CONTENTS

I

A Comparative Feasibility Case Study on Hybrid RCS Moment Frames with Concrete and Steel Frames in Construction and Project Management Point of View

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Abstract: RCS structures are new developed hybrid frames made of reinforced concrete columns connected to steel beams. These frames can provide practical and economical merits by combining longer steel beams with high compression resistant reinforced concrete columns. RCS structures by use a system of reinforced concrete supports and steel frame beams have been recognized to possess several advantages in terms of structural performance and economy compared to pure reinforced concrete, steel and concrete frames. This study aims to investigate a detail compared feasibility study between RCS, steel and concrete structures. The applied procedure is validated through the testing of a real case study in Tehran. Experimental results indicate that the proposed design procedure is effective in controlling deformations and damage, leading to economic and feasible criteria. Obtained results indicate that in equal conditions, RCS frames are shown better circumstance cost, required human resource, physical and financial progress, management and economical condition than steel and concrete structures.

INTRODUCTION

Construction management or construction project management (CPM) is the overall planning, coordination, and control of a project from inception to completion aimed at meeting a client's requirements in order to produce a functionally and financially viable project. Construction managers plan, direct and coordinate a wide variety of construction projects, including the building of all types of residential, commercial and industrial structures, roads, bridges, wastewater treatment plants, schools and hospitals. Construction managers may oversee an entire project or just part of one. They schedule and coordinate all design and construction processes, including the selection, hiring and oversight of specialty trade contractors, but they usually do not do any actual construction of the structure.

Typically the construction industry includes three parties.

1. An owner
2. A designer (architect or engineer) which should execute work inspection, change orders, review payments, materials and samples, shop drawings and 3D image.
3. The builder (usually called the general contractor). Traditionally, there are two contracts between these parties as they work together to plan, design, and construct the project (Halpin, 2006). The first contract is the owner-designer contract, which involves planning, design and construction administration. The second contract is the owner-contractor contract, which involves construction. An indirect, third-party relationship exists between the designer and the contractor due to these two contracts. For planning and scheduling, project management methodology includes the following sub procedures.

- ✓ Work breakdown structure
- ✓ Project network of activities
    - ☐ Critical path method (CPM)
    - ☐ Resource management
    - ☐ Resource leveling

As shown in figure2, a traditional phased approach identifies a sequence of steps to be completed. In the "traditional approach", five developmental completion components comprising initiation, planning and design, execution and construction, monitoring and controlling systems of a project can be distinguished. Not all projects will have every stage, as projects can be terminated before they reach completion. Some projects do not follow a structured planning and/or monitoring process. Projects need to be controlled to meet their objectives and deliver benefits. Objectives are

defined in terms of expectations of time, cost and   quality as shown in figure2.
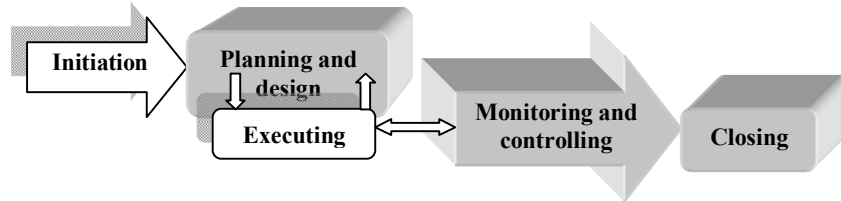
Figure1. Typical development phases of an engineering project
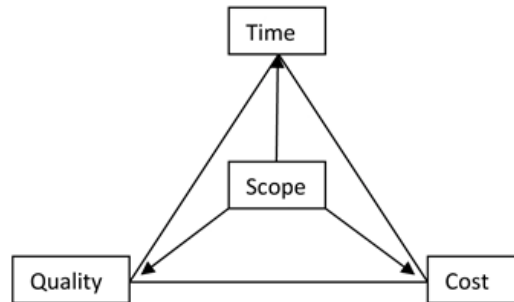
Figure2. Project objectives

The case study of this paper is a hybrid combined structure that called as RCS. RCS frames are one of the most recent practical bending frames in cases of large spans and moderate height as shown in figure3. This type of structure has been used as a cost-effective alternative to traditional structural steel or RC construction (Griffis, 1992).

All of the materials are of the highest quality in order to achieve rational structures, withstand great force and at the same time allow wide spaces between supports. As shown in figure4, this type of construction allows for large open structures like warehouses for heavy loads and shopping centers. To further develop such hybrid structures, it is necessary to determine the strength and ductility of the connection. In this case a program named as DYNAMIX for the dynamic analysis of mixed of 3D steel and RCS frames with capabilities to perform inelastic static and dynamic analyses of has been developed (El-Tawil et al. 1996).

Reinforced concrete frames, due to increasing in depth of beam and loss of architectural space, are not suitable; therefore RCS frames were proposed to improve these systems (Chopra, 1995).

Figure3. RCS frames performance (Tehran-Case study of this paper)

From the construction viewpoint, these systems are usually built by first erecting a steel skeleton, which allows the performance of different construction tasks along the height of the building (Griffis, 1986). Structurally, the connections between steel beams and RC columns have been reported to possess a good strength and stiffness retention capacity when subjected to large load reversals (Kanno, 1993; Parra-Montesinos and Wight, 2000a). Utilizing compressive strength of concrete in columns and stiffness and strength of steel beams which makes them suitable for long spans, results in a cost effective hybrid system, which behave well under both gravity and lateral loads (ASCE ,1994).

In seismic design, reduced forces due to different causes like, damping, ductility, excess resistance and etc are calculated from dividing linear seismic spectra to a factor named is behavior coefficient (ATC, 1996; C.M.Uang, 1991). Several researchers such as Deierlein et al. (1988), Kanno (1993), Kim and Noguchi (1997), Parra-Montesinos and Wight (2000b) were compared the accuracy of design equations to predict the shear strength of RCS joints between ultimate experimental and predicted strength. However, their use has been limited to low or moderate seismic regions due to lack of appropriate design guidelines for RCS frames in high seismic risk zones.

ANALYSIS FRAME WORK
For this study the selected building in Tehran, was modeled for three various kinds of structures (steel, concrete and RCS) for similar conditions. By this consideration that structural steel members, have high second moments of area concrete is a material with relatively low tensile strength and ductility and will reinforced by bars usually embedded in concrete before sets. It means that characteristics of steel structures allow them to be very stiff in respect to their cross-sectional area.
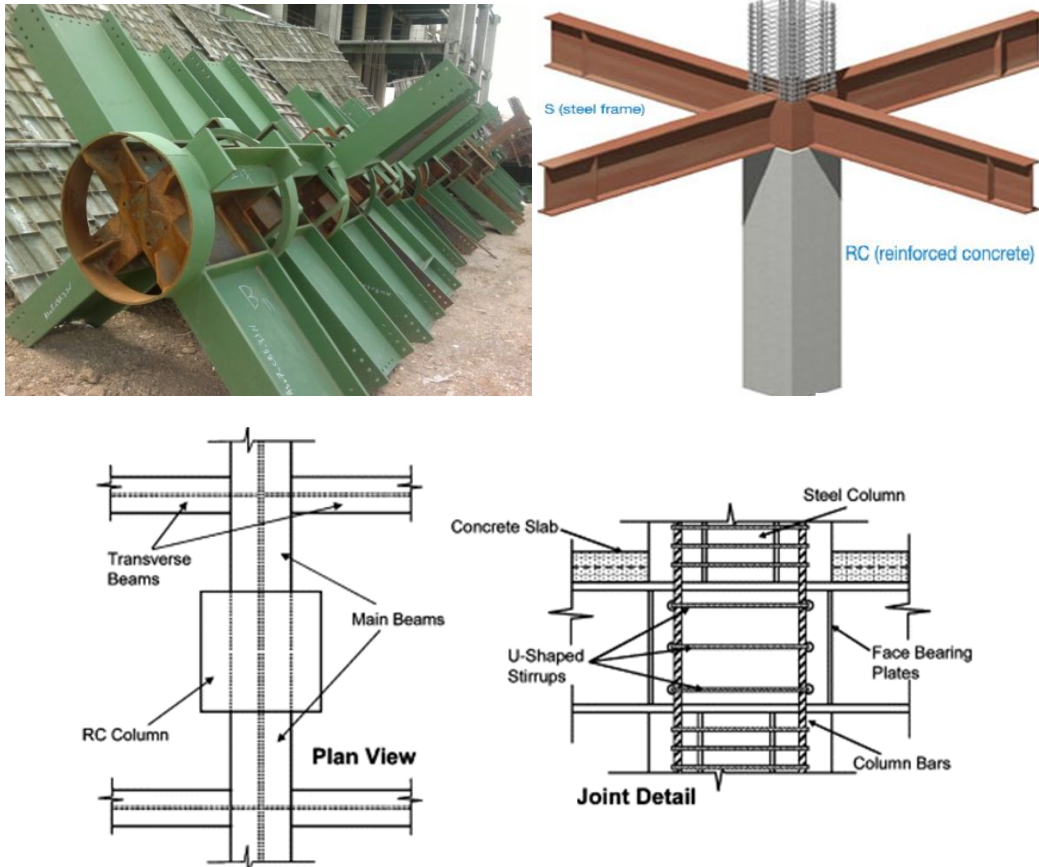
Figure4. Real RCS joints and schematic one with detail

By refer to modular analysis frame work which is given in figure5, and application of ETABS software package the modeling for three kinds of structures by above mentioned assumption was executed and the required data were extracted which is indicated in figures 6, 7, 8 and tables (1), (2) and (3) respectively. By obtained results of the constructed models, the authors would be forced to use MATLAB programming environment to analyze the results of the models and MSP software outputs. The written code is capable to draw the requested diagrams and can analyze the applied loads on the structure. Obtained results of the mentioned code and comparative plotted diagrams are indicated in figures 9 to 11.

By consideration of the performed analysis and to show better resolution of obtained results a detailed separately comparison was executed and the results are given in figure 12 respectively.
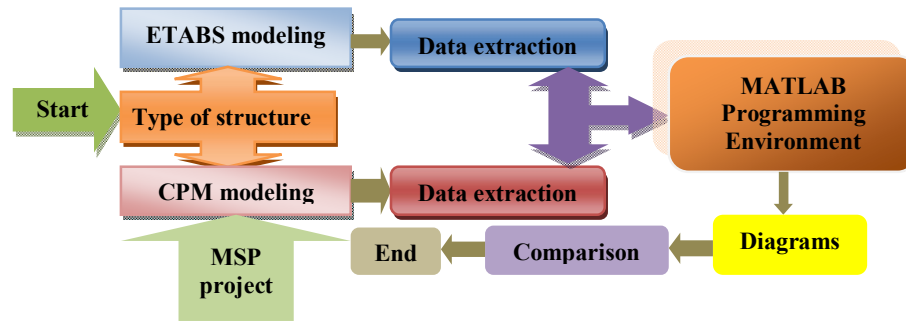
Figure5. Modular analysis frame work of this study
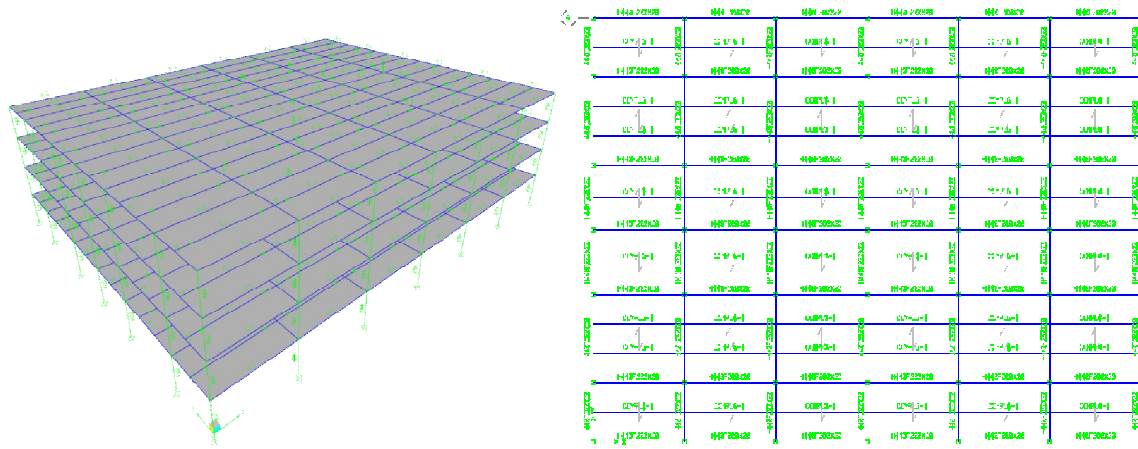
Figure6. ETABS model of steel structure for the case study
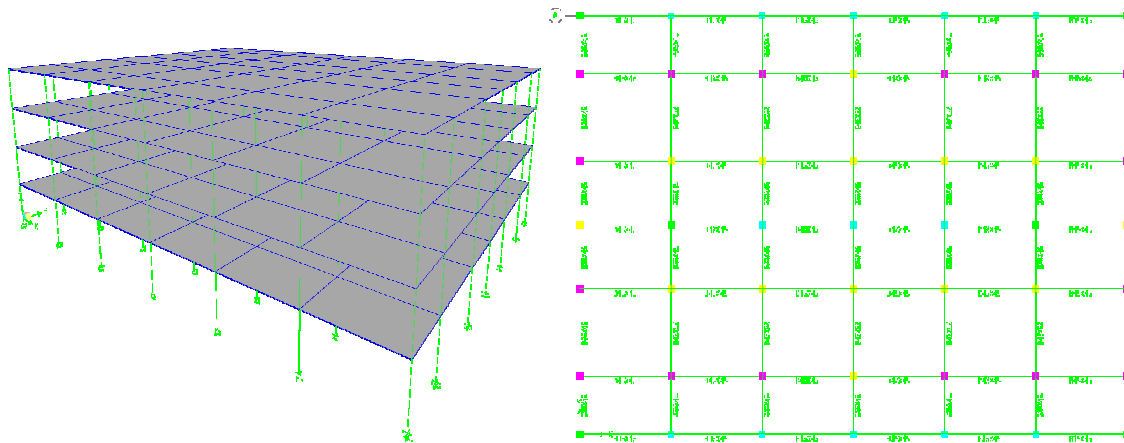
Figure7. ETABS model of concrete structure for the case study
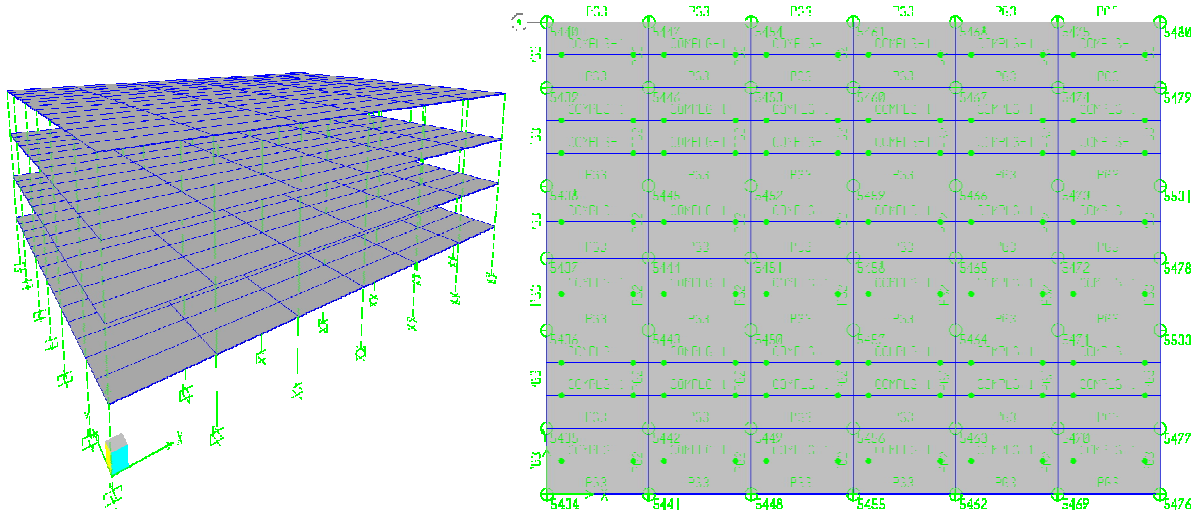
Figure8. ETABS model of RCS structure for the case study

Table (1). Steel structure characteristics extracted by ETABS

		Column	
Column section	**All the frames**	**stories**	
	B_{ox} 350x20	1, 2	
	B_{ox} 300x20	3, 4	

beam			
frames			stories
(B-C) 30 (B-C) 31 (B-C) 32 (B-C) 33 (B-C) 34	(E-F) 30 (E-F) 31 (E-F) 32 33(E-F) (E-F) 34	others	
Beam section	250x20 F 440H	250x20 F 440H		1, 2
	440 H F 200x20	440 H F 200x20		3, 4
			200x20	1, 2, 3, 4

Table (2). Concreter structure characteristics extracted by ETABS

		Beam				
		frames				stories
		31, 32, 33, 35, 29	30, 34	A,C,E,G	B,D,F	
	Beam section	40x45	40x50	40x45	45x45	1, 2
		35x45	40x50	35x45	45x45	3, 4
Column						
frames						stories
29, 32, 35	30, 34	(31-F)(31-G) (31-A)(31-B)	-F)(33-G) -B) (33 (33-A)(33	29, 35	31, 32, 33, 35, 40	30, 31, 32, 29, 33, 34, 35	
Column section	50x50	55x55	55x55	55x55				2
					45x45	50x50		3, 4
							60x60	1

Table (3). RCS structure characteristics extracted by ETABS

	Beam		
	frames		stories
	A, B, C, D, E, F, 29, 35	30, 31, 32, 33, 34	
Beam section	PG_3	PG_2	1, 2, 3, 4
	Column		
	frames		stories
	A, B	C, D, E, F, G	
Column section	C_5	C_6	1, 2, 3, 4
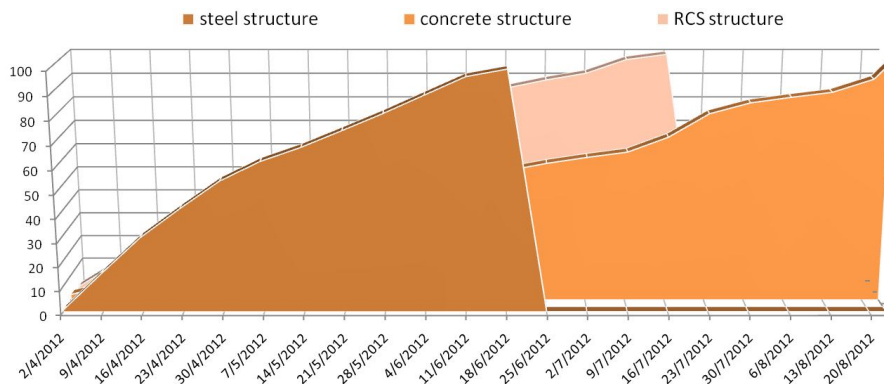
Figure9. Comparison of physical progress for three kinds of structure
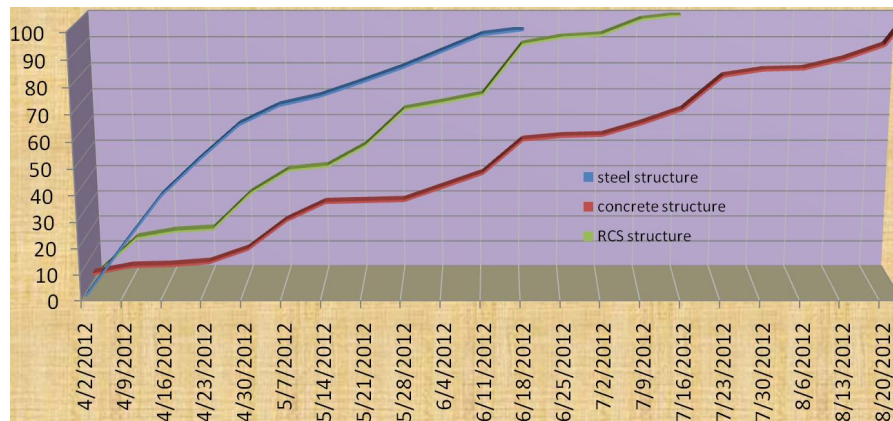
Figure10. Comparison of financial progress for three kinds of structure
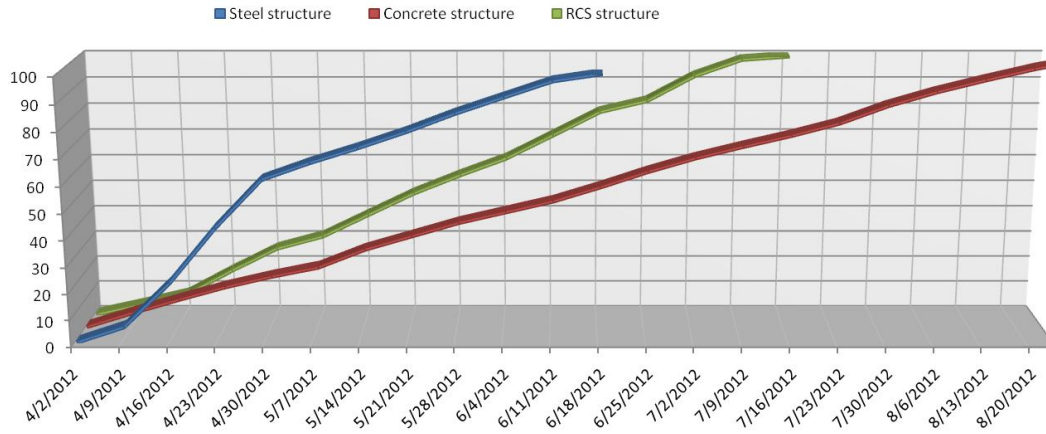
Figure11. Comparison of development of human resources for three kinds of structure
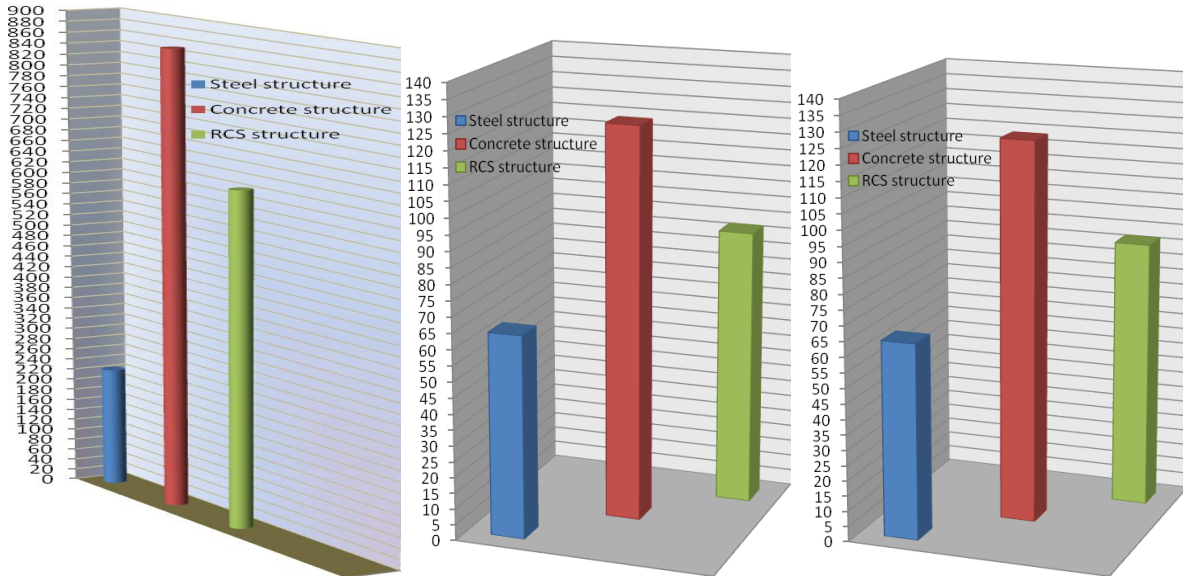
Figure12. Comparative diagrams of the number of required personnel (left), required performance time (middle) and required cost (right) for three kinds of constructed model

CONCLUSION AND DISSCUSION

It can be concluded from the observed general performance that the presented hybrid beam-column connection provides an adequate strength and ductility. The hybrid connection method can be an alternative design in building frame type structures. All of the materials are of the highest quality in order to achieve rational structures, withstand great force and at the same time allow wide spaces between supports. This type of construction allows for large open structures like warehouses for heavy loads and shopping centers.

In this study a practical model to predict the advantages of RCS structures versus steel and concrete ones by regarding a real case study RCS structure in Tehran was presented. The proposed methodology was based on the state of generated computer code, which was defined through the development of a detailed analysis of a case study. A good agreement was found between experimental results and the calculated and predicted by the proposed model. More than the results and resolution of outputs of the generated code in comparison with other available software packages shows good agreement with practical and indicated that this code can employed as a good, strong and reliable tool for this type of analysis.

1. A detailed comparison feasibility study on technical, economical and management conditions between usual structures (steel and concrete) with RCS were performed. At the first by ETABS three mentioned kind of structures with similar basic characteristics were constructed. Then by MSP the performance timing of each of them with total required costs, time and personnel were extracted. At the end to clear the obtained results, by use of MATLAB programming environment a computer code was generated to design the structures and project timing performance. The obtained results showed that the generated code can detect and process of civil operation data and capable to provide higher quality output diagrams with an upper resolution and accuracy.

2. Experimental results indicate that the proposed procedure is effective in controlling joint deformations and damage, leading to economic condition. The obtained number of required personnel for three kinds of model shows that the third place with 224 personnel belongs to steel structure and concrete structure with 852 personnel was the first. In this case the RCS structure with 624 personnel takes the second place. In required performance time (day), the number of 64 (steel structure), 124 (concrete structure) and 87 (RCS structure) was obtained in this study. For required cost (Rials, Iranian unit money), 8660000000 for steel structure, 5160000000 for concrete structure and 7600000000 was computed on base of case condition.

3. Results from the testing of physical progress, required costs and development of human resources in RCS versus steel and concrete structures show that hybrid structures consisting of RC columns and steel beams are suitable for use with lower risk in upper level of construction management.

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Academic Engagement as a Mediator in Relationship between Parenting Style and Academic Achievement among Adolescents in Sirjan-Iran

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Abstract: The major purposes of the presents study were (a) to examine the degree to which academic engagement mediates the relationship between parenting style and academic achievement. Data from 382 participants (191 males and 191 females) were examined using measures of parenting style, academic engagement, and self-reported grade point average. The results indicated that academic engagement mediate relationship between parenting style an academic achievement. Implications for future research are discussed.
[Elham Dehyadegary, Kouros Divsalar, Nooshin Sabour Esmaeili, Azimeh Jafari Sadr, Fatemeh Askari. **Academic Engagement as a Mediator in Relationship between Parenting Style and Academic Achievement among Adolescents in Sirjan-Iran.** *Life Sci J* 2012;9(4):5715-5727] (ISSN:1097-8135). http://www.lifesciencesite.com.851
Keywords: Parenting Style, Academic Engagement, Academic Achievement

1. Introduction

The issue of academic performance among Iran continues to be a matter of great concern for many scholars, researchers, and educators (Fouladi, 2007). Many researchers focus on what has been called the achievement gap (Fathi, 2006), with the general focus on understanding the factors that contribute to differential academic outcomes. In recent years, countries such as Canada, has noted an increase in children with risk factors that may compromise their present achievement and future success, and approximately 27.6% or 1 in 4 students is considered to be at risk for school failure (Jordan, 2006). Also, across the 21.9 million adults in California, 2.19 million males and 1.96 million females (20% of the students) were dropouts (Belfield, 2007). In Iran, a study by Ghasemi (2010) found that

22% of the students in Iran suffer from low academic achievement due to family problems and personal factors.

Halawah (2006) argues that academic achievement has been linked to several influences distinctive to the home environment, such as parenting style and parental involvement. It is generally agreed that parenting is a thankless task and parental responsibility begins from the day the child is born. Parenting is made even harder by the fact that all parents would like to succeed in bringing up their child. The task of parenting is one of the important variables widely investigated in the field of human development (Baldwin, Mclntyre, & Hardaway, 2007). It has been shown that there is a relationship between the way parents bring up their children and adolescents and academic performance. Baumrind's theory

(1971) proposed three styles of parenting that were qualitative in nature: permissive, authoritarian, and authoritative. Parents who are permissive neither exercise control over nor make demands of their children in respect of the way they behave. Although such parents are relatively warm toward their children, their children usually end up not performing well academically (Roche, Ensminger & Cherlin, 2007). In contrast, authoritarian parents who are strict, do not encourage interpersonal dialogue, exercise absolute control over their children's behavior according to a rigid set of standards and demand complete obedience, and in the process do not show much warmth toward their children. As a result, children of authoritarian parents often show poor academic performance (Attaway & Bry, 2004). Parents with an authoritative parenting style make the effort to guide their children by rationalizing their actions, encourage interpersonal communication, and at the same time put their children at ease by being warm toward them. According to Park & Bauer (2002), by using reasoning and emotionally supportive approach, parents help their children to exhibit sound moral reasoning, empathy and self-confidence, which are closely associated with high academic performance.

Parenting Style Theory

Bumrind states that the parenting styles (i.e., authoritative, authoritarian, and permissive) contributes to child behaviour differently. Authoritative parents who are characterized by effectiveness and are supportive of their child, encourage their children to do well academically and explain the need for education in order to become a successful adult. Thus, the child under the authoritative style of parenting performs well in school-related activities and achieves are overall well-being. The authoritative parents also recognize when their child is improving or learning new material and show satisfaction when it is done through hard work. Additionally, these parents are not angered by their children's mistakes; instead they want the children to know that mistakes are part of the learning experience (Baumrind, 1971).

Authoritarian parents are characterized by a strict and rigid disciplinary approach and expect obedience without question. These characteristics negatively affect the child's academic achievement. Stress and anxiety in children may experience higher if their authoritarian parents are involved in their academic activities.

Permissive parents, who allow their children to regulate their own activities, exert no control and rarely use punishment in children's upbringings. Permissiveness style negatively impact children's academic involvement, resulting in their low academic achievement. These parents may not ever visit the school or the classrooms of their children or speak with a school adult involved with their children's educational experience (Baumrind, 1971).It is concluded that various types parenting style will influence academic achievement of adolescents differently.

In light of this, the Baumrind's parenting style theory supports the relationship between parenting style and academic achievement in present study. Academic engagement is individual factor that plays a significant role in students' academic achievement (Ogbu& Davis, 2003; Stewart, 2007). Such engagement can be described as the level of commitment and involvement or the amount of time, energy and effort that students put into their educational learning activities (Greene et al., 2004; Stewart, 2007). Research studies (Carbonaro, 2005; Johnson, Crosnoe, & Elder, 2001; Stewart, 2007; Sirin, 2005; Flowers & Flowers, 2008; Wang & Holcombe, 2010) show that an adolescent student's engagement in academic activities has a significant effect on academic achievement. Students with higher academic engagement, tend to have higher academic performance.

In the present study, involvement in school activities is seen as a mediating construct that connects parenting style with the academic achievement of their children. This belief is supported by a small but steadily growing body of research. For instance, Hedvat (2008) expected and found that children's school engagement mediated the effect of the parental school involvement on adolescent's academic achievement. However, there is still a lack of

evidence to support the assumption that school engagement has a mediating impact on factors like parenting style or peer and school variables as well as personal characteristics. Therefore, the present study also examined the mediate effect of academic engagement on the relationship between parenting style and academic achievement.

2. Objectives
1. To describe the parenting style, academic engagement, and academic achievement of the respondents.
2. To determine the relationships between parenting style, academic engagement and academic achievement of the respondents.
3. To determine the mediating effect of academic engagement on the relationship between parenting style and academic achievement.

3. Method
Research design
This study used a descriptive and correlational research design to examine the relationships between parenting style, academic engagement and academic achievement. Also, it is a cross-sectional study which involves collecting data over a short period of time in order to search for the answer for the outlined research questions.

Population and Sample
The study population in this research is high school students from two districts in Sirjan. The total number of students in two districts is 3,500, with 1,640 (47%) of the population in south district high schools and 1,860 (53%) in north district high schools (Ministry of Sirjan Education, 2010). Table 3.2, shows that the number of student's aged 15-16 years old in two districts is 1,738 while the number of students aged 17-18 years old in two districts is 1,762. Based on Krejcie and Morgan Table (1970), the number of respondents required to represent the population of the present study is 382 by the proportional stratified random sampling technique was used to choose a representative sample from the target population.

4. Measures
Parenting Style

Parenting style was measured by Parental Authority Questionnaire (PAQ: Buri, 1991). PAQ is valid and reliable instruments. PAQ consists of 30 items with three subscales which are permissive, authoritarian, and authoritative parental authority types. There are 10 items for each subscale. Examples of items included in the Parental Authority Questionnaire are as follows:
1. As I was growing up, once family policy had been established, my mother/father discussed the reasoning behind the policy with the children in the family(Authoritative Style).
1. Whenever my mother/ father told me to do something as I was growing up, she/he expected me to do it immediately without asking any questions (Authoritarian Styles)
1. As I was growing up my mother/father did not feel that I needed to obey rules and regulations of behavior simply because someone in authority had established them (Permissive Style)

Parental Authority Questionnaire is rated on five point Likert scale ranging from 1= strongly disagree, 2= disagree, 3= neither agree nor disagree, 4= agree and 5= strongly agree. For each subscale, the score was obtained by summing the score for individual items. Score on each subscale ranged from 10 to 50. High score means high parenting in specific subscale of PAQ.

Buri (1991) reported high reliability for PAQ with Cronbach coefficient alpha values with a range from 0.78 to 0.86 for the mother's parenting style and 0.74 to 0.87 for the father's parenting style. In this study, the subscales within the Parental Authority Questionnaire showed varying degrees of internal consistency ranging from.70 to .76 in a pilot study and .78 to .82 in actual study .PAQ has been tested by Esfandiary (1995) to determine its reliability and validity among Iranian samples. It yielded respectable test-retest reliability.

Academic Engagement
Academic engagement was measured using Academic Engagement Scale (AES) by Short, Fleming, Guiling, and Roper (2002). The AES was developed by Short, Vowels, and Robinson (2002). The AES has 40 items with three subscales. The subscales are cognitive

engagement (10 items), behavioral engagement (15 items), and affective engagement (15 items). A five-point Likert scale from 1= never, 2= seldom, 3= sometimes, 4= often and 5= always was used to rate the items. The score for AES was obtained by summing up the scores for the 40 items after reversing 11 items (items 7, 8, 12, 14, 15, 18, 19, 20, 26, 29 and 34). The total scale score ranged from 40 to 200, with high score indicating high academic engagement among respondents. The AES has demonstrated respectable psychometric properties (alpha =.94).In the current study, alpha reliability for the scale was .87.

Academic Achievement

Respondent's academic achievement was measured by using cumulative grade point average (CGPA) obtained by students' in the academic year of 2009-2010. According to the rules of the Ministry of Education in Iran, the range of academic achievement (GPA) is from 0 to 20, which can be categorized into four levels: fail (scores of 0-9), weak (scores of 10-14.99), moderate (scores of 15-16.99), and excellent (scores of 17-20). In the present study, the cumulative grade point average (CGPA) was utilized in differently. High scores mean high academic achievement.

5. Data Analysis

Data from the present study were processed and analyzed using Statistical Package for Social Science (SPSS) version 16. Three statistical procedures i.e. exploratory data analysis, descriptive analysis, and inferential statistical analysis were utilized for the data analyses. Descriptive statistics such as mean score, standard deviation, percentage and frequency distribution were used to describe the demographic profiles of the respondents. Inferential statistics that was used in the data analysis were Pearson Corelation Analysis, and Hierarchical Multiple Regression analysis.

6. Results

Descriptive findings

Respondents in this study were high school students in Sirjan, Iran. As shown in Table 1, there were equal number of male (50%) and female (50%) students who were involved as respondents of the study. The mean age of the respondents was 16.50 years (SD= 1.11). As shown in Table 1, majority (75.4%) of the respondents reported that their parents were authoritative. The remaining respondents perceived their parents authoritarian (13.6%) and permissive (11.0%). Also, more than half of the respondents have high engagement in school activity and homework (57.1%) and high academic achievement (62.5%).

Table 1: Gender, Age, and Levels of Variables

Variables		n	%	Variables	n	%
Gender (N=382)				**Parenting Style**		
Female		191	50	*Authoritative Parenting Style*	288	75.4
Male		191	50	*Authoritarian Parenting Style*	52	13.6
Age				*Permissive Parenting Style*	42	11
15 Years		95	24.9	**Academic Engagement**		
16 Years		96	25.1	*Low*	164	42.9
17 Years		96	25.1	*High*	218	57.1
18 Years		95	24.9	**Academic Achievement**		
Mean	*16.50*			*Low*	143	37.5
S.D	*1.11*			*High*	239	62.5
Minimum	*15*					
Maximum	*18*					

Bivariate Analysis

The Pearson correlation analysis was conducted to examine the relationships between parenting style, academic engagement and academic achievement.

Relationship between parenting style and academic achievement

Pearson correlation test was conducted to examine the relationship between authoritative parenting style and academic achievement. As shown in Table 2, there was a weak positive and significant relationship between authoritative maternal style and academic achievement (r=.25, p<.01). The positive correlation coefficient indicated that an increase in the score for authoritative parenting style is followed by an increase in the adolescents' academic achievement. Adolescents with authoritative parenting were more likely to perform better in academic aspects. Also, there was a negative correlation between parenting authoritarian style and academic achievement (r=-.037, p>.05). This means that parents who are highly authoritarian, have adolescents children with lower academic achievement. However, the relationship was not statistically significant.

In other hand, there was a negative significant correlation between permissive parenting style and academic achievement (r=-.16, p<.01). This means that parents who are highly permissive, have adolescents children with lower academic achievement. The strength of correlation between maternal permissiveness and academic achievement is weak.

Relationship between parenting style and academic engagement

As shown in Table 2, there was a weak positive significant relationship between authoritative parenting style and academic engagement (r=.35, p<.01). The positive correlation coefficient indicates that an increase in the score for authoritative parenting style is followed by an increase in the adolescents' academic engagement. Adolescents with authoritative mothers were more likely to report higher engagement in academic activities. Also, there was a negative correlation between authoritarian parental style and academic engagement (r=-.053, p>.01). This means that parents who were highly authoritarian, have adolescents children with lower academic engagement. However, the relationship was not statistically significant. In the other hand, there was a negative significant correlation between permissive parenting style and academic engagement (r=-.17, p<.01). Even though the strength of correlation is weak, the result indicates that parents, who were highly permissive, had adolescent's children with lower academic engagement.

Relationship between academic engagement and academic achievement

As shown in Table 2, the result of the present study show that there was a significant positive relationship between academic engagement and academic achievement of adolescents (r=.69, p<.01). This means that respondents who were highly engaged in school activities, reported higher academic achievement. The strength of correlation between academic engagement and academic achievement is strong.

Table 2: Correlation analysis

	Variables	X1	X2	X3	X4	Y
X1	Authoritative Parenting Style	1				
X2	Authoritarian Parenting Style	-.256**	1			
X3	Permissive Parenting Style	.248**	.243**	1		
X4	Academic Engagement	.355**	.053	-.174**	1	
Y	Academic Achievement	.251**	-.037	.162**	-.69**	1

Mediation Analysis

A series of Multiple Regression analyses were conducted to explore the mediating effect of academic engagement on the relationships between parenting style with academic achievement. The mediation test examines the indirect effect of predictor (X) on the outcome (Y) variable through mediator variable (Z). The present study follows the guideline proposed by Baron and Kenny (1986) to test the mediation effect of a mediator on the relationship between the independent and dependent variables. According to Baron and Kenny (1986), there are four steps in establishing mediation:

Step 1: There must be a significant relationship between the predictor and the outcome variable.

Step 2: The relationship between the predictor and the hypothesized mediator is significant.

Step 3: The hypothesized mediator is significantly related to the outcome variable when both the IV and the mediator are treated as predictors and DV as the outcome variable.

Step 4: When the assumptions at step1 to 3 are fulfilled, the mediation test is conducted (step 4). The IV and mediator are treated as predictors and DV as the outcome variable. To establish that the mediator variable completely mediates the relationship between IV and DV, the unstandardized coefficient (path c´) should be zero.

At step 4, if there is a mediation effect, the strength of relationship between the predictor and the outcome is reduced after controlling for the effect of the mediator. Figure 1 shows the mediation model of the relationship between the independent variables and the outcome variable. Path *a* indicates the relationship between the independent variable and the mediator. Path *b* refers to the relationship between the mediator and the outcome variable. Path *c'* indicates the relationship between the independent variable and the outcome variable after controlling for the mediator. According to Baron and Kenney (1986), it is preferable to used unstandardized coefficients in mediating analyses. This is supported by Dugerd, Todman, and Strains (2010).
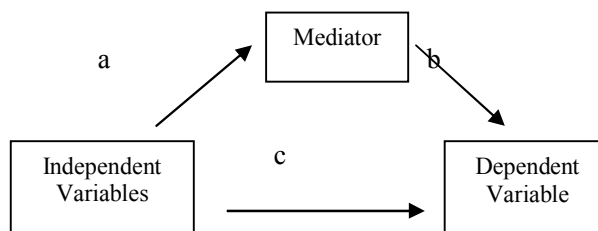
Figure 1: Mediation Model (Center)

When the results shown are consistent with the mediation model (partial or complete mediation), Sobel test was conducted to confirm the significant effect of the mediation. Partial mediation means that path b (relationship between the mediator and the outcome variable) is significant after controlling for independent variable; and path c' is still significant. Complete mediation means that the measured effect in path c' (relationship between independent and the dependent variable after fixing the mediator variable) is zero or at least non-significant (Dugerd, Todman, & Strains, 2010).

Academic engagement mediate relationship between authoritative parenting style and academic achievement

As shown in Table 3, there was a direct significant effect of authoritative maternal style on academic achievement (B=.090, SE=.018, t= 5.047, p<.05) and academic engagement (B= 1.506, SE=.203, t=7.413, p<.05). The relationship between academic engagement (mediator) and academic achievement was also significant (B=.059, SE=.003, t=19.053, p<.05).

Based on the fourth regression step (see Table 3 and Figure 2), the results support the complete mediation model. The relationship between authoritative maternal style and academic achievement after fixing academic engagement is approaching zero and non-significant (Beta=.001, SE=.014, p>.05). The amount of mediation was obtained by deducting the regression coefficient (authoritative Maternal style → Academic achievement) in the fourth regression (when academic engagement controlled) from the regression coefficient (Maternal authoritative

style → Academic achievement) in the first regression (with academic engagement not controlled). The reduction in the maternal authoritative → academic achievement when academic engagement was controlled was 0.090-0.001= .089. The mediation effect was tasted by application of the Soble test (Baron & Kenny,1986) to the unstandardized coefficient and standard error values for maternal authoritative style → academic engagement (2nd regression)and academic engagement → academic achievement when maternal authoritative style is controlled (forth regression). This yielded a value of Z= 6.9170, p<.05, so it was confirmed that academic engagement significantly mediates the relationship between maternal authoritative style and academic achievement.

Table 3: Relationship between maternal Authoritative Style and Adolescents' Academic Achievement Mediated by Academic Engagement

Step I V	DV	B	SE	Beta	t
1 Parental Authoritative. S	Academic Achievement	.090**	.018	.251	5.047
2 Parental Authoritative .S	Academic Engagement	1.506**	.203	.355	7.413
3 Academic Engagement	Academic Achievement	.059**	.003	.699	19.053
4 Parental Authoritative .S	Academic Achievement	.001	.014	.003	.064
Academic Engagement		.059**	.003	.698	17.763

*Note:*B= Unstandardized coefficient; Beta= Standardized coefficient
** p<.05
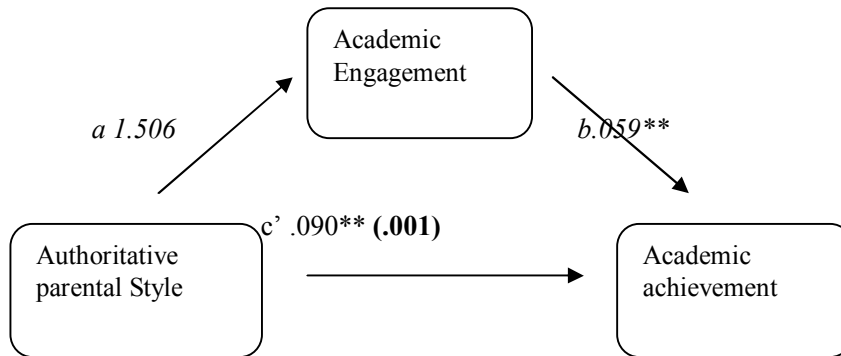
Note: **p<.05

Figure 2: Mediation model

Academic engagement mediate relationship between authoritarian parenting style and academic achievement

The results of the analysis showed that there was no significant relationship between authoritarian parenting style with academic achievement and academic engagement. Therefore mediation analysis was not performed. The results implied that academic engagement does not mediate the relationship between authoritarian parenting style and academic achievement among adolescents.

Academic engagement mediate relationship between permissive parenting style and academic achievement

Table 4 shows that there was a direct significant effect of permissive parenting style on academic achievement (B= -.059, SE=.018, t= -3.196, p<.05) and academic engagement (B=-.747, SE=.217, t= -3.437, p<.05). The relationship between academic engagement (mediator) and academic achievement was

also significant (B= .059, SE=.003, t=19.053, p<.05). The results of multiple regression analysis at Step 4 (Beta=-.015, SE= .014) supported the complete mediation model. The summary of the results is presented in Figure 3. The amount of mediation was obtained by subtracting the regression coefficient (Permissive maternal style → Academic achievement) in the fourth regression (when academic engagement controlled) from the regression coefficient (Permissive parenting style → Academic achievement) in the first regression (with academic engagement not controlled). The reduction was -0.015-(-.059) = .044. Soble test was conducted to check the significance of the indirect effect of the permissive parenting style on academic achievement via academic engagement. The results of the Sobel test confirmed that academic engagement significantly carries the influence of the parenting permissive style to academic achievement (Z = -3.3908, P <.05).

Table 4: Relationship between Permissive Parenting Style and Adolescents' Academic Achievement Mediated by Academic Engagement

Step	I V	D V	B	SE	Beta	t
1	Maternal Permissive. S	Academic Achievement	-.059**	.018	-.162	- 3.19
2	Maternal Permissive. S	Academic Engagement	-.747**	.217	-.174	-3.43
3	Academic Engagement	Academic Achievement	.059**	.003	.699	19.05
4	Maternal Permissive. S	Academic Achievement	-.015	.014	-.042	-1.11
	Academic Engagement		.059**	.003	.692	18.57

Note: **B**= Unstandardized coefficient; Beta= Standardized coefficient** **p<.05**
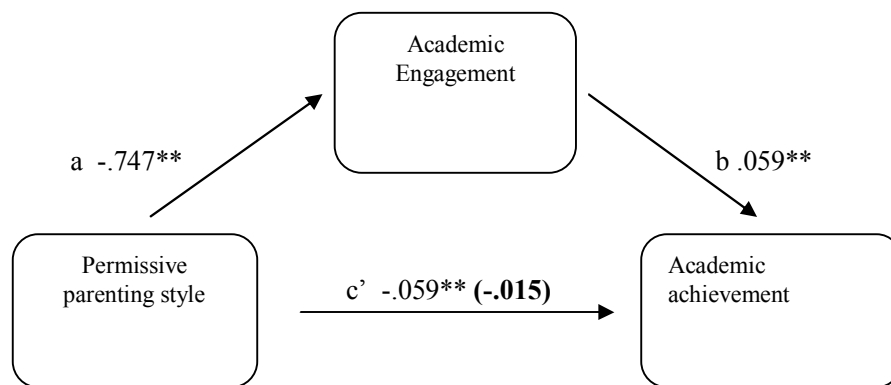
Figure 3: Mediation model

Discussion and Conclusion

Respondents of the present study were adolescent females and males aged between 15 and 18 years old. Results of the study indicated that a majority of the respondents perceived their parents as authoritative. This finding of the present study is consistent with past studies by Nouri (2007) and Saeedi (2003) which indicated that most of the participants of their study viewed their parents as authoritative. Based on Baumrind's (1970), respondents' report indicated that they may perceived that their mother give clear instructions, rationalized reasons for actions, show a sense of control and make their wishes clear in a way that the children see them as caring and warm. More than half of the respondents reported high academic engagement and academic achievement.

Pearson correction test indicated that students who had parents with authoritative style were more likely to have high academic engagement and academic achievement. Authoritative parenting style has positive influence on academic achievement. The present finding support past studies on parenting style and academic achievement (Pong et al., 2010; Ellefsen & Beran, 2007; Assadi et al., 2007; Lee et al., 2006; Park & Bauer, 2002). Authoritative parenting style resulted in several effects on the way adolescents developed and behaved and the positive influence of authoritative parenting style remains substantial during adolescence

(Baumrind, 1991 & Steinberg, 2001). Positive relationships between parents and their children can promote a healthy sense of competence and autonomy within the home which then becomes internalized and used in other settings such as schools (Furrer & Skinner, 2003). There are three possible reasons for the relationship between authoritative parents and children with high academic performance. Firstly, parents with such a parenting style give their children a feeling of being secure and cared for. The children feel comfortable and independent and this helps them to perform well in school. Secondly, such parents make it a point to explain their actions to their children to ensure that they understand the reason for any action. Such efforts by the parents to rationalize their actions to their children allow them to be aware of and to understand why their parents do what they do. They get to understand their parents' intentions, values and objectives in relation to their school work and school. Thirdly, parents who are authoritative have an open communication channel with their children. Due to such interpersonal communication with their parents, children develop their interpersonal skills and interact well with friends and peers. In general, they are well-adjusted and do well academically and socially (Spera, 2005).

Also, result of Pearson correlation showed students who had parents with authoritarian style have low academic engagement and

academic achievement. The finding is in line with Park and Bauer (2002) and Elias and Yee (2009) who didn't find any relationship between authoritarian parenting style and academic achievement. According to the effect of parenting style in childhood may have direct effect but the effect may alter to indirect when the children become adolescents (Glasgow, Dornbusch, Troyer, Steinberg, & Ritter, 1997). In adolescence period, their parents were ready to step away from direct parenting and engagement in the facets of their life such as in their academic (Paulson & Sputa, 1996). During this period, association of adolescents and their parents tend to alter and other factors such as relationship with peers have a stronger effect on their academic performance in schools (Steinberg, Dornbusch, & Brown, 1992).

In light of the relationship between permissive parenting style and academic achievement students under rearing parents with permissive have low academic engagement and academic achievement. Results from the present study support past findings (Pong et al., 2010; Roche et al., 2007; Assadi et al. 2007; Ellefsen & Beran, 2007; Lee et al.2006), which found that permissive parenting relates to more school problems among adolescents. In permissive parents, negotiation and demands are relatively low. Responsive parents provide support and guide their adolescent children towards achieving specific goals (Pellerin, 2005). Therefore, when children feel rejected by their parents and do not get much parental guidance, they are more likely to do poorly in their academic endeavors. Eccles et al. (1983) believe that such children may be affected negatively in their academic work when they have parents who do not show any consistent parenting style or who seem to reject them. Encouraged and guided children who are praised for their achievements develop a sense of confidence and are likely to excel academically and prepared to take on challenges. And when such children succeed in their endeavors, they do their parents proud and in turn motivate them to give continued support to their children. In contrast, children who do not do well in school create strain and disapproval among their parents and this leads to even less satisfactory parenting ways.

Result of present study utilized that that students who were highly engaged in school activities, reported higher academic achievement. The present finding is consistent with the finding of past studies (Fallon, 2010; Haney, 2010; Wang & Holcombe, 2010; Sbrocco, 2009; Flower & Flower, 2008; Stewart, 2007; Sirin & Sirin, 2005) which concluded that school engagement is closely related to academic performance. Children feel that they have the attention and support of teachers and parents in their academic and school activities, they will naturally develop a special sense of belonging and attachment to both school and school-related activities, including academic activities. Thus, there is a tendency for such children to achieve higher grades and generally show better academic achievements. Also, students who value their education and have clear ideas about goals they wish to achieve will exhibit a desire for status attainment and be higher performing students (Carbonaro, 2005).

Finally, academic engagement completely (full) mediated the relationship between parenting authoritative style and permissive style with adolescents' academic achievement. Parenting authoritative and permissive style affects adolescent academic achievement primarily via academic engagement. Based on the Baumrind's Parenting Style Theory (1966), authoritative parents encourage their children to do well academically and explain the need for education in order to become a successful adult. Encouragement and explanation will motivate children to involve in school-related activities and achieves overall well-being. The authoritative parent also recognizes when their child is improving or learning new material and is satisfied most when it is done through hard work. Thus, the engagement in school activities leads to high academic achievement. In addition, authoritative parent is not angered by the child's mistakes; instead the parent wants the child to know that mistakes are part of the learning experience (Baumrind, 1971). Permissive parents do not involve in regulating their children's activities and exert no control on them. These parents may not even visit the school or the classrooms of their children or speak with a school adult involved with their children's educational experience

(Baumrind, 1971). The nature of this parenting style may negatively impact their children's academic involvement, which consequently leads to lower academic achievement.

Implications
In view of the value of academic success for students, parents, teachers and the community, results the appropriate and right techniques to prevent low academic achievement is vital. Based on the finding of this study parenting style, academic engagement has relationship with academic achievement among high school students. In other words, adolescent students with authoritarian and permissive parents are likely to drop out from school. Also, students who have low academic engagement are likely to drop out from school. Hence, methods of training parenting style for parent, implying that interventions designed to increase school engagement that may positively affect students levels of academic achievement, need to receive guidance about their education and occupational from teacher and parent must be devised in order to reduce the students' dropout rate and increase their academic achievement. Thus, it is recommended that parents' parenting in Iran be equipped with appropriate knowledge and strategies. It is further assumed that the information learned in this study has important implications to the Iranian Ministry of Education to better understand the factors which have strong influence on adolescent's academic achievement. It can also be potential input for improving educational policy.

The findings from the present study can also be used by the Ministry of Education should in conducting seminars to motivate and enhance students' academic engagement. The results of the present study will be a useful input to the organizations aimed at promoting family and adolescent development.

The present findings provide empirical basis for future research in academic achievement among adolescents in Iran. This study explains academic achievement in relationship to family and personal factors. Future study may venture into other unexplored phenomenon in academic achievement.

Recommendations for Future Research
This research is limited to these factors: parenting style, academic engagement and academic achievement. Familiarization and information regarding many relevant factors affecting academic achievement are significant for any educational organizations. However, this study was only conducted in Sirjan, thus its scope and generalization not representative other locations. Therefore, to strengthen the validity of the results and also to generalize to the other population, it is recommended separate studies are carried out in more locations. According to the framework of the present study, academic engagement is the mediating variable. However, there are many other variables, such as motivation and self-esteem, self-control, which could become mediating variables that could be studied in future, researches. It is also suggested that studies be carried out to include factors such as psychiatric disorders, family problems, financial problems and school problems such as teaching space, the number of students per class and type of teachers and their behavior which could affect students' academic achievement. In this research, the respondents were adolescents, thus the results were limited to this age group only and mothers. It is suggested that future studies be conducted among other age group. With respect to age, for instance academic achievement is different. This is because there are various problems encountered by the other age groups which are difficult to quantify.

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Synthesis and thermogravimetric analysis of modified glycidyl methacrylate copolymers with oxime groups

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Abstract: In this work the homopolymer and copolymers of glycidyl methacrylate (GMA) with methyl methacrylate (MMA), ethyl methacrylate (EMA), methyl acrylate (MA) and ethyl acrylate (EA) were synthesized by free radical polymerization using azobis(isobutyronitrile) (AIBN) as initiator at 70 ± 1 ºC. Then, copolymers of glycidyl methacrylate have been modified by incorporation of highly sterically hindered demanding benzophenone oxime through the ring opening reaction of the epoxy groups. The polymers were characterized by ^{1}H-NMR and FT-IR. Presence of bulk the benzophenone oxime groups in polymer side chains leads to an increase in the rigidity and glass transition temperature of polymer as shown by DMTA analysis. The thermal stability of all copolymers was determined by thermogravimetric analysis (TGA). This modification increases the thermal stability of polymers. With the incorporation of the benzophenone oxime groups in the polymer side chains, a series of novel modified polymer containing new properties are obtained, that can find some applications in polymer industry.

