 3.1.1 NS-mRNA change

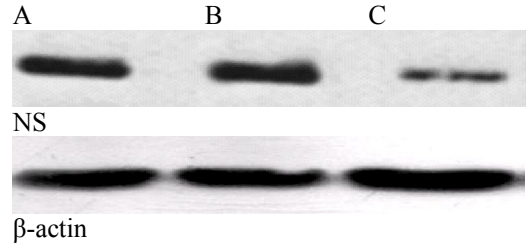
Cells were harvested for RT-PCR after incubated with NS-shRNA for 48 hours. According to scanning gray scale analysis, the related score were 0.826, 0.809 in C1 group and C2 group, and 0.503, 0.207 in R1 (NS-shRNA-1) and R2 (NS-shRNA-2) group. Compared with the control groups, the expression of NS-mRNA was significantly down-regulated after transfected with NS-shRNA-1 and NS-shRNA-2, with the inhibiting rates of 39.10% and 74.94%, and the more effective one was NS-shRNA-2, so it was selected for further experiments (Figure 1).



**Figure 1. RT-PCR result of human leukemia HL-60 cells treated 48 hours with NS-shRNA (A, DNA Mark; B, R1, treated by NS-shRNA-1; C, C1, blank control; D, C2, negative control; E, R2, treated by NS-shRNA-2).**

#### 3.1.2 NS protein change

Cells were harvested for Western blotting after transfected for 48 hours, the expression of NS protein was significantly lower than the blank and negative control group. The figure of Western blotting has been shown as below (figure 2). Then the gel was analyzed by gray-scale scanning. The gray-scale result of the negative control group was supposed 1.000, then the result of the other two groups were detected respectively, and the results were shown in the table below (table 1). Finally, the inhibition rate was calculated by the formula:  $(1.000 - 0.479) \times 100\% = 52.1\%$



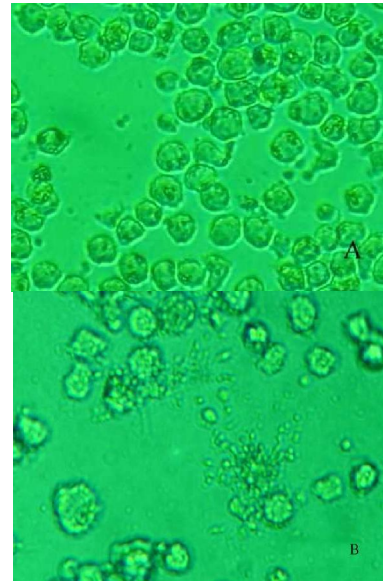
**Figure 2. Detecting of NS protein by Western blotting in HL-60 cells transfected for 48 hours (A, blank control; B, negative control; C, transfected group).**

**Table 1. Inhibition rate of NS protein in HL-60 cells transfected for 48 hours**

Group	Gray-scale result	Inhibition rate of NS protein (%)
Blank control	1.053	\
Negative control	1.000	0
Transfected group	0.479	52.1

### 3.2 Morphology changes observed in culture

Some cells in transfected group were disintegrated or broken 24 hours after incubated with NS-shRNA-2, vesiculose projections could also be seen, the nuclei disappeared, and the fragments spread around radially (Figure 3).

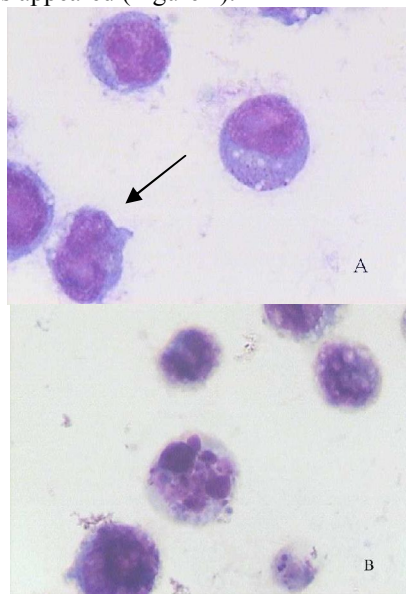


**Figure 3. The cataplasms of HL-60 cells dispersed radially after transfected with NS-shRNA-2 for 24 hours (1 000×) (A, HL-60 cells in control group; B, HL-60 cells transfected for 72 hours).**

### 3.3 Morphology changes detected by Wright-Giemsa staining

24 hours after transfection, nuclear fragmentations and "apoptosis" appeared in a few cells, and the

amount of nuclear fragmentations were larger. By the 48th hour, there were more apoptotic cells and more “apoptosis”, and the amount was larger with time going on. The apoptotic cells showed some features as follows: cells shrunk and became smaller, the nuclei were broken, cell debris with nuclear fragmentations that varying in shape and size could be seen easily, apoptosis appeared (Figure 4).



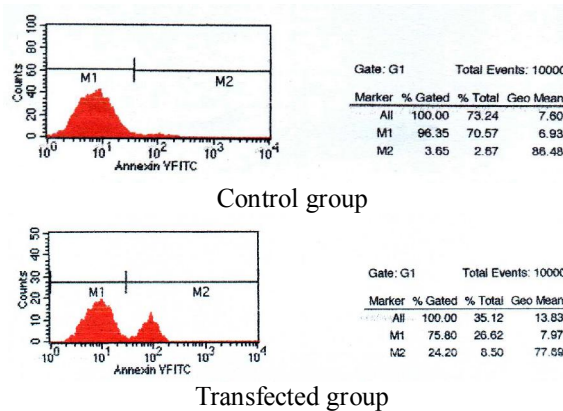
**Figure 4.** The apoptosis characters in HL-60 cells after transfected with NS-shRNA-2 (1000×, Giemsa staining, the arrows indicate the apoptosis characters) (A, HL-60 cells in the control group; B, HL-60 cells transfected for 72 hours).

**3.4 Detection of apoptosis by flow cytometry (FCM)**

Cells were collected for FCM after transfected with NS-shRNA-2 for 24 hours, and FCM Annexin V FITC was used to detect the apoptosis rate (reported as  $\bar{x} \pm SD$  according to three repeated researches) of HL-60 cells. The apoptosis of HL-60 cells was induced by down-regulation of NS protein, which was caused by the transfection of NS-shRNA, and the apoptosis rate increased from (3.12±0.38)% to (25.32±3.06)%,  $P < 0.05$ , the difference was statistically significant between the two groups (Figure 5, Table 2).

**3.5 Detection of apoptosis by TUNEL technology**

Cells both in the transfected group and the control group were harvested and detected by TUNEL technology 48 hours later, and the positive cell number and positive rate were both calculated. The apoptosis inducing effect of NS-shRNA-2 was significant, the difference was statistically significant between the two groups (Figure 6, Table 3).

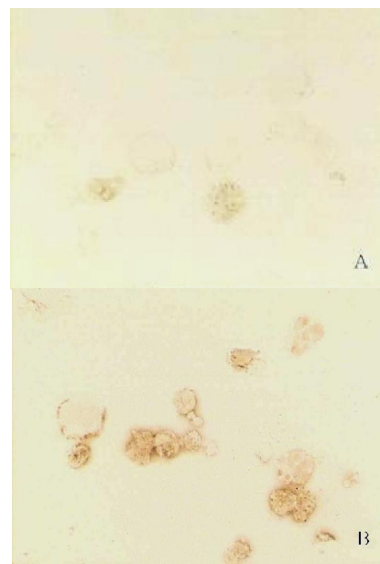


**Figure 5.** The apoptosis rate of HL-60 cells transfected with NS-shRNA-2 for 24 hours (%).

**Table 2.** The apoptosis rate of HL-60 cells transfected with NS-shRNA-2 for 24 hours (%)

Group	Apoptotic cell numbers	Non-apoptotic cell numbers	Apoptosis ( $\bar{x} \pm SD$ ) %
Control group	312	9688	(3.12±0.38)
Transfected group	2532	7468	(25.32±3.06) *

\*  $P < 0.01$ , compared with control group, examined by four table  $\chi^2$  test.



**Figure 6.** HL-60 cells apoptosis induced by NS-shRNA-2 with TUNEL method (400×). A: Control group; B: Transfected group.

**Table 3.** Detection results of cell apoptosis induced by NS-shRNA-2 with TUNEL method

Group	Total cell numbers	Positive cell numbers	Apoptosis rate (%)
Control group	300	10	(3.30±1.52)
Transfected group	300	82	(27.30±3.21) *

\*  $P < 0.01$ , compared with control group, examined by  $t$  test.

#### 4. Discussion

The products of Nucleostemin gene are p53-binding protein with molecule weight of 61 KD. NS protein is probably a specific regulatory factor responsible for stem cells and cancer cells acrossing G<sub>2</sub>/M checkpoint, so NS may play an important role in cell proliferation and differentiation, and keep cells in non-differentiated state<sup>[1,8,9]</sup>. Our previous studies had discovered that continuous overexpression of NS gene and NS protein existed in leukemia HL-60 cells, which has close relationships with the occurrence and development of acute leukemia<sup>[3-7]</sup>. RNAi (RNA interference) is a process of post-transcriptional gene silencing mediated by small double-stranded RNA (dsRNA), which can inhibit the expression of target gene with its specificity, efficiency and rapidity<sup>[10-12]</sup>. We designed and constructed two NS specific shRNA, and successfully transfected them into HL-60 cells, respectively. The RT-PCR showed that NS gene was proved to be down-regulated and NS-shRNS-2 was more effective. Probably this is because the "position effect" which means different NS-shRNA targets different site of NS gene. But this effect is still unclear up to now. Some of the cells treated with NS-shRNA shrunk or broke into pieces, the nuclei were disappeared, karyorrhexis and "apoptosis bodies" appeared, and the amount of "apoptosis bodies" was larger as time went on, observed in culture or by Wright-Giemsa staining. Apoptosis rate increased according to FCM and Tunel methods, showing the existence of apoptosis, which suggested that cell apoptosis could be induced by the blocking of NS gene and NS protein in HL-60 leukemia cells. Tsai and Liu had experimented on solid tumor cells, and discovered that while inhibiting the expression of NS gene and NS protein, the reproductive activity would be weaken in the meanwhile, and part of the cells quit their cell cycles<sup>[1,2]</sup>, and in our previous studies, we treated HL-60 cells with NS-shRNA, and the results were similar, so in such a case, if the weaken of cell reproductive activity is related with the increase of apoptosis, is a question deserves deep consideration. Inducing apoptosis in tumor cells is one of the strategy to treat malignant tumor<sup>[13-14]</sup>. A number of studies have proved that high expression of NS gene is a common phenomenon in malignant tumor, if the apoptosis of tumor cells could be induced to weak cell reproductive activity by blocking NS gene, NS can be established as a candidate gene for the theoretical therapy principle of malignant tumor. But the mechanism of apoptosis caused by NS, and the signal transduction pathway in the process are unclear, and need to be discussed by further experiment.

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## Evaluation of Breast Milk in Lactating Pregnant and Non-Pregnant Mothers. Is it a Crime for the Pregnant Woman to Breastfeed her Infant?

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**Abstract:** The aim of this study was to compare milk composition in lactating pregnant and lactating non-pregnant mothers in order to know to what extent pregnancy could affect milk composition, and how this could be reflected on both child development and health. The study included 64 lactating females attending the Outpatient Obstetrics and Gynecology Clinic of El-Sahel Teaching Hospital during the period from March 2006 to February 2009. The cases were classified into three groups: **Group I** included 23 lactating and amenorrhoeic females, **Group II** included another 23 lactating and menstruating females and **Group III** included 18 lactating pregnant women with a gestational age that ranged from 8-16 weeks. The milk samples obtained from the patients were analyzed for their total protein, lipid and lactose contents. The quantity of milk lactose and lipids were significantly lower in pregnant lactating mothers than non-pregnant lactating women. These two nutrients are essential for the physical development of the newly born in general and for the development of its nervous system in particular. In conclusion, it is our responsibility to strongly advice against pregnancy during lactation, so that the newly born receives the best nutritional support to ensure its full physical and neurological development.

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**Key wards:** Breast Milk, Pregnancy, Breastfeeding

### 1. Introduction

Human breast milk is uniquely suited to our biologic needs and remains the best source of nutrition for the human infant (Kunz *et al*, 1999). It provides the necessary support for the developing immune system (Goldman *et al.*, 1994 ; Garofalo and Goldman, 1999). The powerful anti-infective qualities of breast milk are measured by decreased infant mortality in developing countries where exclusive breastfeeding is the norm (Scariati *et al*, 1997 ; Ball and Wright, 1999).

The human breast milk is composed of about 6% carbohydrates, about 4% fats, about 1% proteins and about 89% water. The composition of human milk is very different than artificial milk or "formula". Most artificial breast milk products use bovine milk as a substrate. The latter has more proteins and less lactose when compared to human milk (Kunz, *et al*, 1999). Lactose levels correlate well with brain size across species. Given their large brain size, it is not surprising that humans have a higher concentration of lactose in their milk than any other species (Newton, 2004).

From the Islamic religion point of view, although it is recommended to prolong adequate lactation for up to two years, Prophet Mohamed pointed that the milk of the pregnant women is harmful for the infants and strongly advised that pregnant mothers should never breastfeed their infants.

The aim of this study was to compare milk composition in lactating pregnant and lactating non-

pregnant mothers in order to know to what extent could pregnancy affect milk composition, and how this could be reflected on both child development and health.

### 2. Subjects and Methods

#### I- Subjects

This study was carried out on 64 urban healthy lactating females attending the Outpatient Obstetric and Gynecology Clinic of El-Sahel Teaching Hospital, Cairo, Egypt during the period from March 2006 to February 2009. All the subjects were in the third to tenth month of their lactation and all the infants were full term. The cases were classified into three groups: **Group I** included 23 lactating and amenorrhoeic females, **Group II** included another 23 lactating and menstruating females and **Group III** included 18 lactating pregnant women with gestational age ranged from 8-16 weeks. Exclusion criteria were age less than 19 years or more than 30 years, body mass index less than 19 or more than 27, smokers, low socioeconomic class, undernourished women, women under diet control, and women with medical disorders.