INTRODUCTION

2,3-Epoxypropyl methacrylate (or glycidyl methacrylate = GMA) is a fascinating monomer exhibiting polymerizable Methacrylic unsaturation and an oxirane function of potential reactivity [1]. Due to the reactive nature of the epoxy group, copolymers containing GMA have led to an interesting class of new materials. This monomer has been homopolymerized and copolymerized by means of free-radical initiators (organic peroxides or azo-catalysts) known to selectively attack methacrylic double bonds [2, 3, 4]. The copolymers based on the potential class of functional polymers. Free-radical random copolymerizations of GMA with conventional monomers have been widely investigated for their potential applications [5, 6]. The interest in these copolymers is largely due to the ability of the pendant epoxy group to enter into a large number of chemical reactions [7]. Copolymers based on glycidyl methacrylate (GMA) have applications in biological drugs and biomolecules and in electronics as negative electron-beam resists materials [8, 9]. The epoxide opening reaction with nucleophiles is generally performed with acidic or basic catalysis and in the absence of such catalysts, the reaction is moderately slow [10, 11]. Oxime and oxime ethers are important intermediates in organic synthesis. O- and N-Alkylated products were obtained by reaction of pyridine-2-, -3-, and -4-carbaldehyde oximes with epoxy compounds (1-chloro-2,3-

epoxypropane, and 1-bromo-2,3-epoxypropane) in the presence of a base and under condition of phase-transfer catalysis [12, 13, 14]. Present research work describes the novel synthesis and properties of copolymers of GMA modified with benzophenone oxime. The thermal stability of the modified polymer was determined by TGA analysis. This scans show that the presence of bulky benzophenone oxime groups leads to higher thermal stability for modified polymers.

MATERIALS AND METHODS

Synthesis of (glycidyl methacrylate-co-ethyl acrylate) copolymer; poly(GMA-co- EA)

In a flask 100 mL, initiator (0.4 mmol, 6.500×10^{-2} g) and glycidyl methacrylate (20 mmol, 2.840 g) and ethyl acrylate (20 mmol, 2.560 g) are dissolved in tetrahydrofuran (THF) solvent 20 mL. The solution is de-oxygenated by nitrogen gas flow. Then, it is stirred in 70 ± 1°C temperature for 24 hours. The resulted sticky solution is poured into a beaker containing cold ethanol 125 mL. While it is stirred by a magnetic stirrer. Gradually, polymeric precipitations are formed, the polymeric precipitations are separated by decantation of methanol and are washed by methanol and are dried under vacuum in room temperature. The resulted polymer weight is 3.9 g and reaction yield is %72. The reaction condition is shown in Scheme 1.

ploy(GMA-co-MMA);X=CH₃ ,Y=CH₃   poly(GMA-co-MA);X=CH₃ , Y=H
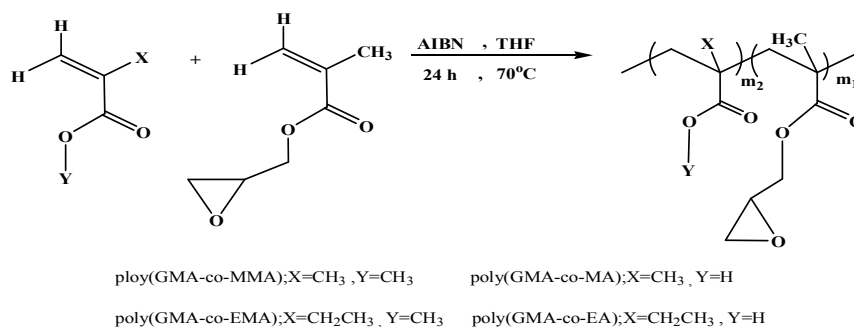
poly(GMA-co-EMA);X=CH₂CH₃ , Y=CH₃   poly(GMA-co-EA);X=CH₂CH₃ , Y=H
Scheme1. Synthesis of glycidyl methacrylate copolymers

Synthesis of (glycidyl methacrylate-co-ethyl acrylate) copolymer containing benzophenone oxime:
In a 100 mL tow-necked flask equipped a dropping funnel and a reflux condenser, benzophenone oxime (3.92 g, 20 mmol) was dissolved in dry DMF solvent (20 mL) under nitrogen gas and guard tube of chloride calcium. After one hour, BTAC (20 mmol, 4.46 g) is added into the flask containing oxime. Then Sodium hydride (20 mmol, 0.48 g) was slowly added to benzophenone oxime. Then, in a 100 mL flask (10 mmol, 2.56 g) of copolymer (GMA-co-EA) was dissolve in 15 mL of DMF, next, flask contents that containing solved copolymer are added dropwise into tow-necked flask(containing oxime group) during one hour. There action solution is stirred in 25 ^{o}C for 3 hours. For

precipitation of flask contents, cold ethanol 150mL is added in to a beaker. Then, polymeric precipitation is formed in the bottom of beaker, next it is preserved in a refrigerator for one night to formed alkoxide is conversed to hydroxide. Polymer is filtered by helping a funnel and filter paper. For washing of unreacted oxime, the resulted precipitation is continued with ethanol 50mL twice and with water 50mL twice. Also, for washing of potassium carbonate, we continue washing with water and the resulted white precipitation was dried under vacuum for one hour and in room temperature for 48 hours. The resulted weight is 4.1 g and the reaction yield is %63. Reaction condition is shown in Scheme 2.

Copolymer	I	II	III	IV
X	CH$_3$	CH$_3$	H	H
Y	CH$_3$	C$_2$H$_5$	CH$_3$	C$_2$H$_5$
	MMA	EMA	MA	EA
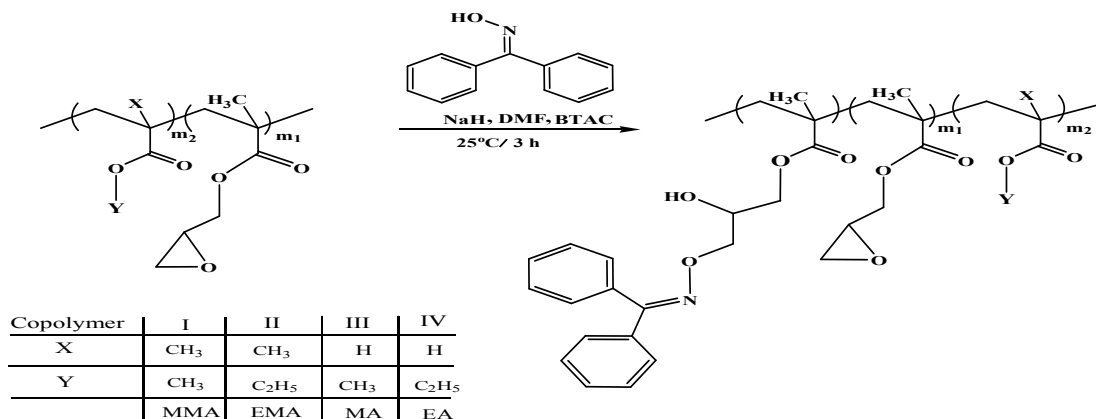
Scheme 2. Synthesis of glycidyl methacrylate copolymers containing benzophenone oxime groups

RESULTS AND DISCUSSION
Study of FT- IR spectra: Poly (GMA -co- EA)
Symmetric and asymmetric stretch vibrations of aliphatic C-H bonds have seen in 3043 and 2980 cm^{-1}. The stretch vibration of

carbonyl ester has seen in 1732 cm^{-1}.The peak in 908 cm^{-1} is related to asymmetric stretch vibration of epoxy ring C–O bonds. The peaks in 1261 cm^{-1} and 1147 cm^{-1} are related to stretch vibration of ester C-O bonds. Figure 1.
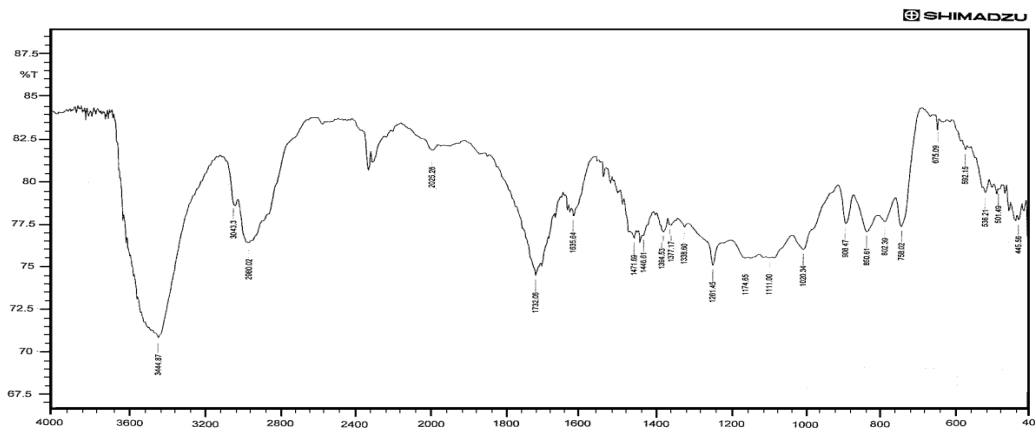
Figure 1. FT-IR Spectra of poly(GMA-co-EA) before reaction with benzophenone oxime.

Study of ^{1}H-NMR spectra: Poly(GMA-co- EA) in CDCl$_3$
In ^{1}H-NMR Spectra, glycidyl methacrylate-co-ethyl acrylate copolymer, CH$_3$ protons of the main polymer chain and CH$_2$ protons of the main chain and CH ethyl acrylate have seen in 0.87-2.19 ppm. Methylen protons of epoxy rings have seen in 2.56 ppm and 2.76 ppm. 3.14ppm peak is caused by methyn

protons of epoxy rings. Two methylen protons through COOCH$_2$ ester group in polymer GMA unit have seen in 4.00 ppm and 4.23 ppm. The absorption of two methylen protons through COOCH$_2$ ester group related to EA units have seen in 3.72 ppm. Figure 2.
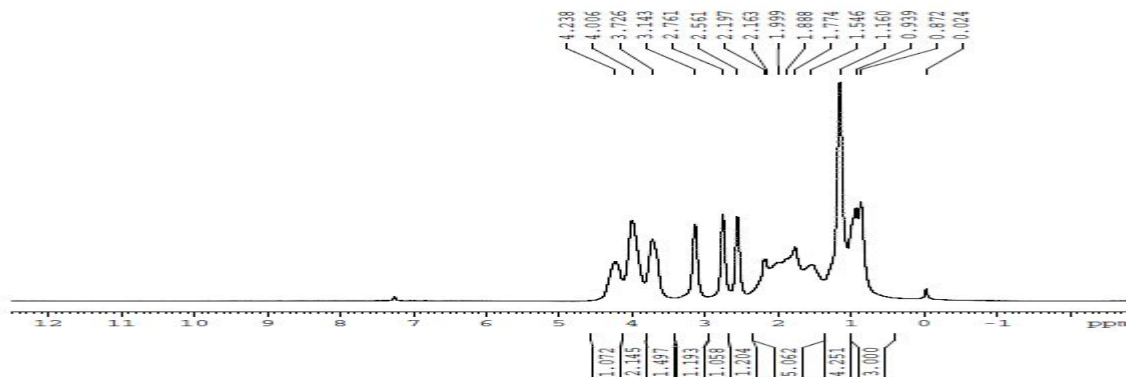
Figure 2. ^{1}H-NMR Spectra of poly(GMA-co-EA) before reaction with benzophenone oxime.

Study of FT- IR spectra: Poly (GMA -co- EA) containing benzophenone oxime group

In FT- IR spectrum, modified polymer of related peak to epoxide ring C-O in 912 cm^{-1} has destroyed peak to epoxide ring C-O in 908 cm^{-1} has destroyed to secondary alcohol functional group in polymeric chain that it is indicative of opening ring reactions in fundamental polymer. The related peak to ester group has stretched from 1732 cm^{-1}, probably it is

formed with generated OH of hydrogen bonding peak to alcohol in 3434 cm^{-1}. The peaks with average intensity that have seen in 1514 and 1465 cm^{-1} are related to aromatic ring C=C after through oxime. The related peak to oxime N-O bond has seen after through in 945cm^{-1}. The remain peaks of IR spectrum similar with polymer peaks have not reacted. Figure 3.
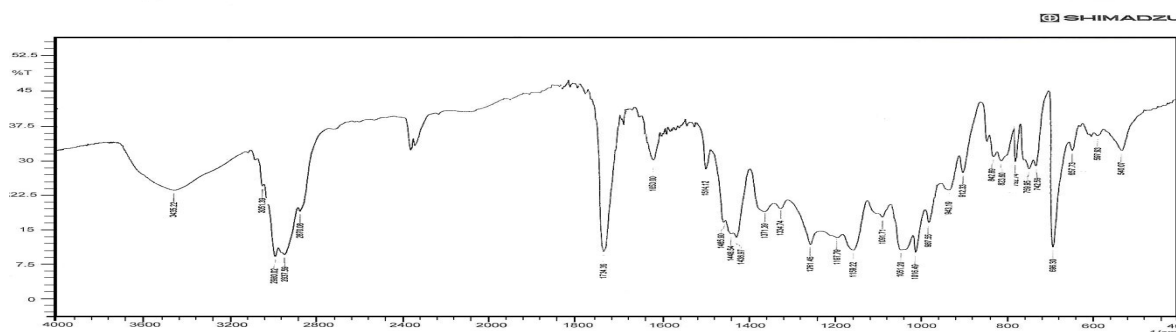
Figure 3. FT-IR Spectra of poly(GMA-co-EA) after reaction with benzophenone oxime.

Study of ^{1}H-NMR spectra: Poly(GMA-co-EA) containing benzophenone oxime in CDCl3.

In ^{1}H-NMR spectra, glycidyl methacrylate-ethyl acrylate modified copolymer hadn't destroyed perfectly due to spatial inhibition of benzophenone oxime groups, CH_2 two protons of epoxide ring in 2.6 ppm. The peak in 3.8 ppm is related to two protons methylen through $COOCH_2$ ester group in EA unit. Related protons to CH_2, CH of epoxide ring overlapped after

opening the amount of epoxides in 4.27-4.34 ppm with related protons to $COOCH_2$ two protons in GMA unit and the peak of OH groups that caused by opening epoxide ring. The peaks in 6.8-7.33 ppm are indicative of benzophenone oxime protons. Other protons in copolymer structure have remained in 0.998-1.96 ppm range. Figure 4.
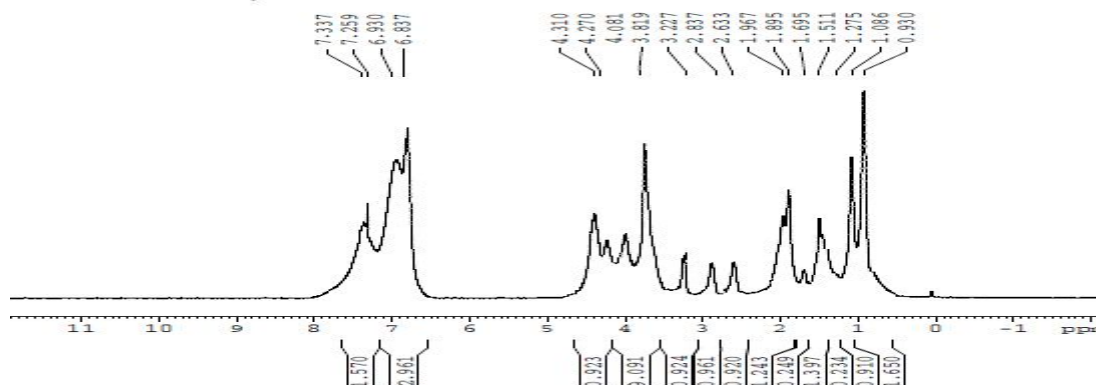
Figure 4. ^{1}H-NMR Spectra of poly(GMA-co-EA) after reaction with benzophenone oxime.

TGA Analysis

The thermal stability of polymers were determined by thermogravimetric analysis (TGA) as it is shown in Figure 5. Thermal gravimetric analysis (TGA) reveals that the framework of polymer before reaction with benzophenone oxime is stable up to 280 ^{o}C, and an abrupt well-defined weight loss follows from 300 to 435 ^{o}C. Poly(GMA-co-EA) after reaction with benzophenone oxime exhibits three relatively well-defined steps for its weight loss. The first weight loss at 25–90 ^{o}C corresponds to the loss of excess water remaining in polymer. Then it's stable up to 280 ^{o}C. Above which, the second weight loss occurs at 290–345 ^{o}C,

corresponding to the loss of oxime-polymer bounds (-N-O-CH$_2$). Above this temperature range, the framework breaks down gradually. The third weight loss occurs at 350-450 ^{o}C. According to the TGA data, the frameworks of polymers have practically the same thermal stability, but their decomposition modes appear to depend on breaking of the new connections after modification. Study of thermal properties of the obtained polymers by TGA curves showed that, thermal stability of acrylate and methacrylate copolymers containing oxime groups was found to be increased(about 25%) with incorporation of oxime groups in polymer structures. Figure 5.
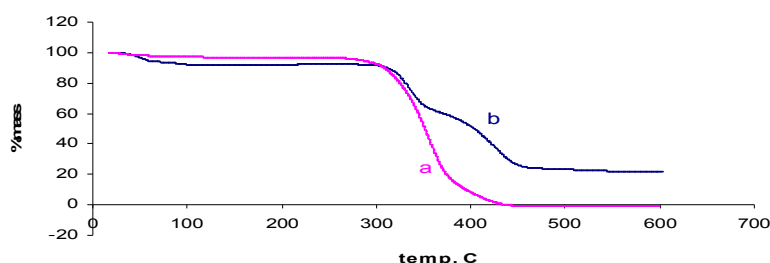
Figure 5. TGA Curves of poly (GMA-co-EA): (a) before reaction and (b) after reaction with benzophenone oxime

The presence of oxime groups in the polymer side chains created new polymers with novel modified properties that may find some applications in polymer industry. For example, the polymer systems with very bulky oxime side chain substituents can be used as fluid separation membranes.

ACKNOWLEDGEMENT

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Structural Relationships of Social and Marriage-Related Factors with the Amount of relationship with the Opposite Sex

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Abstract: In the recent years, one of the issues, which young people have encountered as a social norm, is friendship and relationship between girls and boys particularly at the university while there are legal, social, cultural and religious limitations in this respect, which causes concern and anxiety among parents and authorities. The purpose of this study is to investigate the structural relationships of social and marriage-related factors with the amount of relationships with the opposite sex. The statistical population is single students of Bahonar University of Kerman in the Iranian academic year of 2010-2011. Samples are 384 (212 girls and 172 boys) students. The research approach we have adopted is descriptive ex post facto research. Two researcher-prepared questionnaires have been used to gather data: a ten-item questionnaire intended to measure the amount of relationship with the opposite sex and a twenty-item questionnaire intended to measure social and marriage-related factors. Construct validity of the questionnaires was examined using confirmatory factor analysis approach. To analyze the data and structural equation modeling (SEM), SPSS18 and Amos18 software were used, respectively. The results of structural equation modeling showed that, the indices for measuring the elements have an acceptable validity and reliability and based on these elements a model could be drawn to explain the relationship with the opposite sex among teenagers and youth.

Key words: Social Factors, Marriage-Related Factors, Relationship with the Opposite Sex, Structural Equation Modeling (SEM)

1. Introduction

Sexual needs are a fact. Interest to the opposite sex increases from puberty. The puberty and its positive and negative consequences and the way the important institutions such as family, school and universities deal with are among the critical issues of each community (Ahmadi, 2007). One of the important outcomes of puberty is a tendency to have relationships with the opposite sex. Scientific research also shows that, familiarity, promises and adventures associated with the opposite sex will form an important part of human development (Lotf Abadi, 2001).

Desire to know and interact with the opposite sex, in the context of personal, social, cultural, and familial norms are among the matters, which finally occur foe for both girls and boys after reaching puberty, adolescence, and youth and it may happen many out of moral and religious norms. On the other hand, the skills to communicate with the opposite sex and eventually get through mate choice and completion through intellectual and emotional interactions with the opposite sex have a great importance in each individual's (Kaveh, 2007). The a person, who puts it

well behind, will have an acceptable growth and excellence in the rest of his or her life, while the people who are not equipped with such skills will not only provide problems for themselves, but also damages, problems, and troubles for the opposite sex had relationship with. Therefore, recognizing the nature of this relationship and factors affecting it as well as determining the right limitations in this relationship, particularly in universities, is a major concern of all segments of society.

Although friendships and relationships with the opposite sex in Iran as well as other countries, due to human nature and the mutual desire between the two sexes, has a long history but, in recent years, the increased number of adolescents and youth having such relations, lowered age of first date and considering this issue as a social norm among adolescents and youths, despite all the limitations and restrictions, have concerned social analysts, parents and authorities of educational system and universities.

Nowadays, due to the reasons such as educational and scientific cooperation, exchange of materials and resources, work environment and financial activities,

we cannot generally rule to stop working, organizational or educational relations of women and men. However, it does not mean any unrestrained relationships in the schools, colleges, and workplaces (Ghaffari, 2007). The relationship between boys and girls in all areas and instances that refer to the human identity is a necessity today's world. In addition, the relationships of boys and girls before getting married has been an important but covered with the ambiguity, doubt and anxiety issue. In one hand, the pressures and stresses of adolescence and instinctive tensions lead youths toward relations with the opposite sex and on other hand, social, cultural, and moral considerations in this regard act as barriers (Kothari, 1995). These contradictory situations in the society always had created uncertain and confusing situation for young people in terms of how to cope with this problem. Therefore, in the pathology of male and female relationships, we should pay attention to the origins of this relationship. Ahmadi (2001) divided the factors causing right or wrong behaviors in this relationship into three categories:

- Young people and their unreal thoughts
- Social media and ways of dealing with youths
- family issues and how parents deal with youths

2. Literature Review

One of the obliquities of youths due to the friendship with deviant peer is relationships with the opposite sex. From the view of social motivation, people who have friends of the opposite sex introduce themselves as powerful and socially competent persons and in such a circumstances, girls or boys without such a relation regard themselves as humble and helpless. This attitude plays an important role in stimulating youth to make friends with the opposite sex (Ahmadi, 2001). Results of Connolly (2004) showed that, mixed-gender groups in schools, especially at the youth level, increases the dependence to the opposite sex. The impact of friends of the same sex has the most importance in love affairs.

The effect of mass media in creating intellectual atmosphere, behavior making and in all aspects of life, especially among certain age groups such as adolescents and young adults is clear. Mass media, in a one-way fashion, engage youth's minds and even the dictate their clothing, attitudes, and relationship (Ahmadi, 2003). In a study by Harris, the results indicated that, about two third of American adults believed that, watching TV may encourage adolescents to be sexually active. Teenagers themselves, have had placed the impact of TV in their thoughts and behaviors after their friends and their parents (Giordano, 2004). Moreover, satellite TVs and vulgar images and videos could influence the attitudes,

beliefs, and behaviors about the opposite sex and create false picture from opposite sex and sexual behaviors (Kaveh, 2008). Cell phones not only make male and female relationships easier and more convenient, but also minimize the pressure and control of the family and create a background for deviant behaviors and extramarital relations between male and female (Boostani et al, 2009).

In most cases, the relationship between girls and boys, particularly in the educational areas and universities, initiates from cooperation in the courses and scientific instructions. Studies of Golzari (2005) have shown that, the causes of female students to have relationships with the opposite sex were cognition of opposite sex for marriage, simple friendship, scientific guidance and cooperation; also, their current relationships and friendships have established for marriage, simple friendship, fun, and entertainment. Movahhed and colleagues (2009) conducted a study on the students and concluded that, 46 percent of boys, compared with 10 percent of girls, have a tendency to have relationships and friendships with the opposite sex. 33 percent of boys and 25 percent of girls believed that, in order to better doing exercises, they had to have relationships with the opposite sex. 39 percent of boys and 28 percent of girls stated that interaction with the opposite sex contributes to their academic success. 58 percent of boys and 20 percent of girls stated their goals of having such relationships as a background for further understanding the opposite sex. Results of Peggy and colleagues (2006) indicate that, adolescents who have friendship with the opposite sex with a higher academic degree are most likely to continue their education to higher levels.

Sometimes, a wrong reasons to begin a relationship with the opposite sex before marriage, is age pressure and in many cases, especially for girls, they initiate such relationship with marriage promise. A significant percentage of youth do not have the information and knowledge required within the relationship with the opposite sex and consequently, they start such relations under the pretext of better understanding of the opposite sex (Kaveh, 2009). Ghaffari (2007) investigated attitudes of young people of 15 to 29 years old about friendships with the opposite sex. The results showed that, the agreement among boys with such relations is more than among girls. Respondents with higher levels of education and economic welfare are more in agreement concerning the relationship between friendships of boys and girls. Respondents' reasons for relationship with the opposite sex were response to an emotional need, lack of religious faith, negligence and carelessness of family, friends and peers pressure, sexual needs, introduction to marriage, marriage problems, and family planning, respectively. Studies

by Mirzaei and Baraghmadi (2010) showed that, the need to be loved, loneliness, understanding the opposite sex to marry, experience and the media, respectively, are the most important reasons to have relationships with the opposite sex.

Lotf Abadi (2001) stated that, for most of the youths, the purpose of relationships with the opposite sex before marriage was the possibility to marry with desired person and they believe that via friendships before marriage one can marry with a person with the desired moral characteristics and has a more convenient and guaranteed marriage. Results of Hajilari (2005) showed that, the purpose of establishing relationships with the opposite sex for 70 and 47 percent of students were getting rid of loneliness and the possibility of marriage with the desired person, respectively. In addition, factors such as gaining social prestige, gaining experience, understanding the opposite sex, resolving sexual needs, voluptuousness, and happiness, respectively, are important in establishing these relationships.

3. Methodology and Data

This study is descriptive – ex post facto. The statistical population consisted of all single students of Bahonar University of Kerman in the academic year of 2010-2011. Number of samples, based on the Cochran formula, was equal to 384 persons. Sampling method was random- stratified and the sample size for each category was determined based on gender, discipline, according to the population size.

To investigate the factors affecting the relations between the sexes, several factors were identified; however, we only used two social and marriage-related factors. Questionnaire with 20 items based on the 5-point Likert scale was prepared to collect the date. The questionnaires were developed by researcher in two dimensions; First, societal factors with components such as, cultural invasion, scientific guidance, educational cooperation between peers and friends and second, marriage-related factors with components of delayed marriage, marriage promise, recognizing opposite sex for marriages. According to retest method, reliability of the questionnaire was equal to 0.87. According to Cronbach's alpha test, in general, reliability was equal to 0.92 and specifically, was 0.84 for social factors and 0.68 for marriage-related factors.

The type and the amount of relationship with the opposite sex were determined by nine-item researcher-made questionnaires. According to retest method and Cronbach's Alpha reliability of this questionnaire was equal to 0.92 and 0.87, respectively.

3.1. Structure of social factors

To develop a model for measuring structure of social factors, at first, the number of components were selected and submitted to experts. After considering their comments, using Amos software to determine load factor for each of the components, and statically analyzing their significant differences with zero, we found that, some components have not gained acceptable coefficients. Finally, a model including four components was developed. The correlation of the components is shown in Figure 2 where, Xi is social factors, X1 is media, X2 is Cultural invasion, X3 is scientific guidance and educational-classroom cooperation and finally, X4 is peers and friends.
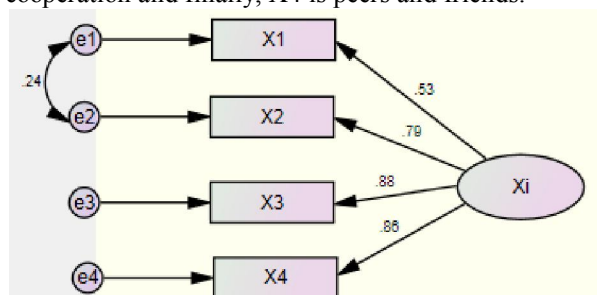
Figure 1: Measurement model for structure of social factors

About the above figure, it is worth mentioning that:

- All positive regression weight or load factor show an acceptable and reasonable conclusion from the theoretical foundations of the above figure.
- Components of scientific guidance and classroom cooperation component along with peers and friends with coefficients of 0.88 and 0.86, respectively, have higher correlation with the structure of social factors. Therefore, they have higher weight in the calculations associated with this hidden variable. In contrast, variables such as cultural invasion and media with coefficients of 0.79 and 0.53, respectively, have lower correlation with structure of social factors and subsequently, have less weight in the associated calculations.

Table 1: estimated general indices for the of social factors' model affecting the amount of relationships with the opposite sex

Index type	Index	Index description	Model	
			Developed model	Independence model
	NPAR	Free parameters of the developed model	9	4
Absolute	CMIN DF P	Non-significant chi-square Degree of freedom Significance level	1.89 1 0.169	302.97 6 0
Relative	RMSEA CMIN/DF	Root Mean Squared Error	0.077 1.89	0.573 50.5

		of Approximation Chi-square to degree of freedom ratio		
Comparative	TLI CFI	Tucker-Lewis fit index Comparative fit index	0.982 0.997	0 0
Parsimony	PNFI PCFI	Parsimony Normed Fit Index Parsimony Comparative Fit Index	0.554 0.562	0 0

Using the overall fit indices, we can answer to the question that, ignoring specific values reported for the parameters, whether, in general, the developed model is supported by experimental data collected or not. If the answer is yes, then the model is acceptable and otherwise it must be corrected. To interpret the values in the above table it should be noted that:

- Existence of the non-significant chi-square (CMIN) equal to 1.89 and significance level of p=0.169 shows a desired result, however, the degrees of freedom (Df) Is also important. The extent the degree of freedom of developed model (equal to 1) is far from zero and close to the degree of freedom of independence model (equal to 6), the more desirable is the model.

- The number of free parameters of the developed model (NPAR) whose value is 9, indicates that, the researcher, while developing the model, did not expended degrees of freedom easily and this is acceptable.

- Chi-square to degrees of freedom ratio (CMIN/DF) is a better index to judge the developed model and determine to what extent the data supports it. For this index, values from 1 to 5 are appropriate and values close to 2 to 3 are very good (Ghasemi, 2011). In this table, the value of relative chi-square is equal to 1.89 indicating that, the model is acceptable.

- Root Mean Squared Error of Approximation (RMSEA) shows that, the developed model can be considered acceptable or not. This index is known as one of the indicators of a bad fit. Its value varies between zero and one. The smaller the value is, the more acceptable is the model. The obtained 0.077 for the developed model indicates that, the model is acceptable.

- Comparative indicators are developed to examine that, based on the comparison with the developed independence model, whether the model is acceptable or not. The index values range from zero to one and the values higher than 0.90 are considered as acceptable. In the above table, the Tucker- Lewis fit index (TLI) is equal to 0.982 and the comparative fit index (CFI) is equal to

0.997 and since these values are higher than 0.90, therefore, based on these parameters, the developed model is acceptable.

- In addition to the above-mentioned indicators, investigating parsimony indices is necessary to determine the acceptance of the model. For Parsimony Normed Fit Index (PNFI) and Parsimony Comparative Fit Index (PCFI) values of 0.5 or higher are acceptable, however, some references reported this value as 0.6. In the above table, the values of PNFI and PCFI are equal to 0.554 and 0.562, respectively, indicating that, both are acceptable. The overall fit indices values in the above table show that, our measurement model is acceptable. Nevertheless, if the overall measure of the fitted model will be acceptable, the estimated individual parameters in the model could be meaningless. Therefore, it is necessary to investigate the individual parameters. Table 2 shows the estimated parameters individually.

Table 2: investigating the significant differences of estimated parameters from zero for the model of social factors

The significance level P	Critical ratio (CR)	Standard error (SE)	Type of estimate		
			Standard	Standard	
***	6.6	0.087	0.531	0.577	X1 Xi
***	11.14	0.079	0.789	0.876	X2 Xi
			0.875	1	X3 Xi
***	12.18	0.068	0.763	0.825	X4 Xi
0.016	2.41	0.712	0.243	1.72	e1 e2

The *** symbol in Amos software is an alternative to 0.000. Thus, the significance levels of 0.016 are reported differently.

- The value of CR (the ratio of non-standard estimated value of parameter to calculated standard error (SE) for the same parameters) shows the probability of error if the null hypothesis that makes this value equal to zero will be rejected. Since that for the significance level, the maximum error of 0.05 is acceptable, therefore it is concluded that, the calculated parameters have been significantly different from zero.

- In the above table, all of the values estimated for p are less than 0.05 indicating that, the error probability is low and subsequently, no component will be excluded and the model is acceptable with them.

3.2. Structure of marriage-related factors

In this research, in order to evaluate the marriage-related factors, we applied a first order factor model with three components of X5 as promise of

marriage, X6 as delay in marriage, and X7 as understanding the opposite sex for marriage.
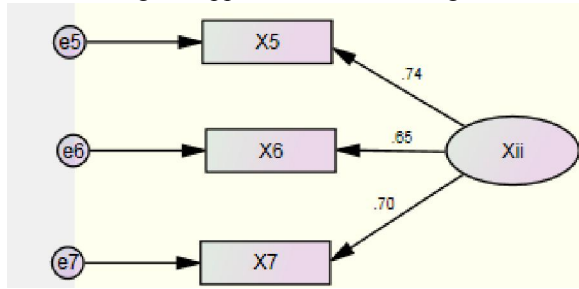
Figure 2: the measurement model for structure of marriage-related factors

It is worth mentioning about the above figure that:
- All positive regression weight or load factor show an acceptable and reasonable conclusion from the theoretical foundations of the above figure.
- Components of promise of marriage and understanding the opposite sex for marriage with coefficients of 0.74 and 0.7, respectively, have higher correlation with the model and marriage-related factors. Therefore, they have higher weight in the calculations associated with this hidden variable. In contrast, the variable of delay in marriage with coefficient of 0.65 has lower correlation with marriage-related factors and subsequently, has less weight in the associated calculations.

3.3. Structure of amount of relationships with the opposite sex

According to the existing theoretical foundations and perspective of community, the relationship with the opposite sex can be evaluated via three components as the amount of face-to-face or absent communications and the number of relationship with the opposite sex. Therefore, in order to evaluate the amount of the relationship with the opposite sex, we used a three-factor model with components of Y1 as in person relationship, Y2 as distance relationship, and Y3 as number of relationship.
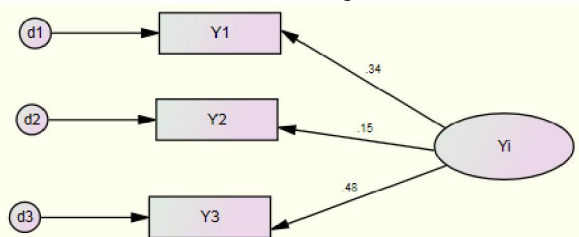
Figure 3: Measurement model for the structure of relationship with the opposite sex

It is worth mentioning about the above figure that:

- All positive regression weight or load factor show an acceptable and reasonable conclusion from the theoretical foundations of the above figure.
- Components of number of relationship and in person relationship with coefficients of 0.48 and 0.34, respectively, have higher correlation with the amount of relationships. Therefore, they have higher weight in the calculations associated with this hidden variable. In contrast, the variable of distance relationship with coefficient of 0.15 has the lowest correlation with the amount of relationships and subsequently, has less weight in the associated calculations. According to these coefficients, the amount of relationships with the opposite sex will be better evaluated with the factors of number of relationship and in person relationship.

4. Results

According to what was mentioned above, the main hypothesis of the research was tested. Therefore, using structural equation modeling, the effect of social and marriage-related factors on the amount of the relationships with the opposite sex was tested and the following results were obtained (Table 3).

The results of the model tests indicate that, the impact of social factors on the relationship with the opposite sex is positive (0.66) meaning that, the more people are affected by social factors (media, cultural invasion, peers, and more classroom cooperation), the more they establish relationships with the opposite sex. In addition, the results show that, the impact of marriage-related factors on the relationship with the opposite sex is positive (0.34) meaning that, the more people affected by the marriage-related factors (marriage delay, marriage promise, and understanding the opposite sex for marriage), the more will be the amount of the relationship with the opposite sex.

- Existence of the non-significant chi-square (CMIN) equal to 58.42 and significance level of p=0.002 shows a desired result, however, the degrees of freedom (Df) Is also important. Concerning that the degree of freedom of developed model (equal to 31) is far from zero and close to the degree of freedom of independence model (equal to 45), the model is desirable.
- The number of free parameters of the developed model (NPAR) whose value is 24, indicates that, the researcher, while developing the model, did not expended degrees of freedom easily and this is acceptable.

- Chi-square to degrees of freedom ratio (CMIN/DF) is equal to 1.88 indicating that, the model is acceptable.
- Root Mean Squared Error of Approximation (RMSEA) for the developed model is 0.077 indicating that, the model is acceptable.
- In the above table, the Tucker-Lewis fit index (TLI) is equal to 0.937 and the comparative fit index (CFI) is equal to 0.957 and since these values are higher than 0.90, therefore, based on these parameters, the developed model is acceptable.
- The values of Parsimony Normed Fit Index (PNFI) and Parsimony Comparative Fit Index (PCFI) are equal to 0.629 and 0.659, respectively, indicating that, both are acceptable. The overall fit indices values in the above table show that, our measurement model is acceptable. These results are consistent with the results of many researches and proposed theories.
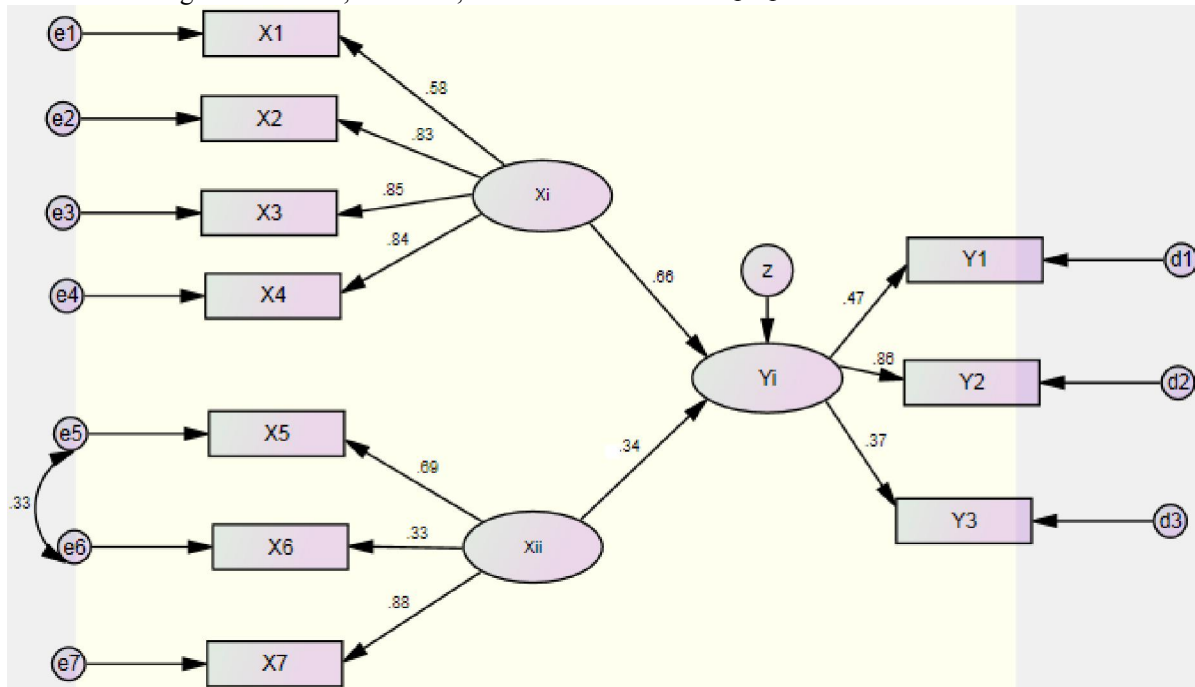
Figure 5: investigating the effect of social and marriage-related factors on the amount of the relationships with the opposite sex

Table 3: Overall fit indices to assess the impact of social and marriage-related factors on the relationship with the opposite sex

Index type	Index	Index description	Model	
			Developed model	Independence model
	NPAR	Free parameters of the developed model	24	10
Absolute	CMIN	Non-significant chi-square	58.42	675.1
	DF	Degree of freedom	31	45
	P	Significance level	0.002	0
Relative	RMSEA	Root Mean Squared Error of Approximation	0.077	0.305
	CMIN/DF	Chi-square to degree of freedom ratio	1.88	15.1
Comparative	TLI	Tucker-Lewis fit index	0.937	0
	CFI	Comparative fit index	0.957	0
parsimony	PNFI	Parsimony Normed Fit Index	0.629	0
	PCFI	Parsimony Comparative Fit Index	0.659	0

5. Conclusion

Feelings, thoughts and behaviors toward the opposite sex are the most exciting feature of human life that engage the mind from mid-adolescence the end of youth and adulthood. Regardless of the customs and social-cultural rules in a given society and apart from hiding this big secret and considering as taboo in our community, dreaming about the opposite sex, establishing relationships with the opposite sex, stories, enjoyments, euphoria and tragedy of love, matchmaking and engagement, marriage, separation and ... are very effective and important parts of everyone's life in the real world with good and evil. These emotions, thoughts and behaviors have

undeniable impacts on the development of gender identity, attitudes toward opposite sex, and marriage. Given the importance of this issue, the authors investigated the influence of social and marriage-related factors and factors on the amount of the relationships between the sexes.

Regression coefficients of the structural equation modeling suggest that, social factors (media, cultural invasion, educational and classroom cooperation, and the impact of peers and friends) have a positive impact on the relationship with the opposite sex. The model results also indicated that, among the social factors, schoolwork and friends have the greatest impact on relations between the sexes. These findings are in agreement with the results of Golzari (2005), Hajilari (2005), Movahhed and colleagues (2009), Miller (1998), and Connolly (2004), who investigated the influence of friends and peer groups in relationships with the opposite sex. To explain these findings we can say that, the youths pay extra attention to camaraderie and friendship with peers. Community of adolescent provides an environment that has a profound effect on them. Peer groups can have a lot of power and can affect group members. One of the obliquities of youths due to the friendship with deviant peer is relationships with the opposite sex. From the view of social motivation, having girlfriends is a personal and social power and for girls, girls it is considered as a higher sex appeal and social prestige to have a boyfriend. For this reason, girls or boys without such a relation regard themselves as humble and helpless. This attitude plays an important role in stimulating youth to make friends with the opposite sex. Sometimes, emotional ties established due to the academic and classroom cooperation, could lead to a profound relationships with the opposite sex. Therefore, it is recommended to the family and the community to control the type and the condition of the relationships and friendships of their youths in a subtle fashion. They can create conditions through witch these relationships could be guided in the right direction. Moreover, it is recommended to the students to have such relations with the classmates of opposite sex only if it is essential and restrict these relations to the accepted boundaries of culture and society.

Based on these results, some TV programs (media) like some of series and movies are encouraging and guiding the youth to have relationships with the opposite sex; since in some of the movies, it is a form of cultural pride to have a relationship with the opposite sex and it increases the tendency of young people to have such relations. Therefore, it is recommended that the authorities consider Islamic patterns in producing TV shows and movies.

The findings of this study showed that, with the increase of cultural invasion and the trend toward the satellite TVs, Internet, foreign vulgar movies etc., the relationship with the opposite sex will be increased. The results of Moayyedi (2000) and Ahmadi (2008) have confirmed the relationship between cultural invasion and the media with the relationships with the opposite sex. In explaining these findings, we can say that, by emerging todays new technologies, greater diversion areas for youths are provided. In addition, we are observing the influence of foreign culture and promotion of the materialistic culture and moral degeneracy among the teenagers and young adults. All these factors play a major role in the relations between the sexes. Therefore, it is recommended to the authorities to deal with cultural invasion and try to reduce the relationships with the opposite sex by improving the quality of domestic media programs as well as informing the youth about the correct use of new technologies such as mobile phones, Internet.

Other results of the model showed that, the marriage-related factors (delay in marriage, promise of marriage, and understanding the opposite sex for marriage) have a significant positive impact on the relationship with the opposite sex. Promise of marriage and the understanding the opposite sex have the greatest impact on relations between the sexes. These results are in agreement with the findings Golzari (2005), Ahmadi (2008) and Meston & buss (2007), which showed that, understanding the opposite sex for marriage is one of the most important objectives of such friendship between girls and boys. In fact, a significant percentage of young people do not have the information and knowledge required to communicate with the opposite sex because the scientific and systematic training to suit the conditions are not sufficiently provided by the experts and still there is no information about the basic life skills among educational contents. These issues cause that the youths to start such relations under the pretext of better understanding of the opposite to start a better life. About relationship between marriage promise and the relationship with the opposite sex, it can be said that, almost all boys give marriage promise to their girlfriends develop this imagination in their minds. Since the nature of this imagination is the freedom from anxiety created by the conflict between their acts and the rules and norms accepted by society, thus in most cases, the imagination remains in the romance state and never is materialized. Presence of the marriage promise in these relationships is only the kind of satisfaction for boys and girls and improves relations between them. The results show that, the age students and marriage postponement have impact on the relationship with the opposite sex is. This finding is in

agreement with the results of Schofield (1968) who showed that, with increasing the age, the relationship with the opposite sex would be increased. It seems logical that, with the increase of age, the tendency to marriage and founding a family would be increased and if the necessary conditions for marriage would not be provided, the marriage will be delayed and this causes the young to have relationships with the opposite sex.

Therefore, it is recommended that, the authorities and families try to provide appropriate and timely marriage conditions for youths. In addition, counseling centers, schools, and universities can create appropriate attitudes and raise awareness about the proper relationship with the opposite sex to help youth. .

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Analyzing the effect of demographic characteristics on compulsive buying: A case study of consumers in Iranian shopping centers

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Abstract: Shopping is a normal and routine part of everyday life for most people, but compulsive buyers have different shopping experience. The inability to control an overpowering impulse to buy pervades their lives and results in significant and sometimes severe consequences. This study aims to investigate the compulsive buying behavior and explore its relationship with some demographic variables. A comprehensive data set has been collected through a survey of 1144 customers who had the experience of buying at various shopping centers in Tehran, Iran. According to the result of a validated questionnaire, a compulsive buying latent variable has been calculated for each costumer using the Structural Equation Modeling. Afterward, the relationship between underlying demographic characteristics and the compulsive buying variable has been analyzed using a number of statistical tests. The results demonstrate that some of these antecedents significantly affect compulsive buying tendency. The findings would assist managers, marketers, policy makers, and health care practitioners in their decisions, because individuals who exhibit negative consumption behaviors variously affect these parties.

Keywords: Consumer Behavior; Compulsive Buying; Demographic Characteristics.

1. Introduction

One of the fundamental objectives of the marketing discipline is to theoretically and empirically analyze and predict the consumer behavior in market place. The consumer behaviors, like other human activities, are not totally the result of rational decision and may be influenced by short-term emotional factors. Such consumer behaviors have been addressed as non-rational (Rook, 1987), abnormal (O'Guinn & Faber, 1989), and even deviant (Donovan, 1988; Hirschman, 1992) behavior. Compulsive consumption is one of the non-rational consumer behaviors in which individuals use a product repetitively or excessively to ease a tension, anxiety or distress aroused by an obsession. Compulsive buying, as a type of compulsive consumption termed in the clinical literature, is a non-rational behavior and has gained remarkable attention among researchers in marketing since 1980. (cf. Black, 2004; Dittmar, 2004; Faber, 2004). Compulsive buying is a label which has been used recently in both psychiatric and consumer research contexts to refer to the inability to shop or buy ''normally'' (Faber, O'Guinn, & Krych, 1987; O'Guinn & Faber, 1989).

Nowadays, an increasing number of people engage in uncontrolled, excessive buying of consumer goods that can lead to psychological distress and serious effects on individuals' lives, such as substantial debt (e.g. Benson, 2000; Dittmar, 2004). Hence, analyzing the factors influencing the compulsive buying is essential to assist individuals avoid the destructive financial and psychological consequences and support decision makers effectively and efficiently establish the marketing strategies. Compulsive buying, like other obsessive and addictive behaviors, may be affected by biochemical, psychological and/or demographical factors (Donovan, 1988; Faber, 1992). This study focuses on demographical factors and aims to examine the relationship between demographic variables and compulsive buying tendency. Firstly, the compulsive buying tendency is measured by a verified scale, referred as Edvards compulsive buying scale (Edvards, 1993), using questionnaire collected from 1144 consumers. Gender, Age, Marital Status and Income were therefore examined as factors that may systematically influence the tendency of buying behavior using statistical tests.

The affluence and welfare has had increasing trend in developing countries like Iran and gigantic shopping centers have become more and more into existence and available in recent decades. Therefore, the people purchasing power has boosted and shopping has become more convenient, and consequently the consumerism and relevant dysfunctions of consumer behaviors has augmented. On the other hand, rising social problems such as budget and career problems, heavy traffic, air and noise pollution and lack of emotion and leisure time in capital cities brings about the psychological illnesses such as depression, anxiety and stress that

may cause the non-rational consumer behaviors. Hence, studying these consumer behavior dysfunctions such as compulsive buying and the factors inspiring them would be significantly valuable to policy makers who are responsible for the society wellbeing. The outcomes of this study would assist decision makers to uncover the demographic factors, which remarkably affect compulsive buying, and launch strategies to prevent this abnormal behavior occurrence.

The rest of paper is organized as follow: Section 2 describes the literature of compulsive buying and the demographic factors. The methodology of this study is explained in Section 3. The empirical results are demonstrated in Section 4. Finally Section 5 concludes the findings and proposes the future studies.

2. Literature Review

In this section the existing studies about compulsive buying and current definitions are briefly presented. Then, the researches that investigated the influence of each demographic variable, namely Gender, Age, Marital Status and Income, on compulsive buying are introduced in four sequential sub-sections.

2.1 Compulsive Buying

Compulsive Buying (CB) is characterized by a preoccupation with buying and shopping, by frequent loss of control over buying, and by excessive purchasing of items that are not needed and not used. Compulsive buyers buy not so much to obtain utility or service from a purchased commodity as to achieve gratification purchased commodity as to achieve gratification through the buying process itself. CB negatively impacts personal relationships, is time consuming, causes marked distress and financial problems and interferes with social and/or occupational functioning (McElroy, Keck, Pope, Smith, & Strakowski, 1994). O'Guinn and Faber (1989) view compulsive buying as an addictive behavior with the following definition: a response to an uncontrollable drive or desire to obtain, use or experience a feeling, substance, or activity that leads an Individual to repetitively engage in a behavior that will ultimately cause harm to the individual and/or others. This definition is similar to that suggested by Valence et al. (1988), who identified three constructs associated with compulsive buying behavior: 1) a strong emotional activation (increase in psychological tension); 2) a high cognitive control (an acknowledgement that buying will reduce the tension); and 3) a high reactivity (looking for tension reduction, rather than owner ship). It also should be noted that the O'Guinn and Faber (1989) definition is sufficiently general to allow for non-purchase consumption (e.g., anorexia/bulimia, or gambling).

D'Astous (1990) argued that all consumers can be fitted along an "urge to buy" continuum and that those in the upper extreme of this continuum can be referred to as compulsive buyers. O'Guinn and Faber (1989) insist that any definition of compulsive buying must include two criteria: (1) the behavior must be repetitive, and (2) the behavior must be problematic for the individual. Using these two criteria, O'Guinn and Faber (1989) defined compulsive buying as "chronic, repetitive purchasing that becomes a primary response to negative events or feeling. The activity, while perhaps providing short-term rewards, becomes very difficult to stop and ultimately results in harmful consequences".

2.2 Compulsive buying and Gender

In recent consumer research, there is an ongoing debate about the relationship between compulsive buying and gender. In research that examines continuous scores on Compulsive buying scales, women typically score significantly higher than men (e.g. Scherhorn, Reisch & Raab,1990). With regard to population-based studies, some authors (Neuner, Raab, & Reisch, 2005) indicated that women are more prone to CB than men whereas others did not (Koran, Faber, Abou jaoude, Large, & Serpe, 2006; Mueller et al., 2010). Shopping is described as a gendered activity, part of ''women's work' in their roles as housewives (Campbell, 2000; Elliott, 1994; Lunt & Livingstone, 1992). Women's propensity for shopping is related to their ''dependent'' role, both in relationships and in society (Scherhorn et al., 1990). Women do most of the shopping because it is one of their main ''leisure activities'' (Campbell, 2000, p. 60; Elliott, 1994) and because shopping provides them with an''opportunity to socialize'' (d'Astous, 1990; Lunt & Livingstone, 1992). Women are taught that shopping is ''pleasurable'' (Faber et al., 1987); they use shopping as a form of ''self-expression'' (Elliott, 1994) and to ''enhance their self-image'' (Dittmar, 2000; Dittmar, Beattie, & Friese, 1995; Krueger, 1988) because society places importance on their outward appearance. Krueger (1988) suggests that ''women who shop compulsively are driven to attempt to restore a depleted self'' (the ''empty self'' noted by Elliott, 1994; Cushman, 1990; and Scherhorn et al., 1990), that is, they seek validation of self-worth through consumption. Despite general agreement that compulsive buying is a gender-related disorder (Campbell, 2000; d'Astous, 1990; d'Astous, Maltais, & Roberge, 1990; Dittmar, 2000; Dittmar & Drury, 2000; Elliott, 1994; Faber & O'Guinn, 1992; Faber, Christenson, de Zwaan, & Mitchell, 1995; Scherhorn et al., 1990), some researchers are not entirely convinced of this gender specificity (Elliott, 1994). Dittmar and Drury (2000) believe that, as roles for

men and women change and an ideal male appearance is increasingly emphasized, excessive buying will be found more frequently among male consumers.

2.3. Compulsive buying and Age

The majority of studies that compare compulsive buyers with ordinary buyers report that the average age of compulsive buyers is lower by between 8–11 years in France (Lejoyeux et al., 1997), Germany (Scherhorn et al., 1990), and the US (Hanley & Wilhelm, 1992; O'Guinn & Faber, 1989), although some studies found small (Lejoyeux, Haberman, Solomon, & Ades, 1999) or no age differences (Black et al., 1998; DeSarbo & Edwards, 1996). Reports from compulsive buyer samples give average ages between 30 and 31 years (Black, Monahan, Schlosser, & Repertinger, 2001; Schlosser et al., 1994). European Union project identified 46% of Scottish 16- to 8-year-olds as showing possible early tendencies towards uncontrolled buying, because they reported being unable to resist advertising stimuli and had a lack of control over their spending habits (Garces Prieto, 2002). There are some indications that younger persons may be more susceptible than older ones to compulsive buying tendencies (e.g., Dittmar, 2005), although several studies found no effect of age on problematic buying (e.g., Black, Repertinger, Ganey, & Gabel, 1998). Taking these findings and arguments together, it is therefore hypothesized that younger respondents are more prone to compulsive buying than older respondents, which makes it important to sample across a wide age range, including adolescents.

2.4. Compulsive buying and Marital Status

Previous research on compulsive buying has suggested that it too may be related to mood state. In sentence-completion tasks, compulsive buyers indicated that they were most likely to buy something when they were in negative mood states (Faber et al. 1987). Recent work on compulsive buying has suggested that these behaviors may be partially motivated by a desire to change or manage moods. These purchases are most common when people are experiencing negative moods such as divorce, low exam grade, personal rejection. Divorce is a common and highly stressful event. Marketing researchers know relatively little about the long term or short term effects of divorce on consumer behavior and attitudes. MCAlexander, Schouten and Roberts (1993) suggest that divorce leads to acts of consumption, which, in turn, are attempts to cope with the stress associated with a divorce.

2.5. Compulsive Buying and Income

Despite the ongoing debate about the relationship between compulsive buying and gender, the literature is clearer about the relationship between compulsive buying and class. No one class seems to be immune to compulsive buying. In those studies in which class is mentioned specifically, subjects are drawn either exclusively from the lower middle to upper middle classes (Rook, 1987), predominantly from the middle to upper middle classes (Lunt & Livingstone, 1992), or from the same unidentified class (O'Guinn & Faber, 1989). In another, the occupations of subjects ranged from unskilled to managerial (Dittmar & Drury, 2000). D'Astous (1990), the only researcher to address the relationship between compulsive buying and class in some detail, found a ''U-inverted relationship between income and compulsive buying tendencies,'' which suggests that the middle class, not the lower or upper classes, is most likely to suffer from such tendencies. D'Astous (1990) also claims that his findings seem to converge with those of O'Guinn and Faber (1989).

3. Methodology

Questionnaire based scales are ideally suited to measuring the relative strength of compulsive buying tendencies in individuals. Edvard (1992) developed a scale to measure the low to high levels compulsiveness in consumers' behavior. According to the Edvards (1992) scale, the range of compulsive buying is not dichotomous behavior, but one that lies along a continuum with ranges that could be designated as non-compulsive, recreational, borderline compulsive, compulsive, and addicted buying respectively. Such a continuum is illustrated in Figure 1.

Continuum				
-				+
Normal/ Non-Compulsive	Recreational	Borderline	Compulsive	Addicted
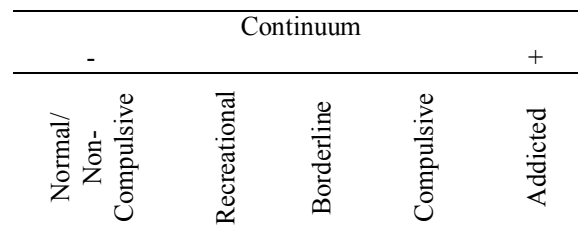
Figure1: The compulsive buying continuum

The scale is comprised of 13 psychological items that best measure the level of respondents' compulsive buying behavior. The questionnaire used in this study applied this 13-item scale to measure the degree of compulsiveness and therefore it asked respondents the following questions:

1) I feel driven to shop and spend, even when I don't have the time or the money.
2) I get little or no pleasure from shopping.
3) I hate to go shopping.
4) I go on buying binges.
5) I feel "high" when I go on a buying spree.
6) I buy things even when I don't need anything.

7) I go on a buying binge when I'm upset, disappointed, depressed, or angry.

8) I worry about my spending habits but still go out and shop and spend money.

9) I feel anxious after I go on a buying binge.

10) I buy things even though I cannot afford them

11) I feel guilty or ashamed after I go on a buying binge.

12) I buy things I don't need or won't use.

13) I sometimes feel compelled to go shopping.

The above-mentioned items were measured by the revised CBS scale which used 7-point Likert-type scales, ranging from 1 strongly disagree to 7 strongly agree. Higher scores indicate stronger compulsive buying tendency and vice versa. The sample is composed of consumers who went shopping in several shopping centers in capital city Tehran. Due to disperse geographical distribution of consumers, this study applied the two-stage cluster sampling. Each shopping center was considered as one cluster and a number of clusters (shopping centers) were randomly selected at the first stage. At the second stage, a sufficient number of respondents then randomly selected from the clusters that we choose at the first stage. According to the (Cochran, W. G. (1977) the minimum sample size n can be computed as follow:

$$n= (Z^2 p(1-p))/d^2 \qquad\qquad (1)$$

This formula provides us with the minimum sample size needed to detect significant differences when Z is determined by the acceptable likelihood of error (the abscissa of the normal curve). The value of Z is generally set to 1.96, representing a level (likelihood) of error of 5%. We want the highest accuracy possible, with the smallest sample size. This level of error, 5%, gives us the best trade-off between these two goals p is the conversion rate we expect to see (estimate of the true conversion rate in the population), and d is the minimum absolute size difference we wish to detect (margin of error, half of the confidence interval). Hence, 1200 respondents were asked to fill the questionnaire during a one-month period. 56 out of 1200 responses are deleted due to either obvious inconsistent response patterns or a large number of missing data within questionnaire. Finally, a sample of 1144 customers who had the experience of buying at various shopping centers in Tehran-Iran was collected. Table 1 presents the demographic profile of the respondents including their Gender, Age, Marital Status and Income Levels and the compulsive buying tendencies for each group.

4. Results

This section presents the statistical tests results, t-test and ANOVA analysis, which were used to examine the impact of demographic variables on compulsive buying. The following four hypotheses are defined to examine the effect of Gender, Age, Marital Status and Income respectively on compulsive buying.

H1a: There is a significant relationship between compulsive buying tendency and gender.

H1b: There is a significant relationship between compulsive buying tendency and age.

H1c: There is a significant relationship between compulsive buying tendency and marital status.

H1d: There is a significant relationship between compulsive buying tendency and income level.

According to the results of Table 2, t = -0.693, so a significant difference between the two groups in terms of gender and compulsive buying not be seen. ANOVA results indicate that the null hypothesis is rejected based on the equality of the groups mean. According Table 3, F=16.929 is significant and average compulsive buying is different in age groups and the highest compulsive buying have been seen in the age group 21 to 26. According Table 4, F=6.325 is significant and average compulsive buying is different in marital status groups and the highest compulsive buying have been seen in the group of divorced. In Table 5, ANOVA results indicate that the null hypothesis is rejected based on the equality of the groups mean. F=5.228 is significant and average compulsive buying is different in income groups and the highest compulsive buying have been seen in the 1000000-1500000 income group.

5. Conclusion

For most people, buying is a normal and routine part of everyday life. For compulsive buyers, the inability to control an overpowering impulse to buy pervades their lives and results in significant and sometimes severe consequences. The present research aims to improve understanding of compulsive buying through examining gender, age, and marital status. The results not confirmed previously documented gender differences, and showed that younger people are more prone to compulsive buying. Also average compulsive buying is different in groups based on marital status and divorce influence on compulsive buying. The consumption implications have not received adequate attention. Added to the earlier work in this area of research, the present study enhances our current understanding of how marital status and age can influences the consumption of people. This is particularly true, given that many Iranians will experience divorce or parental separation in their lifetime. There is an increased risk of compulsive buying. Since consumption compulsivity can be seen as a negative social and cultural phenomenon, social

policy maker should consider this negative impact and design policies to reduce it.

There are ample opportunities and need for future research in the area of compulsive buying. Future research is needed to investigate the impact of other demographic variable such as social class, family structure on compulsive buying.

Table 1: The demographic profile for respondents

	Number of samples	Percentage	Mean	Std. Deviation	Std. Error Mean
Gender					
Female	758	66%	3.23	0.73	0.02
Male	386	34%	3.26	0.76	0.03
Age					
15-20	102	9%	3.17	0.67	.070
21-26	637	56%	3.32	0.74	.03
27-32	232	20%	3.25	0.71	.04
33-38	99	9%	3.21	0.60	.06
39-44	43	4%	2.68	0.51	.08
45-50	31	2%	2.24	0.78	.14
Marital Status					
Single	830	73%	3.25	0.72	0.02
Married	280	25%	3.18	0.81	0.04
Divorced	16	1%	3.96	0.49	0.14
Other	18	1%	2.75	0.47	0.13
Income Level					
<400 RL	358	31%	3.09	0.76	0.05
400-1000 RL	482	42%	3.25	0.85	0.04
1000-1500 RL	192	17%	3.48	0.59	0.06
1500-2000 RL	36	3%	3.40	0.84	0.21
2000> RL	76	7%	2.97	0.59	0.09

Table 2: Levene-test results for gender effect

		Levene's Test for Equality of Variances	
		F	Sig.
Compulsive buying tendency	Equal variances assumed	0.18	0.67
	Equal variances not assumed	Null	Null

Table 3: t-test results for gender effect

		t-test for Equality of Means			
		t	df	Sig. (2-tailed)	Mean Difference
Compulsive buying tendency	Equal variances assumed	-0.69	1113	0.488	
	Equal variances not assumed	-0.68	718	0.494	-0.03278

Table 3: ANOVA results for age effect

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	43.36	5	8.67	16.92	.0
Within Groups	546.17	1066	0.51		
Total	589.53	1071			

Table 4: ANOVA results for marital status effect

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.367	3	3.45	6.32	.0
Within Groups	606.97	1111	.54		
Total	617.33	1114			

Table 5: ANOVA results for Income Level effect

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.906	4	3.227	5.228	.000
Within Groups	415.366	673	.617		
Total	428.272	677			

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Global dynamics of the avian-human influenza with horizontal transmission in human population

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Abstract: The family case clusters of highly pathogenic avian influenza A subtype H5N1 in Thailand (2004) and in Sumatra, Indonesia (May 2006) that were due to human-to-human transmission attracted the attention of the responsible agencies. If the H5N1 virus gain the ability of sustained human-to-human transmission a pandemic could result with potentially high mortality. In order to understand the dynamical behavior of the human-to-human transmittable avian influenza, we develop a mathematical model by taking into account the human-to-human transmission of the avian influenza with the exposed compartment in both human and bird population. We show that by using the basic reproduction number the stability of the equilibria in the proposed model can be controlled. The global stability of both the disease-free and the endemic equilibrium is shown by using the Lyapunov function theory. Finally, numerical results are carried out to justify this work.

Key words: Avian influenza model; Backward bifurcation; Basic reproduction number; Global stability.

1 Introduction

Avian influenza is one of the most dangerous diseases for the wellbeing of animals and humans nowadays. A few years back it was a disease of wild birds and poultry and was of a limited significance [1, 2], but this perspective has changed as the emergence of a strain can infect humans through bird-to-human transmission and can kill about 60 percent of those infected [3]. But its potential to change into an extremely virulent human-to-human transmittable pandemic strain is the real danger for the human health. It requires drastic measure for the control of the spread of avian influenza to reduce such kind of probabilities.

The pathogens of the avian influenza mutates at a very high rate and expands its host range [3]. A large number of wild bird species, species of mammals and species of domestic birds as well as humans have been infected by various strains of avian influenza [4, 5, 6]. It is difficult to control the disease because of the multi-species conglomerate of hosts and because of this difficulty, the researchers directed their efforts to reduce the circulation within the poultry population, as it is the main responsible for the transmission of the disease. In order to control the spread of the disease, only culling was applied in the last decade [3] which caused a significant economic loss by destroying a large number of chickens. Nowadays, to control the spread, multiple control strategies are in attempt like increasing bio security, culling and vaccination of poultry.

A pandemic among humans may cause by the highly pathogenic H5N1 strain, if it acquires a highly efficient human-to-human transmission mechanism, while retaining high pathogenicity. Several reports have so far been made of possible co-infection of humans with an H5N1 strain and a human strain. One of the co-infection reports was of an Indonesian teen in 2008. The other of an Egyptian man, suspected of the co-infection by H5N1 and the pandemic H1N1 strain in 2009 [3]. The reports of such co-infection in humans alarms the threat of future pandemic.

In this work, we combine two nonlinear models, the first one describes the interaction between the susceptible human and the infected human and infected birds populations, and the second one describes the interaction between the susceptible birds and the infected birds populations. We first establish stability results for the proposed model. Analysis of the model reveals that there are two equilibria, the disease-free and the endemic equilibria. Further, it is shown that the model exhibits the phenomenon of backward bifurcation where the locally asymptotically stable disease-free equilibrium co-exists with a locally asymptotically stable endemic equilibrium when the threshold quantity $R_0 < 1$. The local dynamics of the proposed model are completely determined by the basic reproduction

number R_0. For $R_0 < 1$ the disease-free equilibrium is locally stable while for $R_0 > 1$ the endemic equilibrium is locally stable. By using the Lyapunov function theory, we present the global asymptotic stability. Finally numerical simulations are carried out to support the analytical conclusion and illustrate possible behavioral scenarios of the proposed model.

2   Model frame work

In this section we present a compartmental model that divides the human and birds populations into two different classes. We divide the total human population at time t denoted by $N_h(t)$ into five distinct epidemiological subclasses which are susceptible $X_h(t)$ exposed $E_h(t)$ infected $I_h(t)$ treated $T_h(t)$ and recovered $R_h(t)$ and the birds population $N_b(t)$ into three distinct subclasses which are susceptible $X_b(t)$ exposed $E_b(t)$ and infected $I_b(t)$ The model is represented by the following system of differential equations.

$$\frac{dX_h(t)}{dt} = \Lambda - \mu_h X_h(t) - (\alpha_1 I_h(t) - \alpha_2 I_b(t)) X_h(t) + \eth_h R_h(t),$$

$$\frac{dE_h(t)}{dt} = \alpha_1 X_h(t) I_h(t) + \alpha_2 X_h(t) I_b(t) - (\mu_h + \phi_h) E_h(t),$$

$$\frac{dI_h(t)}{dt} = \phi_h E_h(t) - (\rho_h + \beta_h + \mu_h) I_h(t),$$

$$\frac{dT_h(t)}{dt} = \rho_h I_h(t) - (\gamma_h + \mu_h) T_h(t), \qquad (1)$$

$$\frac{dR_h(t)}{dt} = \gamma_h T_h(t) - (\mu_h + \eth_h) R_h(t),$$

$$\frac{dX_b(t)}{dt} = \pi - \mu_b X_b - \alpha_3 X_b(t) I_b(t),$$

$$\frac{dE_b(t)}{dt} = \alpha_3 X_b(t) I_b(t) - (\mu_b + \phi_b) E_b(t),$$

$$\frac{dI_b(t)}{dt} = \phi_b E_b(t) - (\beta_b + \mu_b) I_b(t),$$

with the initial conditions

$$\begin{cases} X_h(0) \geq 0, \ E_h(0) \geq 0, \ I_h(0) \geq 0, \ T_h(0) \geq 0, \\ R_h(0) \geq 0, \ X_b(0) \geq 0, \ E_b(0) \geq 0, \ I_b(0) \geq 0. \end{cases} \qquad (2)$$

The complete descriptions are given in the following Table 1 and the transmission dynamics are given in Figure 1.

Table 1.

Parameters	Description
Λ	Recruitment rate of human population
α_1	Effective contact rate between the susceptible human and infected human
α_2	Effective contact rate between the susceptible human and infected birds
ε_h	The rate of immunity loss
φ_h	Progression rate of human from exposed class to infected class
ρ_h	The treatment rate of human
μ_h	The natural death rate of human class
β_h	Disease induced death rate in humans
Π	Recruitment rate of birds population
γ_h	Recovery due to treatment
β_b	Disease induced death rate in birds
α_3	Effective contact rate between susceptible birds and the infected birds
φ_b	Progression rate of birds from exposed class to infected class
μ_b	The natural death rate of birds
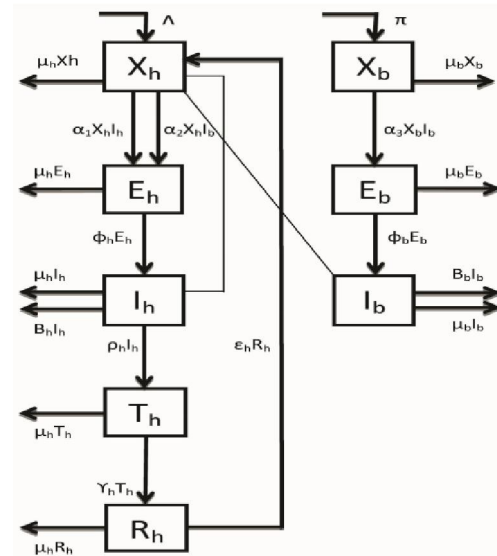
Figure 1: The flow chart represents the transmission dynamics of the avian-human influenza with horizontal transmission.

The total human population dynamics is given by

$$\frac{dN_h(t)}{dt} = \Lambda - \mu_h N_h(t) - \beta_h I_h(t). \qquad (3)$$

By the given initial conditions $N_h(0) \geq 0$, so the total population $N_h(t)$ remains positive and bounded for all finite time t > 0. The total dynamics for the birds population is

$$\frac{dN_b(t)}{dt} = \pi - \mu_b N_b(t) - \beta_b I_b(t). \qquad (4)$$

From equation (3) and (4), we have

$$\begin{cases} \dfrac{dN_h(t)}{dt} \leq \Lambda - \mu_h N_h(t), \\ \dfrac{dN_b(t)}{dt} \leq \pi - \mu_b N_b(t). \end{cases} \qquad (5)$$

Then $\lim\limits_{t \to \infty} Sup N_h(t) \leq \dfrac{\Lambda}{\mu_h}$,

$\lim\limits_{t \to \infty} Sup N_b(t) \leq \dfrac{\pi}{\mu_b}$. Hence the feasible region for

the system (1) is given by

$$\Omega = \{(X_h(t), E_h(t), I_h(t), T_h(t), R_h(t), X_b(t),$$
$$E_b(t), I_b(t)) \in R_+^8, V_1 \leq \frac{\Lambda}{\mu_h}, V_2 \leq \frac{\pi}{\mu_b}\}.$$

3. Disease-free equilibrium

In order to find the dynamical features of the proposed model (1), we set the right hand side
of all equations in the system (1) equal to zero. By the direct calculations we get the disease-free equilibrium point $E_1 = (X_h, 0, 0, 0, 0, X_b, 0, 0)$,

where $X_h^o = \dfrac{\Lambda}{\mu_h}$ and $X_b^o = \dfrac{\pi}{\mu_b}$. The dynamics

of the disease is described by the threshold quantity

$$R_0 = \frac{\pi \alpha_3 \phi_b}{\mu_b(\mu_b + \phi_b)(\mu_b + \beta_b)}.$$

The threshold quantity R_0, is known as the basic reproduction number of the disease and it shows the expected number of new infections produced by a single infective when introduced into a susceptible population. The disease dies out if $R_0 < 1$, as it shows that on average each infected individual infects fewer than one individual. The disease will spread if $R_0 < 1$, as it shows that on average each infected individual infects more than one individual.

Theorem 3.1 For $R_0 < 1$, the disease-free equilibrium poin E_1 of the system (1) is locally asymptotically stable if and only if
$M_1 M_2 > \alpha_1 \phi_h X_h^o$.

Proof. By linearizing the system (1) at the equilibrium point $E_1 = (X_h^o, 0, 0, 0, 0, X_b^o, 0, 0)$,
We obtain the characteristic equation
$[\lambda + \mu_h][\lambda + M_1][\lambda + M_1 M_2 - \alpha_1 \phi_h X_h^o][\lambda + \mu_b] \times$
$[\lambda + M_5(M_1 M_2 - \alpha_1 \phi_h X_h^o)][\lambda + M_5 M_6(M_1 M_2 - \alpha_1 \phi_h X_h^o)]$
$\times [\lambda + M_3][\lambda + (M_3 M_4 + \phi_b \alpha_3 X_b^o)] = 0,$

where

$$M_1 = \mu_h + \phi_h,$$
$$M_2 = \rho_h + \beta_h + \mu_h,$$
$$M_3 = \mu_b + \phi_b,$$
$$M_4 = \beta_b + \mu_b,$$
$$M_5 = \gamma_h + \mu_h,$$
$$M_6 = \mu_h + \grave{o}_h.$$

The eight eigenvalues corresponding to the above characteristic equation are

$\lambda_1 = -\mu_h < 0, \quad \lambda_2 = -M_1 < 0,$
$\lambda_3 = -M_1 M_2 + \alpha_1 \phi_h X_h^o,$
$\lambda_4 = M_5(-M_1 M_2 + \alpha_1 \phi_h X_h^o),$
$\lambda_5 = M_5 M_6(-M_1 M_2 + \alpha_1 \phi_h X_h^o),$
$\lambda_6 = -\mu_b < 0, \quad \lambda_7 = -M_3 < 0,$
$\lambda_8 = -M_3 M_4 + \phi_b \alpha_3 X_b^o = -M_3 M_4[1 - R_0].$

We see that all the eigenvalues will have negative real parts only if $M_1 M_2 > \alpha_1 \phi_h X_h^o$ and $R_0 < 1$. Hence the disease-free equilibrium E_1 is locally asymptotically stable if $M_1 M_2 > \alpha_1 \phi_h X_h^o$ and $R_0 < 1$.

3.1 Endemic equilibria and backward bifurcation

In order to find the endemic equilibria of the proposed model (1), we need to take the following steps:

Let $E_2 = (X_h^*, E_h^*, I_h^*, T_h^*, R_h^*, X_b^*, E_b^*, I_b^*)$
represents any arbitrary endemic equilibrium of the model (1). By solving the equations of the system (1) at steady state, we get

$$X_h^* = \frac{\Lambda\phi_h M_5 M_6 + \grave{o}_h\phi_h\gamma_h\rho_h I_h^* - M_1 M_2 M_5 M_6 I_h^*}{\mu_h\phi_h M_5 M_6},$$

$$E_h^* = \frac{M_2 I_h^*}{\phi_h}, \quad T_h^* = \frac{\rho_h I_h^*}{M_5},$$

$$R_h^* = \frac{\gamma_h\rho_h I_h^*}{M_5 M_6}, \quad X_b^* = \frac{M_3 M_4}{\alpha_3\phi_b},$$

$$E_b^* = \frac{\mu_h M_1 M_2 M_4 M_5 M_6 I_h^*}{\alpha_2\phi_b[\Lambda\phi_h M_5 M_6 + \grave{o}_h\phi_h\gamma_h\rho_h I_h^* - M_1 M_2 M_5 M_6 I_h^*]}$$
$$-\frac{\alpha_1 M_4}{\alpha_2\phi_b},$$

$$I_b^* = \frac{\mu_h M_1 M_2 M_5 M_6 I_h^*}{\alpha_2[\Lambda\phi_h M_5 M_6 + \grave{o}_h\phi_h\gamma_h\rho_h I_h^* - M_1 M_2 M_5 M_6 I_h^*]}$$
$$-\frac{\alpha_1 I_h^*}{\alpha_2}.$$

If $I_h^* \neq 0$, then by putting values in the system (1) at steady state, we obtain after some calculations the following equation:

$$f(I_h) = a I_h^2 + b I_h + c = 0, \quad (6)$$

Where

$a = \alpha_1\alpha_3 M_3 M_4[M_1 M_2 M_5 M_6 - \gamma_h\rho_h\grave{o}_h\phi_h],$

$b = [M_1 M_2 M_5 M_6 - \gamma_h\rho_h\grave{o}_h\phi_h][\pi\alpha_2\alpha_3\phi_b - \alpha_2\mu_b M_3 M_4]$
$\quad - \alpha_3 M_3 M_4 M_5 M_6[\alpha_1\Lambda\phi_h + \mu_h M_1 M_2],$

$c = \Lambda\alpha_2\phi_h\mu_b M_3 M_4 M_5 M_6[1 - R_o].$

The coefficient a is always positive as $M_1 M_2 M_5 M_6 > \gamma_h\rho_h\grave{o}_h\phi_h$ and c is positive if R_0 is less than unity and is negative if R_0 is greater than unity. Since $a > 0$, so the positive solution depends on b and c. For $R_0 > 1$ the above equation gives us two roots, one is positive and the other is negative. By substituting $R_0 = 1$, the equation has nonzero solution $I_h = \frac{-b}{a}$, which is positive in case if and only if $b < 0$. For $b > 0$ there is a positive solution for $R_0 = 1$ It means that equilibria depends upon R_0 and there exists an open interval which has two positive roots

$$I_{h1} = \frac{-b - \sqrt{b^2 - 4ac}}{2a}, \quad I_{h2} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}.$$

If $c > 0$ and either $b^2 < 4ac$ or $b \geq 0$, the above equation has no positive solution, and thus there are no endemic equilibria. For different range of these parameters the following results are established.
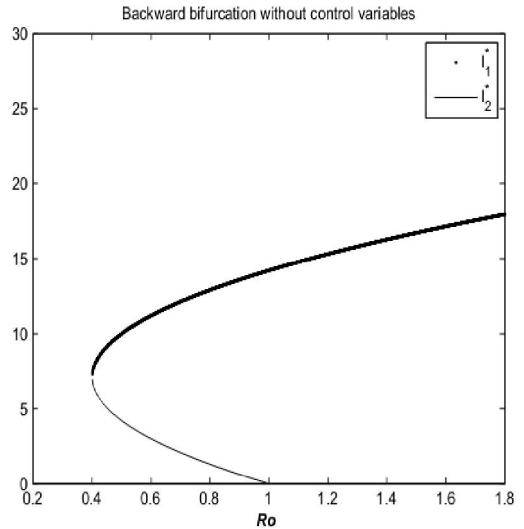
Figure 2. I_1^*, I_2^* versus R_0 shows a backward bifurcation with endemic equilibria when $R_0 < 1$.

Theorem 3.2 The system (1) has
(i) a unique endemic equilibrium in Ω if $c < 0 \Leftrightarrow R_o > 1$;
(ii) a unique endemic equilibrium in Ω if b<0 and $c = 0$ or b^2 - 4ac = 0;
(iii) two endemic equilibria in Ω if $c > 0$, b<0 and b^2 - 4ac > 0
(iv) no endemic equilibria otherwise.
In the above theorem case (iii) indicates the possibility of backward bifurcation in the model (1) when $R_0 < 1$
. To find the backward bifurcation, we set the discriminant b^2 - 4ac to be zero and solved for the critical value of R_0, denoted by R_c is given by

$$R_c = 1 - \frac{b^2}{4a[\Lambda\alpha_2\phi_h\mu_b M_3 M_4 M_5 M_6]}.$$

Hence, $R_c < R_0$ is equivalent to $b^2 - 4ac > 0$ and therefore the backward bifurcation would occur for values of R_0 such that $R_c < R_0 < 1$. We can illustrate it by simulating the proposed model (1) with the following set of parameter values: $\Lambda = 2$,

$\pi = 18$, $\alpha_1 = 0.001$, $\alpha_2 = 0.019$, $\alpha_3 = 0.35$, $\grave{o}_h = 0.01$, $\phi_h = 0.03$, $\rho_h = 0.05$, $\mu_h = 0.085$, $\mu_b = 0.455$, $\beta_h = 0.018$, $\beta_b = 0.6$, $\phi_b = 0.2$, and $\gamma_h = 0.01$. The phenomenon of backward bifurcation is confirmed by Figure 2 as it clearly shows that for $R_0 < 1$ there exist two locally asymptotically stable equilibria.

Theorem 3.3 The model (1) has a backward bifurcation at $R_0 = 1$ if and only if b<0.

Proof: Let us consider for sufficiency the graph of $y = g(I) = aI^2 + bI + c$. Since $c = 0$ for $R_0 = 1$ thus g(0)=0, hence the graph passes through the origin. Further g(I)=0 has a positive root $I = \dfrac{-b}{a}$ if b<0. On increasing the value of c from zero to some positive value, g(I) being a continuous function of c guarantees that there will be some open interval $(0, \grave{o})$ containing c, on which g(I) has two positive real roots. Thus we have shown that for $R_0 < 1$ there exist two endemic equilibria. The necessity is obvious as $b \geq 0$, the equation (6) has no positive real roots when $R_0 < 1$.

Theorem 3.4 When $R_0 > 1$, the unique endemic equilibrium state E_2 is locally asymptotically stable for $M_5 M_6 Q_5 < -\gamma_h \rho_h \phi_h \grave{o}_h Q_2$.

Proof: By linearizing the system (1) at $E_2 = (X_h^*, E_h^*, I_h^*, T_h^*, R_h^*, X_b^*, E_b^*, I_b^*)$, we have the characteristic equation

$$[\lambda - Q_1][\lambda - M_1 Q_1][\lambda - Q_5][\lambda - M_5 Q_5] \times$$
$$[\lambda - M_3 Q_6][\lambda - (M_5 M_6 Q_5 + \gamma_h \rho_h \phi_h \grave{o}_h Q_2)] \times$$
$$[\lambda - Q_6][\lambda - (M_3 M_4 Q_6 + \phi_b Q_7] = 0,$$

where

$$Q_1 = -\mu_h - \alpha_1 I_h^* - \alpha_2 I_b^*,$$
$$Q_2 = \alpha_1 I_h^* + \alpha_2 I_b^*,$$
$$Q_3 = \mu_h \alpha_1 X_h^*,$$
$$Q_4 = \mu_h \alpha_2 X_h^*,$$
$$Q_5 = M_1 M_2 Q_1 + \phi_h Q_3,$$
$$Q_6 = -\mu_b - \alpha_3 I_b^*,$$
$$Q_7 = -\alpha_3 X_b^* Q_6 - \alpha_3^2 I_b^* X_b^*.$$

There are eight eigenvalues corresponding to the above equation. All the eigenvalues will be negative only if $Q_5 = M_1 M_2 Q_1 + \phi_h Q_3 < 0$ and $M_5 M_6 Q_5 < -\gamma_h \rho_h \phi_h \grave{o}_h Q_2$.

After simplification we see that $Q_5 < 0$ only if $R_0 > 1$. Thus all the eigenvalues have negative real parts, which indicates that E_2 is locally asymptotically stable.

4   Global stability analysis

We illustrate the global property of the disease-free and the endemic equilibrium of the system (1) by the following theorems.

Theorem 4.1 The disease-free equilibrium of the system (1) is globally asymptotically stable on Ω.

Proof: We construct the Lyapunov function for the global stability of the system (1) at the disease-free equilibrium E_1 as follows:

$$F(t) = [(X_h(t) - X_h^o) + E_h(t) + I_h(t) + T_h(t) + R_h(t)]^{\frac{3}{2}}$$
$$+ [(X_b(t) - X_b^o) + E_b(t) + I_b(t)]^2.$$

By taking the time derivative, we have

$$F'(t) = \frac{3}{2}[(X_h(t) - X_h^o) + E_h(t) + I_h(t) + T_h(t) + R_h(t)]^{\frac{1}{2}} \times$$
$$[\Lambda - \beta_h I_h(t) - \mu_h N_h(t)] + 2[(X_b - X_b^o) + E_b + I_b]$$
$$\times [\pi - \beta_b I_b - \mu_b N_b],$$

Where

$N_h(t) = X_h(t) + E_h(t) + I_h(t) + T_h(t) + R_h(t)$,

$N_b(t) = X_b(t) + E_b(t) + I_b(t)$

(') denotes the derivative with respect to time t.

Using $X_h^o = \dfrac{\Lambda}{\mu_h}$ and $X_b^o = \dfrac{\phi}{\mu_b}$, we have

$$F'(t) = \frac{3}{2}[(X_h(t) - X_h^o) + N_h(t) - X_h(t)]^{\frac{1}{2}} \times$$
$$[-\mu_h(X_h(t) - X_h^o) - \beta_h I_h(t) - \mu_h(N_h(t) - X_h(t))]$$
$$+ 2[(X_b(t) - X_b^o) + E_b(t) + I_b(t)] \times$$
$$[-\mu_b(X_b(t) - X_b^o) - \beta_b I_b(t) - \mu_b(N_b(t) - X_b(t))].$$

Thus $F'(t)$ is negative and $F'(t) = 0$ if and only if

$E_h(t) = I_h(t) = T_h(t) = R_h(t) = E_b(t) = I_b(t) = 0$ and

$X_h(t) = X_h^o$, $X_b(t) = X_b^o$. Hence by Lasalle's invariant principle [7], the disease-free equilibrium state E_1 is globally asymptotically stable on Ω.

Theorem 4.2 The endemic equilibrium E_2 of the system (1) is globally asymptotically stable on Ω for $\Lambda = \mu_h N_h^* + \beta_h I_h^*$ and $\pi = \mu_b N_b^* + \beta_b I_b^*$.

Proof: We define the Lyapunov function for the endemic equilibrium as

$$L(t) = [X_h(t) - X_h^*] + [V_h(t) - V_h^*] + [E_h(t) - E_h^*]$$
$$+ T_h(t) + R_h(t) + [X_b(t) - X_b^*] + E_b(t) + I_b(t).$$

By calculating the time dependent derivative of the above function along the solutions of the system (1), we have

$$L'(t) = \Lambda + \pi - \beta_h I_h - \beta_b I_b - \mu_h N_h - \mu_b N_b.$$

Using $\Lambda = \mu_h N_h^* + \beta_h I_h^*$ and

$\pi = \mu_b N_b^* + \beta_b I_b^*$,   we have

$$L'(t) = -\mu_h(N_h(t) - N_h^*) - \mu_b(N_b(t) - N_b^*)$$
$$- \beta_h(I_h(t) - I_h^*) - \beta_b(I_b(t) - I_b^*),$$

where $N_h^* = X_h^* + E_h^* + I_h^* + T_h^* + R_h^*$ and $N_b^* = X_b^* + E_b^* + I_b^*$.

Thus $L'(t)$ is negative and $L'(t) = 0$ if and only if $X_h(t) = X_h^*$, $E_h(t) = E_h^*$, $I_h(t) = I_h^*$, $T_h(t) = T_h^*$, $R_h(t) = R_h^*$, $X_b(t) = X_b^*$, $E_b(t) = E_b^*$, $I_b(t) = I_b^*$.

Hence by Lasalle's invariant principle [7], the endemic equilibrium state E_2 is globally asymptotically stable on Ω.

5 Numerical results and discussion

We solve the proposed model by using Runge-Kutta fourth order scheme. Some of the parameter values in the proposed model based on reality, for example the duration of the infectious period, natural death rate, disease induced death rate, etc. (see Table 2). As a person infected with the avian influenza virus is only infectious for almost seven days, the recovery rate should be equal to 0.143 per day and not the inverse of the length of the illness. The natural death rate of

human $\mu_h = 0.0000421$ per day, corresponding to the life expectancy of the human is 70 years. α_1 , α_2 are the effective contact rates between S_h and E_h and between S_h and I_b respectively, α_3 is the effective contact rates between S_b and I_b, we choose these parameters arbitrarily as $\alpha_1 = 0.0002$, $\alpha_2 = 0.0025$ and $\alpha_3 = 0.01$. The values used for numerical simulations are given in Table 2, with $\pi = 10$, $\phi_h = 0.14$ and $\phi_b = 0.4$, which are biologically feasible. Figure 3 represents the human population and Figure 4 represents the birds population.

Table 2. Parameter values used for numerical simulations

Notation	Values	Resourse
Λ	2.5/day	[8]
μ_h	0.0000421/day	[9]
μ_b	0.00137/day	[10]
β_h	0.002/day	[11]
β_b	0.1/day	[10]
μ_h^{-1}	70 years	[12]
ε_h	0.00137/day	[13]
ρ_h	0.4	[14]
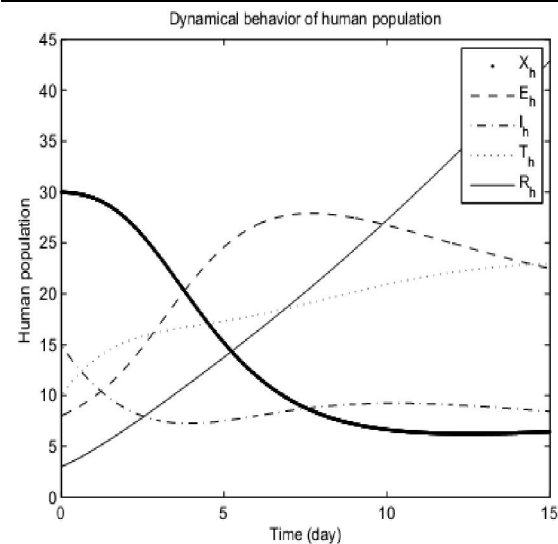
Figure 3. Plot shows human population
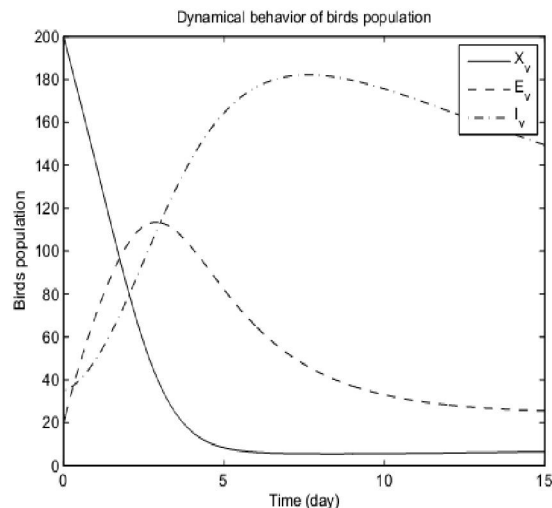
Figure 4.   Plot shows bird population

6   Conclusion

In this work, we discussed the compartmental avian influenza model. As in epidemiological models, our model has two steady states, an uninfected steady state and endemically infected steady state. By establishing the stability results we found both the disease-free and the endemic equilibria. We also presented that the proposed model exhibits the phenomenon of backward bifurcation, where for $R_0 < 1$, the locally asymptotically stable disease-free equilibrium co-exists with a locally asymptotically stable endemic equilibrium. Then to present the global stability of both the disease-free and endemic states, we developed Lyapunov functions. We believe that this new analysis is biologically more plausible than the previous assumptions.

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The Comparison of Depression in Hemodialysis and Renal Transplantation Patients

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Abstract: Depression is the fourth most disabling disease affecting people worldwide and the most common psychological disorder among patients with end-stage renal disease (ESRD) . The objective of this study is to survey and comparison of depression in dialysis and renal transplantation patients. Also examine the association between depression and demographic information in two groups. Cross-sectional was used to conduct the study, which involved 108 patients (61 dialysis and 47 renal transplantation) . Depression was measured using the Beck depression Statistical was done by chi-score and t-test using SPSS win software. The mean age in dialysis and renal transplantation patient was 47.4+/-15.5 and 39.9+/-9.7 years. Statistical analysis of this means was significant difference (P<0.003).in two group, the prevalence of depression was not significantly in females than males. Prevalence of depression in the dialysis patients was nearly %93 and in the renal transplantation was %56. Major depression in dialysis patients was %16.4 and in renal transplantation was %6.4. The Mean number of depression in dialysis and renal transplantation patient was 21/08+/-8/04 and 13/23 +/- 8/31(number of Beck depression above of 9) finally mean value of depression in dialysis samples was significantly different (P<0.000) from that of renal transplantation samples. Depression was not significantly correlated with demographic factors such as duration of treatment, age, gender and married. Prevalence of depression in dialysis patients was more than renal transplantation patients. This high prevalence of depression emphasizes the significance of this disorder among patients on chronic hemodialysis. Due to its impact on mortality and survival rates, early diagnosis of depression in such patients is therefore crucial.

1. Introduction

End-stage renal disease (ESRD) or a very advanced renal disease with a prevalence of one per one million in developed countries [1] is a kind of progressive and irreversible impairment of kidney work in which the body's ability to maintain fluid and electrolyte balance is disrupted. In addition, the available data indicate that at least 60 million people worldwide are suffering from different degrees of renal dysfunction. According to the last data, nearly two millions suffer from renal failure (ESRD) in USA, costing over 70 thousand dollars for treatment and care of patients undergoing dialysis [2]. The rate of increasing renal patients is annually 15 percent in Iran [ref]. Number of renal patients has increased by 11 % during the last 4 years and there are 22376 patients suffering from kidney impairment in Iran. Rate of ESRD patients is principally increasing in recent years [ref].

Number of hemodialysis patients has doubled since 1990. Increasing the number of hospitalization days due to mental diseases in ESRD patients is much higher compared to the total population and timely recognition and treatment of mental disorders is not only effective in promoting the care and

following up the patients treatment, but also increases the life quality of patients[3,4].

Among well-known psychological problems in patients with ESRD includes depression, organic mental disorders, materials abusing, and anxiety [5]. Depression is the most important mental problem that can lead to changes in nutritional status, direct and indirect effects on the immune system, non-compliance with treatment regimen, increasing the severity of the disease and ultimately suicide or stopping treatment if not be identified or treated [6, 7].

Prevalence of depression in ESRD patients has been reported from 10 to 100 percent; this rate of depression is an alarm that its approval and epidemiological studies carried out reveal that suicides occurred in these patients are 10 to 100 times higher than normal individuals [8].

Moreover, depression is one of the disorders, which allocates exorbitant costs. Depression is considered as one of the expensive diseases in America. Several studies show that compared to other patients, physical patients having depression hospitalized in hospital had 40 percent and 35 percent increase in terms of length of stay and the cost of treatment respectively [9]. Perhaps, the reason is that

depression is an important factor in decreasing the patients' tendency to take the treatment and lack of participation of depressed patients in the treatment has increased their medical problems and has endangered their health, even cause premature death [10]. Patients with ESRD are those patients dealing frequently with many social-psychological stress-makers because of changing in lifestyle due to illness, and treatment method, therefore, depression is one of the most common their psychological problems [11].

Using Beck Depression Inventory (BDI), Kamil et al (2000) found a significant relationship between severity of depression and mortality in dialysis patients [12]. In addition, they reported that patients earned higher depression score have experienced more complications during treatment [13]. In a research carried out by Coolins et al (1999), they implied to 25 - 50 percent of depression in hemodialysis patients [14].

Ekman et al (2004) reported that depression in receivers of kidney transplant was significantly lower [15] than patients undergoing hemodialysis and chronic transplant rejection. According to Haily, 20 percent of hemodialysis patients had psychological problems and 43 percent of them had depression [16]. Research conducted in Iran indicate high frequency of depression in dialysis patients; for example, Vafa'i et al investigated the prevalence of depression in hemodialysis patients using the BDI test and found that 53 percent of patients had depression [17]. Zahyraldin et al recorded that the rate of depression in hemodialysis patients was around 69 percent (scoring more than nine) using BDI test [18].

Since compared to other health stuffs, nurses are associated with dialysis patients; they can prevent the negative impacts and devastating effects of depression through identifying depression and its severity in order to assist its timely treatment. Therefore, timely diagnosis and effective treatment of depression would result in improving the life quality, disease prognosis, and patients' survival [19]. The present research was carried out to assess depression rate in hemodialysis and kidney transplant patients in Sanandaj, Kurdistan province, Iran.

2. Material and Methods

This is a cross – sectional, analytical-descriptive study. The samples were all patients treated with hemodialysis in 2005 at Hemodialysis Center, Tauhid Hospital in Sanandaj selected through census, considering rejection and admission criteria. Criteria for admission were that the samples studied were at least six months under treatment with hemodialysis and would like to participate in research and rejection criteria included a history of suffering from psychiatric disorders before treatment with hemodialysis, mourned during the last 6 months, and

physical and mental disabilities. Hence, the total number of patients participated in the study was 108 patients (61 dialysis and 47 hybrid).

In order to collect data, two questionnaires of demography including age, sex, qualification,…, and Center for Epidemiologic Studies-Depression (CES-D) Beck scale (including 21 questions with four options) were used to assess depression rate at wards studied. Validity and reliability of this questionnaire has been confirmed by Rodin (1999) and Beck (2009) [20]. Test-retest was used to determine the reliability of this tool. Scores of depression and severity of depression have been determined based on the Beck Depression Standard as No depression (0-9), Mild (10-18), Medium (19-29), and severe (30-63). For patients who were illiterate or for some reasons were not able to record answers, the questions desired were asked and their answers were recorded without any manipulation. The data collected were analyzed for T-test and Chi-Square, the descriptive statistics and statistical test using SPSS statistical software.

3. Results

108 (61 dialysis and 47 hybrid) patients treated by dialysis and transplantation were assessed and compared. The age mean of patients studied in the dialysis group and the transplantation group was 47.4 $\pm$ 15.5 and 39.9 $\pm$ 9.7 years old respectively with $P<0.003$ showing significant difference.

Higher percentage (more than 30 percent) of patients in both groups had elementary qualification and a very small percentage had a higher education. Illiteracy level in dialysis patients was more compared to transplant patients. Nearly 20 percent of patients in both groups are single. Prevalence of depression in both groups was reversely proportional to the qualification. Moreover, rates of depression in hemodialysis patients increased as duration of hemodialysis increased, whereas this rate decreased with increasing duration of transplantation.

There was no significant relationship between severity of depression and gender, age, duration of treatment and marital status. Separate comparison of depression rate in men and women in each group showed no significant difference. Generally, 93 percent of dialysis patients had depression with different severity (mild to severe). Nearly, 56 percent of the transplant patients had depression with different severity (mild to severe) (Table). 16.4 percent of dialysis patients and 6.4 percent of transplant patients had severe depression. Moreover, the mean and standard deviation of both groups with $P<0.000$ showed significant difference (Table 2) so that rate of depression in dialysis patients was much more than transplant patients. There was no

significant difference in depression rate with age, sex, marital status, and qualification.

Table 1: comparison of absolute and relative frequency in patients studied as dialysis, and kidney transplant group and their depression rate

Group classification	Depression level	Number	Percent
dialysis	No depression	4	6.6
	Mild	24	39.3
	Medium	23	37.7
	Sever	10	16.4
	Total	61	100
transplant	depression	18	38.3
	Mild	18	38.3
	Medium	8	17
	Sever	3	6.4
	Total	47	100
Total	depression	22	20.4
	Mild	42	38.9
	Medium	31	28.7
	Sever	13	12
	Total	108	100

Table 2: Comparison of mean and standard deviation in both dialysis and transplant patients

Dialysis (61 patients)		Transplantation (47 patients)		Total (108 patients)	
Mean	S.D.	Mean	S.D.	Mean	S.D.
21.8	8.04	13.23	8.31	17.15	17.8

4. Discussions

Depression is one of the most common psychological disorders in ESRD patients. Several studies have been reported the depression rate from 10 to 100 percent; this rate is especially higher in hemodialysis patients. Over the last years, more attention has been paid to psychosocial factors affecting the results of treatment. Progressing renal failure, patient feels more the symptoms of diseases day by day, and the best available treatment that can reduce symptoms and partial improvement in patient quality of life is hemodialysis and peritoneal dialysis and kidney transplantation is in final category of the ESRD treatment [7, 8].

The findings showed that 93.4 percent of patients treated with hemodialysis and 61.7 percent of kidney transplant patients have experienced some degree of depression and 16.4 percent of hemodialysis patients and 6.4 percent of transplant patients had severe depression. In these patients due to a lot of social-psychological stresses or problems that they experience, depression has been reported as the most commonly psychiatric diagnosed, associated with high mortality rate. Depression in hemodialysis patients was significantly more than patients transplanted. Many studies indicate that prevalence of depression in dialysis patients is higher that is consistent with the present study. Akman et al found that depression rate in renal transplant recipients in Turkey (Ankara City) was significantly lower than the hemodialysis and chronic rejection patients (P = 0.003) [15]. In another study conducted in Italy, it was shown that transplant patients 4 months after kidney transplantation, their depression rate had been reduced from 45.8 percent to 32 percent and the severity of depression was changed from 16.4 percent to zero percent was [22]. Penkower et al found that the depression rate in kidney transplant patients was significantly lower than the hemodialysis patients [23]. Since the overall goal of our study was comparative study of depression rate in two groups, therefore, the results of the similar studies with various criteria and investigative techniques can be reliable. However, it was attempted to use the same research tools to study the rate of depression in patients.

Anis et al have noted the depression rate in hemodialysis patients in Pakistan was 56.1% [24]. Etemadi also has expressed that the rates of depression in hemodialysis patients is 65% [25]. Koo et al reported that depression rate in dialysis patients was 56.5 percent [26]. Vafa'i has claimed that this percentage was 33.3 [27]. Other studies, including Kimmel et al, have proved that depression results in the incidence of malnutrition, immune system disorders, and suicide risk [7].

In the present study, comparing mean and standard deviation of two groups showed significant differences with P <0.000 so that the average depression in dialysis and transplant patients was 21.08±8.04 and 13.23±8.31 respectively. Other researchers, including Koo et al from Korea, Nordan et al from Turkey, and Robert et al from United States reported the depression rate in hemodialysis patients as 22.7±11.4, 14.1±11.3, 12.1±7.7 respectively [26,28, 29] showing that rate of depression in dialysis patients is quite variable depending on different conditions. With reference to job loss; reduced income; reduced role in family, workplace, and the community; presence of refractory and incurable disease; and stresses resulted during treatment can justify the increase of the depression mean. On the other hand, the majority of patients studied were from poor and low income families with financial ties to the relatives; however, the cultural poverty would not ineffective.

Factors that may undermine the life quality of patients include age, sex, race, level of anemia, nutrition, glomerular filtration rate, quality and techniques effective in dialysis treatment, social factors and depression. Among all the factors

mentioned, depression has been known as one of the factors that has determinative role in prognosis (pish agahi) and survival of pateint. Several studies have proved the relationship between depression and mortality rate of patients [30]. In present study, despite assessing depression rate in these (dialysis and renal transplantation) patients, the possible demographic factors associated with severity of depression has also been discussed. No significant difference was found between depression rate and age, sex, qualification, and treatment duration.

Although similar research works have reported relationship between gender and depression significant, Vafa'i has claimed that the depression in women was more than men having a significant difference [27]. Koo et al have also found significant differences between age and depression rate [26], however, Etemadi did not find a significant relationship between the sex and age with depression [25]. The present study shows that the highest rate of depression was in dialysis patients, in age group of ≥45 years old and the lowest in the age group of 15-24 years old. Moreover, the highest rate of depression was in transplanted patients in the age group of 35-44 years old and the lowest in the age group 25-34shows.

80 percent of dialysis patients and 58 percent of transplanted patients have obtained score equals to or more than 9, increasing with increasing duration of dialysis treatment, but not significant and does not show significant difference in transplanted patients. Koo et al have found significant relationship between depression and dialysis treatment duration [26].

Depression severity was higher in single patients rather than the married one, which is in agreement with the other researches including Akman et al and Vafa'i [27], however, Etemadi has reported that this rate was more in married patients [25]. In the present study, increasing level of qualification, the depression rate decreased, although it is not significant. Several studies suggest that depression in individuals having no higher education, was more than individuals having academic qualification [23].

According to the results obtained in this study and the results of some similar studies mentioned, it seems that the psychological problems of dialysis patients in dialysis wards must paid more attention. For this purpose, applying psychiatric services in hospitals and specialty divisions and activating counseling psychiatric services, which are rapidly growing in developed and developing countries is recommended to provide mental health for patients and real time diagnosis and treatment of psychiatric disorders so that to prevent the negative impacts of these disorders on life quality and the treatment process of background disease. One of the limitations of this study as a preliminary study is low sample volume. A study with a larger size is recommended. One of the other limitations of this study was applying only one psychological tool for assessment; therefore, use of psychiatric interviews and or other psychological tools is recommended to make more ensure and identification of other psychiatric problems of this group of patients

The present study showed that depression in patients with renal failure is considered as a serious threat and can jeopardize the health of patients. Therefore, conducting periodically psychiatric examinations is recommended for timely diagnosis and subsequent treatment of depression in hemodialysis and renal transplant patients. In addition, it is recommended to carry out other studies on more patients with the aim of identifying other factors related such as stressors of hemodialysis patients. Such interventional researches could be designed and implemented to increase life skills to deal with psychological-social stressors, and thus to prevent and to treat depression and to enhance life quality of the patients.

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A Field Wireless Sensor System for Mangrove Ecology Environment in Taiwan: Design and Implementation

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Abstract: This study is the first investigation of using wireless sensor networks (WSNs) for Mangroves protection and observation in Taiwan. In this paper, this study proposes a natural ecology sensing system (NESS), which consists of ecological environment sensor (EES), nature resource integrated computing server (NRICS), mobile user (MU), and ecological information service system (EISS). NESS can improve the traditional long-term observation method by providing a real-time and networked surveillance system. This paper considers the practical issues of deploying ecological environment sensors in the Mangroves protection area. In additions, feasible solutions are proposed to achieve sustainable energy supply and reliable data delivery with high accuracy. We also evaluate system performance in terms of energy consumption and packet loss rate at different duty cycles, and compare lifetime and data accuracy with different energy supply sources. According to implementation results, the proposed system is efficient and feasible for Mangroves observation, protection, and surveillance.

1. Introduction

Mangroves are rare and protected trees and shrubs in Taiwan. They grow within Dan Shiu Mangroves protection area, where are on Dan Shui river mouth and near Mangroves station of Taipei mass rapid transit (MRT) as shown in Figure 1 (circled by dotted line). The main function of Mangroves is to keep the balance of biology and ecology in Dan Shui river. With the rapid development of Taipei city, the Mangroves range is seriously being affected by man-made and environmental effects such as the construction of MRT, tourism, polluted water, and greenhouse effect. Such factors will cause the unbalance of biology and ecology in the river.

Recently, several articles are proposed and used for investigating the Mangroves observation and protection [7]. However, the data collection methodology of these articles adopts field survey. With field survey, researchers have to collect data by themselves in Dan Shiu Mangroves protection area. Therefore, such a method is labor intensive and dangerous, and cannot provide real-time and continuous data collection. To improve above drawbacks, this study designs and implements a natural ecology sensing system (NESS) using wireless sensor networks (WSNs). With the application of WSNs and Internet, researchers can perform long-term observation such as micro-climate and images collection at the remote site. In addition, the system can be used for surveillance of Dan Shiu Mangroves protection area, i.e. real-time invasion detection and alarm.

In recent years, several papers are proposed that WSNs are used for real-time data collection in the field [4, 9, 11, 13]. In [6], the authors proposed and implemented a reactive and robustness event driven WSN for data collection in the field. They adopted SMAC as MAC protocol to address the problems of energy efficient and coordinated sleeping. In [19], the authors present a case study of a WSN. A multi-dimensional analysis methodology was used to reveal trends and gradients in collected data. In [6], a Trio system was proposed. This system is used in large scale and long-term observation using WSNs. This paper also considered several deployment issues such as scalable, sustainable, and fail-safe flexibility. In [5], the authors designed and implemented a system, INSIGHY, which is used for habit monitoring. The goal of this study is to extended deployment lifetime, remote querying configuration, ease of deployment, and reliability. These articles prove the application of WSN is an efficient and challenging way for environment monitoring, and offer many evaluation metrics for deploying in the field.

The adopted ecological environment sensors of this paper are composed of temperature, humidity,

light, water temperature, PH value, and image sensor. In order to deploy these sensors in the Dan Shiu

Mangroves protection area, two design principles must be considered.

Figure 1. Dan Shiu Mangroves protected area

- Energy consumption: Long-term observation needs continuously collected data; hence, deployed sensors must perform data sensing and delivery task all days. Battery is major power supply for ordinary sensors. However, the sensors will not work well because the battery soon runs out. To overcome above challenges, [8] and [17] proposed two energy consumption solutions. However, they did not consider real environmental effects (e.g. sunshine and rainy period). In this paper, we evaluate require power for each sensor and real environmental effects and propose a solution for sensor energy consumption in the Mangroves range.

- Data delivery and accuracy: The field survey is not an efficient way for real-time data collection, but it could guarantee data accuracy. Although WSNs can efficiently perform data collection, data accuracy is a challenging issue. Data accuracy may be degraded by some effects, e.g. low power level that could affect the conversion results of analog to digital converter (ADC), delivery and database error, and so on. To avoid above errors, this paper proposes a solution that can filter error data and dynamically adjust data accuracy.

The remainder of this paper is described as follows. The system architecture and operation flow are described in Section 2. Section 3 depicts practical considerations for deploying sensors in the field.

Performance evaluation and system implementations are demonstrated in Section 4. Finally, this study is concluded in Section 5.

2. Overview of Natural Ecology Sensing System Architecture

　　NESS system architecture includes four parts that are (1) ecological environment sensor (EES), (2) nature resource integrated computing server (NRICS), (3) mobile user (MU), and (4) ecological information service system (EISS). Each part and the detailed operation of NESS are described in Sections 2.1 and 2.2, respectively.

2.1. System architecture

　　NESS is a four-tier system as shown in Figure 2. Researchers and observers can utilize various terminal devices such as PC, notebook, Tablet PC, third-generation (3G) mobile phone and personal digital assistant (PDA) to access services in the system [2-3, 10, 12]. First tier is EES, in which the sensors must perform data sensing task as well as data delivery task via the gateway. Second tier is NRICS which is to store collected data such as micro-climate and image data. Third tier is MU. In this tier, users can acquire collected data in the EISS through wireless local area network (WLAN). EISS is the final tier where system can convert collected data to useful information that could be utilized in further Mangroves observation and protection.

2.1.1. Ecological environment sensor

EES consists of two types of sensors, which are ecological sensors (e.g. temperature, humidity, light, water temperature, PH value sensors) and biological sensors (e.g. image sensors). Ecological sensors are to collect ecology data (i.e., micro-climate, water temperature and PH value). On the other hand, biological sensors, namely IP cameras, are to capture biology data (i.e., crab's movement and Mangroves growing). In this paper, ecological sensors employ two different platforms based on specific sensing tasks. The platforms include Telos sensors [18] and MicaZ sensors [15]. Figure 3 shows practical deployment of both sensor platforms and an IP camera. The sensors of each platform transmit their sensed data to distinct gateway that are Tmote Connect [16] and MIB600 [15]. Both gateways forward sensed data to the local server via wireless access point (AP) that is configured as wireless distributed system (WDS) mode as shown in Figure 4.
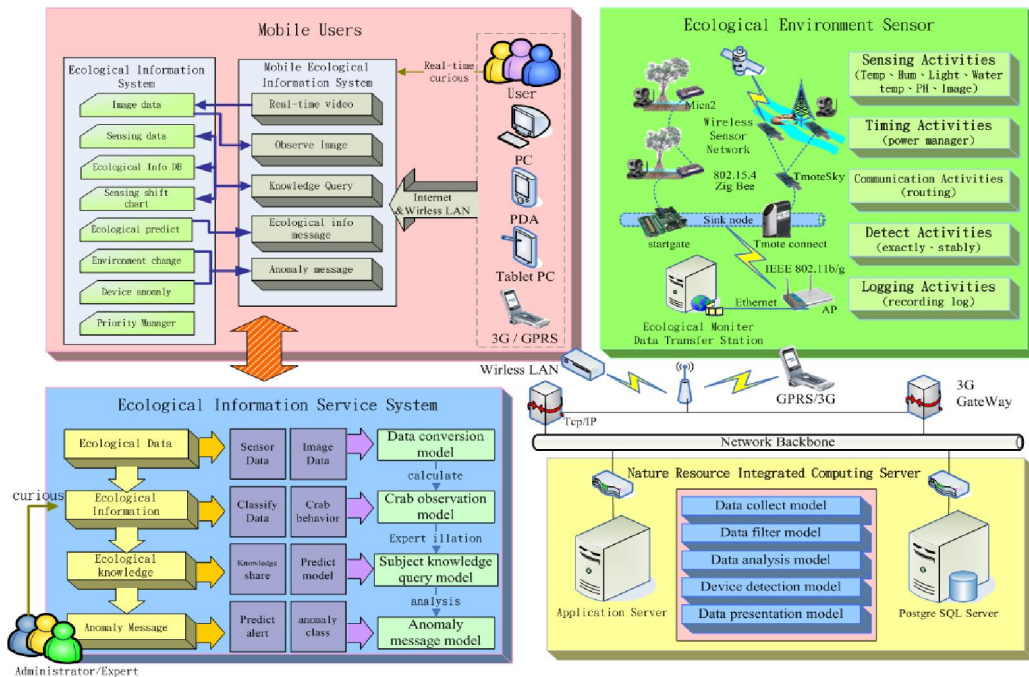
Figure 2. NESS system architecture

Figure 3. The practical deployment of EES and an IP camera in the field
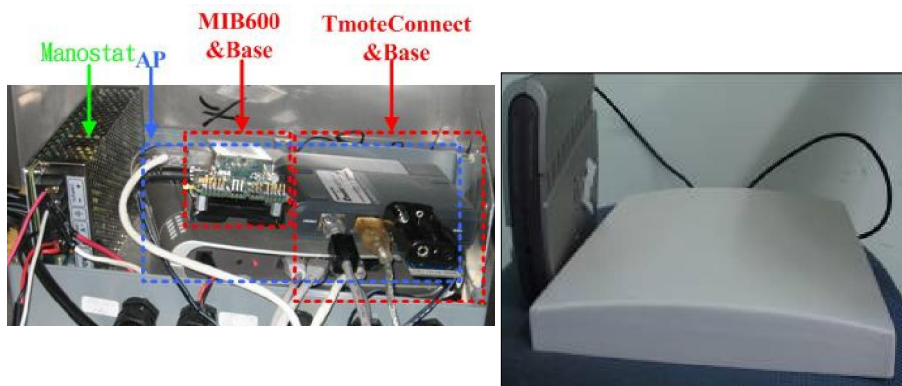
Figure 4. Wireless AP with two gateways and antenna

2.1.2. Nature resource integrated computing server

In NRICS, there are five system models, which are (1) Data collection model, (2) Data filtering model, (3) Data analysis model, (4) Device detection model, (5) Data presentation model. Each model is described in the following.