The study protocol was approved by the hospital ethics committee and informed consent was obtained from the lactating women.

A full history and full general, abdominal and pelvic examinations were done for all cases. Pregnancy was diagnosed by history, and by general and local examinations, and was confirmed by routine

pregnancy test and ultrasonography. Lactating mothers were questioned about the social and economic statuses to exclude patients with low socioeconomic class. The patients were asked to fill a quantitative questionnaire about the main constituents of the diet consumed in the three days before obtaining the milk sample.

Milk samples were obtained in the mid-morning before lunch. About 15-20 ml of human milk were obtained in a sterile glass bottle and stored frozen at -20 C till analyzed later. The samples were analyzed for their total protein, lipid and lactose contents.

## II- Methods

The lactose in milk was estimated using the method of Folin and Wu (1920). The protein free milk filtrate was heated with alkaline copper solution using a special tube to prevent reoxidation of the cuprous oxide formed. The latter was treated with phosphomolybdic acid solution, and the blue colour obtained was compared with that of a standard.

The method of estimation of total lipids in milk was written in detail in the Association of Official Agricultural Chemists, Washington (1960). This method involves the breaking of the emulsion of milk lipids by means of concentrated sulfuric acid (Sp. Gr. 1.82) and amyl alcohol, centrifugation of the solution in the special Gerber's tube, and the subsequent reading of lipids percentage in the graduated neck of the tube.

The dye binding method of Ashworth *et al.* (1960), was used in estimation of the total proteins in milk. In this method, Orange G dye binds to milk protein forming a colour complex that could be measured colourimetrically.

## Statistical analysis

Data were statistically described as mean  $\pm$  standard deviation (SD). Comparison between the three groups was performed using Kruskal-Wallis test, followed by Mann-Whitney U test for two group comparison. A probability value (p value) less than 0.05 was considered statistically significant. Statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, and USA) and SPSS (Statistical Package for the Social

Science; SPSS Inc., Chicago, IL, USA) statistical program.

## 3. Results

There were no differences between the three groups with regard to age, body mass index and duration of lactation (Table 1). Furthermore, there were no significant differences between the three groups with regard to the daily caloric intake or the percentage of the main constituents (complex carbohydrates, proteins and fats) of their diet. In all the patients included in the study, complex carbohydrates (rice, bread and grains) were the main constituent of the diet forming more than 60% of their daily energy requirements (Table 2).

The milk lactose level was significantly lower in lactating pregnant women compared to lactating amenorrhoeic women. Moreover, the milk lactose level was lower in lactating pregnant women compared to lactating menstruating women ( $5.75 \pm 1.34$  Vs  $6.53 \pm 0.77$  g/dl, P value= 0.055), but this difference failed to reach statistical significance because of small sample size of both groups (Table 3).

The milk lipid level was significantly lower in lactating pregnant women compared to lactating menstruating women. Moreover, the milk lipid level was lower in lactating pregnant women compared to lactating amenorrhoeic women ( $3.84 \pm 1.94$  Vs  $4.25 \pm 1.95$  g/dl, P value= 0.092), but this difference failed to reach statistical significance because of small sample size of both groups. The milk protein levels were significantly higher in the lactating pregnant and lactating amenorrhoeic women compared to lactating menstruating women (Table 3).

Comparison of milk lactose, lipid and protein levels (g/dl), between the lactating pregnant participants, and the lactating non pregnant participants (either menstruating or amenorrhoeic) showed a significant decrease of both milk lactose and milk lipid levels in the lactating pregnant females. On the other hand, the level of milk proteins showed no significant difference between them (Table 4).

**Table 1. Demographic criteria of the three groups of patients**

	Group I Lactating amenorrhoeic	Group II Lactating menstruating	Group III Lactating pregnant
Age (years)	25.26 $\pm$ 3.19	25.43 $\pm$ 2.64	24.44 $\pm$ 3.05
BMI(Kg/m <sup>2</sup> )	23.09 $\pm$ 2.02	22.22 $\pm$ 2.15	23.17 $\pm$ 2.26
Duration of lactation(months )	7.57 $\pm$ 2.23	7.61 $\pm$ 2.84	7.94 $\pm$ 2.69

Values are expressed as mean  $\pm$  SD.

There was no significant difference between the three groups



**Table 2. Maternal dietary intake**

	Group I Lactating amenorrhoeic	Group II Lactating menstruating	Group III Lactating pregnant
Energy (Kcal/day)	2377± 454	2315± 553	2334±586
Fat (% of Kcal)	69.91± 5.99	67.43± 5.62	69.11± 6.47
Lipids (% of Kcal)	14.74±3.78	15.57±3.69	15.67±3.41
Carbohydrates(%of Kcal)	14.48± 5.69	16.82± 5.91	15.5±6.16

Values are expressed as mean ± SD

There were no significant differences between the three groups

**Table 3. Comparison of milk lactose, lipid and protein levels between the three groups of patients**

	Group I Lactating amenorrhoeic	Group II Lactating menstruating	Group III Lactating pregnant	P value		
				G1 Vs GII	GI Vs GIII	GII Vs GIII
Lactose(g/dl)	7.56± 0.64	6.53± 0.77	5.75± 1.34	< 0.001	< 0.001	0.055
Lipids(g/dl)	4.25± 1.95	5.13± 1.23	3.84± 1.94	0.092	0.422	0.007
Proteins(g/dl)	2.64± 0.53	2.41± 0.29	2.64± 0.39	0.038	0.989	0.015

Values are expressed as mean ± SD

**Table 4. Comparison of milk lactose, lipid and protein levels between lactating pregnant participants and lactating non pregnant participants (either menstruating or amenorrhoeic).**

	Lactating non pregnant (n=46)	Lactating pregnant (n=18)	P value
Lactose(g/dl)	7.05± 0.87	5.75± 1.34	< 0.001
Lipids(g/dl)	4.69± 1.67	3.84± 1.94	0.044
Proteins(g/dl)	2.52± 0.44	2.64± 0.39	0.154

#### 4. Discussion

According to the best of our knowledge this was the first study to determine the impact of pregnancy on the composition of breast milk. The result of our study revealed that the major nutrients of maternal milk are affected by pregnancy. The quantity of milk lactose and lipids were significantly lower in pregnant lactating mothers than non-pregnant lactating women. These two nutrients are essential for the physical development of the newly born in general and for the development of the nervous system in particular.

The decreased lactose and lipid levels in pregnant lactating women could be explained by the increased demands and the effect of stress of pregnancy (Tucker, 1979). Moreover, anorexia, emesis and even hyperemesis complicating early pregnancy will reduce the dietary and nutritional status of the mother. On the other hand milk proteins are synthesized in the breast and their quantities appear to be little dependant upon the protein intake (Forsum and Lonnerdal, 1980; Nommensen *et al.*, 1991).

Lactose constitutes 90% of carbohydrates in the human milk. Lactose is a disaccharide composed of 2 mono-saccharides: galactose and glucose. Lactose is synthesized by the breasts. In the infants' gut it stimulates the growth of microorganisms which produce organic acids and many of the B-vitamins.

Lactose is specific for newborn growth especially for the brain, as it is the major energy source. Lactose is a ready source of galactose which is essential to the production of galactolipids including cerebrosides that are essential to central nervous system development (Newton, 2004). Galactose is also essential for the synthesis of proteoglycans and glycoproteins. These are constituent of the outer leaflet of the plasma membrane playing an important role in intercellular communication and contact. They also play a role in receptor function and cell permeability to different nutrients (Jensen *et al.*, 1992).

There is a positive correlation between lactose level in the milk and brain size in different mammalian species and the humans have the largest brain size and the highest lactose concentration in the milk (Newton, 2004). Therefore it is reasonable to expect that the reduced lactose level in the milk of pregnant lactating women may affect mental and physical growth of the infants.

Lipids are the second largest constituent of milk. Human milk fat is composed of 98% triglycerides, 0.7% phospholipids and 0.5% cholesterol. Lipids function in at least 3 critical roles, they are an important source for energy as they supply over 50% of the required calories, the digestion of lipids to fatty acids and monoglycerides produces protective effect

against viral infection and parasites, and fatty acids, phospholipids and cholesterol are major substrates for somatic and central nervous system growth (**Jensen et al., 1992; Rodriguez – Palmero et al., 1999**).

Linoleic and linolenic acids are essential fatty acids for the synthesis of long-chain poly-unsaturated fatty acids that are critical in the formation and function of neural tissue. For example; Arachidonic acid and docosahexaenoic acid are characteristic of grey matter; while linoleic and linolenic acids are characteristic of myelin laid down. (**Carlson et al., 1996**). Linoleic acid and other essential fatty acids are also concerned with the integrity of the mitochondrial membranes. Docosahexaenoic acid, which is synthesized from linolenic acid, is present in high concentration in the retina and cerebral cortex and it is particularly needed for brain development. In the first year of life, the brain size nearly triples, 85% of the growth occurs in the cerebrum and 60% of this tissue is lipid (**Newton, 2004**).

Stopping breastfeeding, should pregnancy occur; to avoid its adverse effect on the nursed infants may expose them to many hazards as gastroenteritis and malnutrition diseases. And so, it is our responsibility to strongly advice against pregnancy during lactation so that the newly born receives the best nutritional support to ensure its full physical and neurological development.

This is what exactly recommended by the Islamic religion to prolong adequate lactation, up to two years, to face infant's requirements and at the same time to avoid pregnancy on top of lactation with its bad effects on both the nursed infant and the fetus. It is hoped that further works will be done to study the effect of pregnancy on other milk constituents that could also possibly affect the nursed infant.

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## The Armenian-Iranian Common Ritual Festivals

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**Abstract:** Armenia-Iran has had relations for thousands of years. Their cultural interactions, especially, have not yet been investigated multilaterally and objectively. Some of the celebrations, which were celebrated before Christ, are celebrated by Armenian as their religious celebrations. Some of these are common between Iranian and Armenian. In this paper, the author wants intentionally to investigate these ritual common festivals. The findings of present investigation may describe the relation and accessibility of the rite, religion and history of these two countries during the year longs.

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**Keywords:** Armenian-Iranian, Ritual Festivals, religion, celebrations

### 1. Introduction

The relations between Iran and Armenia have a long precedence from historical, cultural and economical point of view, which, one of the important factor is their neighboring and their neighborhood.

In this regard, the first truthful evidence is Bistoon inscription, which is wrote during Darioush the first Achaemenian and in the concept Armenia is called ad Armina. (Kent, 1952, p115)

Before that time, the Armenian also have close relation with the Media, even at war with Assyrian, they help them during the storming toward the Neinava. Considering to the equality of both races and their relationship and closeness of their countries from geographical point of view, it seems that both races also have common points in various cases, such as their celebrations and rites.

Here, their common ritual celebration will be investigated and each related celebration is compared and discussed according to both countries traditions.

### 2. Iranian Nowsard (Naurooz) and Armenian Navasard

Nowsard or Navasard (Naurooz) festivals have been in ancient Aryan races as an olden common tradition. In ancient Armenia the first month of the year was called Navasard. Navasard was celebrated in the 11<sup>th</sup> August, in the ancient Armenia calendar. Kings, courties and others were attended in this festival and the celebration had a special gloriousness. Some of athletic tournament, such as: horse race, harness racing and foot-race carried out, the poet read their own poets and the musicians blowed out their own music. Releasing doves was another formality in this festival. The Armenian used many kinds of masks in Navasard festival, some people believed that mask was ran the evil spirits off.

The rhapsodists (public musicians) were reading and dancing.

Navasard is just celebrated between some Armenian societies. The Armenian come together and compete with each other, the winners are the victors of the Navasard. It says that the Armenian gods and the Goddess had their own special rites. The proper rite for Armazd (Ahura Mazda) had established at the beginning of Navasard (the first month of the year).

To the Zoroastrians, the sixth day is called the "Naurooz Bozorg" or "greater Naurooz" as it is celebrated as the birthday of Holy Zarathushtra. The sixth day of the month of Farvardin is the day of Ourmazd.

Anania Shirakasi (Seventh Century) and Johannes Imastaser (1129-1045) say:

Nowsard is the name of one of the Hig daughter. Hig is the Father of Armenian mythos.

The day after Amanor, they celebrated one of their most important and gloriousness celebrations and all of people of all ages attended to the festival and drench each other with water.

Taghizadeh believed that the Armenian adopted Nowsard festival from Iranian and they changed in to John the Baptist festival after believe in Jesus Christ. He advocates: "in case of adoption of the Iranian festivals by Armenian, it can be said that it will be truth in the first celebration of Nowsard or Vanature festival" Armenian celebration which will be changed into John the Baptist celebration and also Nowsard 15<sup>th</sup> celebration with Anahid festival and may be in seventh parabola festival or "Vah Vah" festival "likely is another type of Vahagan", which is believed that is the same as Iranian Varsarghaneh.

He added that after adoption of the Jesus Christ Armenian used their calendar but they changed their paganism festivals into Christianity festivals. It

is understood from "Agatankghos" that the Gregore was chosen the first day of Nowsard which was "Amanor" festival as John the Baptist reminiscence. He expressed that Gregor is determined the seventh day of the third month of the Armenian for the same apostles and this was encountered with the day which Gregor established some churches in order to infix the sacred relics remaining from these Saints and was brought from Qeysarieh.

The other author "Gregor Arozroni" about 690 BC declared that this second feast was one of the Armenian Gods fest from the first, "Vah, Vahan" and Zarbar Demon, was Zarmad and the first feast (first of Nowsard) was Armazd feast, he added two other feasts into the list of the Armenian church which were altered from the Armenian pagan time.