(1) Data collection model: The purpose of this model is to execute data sensing, convert analog data to digital data, integrate sensed data from two distinct sensor platforms, and store integrated data in the PostgreSQL server. The operation procedure of the model is shown in Figure 5.

(2) Data filtering model: According to our investigation, low power level, namely low voltage (V), could affect the accuracy of the ADC conversion results. In addition, data delivery and storage procedure may degrade data accuracy as well. Therefore, this model set two voltage thresholds ($2.1<V$ and $V>3.1$) to filter wrong data. The thresholds evaluation is in Section 4.1. If voltage is out of compass or received data is below zero, the sensed data will be deleted. After deleting data, an entry is added in the log, and the system sends an email to the manager; otherwise, sensed data is saved

in the database (DB). The operation of this model is shown as Figure 6.

(3) Data analysis model. Due to continuous data collection in NESS system, there is going to be a large number of data in the DB. Hence, data analysis also is an important step in the system. This model is able to convert raw data in the DB into applicable information. We also compare such information with central weather bureau of Taiwan to verify the information accuracy.

(4) Device detection model. This model is to determine whether NESS system and other appliances such as access point, gateways, and sensor nodes work well. If anyone device fails, the model will automatically send an email to managers. The email is encoded by XML language.

(5) Data presentation model. For presentation types, this model provides a various presentation types for acquired information, i.e. a bar chart, a figure, or a table. For collection timeliness, the users could view acquired information with hour, day, month or year. These users include researchers, observers, managers or the public. They own distinct limits of authority. According to distinct authority, the system can present adequate information to them.

Figure 5. Sensing data collect model
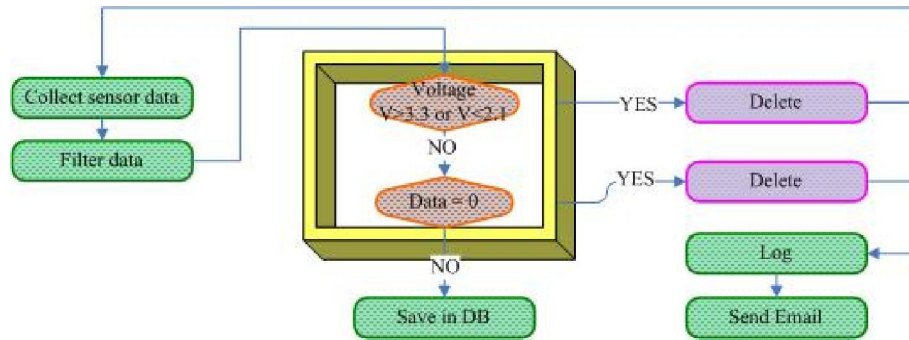
Figure 6. Data filtering model

2.1.3. Mobile user

MU can utilize mobile appliances to access service in NESS. The models of MU are described below.

(1) Real-time/history multimedia receiver model: This model is to receive real-time or historical biology images. Therefore, users can realize biology growing environment in Mangroves range according to real-time and historical image data.

(2) Customized information service model: This model provisions customized services to users, in which users can view different system interfaces based on the appliance platform, and obtain the personal functions and information.

(3) Real-Time/history information receiver model: This model implements a push technology. Hence, the system will automatically push real-time Mangroves information and images to users. This model also can transmit current alert messages to the user when an error occurs.

2.1.4. Ecological Information Service System

The system models of EISS are described as follows.

(1) Data conversion model: This model assists to convert raw data to applicable information with data analysis model, and provides converted information to users.

(2) Crab observation model: Crab is one kind of biology in the Dan Shiu river. This model provisions an interface, in which users can observe Crab's activity every day.

(3) Subject knowledge query model: This model provides a subject query function. Users are able to search related knowledge based on specific subject with this function. For instance, the keywords can be "spring in the mangrove" or "the day life of crabs" .

(4) Anomaly message model: This model is to store device condition at each detection period and find relationship between regular data or anomaly data.

2.2. System operation flow

This section shows the system operation flow of NESS. As depicted in Figure 7, the system flow is composed of three stages. Stage one is data collection, stage two is data application, and last stage is for anomaly detection of WSN data.
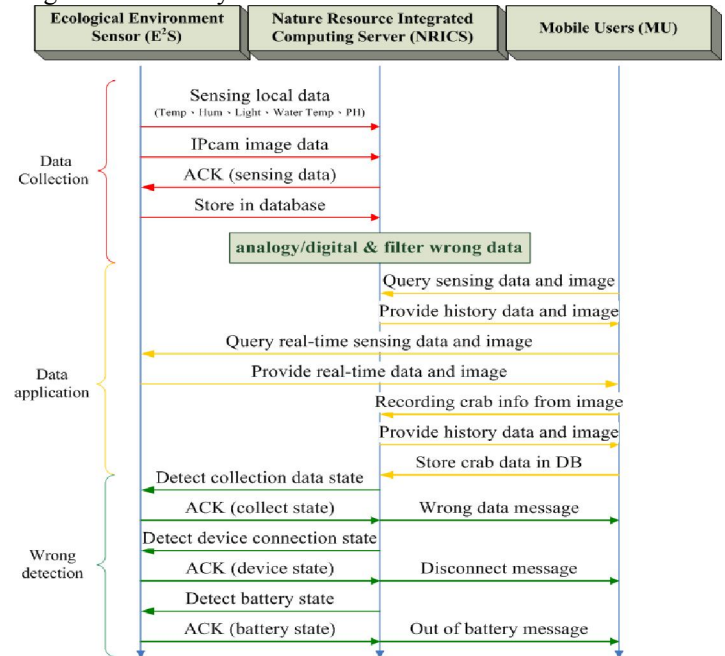
Figure 7. NESS system operation flow

2.2.1. Stage 1: data collection

In this stage, each sensor of EES is to execute specific sensing tasks such as micro-climate and image sensing, and has to perform ADC conversion and then transmitting converted data and images to NRICS server. When NRICS server received sensed data, it is able to response an ACK message to the sensor. Finally, the sensed data is stored in the database.

2.2.2 Stage 2: data application

MUs are able to send a query message to request historically sensed data in NRICS server. When NRICS has received the message, the server

provisions sensed data to users. If users request real-time data, NRICS will forward the query message to EES. When EES has received the message, it provides real-time data to users directly.

2.2.3. Stage 3: wrong detection

To prevent whole system failure, NRICS system needs to periodically execute failure detection and check energy level of each sensor. If a failure occurs or batter runs out, NRICS will response the failure status to managers immediately.

3. Practical Deployment of Ecological Environment Sensor

In this section, we discuss practical deployment of EES in Mangroves range. Energy supply is a challenging issue for practical deployment of EES in the field and long-term observation. In order to provide continuous energy supply for each sensor and equipment, we adopt solar panel and rechargeable battery as our energy solution. First, we have to choose two observation points in Mangroves range, and place a gateway in these points, respectively. The gateway is responsible for relaying query message and sensed data between sensors and the server end. As Figure 1 shows, first observation point is near river side (SS1) that is near the Mangroves station of Taipei MRT, the other is far river side (SS2) that is on the Dan Shiu river.

Table 1 shows all equipments and energy requirements at both observation points. In order to offer enough energy, we estimate energy consumption for each sensor and equipment, and consider environmental effects such as weather,

location, and branches and leaves of Mangroves. In North Taiwan, the mean of sunlight is four hours every day, there is higher rainy probability in winter. Therefore, we have to prepare more energy supply than estimation value in Table 1. In SS1 and SS2, there have 1.9Ahr and 12.5Ahr voltage and 130W and 480W solar panel with 3 cells battery, respectively. The practical deployments are shown in Figure 8.

Table 1. Equipment list and voltage at both observation points

Location				SS1
Item	Current (Ah)	Voltage (V)	number	Watt (W)
AP	2	5	1	10
Antenna panel	4	5	1	20
Tmote Connect	2	5	1	10
Tmote	0.4	3	4	4.8
IP-CAM	1.5	12	1	18
IP-CAM shell	2	12	1	24
Total	11.9			86.8
Location				SS2
Item	Current (Ah)	Voltage (V)	number	Watt (W)
AP	2	5	1	10
Antenna panel	4	5	1	20
MIB600	1	5	1	5
IP-CAM	1.5	12	1	18
IP-CAM shell	2	12	1	24
Water temp and PH	2	9	1	18
Total	12.5			95

Figure 8. Power module and Gateway node in SS1 and SS2

4. Performance Evaluation and System Implementation
4.1. System operation flow

In this section, we evaluate system performance in terms of energy consumption and packet loss rate at different duty cycles, and compare lifetime and data accuracy with different energy supply sources. In Figures 9 (a) and (b), we find that

there are long lifetime and low packet loss rate at duty cycle 5 and 10 minutes, respectively. Hence, duty cycle is set 10 minute within deployed sensors.

Figure 10 shows the lifetime comparisons with different energy supply sources such as Aklalin, NiMH, and solar panel. In this experiment, duty cycle is set zero. In other words, the tested sensor works all days. As the figure shows, Aklalin and NiMH

batteries are renewable energy. Lifetime of both batteries is 90 hours and 127 hours, respectively. In contrast, solar panel is not renewable energy, in which power is cut at three intervals that are 19-31 hours, 67-79 hours, and 97-102 hours. In these intervals, battery box of solar panel is broken. After fixing the box, power supply resumes work. As a result, solar panel can provide a continuous power supply solution for long-term observation, but the protection of the battery box is another critical issue. Observe from Figures 10 and 11, sensed data is anomaly using solar panel at time points 7, 13, 35, 63, 86, 91, and 104 hours. At these time points, the sensor voltages are 2.1 volts and 3.1 volts. Consequently, we set two filter thresholds based on voltage (V) are V<2.1 and V>3.1.
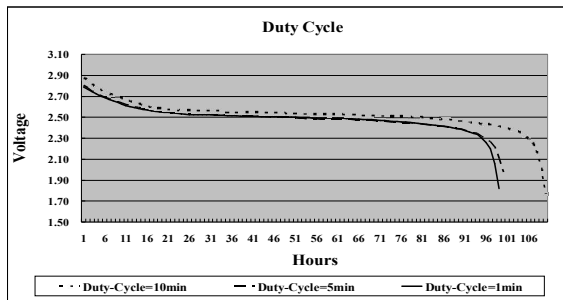
(a)
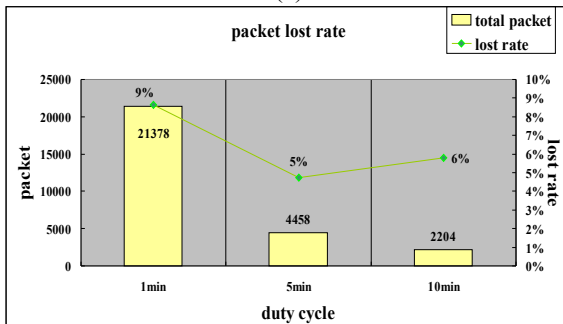
(b)

Figure 9. (a) duty cycle and (b) packet lost
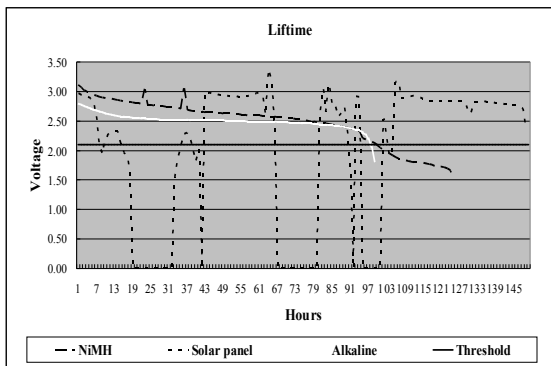
Figure 10. Lifetime
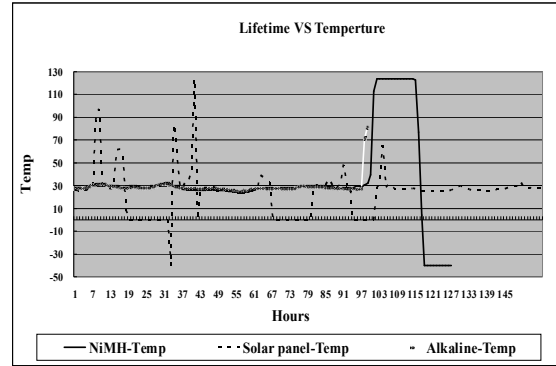
Figure 11. Voltage vs. temperature

4.2. System implementation

This section demonstrates NESS system implementation. Figure 12 depicts portal interfaces of PC and PDA version, respectively. Users in MU are able to access services in NESS via these interfaces. The system will automatically provision a suitable interface based on users' appliance, when users enter NESS system. In the interface, users can choose query parameters such as observation location, required micro-climate information, images, and presentation types. As Figure 13 shows, users can view acquired information with a table or line chart.

4.3. Summary and comparisons

In this section, we summarize and compare the existing systems and our proposed system (NESS) as shown in Table 2. Our proposed system, NESS, has higher deployment coverage with single-hop transmission, and longer monitoring period. In addition, NESS adopts two error detection methods that are fail-safe flexibility and device error detection, and equips with a variety of sensors such as temperature, humidity, light, voltage, image, PH value, Water temperature sensor. NESS also integrates both Tmote and MicaZ sensors.

5. Conclusions

This study designs and implements a four-tier system, NESS, which consists of EES, NRICS, MU, and EISS. NESS can solve drawbacks of using field survey for Mangroves protection and observation. With application of WSNs and the proposed system, users can efficiently perform data collection, real-time surveillance, and ecology and biology information acquirement. The main contributions of this study are as follows.

(1) This study is a first investigation with the application of WSNs and Internet to enhance Mangroves protection and observation of Taiwan, and provisions a portal website for real-time surveillance and information query.

(2) Practical sensor deployment issues in Mangroves are considered in this study, and two solutions to overcome these issues are proposed.

(3) We evaluate two system performance metrics in terms of energy consumption and packet loss rate and observe suitable setting parameters in the proposed system.

Figure 12. NESS portal websites for PC and PDA version

Figure 13. NESS portal websites for PC and PDA version

Table 2. Summary and comparisons of the existing systems and the new proposed system

Author / Item	Prabal et al. [6]	Murat et al. [14]	Rachel et al. [1]	Gilman et al. [20]	NESS
Deployment coverage	50,000 square meters	Indoor	30 meters	55 meters	1 km
Energy source	Solar	Battery	Battery	Battery	Solar
Monitoring Period	4 month	6 month	11 day	44 day	1year
Topology	Muilt-hop	Single-hop	Muilt-hop	Muilt-hop	Single-hop
Error detection	Fail-safe flexibility	Threshold	-	Backup in interrupt	Fail-safe flexibility, and data and device error detection
Sensor types	Voice, voltage, Magnetometer, infrared	Temperature, Humidity, Light, voltage	Soil moisture, rainfall	Temperature, Humidity, Light, voltage	Temperature, Humidity, Light, Voltage, Image, PH value, Water Temperature
Duty-cycle	20%-40%	20 times per 1 min	1% in the dry, 100% in the raining	1 time per 5 mins	1 time per 5 mins
Sensor hardware	Telos	Telos	Mica2	Mica2	Tmote and MicaZ
Delivery rate	-	-	63.8%	49%	57.2%

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Comparison of the effects of aquatic exercise in shallow and deep water on Postural Control in elderly women with chronic knee Osteoarthritis

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Abstract: Knee osteoarthritis (OA) is related to poor balance and impairment of the proprioceptive system. Exercise in the water recommended for the rehabilitation of these patients. However, the depth of water influences some physiological and biomechanical factors. Hence, the objective of present study was to Comparison the effects of aquatic exercise in shallow and deep water on Postural Control in elderly women with chronic knee OA. 43 elderly women over 60 years old with knee OA on preferred foot voluntarily participated in present study. The RPE scale was used to measure and control the exercise intensity after each exercise session. Using Romberg test, the postural control was evaluated. Data were analyzed using Two Factor Repeated Measure ANOVAs and follow up tests included LSD and paired samples t test at p<0.05 significance level. The results showed that postural control improved significantly in shallow water group more than deep water group (p=0.02). We conclude that water exercise, especially shallow water is recommended to rehabilitate the patients suffering from knee OA.
[Zamanian, F. Vesalinaseh, M. Nourollahnajafabadi, M. Asadysaravi, S. Haghighi, M. **Comparison of the effects of aquatic exercise in shallow and deep water on Postural Control in elderly women with chronic knee Osteoarthritis.** Life Sci J 2012;9(4):5768-5771] (ISSN:1097-8135). http://www.lifesciencesite.com. 858

Keywords: Postural control, shallow water, deep water, elderly patient, knee OA

1.Introduction

Osteoarthritis is a widespread disease among patients over 60 years (Davis, Ettinger, Neuhaus, & Mallon, 1991; Felson et al., 1987; Hochberg, 1991). Knee OA is also the most common form of arthritis in the old adults especially in women (Felson DT, Naimark A, Anderson J, Kszis L, Castelli W, Meenan EF., 1987; Chaiammuay P, Darmawan J, Muirden KD., 1998). Knee OA associated with pain and stiffness of the joint, disability (Bruce ML, Peck B., 2005; Brosseau L, Pelland L, Wells G, et al., 2004; Foley A, Halbert J, Hewitt T, Crotty M., 2003), impairment of the proprioceptive system (Garsden LR, Bullock-Saxton JE,1999; Sharma L, Pai YC, Holtkamp K, Rymer WZ,1997) difficulties of the knee motion and postural control and then decline ability to perform simple daily activities(Kaufman, Hughes, Morrey, Morrey, & An, 2001; Mangione, Axen, & Haas, 1996). It has been demonstrated that the most falls in elderly people with knee OA are related to poor balance (Sturnieks DL, Tiedemann A, Chapman K, Munro B, Murray SM, Lord SR., 2004; Jones G, Nguyen T, Sambrook PN, Lord SR, Kelly PJ, Eisman JA., 1995).

Furthermore, lack of proprioceptive feedback due to knee injuries could lead to improper and/or overload to joints. In addition, a capsule injury not only cause distortion in transmission of afferent signal from joints, but also more importantly could lead to change in coding of afferent nerve signal to central nervous system. (Prentice, 2001)

Studies showed that various kind of exercise are important for balance improvement in knee OA patients (Messier SP, Royer TD, Craven TE, O'Toole ML, Burns R, Ettinger WH Jr.,2000; Wang TJ, Belza B, Thompson FE, Whitney JD, Bennet K.,2007). However, because of advantages of water, exercise in the water recommended for the rehabilitation of these patients(Cochrane T, Davey RC, Matthes Edwards SM., 2005; Foley A, Halbert J, Hewitt T, Crotty M., 2003). Yet, the depth of water influence some factors such as gravity, hydrostatic pressure and touching the floor of the pool in which makes some different biomechanical and physiological conditions for exercises in the water. Therefore, exercise in two different depth of water may have different effects on patient with knee OA. However, no study was found to comparison the effects of exercise in shallow and deep water on patients with knee OA. In the present study we investigate Comparison of the effects of aquatic exercise in shallow and deep water on Postural Control in chronic Osteoarthritis female elderly.

2. Material and Methods

Originally, 43 women elderly patients who had knee OA on preferred foot participated in present study. They were selected from among elderly patient who had been diagnosed with chronic degenerative OA that suffered over 8 months. Based on the preliminary examination and pretest, the subjects were divided by random matching into 3 homogeneous groups: 1) shallow- water exercise program (n=14, age: 62.41 ± 5.16 year, height: 154.92 ± 4.63 Cm, weight: 59.84 ± 10.45 kg, Body Mass Index (BMI): 25.71 ± 3.96 kg/m2, Body Fat Percentage (BFP): 30.69 ± 7.24), 2) deep- water exercise program (n=14, age: 63.11 ± 5.37 year; height: 155.22 ± 4.03 cm. weight: 61.03 ± 11.20 kg; BMI: 26.11 ± 4.09 kg/m2, BFP: 31.83 ± 6.88), and 3) control group (n=15, age: 63.41 ± 5.16 year; height: 154.85 ± 3.99 cm; weight: 60.13 ± 10.86 kg; BMI: 25.83 ± 4.21 kg/m2, BFP: 31.17 ± 7.55) without intervention.

Multifactor ANOVAs showed that there was no statistical significant between groups on BMI and BFP (wilks's Lambda=0.897, F (4, 78)=1.08, p=0.428). It means three groups are homogenous. The RPE (Rating of the Perceived Exertion) scale was used to measure and control the exercise intensity (Borg G., 1970).

It was evaluated after each exercise session. The Rating of the Perceived Exertion (RPE) was in range of 4 to 6 (from mild to severe). Postural control was evaluated using a One-Leg Balance with open eyes test (Romberg test). The participant stands on the preferred foot with knee OA while resting the hands at waist level and then raises the other foot approximately 10 cm off the floor. Balance is scored by the number of seconds for which the foot is kept raised or until balance is lost. Timing is terminated with touch the floor by the free foot, take hands away from the hips, move the support foot from the initial place, and hook the free foot behind the supporting foot (Rogers, M. E., Rogers, N. L., Takeshima, N., & Isam, M. M., 2003).

Intervention

The water exercise program include one hour of exercises was performed three session per week on experimental groups (shallow water and deep water) underwent a 12 weeks period (figure 1).

Data Analysis

Data were analyzed using Two Factor Repeated Measure ANOVAs and follow up tests included LSD and paired samples t test at p<0.05 significance level.

3. Results

Table 1 shows the descriptive data of postural control in pretest and posttest of three groups.

Table1. The descriptive data of postural control (second) in pretest and posttest

Groups	Measurements		Percentage of changes
	Pretest	Posttest	
Shallow water	7.90± 6.29	9.83 ± 5.02	% 24.43↑*
Deep water	8.03± 6.54	9.36± 5.91	% 16.56↑*
Control	7.55 ± 8.11	7.38 ± 7.58	% 2.25↓

* Significant at p≤0.05

With regarding to the significant interaction between two independent variables, it used paired-sample T test for post hoc comparison. As there is a significant main effect of depth of water, LSD test was used for multiple comparisons. Results show in table 2 and 3 respectively.

Table2. The results of paired t test for within groups' comparison of postural control

Groups	Mean difference Pretest-Posttest	T	df	p
Shallow water	-1.93	-11.73	13	<0.001*
Deep water	-1.33	-8.96	13	<0.001*
Control	0.17	0.230	14	0.856

* Significance at p<0.001

Table3. The results of LSD test for between groups comparison of postural control

Groups		Mean difference	Standard error	p
Shallow water	Deep water	0.47	0.21	0.02*
	Control	2.45	0.63	<0.001**
Deep water	Control	1.98	0.66	<0.001**

*significance at p≤0.05, ** significance at p≤0.001

4. Discussions

This study showed that a program of aquatic exercise in both shallow and deep water over a period of 12 weeks was effective in improving the postural control (open eyes position) in the elderly women with knee OA. However, the improvement of static balance was significantly higher in shallow water exercise group compared with the deep water exercise group. Numerous studies revealed that

physical exercise in shallow water could lead to a improvement in static balance (e.g. Roth et al., 2006; Yennan et al., 2010; Geigle, Cheek, Gould, Hunt & Shafiq, 1997; Sadeghi & Alirezaei, 2007). However no significant study was found comparing different water depths. It seems that as liquid makes movements slow; the patient is given more time to control their moves. Therefore it is thought that the patient takes more advantage in water, especially patient with the lower body injuries, to regain balance and proprioception.

Extension in the reaction time (winter, 1996), as well as a safe environment in which there is little fear of falling down, enables the patient to regain their already lost proprioception. Also, it allows them (people unable to maintain balance) to get to understand their posture mistakes. (Simmons & Hansen, 1996).

Furthermore, touch stimulation, caused by turbulences of water while moving, as well as ongoing losing and regaining balance, provides a proper feedback which helps return the proprioception and balance (Sadeghi & Alirezaei, 2007).

On the other hand, the protective environment of water allows the elderly to independently maintain a straight posture (Era & Heikkinen, 1985; Geigle & et al., 1997). This may in turn lead in improvement of the elements which bring upon balance. In fact, sense of hydrostatic pressure of water all over the body creates a feeling of stability (Genuario & Vegaso, 1990; Thein & Brody, 1998) for the patient. Therefore, they keep doing exercises fearlessly in the water. Regarding the advantage of shallow to deep water on postural control, most probably due to patient's feet touching the floor and the close chain in shallow water, and consequent benefits for the patient to regain balance and somatosensory. Central nervous system intervention in keeping a proper posture is done in two domains. Firstly in sense organization (i.e. data collected from eyes, ears and the somatosensory system) and secondly in muscular coordination. For the first domain among adults a preferable source of data to control balance is the data coming from the somatosensory system (Understanding of movement caused by feet touching the floor).

Due to available support, all movements are made in a close chain in shallow water which leads to more stimulation in somatosensory information. Features of close chain include increase in joint pressure and joint coordination which in turn result in more stability, decrease acceleration, more resistance, proprioceptors stimulation and more dynamic stability all with weight tolerance. Therefore, it could be biomechanically suggested that exercises done in a close chain are more secure and can create forces and pressures which does less harm to tissues being recovered. Weight tolerance exercises increase joint pressure forces leading to more joint stability. It is also recommended that close chain exercises, specifically in lower body are more practical compared with open chain ones. That's because close chain exercises include weight bearing exercises which are similar to real life activities a person does on a regular basis. As for muscular coordination, movements are controlled by a central nervous system. The system coordinates the signals transmitted by mechanical receptors from the joint and muscles in motor chain. A well-balanced movement needs ongoing coordination of receptors, feedback and the data from the control center. Therefore a well-balanced weight bearing movement needs all joints to work in accordance with each other. Therefore, coordination exercises should be done in a close chain movement (shallow water with feet touching the floor). It seems that close chain exercises, using muscles in feet, ankle, knee and hip, create normal pressures and forces on joints which have more biomechanical advantages. Using multi-joint and multi-dimension movements, close chain exercises coordinate the proprioceptive feedback coming from pacinian corpuscle, ruffini end organ, golgi tendon apparatus (Prentice, 2001). Therefore, regarding the findings of the present study, water exercise, especially in shallow water is recommended to rehabilitate the already lost balance in patients suffering from knee OA.

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Correlation between the lipid and cytokine profiles in patients with coronary heart disease (CHD)
(Review article)

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Abstract: Many population-based studies consistently demonstrate some significant correlation between plasma levels of TG, LDL cholesterol, HDL cholesterol, different inflammatory cytokines and the prevalence of coronary heart disease (CHD). This article aimed to analyze the correlation between the occurrences of CHD with profile of lipid and cytokines.This is a meta-analysis study evaluating all pubmed, web of sciences, science direct, Scopus and Google scholar articles about the CHD and lipid and/or cytokine profiles from 2010 to 2012 using analytical statistical nalysis. Data were collected and the related information extracted and put in statistical package and analyzed.According to the analysis of many studies healthy individuals have higher levels of HDL lipoprotein than those with CHD so that An estimated of 1 mg/dl higher HDL-C is associated with a 2% lower risk of CHD for men and a 3% lower for the women. The plasma levels of Interleukin-6, CRP, TNFα, IL-18, IL-15, complement C3, colony stimulating factor (M-CSF), ICAM-1, CD54) on CD14+ CD16+ all are increased among CHD patients and therefore considered as risk markers of MI-coronary death. Profiles of lipid and cytokines are good predictors for CHD particularly if they to be evaluated and analyzed simultaneously and management of both groups can change the severity of the disease.

1. Introduction

Evidences now indicate that inflammation contributes considerably to the initiation and progression of atherosclerosis, (1, 2). and histopathological and immunochemical observations suggest that active inflammatory processes may trigger plaque rupture and enhance the risk of coronary thrombosis leading to a clinical ischemic event (3) . Inflammation is characterized by a local reaction that may be followed by the activation of an acute phase reaction (4). Some systemic inflammatory markers can indicate the severity of inflammation, and their levels have actually been associated with coronary disease for example the fibrinogen, which was previously recognized as an independent coronary heart disease (CHD) risk factor,(5-6) is now considered an inflammatory marker and not only a coagulation component (7). On the other hand, prospective epidemiological studies have shown a strong and consistent association between the clinical manifestations of atherothrombotic disease and systemic markers of inflammation, including white blood cell count (8) and various hemostatic proteins that are also acute phase reactants such as fibrinogen (9) plasminogen-activator inhibitor type-1, and von Willebrand factor (10-11). Evidences now indicate that inflammation contributes considerably to the initiation and

progression of atherosclerosisand histopathological and immunochemical observations suggest that active inflammatory processes may trigger plaque rupture and enhance the risk of coronary thrombosis leading to a clinical ischemic event (12,13). The measurement of acute phase proteins in blood provides a measure of inflammation activity (14). Numerous plasma proteins have an acute phase response including proteins involved in coagulation(e.g., fibrinogen and factor VIII) and plasminogen, complement proteins and transport proteins (e.g., ceruloplasmin, ferritin), and other proteins such as C-reactive protein, serum amyloid A protein, A Acid Glycoprotein (AAG), and albumin (15). Some changes are positive (e.g., fibrinogen) and some are negative (e.g., albumin). The changes in acute phase proteins are related to their hepatic production in response to inflammatory mediators such as cytokines. The changes in the levels of acute phase proteins may provide an indirect measure of inflammation or injury to the arterial wall and the associated increases in cytokine production, especially by the macrophages (1).

It has been proposed that inflammation of arteries results in an increased production of cytokines, especially IL-6, and activation of clotting factors, increased platelet aggregation, and smooth muscle cell proliferation. Interleukin 6 (IL-6) is the

major initiator of acute phase response by hepatocytes and a primary determinant of hepatic CRP production as suggested by IL-6–deficient animals showing impaired acute phase reaction (16,17, 18). Experimental studies indicate that vascular endothelial and smooth muscle cells produce IL-6 and that IL-6 gene transcripts are expressed in human atherosclerotic lesions (19, 20, and 21). Given the role of IL-6 in CRP regulation and the hypothesis that atherosclerosis fundamentally represents a chronic inflammatory disorder (22, 23), the predictive value of IL-6 for cardiovascular ischemic events can has been evaluated showing an association of it with increased risk of future myocardial infarction (MI) in healthy middle-aged men (24) . Also it is to say that IL-6 and TNF-α as the inflammatory cytokines are the main inducers of the secretion of C-reactive protein in the liver (25). C-reactive protein is a marker of low grade inflammation, and recent studies suggest that this protein has a role in the pathogenesis of atherosclerotic lesions in humans (26, 27).

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an emerging biomarker of CV risk that is pharmacologically modifiable and therefore well positioned to address novel mechanisms of atherosclerotic vascular disease (32). Although this enzyme has been referred to as platelet activating factor-acetyl hydrolase (PAF-AH), Lp-PLA2 exhibits much broader substrate specificity (33-34). In particular, Lp-PLA2 rapidly degrades polar phospholipids present in oxidized LDL-C, releasing downstream products such as lysophosphatidylcholine species and oxidized nonesterified fatty acids (35). These products of the Lp-PLA2 reaction exhibit a wide range of pro-inflammatory and pro-apoptotic effects in experimental settings (32). In this context, Lp-PLA2 could be proposed as the enzyme that links oxidized LDL-C with atherosclerosis progression and plaque vulnerability. Lowering the low density lipoprotein cholesterol (LDL-C) therapy for lipid modification in atherosclerosis treatment and prevention has increasingly been at the focus of many studies. Lipid-lowering treatment directed at LDL-C with standard doses of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ("statins") has resulted in a relative risk reduction of one-third in major vascular events as compared with placebo (36). In patients at very high risk for vascular events, intensive lipid-lowering has been shown to be beneficial compared with standard therapy. The Framingham Heart Study in the 1980s demonstrated that the risk of coronary heart disease (CHD) was significantly lower among persons with higher levels of high-density lipoprotein cholesterol (HDL-C) (normal range 40 to 60 mg/dl).

A number of studies have supported this inverse correlation between HDL-C and CHD (37, 38); hence, HDL-C has quickly evolved as one of the "traditional" risk factors used by clinicians to predict risk of incident CHD (39). As there some strong evidence about the relationship of cytokines and the biochemical risk factors among the patients suffering from heart disease the current review aimed to perform a systematic review to elucidate further aspects of such relationship.

2. Review process

A systematic review on relevant studies published in Web of Science, PubMed and Google scholar between 1976 and March 2012 reporting the associations between CHD risk and cytokine/biochemical risk factors was performed. The review was restricted to studies carried out on human subjects and written in the English language. Results were evaluated according to the date and subject and using the matrix model in which the analyze was based on both the chronologic theme and the subject and the report was written accordingly.

3. Body text
3.1. Cytokines and the CHD risk

Many immunological risk factors such as C-reactive protein, interleukin-6, fibrinogen, interleukin-8, interleukin-15, interleukin-18, plasma C3, FcγRIIIA, Lp-PLA2, TNF-α, NFKB1 and… for the prediction of coronary heart disease were evaluated .C-reactive protein is one of the most important immunological risk factors for the CHD prediction and majority of researchers believed that there was positive and direct relation between CRP and CHD. Some studies show that there was some associations between the CRP, interleukin-6 and fibrinogen some others have measured the hazard rate of CHD by the CRP that was 1.67. Evaluating the association between the CRP, IL6 and fibrinogen only interleukin-6 remained significantly associated with MI-coronary death when the 3 inflammatory markers were included in the model. Interleukin-6 appeared as a risk marker of MI and coronary death, and it improved the definition of CHD risk beyond the LDL cholesterol (41). In another Coronary Prevention Study 6447 men were evaluated to predict the CHD risk and also 5974 men to predict the incidence of the diabetes over the 4.9 years of follow-up. The mean LDL cholesterol was similar but the C-reactive protein was higher (P<0.0001) in the 26% of men with the syndrome compared with those without. Metabolic syndrome increased the risk for a CHD event {(HR) 1.76}). C-reactive protein enhanced prognostic information for both outcomes. Men with the syndrome had similar risk reduction for CHD as

compared with those without (43). The plasma levels of C-reactive protein and serum amyloid A protein have showed a strong association (P<0.00001) while they were inversely related to the levels of serum albumin (P<0.00001). Also a significant associations of plasma CRP concentrations with cigarette smoking and obesity is reported (P<0.00001). Serum albumin levels raised a strong association with blood pressure (P<0.0001) and plasma lipids (P<0.001), while the serum amyloid A protein showed a strong correlation with obesity (P<0.0001). The strong associations of plasma levels of CRP with cigarette smoking and obesity indicate that this particular protein can mediate some of the effects of risk factors of coronary heart disease. CRP has been proposed as an independent risk factor for CHD (44). The significantly higher level of interleukin-8 in unstable coronary heart disease patients in comparison to the stable coronary heart disease patients ($P \leq 0.01$) and the control group ($P \leq 0.02$) indicated the effective role of this cytokine on CHD. These findings suggest that the soluble form of P-Selectin and interleukin-8 may be considered as some useful clinical predictors of unstable coronary heart disease (46). Cahide Gokkusua who is the first person that showed the relation between the IL-15 and the CHD, reported the influences of IL-15 gene variants and IL-15 levels on CHD. The results of this study showed that the serum levels of IL-15 were significantly higher in both acute and chronic patients than in controls. Also the genetic variants of IL-15 gene and IL-15 levels were associated with CHD. This study supports the hypothesis that genetic variation in IL-15 gene and IL-15 levels influence the risk of CHD (table-1) (47). IL-18 and TNFα were shown as the risk factors of CHD, as was reported that the baseline plasma levels of IL-18 and TNFα were significantly elevated in CHD cases versus the controls. Using univariable models IL-18 was associated with CHD risk (odds ratio [OR] upper third to lower third, 1.63; 95% CI 1.08, 2.46), but TNFα was not (OR 1.33; 95% CI 0.87, 2.02). After adjusting for major CHD risk factors and CRP, the association of IL-18 with CHD risk was attenuated (OR 1.69; 95% CI 0.94, 3.03). IL-18, but not TNFα, had a non-negligible association with CHD risk, although the association of IL-18 with risk was weak after full adjustment (48).

3.2. Complement components and CHD

The higher plasma levels of C3 were associated with a higher CHD prevalence, and there was a significant interaction with heavy smoking (p<0.01). In never & light smokers, the univariate OR for CHD per 1 smoking daily (0.33 g/L) increase in C3 was 1.09 [95% confidence interval (CI) 0.85–

1.41] (p<0.505) whereas in heavy smokers it was 2.05 [1.43–2.93] (p< 0.001). Human plasma complement C3 was reported to be associated with prevalent CHD, but only in heavy smokers, and this association was independent of important metabolic cardiovascular risk factors (49).

3.3. Receptors involved in immunity to CHD

Ye Huang et al demonstrated a significant increase of FcγRIIIA at the mRNA level in leukocytes, and at the protein level for both soluble CD16 in sera and membrane CD16 on monocytes of CHD patients compared to the healthy control. Similar to the soluble CD14 (sCD14), the sera level of macrophage colony stimulating factor (M-CSF) was elevated in CHD patients than compared to the controls. Furthermore, the levels of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), in sera and the mean fluorescent intensity of intercellular adhesion molecule 1 (ICAM-1, CD54) on CD14+ CD16+ monocytes were increased in CHD patients. The significant increase of CD14+ CD16+ monocytes in CHD patients therefore suggested that the increase of the FcγRIIIA level might be a sensitive marker for the CHD diagnosis (50). The NFKB1 promoter variant, previously shown to cause partial depletion of NF-κB p50, was associated with a higher risk of CHD in three independent prospective studies of generally healthy Caucasians (55). For every inherited copy of 358Ala the mean concentration of IL-6R was increased by 34.3% (95% CI 30.4–38.2) and of interleukin 6 by 14.6% (10.7–18.4), and mean concentration of C-reactive protein was reduced by 7.5% (5.9–9.1) and of fibrinogen by 1.0% (0.7–1.3). For every copy of 358Ala inherited, risk of coronary heart disease was reduced by 3.4% (1.8–5.0). Asp358Ala was not related to IL-6R mRNA levels or interleukin-6 production in monocytes. Large-scale human genetic and biomarker data are consistent with a causal association between IL-6R-related pathways and coronary heart disease (41).

3.4. Lipid Profile and CHD

The Lp-PLA2 activity has been shown to be significantly associated with myocardial infarction. Levels of Lp-PLA2 activity were shown a significant association with incident of CHD among women. The Lp-PLA2 levels was positively correlated with age, body mass index, low-density lipoprotein, triglycerides, and C-reactive protein, and negatively correlated with high-density lipoprotein (52).
Among the biochemical risk factors for CHD, the LDL and HDL are important than the other biochemical risk factor. Jonathan C examined the effect of DNA-sequence variations that reduced the

plasma levels of LDL cholesterol on the incidence of coronary events in a large population and compared the incidence of CHD (myocardial infarction, fatal CHD, or coronary revascularization) over a 15-year interval in the Atherosclerosis Risk in Communities study according to the presence or absence of sequence variants in the proprotein convertase subtilisin/kexin type 9 serine protease gene (PCSK9) that were associated with the reduced plasma levels of LDL cholesterol. Of the 3363 black subjects examined, 2.6 percent had nonsense mutations in PCSK9;these mutations were associated with a 28 percent reduction in mean LDL cholesterol and an 88 percent reduction in the risk of CHD (P <0.008). Of the 9524white subjects examined, 3.2 percent had a sequence variation in PCSK9 that was associated with a 15 percent reduction in LDL cholesterol and a 47 percent reduction in the risk of CHD. These data indicate that moderate lifelong reduction in the plasma level of LDL\ cholesterol is associated with a substantial reduction in the incidence of coronary events, even in populations with a high prevalence of non–lipid-related cardiovascular risk factors (58).

4.Conclusion-

The association of different Immunological and biochemical risk factors for the CHD disease has been the focus of many studies but such analysis of these risk factors in a systemic review is a matter of necessity. Some findings have showed the association of the soluble form of P-Selectin and interleukin-8 with unstable coronary heart diseases and some other hypothesized that genetic variation in IL-15 gene and IL-15 levels can influence the risk of CHD. Still another cytokines levels such as IL-18 and TNFα were stablished as predictors of CHD. Human plasma complement C3 and prevalence of CHD is been reported too. There is also strong association reported for the increasing level of FcγRIIIA at the mRNA and at the protein level for both soluble CD16 in sera and membrane CD16 on monocytes of CHD patients. Sera level of CD14 and macrophage colony stimulating factor (M-CSF) was elevated in CHD patients as was seen for the inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) , interleukin-1 (IL-1) and intercellular adhesion molecule 1 (ICAM-1, CD54). Also the increase of the FcγRIIIA level has been considered as a sensitive marker for the CHD diagnosis. There has also been proven that the partial depletion of NF-κB p50, is associated with a higher risk of CHDis some population like the Caucasians. The relationship of lipids such as Lp-PLA2 levels was also been proved to be positively correlated with age, body mass index, low-density lipoprotein, triglycerides, and C-reactive protein, and negatively with high-density lipoprotein

while moderate lifelong reduction in the plasma level of LDL\ cholesterol is associated with a substantial reduction in the incidence of coronary events. These data all together indicated that a rise in Immunological and lipid risk factors is proportional to high prevalence of cardiovascular diseases.

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Evaluation of Medical Ethics among patients with Nose and Abdominal Surgery

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Abstract: Background-Medical ethics in surgeries is counted as great importance according to the surgical results that might have irreversible side effects or cause patient's death, or bring the patient back to life and health. Especially in recent years, in addition to indispensable surgeries, surgeries with cosmetic purposes have increased and of course many reasons are claimed for these without indication for surgeries, including economic factors and educational purposes. **Materials and Methods**: This study intended to investigate the role of medical ethics in nose and different abdominal surgeries among 100 patients and 70 hospital personnel in different parts of Ilam City in 1386. **Results and Conclusions**: The results state a lack of patient's awareness of medical ethics committee, lack of awareness of their rights and lack of medical staff's awareness of the way of dealing with medical ethics issues while facing such cases during treatment of patients and during working in therapeutic environment. On the other hand, patients' awareness of their rights help them endure health problems and may lead to their satisfaction from treatment team.

1. Introduction

The word akhlagh (ethics) is the plural form kholgh (ethic) which means morals. Ethics is a branch of philosophy and it is also known as moral philosophy. Ethics is a science dealing with the way of humans thinking and acting and behaving and it is not about the ethics humans already have, but it is about the ethics humans should have and is proper for humanity. In fact, ethics is the science of determining principles for human beings life and that's why it is also known as science of duties or good deeds. Medical ethics is a branch of ethics and medical science which deals with moral philosophy in the field of medicine, daily practice medicine and the rules, and medical researches. In other words medical ethics is a set of moral principles and good manners that doctors must follow them or the improper behaviors they must avoid. Ethics are more personal and cannot be delegated to someone else. The rules clarify the minimum or necessary standards for us. Although obeying the labor law is reasonable, but it is not enough. Morality asks us to do more than the minimum standards and in fact to do the best. The ethics attends specially to beneficence (goodness), justice (treating equally to every one), and informed consent (the right that the patient become aware of the consequence of his decision before obtaining consent). Scientific advances are useful and effective for mankind when they are appropriately and morally supported. Medical movements have been along ethical axis mainly in the field of medicine and medical efforts for saving human lives and bringing them back to life and health and treating diseases and relieving pain are enormous efforts for human survival and enriching generational environment of moral values. Thus the nature of medicine is piety and medical ethics is in fact a kind of commanding what is good and forbidding what is bad in medical science. Like commanding to dignity, not ravening, having pure visions, chastity, compassion, kindness, friendship, Patience, sympathy with people and patients, the confidentiality, the integrity, command to study and update one's knowledge and they are forbidden from non-responsibility due to patients and society, from wrong treatment and wrong decisions, forbidden to tell patient's secrets, forbidden from unnecessary violence and humiliation the doctors and colleagues, forbidden to see a patient as a client, Forbidden from illegal certificate issuance , from sending patients to unprofessional colleagues, from receiving high and disproportionate salary etc.

If the result of medical operators' effort and diligence is to provide valuable services based on medical ethics and proportional to the science progress then medical services are the upmost emerged human feelings and the beautiful combination of commitment, knowledge and specialty with the approach of sacrifice. The influence of this ethical integrity in medical world is shown in the sensitive positions, doctor and patient. The more

human, ethical, sincere, and honorable this behavior is, the result is nothing but mental and physical secure and satisfaction for the patient and the people around. One of the reasons of bolding the discussion of medical ethics is the increasing demands of society and the right of choosing by the patient. Certainly the community attitudes are changing towards doctors, and the best for the patient is not only determined by the doctors. So the doctor should inform the patient in decision making and any action must be held with the patient's satisfaction. Medical ethics in surgeries is counted as a great matter according to the surgical results that might bring the patient back to life and health, or may cause irreversible side effects or patients' death. Especially in recent years that in addition to indispensable surgeries, surgeries with cosmetic purposes have been increased and of course many reasons are claimed for these without specific indication surgeries, including economic factors and educational purposes. According to what is being said, research on the relationship between doctors and patients, the patients' awareness of all the side effects of the surgery, being aware of different ways of dealing with illness and awareness of the aims of the surgery, determining the compliance of the patients' rights to make decisions. The doctors' reliability of treatment and surgery, existence or absence of alternative methods are the cases which considered in connection with nose and abdominal surgeries. Accordingly with the aim of investigating the role of medical ethics in nose and various abdominal surgeries and to determine the patient's awareness of surgical procedures and treatments or alternative methods, and to determine the patient's information provided by the doctor, and to determine the level of patient satisfaction after surgery, to determine the level of doctor's reliability from the result of surgery based on patient's information (patient's questionnaire), to determine the level of compliance of medical ethics in relation with the doctor and the patient based on the observance of patient rights by doctor and to determine the awareness level of medical staff, the present study was designed and performed.

2. Materials and Methods

The type of study is cross-sectional and descriptive. 100 patients admitted to Ilam hospitals were selected randomly according to statistical rules in 1386. 100 questionnaires were returned from 250 questionnaires distributed among the patients and in the second part of the study 70 questionnaires were distributed among the male and female surgery department personnel, operating room personnel, specialists and doctors with the aim of awareness survey and finally 50 questionnaires were returned. The obtained data were analyzed via software SPSS.

3. Results

The obtained results from this study show that most of the people (about 64 percent) followed their doctor's advice and did the surgery, in other words the patients did not play a special role in making decision and more than half of them had the doctor's decision as their treatment basis. The reason that doing the surgery has been chosen as the optimal decision by the doctor advice, needs more investigation and it is not clear if it had been the best choice. As **Porthovoe** et al found out in their survey conducted in 2000, 61 percent of people in the study did not participate in making decision and their doctors took the main decision and also the results of a survey conducted by **Entwist** in 2001were similar to this study (tables 1-4). The results show that only 13 percent of patients are aware of the consequences of their surgeries, in a more simple way more than 87 percent of patients were not aware of what would happen to them finally and it means weather the patients were not aware of their rights and did not know it was a natural right to be aware of the consequences of the treatment method or the surgery, or the doctor or the medical team were not aware of the fact that their patients must be aware of their right of knowing possible side effects during or after surgery or even they knew but did not tell the patient. People who had a higher education than a bachelor's degree believed that the presented information by doctor and their medical staff helped them in taking the surgery; As if this group of patients took these information better or the doctor and medical staff could have justified them better, or these patients could make the medical staff give them the necessary information better because they were aware of the surrounding issues or they had done the necessary researches before the surgery or even decision making and after that they proceed to have surgery (tables 4, 5). Those who had a diploma or illiterate people have benefited somewhat less than this and about 20 percent of them believed that the taken information from the medical team was useful enough to sustain their operation. One of the most interesting findings of this study is to answer this question that if you had the current information, would you do the surgery or not? People, to whom the obtained information from medical staff was useful to sustain the surgery, believed that they would still sustain the surgery with the current information, in a more simple way they are people who have been really justified (tables 3, 4). The information obtained from the medical staff also helped them in sustaining the operation and also they were satisfied with the surgery result and the treatment process was as their expectations.

Table 1. Frequency of reasons mentioned by the admitted patients for their referring to hospital

Reasons	NO	Percent	Total
Sickness	78	78	78
Beauty	22	22	22
Total	100	100	100

Table 2. Frequency distribution of the responses to the question" What will you do if you have a medical problem?"

Responses	Frequencies	Percent	Total
Consult with physician	73	73	73
Consult with nurse	6	6	6
Consult with family	10	10	10
Consult with social nurse	11	11	11
Total	**100**	**908**	**90**

Table 3. The relationship between educational level and the patients' ideas about whether they do the surgery by having the current ethics knowledge?

tendency for surgery/ Education level	Yes, not sure(N/P)	Yes certainly	No	Total
ILITERATED	13(56.5)	8(34.8)	2(8.7)	23(100)
HIGH SCHOOL	17(56.7)	7(33.3)	6(20)	30(100)
BSc level	17(5.8)	10(32.3)	4(12.9)	31(100)
MSc and higher	13(81.2)	3(18.8)	00(0)	16(100)
Total	**60(60)**	**28(28)**	**12(12)**	**100(100)**

Table 4. The relationship between satisfaction with the surgery outcomes and undergoing the surgery if they have the present ethics knowledge.

Satisfaction of surgery/ tendency for surgery	Yes (N/P)	No(N/P)	Total (N/P)
Doing the surgery	41(100)	00(0.0)	41(100)
Not doing the surgery	6(50)	6(50)	12(100)
Don't know	15(100)	00(0.0)	15(100)
No any other options	32(100)	00(0.0	33(100)
Total	**94(94)**	**6(6)**	**100(100)**

Table 5. Frequency of medical staff based on their specialties

Medical staff	Frequency(percent)	Total (N/P)
Nurse	7	7
Student	1	1
Anesthesia technician	2	2
ENT specialist	3	3
Administrative	2	2
Assistant nurse	6	6
Anesthesia specialist	2	2
Surgeon	4	4
GP	13	13
Obstetrics and Gynecologist	4	4
Operation room technician	3	3
Urologist	2	2
Orthopedist	1	1
Total	**50(100)**	**100(100)**

44 percent of studied medical staff believed that the behavior and the moods of the patients are effective in their treatment and if these people are decision makers for patient's treatment and their care, it cannot be expected that the patient's rights must be fully respected and if the patient has a violent temper, he/she should forget some part of his/her rights and this means that the matter of medical ethics is ignored. 54 percent of people have claimed that they have never dealt with the problem of medical ethics so far and the analysis of this response is a complex. If we assume that they were aware of medical rights and they have followed them with their patients we went wrong because they have confessed in their questionnaires that they did not know the medical ethics issues as well and if we accept that the problems of medical ethics have not existed then we went wrong again because such issues exist in our communities, on the other hand the response to different questions prove the fact. The last point worth mentioning is that the medical staff itself did not recognize that such issues as medical ethics exist or not and they have neglected them. It is not in consistent with the researches conducted by **Harihavan** et al in 2006 which suggests that more than 90 percent of people have dealt with medical ethic issues while a smaller percentage have not dealt. Patient participation in decision-making is based on medical ethics and since the patient and the doctor are participated, decision making is done in a more optimal way. One of the investigating ways of this issue is to ask medical staff: how important is the patient's desire in decision making? More than half of people have considered it somewhat important while we know how essential it is. And the decisions must be based on the patient's desire. These results were much similar to the results of the researches conducted by **Entwistle** et al in 2001. It is an important matter the way you consult when there are medical ethics problems and in this regard it seems that some centers to obtain medical ethics consultations can be very helpful. Over 75 percent of people responded that they took advice from their colleagues and this fact is a lot consistent with the research done by **Mosely** et al in 2006 (Table 2). And in a survey conducted by **Harihavan** in 2006, doctors and nurses preferred to consult with their colleagues. Although the colleagues and the people we ask are in similar situations. Table (1) Frequency distribution of reasons to refer hospitals in admitted patients.

Conclusions

The discussion of medical ethics is of current human societies needs and considering this role and also medical staff's awareness of medical ethics can result in a better interaction with the patients and help the patients undergo the surgery better; on the another hand, it is more important to know what patient's rights are and in what cases their rights must be considered and what mechanisms needed to secure these rights. There is not enough awareness about medical ethics among medical staff and also there are not provided with the necessary resources. On the other hand, the patient's rights cannot be secured totally due to the lack of awareness and knowledge of patients and medical staff. Primarily those who have enough knowledge about their rights and their treatment and are active in decision making would have more successful surgeries. Both groups emphasize the need to learn about medical ethics and be active in this field.

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Effects of Temporal and Spatial Evolutions on Religious Punishments (Sharia)

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Abstract: Nowadays, punishment has become the most challenging subject in Iranian Law as one of the most important discussions in jurisprudence. Following the Islamic jurisprudence, Iranian Law has recognized a wide range of physical punishments in its legal system which the possibility or impossibility to review its principles is propounded according to increasing influence of human rights discussions in legal and intellectual societies. Apart from the issue of possibility or impossibility, the necessity to review these rules is essential regarding some special conditions of these days, but important arguments may be raised about possibility or impossibility too. This study considers legal evidences of these potential changes.

1. Introduction

The problem of compatibility and coordination between legal ideas and foundations with social developments and changes is the most important and fundamental issue in all legal systems. Criminal jurisprudence of Islam has the characteristics of a legal system, so it confronts with such a problem, even harder and more sophisticated than other criminal systems because it claims to be universal, immortal, stable and an equivalent for natural rules, but other criminal systems have emerged as a social phenomenon and follow social developments. Yet, the process of encounter between legal theories and social developments in criminal system of Islam is obviously considerable.

After Islamic Revolution and following legal system of Islam, Forth Principe of Constitutional Law was approved in order to adaptation of all approvals with Islamic Rules and not inconsistence of them with religious principles in legal system of Iran. Law-maker have tried to correct and improve these rules (especially criminal rules) since 1982 and has considered religious criteria to reapprove them to have no inconsistence with legal rules and jurisprudence as much as possible, such as approval of Islamic Punishment Law, penance, blood money and retaliation (1982) and approval of suspended sentences (1983) by Commission of Legal and Judicial Affairs of Parliament of Islamic Republic of Iran. Revitalization of some Islamic punishments such as stoning, retaliation, amputation of hand, rigid punishment, etc and also principles of swearing, the right to select criminal for blood money, lowering the age of criminal, etc are the most important changes to provide above-mentioned goal.Whatever is important

to restore these punishments is the compliance or noncompliance with legal viewpoints of global society. According to increasing influence of human rights discussions in legal and intellectual societies and regarding protection of world powers and western countries of development of criminal rules according to philosophy of human rights and also development of activities of western organizations, there is a great pressure on Iran about execution of these principles, so that Iranian judicial authorities are forced to repair these principles unofficially and it is possible only by elimination of these punishments. For example, Head of Judiciary Power prohibits performance of some punishments such as rigid punishment and limits the right to select criminals from six to three people according to article 297, and or members of parliament try to eliminate stoning punishment. Such these efforts, cross these questions in the mind that this paper is trying to one of the most important problems, namely the ability or lack of ability to adapt Islamic rules about punishments with temporal and spatial circumstances. Iranian law-maker will pass these problems powerfully according to requirements of the Forth Principle of Constitutional Law and based on requirements of temporal and spatial changes. Historical Origin of Temporal and Spatial Problem: One of the methods to recognize any problem accurately is to consider its historical background. Researching evolution of an opinion along the history provides many hidden angles to try to complete and probably correct it. It may be claimed that while the earth and its inhabitants were deprived of inspiration and prophecy was finished, the problem of temporal and spatial evolutions appeared.