Changing of the feast of Armenian pagan time into Christianity feast is actual in Taqizadeh point of view, but the factor of this changes was Gregor Noor is not sure.

### 3. Iranian Nowsard Tradition

Nowsard is one of the most ancient traditions that is celebrated in Iran and in many other countries from Ma'ad era. Now means bright and Sard means year, then Nowsard means New Year.

Nowsard tradition is one of the especial and common Aryan tradition which is called Nowsard by Iranian, Navasard by Indian, Nosarej or Navsarji by Kharazmis, Nowsard by Soqdis and Navasard by Armenian.

Abo-Reyhan Biruni wrote about Nowsard in Soqd:

"Soqdis divided their months in accordance with the fourth part of the year. First of Nowsard from the month of Soqdi was the first of summer; the first day of Nowsard was new year for Soqdis which is the Great Naurooz. He also wrote about Nowsard in Kharazm: "Navarsachi, the first day is the beginning of the year and it is new day."

Taqizadeh wrote about Nowsard: "Nowsard is not the name of the first day of Iranian year or is not the well-known Naurooz (the first day of Farvardin = small Naurooz), but it is the name of the Great Naurooz, which celebrated on sixth of Farvardin and it was equal with the first day of Armenian Navasar and Nowsard, the Soqdis month and Nowsarej the month of Kharazm. This day was very great by Iranian and it was really the first day of Iranian year. After first leap year, beginning of the year back 5 days off, this actual Naurooz, became the sixth day of the year. Iranian were celebrated this day as second Naurooz (Great) and it showed continuously the actual Naurooz. Beginning of summer, or perhaps Nastoris (beginning of year) and their ritual tradition "12 apostle" is not denominated from Iranian

Naurooz (small), but it denominated from Iranian Nowsard (or Great Naurooz) which was coalesced with it on that year, whether on that time Iranian tradition encountered with Sunday June 28, just the seventh Sunday after the first festival. It was Nastoris custom that splashing water on each other, whether Iranian had the same custom in sixth day (day of Mazdad) of Farvardin (Great Naurooz or Nowsard Naurooz).

### 4. Iranian Baregandan and Armenian Barekandan

The other common festival between Iranian and Armenian is the Baregandan or Barekandan. In addition to the similarity of the name, this festival is similar in tradition and its pursuance too. According to calendar, the prior to weekly feasts, as Greater Lent, (with the exception of the feast preceding Holy Nativity) are called Barekandan. And Iranian and Armenian are celebrated it the day before Lent begins. Baregandan is celebrated on Sunday and run on 8-10 days. The word Baregandan means "good living" or "good life", as are called to live cheerfully, joyfully and to be happy on these days preceding fasting period. In Baregandan all people are uninhibited in their actions.

Armenia Great Lent begins on Sunday and it will be continued for seven weeks, and Easter will come after these seven weeks. Due to variability of Easter, pursuance of Baregandan is variable in different years.

The latest Saturday before Great Lent is called "Bon-Baregandan" by Armenian. Baregandan was celebrated in ancient Armenia before adoption of Christianity. This day is celebrated because winter and the problem of winter time were finished from one side and the glad tidings of beginning of spring together with happiness are started from the other side.

Armenian was celebrated this day together with their friends and family one week before the Great Lent and they lay the cloth and prepare some amusement in relation with this festival. The actors use masks and some athletic competition effectuated and actor showdown.

Armenian was used mask during hunting, because they believed that mask can escape the unmanageable spirits. Bargendan was celebrated before the fasting time in Iran. In ancient time Iranian were celebrated the last days of the month of Shaaban and Bargendan was celebrated in seven to ten days. Alaameh Dehkhoda declared that:

"Bargendan, peg a stone or carnival is the feast which is celebrated in Shaaban due to arrival of Ramadan"

He described that: Peg a stone, Bargh Anaz, or carnival is a feast which is celebrated at the end of

Shaaban. The last day of Shaaban is called Carnival by Ali Akbar Nafisi and he described:

"Bargendan is a festival which is celebrated in the last days of Shaaban and it is called carnival and it means the feast of the end of fasting time."

### 5. Iranian Tirgan and Armenian Vartavar

Tirgan or Vartavar is one of the most ancient festival common between Iranian and Armenian. It is celebrated from the time of the Ma'ad (Media) era. Tirgan or Vartavar was celebrated during harvest time. Armenian was splashing water on each other and were prepared athletic completion. It seems that Tirgan or Vartavar is Aryan especial festival and it doesn't any relation with any religion.

Armenian interested in Vartavar and all Armenian of around the world celebrate it. Armenian calendar during ancient Armenia was like Iranian calendar and a year had 12 month and each month consists of 30 days; each day had an especial name. the five remaining days was called "Havliast" by Armenian and was added to the end of the 12<sup>th</sup> month of the year and by these five days the year had become 365 days. Armenian was celebrated vartavar during the first day of "Havlitas" and during the festival they were released doves and sprinkled water on each other.

The Armenian researcher have been attributed Vartavar to Astghik and Anahid. Astghik was the goddess of water, beauty, love and fertility. One of the Armenian researcher in Israel expressed that people celebrated Vartavar and they offered her red roses as a celebration ("Vart" means "rose" in Armenian).

Agatangoes and Khurnansi expressed that "Majan" was son of Ardashes, the Armenia King. He was fought by "Meeds" and "Krows" in Ararat campaign and won them. Majan became the priest of "Aramazd" temple and after death he was buried in "Bagauan" province and he was adored as God of hospitality and patron. On this occasion a glorious festival was celebrated in Bagavan region every year and Armenian from whole the world were gone there and were attended to the feast; and this is Vartavar.

Hayo alishan stated that the Armenian who were resided in around of Ararat mountain, after Noah rak was gotten stuck in mud, were cherished and celebrated memory of Noah storm, after they were adopted Christinaity. They superposed Vartavar with transfiguration of Jesus Christ. He stated that Tirgan and Vartavar was celebrated in Iran and Armenia simultaneously since long time ago, but Vartavar is celebrated 98 days following Easter from 28 of July till the first of August.

According to a tradition, Armenian did not eat apple the days before Vartavar and they offer apple to each other during the feast.

Tirgan or water splashing feast is celebrated from the time of the Ma'ad (Median) era. A year was divided into 12 months in Iranian calendar, which was 360 days. The remaining 5 days were added to the 8<sup>th</sup> month and were called "Khamseh Mostaraghe". Each day of a month had an especial name of a God. Whenever name of a month and a day was similar, Iranian were celebrated that day. Bable Talmud and Jerusalem Talmud are the ancient documents which refer to celebration of Tirgan in Ma'ad era.

In Zoroastrian religion, tir means the goddess of rain. There is a hymn in Avesta which is praised the rain goddess; due to her efforts, the earth is blessed with rain and the farmlands are irrigated.