In fact, confronting of newly established Islamic society with legal problems was the ground of jurisprudence and deliberation in many Islamic texts, and the principles derived from Islamic texts turned to various philosophies and faiths gradually and along the history, namely jurisprudence and its principles were appeared in different tendencies.

The approach of Sunnites toward syllogism, charity, sent interests and reunion as resources of religious jurisprudence is the result of confrontation with these legal problems and issues, some difficulties such as the problem of … about new subjects and events, appearance of the hidden Imam, executive obstacles against anticipated principles and rules in Sharia. Historically, Sunnites faced legal and religious problems sooner than Shiites because first of all, religious texts did not include all issues and affairs, and second of all, jurisprudence of Sunnites was involved with governing the society and exercising political sovereignty continually [1]. Apart from this historical antecedence, it may be said that both Shiism and Sunnah found the reply of modern legal problems in "religious jurisprudence and duties of jurisprudent". According to both philosophies, the jurisprudent should remove the conformity problem of jurisprudence with temporal and spatial circumstances with his jurisprudence. The differences are about the method of jurisprudence and its resources referred by any jurisprudent and also the validity of these methods and resources, not about necessity of jurisprudence.

2.Description of Religious Rules

It is said to describe a religious rule: Invention of lawgiver for a specific subject including human, items and contexts [2]. It is also said [3] namely, it is religious obligations with verification and negation (necessity and reverence) which usually comes with "must" and "must not" or other words. Anyway, religious rule defines viewpoint and special position of religion about a special subject. These viewpoints are mentioned as "lawful", "unlawful", "obligatory", "recommended" and "disapproved".

The meaning of "rule" differs from the meaning of "law" in expression of jurisprudents, and the meaning of "law" for jurisprudents differs from the meaning of law in Law Books. The purpose of

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[1] Vaezi, Ahmad; stable and unstable bases in Islamic jurisprudence; series of papers in eighth international conference of Islamic Unity, 1995, p. 3

[2] Gorji, Abolghasem; Papers of congress to consider judicial bases of Imam Khomeini, volume 14; p. 277

[3] Al-Asefi, Mohammad-Mehdi; Opinion of Imam Khomeini about Spatial and Temporal Effects on Ijtihad

jurisprudents from "Law" is permanent and stable general rules which are enacted by God based on real interests and mischiefs, and are according to numerous evidences, but "rule" includes real general rules of Sharia and discoveries of jurisprudents which may be general or partial. According to lawyers, law means necessary principles to regulate social relationship and may exist for a long time but it is not permanent and stable.

3. Division of Rules:

Jurisprudents divide religious rules according to various expressions. They categorize religious rules in three groups according to their constancy and changeability:

A) Real divine rules as the texts of Holy Koran and traditions (of course jurisprudence tradition) are not changeable in the manner of nullification. These rules are called explicit text rules.

B) Apparent religious rules or in other words, statements of jurisprudents which can be interpreted according to explicit texts and are changeable.

C) Government Rules are enacted by Vilayet (Supreme Religious Authority) as expedient rule which are changeable by him or by the authorities who are appointed by Vilayet.

Some other jurisprudents divide religious rules in primary and secondary. Secondary rules are those which jurisprudents may change or invent them according to some exceptional conditions. Secondary rules have causal relationship with personal and social circumstances of people and their conditions, so they are a way for temporal and spatial effect on Islamic rules through secondary pretexts.

3. Applicability of secondary rules

1- The most important issues: As Islamic rules follow the real interests and mischiefs and these interests and mischiefs may lead to practical conflict while performance, Islam has authorized religious experts to relinquish the least important issues due to save the most important ones. Holy Prophet of Islam says: "When two affairs are intercourse, both are worthy of respect, the lesser should be relinquished due to the greater"[4].

2- Reluctant and distress issues: Duties are the rules which are propounded for those who are empowered and authorized and are not distressed. In this way, when somebody is distressed or has to do something reluctantly, its performance is authorized[5].

3- Governing and adjusting rules: Among Islamic rules, there is a series of rules which are adjusting

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[4] Mottahari, ؛اذا اجتمعت حرمتان طرحت الصغرى للكبرى Morteza; Conclusion of Prophecy

[5] Surah Baghara; verse 173

and govern other rules. Namely, when performance of a religious rule harms specific people or puts Muslims or Islamic society in a critical situation of difficulty and fault, the rule will be set aside until such a condition exists, and a secondary rule will substitute with it, because the subject is related to fault and difficulty not the essence of subject.

Sometimes the punishment of a crime will turn to other kind of punishment due to temporal and spatial Evolutions. A brief review of criminal rules, approved by Parliament and Expedience Council of Iran, shows that for some reasons, law-makers have lessened punishment of whipping and have tried more to exercise financial punishments or imprisonment or other complementary and preventing punishments. This effort is a kind of punishment changing. Substitution of camel with money by Imam Ali (PBUH) to pay blood money confirms this principle [6], so in our country the payment of blood money includes the currency of the country instead of six various materials, mentioned previously in Islam. By the way, changing punishments is sometimes to harden them and sometimes to lessen them, and may be for internal and external situations of country, or it may be to perform new criminal policies which are more effective that punishments in texts. In all instances, the punishments may be changed and altered such as changing of whipping for fraudulence, theft, treachery in trusts and so on. Sometimes, penances may be changed, for example, suppose the performance of stoning is not in a good interest in present times or amputation of the hand may weaken Islam and Muslims around the world, so the Islamic Ruler changes them to other punishments. Criteria to avoid changing penances during a war an along the land of enemies include all these punishments too.

Of course, those who believe that penances are not required to be performed during the Age of Absent Imam surely do not mean to leave the criminals free and not be punished anyway. They believe in substitution of other punishments with penances and to change penances to lawful punishments. Problems Encountering Criminal System of Islam about Changes of Rules Today, the speed of changes and social developments is so high that sometimes legal symbols and concepts are being changed subsequently, so that, it sometimes seems that the only way to exist a legal system is to set aside some of the old concepts and structures. The process to encounter of legal opinions and social developments as one of the most fundamental issues in philosophy of law is considerable in the realm of

Islamic criminal system. It should be mentioned that the essence of existence of such a problem is not deficiency or imperfection, but its existence is unacceptable. Confronting a problem is the base to offer a "theory" and interaction and investigation over a theory is the base to form and complete a scientific theory. These theories change to a mental and practical "system" in their evolutional process. This is a rule for all humanities. Among Islamic sciences, the history of jurisprudence is a clear and rational instance of this rule. The texts of Holy Koran and traditions caused the creation of jurisprudence due to their variation, and the science of essentials was born of jurisprudence.

According to the history of jurisprudence and Ijtihad, Islamic jurisprudence has faced a great development such as other legal systems. One of the main reasons of these developments is to expand the relations between humans and various changes in human life. Of course these changes have made a deep influence on logic of jurisprudence, namely science of essentials. Basically, the creation of science of essentials has been due to these problems and issues; just as, the systematic development, deepening and completion of this science has been the same [7]. Sensitiveness of jurisprudents to employ the element of time has regarded the intellect as the fourth resource of Shiite jurisprudence in the width, not in the length, of other resources (Holy Koran, traditions, and unanimity). Islam is a natural religion, and one of the natural and instinctive goals and ideals of human is to achieve interests and advantages, either personal and private interests or public and general ones. Islam, due to its nature, pays a complete attention to personal interests and advantages of humans and enacted the rules to obtain these goals and high interests. The attention of the Great Lord (the great law-maker) to these interests and advantages is obvious through traditions about Vilayet, government and business such as contracts, cadences and all rights including what is due to God and what is due to men[8].

Following issues justify the attention to social developments to enact any rule and religious statement:

A. Changing the Subject

Rule is the cause, and subject is the effect. So while the subject is not practical, the rule will not exist. Consequently, if subject is changed due to temporal and spatial evolutions, the rule will

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[6] Dehghan, Hamid; Effects of time and place on criminal law of Islam, Madyan publications, 1997

[7] Mehr-Rizi, Mahdi; problem of jurisprudence and time

[8] Marashi, Seyed Mohammad Hassan; New viewpoints about criminal law of Islam, Mizan publications, 1994

naturally changed too. For example, sculpture is a subject which was changed [9].

B. Changing Performance Outline of Law

Performance outline for many jurisprudential rules has been changed in comparison with one thousand years ago. A jurisprudent should consider these changes while he states his statements, for example the subject of slavery. "During the wars occurred in the beginning of Islam, slavery was authorized and allowed due to retaliation, and any fighting party would take the forces of the opposite party as its slaves and sell them. Islam was not able to nullify this rule; if so, this nullification was unilateral and would cause the weakness of Islam and strengthening of enemy" [10]. While there is no performance ground, the rule of slave selling should be nullified along with international contracts.

C. Scientific and Industrial Developments

The development of industry and technology may change a rule. For example, alphosis is an illness which gives the husband the right to divorce his wife (article 1123 of civil law), but if medical developments be able to treat this illness during a short time, will the husband rightful to divorce his wife again? Apparently no, because when such a rule was enacted, alphosis was an epidemic illness which might penetrate to the husband and it was also a difficult-to-treat illness. If these circumstances are removed, the rule will be nullified too.

New thinker jurisprudents always consider social changes when enacting their statements such as followings:

1- Ibn-Timieh says: During the era of Tartars, I met some of them when walking with my followers and I said them: Great Lord has prohibited wine because it deceives people to remember God and to pray, but drinking wine prohibits them (Tartars) to kill people and to plunder them, so leave them in peace".

2- Based on convenience, Abu-Hanifeh had believed that the duration to rent an estate from orphans or an endowed property should not exceed one year and the duration to rent lands should not exceed three years, but other jurisprudents after him let longer period based on convenience of their time.

4. Conflict between Islam and Human Rights

Critics believe that rough and harsh Islamic punishments are one of the bases of conflict between Islam and human rights. In fact, they have problem with criminal law of Islam. Fifth article of International Letter of Human Rights (General

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[9] Mousavi Bojnoordi, Seyed Mohammad; Role of time and place to change rules

[10] Mohaghegh Damad, Seyed Mostafa; the same source

Assembly of United Nations, 1948) says: "Nobody should be tortured or be punished or be behaved cruelly and contrary to humanities and human authorities or based on humiliating acts". The sixth article of International Pact of Civil and Political Rights (General Assembly of United Nations, New York, 1966) recognized the right of living as a natural right for human and specifies that nobody should be deprived of life unlawfully. The punishment of execution is specific for very important crimes under the law and by the explicit order of authorized courts. Punishment of execution may not be performed for youngsters under eighteen. The seventh article of this Pact prohibits torture or cruelly and savagely punishments and behaviors. The Convention to prohibit torture or cruelly and savagely punishments and behaviors is one of the most important international documents which is approved in 1984, describing and explaining any torture (article 1) and making member governments bound to avoid any kind of torture and to guarantee this prohibition by effective legal and executive measures. The convention recommends that no exceptional circumstances such as war, war threat, internal political disorder and any other emergency situation is a justification for torture (article 2). Some religious punishments in Islamic jurisprudence can be performed by people without order of court and judge, even the punishment of execution which may be performed as a religious duty. One of the conditions for retaliation is that killing the criminal is not reverenced and prohibited. So, natural apostates, cursers to Holy Prophet of Islam and those who claim to be a prophet can be killed without permission of authorized court, defense of guilty and the judgment of the Jury and other formalities. In authorized killing, retaliation is not allowed. It is true about killing those who should be executed for retaliation (with permission of relatives of criminal) or penance. If somebody kills such a criminal because his killing is authorized, he will not be retaliated. If a man finds his wife making sexual relation with another man, he is authorized to kill both of them religiously.

The second point in this conflict is the way to perform religious punishments. Today, some punishments such as burning the criminal in the fire, throwing the criminal from the height on the mount with closed hands and feet, beheading by the sword, stoning, imprisoning with hard work such as beating at the time of praying or shortening the amount of food and water, hanging on a cross, cutting right hand and left foot, cutting four fingers of a hand or whipping are the evidences of rough punishment and against humanity, so all religious penances which order to above punishments are declared as punishments against principles of human rights.

Definition of such heavy punishments by the law has been for eradication of heavy crimes. But the opinion of human rights goes forward to eliminate physical punishments completely, to limit execution and to lessen it up to elimination, and to remove any rough method of execution, generally toward elimination of rough punishments. So, according to religious punishments all religious penances and whipping are against principles of human rights. The third point in this conflict is to force offender to give information and to break his or her resistance to cooperate with the judge. Human rights believe that any kind of physical and mental pressure on the criminals is a torture even by judicial officers and to force them to confess, and the convention of human rights has approved prevention of torture exactly to avoid these measures. Although the conflict between human rights and legal law of Islam is not limited to above items and includes many other subjects, so we point at them briefly:

4.1. Punishments in Islamic rules

Generally, crimes are divided into five groups according to criminal system of Islam and due to policies of great Law-Maker (Great Lord):

1. **Crimes against religion:** Apostasy, religious innovations, disrespecting Holy Koran
2. **Crimes against the intellect:** Drinking wine, taking drugs
3. **Crimes against body:** Murder, beating and injuring
4. **Crimes against generation:** fornication
5. **Crimes against properties:** Theft, unlawful profit[11].

Other categorizations can be represented beside the above categorization such as categorization based on the rate, quality and type of punishments for performed measures such as:

1- Crimes deserving penance: The punishment for these crimes is defined. These crimes are the sin against God and never can be forgiven such as fornication, fighting with the religion of God and theft.

2- Crimes deserving retaliation and blood money: The amount of punishment is defined. These crimes are the sin against people and can be forgiven such as murder, beating and injuring.

3- Under-law punishments: The punishment of these crimes is not defined in law and if they are the sin against people, they may be forgiven. The judge is capable to define these crimes and the limits of their punishments. Administrative punishments and disciplinary breaches are among this category. Of course it should be said that the authority of judge is

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[11] Gorji; the same source

valid until his decisions are according to real interests and mischiefs in viewpoint of religion, because the goal of religion to give these authorities to the judge is to capable him to regulate social relations and to guide the members of society toward appropriate directions and to save the interests of society and to prevent circumstances[12].

4.2. Effects and consequences of performance of religious physical punishments

Performance of physical punishments during the initial years after Islamic Revolution of Iran, under the name of religious punishments, was one of the consequences of Islamic Revolution which had too many internal and international interactions, different from what law-makers and performers expected. Inside the country, creation of a kind of distaste about performers of these punishments and even pessimism about religious rules among various social classes especially educated people and the youth on one hand, and doubtful efficiency of these punishments to prevent repetition of crimes especially about those criminals who were addicted to crimes on the other hand, were the consequences of these punishments. The newly-established government which strongly needed the support of various social classes and had to attract the people especially during the unwanted imposed war to stand against external pressures, faces serious problems to perform physical punishments. Although these decisions were supported by people, but some punishments such as stoning and whipping in the squares of cities had excited people. On the other hand, leaving criminals after physical punishments and the feeling of addiction of criminals to punishments had made the judicial authorities too worried about the security of society and efficiency of physical punishments. These problems were not all the difficulties arose by physical punishments. The problem of opposition of international societies and institutions and some political and legal authorities in various countries with physical punishments were added to internal problems. Especially when photos and videos were taken by some foreign agents from the scene of whipping and stoning in the public, and were demonstrated for the people of foreign countries, negative injurious effects were brought up for the Islamic Revolution among the people and authorities of those countries, and reduced international support and public opinion of other countries to which, our country required hardly especially during the initial years after the Revolution. Although supreme authorities of country

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[12] Audah, Abdolghader; Islamic Criminal Jurisprudence, First edition, Beirut

at first resisted against international oppositions and public opinion inside and outside the country and tried to show that these oppositions are unimportant, but continuation of this situation brought political and social problems, and authorities of government could not be indifferent against these consequences especially those who were more anxious about the future of revolution and Islamic rules. Of course if there was no doubt about the efficiency of physical punishments, and great reduction in the statistics of crimes was obtained, and the cost of insistence to perform these punishments was proportional with obtained advantage, the performance of these punishments was more defendable. But such a thing never happened and political and social costs of these punishments were increasing inside and outside the country about the obtained advantages. The major reasoning of adherents of these punishments was that the performance of these religious punishments would have cost for the revolution as the victory of revolution had heavy costs for people, and performance of divine rules is valuable against political and social costs. In addition, if performance of criminal rules of Islam may be endangered due to opposition of some opponents, the base of Islam will be endangered too. To escape from this problem, these punishments can be reviewed by jurisprudents and under continuous jurisprudence of skillful jurisprudents of Islam, just like hundreds of religious problems. So, it is possible to save the essentials of religious rules and to create new and effective frames according to scientific criteria to substitute new punishments with physical punishments. In this case, performance of divine rules was saved as a major goal of Islamic Revolution and negative consequences were reduced. With due consideration that jurisprudence of Imams has been shortly performed in the past, executive problems of such these rules have been occurred less for jurisprudents of Imams and for this reason, there is little judicial innovation in this area of jurisprudence as the continuous and updated jurisprudence. After Islamic Revolution during which, performance of criminal rules was dismissed from merely scientific and mental subjects and the possibility of scientific fulfillment of them was prepared, executive problems were appeared, in a way it required multiple scientific efforts to remove these problems and to offer more scientific solutions with less political and social costs. The jurisprudents will perform these reforms that insist on criminal law of Islam like other parts of jurisprudence and believe that these rules are both sufficient and practical and on the other hand, are sensitive and feel commitment to reduce internal and external oppositions and various political and social costs. Offering new judicial viewpoints about under-

law punishments have created major developments in religious punishments gradually during two decades, and in Islamic Criminal Law, approved in 1996, in many cases the same punishments of previous law was substituted again with punishment of whipping, or the punishment of imprisonment and financial penalties were added to whipping. Judges are allowed to select appropriate punishment according to the situation of people and their states. It should be said about penances, blood money and retaliation that although these rules are approved by Islamic texts and all of them aim to avoid mischiefs and crimes to provide a healthy society, doubtlessly they conflict with other interests of society and cause many disadvantages such as representation of a rough face of religion and application of hidden methods to perform evil deeds. But avoiding crimes which are the main target of these religious texts are practical in new methods and brings no mischiefs of text punishments. In other words, it does not include many of penances as it is said in Holy Koran: The advantage of an evil deed is an evil deed [13].

Many traditions say that religious rules have not been stable during the time and have been changed according to interests of society. Of course these changes have not been for interference and possession of Islamic rules, but they knew that divine rules are not stable and depend on a specific time and should be changed if other interests occur[14]. So, jurisprudent not only should argue about document of traditions and its accuracy, but also should know the difference between stable and unstable rules. He should know which rule is stable and which one is not[15]. Punishments are enacted for some interests and these interests are generally for people and society, but as we know the interests of people are changeable because human finds new interests during the time and previous interests will be insufficient and repetitive for him. Allameh Helli says: The existing rules are the orbit of interests and interests are changeable and various according to the power of those who are responsible to perform them, so a specific rule may be in interest of a nation in a specific time. In such a case, this rule is honored and should be performed. This specific rule may not be in interest for another nation in another time. In such a case, the rule is prohibited and should not be performed. So stability of rules in all times and places is not a strong statement [16].

As the Islamic rules follow the real interests and mischiefs and these interests and mischiefs may

[13] Surah Shora, verse 40: جزاء سيئة، سيئة مثلها

[14] Audah, Abdolghader; the same source, p. 176

[15] Audah, Abdolghader; the same source, p. 211

[16] Helli, Jafar-Ibn-Hassan; Jurisprudence of Islam

have conflict in practice, Islam has het experts of religion to relinquish less important issued due to the most important ones [17]. Great Lord has strictly prohibited the weakness of Islam and Muslims. For example presence of non-Muslims is prohibited while performance of penance and performance of penance in the land of enemies is not allowed. So if performance of penances weakens Islam and Muslims, they should be given up. So, the attention should be paid that principles and rules of Islam are the effect of interests of believers to God. Islam as the most complete religion aims the prosperity and honor of Muslims and Islamic system and always tries to invite the followers of other religions to Islam, so enough efforts should be taken to show a kind and advancing face of Islam. If any disadvantage is bound to performance of penances or if Islam and Muslims are being weakened, the performance of penances will be relinquished due to the principle of conflict and Ijtihad, in order to provide interest of Muslims according to temporal and spatial evolutions.

6.   Conclusion

It can be concluded according to all propounded subjects that due to naturality of Islam and due to temporal and spatial effects on this human essence, Islamic rules have nothing to do instead of accepting these changes and developments. This development and impression is not a new and strange issue in Islam and has been discussed since the appearance of Islam by creation of the rules in Islamic manuscripts. Today, the necessity to make changes in rules of punishment especially in physical punishments is obvious for any body, and logical judicial justifications can be offered. Following this authority, the Iranian Law-Maker can bring up himself as the pioneer authority for these changes in the Islamic world. So insightful and provident jurisprudents with their supreme Ijtihad should introduce an acceptable and practical appearance of practical rules of Islam, while avoiding any deviation and distortion, and try to discover interests of rules and their philosophy and to understand the real opinion of Great Lord about temporal and spatial evolution instead of relying on obligatory rules and following Islamic texts in social affairs. They should enter the concept of criminal policy in criminal law and insist on removing punishments and administrative and disciplinary punishments and rely on civil solutions to provide new requirements of society.

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[17] Dehghan, Hamid; the same source; p. 162

The Effect of Employing Paradoxes in Teaching and Learning Mathematics in Correcting Students' Common Mistakes in Solving Equation

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Abstract: Paradoxes create a special challenge to learners and the incoherence involved causes great problems in their mind. Students' facing paradoxes provide an invaluable chance for the teachers to direct their attention towards the point and help them resolve the problem of concern. Therefore, because of the importance of paradoxes, the present paper aims at studying the effect of paradoxes in teaching and learning mathematics, specially, in cases in which students make frequent mistakes which are here referred as "common mistakes". To do this, 150 students 14-15 years old, participated in the study to investigate the efficiency of paradoxes in two most frequent errors: "simplification in equations" and "eliminating the radical from square expressions". The study lasted about four months during which the researcher taught relevant concepts in relation to the problems through paradoxes. The analysis of the data collected from a pre-test and a post-test showed that employing paradoxes is quite efficient in teaching and learning mathematics as for correcting "common mistakes.[Ahmad Shahvarani, Ali Barahmand , Asghar Seif, **The Effect of Employing Paradoxes in Teaching and Learning Mathematics in Correcting Students' Common Mistakes in Solving Equation**. *Life Sci J* 2012;9(4):5789-5792] (ISSN:1097-8135). http://www.lifesciencesite.com. 862

Key words: paradox; learning mathematics; teaching mathematics; common mistakes in solving equation

1. Introduction

Mathematics teachers and educators all over the words look for means for integrating of contents with teaching of psychological and pedagogical issues [7]. A popular trend in mathematics education advocates connecting mathematical instructions to students' intuitions and prior experiences [6]. For example, the Principles and Standards for School Mathematics (NCTM 2000) suggests that ''a pattern of building new learning on prior learning and experience is established early and repeated'', and that ''students of all ages have a considerable knowledge base on which to build, including ideas developed in prior school instruction and those acquired through everyday experience''. [8]
Paradoxes create a special challenge to learners and the incoherence involved causes great problems in their mind. Students' facing paradoxes provide an invaluable chance for the teachers to direct their attention towards the point and help them resolve the problem of concern.

2. The relationship between paradoxes and learning

Cognitive conflicts have long been a part of psychological theories of cognitive change. According to [6] engaging learners in resolving paradoxes could trigger a state of cognitive conflict which, for some learners, resulted in the construction of new cognitive structures. Cognitive conflict is regarded as a state in which learners become aware of inconsistent or competing ideas. It was found the role of cognitive conflict in enhancing a change from instrumental to relational understanding of mathematics [10]. Based on the [7] a cognitive conflict is strongly related to paradoxes. Using paradoxes, in their simplest forms, as a puzzle, can cause learning by incoherence. A special challenge is created between what a person knows and what they see, such that they cannot discover the reason of duality. The person may even doubt his/her knowledge. And it is at this moment when the learner comes to gain more knowledge by improving incomplete knowledge. Some researchers believe that paradoxes have helped forward mathematics in the history. For example, the Zeno's paradox of infinity in the 5th century B.C., caused the concept of limitation and calculus to appear in the 17 th century.

3. Research Context

Usually, mathematics teachers, based on their experiences, know that many students have problems in some areas. These highly frequent problems are referred to here as "common mistakes". Teachers always seek methods for decreasing these problems such as using different teaching methods or providing more examples, or even predicting and introducing the mistakes the students are supposed to do. Nevertheless, a number of research report that some students still have difficulty with these concepts

and these problems cause other problems in cases too. So far, however there has been little discussion on the effect of paradoxes in teaching mathematics for improving the mistakes.

4. Methodology
4.1. Purpose
In this paper, accepting the relationship between paradoxes and learning, the effect of paradoxes in improving and correcting two common mistakes by students in relation to "simplification in equations" and "eliminating the radical from square expressions", will be studied.
4.2. Participants
The research subjects were 150 students 14-15 years old from a public school who had already been taught solving quadratic equations by formula, and the study lasted about four months.
4.3. Procedure
First, a pre-test consisting a set of three carefully chosen questions based on research literature and authors' teaching experience in mathematics classes (see figure 1) was performed and after analyzing the mistakes in the students' responses, the researcher began to teach the concepts problematic through paradoxes. Then, the researcher designed a post-test based on the concepts, consisting five questions (see figure 2), for investigating the influence of the method.
4.3.1 The Pre-Test
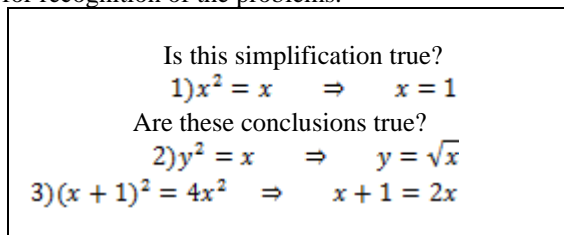The researcher conducted a pre-test (see figure 1), for recognition of the problems.

Is this simplification true?
$$1) x^2 = x \quad \Rightarrow \quad x = 1$$
Are these conclusions true?
$$2) y^2 = x \quad \Rightarrow \quad y = \sqrt{x}$$
$$3) (x+1)^2 = 4x^2 \quad \Rightarrow \quad x + 1 = 2x$$

Figure 1: Pre-Test

4.3.2 The Paradox' Method
According to [6] paradoxes provide educators with an important instructional tool that can help bridge the gap between mathematics and education by provoking discussion and controversy. Thus two important points are worth mentioning here:
1- Students should be given enough time for thinking, and
2- Students should be taught step by step via dialogue and discussion.
4.3.3 Simplification in equation
According to [5] some students simplified the variable m from both sides of the equation $4m=2m$ and concluded that this equation does not have any

answer. Regarding [1], many students facing the equation $(n-1).(n-2)=(n-1).(n-3)$, immediately simplified the same expressions, before anything.
The paradox used in this section was as following, (Quoted from [11]):
Look at my solution at the board carefully. Please tell me if you see any mistakes:
Let x=0 , so this relation is true , $x^2 = x^2 + x$
$$\Rightarrow \quad x^2 = x(x+1)$$
$$\Rightarrow \quad x = x + 1$$
$$\Rightarrow \quad 0 = 1$$

The students were surprised by the solution. Some students thought the mistake was due to the decomposition and the rest attributed it to the end of the solution. This was a suitable time for thinking, and the author asked to the students to write the question and think about the reason for the mistake. After 50 minutes, none of them could find any solutions and they were eager for understanding the reason for the paradox. This was suitable time for teaching via guidance as follows:
Please listen to my questions and answer carefully:
    1- *Is this conclusion true?*
$$2x = 2y \quad \Rightarrow \quad x = y$$
Most answers were positive.
    2- *If I put each number instead of 2, is the conclusion true again? For example:*
$$7x = 7y \quad \rightarrow \quad x = y$$
$$-5x = -5y \quad \rightarrow \quad x = y$$
$$\cdot$$
$$\cdot$$
$$\cdot$$
$$cx = cy \quad \rightarrow \quad x = y$$
Again, the answer was positive.
    3- *If I put 0 instead of 2, what happens? Is the conclusion true?*
$$0 \times x = 0 \times y \quad \rightarrow \quad x = y$$
Still, some of the students thought the relation was true and could not realize the problem.
    4- *This relation is true:* $\underbrace{0 \times 2}_{0} = \underbrace{0 \times 3}_{0}$. *If I simplify zero from both sides, I will have* $2 = 3$. *This equality is obviously wrong. But, where is the source of the mistake?*

This time, some of the subjects pointed to the particular property of zero and in response to the reason of the paradox, they could understand the source of the mistake. Then, the author stressed the "zero factor" which functions as zero number, such as:
$(n-1).(n-2)=(n-1).(n-3)$.

4.3.4. Eliminating the radical from square expressions

This problem is a common mistake too. The results of the pre-test showed that 73% of the subjects solved the equation $(x + 1)^2 = 4x^2$ as follows:

$$(x + 1)^2 = 4x^2 \quad \rightarrow \quad x + 1 = 2x \quad \rightarrow \quad x = 1$$

In this case, firstly, it was necessary for the students to understand their mistake. So, they were asked to check if $x = -\frac{1}{3}$ was the answer to the equation. It was known that $x = -\frac{1}{3}$ was an answer. Then, the author asked them "why could your method not find this number as an answer?".

The paradox used in this case, was taken from [6] as the following:

"Look at the board carefully, if you notice any mistakes at any stage, please tell me:

$$-8 = -8$$
$$\Rightarrow \quad 16 - 24 = 4 - 12$$
$$\Rightarrow 16 - 24 + 9 = 4 - 12 + 9$$
$$\Rightarrow 4^2 - 2 \times 4 \times 3 + 3^2 = 2^2 - 2 \times 2 \times 3 + 3^2$$
$$\Rightarrow \quad (4 - 3)^2 = (2 - 3)^2$$
$$\Rightarrow \quad 4 - 3 = 2 - 3$$
$$\Rightarrow \quad 4 = 2$$

This paradox was very surprising for all. Like the previous one, the students were providing with enough time for thinking and the source of the mistake was found via discussion step by step by themselves. Finally, after resolution, they achieved $x = -\frac{1}{3}$ which was again surprise for them.

4.3.5. The Pos-Test

After the treatment the effects of teaching mathematics using paradoxes were tested by administrating a carefully designed post-test (see figure 5 below).

Are these conclusions true? Please explain your reason in each case.

2)$(x^2 + 1)x^2 = (x^2 + 1)(2x - 1) \quad \Rightarrow x^2 = (2x - 1)$
1)$(2x + 1)^3 = 2x + 1 \quad \Rightarrow \quad (2x + 1)^2 = 1$

3)$(x + 2)^2 = 9x^2 \quad \Rightarrow \quad x + 2 = 3x$

Solve these equations and write your solution please.

4)$x^4 = 16$

5)$(x + 3)^2 = (x + 2)^2$

Figure 2: Post-Test

5. Data Analysis

Analyzing the data from the pre-test reveals that a great number of the students have problems and they commit the mentioned mistakes. The following table shows the percentage of the students' mistakes concerning each question.

Table 1: the Pre-Test Results

Question	Incorrect Response
Q 1	69%
Q 2	73%
Q 3	75%

Even, a brief look at the results revealed the necessity of emphasizing the points. Following the treatment and analyzing the data obtained through the post-test pointed out the significant role of using paradoxes to improve students' common mistakes (see table 2 below).

Table 2: the Post-Test Results

Question	Incorrect Response
Q 1	19%
Q 2	22%
Q 3	28%
Q 4	21%
Q 5	24%

According to Kolmogorov-Smirnov test the data were not normal, thus, Wilcoxon test were used. Because of $z=-7.02$ and p-value was less than *0.0005*, the effect of the method is significant with error $\alpha = 0.05$ (see table 3)

Table 3: Wilcoxon Signed Ranks Test

		N	Mean Rank
posttest – pretest	Negative Ranks	9[a]	23.50
	Positive Ranks	72[b]	43.19
	Ties	19[c]	
	Total	100	

a. posttest < pretest
b. posttest > pretest
c. posttest = pretest

Moreover, according to Mean Rank presented in table 4, the post-test is more successful than pre-test.

Table 4: Test Statistics[b]

	posttest – pretest
Z	-7.023[a]
Asymp. Sig. (2-tailed)	.000

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

6. Discussion

An aha-moment can occur when students face a paradox. This state can be effectively used for teaching mathematical concepts related to the essence of the paradox [4]. A desirable outcome is to make the learner to apply the concepts to resolve the paradox. A whole lesson can be designed to lead the discoverer to her goal [4].In this study we attempted to show that mathematical paradoxes carry an impornant message, thinking about which provides the learner with an opportunity to refine their understanding.

7. Conclusion

The most obvious finding emerging from this study seems to be that employing paradoxes is effective for teaching and learning mathematics in highlighting and correcting common mistakes. Teaching through this method is attractive and because the students themselves participate in the learning process, it is important from educational viewpoint as well.

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Gradual diminish of post Lasik corneal ectasia with a rigid gas permeable lens

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Abstract: Corneal ectasia following Laser in situ keratomileussis (Lasik), a known complication can only be treated with the use of rigid gas permeable contact lens, penetrating keratoplasty or corneal cross-linking surgery. A 33 year old Asian male was diagnosed with corneal ectasia in the right eye several months after bilateral Lasik and was fitted with a rigid gas permeable contact lens in our center. During weeks and months of following visits, the ectasia gradually diminished to protrusion-free corneal shape with the aided visual acuity of 20/20 by the 11 months' time. Patient's cornea however did return to its ecstatic state in about 9 days once the rigid lens was ceased to wear as a test of corneal stability. During the follow up visit in 5 years later, without the trace of ectasia, a steeper base curve of rigid lens was needed for a better fitting pattern, suggesting the cornea had protruded further even under the event of lens wear. The rigid lens seems to have a biomechanical influence on patient's ectasia condition following Lasik.
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Keywords: Post Lasik corneal ectasia, Rigid gas-permeable lens, Corneal changes

1. Introduction

Corneal ectasia is progressive steepening and thinning of the cornea after excimer laser corneal refractive surgery that reduces uncorrected and often best spectacle-corrected visual acuity.[1] Management of keratectasia requires the use of rigid contact lenses or penetrating keratoplasty.[2] In this paper, we fitted a rigid gas permeable contact lens on a patient with post Lasik ectasia in his right eye. During the course of following visits, the ectasia was gradually diminished by our daily wear rigid lens. To our surprise, by the 11 months' time, the ectasia disappeared completely and there was no sign of any corneal protrusion remains. To test the stability of cornea, patient was told to cease lens wear for 2 weeks. The ectasia returned when patient came back at 9th day follow up. In view of the return of ectasia, the patient was instructed to continue wearing of the same lens until a steeper design of rigid lens was fitted 5 years later.

2. Material and Methods

Best corrected visual acuity was done by using Topcon phoropter. Documenting ectatic condition was done by Dicon topographer (Paradigm Medical, USA) and keratometry reading via Topcon keratometer, Contact lenses used were traditional tri-curve rigid gas-permeable lens.

3. Results

A 33 year old Asian male with unremarkable health condition had a Lasik refractive surgery done to his both eyes. The pre-operation manifest refraction were–5.25– 2.50 x 042 in the right eye, and –5.50 – 0.75 x 175 in the left eye. Both eyes had the corrected vision of 20/20. The keratometric values were 43.50@032/45.75@122 in the right eye and 43.25@170/44.25@080 in the left eye. Corneal pachymetries (Orbscan) were 568 μm in the right eye and 562 μm in the left eye. Surgery was performed with the Moria microkeratome and the VisxStar S4 excimer laser. During the post operation follow-ups, the unaided acuities were 20/20 OD and 20/20 OS for 1 week and 1 month time. At 10 week follow up visit, the acuity was 20/30 OD and 20/20 OS. At the 19 week follow up visit, the patient had noticed significant worsen of his OD vision. The acuity was 20/200 OD and still 20/20 OS. Corneal topography was taken (Fig. 1) and confirmed corneal ectasia for his right eye. Keratometry readings were 42.75@044/44.50@134. After many trial fitting lens, the first rigid lens with parameters 7.80/9.6/-7.25 giving an acuity of 20/40- was ordered and given to the patient. After a couple of days of lens adaptation, due to unacceptable lens sensation, a second steeper rigid lens with 7.75/9.6/-4.00 was given to patient to wear as a fitting trail. The patient returned 1 week later. The patient was pleased with sensation and the

corrected vision of the trial lens which gave a visual acuity of 20/30 with an over refraction of -0.75 D only. A topography map and keratometry (43.00@ 005/43.50@095) were performed and both showed a less ectatic condition than in the previous visit. The patient was told to continue wearing the same lens until next follow up visit.
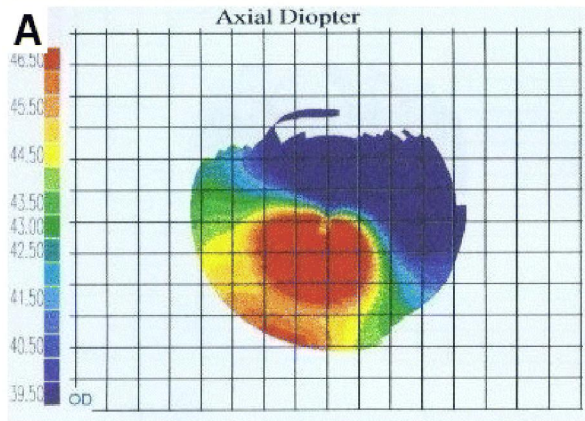
Fig. 1. Corneal ectasia found at 19 weeks after LASIK surgery, before rigid lens fitting.

At the follow up visits of 3 weeks, and 4 months (Fig. 2), corneal topography maps continued to show reduced protrusion although no much change were found on Keratometry readings. At 11 months post RGP treatment follow up visit, the patient's Ks were 44.00@170/44.75@080, over refraction was +0.25D, and VA was 20/20–. Manifest spectacle refraction was –3.75 –1.25 x 127 giving an acuity of 20/30. A topographic map (Fig. 3) showed no remaining trace of corneal ectasia.
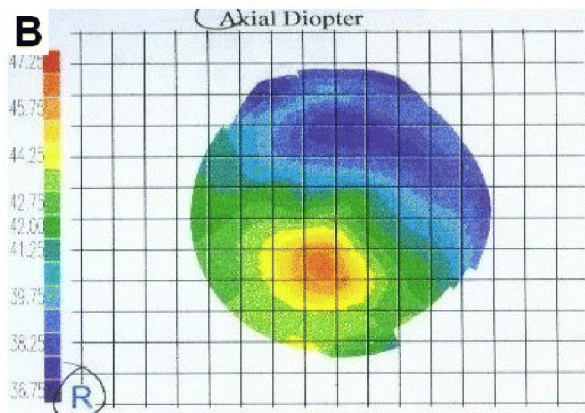
Fig. 2. Four months post rigid lens treatment.

The patient was then told to stop wearing the rigid lens as a test of corneal stability. Without too

much delay, the patient's right cornea returned to its ectatic state in the region where the protrusion had previously presented (Fig. 4). Ks were 46.75@010 /47.75@100, and manifest refraction was –7.75 –2.50 x 065, giving an acuity of 20/40. This indicates that the patient's corneal shape is unstable and it requires a rigid lens to help maintaining its curvature. The patient was instructed to resume lens wear.
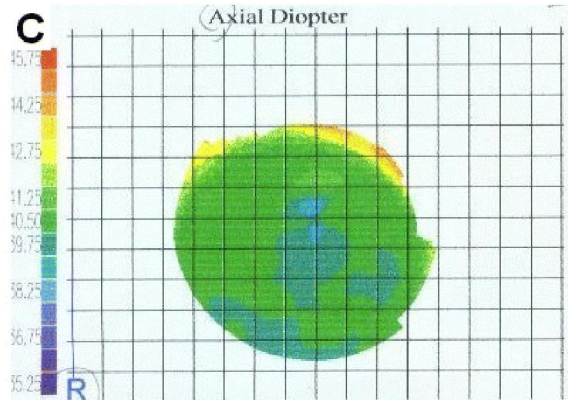
Fig. 3. Eleven months post rigid lens treatment.
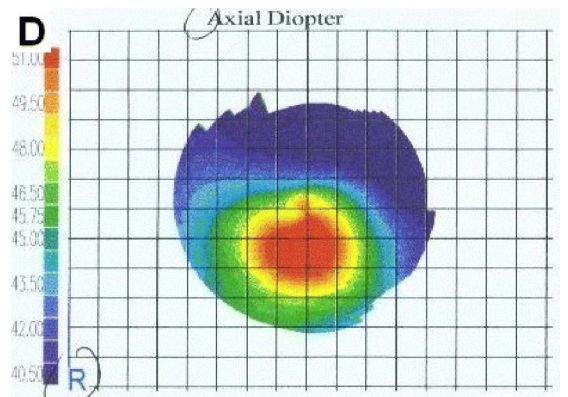
Fig. 4. Nine days after cease of rigid leans
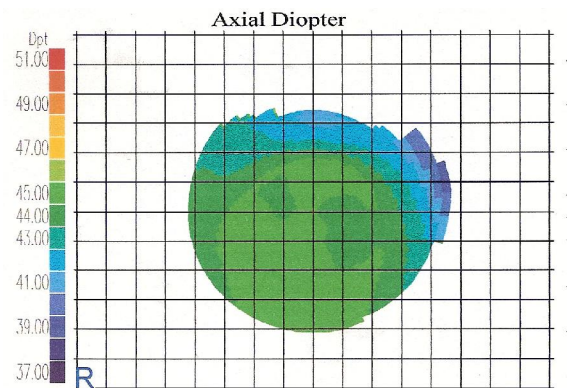
Fig 5: Five years post rigid lens treatment.

At the follow up visit of 5 years later, although still no trace of corneal ectasia on the topography map (Fig. 5), the patient's corneal curvature had steepened and was refitted with a steeper base curve of rigid lens for a better fitting pattern. The new lens design was 7.65/9.6/-4.50.

5. Conclusion

Corneal ectasia after LASIK represents a unique alteration in corneal integrity that presents as progressive inferior corneal steepening, an increase in myopia, an increase in astigmatism loss of uncorrected visual acuity, and often loss of BCVA.[6] In views of corneal curvatures changes in our patient's post Lasik ectatic eye, we conclude that the use of rigid gas permeable contact lens may help to restrict, slow down or reverse the corneal protrusion following Lasik surgery.

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Effects of Auxin and Cytokinin on Morphological and Physiological Factors in Soybean *(Glycin max L)*

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Abstract: Auxins and Cytokinins are of plant growth regulators. These two hormones are interfered in various physiological growth processes such as (activation and meiosis, tropism and morphology and …). Soybean is the most important sources providing world protein and plant oil and considering to food consumption for human and animals and medicine functions has high economic importance. For this reason, determining affects of plant hormones, Auxins (NAA) and Cytokinin (BA) was done on growth factors and then affect on the level of chlorophyll carotenoid and sugar and protein. Three different concentration of Auxins (NAA), Cytokinin (BA) hormones and three different concentration of equal combination from these two hormones were used. The studies showed that in the treated plants due to using different levels of (NAA) and (BA) concentrations as independently and treatment (NAA + BA) it was seen to increase in wet weight because of enhancing the concentration of hormone up to 1.5 mg/l and then to decrease wet weight. The dry weight verses wet weight increased by using hormones with enhancing hormones concentration, but through using combination of these two hormones along with enhancing concentration, it has been decreased. So, the most wet and dry weight of stem to treatment (NAA + BA) was 0.5 mg/l and the least wet and dry weight of stem to treatment (NAA) was 0.5 mg/l. In associated with dry weight of root, it was seen increasing hormone concentration NAA caused decreasing dry weight of root. But in treatments (BA and NAA + BA), it was seen increasing up to 1.5 mg/l and then decreasing dry weight. In associated with increasing treatment concentration (NAA), it was seen decreasing wet weight of root, and by increasing concentration, it was seen decreasing and then increasing and at last by increasing concentration (NAA + BA), it has been seen increasing wet weight. Also, the most wet weight root related to NAA treatment was 0.mg/l and the least was 1.5 mg/l for (NAA) treatment and the most dry weight of root was 1.mg/l for (NAA + BA) treatment and the least amount was 0.5 mg/l for (NAA + BA) treatment. In general, by increasing treatment concentration it was seen to shorten stem length and also in associated with root length, by increasing treatment concentration, it was seen increasing trend in root length, therefore the most stem length and root was dedicated to (NAA) treatment, 0.5 and (BA) 2.5 mg/l and the least stem and root length to (NAA + BA) treatments, 2.5 and (NAA) 0.5 mg/l, respectively. By increasing hormone concentration NAA and combination hormone, first increasing sugar concentration to treatment 1.5 mg/l and then decreasing sugar concentration amount were seen and in treatment with BA, by increasing hormone concentration, first decreasing and then increasing sugar concentration were seen, the most amount of carbohydrate in NAA treatment, 1.5 mg/l and the least amount in (NAA + BA) treatment, 0.5 mg/l were seen and in related with protein, by increasing hormones concentration of (NAA + BA), increasing protein concentration was seen. But by increasing combination hormones concentration, decreasing concentration amount has been seen, so the most amounts was seen in (BA) treatment, 2.5 and the least amount in control treatment. In associated with protein there was a statistically significant difference among control and all used treatments except (NAA + BA), 2.5 mg/l. The studies has been shown that in related to photosynthetic pigments, by increasing NAA hormone concentration, the amount of chlorophyll a has increased and in BA treatment up to 1.5 mg/l it was seen increasing and then decreasing and in combination treatment of two hormones, by increasing hormones, the amount of chlorophyll a has been decreased and about chlorophyllb and total chlorophyll and carotenoids, by increasing hormones concentration used, increasing was seen, respectively.

Introduction

Soybean is from class Faboidiea, Fablese row, Fabaceae or Leguninoea family and Papilionoidea and sub-family Glycinmax species. This plant has branched branches, with to purple flowers and one to five seeds in each sheath, this plant is native of tropical areas but is planting in moderate areas also and naturally it is very sensitive to low temperatures and easily damaged against cold weather. Its chromosomes number is 40 (2n = 40). The flower arrangement is clustery. Russian researcher are among first individuals who found the beneficent of soybean, these researchers realized that soybean has more protein necessary amine acids for

body, calcium and fiber, iron, phosphorus, magnesium, zinc, vitamin group B and the called the following beneficent for soybean: heart health and decreasing cardiac diseases, bones health, preventing from cancer, decreasing menopause complications, skin and hair health, preventing from anemia, fiber caused in decreasing weight, decreasing cholesterol and triglyceride and increasing function of colon and preventing cancer in colon. Scientists succeeded to make a kind of cheap anti biotic from soybean that called Moro mix act for killing harm bacteria of intestine. The plant is found with concentration range 1 to 100 mg auxin per 1 kg plant fresh weight, the young tissues and plantlets that fast grow and elongate contain high concentration of auxin than adult tissues and it is believed that the younger tissues are more sensitive than this growth regulator, physiological effects of auxin are complex and different tissues differently response to them and their reasons are unknown. In addition, separating primary effects from secondary effects are often difficult. The classic effect of auxin is to increase growth or stimulate length growth of cells formed a certain tissue. Additionally, when the effect of its different concentrations is tested, the straight line of this effect becomes complex very soon. The diagrams of different tissues response to concentrate show that addition to their role in elongation growth of cells, the influence on cell mitoses, so it stimulates cambium activation and causes to distinguish vena in vertex region of new branches, they are factor of establishing in the lateral roots and stimulate forming abnormal roots in stem and leaf cuttings. However, cytokinines were identified as cell division factors, but these hormones can have stimulus and inhibitor effects on different physiological, metabolic, biochemical and growth processes. And it is clear that endogenous cytokinines have significant role in regulating these processesin healthy plants. Cytokinines are defined as compounds that have bioactivity similar to Transzatin that interfere in some activities such as inducing cell division in the cell of callous in presence of auxin, stimulating formation of root and bud in planting callous when moll ratio to auxin is proper, delaying to become old leaves an stimulating to enlarge cotyledon in dicotyledonous plants, inducing formation bud in moss and genetic glands, more chemicals that have cytokinineial activity have been tested the analysis of these compounds was provided a good view about necessary structure for activating cytokinine. Almost all active materials as cytokinine are amino purines that their azoth number 6 has been substituted such as Banzil Adenin (BA).

Materials and methods

In order to implementing this research, the seed of soybean plant (Glycin max) canola species has been provided from the agriculture office of Babolsar city. Expected survey were done in plant sciences laboratory of Islamic Azad University in 1391 and has been independently examined the effect of two hormones, auxin (NNA) and cytokinine (BA) and a compound of both of them with concentrations (0.5- 105- 2.5 mg/L) on photosynthesis pigments and amount of carbohydrate and protein of soybean plant.

Preparation and culturing seeds

First the healthy seeds with same size were chosen and sterilized with 5% Hypochlorite solution (bleach) for 3 minutes and well washed by distilled water. Then sterilized seeds were cultured on two layers filter of Vatman number 1 in medium Petri dish (number 8) and added about 20 ml stilled water to each Petri dish in order to occur its primary growth and transferred to germinator with temperature 25℃ to bud seeds under dark conditions and fixed temperature. After spending that time and implementing bud process, they are transferred to a light environment to occur natural growth, when the roots of seed reach 1.5 cm and the color of cotyledon became green, they were transferred to pots with washed sand. After this step, they watered 5 to 7 days and then the sand environment has no food ingredients for faster growth and strengthening seedlings, it was added Hugland to food environment.

Surveying growth factors

After, the soybean has 13 to 15 leaflets or 24 to 5 leaves, the growth parameter include root length, stem length, wet weight and dried weight and seedlings' root were measured by three replicate of each treatment. Stemle and rootlet length were measured by millimeter ruler and wet and dry weight of stem and root by digital scale. For measuring dry weight, samples were put for 24 hours in temperature 70℃ in oven and then the dry weight of samples was measured by digital scale.

Determining the amounts of photosynthesis pigments

For measuring chlorophyll, it was used the Lichtenthler's method (1987), for this purpose 0.1 gr from the green leaf of plant was taken and scaled and well-grinned in porcelain mortar with acetone 80% in order to extracting its pigments. The grinded materials filtered by vatman's filter paper N. one and the filtered sample was transferred to test tubes. Then attraction in wave length of 663 nanometers for chlorophyll and wave length 646 nanometers for b chlorophyll and 470 nanometers for carotenoid were read by spectrophotometer. The control (blank) is acetone in this measurement. After reading waves length, the resulted numbers were put in the formulas and related calculations were done: (concentration in

microgram on milliliter from plant extraction was determined).

Measuring carbohydrate

For measuring carbohydrate, it was used Nelson's method (1943). 0.05 gr from dried leaves grinded with 10 ml distilled water and the grinded samples poured in test tube then filtered by vatman's filter paper N. one. After adding solutions of copper sulphate and acid molybdic, the tubes were shacked to appear the blue color in them, after this step, their absorption was read in wave length 600 nanometers in spectro photo meter apparatuand concentration of carbohydrate was calculated by standard curve of glucose in mg on gr.

Measuring protein

For measuring protein, it was used Lowery's method (1951). Based on this method, it was used 0.05 gr from fresh leaves of soybean and grinded by 7 ml Tris buffer in a porcelain mortar and transferred in centrifuge tube, the higher solution transferred to test tube for extracting protein. After adding D and E reagents it was read in wave length 625 nanometers by spectro photo meter apparatus, for providing standard curve and calculating samples protein, the serum of cow albumin was prepared in different concentrations and all process were repeated on them and their absorption was read.

Statistical Analysis

The tests were implemented with three replications in a full-accidental plan form. The amounts of three replicate were obtained and calculated variance and the standard deviation of means and the difference among means by variance analysis. Surveying the result of tests and drawing curves were implemented according to comparing means and standard deviation (Mean ± SE). Grouping treatments in the level of 5% ($p \leq 0.05$) was done by Tukey test. Statistical analysis of data was done by SPSS software and for drawing diagrams, it was used EXCEL software.

Results

The wet weight of stem: by using different levels of Auxin (NAA) and cytokinine (BA) concentrations as independent and compound treatments (NAA + BA), it was seen increasing in wet weight by increasing hormone concentration amount to 1.5 mg in liter and decreasing weight in 2.5 mg in liter as in (NAA + BA) 0.5 mg was the most wet weight and the least amount in (NAA) treatment was seen 0.5 mg in liter and in the it has been seen a significant difference in statistical level. The difference of wet weight among control and all treatments of (NAA) have been meaningful. (Table 1, Diagram 1).

Table 1: Analyzing variance of hormones effect (NAA), (BA) and (NAA + BA) on soybean's morphological parameters

Source of variations (SOV)	Degree of Free (df)	Mean square					
		Wet weight of stem	Wet weight of root	Dried weight of stem	Dried weight of root	Stem length	Root length
total	29	13.6**	6.9**	75.62**	31.42**	184.91**	16.957**
treatment	9						
repeat	2						
Test error	20	1.17	0.39	0.728	0.0163	39.9	4.9
Coefficient of variations		16.12	46.29	37.03	13.12	29.14	22.07

The dried weight of stem: the dried weight of seedlings opposite of wet weight is increased by using auxin and cytokinine hormones and increasing in hormones concentrations, but by simultaneous action or compound of two hormone as (NAA + BA) has been decreased by increasing concentration so in concentration of (NAA) 0.5 mg in liter was the least amount and in (NAA + BA) treatment, 0.5 mg in liter was the most amount, they had a significant difference in statistical level of 0.001. The difference of dried weight among control and all treatments has been meaningful. (Table 1, Diagram 2).

The wet weight of root: The wet weight of seedlings root in (NAA) treatment has been reached the least amount 2.5 mg/l and the most amount 0.5 mg/l. in general, by increasing concentration of (NAA) treatments, it has been seen to decrease in wet weight andby increasing in (BA) concentration first decreasing and then increasing and at last by increasing (NAA + BA) has been seen to increase wet weight. There has been a significant difference in the statistical level of 0.01. The difference of wet weight of root among control and all treatments except (NAA + BA) 1.5 mg/l has been meaningless (Table 1, Diagram 3).

The dried weight of root: the least dried weight of root was seen 0.5 mg/l in (NAA + BA) treatment, so the most amounts were 1.5 mg/l in (NAA + BA). Totally, by increasing hormone concentration (NAA) occurs decreasing the dried weight of root, but in (NAA + BA & BA) treatments till concentration of 1.5 mg/l and in concentration of 2.5 mg/l was seen increasing and decreasing, respectively. They had a significant difference in the statistical level of 0.01. The difference of the dried weight of root among the control and all treatment except (NAA + BA) treatment in 1.5 mg/l and (BA) treatment in 0.5 mg/l has been meaningful. (Table 1, Diagram 4).

The stems length: it was seen the least amount of stem length related to (NAA + BA) 2.5 mg/l and the most amount related to (NAA) 0.5 mg/l. in general, by increasing treatment concentration, it has been seen to increase in stem length, so compound treatment of (NAA + BA) in all concentrations related to this treatment (0.5 – 1.5 – 2.5 mg/l) than the other treatments had shorter size. They had significant difference in the statistical level of 0.01 and the difference of stem length among control and all treatments except (NAA + BA) treatment has been meaningful in 0.5 mg/l. (Table 1, Diagram 5)

The root length: the least amount of the root length related to (NAA) treatment was 0.5 mg/l and the most amounts related to (BA) was 2.5 mg/l. in general, by increasing treatment concentration, it has been seen increasing trend by increasing the concentration of treatments. They had significant difference in the statistical level of 0.01 and the difference of root stem among control and all treatments except (BA) treatment with 1.5 mg/l and in (NAA + BA) treatment with 0.5 mg/l were meaningful. (Table 1 and Diagram 6)

The amount of chlorophyll (A): by increasing different concentration of hormone of NAA, the amount of chlorophyll A was increased in NAA treatment and in BA treatment increased to 1.5 mg/l concentration and then decreased in concentration of 2.5 mg/l and in the compound treatment of both hormones by increasing hormones concentration, the chlorophyll amount A decreased and it was shown to increase in chlorophyll amount A in the concentration of 2.5 mg in liter. So, they has been dedicated the least amount of chlorophyll in control sample and the most amount in NAA treatment of 2.5 mg/l. they had a significant difference in the statistical level and there has been a significant difference in the difference of Chlorophyll level A among control and all treatment levels. (Table 1 and Diagram 3)

The amount of chlorophyll (B): by increasing the concentration of used hormones, the concentration of existing chlorophyll increased in leaves, so the BA treatment with 2.5 mg/l has dedicated the least amount of chlorophyll B and (NAA + BA) treatment with 2.5 mg/l has had the most amount of chlorophyll. They had significant difference in statistical level of 0.01and difference was meaningful in the chlorophyll levels B among control with all treatments level except (0.5 BA mg/l) and 0.5 mg/l) and (NAA 0.5 and 1.0 mg/l). (Table 1 and Diagram 4)

The total amount of amount: the least amount of total chlorophyll was related to control treatment and the most total of chlorophyll was related to NAA 2.5 mg/l. by increasing the concentration of hormones, the total amount of chlorophyll has been also increased. They had significant difference n statistical level of 0.01 and the difference of chlorophyll level of all control samples with all treatments has been meaningful. (Table 1, Diagram 2)

The amount of Carotenoid: by increasing the concentration of hormones, the amount of carotenoid was also increased, so the most amount was related to (BA) treatment with 2.5 mg/l and the least amount related to (NAA) treatment with mg/l and they had significant difference in statistical level of 0.01and the difference of carotenoid level in control sample with all treatments except NAA and BA and (NAA + BA) with mg/l has been meaningful. (Diagram 5)

The amount of carbohydrate: by increasing the concentration of NAA hormone and compound hormone (NAA + BA), first increasing glucose concentration to treatment 1.5 mg/l and then decreasing glucose concentration was seen and in treatment with BA hormone, by increasing concentration of hormone, first it was seen decreasing and then increasing glucose concentration. The most carbohydrate amount was related to

(NAA) 1.5 mg/l and the least amount of carbohydrate was related to (NAA + BA) 0.5 mg/l, and the difference in the amount of carbohydrate has been meaningful among control and all treatments. (Table 1 and Diagram 1)

The amount of protein: by increasing in the concentration of BA and NAA hormones, it was seen to increase in concentration of protein, but by increasing the concentration of compound hormones, it was seen to increase the concentration of protein. The most amount of protein was related to (BA) treatment with 2.5 mg/l and the least amount of protein was related to the control treatment. And there was no any meaningful difference in statistical level of 0.01. There has statistically been a significant

difference among control and all level of treatments used except (NAA + BA) with 2.5 mg/l (Table 1 and

Diagram 2).