Formerly, the researcher of 4<sup>th</sup> century were believed that celebrating of Tirgan is related to an occasion in Sassanian era and this was because of the importance of Tirgan feast and Iranian favorite to celebrating of Tirgan feast at that time. Abo-Reyhan Biruni stated that the fought between Touranian Afrasiab and Iranian Manouchehr and thrown an arrow y Arash Kamangir in order to determine the Iranian and Touranian speech was the reason of celebrating Tirgan.

During Safavid era Tirgan was celebrated in most part of Iran and the King Abbas was interested in this celebration and attended to the feast. Pieter, the Italian torist Dolavale and Don gracia De Cielo Ofigo were Spanish ambassador to oversee the celebration in attendance of King Abbas.

The Dr. stated that "Tirmysner (13<sup>th</sup> of Tir), Tirgan feast was the most well-known feast after Naurooz, which was celebrated in all around the Iran on 13<sup>th</sup> of Tir in month of Tir.

Tirgan feast was an interested festival for some Giulani and Mazandarani poets. Now it is only celebrated amon some villages in the provinces of East of Guilan and wes of Mazandaran. One of the traditions of this festival was fortune telling. It was in this manner that a first child of a family and the last child of another family, who know each other for a long time, went to spring bank and took a bowl of water and brought back home. Women and men of each family made a wish and threw a sign such as earing and bracelet into the water. During the night they came together and a minor girl sat close the bowl and ceremony is started, Tabari Khan should sing a song at least in the number of each attended. Tabari Khan is sang a song and themarker who made a wish can understand his answer about his wish by the singer hint.

Iranian and Armenian ancient tradition is due to ethnic, national and geographic closeness of both countries, then naturally Armenian Vartavar and Iranian Tirgan was celebrated simultaneously from long time ago.

Iranian Sada and Armenian Diarend Araj festival is one of the Iranian-Armenian common festival. "Fire Festival" is called Sada or Sade by Iranian and Diarend Araj by Armenian. This is one of the Aryan especial festivals from the ancient time.

Armenian is celebrated the festival forty days after took birth of Jesus Christ on February 13<sup>th</sup>, and it means look forwarded to. Pagan spring festival in honor of Mihr, the God of fire, was taken over by the church to commemorate the bringing of the Babe Jesus to the temple, where Mary sacrificed two doves according to the custom of purification. There was an old devotionalist man, Simon, who lived in Jerusalem. He required God had long life to see Jesus. When Mary arrived Jerusalem temple, he came to the temple and he fondled Jesus and praised to God. Owing to the fact that the sky was darkening, when Mary arrived Jerusalem, people look forwarded her and Jesus by lamplight and fire burning. In this manner Armenian church is celebrated Diarend Araj on February 13<sup>th</sup> together with burning fire.

Due to deletion of the Armenian pagan festivals effects, there is not enough information about "feast of fire" from ancient Armenia. Fire had women characteristics and water had men characteristics, due to ancient Armenia traditions, and they had deep aid with each other. In accordance with some Armenian stories, there was "Aramazd", "Astghik" and "Ourmazd" home in the top of "Pagat" mountain and a little inferior there was house of eternal fire. There was a big spring at the mountain slope. Fire and water were sister and brother. Thus nobody wasted the ash, but they clear by brother's "water" treas. In addition to water and fire, ash was also divine.

On the afternoon of the 13<sup>th</sup> of February, which is the day before the church festival of the purification, a pile of wood consisting usually of thorn-wood, cane, and straw is gathered in the churchyard. The entire community comes together in the church on the night of the same day, each person provided with a candle. The candles are lighted from the church light, and after the priest has blessed the pile, it is set ablaze from all sides, after which the candles are put on. As soon as the fire has died down, the candles are relighted from the glowing embers which are regarded as sacred, and carried a small portion of the fire back to their homes to make new glowing fire from the "blessed fire" of the temple.

Jashn-e Sade (feast of fire) is similar to Armenian Diarend Araj festival among Iranian. Sade

fell not on the winter solstice, but forty days after it, namely on the 10<sup>th</sup> (Aban) day of the month of Bahman. Ancient Iranian divided the year into two parts: 7 months as summer and 5 months as winter.

Ferdowsi the famous Iranian poet expressed that King Hushang, the 2<sup>nd</sup> King of the Mythological Peshdadian dynasty established the Sade tradition. It is said that once Hushang was climbing a mountain when all of a sudden he saw a snake and wanted to hit it with a stone. When he threw the stone, it fell on another stone and since they were both flint stones, fire broke out and the snake escaped. This way he discovered how to light a fire. Hushang cheered up and praised God who revealed to him the secret of lighting a fire, and celebrated this discovery. Sade is celebrated until 7<sup>th</sup> century and Mardavij, one of the Iranian King, was celebrated Sade very gloriously. After Mogul attack, this festival passed into silence gradually, but Zoroastrian and some villages in Khorasan are celebrated it now.

## 6. Mehregan in Iran-Armenia

Mehregan is one of the common Iranian festivals. In the Zoroastrian religious calendar, Mehregan is celebrated on the sixteenth day of the seventh month. In Armenian calendar Mehregan was the 8<sup>th</sup> day of the 7<sup>th</sup> month, which was changed by changing the calendar into 21 days. The last day is called Great Mehregan.

Mehr was one of the Gods in ancient Iran. He called Mθra in Avesta and Mihr in Pahlavi, which draw more attention due to Mithra religion.

According to Pahlavi text, he was ethereal referee and interface. He was eminent in ancient Iran and his name was mentioned together with Ourmazd, the Greatest God, and Anahid. In Armenia Mithra was very beloved and the Armenian like him and to hold him in respect. He was so beloved in Armenia and his especial feast was celebrated very gloriously in the fourteenth of the month of Mahgan which was equal to February. One of the Armenian day is called like this.

## Conclusion

It can be said that due to neighboring and congenerous of Iran and Armenia, they have more cultural similarity in their religion. These common cases can be seen in their ritual festivals, which is shown the impressionability of the Armenian from Iranian, whether in culture, religion, language and ritual festivals.

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## Combined 20 G with 23 G Transconjunctival Vitrectomy For Complicated Vitreoretinal Cases

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**Abstract: Purpose:** Sutureless posterior segment surgery has the advantages of faster wound healing, minimal surgical trauma, and reduced postoperative astigmatism; however, it is still difficult to deal with cases of advanced PVR where peripheral vitreous dissection and silicone oil injection are needed. We combined 23 – gauge and 20 – gauge vitrectomy to improve the outcome of vitreoretinal surgery. **Settings:** Accurus 800 (Alcon) vitrectomy machine, Alcon disposable 23- G trocar and cannula set, normal 20- G vitrectomy surgical set. **Patients and Methods:** The study included 20 cases of combined 20 and 23 G pars plana vitrectomies. The indications for surgery included proliferative vitreoretinopathy (PVR) grade C and proliferative diabetic retinopathy (PDR) with tractional retinal detachment. Eyes being injected with silicone oil had 1000 cs silicone oil injected manually through the 20 G port. At the end of the surgery, the single 20 G opening was sutured with 7-0 vicryl. **Results:** 20 Cases were operated upon, 10 had PVR grade C, and 10 had PDR with tractional retinal detachment. 7 cases with PVR needed single operation while the other 3 cases needed second interference. 8 Cases with PDR with tractional retinal detachment needed single operation and the other 2 cases needed re-interference. **Conclusion:** Combination of 20-G with 23-G vitrectomy appears to be an efficient technique in dealing with complicated vitreoretinal cases. Besides, being viable from the economical point of view.