Table 2: Analyzing variance of hormones effect (NAA), (BA) and (NAA + BA) on soybean's Physiological parameters (n.s: it is meaningless, there is no difference. *: It is meaningful in the level of 1%.   **: It is meaningful in the level of 5 %.)

Source of variations (SOV)	Degree of Free (df)	Mean square					
		Chlorophyll (A)	Chlorophyll (B)	Chlorophyll (T)	carotenoid	protein	carbohydrat
total	29	13.10^{**}	205.2^{**}	32.41^{**}	11.24^{**}	$1.66^{n.s}$	279.73^{**}
treatment	9						
repeat	2						
Test error	20	19.76	7.15	9.022	6.13	0.231	2.41
Coefficient of variations		15.54	15.81	13.30	32.23	10.12	89.94

Discussion

By increasing the amount of NAA hormone concentration, the amount of wet weight of stem was increased and also by increasing the amount of BA hormone concentration, the amount of wet weight of stem was increased, so the amount of this increasing was more than the case of treating with NAA. By applying compound of both hormones, in case of increasing it was seen to decrease and then increase in the amount of wet weight of stem. The amount of wet weight of root has been decreased by increasing NAA hormone concentration and by increasing BA hormone to 1.5 mg/l has had increasing and then decreasing and in compound treatments of both hormones it has been seen to increase in wet weight of root by adding concentration. By using NAA and BA hormones and by increasing hormones concentration, the dried weight of stem also has increased and in the case of using compound hormones of both of them, it has been seen the dried weight of stem. By increasing NAA hormone concentration it was seen to decrease in the dried weight of root and about treatment with BA hormone first it was seen to increase in dried weight and then decrease and by simultaneously using both hormones, first it was seen to increase and then decrease. By applying treatments NAA and BA and NAA+BA, it has been seen to decrease the stem length by increasing concentration. Increasing the amount of NAA and BA and NAA+BA, it was seen to increase in the length of root. It was implemented several research about this parameter on different plants that as an example it can be pointed to a research in rapidly sinking in 30 mm concentration of nitrogen along with 500 mg/l NAA that has the highest amount of creating root (salehi Najaf Abadi 1378, planting miniature Rose into glass, Iran agricultural

research, N. 15, 15-67), so in the present research, NAA with 0.5 mg/l has dedicated the highest wet weight of root and with 1.93 mg/l had the lowest amount of wet weight of stem and with 0.482 g had the least amount of dried weight of stem that indicate the opposite auxin effect in aerial and ground organs, so has been consistent with this research. (Davies – 1995) reported that Auxin is able to stimulate cell division similar to cytokinine and or in partnership with it and the high concentration of Auxin in seeds can produce more cytokinine in seeds growing and or in the other research the highest directly creating new branch lets were seen in NAA medium with 0.1 mg/l and 0.5 mg/l BAP and also creating root from separate parts was seen in NAA medium with 0.5 mg/l. Cytokinine treatment accelerates to extend additional cell without causes to increase in dried weight of treated cotyledon. By increasing the amount of BA from 0.5 to 1 mg/l, the percentage of branching will increase and then adding it to 2 mg/l will not alter branching studying the effect of micro-samples and the concentration levels of Auxin and cytokinine in de-morphology of Soybeen, SHarifi – Bageri – Vesal, 1382). Increasing the amount of cytokinine from 0.5 to 2 mg/l will increase the number of branches, but decrease the length of branchlets and wet and dried weight of aerial organs, so in this research it has been dedicated the highest amount of wet and dried weight that has been consistent with this research.

Cytokinines cause to increase food movement in leaves and their accumulation and increasing in dried weight. Since that the main weight of cells is related to cellulose existing in their cells and water, therefore it is natural that the wet weight and also the amount of dried material in 0.5 mg/l BA that its cells made sediment on its walls become

more. In concentration of 1.5 and 2.5 mg/l bA, also division cell causes to increase the number of cells and total weight of micro-samples through water absorption rather than increasing the dried material (Lesani- Mojtahedi- 1367- plant physiology of Tehran University). Accumulation of statolyts in root affected Auxin and geo tropism phenomenon are following by increasing dried weight of root (Slevers.Volkman 1976), so decreasing the wet weight of root was seen by increasing Auxin concentration to 2.5 mg/l.

Rafeekher et al (2001) showed that GA_3 and NAA increased the length of internodes and caused elongation of stem in cucumber. The highest mean number and the length of branch in treatment with 0.5 mg/l concentration BA and 0.05 mg/l NAA were obtained, in a test on miniature Rose that was implemented by Salehi Najaf Abadi, the concentration of 2.25 and 0.1 mg/l NAA and BA has been dedicated itself the highest mean number and the lengtj of branchlet (Salehi Najaf Abadi, 1378, planting miniature Rose into glass, Iran agricultural research, N. 15 – 15 -67). Surveying the effect of different concentrations of cytokinine hormone in micro proliferation of zinnia flower in 1388 showed that the most number of leaves was in BA hormone with concentration of 3 mm and the highest concentration in increasing the leaf length was BA hormone with concentration of 2 mm, and the most increasing stem length was related to BA hormone with concentration of 1 mm (Surveying the effect of concentrations and different kinds of cytokinine hormone on micro proliferation of zinnia flower, Mahmoodzade – Abbasi, 1388). Cytokinines can expand cell in some tissues and organs. This effect is clearly seen in bi- cotyledons with wide leaves such as mustard, cucumber and sunflower. Cotyledons of these species grow due to expanding cell during growth of seedling (Ryle et al, 1982). Kinetin more strongly acts in growing stem and root length and BA has had stronger effect on branching (Babaei, A – R – 1381, surveying proliferating two kinds of Antrium flower by using tissue culture system, Gilan University). Cytokinines like as Auxin does not increase formability by acidifying cell wall (Rulan and Ryle, 1982). BAP as a cytokinine more actively acts in branching (Goerge – 1993). Using 2 mm BAP in MS medium causes to create branch and increase its elongation in Zelegans (Lakshni- Sita G 1993). Auxin is usually known as main responsible of increasing cell length and consequently elongating aerial parts of plant, but in real hormone balance obtained is effective in cell responding, so when the ratio of Auxine is more than cytokinine, regeneration process is following by creating branchlet and in the

opposite case causes to increase in organs length and to produce root (Banki – T. J & A.A Boe 1975).

In the high concentration of BA, the length of branch let is less than the low concentration (Bertrand.A.m 1999). Simultaneously using Auxin and cytokinine cause to create lateral buds and branches and remove the apical dominance and cause to shorten the plant. Auxines increase the growth of stems and stems sheath, so Auxin will increase expandability of cell walls by withdrawing Protom and acidifying walls. (Jacobs & Ray, 1976) Auxin suppresses the growth of lateral bud in the bean plant and is following by increasing stem length (Wilkins).

Auxin is necessary to begin cell proliferation in environmental circle in order to creating root and growing root (Celenza.et.at. 1995)

Protein: in the soybean, in the case of applying hormone treatment, the level of leaf protein has been increased by increasing NAA concentration. Auxin cause severe cell elongation that are placed under the apical (materials and regulators of plant growth, translated by Ghodratolah Fathi – Behrooz Ismaeil poor). Cytokinines along with Auxin cause cell division in erial organ and root and regulating cell cycle, so the low concentration of cytokinine and the high concentration of Auxin cause to create root that in this research (NAA+BA) 2.5 mg/l has been dedicated the least amount of stem length that is 30 cm among treatments, so are completely consistent with this case.

Growing root will occur in the low concentration of cytokinine that in this research, BA with 2.5 mg/l has been dedicated itself the highest stem length that is, 11.8 cm, so it is seen unconformity in this case. In general, elongation of cell will occurs only by presenting Auxin. Auxin has inhibitory effect on this step of growing, for example some amount concentration of Auxin that causes elongation stem, to decelerate elongation of root. Therefore in this research concentration of 0.5 mg/l Auxin has been dedicated the highest amount of stem length that is, 72.33 and in his concentration, the root length was 6.133 that was completely consistent.

Also, by increasing BA concentration, the leaf protein level will increase, too, so this increasing was more than increasing with NAA treatment, but by simultaneously applying both hormones, the level of protein will decrease. Studies shows that the proper concentration of elements especially elements that have important role in making protein cause to alter stored protein (Randall-P.J.A. Thomps-n.j.A 1979). Auxin is effective in biosynthesis some enzymes such as cellulose - peroxisase. Auxin interferes in the step of copying and in this step it activates RNA polymerase enzyme to make DNA from RNA, in real Auxin does not interfere in

biosynthesis DNA, but affects on the step of copying. In general, Auxin causes to copy RNA from DNA and consequently to increase to synthesis protein. Cytokinines are effective on tn the general metabolism of plant especially enzymes – coenzymes and in appearing intra-organs of cell and transferring for synthesizing RNA and DNA and protein and generally for whole parts of plant. Cytokinines can regulate synthesis of protein: there are good evidences that show cytokinines play important role in synthesizing protein, when cultured soybean cells treated with cytokinine (Zeatin), during 15 minutes, increasing in the content of cell polyribosome. This increasing occurs due to movement of single free ribosome (monosomes)of polyribosome. (Tapfer and Fosket, 1978). Since polyribosomes contain such ribosomes that actively make protein, the results show that cytokinines cause to synthesis total protein in these cells (Tapfer and Fosket, 1978). Cytokinines not only are able to increase the speed of synthesizing protein, but also alter the spectra of made protein by plant tissues, this case proves by marking proteins of culture soybean cell with mytonin { S^{35} } and separating them by electrophorus of polyAcryl Amid gel. Gel auto-radiograms showed that treatment with cytokinines will increase to synthesis some proteins, so inhibits to synthesis the other protein (Fosketo and Tapfer, 1978). The tissues of treated to baccowith cytokinine indicates to alter synthesizing protein paradigm that shows hormones are necessary for synthesizing some proteins and inhibiting to synthesis the other protein (Aik Holes et al, 1983). Cytokinines of tRNA can play important role in synthesizing protein, tRNA that contain these compounds, cytokinines occupy sensitive location near to Anti Codones that may influence in tRNA to mRNM (Hal, 1970). In general, cytokinines has sensible effect on the speed of protein making and any kind of made protein by plant cells, especially cytokinines stimulate to synthesis certain chloroplast protein that code by nuclear genes and make by cytoplasm ribosome. Based on existing evidences and results obtained from different research, cytokinines have a significant role in synthesizing proteins that will increase by adding concentration of this hormone. In this research the highest protein was seen in BA with 2.5 mg/l and the lowest amount in the Control sample that are completely consistent with the results of other researches.

Carbohydrate: by increasing the amount of NAA concentration to 1.5 mg/l, the amount of leaf carbohydrate increased and in the case of applying hormones with BA, increasing the amount of hormone concentration has decreased the amount of carbohydrate, by using simultaneously both hormones also was seen to increase up to 1.5 mg/l

concentration and then decrease. Sugars cause to regulate osmosis pressure and also stability in membranes and existing proteins in cell, this action can occur by forming hydrogen bonds between carboxylic groups of sugar and polar chains of proteins and at last stabilizing proteins (Koster.K.L-Leopold A.C 1988). Whatever the process of photosynthesis is done with more speed in plants, primarily metabolites including proteins and carbohydrates increase in the same proportion. Cytokinines existing in the wooden vena often are nucleotides, as soon as nucleotides reach leaves, may alter to free base or glycosides. Glycosides of cytokinine accumulate in large amount in leaves and there are even existing considerable amounts of them in old leaves. Cultured Cotyledons of radish in medium containing cytokinine with Banzyl Adenin base of cultured cotyledon easily destroy hormone and alter it to different glycosides, BAP, Ribonocloizides BAP, and Ribonocloides BAP. When cotyledons reentered to medium lack of cytokinine, the speed of growth, BAP concentration, ribonocloizid and Ribonocloitid BAP decrease in tissues and in this case the amount of glycoside BAP remains constant. Generally, cytokinines accelerate to get chloroplast, so the result of this action is to increase photosynthesis process and then more providing the photosynthesis production including sugars that in this research the highest amount of protein was seen in BA treatment with 2.5 mg/l that was consistent with this principle.

Photosynthesis pigments: By increasing the amount of NAA hormone concentration, the amount of chlorophyll has been increased and by treating with BA hormone and increasing the concentration of hormone to border of 1.5 mg in liter, it was shown first to increase in the amount of chlorophyll and then decrease in the amount of chlorophyll. It was shown to decrease in the amount of chlorophyll with increasing the hormone concentration to the border of 1.5 mg/l and with concentration of 2.5 mg/l to increase by applying compound of both hormones. In related to carotenoid, it has been seen to increase in the amount of carotenoid in leaves by increasing concentration of BA and the by increasing VAA hormone concentration first it was seen to decrease and then to increase and by applying compound of two hormones. It was seen to increase in the amount of hormones concentrations. Cytokinines causes elongation of cells in leaves and cotyledons that this case itself causes to increase in wet and dried weight and to increaseinthe amount of photosynthesis micro pigment including chlorophyll (Koochaki–GholamhossainSarmadnia). Grow thre gulators, especially cytokinines are responsible of keeping

pigments of petal and leaf, Jibrolines also interfere in biosynthesis carotenoids and Antosyanines and delaying chlorophyll decomposition. (Mutui et al, 2001) researchon chrysanthemum morifolium showed that Banzyl Adenin caused to keep the color of petals. (Petridoe et. al 2003) In the old leaves the ratio of chlorophyll a up to b will increase due to decomposition of chlorophyll b and its alteration to chlorophyll a that indicates the activity of pre-Enzymes of the path of breaking chlorophyll. (Harpaz – saod.al 2007) cytokinines affected on many growth aspects of regulating with light includes differentiation of chloroplast and simultaneously with it making chlorophyll- growth and self culture metabolism- enlarging cotyledon of leaf. (Tyze Zyger) Tobin et al studied on the role of cytokinines in chloroplast maturity of humpty blue lenticels. In this research, the most amount of carotenoid pigments was seen in BA treatment with 2.5 mg/l equals with 9.258 mg/l that were consistent with this research, but about chlorophyll pigments, the most amount is seen in NAA with 2.5 mg/l equals with 29.828 and the least amount in BA with 2.5 mg/l.

Diagram 1: The effect of difference treatments (NAA; BA; NAA+BA) on the wet weight of stem (g)

Diagram 2: The effect of difference treatments (NAA; BA; NAA+BA) on the wet weight of root (g)

Diagram 3: The effect of difference treatments (NAA; BA; NAA+BA) on the dried weight of stem (g)

Diagram 4: The effect of difference treatments (NAA; BA; NAA+BA) on the dried weight of root (g)

Diagram 5: The effect of difference treatments (NAA; BA; NAA+BA) on the length of stem (cm)
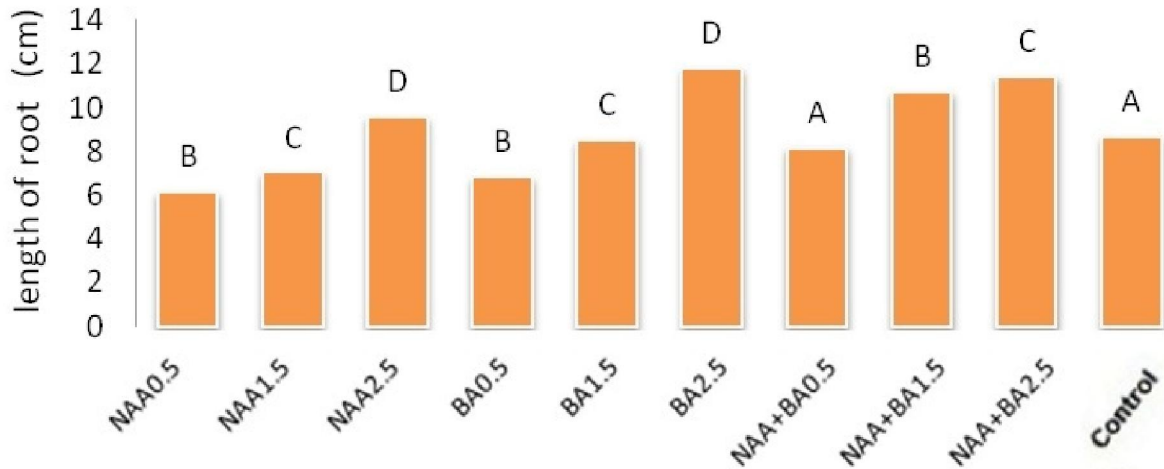
Diagram 6: The effect of difference treatments (NAA; BA; NAA+BA) on the length of root (cm)
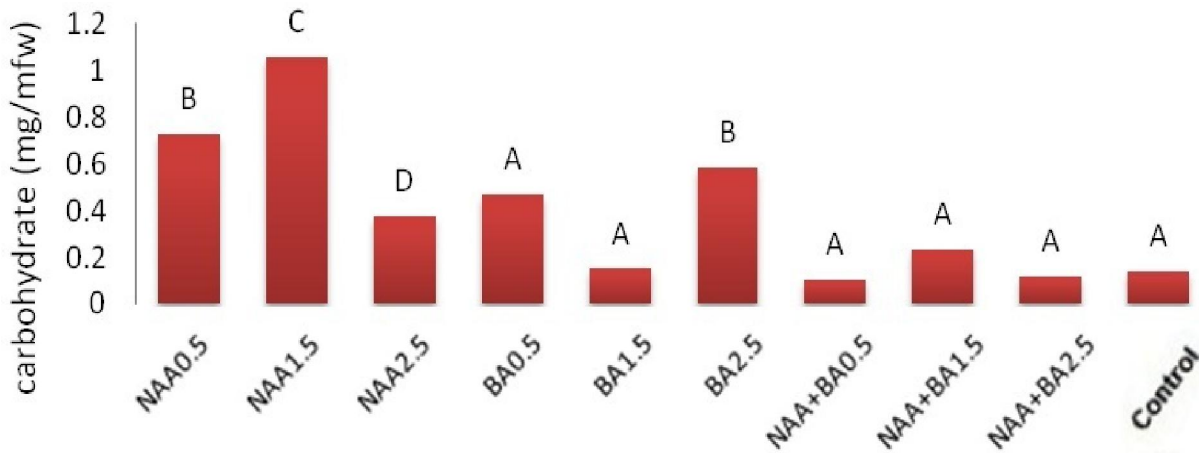
Diagram 7: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of carbohydrate (mg/mfw)
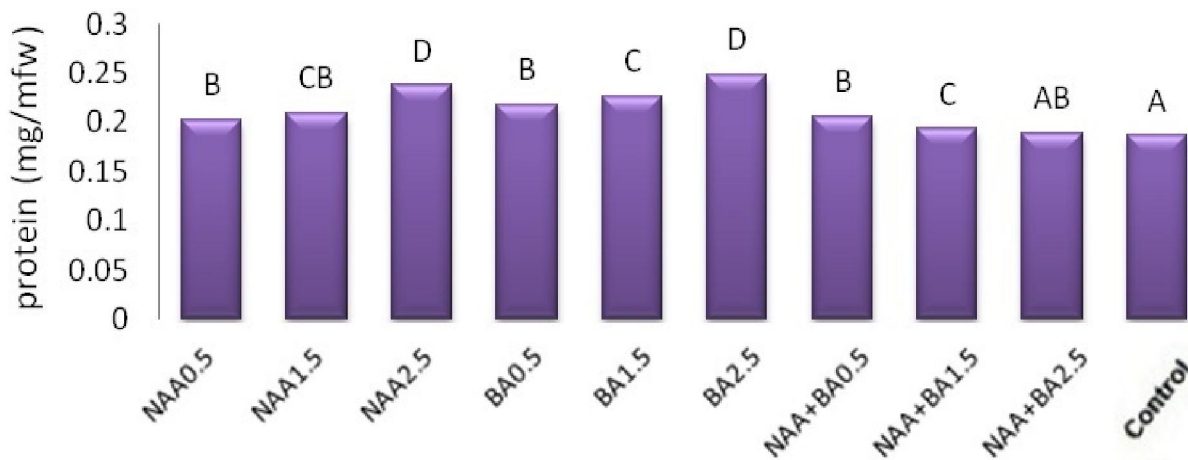
Diagram 8: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of protein (mg/mfw)
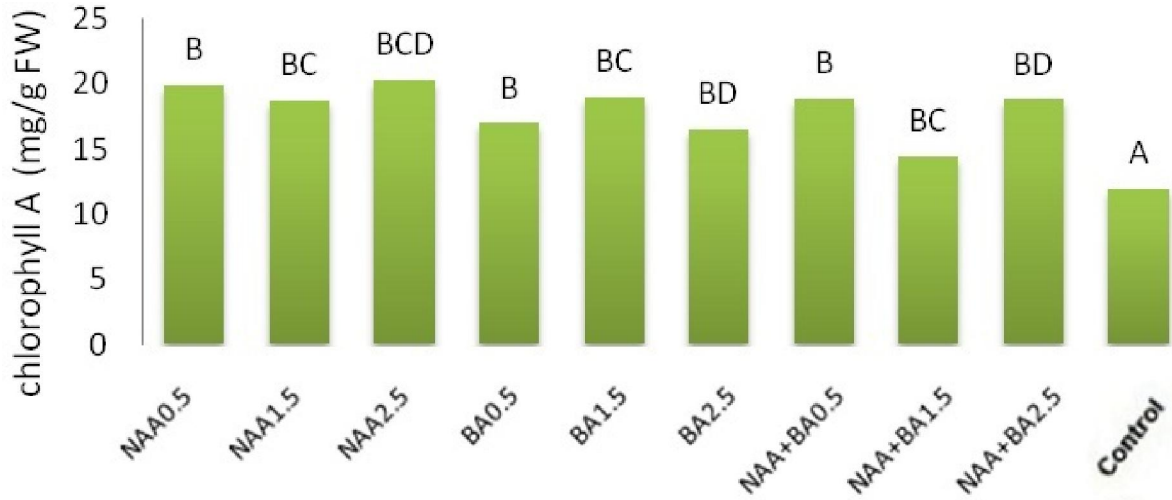
Diagram 9: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of chlorophyll A (mg/g FW)
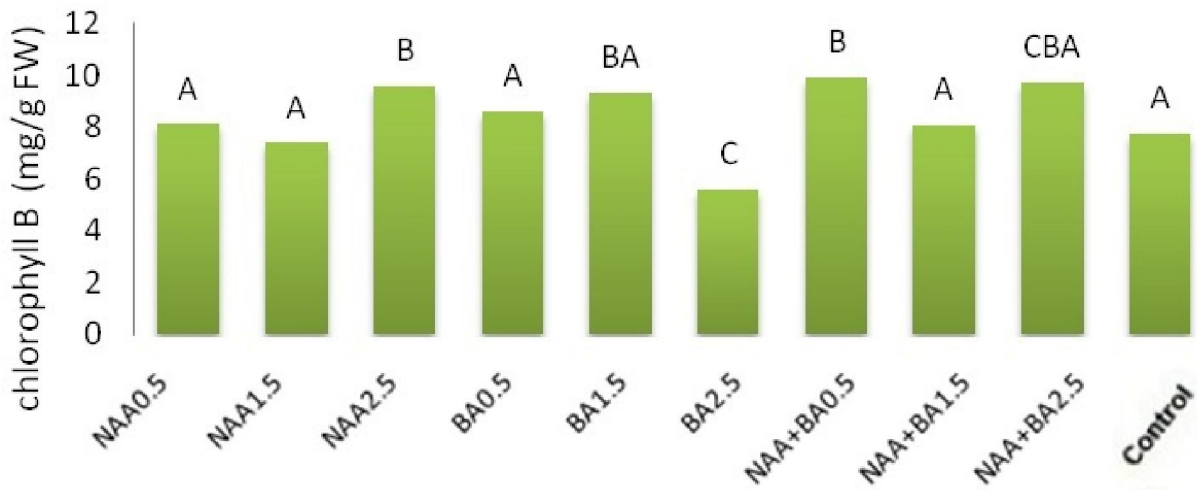
Diagram 10: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of chlorophyll B (mg/g FW)
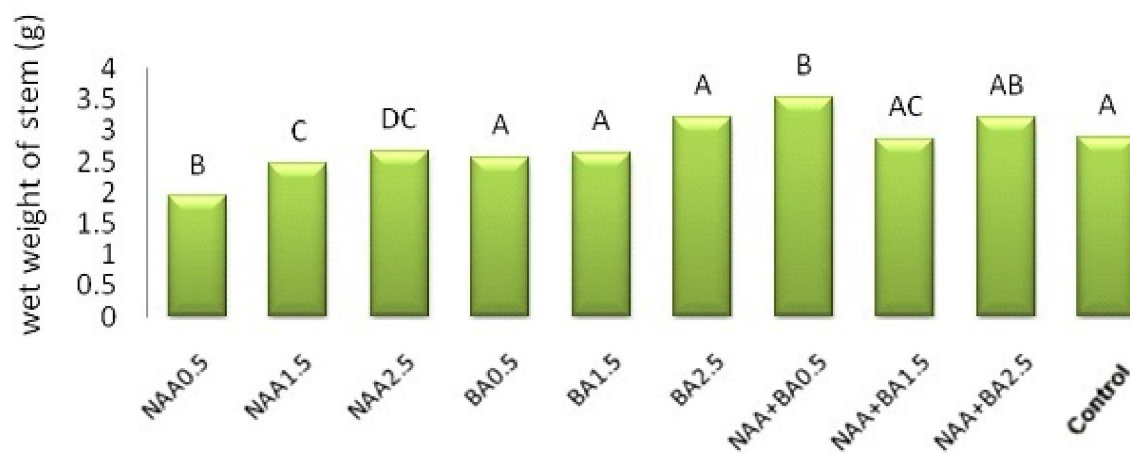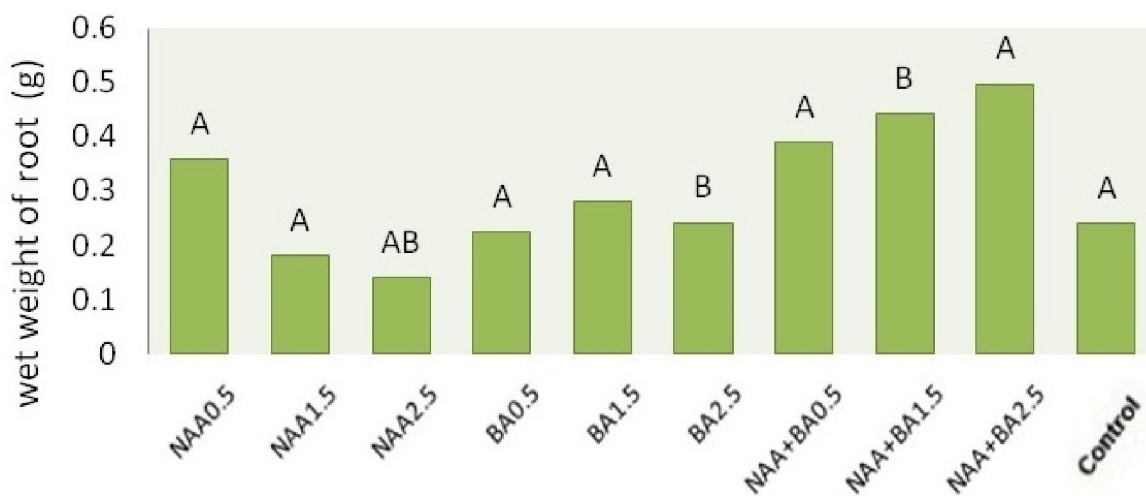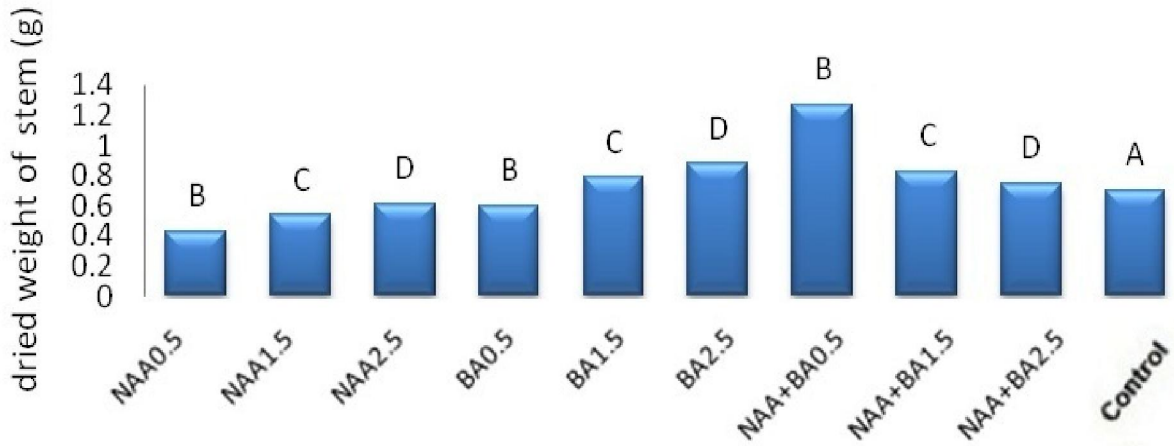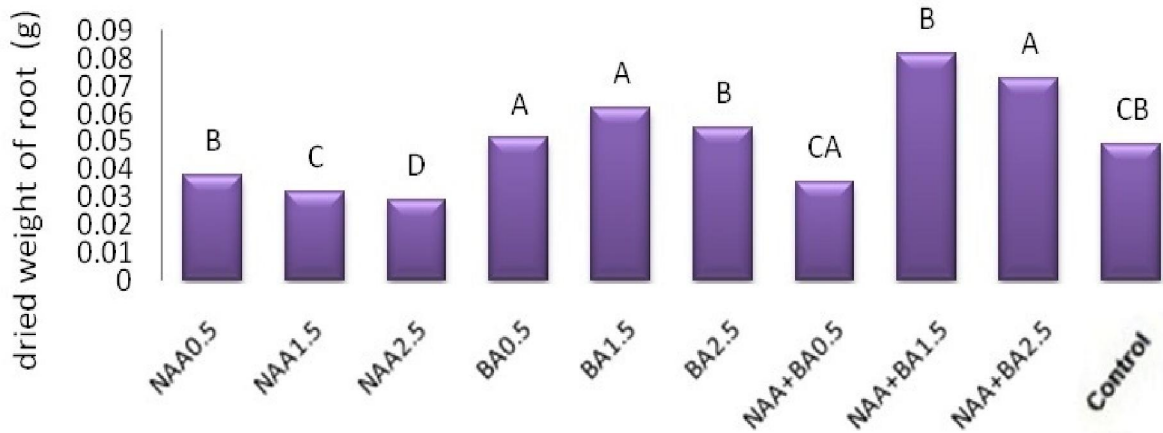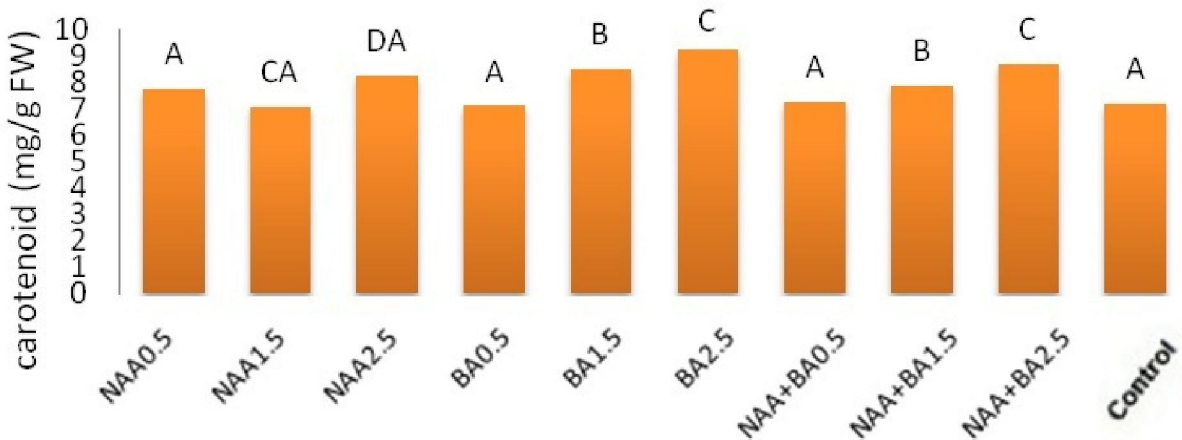

Diagram 11: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of carotenoid (mg/g FW)
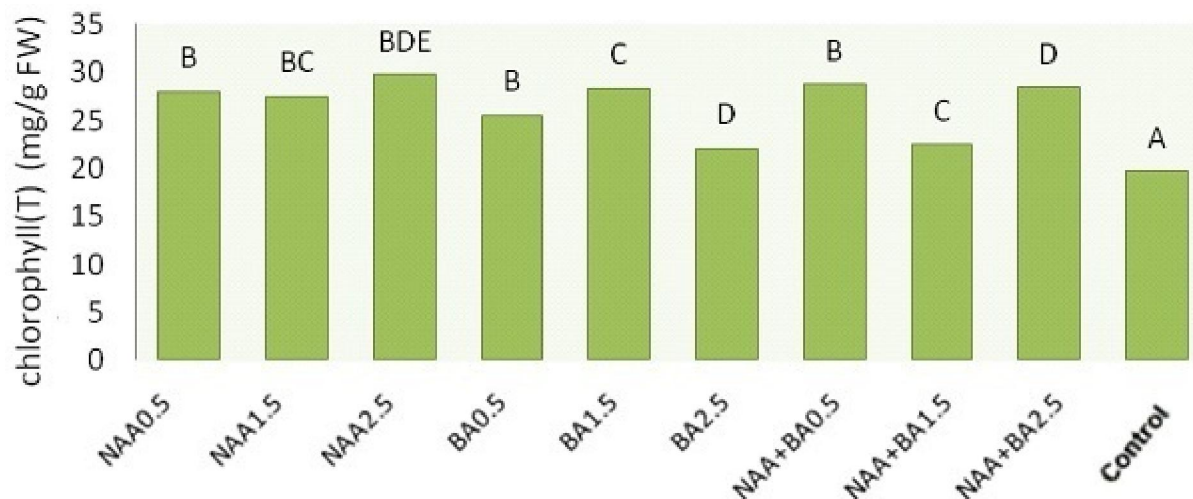
Diagram 12: The effect of difference treatments (NAA; BA; NAA+BA) on the total amount of chlorophyll (mg/g FW)

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Environmental Studies on the Effect of Air Pollution on Natural Forests Deterioration of Juniperus (*Juniperus procera*) in Saudi Arabia

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Abstract: This study aims to evaluate the effect of air pollution by exhausts of different transportation means on *Juniperus procera* forest. Several forests were selected at Al-Baha region (western-south portion of KSA) which differs in traffic density. The study focused on air organs (leaves and branches) of Juniperus and the soil of the plant. Photosynthesis pigments, estimation of some minerals (Na, Ca, Mg, Fe, K, Zn, Pb), soil texture, soil EC, soil pH, estimation of mineral nitrogenous elements (Na, Ca, Mg, Fe, K, Zn, Pb) and amounts of sulfates, chlorides, bicarbonates salts .results revealed that there are significant differences between sites in the amount of photosynthesis pigments, increase in chlorophyll a and b with the increase of pollution degree. There are high significant differences between minerals in the plant, calcium recorded the highest values while sodium recorded the lowest values in the plant. Soil results, on the other hand, indicated that, there are high significant differences between sites in the soil texture, Ec and pH. Raghdan forest soil has the highest Ec, lowest alkalinity compared to other sites. The amount of Pb, sulfates, chlorides, Ca, Mg, Na and bicarbonates salts in the soil of Raghdan forest (of high traffic density) is significantly higher than other sites. This study has shown that (*Juniperus procera*) is of environmental importance has high potential for Adaptation to environmental stresses such as Traffic emissions, these plants used different methods to adapt such as the increase of photosynthesis pigments and accumulation of some elements in the plant tissue in sites with high traffic density, the main source of pollution and important heavy elements especially lead caused by emissions from vehicle exhausts.

Keywords: forests deterioration-air pollution-forests- Juniperus**.**

1. Introduction

The deterioration in ranges and forests leads to shrinkage and sometimes extinction of the plant cover for wide areas leading to reduction of wood and energy production leading to increase pressure over the natural resources (Alserwi, 2009). The Western South mountains represents the most variable areas in Kingdom of Saudi Arabia with high density of plant cover due to the elevation that reaches 3000m with abundant rains, and moderate temperature (Alnafie, 2004). Spread of mountain Juniperus forests which represents 95% from the areas of forests in the Western South part of the kingdom (Abu Alhasan, 1999). Contamination with heavy minerals is one of the factors affecting the world (Sidhimol *et al*., 2011). The means of transports are considered the most important sources of environmental pollution and found that the highest percentage of lead emission in air is from burning of the cars fuels (Alserwi, 2009). Lead in the current time is one of the most important heavy mineral polluting the environment and threatening the human health (Alodat, 2997).

The present research aims to evaluate the effect of air pollution with gases emitted from different means of transport on some features of *Juniperus procera* plants (Fig.1), the most abundance

type in forests in western south part from the kingdom. In addition, the effect of traffic pollution on some features of soil that affect the growth and the productivity of the plant was also studied. By fulfilling theses aims, highlighting one of the most important environmental factors leading to extinction and deterioration of plants can be overcome.

2.Material and methods:

Three forests are selected in Al-Baha area, Al- Baha area (Fig. 2) lies in the Western South part from Kingdom of Saudi Arabia lines (41/42 vertical and 19/20 horizontal). Al- Baha area is characterized by moderate mountain weather in summer and cold in winter with presence of clouds, fog, however, the coastal part characterized by hot weather in summer and moderate in winter, the average temperature in mountain area reaches 17°C and the average rain is 500ml, as the mountains heights decrease gradually towards the desert in east part, the area becomes more dry. Al- Baha area is famous of natural forests that covers most Alsrah mountains. The selected forests were: Raghdan forest (Fig.3) is a famous forest about 5km north from down town of Al-Baha city and is considered one of the best forests in Al-Baha area and one of the largest forests in Kingdom of Saudi

Arabia; Shahbah forest lies in east north of Al-Baha and is about 6 km away from city of Al-Baha and Alghemdah forest which is about 2km away to the south from Al-Baha city. Difference in traffic intensity has been taken into account. Transport intensity varies from being high in Raghdan area (site 1) to be less in Shahbah forest (site 2) and Alghemdah forest (site 3), Fourth site, away from traffic roads, (site 4) was selected to represent the least pollution with gases emitted from different means of transport.

Samples of plants from the upper parts of the plants (leaves and branches) from the trees lying on the traffic road in the three forests are collected together with soil samples from under the plant and from the root complex of the plant (from surface till depth of 30cm) . The soil samples were collected ,air dried then sieved with sieve which pores diameter is 2mm to separate stones and rocks, and estimate the form of the soil using different pores sieves according to system of the international association of earth sciences (Futh, 1985). Then measure the pH for the extract using pH meter (WTW mode 512), while the electrical conduction (Ec) is measured using EC-meter (Matter Toledo- AG) and mineral elements (sodium, calcium, potassium, magnesium, , iron, zinc, lead) were measured in the extract of soil using Atomic absorption Variance Spectr AA 10- Perk in Elmer Spect AA 3100 , using Photo Lab Spektral WTW to measure phosphorus , Nitrogen is measured using ionic separation using the apparatus (Ion chromatography Dionex 50), then determine amount of chlorides dissolved in aquatic extract off the soil using titration method with silver nitrate (Tilrator Mettler DL 55 (0.01N), while using ionic separation to determine amount of sulphates using ionic separation apparatus (Ion Dionex 50 chromatography), then determine amount of bicarbonates in the aquatic extract of the soil using titration method with hydrochloric acid (Tiltrator Mettler DL 55 (0.01N)).

Moreover, sodium calcium, potassium, magnesium, iron, zinc, lead were measured in the plant extract after digestion of plants according to method of Allen 1975 (Westrman, 1990). Determine the amount of stains of photosynthesis as the amount of chlorophyll is determined using the method of (Lichtenthaler, 1987) extracting it from known weights of fresh plant leaves (0.2) and putting them in test tubes then add 5ml (dimethyl formamid) then wrap the tubes with aluminum papers and cover with parafilm papers and leave in refrigerator for 24 hours then filter the extract and measure the degree of the absorption for this extract using spectrophotometer at the wave lengths 664.5, 647, 452.5 nanometer, using the following equations then measure the stains of the photosynthesis:

Chlorophyll $a = 12.7\ A664.5 – 2.79\ A647.0 = \mu/ml$
Chlorophyll $b = 20.7\ A647.0 – 4.62\ A664.5 = \mu/ml$
Carot. $= 4.2\ A452 – (0.0264$ Chlorophyll $a + 0.426$
Chlorophyll $b) = \mu/ml$

These results is estimated from the original sample weight and express the amount in milligrams for each gram of the fresh material and care for estimation of dilution.

Statistical analysis:

The statistical analysis of the results was done in terms of means and standard errors (± SE). In addition one way analysis of variance (*ANOVA*), using SPSS (SPSS, 2012) computer program.

3.Results :
Soil Characters :

The results of chemical and mechanical analysis (Table 1) for the soil in different sites shown that silt represents the highest percentage between soil granules in all places of study, the percentage in Raghdan forest soil was 46% and increased in other sites, reaching 71% in Shahbah forest, 61% in Alghemdah forest and 55% in site 4. On the contrary, sand particles percentage was low. It was 44% in Raghdan forest, 13% in Shahbah forest, 24% in Alghemdah forest and 27% in site 4, while for amount of silt ranged between (10, 16, 15.2, 17%) in Raghdan, Shahbah, Alghemdah and site 4, respectively. Therefore, the soil considered as silt soil in all site in the study area, even it differs from site to other. except in soil of Raghdan forest, wherebit is considered as sandy slit.

Also clarify that that the soil was slightly alkaline in Raghdan forest and site 4 (6.8) and (6.9) respectively, alkalinity rises more in forests of Shahbah and Alghemdah, (7.1) in both sites (Table 1). It is shown that the highest degree of electrical conduction (3385 milimos/cm) was recorded in soil of Raghdan forest, compared to other sites

Soil mineral elements (Table, 2), showed highly significant differences in contents of sodium, potassium, magnesium, calcium and zinc, also amount of phosphate, sulfate, chloride, while there is no significant difference between sites in contents of lead, iron, and nitrogen the values generally are high in soil of Raghdan in terms of calcium (657 mg/liter), magnesium (68.4 mg/liter), sodium (65.6 mg/liter), lead (0.685 mg/liter), amounts of both chlorides (360.5mg/liter), sulfates (787.5mg/liter), bicarbonate (103mg/liter) compared to other sites.

plants Characteristics:

The Results in Fig.(4) indicates that there is significant difference in the amount of chlorophyll A, B and the percentage of chlorophyll A to chlorophyll

B between the different sites, while there are no significant difference in carotene content in plant in all different sites. Unexpectedly, the results showed an increase in chlorophyll A and chlorophyll B with the increase in concentration of pollutants that accompanied with the increase in traffic movement. This was clear in Raghdan forest, where the amount of chlorophyll A, B were (26.3mg/g), (19.1mg/g) and

reaching the lowest value in site 4 (17.3mg/g), (11.4mg/g).

On the other hand, the result in Fig. (5), showed high significant differences between different sites of elements. Moreover the increase in the amount of elements in plants followed the increase in concentration of pollutants, with the calcium elements recorded the highest values, compared to other elements.

Fig.(1):Juniperus procera in Raghadan forest.

Fig.(2): Site Al- Baha area (the study area) in the Western South part from Kingdom of Saudi Arabia.
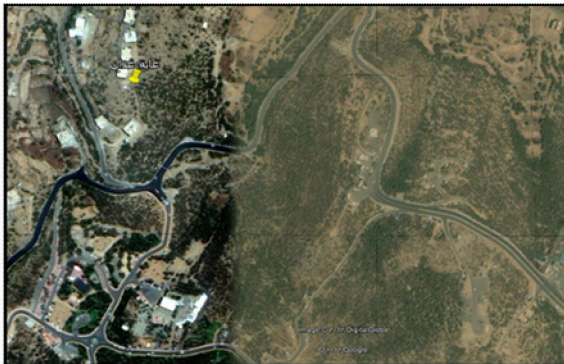
Fig.(3): Raghdan forest and It seems clear impact road construction traffic on the deterioration of forest
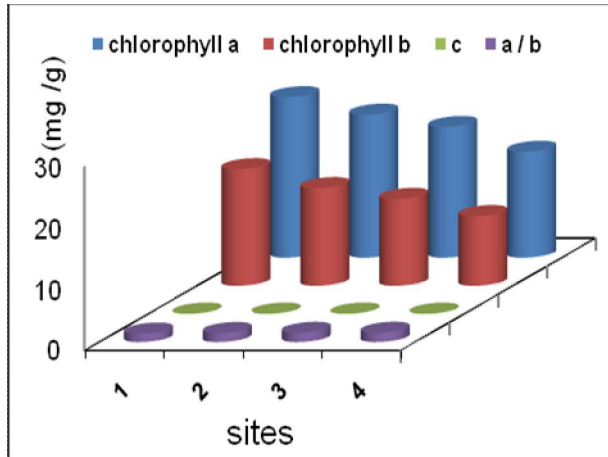
Fig.(4):the amount of photosynthesis pigments of *Juniperus procera* from different forests.
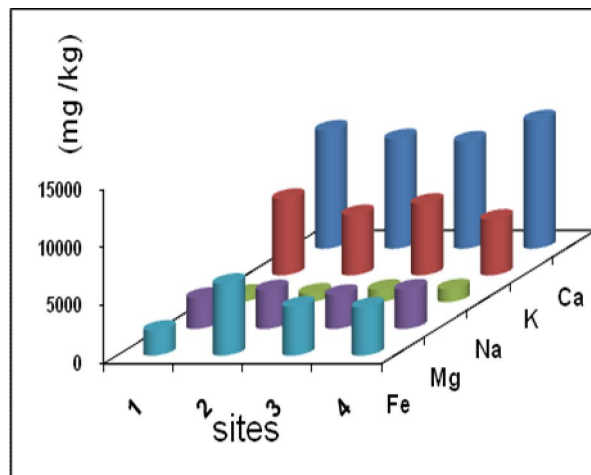
Fig.(5): Chemical analysis of *Juniperus procera* from different forests.

Table (1): Soil Fraction, ECe and pH of soil for *Juniperus procera* from different forests.

Sites	Soil Fraction (%)			EC mmohs/(cm)	pH
	Clay	Silt	Sand		
1	10 ± 0.27	46 ± 1.02	44 ± 1.23	3385 ± 598	6.8 ± 0.00
2	16 ± 0.93	71 ± 0.93	13 ± 0.52	336 ± 48.8	7.1 ± 0.03
3	15.2 ± 0.22	60.4 ± 1.07	24.1 ± 1.11	563 ± 6.06	7.1 ± 0.03
4	17.3 ±1.11	55 ± 1.32	27.3 ± 1.47	341 ± 18.19	6.9 ± 0.06
Sig.	**	**	**	**	**

*   significant   **   high significant

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Table (2) : Chemical analysis of soil for *Juniperus procera* from different forests.

Sites	Mineral elements (mg/L)											
	Ca	K	Na	Mg	N	P	Fe	Zn	Pb	HCO$_3$	Cl	SO$_4$
1	657± 121	1.3± 0.52	65.6±10.2	68.4 ± 12.5	1.86± 0.84	1.56± 0.11	<0.10	6.15± 0.20	0.685± 0.08	103 ± 12.1	360.5± 72.5	787.5± 96.7
2	41.3± 5.20	1.8± 0.26	18.7± 5.11	3.6 ± 0.41	1.13± 0.10	0.76± 0.17	0.63± 0.27	1.51± 0.27	0.515± 0.11	66 ± 2.31	11.5 ± 4.91	122.5±1.44
3	80.7± 5.40	23 ± 0.61	3.1 ± 0.23	13.9 ± 0.09	2.63± 0.91	13.4± 1.07	0.27± 0.07	6.68± 0.41	0.540± 0.11	77.5± 6.64	< 1.0	147.5± 1.44
4	45.8± 2.08	1.8± 0.03	5.25±0.03	6.4 ± 0.09	1.13± 0.07	0.45± 0.08	0.22± 0.03	1.68± 0.03	0.485± 0.03	59.5± 8.37	< 1.0	132.5± 1.44
Sig.	**	**	**	**	N.S	**	N.S	**	N.S	*	**	**

* significant   ** high significant   N.S non significant

4.Discussion

The present study was an attempt to evaluate the effect of traffic activity and The air pollutants emitted from different means of transport on some forests which differ in traffic density at Al-Baha region (western-south portion of Saudi Arabia). Cars and different means of transport are the main source of air pollution especially in the towns (Alodat,2007). In addition, (Harrison *et al*., 1981; Roy *et al*., 1981; Zhang *et al*., 1995 and Badino *et al*., 1998) reported that the highest concentrations of heavy elements such as lead ,zinc, manganese and iron for all of their studies samples connected with high traffic movement and density. This is an important indicator for accumulation of elements emitted from vehicles engines on long-term leading to increase of pollution rate.

This study was made at Juniper forests, where more vegetation patterns are affected by human-induced pollutions (Migahid, 2006). Juniperus plant is one of the main vegetation groups in Saudi Arabia (Ministry of Agriculture and water, 1984), spreads at Alsarawat mountains constituting juniper forest which representing 95% of forests area. Juniperus grows in mountains above 1700m above sea surface, increase intensity in mountains higher than 2000 m above sea surface level (Zohary, 1973). follows the species of (Cupressaceae) which is one of the oldest tree living in Kingdom of Saudi Arabia as some trees reaches thousands of years (Chaudhry, 1999). The plant study (*Juniperus procera*) is a long living tree present in the kingdom in the western mountains south of Taif, and present in the world in Yemen and mountains in east Africa in Sudan and Ethiopia, then south to east Congo, and east north Zambia (Alnafie, 2004).In addition to the plant medical value , its uses in folk medicine and modern medicine are widely known as is mentioned by several researchers (Alshanawani,1996; Rifaat,2003; Shams Al-din, 2006 and Alkahtani, 2008).

The soil pH ranges from weak acidic at sites 1 and4 to weak alkaline at sites 2 and 3. Nevertheless, it was near the neutral point ,the optimum pH for plant growth (6.5-7.5), as was reported by (Migahid, 2009). The study clarified the high significant variation in electrical conductivity at different sites, where it is highest level in Raghdan forest soil compared to other different sites. There is a positive correlation between the increase of electrical conductivity of soil's solution and all mineral elements in addition to chlorides which act as anions connecting with the elements in the soil leading to increase of soil salinity (electrical conductivity).

The results showed the difference of mineral elements concentration between plant and soil in all studied sites. The outcome of mineral elements in the plant indicates that calcium is the most accumulative element inside the plant and in the soil. *Juniperus procera* contains many compounds around 80 compounds , alkaloids , organic acids and cardiac glycosides in addition to salts ,calcium is the most Important one(Alkahtani,2008). The potassium level recorded decreased at all sites but the concentration of potassium in the air branches was higher than its concentration in the soil which indicates necessity of potassium to plants in the different vital activities. Plants need potassium at high levels to activate some enzymes and act an essential role in water balance inside the plants ,in conversions of some carbohydrates. Despite of potassium availability in the crust but almost it is unavailable for plants (Omar, 2009).

The present results clarify that the decrease in sodium accumulation in the plant tissues comparing with other elements may be due to the high potassium concentrations which caused antagonism between them . It is noticed the lack of iron and magnesium in the plants of Raghdan forest which may be due to the plant need for both elements and consumes them in photosynthesis process. Iron plays an important role in chlorophyll construction and lack of this element causes mosaic leaves (Ingested, 1973). Magnesium, on the other hand, is important constituent of chlorophyll (Buran , 2009). Generally, magnesium is available to the plants through ionic exchange in the organic compounds or the mud which contains high level of magnesium, the lack of this element in the soil is due to presence of

high sodium, potassium and calcium concentrations (Omar, 2009) .

The present results indicate the significant variations of zinc concentrations among the different sites soil which may be contributed to role of weather factors especially wind that can help in transmitting and spreading the pollutants. The same is true concerning zinc amounts inside the plant leaves.

Moreover, the presence of highest level of lead element presents in the soil of Raghdan forest where the highest traffic density among the sites .some lead compounds are added to car fuels(benzene)to relieve the intensity of engine Explosion which are used at wide range globally. the lead percent in the air depends upon the traffic motion and density (Buran, 2009). Lead accumulates easily in the soil and different plant parts (Sharma and Dubey, 2009). but the lead accumulation highly in the roots and definite amount transmit to the other plant parts due to presence of natural plant barriers in the root (interior epidermis) (Sengar *et al*., 2008). The concentrations of the lead is significantly high in the airy branches of *Juniperus procera* growing at Raghdan forest more than other sites. the amount of lead is relatively high in the green leaves due to the deposits of air pollutants (Koeppe,1977, Zimdahl and Koeppe, 1979, Adriano, 1986). Lead exists naturally in all plants ,soil, water and air. The normal concentration in the leaves and small branches of wooden plants reaches 2.5 mg/kg dry matter (Who, 1977 . Singh *et al.*, (1997) Stated that some plants are able to tolerate excess amount of lead by different ways such as elimination, reservation or construction of antagonistic elements against the toxicity of it as plant chelating elements.

Many researchers studied the effect of lead on the photosynthetic pigments content in the plants by various concentrations, the researches indicate great decline of photosynthetic pigments content at high lead concentrations (Sidhimol *et al*., 2011). The total photosynthetic pigments content deceases with high concentrations of heavy elements such as lead (Zengin and Munzuriglu, 2005). Lead decreases photosynthesis rates of plants through the decline of chlorophyll construction due to Chloroplasts malformation, inhibition enzymes of Kelvin cycle (Sengar *et al*., 2008). But the results of the present study showed an increase of photosynthetic pigments (chlorophyll a and b) with the combination of high lead concentrations in the soil and plant which indicate the tolerance ability of this plant to this pollutant. There is significant variations in photosynthetic pigments amount, the highest percent at Raghdan forest and the lowest percent at site 4. There was significant variation in the amount of chlorophyll (a / b) at the traffic roads which indicates

the effect of pollutants on both chlorophylls types in the plants at different sites. There isn't significant variation in carotenes amount at all sites. the scientists (Zhang *et al.,* 2011) found that there is high amount of chlorophyll a, chlorophyll b and carotenes at high concentrations of lead and cadmium . Abdel Ghaffar *et al*. (2000) Concluded that most copper and lead factors lead to increase in photosynthesis pigments in wheat but do not affect the inter-correlations among chlorophyll a, chlorophyll b and carotenes.

5. Conclusion:

The *Juniperus procera* plants were high environmental value and the main plant at Western-South forests in Saudi Arabia. This plant has high ability to adapt the environmental stresses in terms of traffic emissions, by using different adaptation strategies such as increasing the photosynthetic pigments and accumulating reasonable amount of heavy metals despite the heavy traffic nearby. Accumulation of heavy metals inside the plant tissues such as zinc and lead at Raghdan forest plants at the sites of high traffic density, indicates that air pollution by vehicles exhausts emissions is the main source of pollution. the traffic activity is the main source of pollution by heavy elements especially zinc and lead.

The study recommends to do more investigation of the physiological studies about how *Juniperus procera* can resist environmental stresses such as the traffic emissions that increase the concentration of heavy elements in the plant, and put an effective laws to protect forests from vehicle exhausts . And the most important of them, do not construct roads through forests so that we can control one of the most important environmental factors that affecting in degradation and extinction of forest.

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The Mean first Birth Interval and Popular Contraceptive Method Use Before First Pregnancy in Women Attending Public Health Center of Ahvaz City, Iran 2010

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The period between the marriage to first pregnancy is one of the most important indicators for fecundability. The aim of this study was to estimate spacing from marriage to first pregnancy and popular contraception methods which used in women at the beginning of marriage. In a cross- sectional study 900 primiparus   women attending health centers after first delivery were studied.   Filling a questionnaire by trained questioner was used for data collection. SPSS 17 used for data entering and analysis. Chi square, Pierson correlation test and logistic regression were done. The mean age of marriage was 21.9 ± 4.21 years. Short interval between marriage to first pregnancy was reported in majority (62.7%) of women. The mean interval between marriage to first pregnancy was estimated 11.8 ± 13.5 months (median interval 8 months). 68.3% of women had used at least one type of contraceptive methods before pregnancy. Prevalence of unwanted pregnancy was 19.5%. Logistic regression showed a significant association between use of contraceptive methods and educational level of women and her husband, women age, ageat marriage and unwanted pregnancy. More continues education at the beginning of marriage help these couples make better decisions about the timing of their first pregnancy.

Keywords: first birth interval, newly married couples, contraception, family spacing, health centers

1. Introduction

Population change is a global pattern. This phenomenon varies even among countries within a region (1).In Iran many aspects of family and gender have changed. Rapidly decline of fertility during 1985-2000,increased women's educational level from 36% in 1976 to 80% in 2006,inspte of traditional pattern in marriage(getting marred eventually, consanguinity, marriage in relative younger age) make a mixture of family change in Iran(2).

Although total fertility rate (TFR) estimates   the effect of population policies, but it seems we need more indicators for estimation of fertility parameters. The period between marriage and first pregnancy has served for the estimation of fertility indicators, especially fecundability(3,4).Without use any method of contraception, based on normal physiology of women, About 25% of women will be pregnant in each cycle and 80-90 of women will experience pregnancy in the first year of marriage if couple don't use any contraceptive method. (5)

As an important human reproductive right, couples must able to decide freely and responsibly timing of birth, number of children and spacing between of their birth. Despite decrease the total fertility rate in Iran, Millions of children who were born before approval rules rated to population control in Iran are now reaching the age of marriage and childbearing (3, 4).Short interval between marriage and first birth is a potential risk factor for costly and excessive increase in the growth of population (3).Fertility as an essential population dynamic's component play an important role in changes in combination and size of a given population. Many direct and indirect factors affect on fertility. Age of being sexually active, use of contraceptive methods, abortion and sterility are direct factors which are related to biology. Socio- cultural factors affect on fertility indirectly by affect on bio-behavioral factors. Longer intervals between marriage to first pregnancy decrease the number of children a woman can have (6).Beneficial effects of longer interval on population size and on mothers and children health is well documented. Short spacing between marriage to first birth introduced as a risk factor for duration of breast feeding, physical and psychological development of children (7).

In the other site, annually approximately 200 million pregnancies occur in the world, which more than one third of them are unwanted (8). Each year more than half a million women die of pregnancy-related illnesses or lose. Unwanted pregnancy and illegal abortion result in unwanted pregnancy is cause of about one fourth of maternal deaths , In addition to unwanted pregnancy associated with many poor outcomes in new born(9,10). Studies

have shown that first unwanted pregnancy is associated with many problems including increase in divorce (7).

Proper use of contraceptive methods improves the quality of life for of couples and have the freedom and to decide about number of children and proper interval between their pregnancies. Use of contraceptive methods in newly married couples, not only is very important for population growth control but also will be given an opportunity, to adjust with new conditions (3).Necessitates the use of contraceptive methods at the beginning of the marriage was the most important objective of approval of premarital counseling centers in 1999 in Iran (7).

Due to importance of use of contraception in the first years of marriage, no existence data about first birth interval in this region, we designed this study to determine the mean interval between marriage to first pregnancy and popular contraceptive method use before first pregnancy in women attending health center of Ahvaz city.

2. Material and Methods

In this cross- sectional study, 900 married primiparus women attending west and east health centers of Ahvaz after first delivery(Interval<6 month) were studied in 2010. Ahvaz is capital of Khuzestan province where is located in southwestern of Iran. The study was carried out from June to December of 2010.Every women seeking routine care for children or family planning services during first 6 months after delivery included study after acceptance. Women were assured of the confidentiality of their information. We used an interviewer-administered questionnaire for data collection. Trained investigators attended the selected health centre for3 days a week.

The data collection instrument was a questionnaire consisting of 28 questions. Validity of the questionnaire was evaluated by expert instructors and reliability by Cronbach's alpha (0.81). Variables included female's age, age at marriage, employment status and educational level of women, husbands education and reproductive behavior before first pregnancy. SPSS 17 used for data entering and analyzing. Chi square, Pierson correlation test and logistic regression were done.

3.Results

The results of the study on 900 primiparus mothers attending health centers showed that the mean age of women was 24.5 (standard deviation 4.8) years. Demographic characteristics of the women in this study are presented in Table 1. The mean age of the husbands of the participants was 28.9 (standard deviation 5.5) years. Of couples 5.9% of them were in same age and 65.8% had less than 6 years differences in age. The mean age of women at marriage was 21.9 (standard deviation 4.21) years. The mean duration of marriage was 31.69 months.

Table 1. Demographic characteristics of women under study

	Number	Percent
Women's age		
15-18	100	11.1
19-29	664	73.8
30≥	136	15.1
Husband's age		
17-19	47	5.2
20-29	558	62.0
30≥	295	32.8
difference between couple's age at marriage(year)	53	5.9
No difference	539	59.9
1-5	249	27.7
6-10	48	53.0
11-15	7	0.8
16-19	4	0.4
20≥		
Women's age at marriage		
13-17	139	15.4
18-24	515	57.2
25-29	205	22.8
30-36	41	4.6
Duration of marriage(month)		
1-11	115	12.9
12-23	203	22.7
24≥	575	64.4
Women's ethnic group		
Fars	354	39.3
Arab	381	42.3
Lor	165	18.3
Women's education		
Less than high school	234	26.0
High school	433	48.1
University	233	25.9
Interval between marriage to first pregnancy		
No space	103	11.4
1-11 months	462	51.3
12-23 months	178	19.8
≥24 months	157	17.4
Duration of contraception use(months)	355	57.7
1-11	171	27.8
12-23	89	14.5
≥ 24		
Type of pregnancy		
Wanted	725	80.5
Unwanted	175	19.5
Women's Job		
Housewife	717	79.5
Occupied	185	20.5
Use of contraceptive methods		
Yes	615	68.3
No	285	31.7
Type of contraception		
OCP	365	40.6
Condom	188	20.9
Natural	171	19.0

*All of data less than 900 is related to missing data

The mean interval between marriage and the first pregnancy in this study was estimated 11.9(standard deviation1.35) months. 11.4% of women experienced pregnancy at the first month of marriage. 62.7% of mothers experience first pregnancy before the end of first year of marriage. The mean interval of marriage to first pregnancy increased from 5.9 months to 17.01 months with increase age of women. The lowest mean interval between marriage to first pregnancy were estimated in 2 aged groups, women getting married less than 18 and more than 30 years. The lowest mean interval of marriage to first pregnancy was reported in Arab ethnic group. Increasing level of education in women result in increase interval between marriage to first pregnancy from 9.2 months to14.05 months. With increase differences in age of couples, interval between marriage to first pregnancy reached from 13.7 months to 4.2 months (table 2).

Table 2. Mean (standard deviation) interval between marriage to first pregnancy based on women's characteristic

Women's characteristic	Mean($\pm$ SD)
Women's age 15-18 19-29 ≥30	5.9($\pm$ 7.5 11.6($\pm$ 11.6) 17.01($\pm$ 21.2)
Husband's age 17-19 20-24 ≥30	3.91($\pm$ 5.3) 11.01($\pm$ 11.3) 14.5($\pm$ 17.0)
Women's age at marriage 13-17 18-24 25-29 30-36	9.5($\pm$ 12.3) 12.7($\pm$ 14.4) 11.5($\pm$ 12.1) 9.19($\pm$ 10.5)
Women's ethnic group Arab Lor Fars	9.17($\pm$ 12.07) 13.7($\pm$ 14.07) 13.7($\pm$ 14.2)
Women's education Less than high school High school University	**9.2($\pm$ 12.7)** **11.04($\pm$ 3.9)** **14.05($\pm$ 13.1)**
Type of pregnancy Wanted Unwanted	**13.6($\pm$ 14.1)** **4.5($\pm$ 6.9)**
Use of contraceptive methods No Yes	**4.6($\pm$ 10.3)** **15.09($\pm$ 13.5)**
Participate in premarital consuling No Yes	**11.5($\pm$ 12.1)** **14.5($\pm$ 22.6)**
Type of contraceptive methods OCP Condom Natural	**17.4($\pm$ 14.9)** **14.1($\pm$ 9.6)** **12.3(10.6)**

The mean duration of contraceptive use before first pregnancy was 11.8±9.6 (range 1-66)   months. The minimum duration of contraceptive methods use was reported in the Arab women 10.7 months. With increase level of education of Spouse, the mean duration of use of contraceptive methods was 12.8 months vs 10.7 months (Table 3). . With increase level of education of Spouse, the mean duration of use of contraceptive methods was 12.8 months vs 10.7 months (Table 3).

Table 3. Mean and Standard Deviation of Duration of contraception use at beginning of marriage

Women's characteristics	Mean($\pm$ SD)
Women's age 15-19 19-29 ≥30	8.9($\pm$ 8.09) 11.7($\pm$ 9.3) 14.05($\pm$ 11.05)
Husband's age 17-19 20-29 ≥30	5.9($\pm$ 4.9) 11.6($\pm$ 9.1) 12.7($\pm$ 10.6)
Women's age at marriage 13-17 18-24 25-29 30-36	11.5($\pm$ 11.3) 12.3($\pm$ 9.6) 11.2($\pm$ 8.7) 8.2($\pm$ 8.4)
Women's ethnic group Arab Lor Fars	10.7($\pm$ 10.07) 12.5($\pm$ 10.1) 12.4($\pm$ 9.07)
Women's education Less than High school High school University	9.8($\pm$ 10.03) 11.96($\pm$ 9.07) 12.9($\pm$ 10.1)
Type of pregnancy Wanted Unwanted	12.8 ($\pm$ 9.8) 6.7($\pm$ 7.2)
Participation in premarital counseling No Yes	11.8($\pm$ 9.4) 12.3($\pm$ 12.5)
Type of contraception OCP Condom Natural	13.3($\pm$ 9.9) 12.6($\pm$ 9.1) 10.6($\pm$ 8.8)

Pearson's test showed a significant correlation between interval between marriage to first pregnancy and women and their husband's age and duration of contraceptive method use in couple(p<0.5) .Logistic regression showed a significant association between use of contraceptive methods and educational level of women and her husband, women age, age at marriage and unwanted pregnancy.

4. Discussion

In present study the mean interval between marriage and the first pregnancy i estimated 11.9 months. Rindfuss and morgan reported shorter duration

between marriage and first birth in Taiwan, Malaysia and South Korea (11, 12). In a cross- sectional study in Sanandaj city in Iran 21% of women were pregnant in the first year of marriage and 11% had a child (7).

We estimated use of at least one method of contraception before first pregnancy in this population, 68.3%.   In contrast with our results, Rindfuss and Morgan, Wang et al in Asian women, reported no contraceptive method use before first pregnancy. They concluded increase in intercourse in early years of marriage is cause of short space between marriage and first.

Pregnancy (11, 12). Similar pattern were reported by Wang and Quanhe in China(2,12).

Singh et al showed use of contraception in 19.7% of Indian women before first pregnancy (4).9.9%   of women   in Pakistan used   birth control methods between marriage and first pregnancy(13). Ahmed et al reported use of contraception in 41% of women before first pregnancy (14). Che et al in 50% of newly marred in china reported use of contraceptive methods (15).

In a demographic study in Iran in 2002 use of contraception in the first year of marriage reached from 3.4% to 20% with Different pattern in cities of Iran (16). Abbasi-Shavazi et al reported use of contraceptive methods in newly married women in Iran as a common pattern (2). Socio- cultural context may affect on variation of this pattern.

In this study popular contraceptive method in women before first pregnancy was OCP, Condom and natural respectively. Ahmed et al reported OCP as the most common method (69.9%) in newly married women in Bangladesh (14). Popular contraceptive method in Turkish women was condom (17). Che et al reported most popular contraceptive method condom, natural and rhythm respectively (15).Prevalence of unwanted pregnancy in this population was 19.55. Unwanted pregnancies were reported as a common phenomenon (21-40%) in married couples in Shanghai, due to nonuse of effective contraceptive methods (18).

Conclusion: The use of contraception in newly married couples regardless of the duration of use is increasing. Prevalence of unwanted pregnancy in women after first delivery attending health center is high. Understanding interval between marriage to first birth and its determinants is helpful to design interventional strategies in women attending health centers. More continues education at the beginning of marriage help these couples make better decisions about the timing of their first pregnancy.

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The production of nanocrystalline Titanium carbide compound via mechanical alloying

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Abstract: A nanocrystalline/nanoparticleTiCrefractorycompound has been produced by mechanical alloying (MA) of the elemental powders. The phase evolution and microstructural changes of the powders during mechanical alloying were investigated by means of X-ray diffractometry, field-emission scanning electron microscopy, high-resolution transmission electron microscopy and microhardness measurements. The results showed that after an optimum mechanical alloying time of 15 hours, a TiCrefractorycompound with thecrystallite size of less than 10 nm, the particle size of about 80nm and the latticestrainof 1.8% was achieved.The obtained TiC refractorycompound exhibited high microhardness value of about 708 Hv.

1. Introduction

Titanium carbide (TiC) is thermodynamically stable which has a very high melting point (3100 ℃) and does not decompose or undergo phase transformations in any temperature range of interest[1, 2]. It has metallic characteristics (luster and metallic conductivity), extraordinary hardness and toughness, and excellent resistance to wear, abrasion and infusibility. Therefore, TiC has been widely produced for some industrial applications such as hard coating to protect the surface of cutting tools from wear and erosion[3]. Normally, titanium carbide is produced by high-temperature processes such as the displacement reaction of titanium oxide and carbon, direct reaction of elemental Ti and C, and self-propagating hightemperaturesynthesis (SHS)[4]. In most cases, these techniquesrequire expensive furnaces and high temperature which should behigher than the melting point of TiC. Moreover, it is difficult toobtain nano-sized TiC powders by these methods.Mechanical alloying (MA) is a rapidly developing technology capable of producing a variety of materials, such as refractorycompounds, metastable solid solutions, amorphous alloys, nanocrystalline materials, etc.[5-7]. In comparison to other fabrication techniques, MA process has the advantage of the final product possessing a nanocrystalline structure with superior properties rather than conventional coarse-grained structure [8-10]. Up to now, several compoundssuch asWC[11], AlNi[12], FeAl[6, 13], NiTi[14], TiFe[15], FeNi[16],AlRu[17], CoAl[18], MoSi$_2$[8],Ni$_3$Al [9, 19, 20],NbAl$_3$[21], Ni$_3$Fe [16, 22] and Ni$_2$AlTi[23]have been fabricated by mechanical alloying. Thisstudyaims to investigate the feasibility of producingsimultaneous nanocrystalline and nanoparticleTiCrefractory powdervia mechanical alloying process. Phase transformation and structural and morphological evolutions occurring during the mechanical alloying process have also beeninvestigated.

2. Experimental procedure

In this investigation, Ti (99.8% purity) and graphite as a source of carbon (99% purity) powders were used as starting materials. Both Ti and C powders had an irregular shape with particle size of less than 10 μm.Mechanical alloying was performed for different milling times using a high energy planetary ball mill machine (Retsch PM400).The milling media consisted of 15balls with the diameter of 20mm confined in a 500 ml bowl. Both the ball and the bowl materials were hardened chromium steels. In all MA runs, the ball-to-powder weight ratio was 10:1 and the bowl rotation speed was approximately 350 rpm. In all experimental operations, Ti and C powders in 1:1 molar ratio (Ti$_{50}$C$_{50}$) were mixed together and then milled inargon atmosphere at the room temperature. Stearic acid (CH$_3$(CH$_2$)$_{16}$COOH) in the quantity of 3 wt.% was utilized as Process-Control Agent (PCA) material to avoid sticking of the powders to the vial and balls.Small amounts of the powders were taken out at selected times as samples for the structural analysis. Samples structures were characterized by a Philips X'PERT MPD X-ray diffractometer with CuK$_\alpha$radiation (k = 0.1542 nm).The crystallite size and internal strain of the samples was calculatedfrom the XRD patterns applying the Williamson–Hall method[24].

$$\beta \cos \theta = \frac{K\lambda}{d} + 2A\varepsilon \sin \theta \qquad (1)$$

where θ is the Bragg diffraction angle, d is the crystallite size, ε is the average internal strain, λ is the wavelength of the radiationused, β is the diffraction peak width at half maximum intensity, k is the Scherrer constant (0.91) and A is a coefficient whichdepends on the distribution of strain being near to unity for the dislocations. The average internal strain can be estimated from the linear slope of $\beta\cos\theta$ versus $\sin\theta$, and the average crystallite size can be estimated from the intersection of this line at $\sin\theta = 0$.The microstructures of the powders were characterized by field-emission scanning electron microscopy (FESEM) (Philips XL30) and high-resolution transmission electron microscopy (HRTEM) (JEOL JEM2100). The microhardness values of the powder particles were determined using a Vickers indenter (LeitzWetzlar microhardness tester) at the load of 50 g and dwell time of 10 s. For the microhardness measurements, small amounts of powder particles were mounted and their cross-sectionswere prepared by conventional metallographic techniques. Ten indentations were then performed on each sample to obtain itsaverage microhardness value.

3. Results and discussion
3.1. Structural evolution
The Ti-C phase diagram is depicted in Fig. 1. As is seen, the reaction of Ti and C in solid state (with distinct composition in the figure) can take place according to the following reaction[25]:

$$Ti + C \rightarrow TiC \quad \Delta G^{\circ}_{298\,^{\circ}C} = -180.296\,J/mol \quad (2)$$
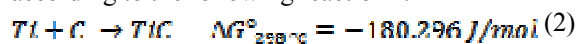
The negative free energy change of reaction (2) suggests thatthis reaction can thermodynamically occur during the room temperaturemechanical alloying of elemental Ti and Cpowdermixture, leadingto the formation ofthe TiCrefractory compound. Furthermore, it is generally believedthat the ball milling process can effectively facilitate the diffusion processby providing high diffusivity paths like dislocation lines and grain boundaries and thus acceleratethe Ti-C reaction [12, 26]. Therefore, it would be possible to produce TiC compound directlyby the MA of the elemental constituent powders. Fig.2 shows the XRD patterns of Ti-C powder mixture prior to the milling and after different milling times. According to the XRD patterns of the as-received powder,the

diffraction peaks of the pure crystalline Ti and C are evident. In the first 5 hours of the milling process, only the broadening of the Ti and C peaks and a remarkable decrease in their intensities are observed and no TiC peak can be detected.The absence of any shifts in the positions of Ti and C peaks leads to the conclusion that no reciprocal solid solution has been formed.The continuation of the milling for another 5hoursleadsto the disappearance of the most individual Ti andC peaks and subsequently appearance of TiC peaks. The transformation of elemental Ti and C powder mixture to the TiC refractory phase is completed after 15 hours of ball milling. The continuation of the milling process for an extra 5 hours brings no further structural changes in the powder. It, however, decreases the intensity of TiC peaks and increases their width as a result of crystallite refinement and lattice strain enhancement [8, 12].Up to date, two kinds of reaction mechanisms have been accepted in MA[2, 27]: (1) The colliding balls undergo severe plastic deformation, causing the flattened particles contact with clean surface, and alloys form through gradual diffusion of thin layers, which is called the gradual diffusion reaction (GDR); (2) The alloys form through a reaction taking place within a short period with the liberation of large heat after certain milling time, which is called self-sustained reaction during mechanical alloying, and it is suggested that mechanical impact plays an important role in igniting the reaction. So, the reaction mechanism is usually named mechanically induced self-propagating reaction (MSR). The reaction mechanism of Ti-C system is GDR[1, 25]. It has beenextensively reported that during GDR mechanism, initial powder particles (such as Ti and C) suffer from very strong high energy impacts attributed to collisions between balls themselves and container wall. These strong impacts will cause large amounts of microstructural and structural defects into the milled powder particles.The presence of such defect structure enhances the diffusivity of solute elements into the matrix. Furthermore, the refined microstructural features shorten the diffusion paths [27, 28]. The slight rise in temperature during the milling process alsohelps diffusion [26, 29]. Therefore, the diffusion of Catoms in some of the Tidefects reduces the stress induced by MA. In other words, the solution of C in the Ti lattice compensates the stress which is stored in the Ti lattice due to the defects induced bymechanical alloying.Considering the above results, it seems that 15 houris an optimum milling time for the production of TiCrefractory compound via mechanical alloying process. This is in agreement with previously reported results [10, 12, 19-21, 30-32]implying that it is possible to synthesizerefractory compounds directly by mechanical alloying of the elemental constituent powders.

Fig. 3 schematically shows the formation process of nanocrystallineTiC by MA of Ti and C powders. At the initialstage of milling, the carbon powders are easier to be crushed intofiner particles, while the large Ti particles are inclined to be plasticdeformed and fragmented (Fig. 3(a)). At this stage, no TiC can bedetected in the as-milled powders. With increasing milling time,the size of the carbon powders continuously decreases. Fine carbonparticles are entrapped between the Ti flakes and the particlesare brought into intimate contact (Fig. 3(b)). The intimate contactmay lead to the formation of a Ti solid solution Ti(C). The Ti(C)particles are gradually refined during the milling process. The refiningof the particles increases the contact area and activities of thereactants. With the milling proceeding, internal stress, and storedenergy increase gradually, and when the stress and energy reacha certain degree, the transformation from Ti(C) to TiC will occur[6, 27]. In this process, the transformation is believed to depend onatomic diffusion, especially the diffusion of carbon atoms[1]. Furthermore,ball milling will induce a severe internal strain, heavydefects and large volume fraction of grain boundaries, providing anexcess short-circuit diffusion pathways and enhancing the reactionrate. A little amount of TiC firstly forms on the surface of the Ti particles (Fig. 3(c)). In addition, the heat generated from the formationof initial TiC raises the temperature in the milling vial, which willaccelerate the diffusivity of atoms, especially the C atoms[24, 31]. Finally, the as-milled products are fully composedof nanocrystalline TiC and most of TiC nanocrystals tend toagglomerate (Fig. 3(d)).

The crystallite size and internal strain of TiCrefractory compound are estimated from the broadening of XRD peaks using Williamson-Hall method.In this method, both of broadening contributions, due to the strain and crystallite size,are taken into account. Fig.4 plots the TiC crystallite size and internal strain as a function of milling time. Upon ball milling,the TiCrefractory compound is readily reached toa nanocrystallinestructure. It is important to note thatthe MA treatment is an effectiveprocedure to reduce the average size domainto nanocrystalline scalein a relatively short time. After 15 hours of milling, the TiC crystallite size and internal strain reaches to 7.5 nm and 1.8%, respectively.As can be observed in Fig. 4, the crystallite size has beensharply decreased at the initial stages of the milling process while it follows aslightly decreasing trend at longer milling times. This phenomenon has also been reported by many researchers [8, 10, 19]. Ball milling is characterized by severe plastic deformation of powder particles under extremely high strain rates resulting in the generation of high density of lattice defects which areprimarily dislocations.The progressive accumulation and interaction of dislocations lead to the refinement of crystallite size as well as increase of the lattice strain[20, 21, 26]. Fig. 5illustrates the HRTEM bright field image of the powders collected after 15 hours of milling. The crystallite size of the TiCrefractory compoundisless than 10 nm, which is in accordance with that calculated by the Williamson-Hall method (see Fig. 4).

3.2. Microstructural observations

The FESEM images of the starting material and milled powder for 10 and 15 h are shown in Fig. 6. Changes in particle shapes and sizes caused by MA process are obvious. The TiC nanoparticles are slightly agglomerated. This could be related to high surface energy of nanosized particles. As can be seen from the morphologies of the powders after 15 hours of milling (Fig. 6(d)), the average size of the TiC particles is reduced to 80 nm. When MA is applied, the TiC particles undergo very strong high energy impacts and fractures resulted from collisions during process. Fragments generated by these impacts may continue to reduce the particle size in the absence of strong agglomerating forces [12]. On the other hand, the TiC is a brittle compound. Hence, the fragmentation of this fragile compound is another reason for the observed decrease in the powder particle size and less agglomeration.

3.3. Microhardness

Fig. 7plots the average microhardness values of powder particles at different MA times. In the early stages of milling, the microhardness values increase due to the formation of fine crystalline grains and high density of defects in the powder [19, 27, 31]. For the MA times between 10 to 15 hours, a considerable increase in hardness value of powder particles is observed, which is attributedto the formation of TiC phase. This is in agreement with the XRD results. After complete formation of TiCrefractory compound, increasing the MA time from 15 to 20 hours causes the hardness value to increase with a much lower rate mainly due to the decrease in the crystallite size of the TiC phase.It is worth noting that the TiC refractory compound obtained after 20 hours of milling exhibited high microhardness value of about 708 Hv.

As a result of this investigation, in contrast with other similar manufacturing processes and technologies, working at high temperatures or complex reaction conditions, mechanical alloying process could be a very efficient, practically simple and low-cost process for synthesis of pure nanocrystalline/nanoparticle TiC refractory powder with high microhardness value.Usingsuch a process to commercially producenanocrystalline/nanoparticle TiC has the potential forsignificant energy and capital

expenditure savings as compared toexisting high-temperature production processes currently used toproduce TiC.
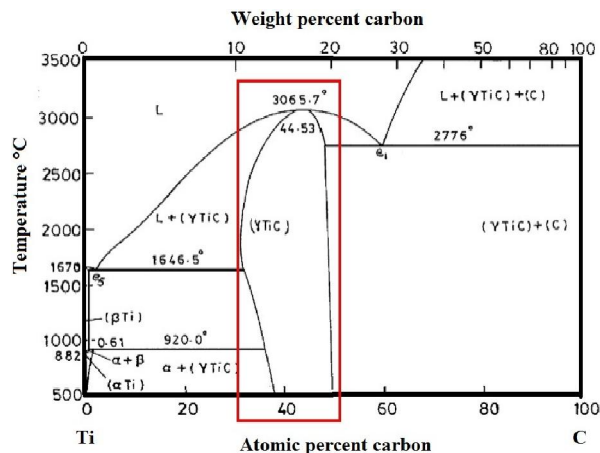
Fig. 1. The Ti–C phase diagram.
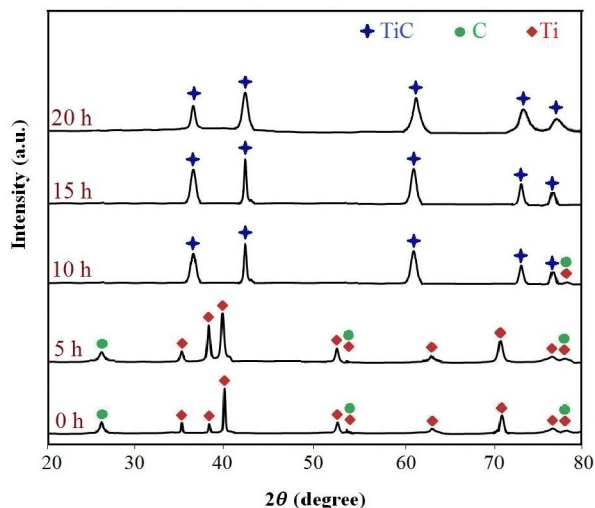
Fig. 2. The XRD patterns of Ti-C powder mixture milled for different times.
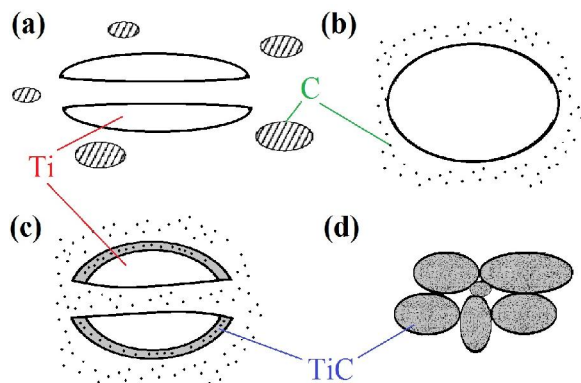
Fig. 3. A schematic showing the reaction progress of Ti and C powders during MA process.
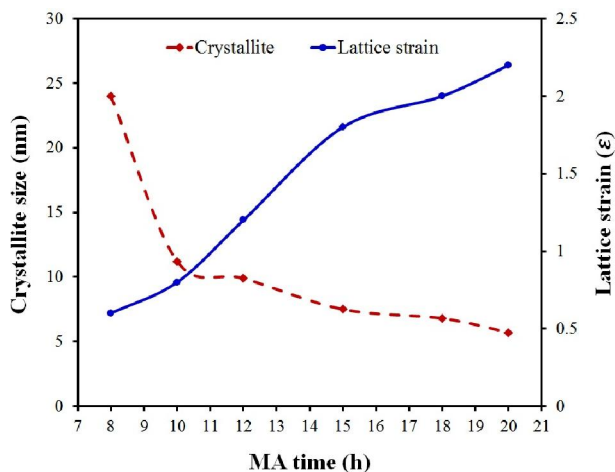
Fig. 4. The variations of the crystallite size and lattice strain of TiC powder at various MA times.
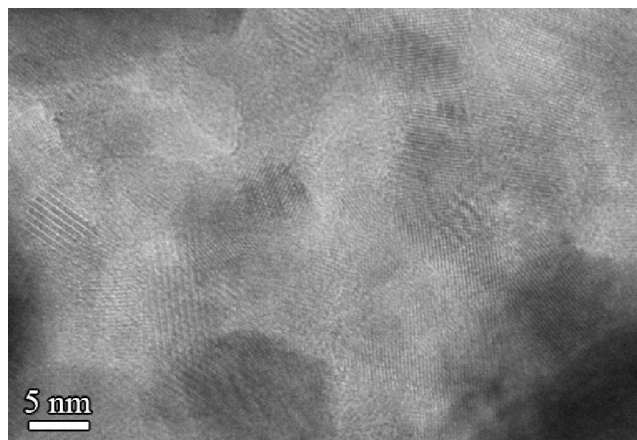
Fig. 5. The high-resolution TEM image of the TiC refractory compound prepared by 15 hours of MA.
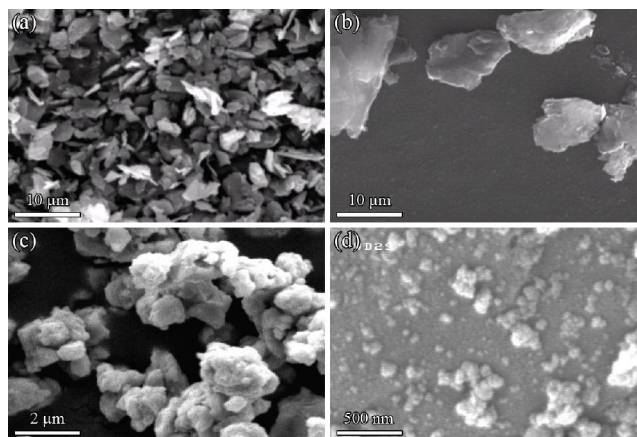
Fig. 6. Typical FESEM images of powders: (a) Ti, (b) C, (c) TiC milled for 10 h and (d) TiC milled for 15 h.
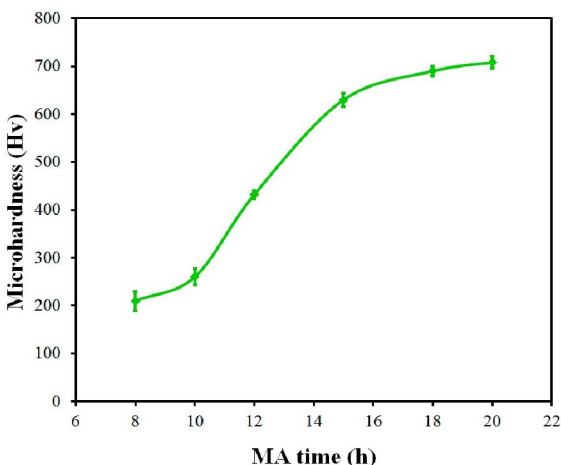
Fig. 7. The microhardness values of powder particles as a function of MA time.

4. Conclusions

Mechanical alloying of $Ti_{50}C_{50}$ powders led to the gradual formation of nanocrystalline/nanoparticleTiCrefractory powder. Results indicated that 15 hours ofmilling was the optimum time for producing the TiCrefractory compound via the mechanical alloying process. After 15 hours of mechanical alloying, a TiCrefractory compound was obtained with the crystallite size of less than 10 nm, the particle size of about 80 nm and the lattice strain of 1.8%.The achieved TiCrefractory compound exhibiteda high microhardness value of about708Hv.

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Domestic Violence Prevalence and Related Factors in Disabled Women: A Pilot Study in Women with Multiple Sclerosis

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Abstract: Domestic violence against women as a leading cause of injury in women is associated with a range of adverse physical and psychological health effects. Few studies focused on experience of domestic violence in women with disabilities. In a descriptive-analytical pilot study 150 married women 19-59 years who attending to Multiple sclerosis society of Khuzestan were studied in 2010-2011. The overall prevalence of physical, psychological, sexual and any form of violence in lifetime of women with multiple sclerosis were 17.8%, 38.4%, 6.8% and 41.5% respectively. Prevalence of domestic violence in women with multiple sclerosis is high as general population. The finding suggests the need for a study with a larger sample size in women with multiple sclerosis and other disabled women.

Keywords: Women, Domestic Violence, Disabilities, Multiple Sclerosis, Sexual dysfunction

1. Introduction

Domestic violence as one of the most common forms of gender –related violence is defined as the threat of physical, psychological, sexual and any type of force against another person with the intent to control over them(1,2).This phenomenon can occur in all socio cultural classes, regardless of any boundaries (1).

Many physical, psychological and reproductive adverse effects of violence in the nondisabled population are well documented (3).Higher risk of cardiac complaint, digestive problems, Arthritis, migraines. Sexually transmitted disease and Pelvic pain is reported in victims of violence. Anxiety, Depression, insomnia and loss of self-esteem are some psychological consequences of domestic violence. (1,4-8). greater utilization of medical services and more likely to access primary and specialty care , emergency departments   and   substance abuse services   is reported   in victim of violence than women without a history of partner violence(4). But little is known about direct and indirect negative impact of abuse on health of disabled victims (3). Disable women are at risk of exposure   to violence by family members, caretakers, their friends and others. (9) Higher risk of abuse is reported in women with disability as compare to disabled male. Disabled women experience violence higher or similar than other women(10).In spite of high rate of abuse in

disable women, studies on experience of domestic violence, common forms of violence based on type of disability is limited(3).

Multiple sclerosis (MS) is an inflammatory, demyelinating, neurological disease of the central nervous system and disabling young adults. Women are affected about twice as often as men (11, 12). Limited information exists about the effect of multiple sclerosis on women's life.

Using Kurtzke classification, Iran is located in a low-risk area for multiple sclerosis. However, recent studies   showed the prevalence of MS has increased to a medium-to-high risk level in Iran (13, 14).

Multi-country WHO study showed   between 15% and 71% of the women experienced physical and/or sexual violence by an intimate partner in their lifetime (1).Prevalence of domestic violence within Iran ,in non disabled women vary from 10 % to 55% based on different populations and various forms of violence (15,16). To our knowledge no information has been recorded in women with MS or other disabilities in Iranian population. Due to high prevalence of domestic violence, increased prevalence of MS and non existence data about experience of violence in Iranian women with physical disability particularly in women with multiple sclerosis we designed this pilot study.

2. Material and Methods

In a descriptive-analytical pilot study 150 married

women 19-59 years who attending to Multiple sclerosis society of Khuzestan were studied in 2010-2011.Khuzestan province is located in south western of Iran.MS society of Khuzestan is located in Ahvaz. Khuzestan is one of the 31 provinces of Iran. It is in the southwest of the country. Its capital is Ahwaz and covers an area of 63,238 km².

Overall 365 female patients (single and married) have registered in MS society of Khuzestan .205 women entered to study after acceptance Analysis was done on 150 married women. Every female patient, during the time of sampling, who seek for receiving disease services was interviewed for participation in the study. Patients were assured of the confidentiality of their responses.

We designed a questionnaire based on abuse assessment screening (AAS) questionnaire, without questions related to pregnancy violence, and female sexual function index (FSFI). The questionnaire included demographic details and domestic violence experience in any point of life. Variables in this study included age of patients, education level, and ethnicity, family history of MS, Age at diagnosis, stage of disease, symptoms of disease, complaint related to disease, sexual dysfunction and behaviors related to violence. We asked questions about some behaviors related to physical violence, physical injuries, hospitalization, psychological and sexual violence. Experience of at least one type of three forms of violence was classified as any form of violence.

Using qualitative approach, we checked validity of questionnaire. Use of scientific resources, neurologist and obstetrician evaluation and interview with 10 women with multiple sclerosis were used for evaluation of validity. Cronbach's alpha was used for reliability's evaluation. We trained questioners based on ethical and security recommendations for domestic violence researches (17).SPSS (version19) was used for data entering and analysis. Logistic regression model for the calculating the Odds Ratio and 95% confidence interval was used.

3. Results:

The mean age of patients was 34.2 (standard deviation 8.1; range19–59) years.10% of patients were in age group 19-24, 26.7% and 63.3% in age group 25-29 and more than 30 respectively. Of women 30% had less than high school education level , 38.7% and 31.3% had high school and collage level education respectively . The mean age of patients at diagnosis of MS was 28.4(standard deviation 7.4) years.

32.7% of patients had emotional problems related to disease. At least one symptom of sexual dysfunction was reported in 43.6 % of patients. In 38.1% of patients, multiple sclerosis had a negative effect on their sexual relationship.

The most common complain related to sexual dysfunction was decreased libido (54.4%) and difficulties in achieving orgasm (45.6%).

The overall prevalence of physical, psychological, sexual and any form of violence in lifetime of women with multiple sclerosis were 17.8%, 38.4%, 6.8% and 41.5% respectively.

Higher prevalence of physical violence was reported in patients aged<25 years, educational level less than high school, age at marriage<18, non Fars Ethnic groups and history of emotional problem. Patients suffering from progressive type of the disease had a significantly higher experience of violence (p<0.01).

Experience of all forms of violence was higher in patients with history of sexual dysfunction (p<0.01).Results of binary regression logistic showed experience of psychological violence [OR= 4.3; CI(2.003-9.611)] and any form of violence [OR =4.99;CI(2.28-10.9)] were significantly associated with history of sexual dysfunction(p<o.oo1).

Table 1. Prevalence of violence experience in any time of life by patients characteristic

Patients Characteristics	Physical violence Number (%)	Sexual violence Number (%)	Psychological violence Number (%)	Any forms of violence Number (%)
Woman's Age				
<25	3(20)	0(0.0)	2(13.3)	3(20)
25-44	21(17.8)	9(7.6)	48(40.7)	53(44.5)
45=>	2(15.4)	1(7.7)	6(46.2)	5(38.5)
Woman's education				
Less than high school	9(20.9)	*3(6.8)*	21(48.8)	21(47.7)
high school and	17(16.5)		35(34.0)	40(38.8)
Collage education		*7(6.8)*		
Woman's age at marriage				
(years)	10(30.3)	4(12.1)	15(45.5)	18(54.5)
18<	12(13.5)	6(6.7)	32(36.0)	34(37.8)
18-24	3(13.0)	0(0)	8(34.8)	8(34.8)
25=>				
Stage of disease				
Remitting	19(15.4)	7(5.7)	46(37.4)	49(39.8)
Progressive	7(30.4)	3(12.5)	10(43.5)	12(50.0)

Ethnicity				
Arab	8(21.0)	4(10.5)	17(44.7)	18(47.4)
Lor	9(21.4)	2(4.8)	20(47.6)	21(50.0)
Fars	9(13.6)	4(6.0)	19(28.8)	22(32.8)
Emotional problem				
Yes	12(26.1)	2(4.3)	26(56.5)	27(57.4)
No	14(14.0)	8(8.0)	30(30.0)	34(34.0)
Sexual dysfunction				
Yes	17(27.0)	10(15.6)	38(60.3)	41(64.1)
No	9(10.8)	0(0.0)	18(21.7)	20(24.1)

4. Discussion

Experience of any form of violence in any point of life in women with MS was 41.5%. Findings of this study emphasized on high prevalence of domestic violence around the world (1, 3). Nouhjah and colleague (2008) reported experience of any forms of violence in healthy women attending health centers of Khuzestan province 47.3 % (18).Although research on experience of violence in women with multiple sclerosis is nearly nonexistent, but few information is reported about association of other disabilities with domestic violence.

Disability as a risk factor of domestic violence has been reported by Young et al In 1987. Results of their study showed that Women with physical disabilities are at risk for emotional, physical, and sexual assault to the same extent as women without disabilities. Prevalence of violence by husbands or partners was similar to estimates of occurrence of domestic violence in any point of life for women residence in the United States. Also Women with physical disabilities were more at risk for experience violence in longer period in comparison with non disable women (19). Nosek et al (2001) reported that women with disability experience violence similar or higher than general population (20).

Hughes et al (2010)In a systematic review and meta-analysis, searched 12 electronic databases published between Jan 1990 and Aug 2010, reporting prevalence of violence against adults with disabilities. Pooled prevalence of any violence (physical, sexual, or intimate partner) was 24•3% (95% CI 18•3-31•0) in adults with mental illnesses, 6•1% (2•5-11•1%) in those with intellectual problem, and 3•2% (2•5-4•1%) in people with non-specific disability. In their study, pooled crude odds ratios for the risk of violence in people with disabilities compared with non-disabled were 1•50 for all studies combined, 1•31for people with non-specific disability, 1•60 for adult with intellectual impairments, and 3•86 for people with mental diseases. The results of their research also showed, available studies in the world have weaknesses in methodology of research in this field (21).

Lin and colleagues (2009) analyzed data from Domestic Violence Report System of Taiwan, and reported significantly changes in prevalence of violence in people with disability. Rate of violence in people with disabilities was 3.7 times of the non disabled people (9.79% vs. 36.08%). Intellectual disability (41.52%), vision or speech disability (38.59%) and chronic psychosis (37.96%) were the most increasing disability types (22).

Jones et al (2010) in a systematic review and meta-analysis estimated prevalence of violence against children aged under or equal 18 years with disability. Pooled prevalence calculated were 26.7% for combined violence measures, 20•4% for physical assault, and 13.7% for sexual abuse. Odds ratios for pooled risk estimates were 3.68 for combined abuse measures, 3.56 for physical assault, and 2.88 for sexual abuse(23)

Leary et all(2006) in only existence study which specifically has focused on experience of domestic violence in women with multiple sclerosis reported psychological violence was experience in all of 150 women with multiple sclerosis participating in REACH(respite care, educational, awareness, change, and hope for people with multiple sclerosis) program. Prevalence of physical and sexual violence was approximately 31% and 20% respectively. In 68.6 % of victim cases, Perpetrators of domestic violence were current or former intimate partners (3).

In the present study prevalence of sexual dysfunction in women with multiple sclerosis was high and experience of all types of violence had a significant association with history of sexual dysfunction. Sexual dysfunction is a prevalent destructive complication of multiple sclerosis that affects quality of life of patients. A complex set of anatomic, biologic, medical and psychological changes may result in sexual dysfunction in women with multiple sclerosis. changes which directly affect libido and orgasm due to damage of the nervous system, complaints which are related to the physical disability of the disease, include fatigue , weakness , rigidity and spasms of muscles and emotional, social problem of MS(24,25).Hastuti and colleagues reported a significant relationship between sexual dysfunction and domestic violence (26).

Small sample size, cross-sectional design of study, conclusion based on patient self-reporting and recall bias in reporting of violence during the life time of patient, were some limitation of the present study.

In spite of limitations, this is the first study in this population in Khuzestan province and in Iran. Our study can provide a guideline for practitioners and

neurologist with useful information for planning and screening domestic violence in women with multiple sclerosis specifically women with history of sexual dysfunction.

Due to limited time in medical routine visits, screening for domestic violence may not possible. It is important that medical practitioners be alerted to this problem. Screening and prevention program providing appropriate services for domestic violence should be a priority for women with multiple sclerosis and other disability.

Conclusion

It can be concluded that Prevalence of domestic violence in women with multiple sclerosis is high, as similar as general population. The finding suggests the need for a study with a larger sample size in women with multiple sclerosis and other disabled women.

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Prevalence and Distribution of Venous Insufficiency in Patients with Post-Thrombotic Scar

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Abstract: Chronic venous insufficiency of deep venous system in most cases is an acquired disorder which occurs following deep vein thrombosis, and can subsequently followed by skin changes and disability. Color Doppler sonography is the main diagnostic tool for chronic venous insufficiency, and also is a useful tool for identifying the distribution and extent of venous reflux. The aim of this study was to evaluate the prevalence and distribution of venous insufficiency in patients with post-thrombotic scar at lower extremity venous system. Fifty three limbs of 48 subjects were evaluated. Patients with previous deep vein thrombosis of the lower limbs presented with skin ulcers, variceal veins or leg edema were included in the present study. Venous Doppler scanning was commenced at external iliac vein (EIV) and moved to common femoral vein (CFV), saphenofemoral junction (SFJ), superficial femoral vein (SFV), greater saphenous vein (GSV), popliteal vein (POPV), saphenopopliteal junction (SPJ), lesser saphenous vein (LSV), calf veins (CV), and perforator veins (PV), respectively. The results showed that prevalence of thrombotic scar was more common in proximal deep veins of the limbs. Moreover, popliteal vein was the most common insufficient vein (58.5%). Perforator veins also had no thrombotic scar but they were insufficient in 3 patients (5.66%). This study also showed that severe stenosis or occlusion was most common in external iliac veins (12 patients). In conclusion, the chronic post-thrombotic scar was common at proximal veins. Moreover, deep vein insufficiency was more common than superficial system. In addition, POPV was found as the most common insufficient vein.

Keywords: Venous insufficiency; post-thrombotic scar; Doppler ultrasound; deep vein thrombosis; venous reflux

1. Introduction

Chronic venous insufficiency (CVI) in the lower limbs is a common and progressive disease. It occurs as a result of post-thrombotic valve destruction or primary valvular incompetence (Callam, 1994; Chiesa et al., 2005). Insufficiency of the saphenous system is the most common form of chronic venous insufficiency giving rise to primary varicose veins. Chronic venous insufficiency of deep venous system in most cases is an acquired disorder which occurs following deep vein thrombosis, and can subsequently followed by skin changes and disability (Burnand, 1988).

Color Doppler sonography (CDS) is the main diagnostic tool for chronic venous insufficiency, and also is a useful tool for identifying the distribution and extent of venous reflux (Baker et al., 1993). The factors affecting the development of post-thrombotic scar are not fully understood. However it might be caused by gravitational venous reflux, venous stenosis or occlusion (Meissner et al., 1998; Haenen et al., 1998). The aim of this study was to evaluate the prevalence and distribution of venous insufficiency in patients with post-thrombotic scar at lower extremity venous system.

2. Material and Methods

From January 2010 to September 2012, 53 limbs of 48 subjects (22 men and 26 women) aged 20 to 78 years (median age of 51 years) were evaluated. Patients with previous deep vein thrombosis of the lower limbs presented with skin ulcers, variceal veins or leg edema were included in the present study. Examinations were performed using a HITACHI EUB-525 color Doppler ultrasonography with 5-7.5 MHz linear probe (Ghabili et al., 2009; Ardalan et al., 2009; Nemati et al., 2010; Ansarin et al., 2010; Farhoudi et al., 2011; Bavil et al., 2011; Bavil et al., 2012). All examinations were performed with the patient on supine and stand positions. The Doppler settings of duplex sonography were optimized routinely and an angle ($<60°$) of insonation was ensured. Reflux is usually defined as reverse flow that lasted longer than one second for deep and superficial veins and 0.35s for perforator veins (Labropoulos et al., 2003). Venous Doppler scanning was commenced at external iliac vein (EIV) and moved to common femoral vein (CFV), saphenofemoral junction (SFJ), superficial femoral vein (SFV), greater saphenous vein (GSV), popliteal vein (POPV), saphenopopliteal junction (SPJ), lesser saphenous vein (LSV), calf veins (CV), and perforator veins (PV), respectively.

Data were presented as mean ± standard deviation (SD) or percentage. Statistical analysis was performed with SPSS for windows (v 13.0; SPSS Inc, Chicago, IL). A *P* value <0.05 was considered statistically significant.

3. Results

Table 1 shows the anatomic distribution of chronic thrombotic scar in lower limbs. It shows that prevalence of thrombotic scar is more common in proximal deep veins of the limbs.

Table 1. Anatomic distribution of chronic thrombotic scar in the studied patients (CFV, common femoral vein; SFV, superficial femoral vein; POPV, popliteal vein; CV, calf veins; EIV, external iliac vein)

Sites	Number	%
CFV + SFV + POPV + CV	30	56.6
EIV + CFV + SFV	13	24.5
POPV + CV	3	5.6
SFV	1	1.8

Table 2 shows the anatomic distribution of venous insufficiency in lower limbs. It shows that popliteal vein is the most common insufficient vein (58.5%). Perforator veins also had no thrombotic scar but they were insufficient in 3 patients (5.66%).

Table 2. Anatomic distribution of venous insufficiency in the studied patients (CFV, common femoral vein; SFV, superficial femoral vein; POPV, popliteal vein; CV, calf veins; EIV, external iliac vein; PV, perforator veins)

Sites	Number	%
CFV + SFV + POPV + CV	15	28.3
EIV + CFV + SFV	12	22.6
POPV + CV	5	9.4
POPV	11	20.7
PV	3	5.6

This study also showed that severe stenosis or occlusion was most common in external iliac veins (12 patients), causing reverse of flow at SFJ, and the extremity venous blood draining through the collateral veins of pelvis. Only in one patient acute thrombosis in deep veins was detected. Moreover, in two patients insufficiency was noted at GSV, while no insufficiency was seen at LSV.

4. Discussion

Color Doppler ultrasound can accurately show the extent of chronic or post-thrombotic venous scar and also the extent of venous insufficiency in the lower limbs. In the majority of the healthy controls, the reflux time is <0.5s and usually the criterion for reflux in incompetent veins is >1s (Campbell et al., 1996). In this study, the chronic post-thrombotic scar was common at proximal veins and it is in competent of more prevalence of acute thrombosis at proximal veins of the lower limbs. Cogo and associates (1993) found that 88% of their patients had thrombi in proximal veins. However, in the previous studies, deep vein thrombosis was considered to originate from distal veins and then extend to proximal veins (Weinmann and Salzman, 1994). In a recent study, Yamaki and Nozaki (2005) showed that the overall incidence of DVT involving the proximal veins was 67%. In our study, deep vein insufficiency was more common than superficial system (96.2% vs. 3.8%). In the study of Magnusson and coworkers (2001), deep vein incompetence was common (38%) and isolated superficial veins (7%) but mixed deep and superficial venous insufficiency was more common (49%) and it was in contrast with our study.

In this study, POPV was the most common insufficient vein (58.5%). Haenen and associates (1999) demonstrated that advanced chronic venous insufficiency was found in FV and POPV with reflux. Yamaki and coworkers (2005) found that the proportion of venous insufficiency was significant in patients who had multisegment disease. In this study we found PV insufficiency in 5.6% of patients. In the study of Delis (2004), the presence and number of PV insufficiency both increased with CVI severity and it was inconsistent with of our study.

In conclusion, the chronic post-thrombotic scar was common at proximal veins. Moreover, deep vein insufficiency was more common than superficial system. In addition, POPV was found as the most common insufficient vein.

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Conventional multiplex polymerase chain reaction (PCR) versus real-time PCR for species-specific meat authentication

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Abstract: Simple and reliable conventional multiplex-polymerase chain reaction (multiplex-PCR) as well as real-time PCR approaches for the partial-length *cytochrome b* or *cyt b* gene of mitochondrial DNA (mtDNA) were compared to identify seven animal species for the authenticity of low non Halal (pig, dog, cat and donkey) and high value Halal (cattle, sheep and goat) meats. Either analysis indicated the successful detection of as little as 0.05 pg (5%) adulteration in cattle meat. As compared to real-time PCR, the approach of conventional multiplex-PCR can also be applied to detect authentication with equal efficiency to fresh, cooked or putrefied mixed samples of cattle meat.
[S. Edris, M.H.Z. Mutwakil, O.A. Abuzinadah, H.E. Mohammed, A. Ramadan, N.O. Gadalla, A.M. Shokry, S.M. Hassan, R.M. Shoaib, F.M. Domyati and A. Bahieldin. **Conventional multiplex polymerase chain reaction (PCR) versus real-time PCR for species-specific meat authentication**] *Life Sci J* 2012;9(4):5831-5837] (ISSN:1097-8135).http://www.lifesciencesite.com.870

1. Introduction

Identification of the species of origin in meat samples is relevant to consumers for the possible economic loss from fraudulent adulterations, medical requirements of individuals that might have specific allergies and for religious reasons (Asensio *et al*., 2008a). For some consumer groups, such as vegetarians, the contamination of food with meat residue is strictly prohibited. Another example of meat identification is the Halal food for the Muslim consumers, who are prohibited from consuming pork (Unajak *et al*., 2011). The extensive development of nucleic acid-based technologies over the past decades reflects their importance in food analysis. Various polymerase chain reactions (PCR)-based approaches were attempted for meat authentication. In recent years, PCR coupled with molecular techniques, such as hybridization, nucleotide-sequencing, single-strand conformation polymorphism (SSCP) or forensically informative nucleotide sequencing (FINS), were used for differentiation of buffalo meat from cattle meat (Murugaiah *et al*., 2009). The advantages of DNA-based analysis include the ubiquity, abundance and stability of DNA in all cell type (Jain *et al*., 2007).

PCR analysis of species-specific mitochondrial DNA (mtDNA) is the most common method currently used for meat species identification. The method is more sensitive in the identification of closely-related species as compared to nuclear DNA because each cell has only one set of genomic DNA in the nucleus, but bearing 10^4 copies of mtDNA (Parodi *et al*., 2002; Rastogi *et al*., 2007) with high mutation rate due to the poor corrective replication of polymerase and lack of proof-reading system in mitochondria. Following this approach, species-specific DNA in picograms (pg) can be detected in both processed and unprocessed meat samples. Genes to be targeted for amplification can be *12S*, *16S* and *18S rRNA*, *actin*, *cytochrome b*, *cytochrome oxidase*-II, *NADH dehydrogenase* 5/6 and *mtD*-loop (Kesmen *et al*., 2009; Singh and Neelam, 2011; Unajak *et al*., 2011). Very old samples of more than 100 million years can also be identified (Girish and Nagappa, 2009). Using conventional multiplex PCR, many targets can simultaneously be amplified, which helps in detection of many species in a short period of time (Bai *et al*., 2009; Ghovvati *et al*., 2009; Girish and Nagappa, 2009).

Heat-stable proteins have been reported to be useful targets for both the detection of animal remains and species identification in foods of animal origin, such as meat (Chen and Hsieh, 2000). However, methods based on DNA

amplification are still preferred, as they are less affected by industrial processing (Pascoal *et al.*, 2005). Generally, mitochondrial DNA (mtDNA)-based PCR methods were proved to be good option for the analysis of putrefied samples or those submitted to heat treatment in which DNA has been partly degraded (Rodríguez *et al.*, 2004). With the emergence of real-time PCR technology, PCR methods for mitochondrial encoded targets (Lahiff *et al.*, 2002) have been reported for the detection of bovine material in feedstuffs (Fajardo *et al.*, 2008; Mafra *et al.*, 2008).

In the present work, conventional multiplex-PCR was proposed to identify seven meat species for the authenticity of low non Halal (pig, dog, cat and donkey) and high value Halal (cattle, sheep and goat) animal meats species. The study also aimed to establish the detection limit (sensitivity) and specificity of the PCR methods developed. Application of these species-specific PCR assays for detection of contaminating material from these species in industrial processed meat samples was also evaluated. In addition, real-time PCR was used for the detection of meat species to compare sensitivity to the conventional multiplex-PCR.

2.Materials and Methods
Meat samples
Thirty five meat samples, five from each of the seven species were collected. Cattle (*Bos taurus*), sheep (*Ovis aries*) and goat (*Capra hircus*)

were collected from slaughterhouse, while pig (*Sus scrofa*) was obtained from meat markets in Egypt. Meat samples of cat (*Felis catus*) and dog (*Canis familiaris*) were collected from Veterinary Hospital, Faculty of Veterinary Medicine, Mansoura University, Egypt and donkey (*Equus asinus*) meat from Mansoura Zoo, Egypt. Samples from each species were collected under aseptic conditions in sterile plastic bags, then, stored at -20°C until processed.