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**Keywords:** Transconjunctival vitrectomy, proliferative vitreoretinopathy, proliferative diabetic retinopathy.

### 1. Introduction

The 23 – gauge (23-G) sutureless vitrectomy technique is becoming increasingly popular because of the decreased surgical trauma, faster wound healing and improved postoperative comfort associated with this technique (1-5). Small gauge sutureless vitrectomy offer several advantages over 20-gauge vitrectomy, including improved operative efficiency, faster visual rehabilitation, decreased convalescence period reduced postoperative astigmatism, reduced operative time and finer instrumentation for working close to retinal surface (6,7).

Still yet, the access to using instruments with 20 G vitrectomy is faster in gel removal, more efficient in peripheral vitreous dissection and easier in silicone oil injection (6,7).

In this study, a combined 20 G and 23 G transconjunctival vitrectomy was performed on complicated cases undergoing proliferative vitreoretinopathy (Grade C) and proliferative diabetic retinopathy with tractional retinal detachment.

### 2. Patients and Methods:

After obtaining the approval of Ethical Committee in the Research Institute of Ophthalmology, all patients were given detailed explanations of the procedure and its potential benefits and risks and an informed consent was obtained from them.

The prospective study was conducted on 20 eyes of 20 patients allocated in two groups, 10 patients in each group.

Group I (PVR group) included 10 patients with PVR, Grade C; group II (PDR group) included 10 patients with PDR with tractional retinal detachment. All surgeries were performed with an Accurus 800 (Alcon) vitrectomy machine and Alcon disposable 23-G trocar and cannula set. All surgeries were performed by a single surgeon between January 2010 and July 2010. Each patient had a complete preoperative ophthalmic examination which included measurement of best corrected visual acuity (BCVA), slit-lamp examination, intraocular pressure (IOP) measurement and fundus examination

Exclusion criteria included: patients with significant cataract which required combined surgery, glaucoma patients and those with corneal disorders.

### Surgical Technique

All patients received preoperative sedation and local anesthesia consisting of a peribulbar injection of 10 ml of a 80: 20 mixture of 0.5% bupivacaine and 2% lidocaine 100 units of hyaluronidase was added to the mixture.

Two 23-G transconjunctival sclerotomy ports were created for infusion and illumination (Alcon laboratories Inc, Fort Worth, TX, USA), and a third 20 G sclerotomy port was created for introducing the vitrectomy probe. For the 23- G opening, the



conjunctiva was displaced by approximately 1-3mm with a pressure plate. A 23-G trocar– cannula was first inserted through the conjunctiva and sclera, parallel and 3.5 mm posterior to the limbus, and then at an angle of approximately 5° until it just passed the end of the bevel. At that point, the handle was raised slightly to an angle of approximately 30° and the cannula was then inserted into The hub. The trocar was removed while the cannula was stabilized with forceps. Another similar port was made for the illumination probe. The third port was created via a localized periotomy and sclerotomy with a 20-G microvitrectoretinal blade. Infusion bottle height during surgery was 60 cms to avoid hypotony and the suction settings were from 80-100 mm Hg. (Fig.1)

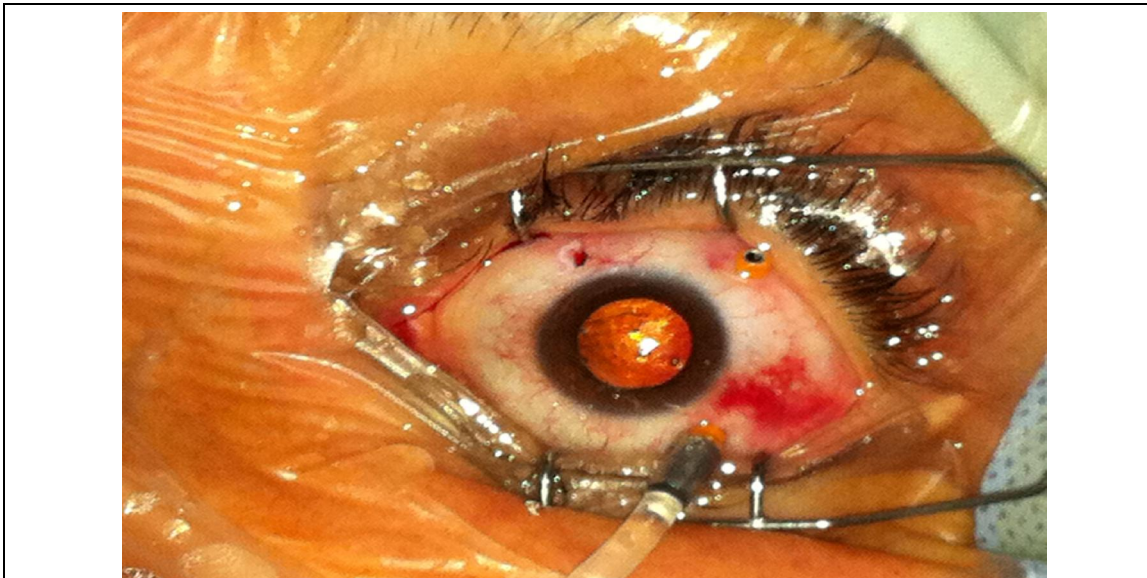
Eyes being injected with silicone oil had 1000 cs silicone oil injected manually through the 20-G port. At the end of the surgery, the IOP was fine tuned through injection of silicone oil through 23-G infusion cannula and the single 20 opening was sutured with 7-0 vicryl, the overlying conjunctiva was closed by bipolar

cauterization. Complete ophthalmic examinations including BCVA measurement, slit- lamp examination, tonometry and fundus examination were performed one day, one week, one month, three months, and six months after the operation.

### 3. Results:

The above surgical procedure was performed on 20 eyes of 20 patients in the 2 groups. Demographic data showed no statistical significance between the 2 groups (Table 1). The preoperative and postoperative data are summarized in (Tables 2, 3).

The ten patients in group I (PVR, Grade C), underwent parsplana vitrectomy using the combined 20-G and 23-G technique, where 8 patients out of the 10 needed the usage of 20-G vitreoretinal forceps to remove the star folds and epiretinal membranes. Two patients out of the ten needed the usage of curved 20-G scissor to perform relaxing retinotomies to overcome the problem of shortened retinae.



(Fig.1) Showing the combined technique: two openings for the 23 gauge and the 20 gauge opening which is closed by a stitch.

**Table 1: Demographic data. Values are mean (SD)**

	<b>Group I PVR Grade C</b>	<b>Group II PDR with retinal detachment</b>
<b>Sex (M/F)</b>	7/3	2/8
<b>Age (years)</b>	48 (4.8)	52(5.3)

**Table 2: The measurement of Best Corrected Visual Acuity (BCVA) in both groups throughout the time of the study.**

Patient number	Preoperative BCVA	Postop BCVA (1day)	Postop BCVA (1week)	Postop BCVA (1month)	Postop BCVA (3months)	Postop BCVA (6months)
*1	HM	HM	C.F (30cm)	0.05	0.1	0.1
*2	CF (10cm)	HM	C.F (20cm)	0.1	0.1	0.2
$\beta$ *3	HM	HM	HM	HM	HM	HM
*4	HM	C.F (20cm)	C.F (1meter)	C.F (2meters)	0.05	0.05
*5	CF (20cm)	C.F (20cm)	0.05	0.3	0.3	0.3
$\beta$ *6	HM	HM	HM	PL	PL	PL
*7	HM	HM	0.1	0.1	0.2	0.2
$\beta$ *8	HM	HM	HM	HM	HM	HM
*9	CF (10cm)	HM	HM	0.05	0.1	0.1
*10	CF (10cm)	HM	HM	0.05	0.2	0.2
$\varepsilon$ 11	CF (30cm)	HM	0.05	0.05	0.1	0.2
$\varepsilon$ 12	HM	C.F (20cm)	0.05	0.05	0.1	0.1
$\beta \varepsilon$ 13	CF (10cm)	HM	HM	HM	HM	HM
$\varepsilon$ 14	HM	HM	C.F (50cm)	0.05	0.05	0.1
$\varepsilon$ 15	HM	C.F (20cm)	C.F (50cm)	0.05	0.1	0.2
$\beta \varepsilon$ 16	CF (30cm)	HM	HM	HM	HM	HM
$\varepsilon$ 17	HM	C.F (30cm)	C.F (80cm)	C.F (1 meter)	0.1	0.2
$\varepsilon$ 18	CF (40cm)	C.F (1 meter)	0.1	0.1	0.2	0.3
$\varepsilon$ 19	CF (10cm)	C.F (30cm)	0.05	0.1	0.2	0.2
$\varepsilon$ 20	HM	HM	C.F (40cm)	C.F (1meter)	0.05	0.1

\* Group I PVR Grade C

 $\varepsilon$  Group II PDR with tractional detachment $\beta$  Patients who needed re-interference

HM = hand movement

CF = counting fingers

PL = perception of light

**Table 3: The measurement of Intraocular Pressure (IOP) in both groups throughout the time of the study**

Patient number	Preoperative IOP	Postop IOP (1day)	Postop IOP (1week)	Postop IOP (1month)	Postop IOP (3months)	Postop IOP (6months)
*1	5	24	18	18	19	18
*2	6	22	19	19	18	18
$\beta$ *3	5	11	6	23	16	16
*4	9	16	15	15	16	16
*5	8	14	13	14	14	16
$\beta$ *6	5	24	21	17	16	16
*7	8	19	17	17	18	19
$\beta$ *8	7	21	20	20	19	20
*9	9	27	22	20	18	19
*10	11	30	24	21	18	17
$\varepsilon$ 11	10	22	19	18	16	16
$\varepsilon$ 12	6	13	12	12	13	15
$\beta \varepsilon$ 13	8	15	14	14	14	15
$\varepsilon$ 14	7	18	17	17	16	18
$\varepsilon$ 15	8	16	16	15	19	18
$\beta \varepsilon$ 16	5	8	6	6	29	24
$\varepsilon$ 17	9	15	13	13	14	14
$\varepsilon$ 18	8	12	12	13	12	12
$\varepsilon$ 19	6	11	12	11	11	12
$\varepsilon$ 20	6	19	17	16	17	17

\*Group I PVR Grade C

$\varepsilon$  Group II PDR with tractional detachment

$\beta$  Patients who needed re-interference IOP measured in mm Hg

Postoperatively, 3 patients out of the ten needed second interference to flatten the retina.

The ten patients in group II (PDR with retinal detachment) underwent parsplana vitrectomy using the combined 20- G and 23- G technique, where 6 patients out of the ten needed the usage of 20- G vitreoretinal forceps to remove adherent membranes to the retina while the other 4 patients needed the usage of 20 G different types of scissors (Vertical, horizontal and curved) to dissect tightly adherent vitreoretinal membranes. Postoperatively, two patients needed second interference to manage complications that happened intraoperatively in the form of subretinal haemorrhage.

#### Visual Acuity outcome:

In group I (PVR Grade C group n = 10), the visual acuity initially worsened at first day postoperatively compared with baseline in three patients, remained the same in six patients and improved in one patient.

In group II (PDR with tractional retinal detachment group n = 10), the visual acuity initially worsened at first day postoperatively compared with the baseline in three patients, remained the same in two patients and improved in five patients.

But vision in both groups gradually improved through each of the remaining time points. Improvement in visual acuity at 3 months in both groups was obvious. At 6 months, in group I, the visual acuity compared with baseline was improved in seven patients, remained the same in two patients and worsened in one patient, and in group II, the visual acuity compared with baseline was improved in eight patients and worsened in two patients.

#### Intraocular pressure analysis (IOP):

All patients in the study in both groups showed an increase in IOP in the first postoperative day compared to baseline. Throughout the 6 months, in group I, six patients required the usage of anti-glaucoma treatment in order to adjust IOP, while in group II, only two patients needed the treatment.

#### 4. Discussion

Proliferative diabetic retinopathy associated with tractional retinal detachment and proliferative vitreoretinopathy grade C, are challenging cases.

Initial reports of such cases were associated with significant intraoperative and postoperative complications. 20- Gauge parsplana vitrectomy in these cases was discussed in literature (8). With the 23- G sutureless vitrectomy technique becoming increasingly popular because of the decreased surgical trauma, faster wound healing and improved postoperative comfort associated with it and decreased postoperative astigmatism, it was logical for vitreoretinal surgeons to proceed with it (2).

A combination of 20 G and 23 – G also was introduced in many studies. A study done by Kongsap, used the combined technique for management of posteriorly dislocated lens (9). Sutureless self-sealing pars plana vitrectomy was first described by Chen in 1996 (10). Combined 20-G and 23-G vitrectomy allows better vitreous base dissection in cases of PVR and the usage of complex instruments to relieve traction in cases of PDR (7). In our study, the advantages of the combined technique used over the pure 23-G technique were faster gel removal and better manipulation with the 20-G instruments. Moreover, the presence of two sutureless ports allowed for less postoperative complications such as inflammation, conjunctival scarring and astigmatism. 20-G cutters are comparable to 23-G cutters in terms of fluidics, port distance and flow rates but the 20 G cutter has the upper hand over the 23 G cutter in peripheral vitreous dissection owing to the fact that it has a stiffer shaft (3).

In some studies, interchanging of hands and hence enlargement of the second superior port was required (7). This disadvantage was not applied in this study.

To summarize, the combined 20-G with 23-G vitrectomy technique could be used in various vitrectomy cases with the advantage of being economically viable for the surgeon. Further studies are recommended to use this combined technique for other various indications.

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