DNA extraction
DNA was extracted from skeletal muscular fresh tissues of the seven different animal species using the commercial AxyPrep Multisource Genomic DNA Miniprep kit (cat. no. AP-MN-GDNA-50, Axygen Bioscience, CA, USA) following the manufacturer's manual.

Species-specific primers and PCR amplification
The primer sequences were derived from the *cyt b* gene sequences of various species. The primers were synthesized from Metabion, Germany to amplify partial-length *cyt b* gene. Primer sequences designed by Matsunaga *et al.* (1999) were used as one universal forward primer (SIM) and five different reverse primers (R) for amplifying species-specific mtDNA segments of the gene from goat, cattle, sheep, pig and donkey. Primers designed by Abdulmawjood *et al.* (2003) were used as two forward (F) and two reverse (R) primers for amplifying the gene from cat and dog (Table 1).

Table 1. Primer sequences utilized for PCR to amplify partial-length *cyt b* gene from DNAs of different animal species.

Name	Primer*	Sequences (5' – 3')
SIM	F	GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA
Sheep	R	CTA TGA ATG CTG TGG CTA TTG TCG CA
Goat	R	CTC GAC AAA TGT GAG TTA CAG AGG GA
Cattle	R	CTA GAA AAG TGT AAG ACC CGT AAT ATA AG
Pig	R	GCT GAT AGT AGA TTT GTG ATG ACC GTA
Donkey	R	CTC AGA TTC ACT CGA CGA GGG TAG TA
Dog	F	GGA GTA TGC TTG ATT CTA CAG
	R	AGA AGT GGA ATG AAT GCC
Cat	F	CTC ATT CAT CGA TCT ACC CA
	R	GTG AGT GTT AAA ACT AGT ACT AGA AGA

*See Matsunaga *et al.* (1999) and Abdulmawjood *et al.* (2003).

Original conventional PCRs for different species were performed in reaction volumes of 50 µl using 1 ng of genomic DNA of each species (gathered from five replicates), 25 pmoles of each primer, 1x Taq DNA polymerase buffer, 2 mM MgCl$_2$, 0.2 mM dNTPs and 0.2 Taq DNA polymerase (Finnzymes, Thermo Scientific, Finland). PCR (Mastercycler Gradient, Eppendorf, Hamburg, Germany) was carried out by initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 60 s, annealing temperature at 48 to 58°C for 60 s, polymerization temperature

at 72°C for 60 s and final extension at 72°C for 10 min, then, the samples were held at 4°C. The amplified mtDNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Real time-PCR was carried out using the Agilent Mx3000P qPCR Systems (Agilent technologies, Palo Alto, CA, USA) with different primers. The reaction components were 12.5 µl

Maxima™ SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Fermentas, Lithuania), 25 pmoles of each forward or reverse primer, and water (nuclease-free water) up to 22.5 µl. Then, 2.5 µl of diluted cDNA template (1/10) was added. Amplification was carried out in triplicates along with a no-template negative control (nuclease-free water). To avoid false positives due to DNA contamination, PCR reaction was carried out for all RNA samples (data not shown). The thermal cycling conditions were similar to those for

Table 2. Binary mixtures (in ng) of different DNAs for conventional multiplex PCR.

Cattle	Goat	Sheep	Pig	Donkey	Cat	Dog
0	1	1	1	1	1	1
0.75	0.25	0.25	0.25	0.25	0.25	0.25
0.9	0.1	0.1	0.1	0.1	0.1	0.1
0.95	0.5	0.5	0.5	0.5	0.5	0.5
1	0	0	0	0	0	0

Preparation of heat-treated and putrefied meats
        Twenty milligrams of meat were wrapped from each species (cattle, goat, sheep, pig, donkey, cat or dog) in aluminum foils and heated in autoclave at 120°C for 30 min. Other meat samples were allowed to putrefy in a natural condition at room temperature by leaving 10 g from samples of each species (cattle, goat, sheep, pig, donkey, cat or dog) in seven different small Petri dishes for seven days. Then 20 mg meat samples were taken from

conventional PCR. Data were collected and amplification plots of ΔRn versus cycle number were generated for analysis.
Preparation of meat binary mixtures
        Binary mixtures were prepared for multiplex PCR by adding DNA from each species (goat, sheep, pig, donkey, cat or dog) to DNA of beef (cattle). The earlier DNAs were added in the percentages of 100 (1 ng), 25 (0.25 + 0.75 ng cattle DNA), 10 (0.1 + 0.9 ng cattle DNA), 5 (0.05 + 0.95 ng cattle DNA) or 0 (1 ng cattle DNA) (Table 2). each species for each treatment. DNA extractions and PCRs were done as previously indicated for fresh samples.

3. Results and Discussion
Original amplification of partial-length cyt b gene from different species
        Original PCR was planned to amplify partial-length *cyt b* gene differing in amplicon sizes for the identification of different meat species samples. The results indicated successful amplification of the target *cyt b* gene sequences with the expected amplicon sizes (157, 274, 331, 398, 439, 672 and 808 bp for goat, cattle, sheep, pig, donkey, cat and dog, respectively (Figure 1). PCRs to detect cross species amplification were negative, that is, goat-specific primers gave amplicons only in goat mtDNA and not in any mtDNAs of the other species (data not shown).
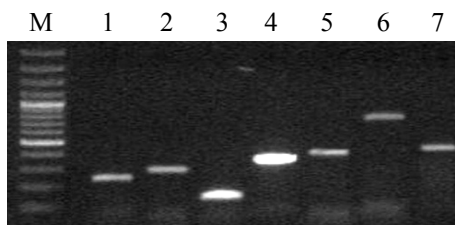
Figure 1. Electrophoretic analysis of partial-length *cyt b* amplicons of DNAs of different meat species samples. Lanes 1 to 7 represent cattle, sheep, goat, pig, donkey, dog and cat DNA samples. Lane M represents molecular size marker (100-bp DNA ladder, New England Biolabs, Ipswich, MA, USA, cat. no. N3231S).

PCR profiles of binary mixtures of different foreign meat samples with cattle
        The PCR sensitivity test of binary meat mixtures shown in Figure 2 indicated that contaminants with as low as 5% DNA (5 pg) of different foreign meat species (goat, sheep, pig, donkey, cat or dog) mixed with 95% DNA from cattle (0.95 ng) were successfully detected (Figure 2a to f, respectively) with the expected amplicon sizes. As for the different reactions with 100% (1 ng) cattle (Figure 2a to f, lane 1), the results indicated no cross species amplification has been found for any species-specific pair of primers.

PCR profiles of heat-treated and putrefied meat samples
Results of PCR with the meat samples subjected to heat treatment to simulate cooking (120°C for 30 min) as well as those subjected to putrefaction before DNA was extracted (from 20 mg muscle samples) and tested via conventional PCR to amplify partial-length *cyt b* gene are shown in Figure 3. Amplicons resulting from either treatment were quite similar to those of the fresh meat samples. In other words, heat or purification did not affect efficiency of amplification of partial-length *cyt b* gene of different meat species.
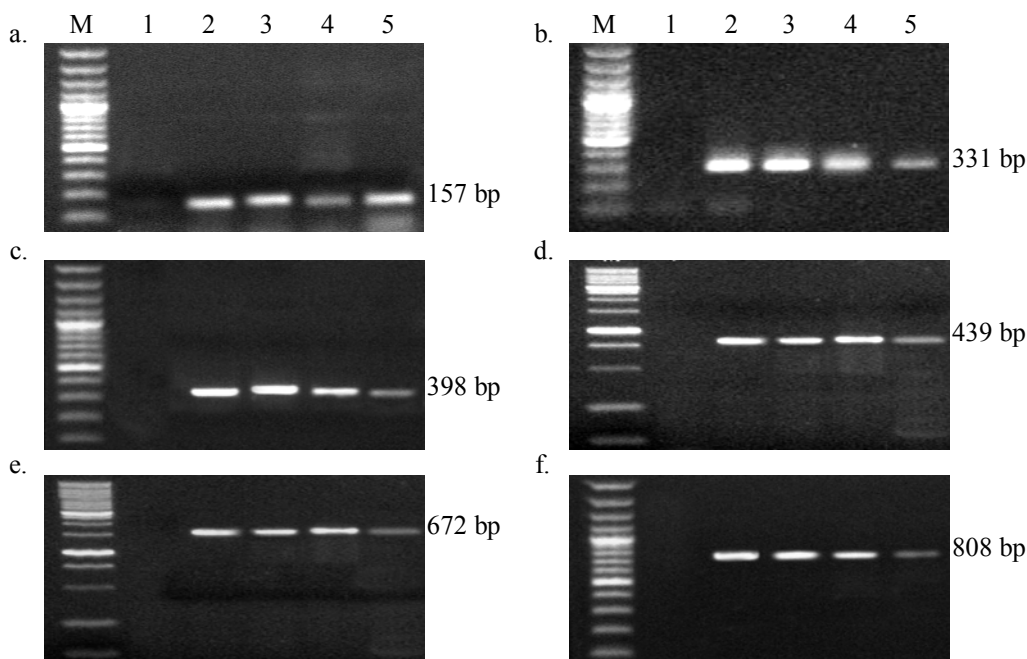
Figure 2. Electrophoretic analysis of partial-length *cyt b* amplicons (Lanes 1 to 5) from binary DNA mixtures (0, 1, 0.25, 0.1 and 5 ng, respectively) of different meat species samples mixed with cattle (1, 0, 0.75, 0.9 and 0.95 ng, respectively). A to f represent mixtures involving goat, sheep, pig, donkey, cat or dog DNAs, respectively.
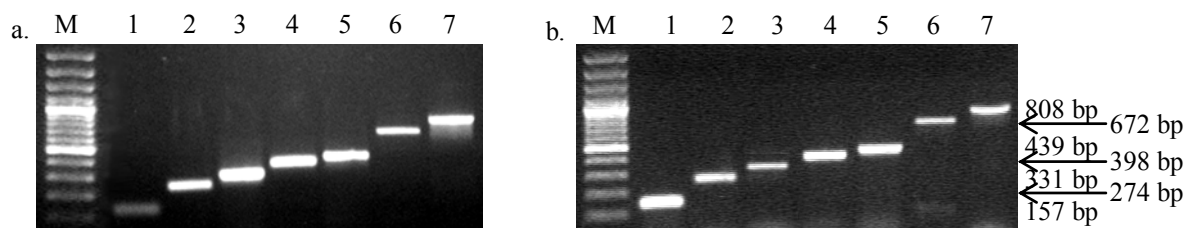
Figure 3. Electrophoretic analysis of partial-length *cyt b* amplicons from DNAs of heat-treated (a) and putrefied (b) meat samples of the seven animals species. Lanes 1 to 7 represent DNAs of goat, cattle, sheep, pig, donkey, cat and dog.

Conventional multiplex PCR

Multiplex PCR of individual animal species was conducted using the seven species-specific primer mixtures. All the primers were mixed in the following proportions (5:1:1:1:1:1:1:1:1:1) for SIM-F : goat-R : cattle-R : sheep-R : pig-R : donkey-R : cat-F : cat-R : dog-F : dog-R, respectively. Two microlitres of this mixture was incorporated in the PCR to give 10 pmoles of each primer except for SIM-F that was represented by 50 pmoles. This multiplex PCR (1 ng/reaction) was tested on DNA samples from each species as well as DNA mixture of the equally mixed seven species. The electrophoretic banding pattern is shown in Figure 4. The results indicated the presence of intense target amplicon for each single species with the absence of any cross reaction (Figure 4, lanes 1 to 7) regardless of primer multiplexing. Additionally, multiplex PCR with the seven DNA samples was successful in detecting the target seven amplicons in the reaction, however, with lower band intensities (Figure 4, lane 8) probably due to multiplexing of DNAs and primer pairs of different animal species.
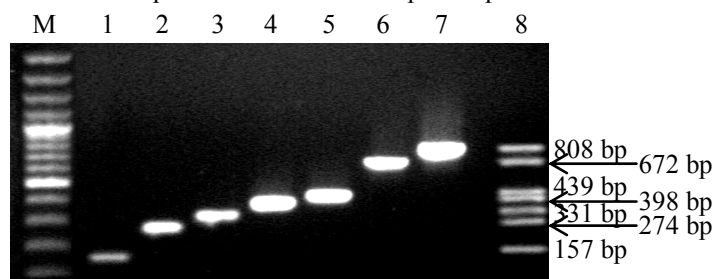
Figure 4. Electrophoretic analysis of conventional multiplex PCR products from DNAs of meat samples from the seven animals species individually (1 to 7) and mixed. Lanes 1 to 7 represents goat, cattle, sheep, pig, donkey, cat and dog DNA samples. Lane 8 represents DNA mixture of the seven animal species samples.

Real-time PCR

In the present study, real-time PCR was supplied with dye detection software (Agilent, USA) to determine the SYBR Green I dye spectrum using a multicomponent algorithm. The Rn value expresses the magnitude of the normalized fluorescence signal generated by the dye for each cycle during PCR amplification. The point at which the amplification plot crosses the threshold is defined as C_t (threshold cycle). The specificity of real-time PCR was evaluated by the amplification of *cyt b* partial-length gene from the mixed mitochondrial DNAs (1 ng/reaction) of different animal species with the seven sets of primers. The results indicated the presence of the target species-specific amplification curve with different C_t value specific for each animal DNA (Figure 5 and Table 3).
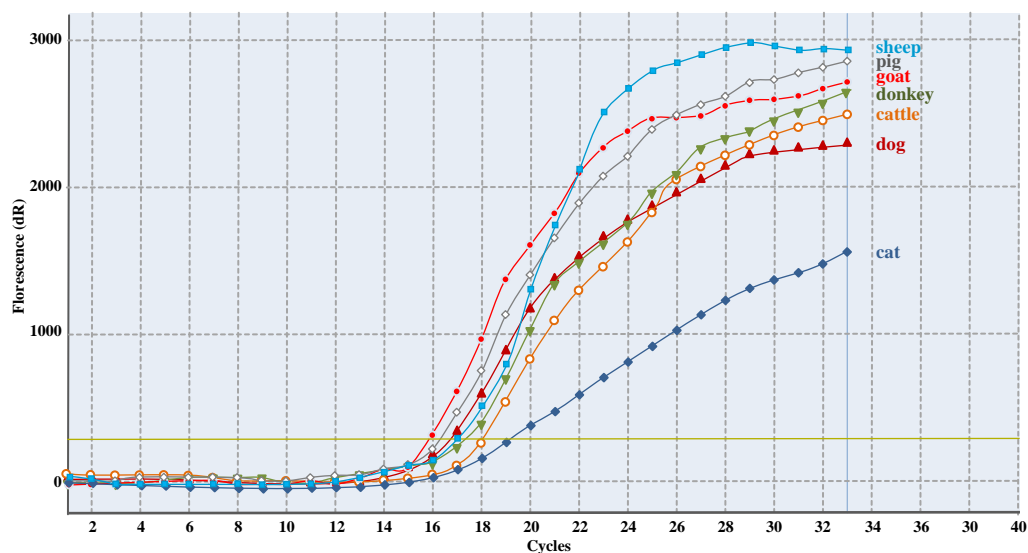
Figure 5. Real-time PCR amplification curves of *cyt b* gene fragments of mtDNAs of the seven animal species.

Table 3. Real-time PCR results for the partial-length *cyt b* gene amplification with DNAs of different meat species.

Animal species	Fragment size (bp)	Threshold (C_T) value	Melting temperature (T_m) (°C)
Goat	157	15.79	78.45 - 81.75
Cattle	274	17.55	82.25 - 84.95
Sheep	331	17.09	80.75 - 83.20
Pig	398	15.94	80.35 - 83.65
Donkey	439	17.28	84.15 - 86.45
Cat	672	19.54	81.75 - 84.55
Dog	808	16.48	81.25 - 84.55

Generally speaking, PCR has proved to be reliable, sensitive and fast technique in detecting species-specific mtDNA fragments (Fajardo *et al*., 2007; Kesmen *et al*., 2007; Martín *et al*., 2007). We successfully developed a species-specific multiplex-PCR assay to examine seven kinds of animal species in which four of them are commonly used in meat adulteration. In the present work, the universal specific primers in combination with species-specific primers for *cyt b* gene vastly increase the specific sensitivity. The results for mtDNA templates coming from fresh, cooked or putrefied meat were optimistic. The present method has similar sensitivity with that of previous methods (Bai *et al*., 2009; Mane *et al*., 2009) in which no cross-reactivity was shown with mtDNAs of other animal species.

Food composition and authenticity assessment is becoming a very important issue by avoiding unfair competition among producers, allowing consumers to have accurate information about the acquired products. Following the European Union labeling regulations, meat products should be accurately labeled regarding their species content (European Commission, 2001). Quality evaluation in these products encompasses many issues, such as the fraudulent substitution of higher commercial value meats by lower value meats (Fajardo *et al*., 2008), the presence of undeclared species (Aida *et al*., 2005) and the use of vegetable proteins, since they have a considerably lower price than muscle proteins (Belloque *et al*., 2002). Furthermore, the presence of undeclared ingredients may be troublesome for health reasons, such as in the case of bovine spongiform encephalopathy due to the addition of infected neurological tissue and because of allergic reactions in sensitised individuals (Asensio *et al*.,

2008b). The most important problem to be considered in meat species adulteration is related to religious practices as in some religions, such as Islam and Judaism pork meat consumption which is forbidden. Although, the species of origin in raw meats can be identified by using most of protein-based methods, some authors showed that they are significantly less sensitive in the evaluation of thermally processed foods because of specific epitopes alterations (Rodríguez *et al*., 2004).

Mitochondrial DNA molecules coupled with polymerase chain reaction (PCR) represents a fast, sensitive and highly specific alternative to protein-based methods (Mafra *et al*., 2008). Conventional PCR techniques are generally able to produce qualitative results of the identified species, while real-time PCR has demonstrated to be a useful tool for the determination of minute amounts of different species, even in complex foodstuffs (Fajardo *et al*., 2008; Mafra *et al*., 2008). Real-time PCR is probably the most used quantitative mtDNA-based method, however, the high cost of the equipment and reagents is still a drawback for the application of this technique in most laboratories. Alternatively, other approaches based on conventional multiplex PCR for quantitative analysis have been tested (Mafra *et al*., 2007) and recommended (the present work). We claimed that the latter process is efficient enough to detect as little as 5% (5 pg DNA contaminants) of other animal species. When committing adulteration of meat, it is not logical to mix low quality meats at percentages lower than 5%.

In conclusion, this study suggests an accurate analytical technique for detecting meat adulteration by conventional multiplex PCR analysis of the *cyt b* gene of animal mtDNA. This technique was used to detect and trace meat adulteration and to differentiate species present in meat mixture. The test could also be used and applied by researchers and quality control laboratories for verification and control of industrial meat products, such as Halal authentication and raw material origin certification

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Molecular markers for salt tolerant wild barley *Hordeum spontaneum*

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Abstract: The present study aims at detecting molecular markers, based on RAPD, ISSR and AFLP, for the salt-tolerant wild barley *Hordeum spontaneum* as a little is known about its genetic structure and function. Barley is one of the most important cereal crops all over the world. Therefore, the identification of molecular markers for the salt-tolerant wild species is crucial for the future development of tolerant domesticated varieties of *H. vulgare*. For comparison, the study involved seven domesticated barley cultivars. Across the three types of molecular markers, a total of 26 species-specific distinguished *H. spontaneum* from *H. vulgare*. RAPD markers revealed the highest expected heterozygosity H_e, E and marker index (MI) values, while ISSR indicated the lowest values. These results indicate the reliability of ISSR as a molecular marker in distinguishing wild and cultivated barley species. Some of these markers can be linked to salt stress tolerance genes in *H. spontaneum* that can be transferred to domesticated barley (*H. vulgare*) through marker-assisted selection (MAS).

1. Introduction

Barley is one of the most important cereal crops all over the world, which ranks fourth in terms of productivity and area of cultivation (Schulte *et al*., 2009). In comparison to other cereals, barley species typically grow in low input and climatically marginal areas (Ceccarelli, 1996, Kausar *et al*., 2012) because of high water use and transpiration efficiencies (Lopez-Castaneda & Richards, 1994). Cultivated barley is a member of the genus *Hordeum* namely *H. vulgare*, which is descended from wild barley (*Hordeum spontaneum*). *H. vulgare* is well-studied in terms of genetics, genomics, and breeding, however, a little is known about the genetic makeup and genome function of its wild descendant (*Hordeum spontaneum*). Identifying molecular markers for this salt-tolerant wild species is essential in breeding programs for the future development of salt stress tolerant crop cultivars. The genome of barley genus (*Hordeum*) has been estimated to weight about 5.5 picograms (pg) of DNA in the haploid (n = 7) nucleus (Bennett & Smith, 1976) in which CG comprises about 41% (Chakrabarti & Subrahmanyam, 1985, Bahieldin *et al*., 2006). Barley genome consists of a complex mixture of unique (20-30%) and repeated nucleotide sequences. The latter is subdivided into several classes, where about 6% exists as inverted repeats of 300-3000 bp, while the rest ranged in size from 400-700 bp. About 20% of repeated sequences arranged in random, while they are mostly interspersed among themselves and/or among unique sequences (Bahieldin *et al*., 2006).

Progress in plant breeding and cultivar identification mostly relies on morphological characteristics that require extensive observations of individuals (Wrigley *et al*., 1987). Factors, like the environment, multigenic and quantitative inheritance or partial and complete dominance virtually confound gene expression. Although protein and isozyme markers were used in many crops, major limitations are the lack of polymorphism among closely-related genotypes and the variation of protein content and type among different tissues and developmental stages under different environmental conditions (Beckmann & Soller, 1983). DNA-based genetic markers are recently integrated into several plant systems and expected to play a very important role in the future of plant

breeding (marker assisted selection or MAS) and molecular genetics analysis.

Polymerase chain reaction (PCR) was initiated as a genetic assay based on selective DNA amplification (Saiki *et al*., 1988, Innis *et al*., 1990). Among the different types of PCR-based molecular markers, random amplified polymorphic DNAs (RAPDs) are useful for the assessment of genetic diversity among rare species (Williams *et al*., 1990) because of their simplicity, speed and relatively low cost as compared to other molecular markers. RAPDs are used extensively in analyzing genetic diversity (Artyukova *et al*., 2004, Sureja *et al*., 2006, Guerra *et al*., 2010). Also, inter simple sequence repeats (ISSRs) were developed as an anonymous approach accessing variation in the numerous microsatellite regions dispersed throughout the genome (Zietkiewicz *et al*., 1994). ISSRs are based on the amplification of DNA regions between inversely oriented SSRs or microsatellites (Bussell *et al*., 2005). The ISSR markers are simple and reproducible. They require small amounts of DNA and do not require information on DNA sequence. ISSR primers are designed from SSR motifs and can be undertaken for any plant species containing a sufficient number and distribution of SSR motifs in the genome (Buhulikar *et al*., 2004). Therefore, ISSRs are widely used in many respects such as the study of genetic diversity in barley (Brantestem *et al*., 2004) and cultivar identification in tobacco (Denduangboripant *et al*., 2010). Microsatellites are very short stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. They are characterized by mono-, di- or trinucleotide repeats that have 4-10 repeat unit side-by-side (Morgante and Olivieri, 1993). Amplified fragment length polymorphism (AFLP) utilizes fragments of DNA amplified using primers from restriction digested genomic DNA (Vos *et al*., 1995). AFLP provides the highest levels of resolution to allow delineation of complex genetic structures, to differentiate individuals in a population in gene flow experiments, and also to register plant varieties (Powell *et al*., 1996, Law *et al*., 1998, Barker *et al*., 1999, Aparajita & Rout, 2010, Misra *et al*., 2010).

The present study aims at the evaluating the usefulness of molecular markers, i.e., RAPD, ISSR and AFLP, in characterizing the salt-tolerant wild barley (*H. spontaneum*) as compared to the cultivated barley (*H. vulgare*) and in detecting possible species-specific markers to be utilized in the future breeding for salt tolerance in barley.

2. Materials and Methods
Plant material
The study involved the wild barley of *H. spontaneum* as well as seven domesticated cultivars

(Table 1) of *H. vulgare* of Egyptian origin to be compared on the molecular levels. Genotype- or species-specific molecular markers for the salt-tolerant genotype were also detected. Names and some pedigrees of the domesticated cultivars are shown in Table 1. Relatedness of the cultivars with no available pedigrees will be detected based on the molecular analyses. Seeds of the wild species were collected from wild habitat at Rafah region near the north coast of Sinai, Egypt. Seeds of each genotype were collected from plants in three locations (populations). Ten plants of different genotypes were selected in each location based on morphological homogeneity.

Table 1. Names and pedigrees of the tested genotypes.

Serial no.	Genotype name	Pedigree
1	*H. spontaneum*	Wild barley
2	Giza 123	Giza 117/FAO 86
3	Giza 124	Giza 117/Bahteem 52// Giza 118/FAO 86
4	Giza 125	Sister line to Giza 124
5	Giza 129	N/A
6	Giza 130	N/A
7	Giza 131	N/A
8	Giza 2000	Giza 117/Bahteem 52// Giza 118/FAO 86*Giza 121

Genomic DNA extraction and purification
Extraction of total DNA was performed using the modified procedure of Gawel and Jarret (1991). The minimum number of plants to be bulked for each genotype to saturate polymorphisms within each cultivar was determined (data shown upon request). To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added to the DNA solution and incubated at 37°C for 30 min. Estimation of the DNA concentration in different samples was done by measuring optical density at 260 nm according to the following equation:

Concentration (ug/ml) = OD_{260} X 50x dilution factor

Random amplified polymorphic DNA (RAPD)
A set of 20 random 10*mer* primers (Operon Technology, USA) from groups A, B, C and O was used in detecting polymorphism among different genotypes but three only were successful in generating reproducible and reliable amplicons. Therefore, triple primer RAPD was used to recover polymorphic and reliable amplicons. The amplification reaction was carried out in 25 µl reaction volume containing 1x PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 21 pmole primer(s), 2 units Taq DNA polymerase and 25 ng template DNA. Names of primers for single primer RAPD are OP-A05, OP-C16 and OP-O07. Combinations of primers for triple primer RAPD are OP-A02/OP-C08/OP-C10, OP-A03/OP-B10/OP-C10, OP-A18/OP-B03/OP-C06, OP-A09/OP-B04/OP-O09,

OP-A17/OP-B09/OP-C07, OP-A08/OP-C14/OP-O06, OP-A16/OP-B07/OP-C16 and OP-A15/OP-C02/OP-O07.

PCR amplification was performed in a Perkin Elmer 2400 thermocycler (Germany), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 37°C for 2 min, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

Inter simple sequence repeat (ISSR)

Thirty primers for ISSR were used in the study but only 14 were successful in generating reproducible and reliable amplicons for different genotypes. Names and sequences of the selected primers are shown in Table 2. PCR analysis was performed in 25 µl reaction and amplification was programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 40°C for 2 min, and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

Table 2. List of ISSR primers and their nucleotide sequences.

No.	Name	Sequence	No.	Name	Sequence
1	814	$(CT)_8TG$	8	HB8	$(GA)_6GG$
2	844A	$(CT)_8AC$	9	HB9	$(GT)_6GG$
3	844B	$(CT)_8GC$	10	HB10	$(GA)_6CC$
4	17898A	$(CA)_6AC$	11	HB11	$(GT)_6CC$
5	17898B	$(CA)_6GT$	12	HB12	$(CAC)_3GC$
6	17899A	$(CA)_6AG$	13	HB13	$(GAG)_3GC$
7	17899B	$(CA)_6GG$	14	HB14	$(CTC)_3GC$

Amplified fragment length polymorphism (AFLP)

AFLP analysis was performed using the AFLP Analysis System I (Invitrogen, cat. no. 10544-013) according to the manufacturer's protocol. Genomic DNA samples were digested with *Eco*RI and *Mse*I restriction enzymes in which *Eco*RI and *Mse*I adapters were ligated to the digested DNA fragments. Pre-amplification was carried out using *Eco*RI primer plus one extension base at the 3' position (A) and *Mse*I primer plus one extension base at the 3' position (C) to amplify fragments that contain complementary sequences. Five combinations of *Eco*RI primers plus three extension bases and *Mse*I primers plus three extension bases were used to selectively amplify the DNA fragments matching the primer-extension sequence. Only, two combinations succeeded to recover good quality polymorphic patterns. These two combinations are M-CCA/E-ACT and M-CAC/E-ACA.

Detection of PCR products

The products of both RAPD and ISSR were detected using electrophoresis on agarose gel (1.2% in 1x TBE buffer), stained with ethidium bromide (0.3 ug/ml), then visually examined with UV transilluminator and photographed using a CCD camera (UVP, UK). AFLP products were detected by capillary electrophoresis and virtual gels were prepared and analyzed. Fragments were separated and sized on an ABI 3500 DNA sequencer (Applied Biosystems, Foster city, California). Using the program Genemapper 4.1 (Applied Biosystems), a genetic fingerprint was produced for each individual sample by scoring for the presence or absence of a standardized

set of markers between 50 and 600 base pairs in size (Rogers, 2008).

Data analysis

The bands recovered by different techniques were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear, unambiguous and reproducible bands recovered through different techniques were considered for scoring. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. Band size was estimated by comparing with 100-bp ladder (Bioron, Germany) using Gel Works 1D advanced gel documentation system (UVP, UK). The binary data matrices were entered into the TFPGA (Ver. 1.3) and analyzed using qualitative routine to generate similarity coefficient. Dissimilarity coefficients were used to construct a dendrogram using un-weighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine.

Matrix comparison

Similarity matrix produced by RAPD, ISSR and AFLP were compared based on the genetic distance of the TFPGA, the normalized Mantel statistic (Mantel, 1967). The PIC (polymorphism information content) was calculated by applying the following formula given by Powell *et al*. (1996) and Smith *et al*. (1997):

$$PIC = 1 - \sum_{i=1-n} fi^2$$

Where, *fi* is the frequency of the *i*[th] amplicon. The number of amplicons refers to the number of scored bands. The frequency of an amplicon was obtained by

dividing the number of cultivars, where it was found, by the total number of cultivars. The PIC value provides an estimate of the discriminating power of a marker. Marker index (MI) was calculated for each primer as the product of PIC and the number of polymorphic bands.

Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) is a method of estimating population differentiation directly from molecular data and testing hypotheses about such a differentiation. A variety of molecular marker data (for example, RAPD or AFLP), direct sequence data, or phylogenetic trees may be analyzed using this method (Excoffier *et al*., 1992). AMOVA was performed using GENALEX 6 (genetic analysis in excel, Peakall & Smouse, 2006) in RAPD, ISSR and AFLP to partition the total molecular variance between and within populations.

3. Results and Discussion

In this work, RAPD, ISSR and AFLP molecular techniques were utilized to analyze germplasms of wild (*H. spontaneum*) and the seven cultivated (*H. vulgare*) barley plants (Table 1, Figures 1 & 2). Generally, the optimal number of primers required to discriminate among genomic DNAs of different plant genotypes depends on the reproducibility of data and level of polymorphism obtained by the type of molecular analysis (e.g., RAPD, ISSR, AFLP, RFLP, etc.). Arguments about the value of genetic distance required to classify correlated plants accessions as distinct cultivars have been raised by several authors (Cabrita *et al*., 2001, Papadopoulou *et al*., 2002). In the present study, primers (11 single and triple for RAPD, 14 for ISSR and two combinations for AFLP) with informative patterns were selected. Selection of primers was based on the number of amplicons recovered through PCR and the stability (reproducibility) of the patterns. These primers were used in the characterization of eight genotypes belonging to the two plants species as well as the level of polymorphism. Less than 10% intra-plants polymorphism (within) was found across the three types of analyses for the plants of the same genotype (data provided upon request). As being dominant markers, pooling (bulk DNA) strategy in ISSR, RAPD and AFLP analyses is ideal to saturate such an intra-plants polymorphism with no effects on the accuracy of the obtained results. Mengoni *et al*. (2000) indicated that this level of intra-plant polymorphism, following the procedure of AMOVA (Excoffier *et al*., 1992), is statistically insignificant and acceptable.

Identification of species- and cultivar-specific molecular markers

A high level of polymorphism was generated utilizing the 11 RAPD with single and triple primers. A total of 531 amplicons, across genotypes and primers, were separated on agarose gel electrophoresis across genotypes. Of these, 222 bands were polymorphic (42%) and the rest were monomorphic (58%). The highest number of amplicons was generated from G2000 (51 amplicons), while *H. spontaneum* generated the lowest (37 amplicons). The highest number of genotype-specific markers (9, see Table 3), due to the presence of a unique band for a given plant species (positive marker), was scored for *H. spontaneum* (species-specific markers), while the lowest number was scored for G124 (4) followed by G125 (6) (cultivar-specific markers).

ISSR is a relatively more recent class of molecular markers, which is based on inter tandem repeats of short DNA sequences. Such repeats were proven to be highly polymorphic even among closely-related genotypes due to the lack of functional constraints in these non-functioning DNA regions that was thought to result in the evolutionary changes in their DNA structures. Accordingly, a high level of polymorphism was generated utilizing the 14 ISSR primers. A total of 736 amplicons were obtained in which 418 of them were polymorphic (57%) and the rest were monomorphic (43%). The highest number of amplicons was generated from G131 (109 amplicons), while *H. spontaneum* plant gene rated the lowest (75 amplicons). The highest number of genotype-specific markers (Table 3) was scored for *H. spontaneum* (13), while the lowest number was scored for G129 (6).

Two combinations were used in the AFLP analysis and revealed 789 amplicons, as little as 355 of them were polymorphic (45%) among the different genotypes. The highest number of amplicons was generated from G123 (112 amplicons), while G2000 generated the lowest (98 amplicons). The highest number of genotype-specific markers (7) was scored for G123, while the lowest number of genotype-specific markers (4) was scored for *H. spontaneum*. In conclusion, the 14 ISSR primers used in the present study allowed for the highest rate of distinction, as compared to AFLPs and RAPDs, between the two barley species, on one hand, and among the different cultivars, on the other hand. A number of 26 species-specific markers were detected that can be used in distinguishing *H. spontaneum* from *H. vulgare* (Table 3).
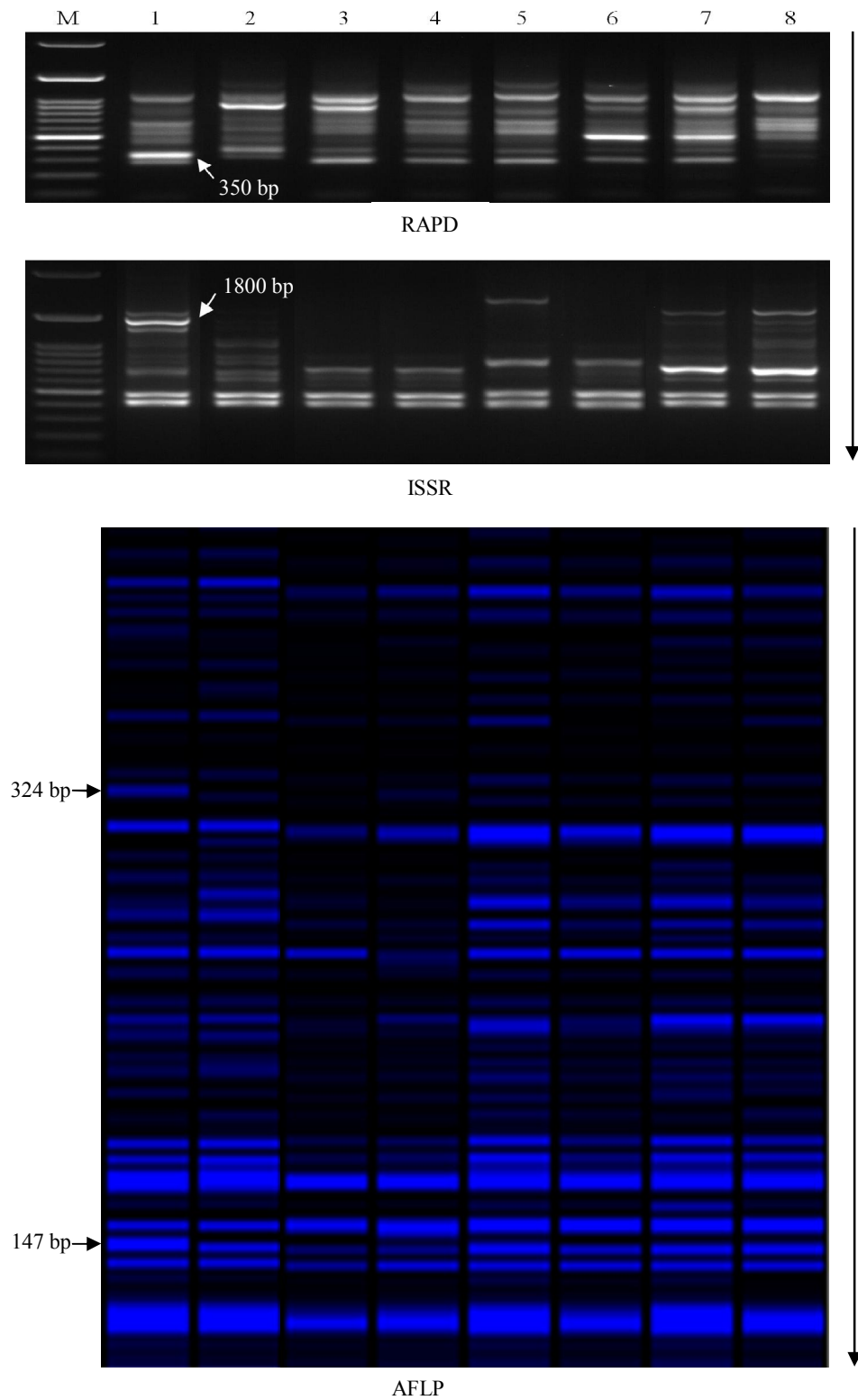
Figure 1: Models of different marker profiles (RAPD with triple primers OP-A02/OP-C08/OP-C10 (350 bp), ISSR with primer 844B (1800 bp) and AFLP with primer combination M-CCA/E-ACT (147 and 324 bp) of the eight genotypes (1-8, see Table 1). M refers to DNA standard (100-bp ladder, Bioron). Arrows indicate direction of migrated PCR products.
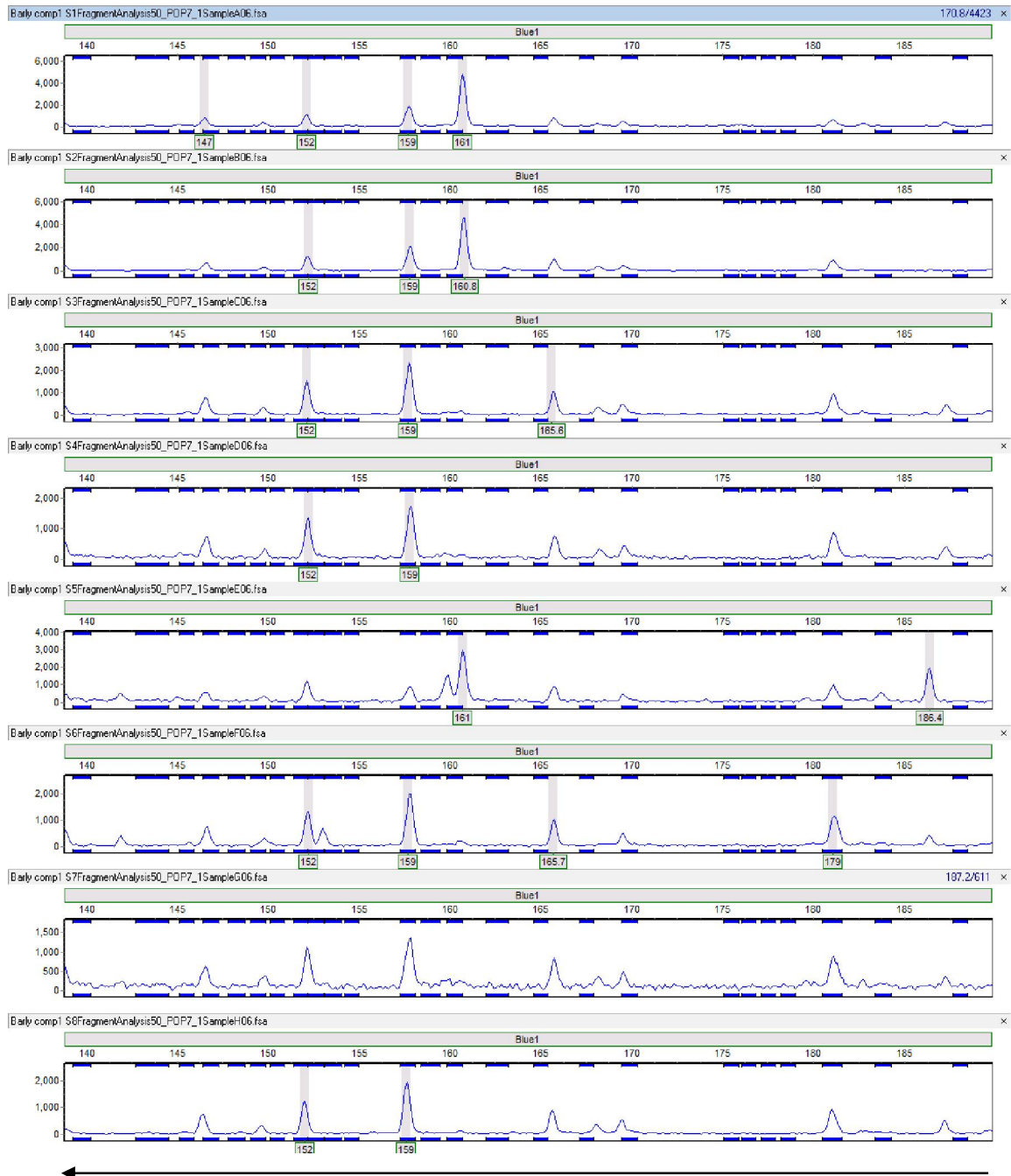
Figure 2: A section of the electrophoregram (140-185 bp) of the AFLP capillary run with primer combination M-CCA/E-ACT showing a specific marker with MW of 147 bp for *H. spontaneum* (1-8, see Table 1). Arrow indicates direction of migrated PCR products.

Genetic relationships and cluster analysis

The genetic similarities between the two species and among the seven cultivars of *H. vulgare* species, based on Nei's method (Nei's, 1978), within and across markers are shown in Table 4 and Figure 3. The results of similarity indices and dendrograms within RAPD, ISSR, AFLP and across markers indicated mean distances between the two species of 82.9, 75.0, 80.3 and 79.7, respectively. It was obvious that ISSR marker was the type of markers resulted in the higher number of positive species-specific markers (13 for *H. spontaneum* and 12 for *H. vulgare* cv. G123) when compared to the other two types of markers. However, the results of the other two marker types as well as across types of markers were able to separate the two species in two clusters. The results of the domesticated

cultivars indicated that G124/G125 were the most closely related genotypes, followed by G129/G130 and G129/G131 within and across types of markers. Although information on the ancestors for G131 is not available, it was shown that this cultivar is closely related to G2000. These results are unexplained as G2000 is known to be closely related to G124, G125, then G123. The three cultivars G129, G130 and G131 seem to be closely related. We can conclude that use of more RAPD primers and AFLP combinations might result in more polymorphic and species-specific markers.

The partition of variation within species was studied with the analysis of the Dice's distance matrix by the analysis of molecular variance (AMOVA) approach. A hierarchical analysis of genetic diversity using a two-way nested AMOVA was performed. Results from AMOVA within and among population are shown at Table 5. Data indicated that 94% of the genetic variation is attributed to differences among populations, while 6% of the genetic variation is attributed to differences within populations. The values of MS indicated the high level of polymorphism among genotypes and the low level of experimental error. This reflects the homogeneity in leaf samples collected for the study as a perfect representative of the target genotypes.

The average of heterozygosity (H_e), the effective multiplex ratio (E), and the marker index (MI) were computed for each assay based on experimental data (Table 6). RAPD revealed the highest H_e (0.48) as compared to AFLP (0.38), then ISSR (0.36). The obtained results in the present investigation agreed with these of Powell *et al.* (1996). Muzher (2005) found that H_e of RAPD was more than AFLP and ISSR. With regard to the E value, ISSR indicated the lowest value (15), while AFLP (28) and RAPD (45) indicated higher values. Concerning MI, ISSR revealed the lowest value (4.33) compared with AFLP (9.21), then RAPD (16.35). In general, the results of ISSR unexpectedly can be considered more reliable than AFLP and RAPD. Reliability of AFLP can be improved if more combinations were used in characterizing species or cultivars on the molecular levels. It could be concluded that markers differ in their ability to differentiate individuals, the mechanism of detecting polymorphism, genome coverage, and the ease of application. They can be complementary to each other depending on technical availability. Some of these markers can be linked to salt stress tolerance genes in *H. spontaneum* that can be transferred to domesticated barley (*H. vulgare*) through marker-assisted selection or MAS (Ribaut & Hoisington, 1998, Zhong *et al.*, 2006, Miedaner & Korzun, 2012). There are some efforts towards breeding salinity tolerance in plant via MAS (Thomson *et al.*, 2010, Singh *et al.*, 2011, Ashraf *et al.*, 2012) that can be duplicated for the development of salt-tolerant domesticated cultivars of barley.

Table 3. List of specific markers for *H. spontaneum* for different marker types. The table indicates the type and number of markers along with their molecular weights (MW) in bp.

Marker type	Marker name	No.	MW (bp)
RAPD	A02/C08/C10	1	350
	A03/B10/C10	1	560
	A18/B03/C06	-	-
	A09/B04/O09	2	420, 890
	A17/B09/C07	1	730
	A08/C14/O06	1	520
	A16/B07/C16	1	1130
	A15/C02/O07	2	1400, 1240
	Total	**9**	
ISSR	814	-	-
	844A	1	810
	844B	1	1800
	17898A	1	550
	17898B	1	1030
	17899A	-	-
	17899B	1	1270
	HB8	1	860
	HB9	1	1330
	HB10	1	1110
	HB11	-	-
	HB12	2	370, 1200
	HB13	1	460
	HB14	2	650, 1440
	Total	**13**	
AFLP	M-CCA/E-ACT	2	147, 324
	M-CAC/E-ACA	2	190, 330
	Total	**4**	

Table 4. Similarity matrixes based on molecular data for the eight barley genotypes.
a. RAPD

Genotype	*H. spon*	G123	G124	G125	G129	G130	G131	G2000
H. spon	1.00							
G123	0.80	1.00						
G124	0.86	0.92	1.00					
G125	0.83	0.91	0.90	1.00				
G129	0.80	0.84	0.85	0.80	1.00			
G130	0.85	0.85	0.87	0.84	0.89	1.00		
G131	0.85	0.88	0.89	0.87	0.93	0.90	1.00	
G2000	0.81	0.87	0.88	0.90	0.83	0.84	0.85	1.00

b. ISSR

Genotype	*H. spon*	G123	G124	G125	G129	G130	G131	G2000
H. spon	1.00							
G123	0.74	1.00						
G124	0.72	0.87	1.00					
G125	0.71	0.86	0.90	1.00				
G129	0.82	0.83	0.84	0.82	1.00			
G130	0.82	0.84	0.86	0.84	0.89	1.00		
G131	0.74	0.87	0.88	0.87	0.87	0.90	1.00	
G2000	0.70	0.86	0.87	0.88	0.81	0.80	0.91	1.00

c. AFLP

Genotype	*H. spon*	G123	G124	G125	G129	G130	G131	G2000
H. spon	1.00							
G123	0.83	1.00						
G124	0.77	0.88	1.00					
G125	0.79	0.89	0.91	1.00				
G129	0.80	0.84	0.82	0.84	1.00			
G130	0.81	0.80	0.85	0.83	0.81	1.00		
G131	0.81	0.86	0.84	0.86	0.87	0.78	1.00	
G2000	0.81	0.89	0.89	0.88	0.80	0.86	0.86	1.00

Overall

Genotype	*H. spon*	G123	G124	G125	G129	G130	G131	G2000
H. spon	1.00							
G123	0.79	1.00						
G124	0.76	0.87	1.00					
G125	0.77	0.89	0.91	1.00				
G129	0.82	0.81	0.80	0.79	1.00			
G130	0.83	0.84	0.82	0.82	0.88	1.00		
G131	0.81	0.81	0.89	0.84	0.85	0.84	1.00	
G2000	0.80	0.83	0.88	0.85	0.81	0.81	0.88	1.00

Table 5: Analysis of molecular variance (AMOVA) of the different barley genotypes.

Source	d.f.*	S.S.**	M.S.***	%
Among Pops	7	5.238	0.748	96
Within Pops	664	122.738	0.185	4
Total	671	127.976	0.198	

*d.f. = Degrees of freedom, **S.S. = Sum of squares, ***M.S. = Mean square

Table 6: Polymorphism information content (PIC), expected heterozygosity for polymorphic products (He), effective multiplex ratio (E) and the marker index (MI) of each marker type used across genotypes.

Marker type	PIC	He	E	MI
ISSR	0.30	0.36	15	4.33
RAPD	0.36	0.48	45	16.35
AFLP	0.31	0.38	28	9.21
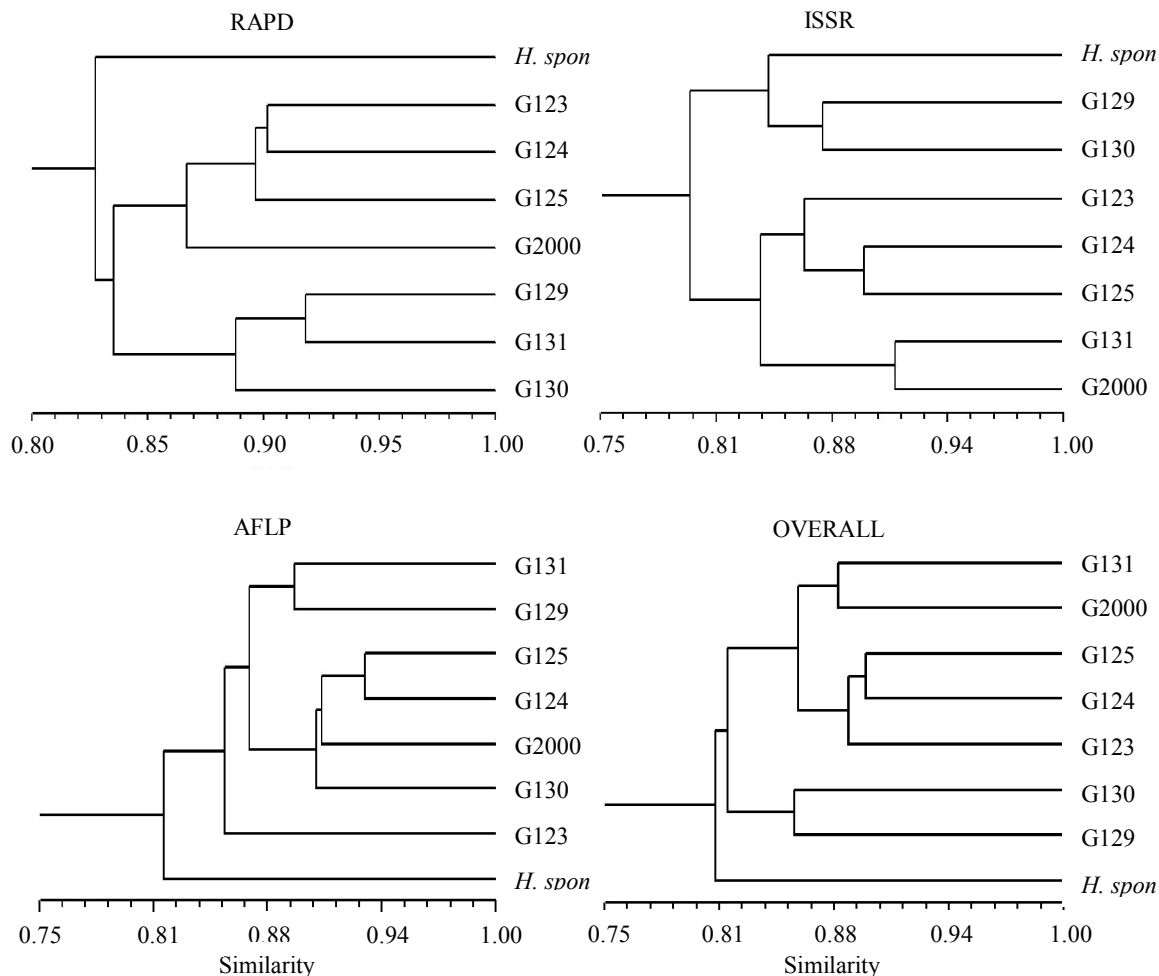
Figure 3: Dendrogram based on algorithm of unweighted pair group method with arithmetic averages between species and among cultivars.

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Identification of Two Honey- Sensitive Pathogens Isolated from Musk Lake, Jeddah

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Abstract: Two bacterial pathogens, *Exiguobacterium* ET and *Staphylococcus aureus* ET, have been isolated from wastewater, Musk lake, Jeddah, KSA, biochemically characterization using the API system, and molecularly identified using the partial sequencing of the 16S rRNA gene (approx. 1000 bp). The 16S rDNA nucleotides of the orange colonies showed 98% similarity to *Exiguobacterium sp.*, while those of the yellow colonies exhibited 99% similarity to *S. aureus*. API system differentiated between them in 3 biochemical tests, arginine hydrolysis, urease production and utilization of raffinose. Both of them are sensitive to Amikacin (30 µg/disc) and Amoxycillin (30 µg/disc). On the other hand, their resistance and sensitivity to Nalidixic acid (30 µg/disc) and Sulfamethoxazole (50 µg/disc) are distinguishable. Diluted honey (60- 80%) was highly potential to inhibit the growth of the isolates. Neat honey (100%) reduced the optical density (OD_{550} is not less than 0.28 for both strains) of the pathogens but not as much as the diluted one (OD_{550} is not more than 0.03 upon using 70% of honey for both strains).

1. Introduction

Wastewater offers a source of water that could drastically reduce the utilization of precious natural water resources. Sources of wastewater can include collected storm water, runoff, industrial wastewaters, domestic grey water and sewages (Bahlaoui et al., 1997). Polluted and/or untreated waters have a large health risk by causing water born disease (Anderson, 1996). Despite large advances in water and wastewater treatment, world born diseases still pose a major world-wide threat to public health. It has been reported that water-born pathogens infect around 250 million people each year resulting in 10-20 million deaths (Anderson, 1996). Many of these infections occur in developing nations which have lower levels of sanitation, problems associated with low socioeconomic conditions and less public health awareness than in more developed nations. However, the health risk for the public comes from microbial pathogens, toxic chemicals and heavy metals (Fliermans, 1996).

The contamination of foods by water containing known toxin producing *Staphylococcus aureus* and other *Staphylococcus* spp. can cause outbreaks of food poisoning, as this genus is one of the major bacterial agents causing food born disease in human worldwide (Le-Loir et al., 2003; EFSA, 2010). The genus *Exiguobacterium* is a newly described bacterium which was explained by Collins *et al*., 1983. This genus has 17 described species and more than 294 strains and new members are still adding to it. Members of this genus had firmly been identified as the genera such as *Corynebacterium, Brevibacterium*

and *Staphylococcus* (Rodrigues et al., 2006) because of some similarities in peptidoglycan structure or fatty acid profile (Karami et al., 2011). *Exiguobacterium* can also be found in many sources of water bodies (Kim et al., 2005; Karami et al., 2011).

One of the most effective antimicrobial agents is honey. It has been shown to exert antimicrobial action against a broad spectrum of fungi and bacteria (Cooper et al., 2002). The reasons for this antimicrobial activity include a relatively low water activity (0.56-0.59), low pH (3.2-4.5), the production of hydrogen peroxide on dilution (as a result of the presence of the enzyme glucose oxidase) and the presence of phytochemical components including flavonoids and phenolic acids (Cooper et al., 2002). Honey has been used from ancient times as a method of accelerating wound healing (Zumla and Lulat, 1989) and has been mentioned in the Torah, Bible and Quran (Namias, 2003).

Traditionally, bacterial identification is performed by isolating the organism and studying it phenotypically to elucidate its Gram staining, culture and biochemical characteristics (Woo et al., 2001). Nowadays, PCR based techniques are commonly used for typing, as they are easy, fast and cost effective (Vázquez-Sánchez et al., 2012). Among such techniques, amplification and sequencing of the 16S rRNA gene, which had proved to be a sufficient successful tool for identification of many bacterial species including members belonging to the genus *Exiguobacterium* (Chaturvedi and Shivaji, 2006) and

Staphylococcus (Woo et al., 2001; Kajikazawa et al., 2007).

In the current study, two different bacterial isolates, *Exiguobacterium* ET and *Staphylococcus aureus* ET, have been isolated from Musk lake (Jeddah, KSA), characterized and molecularly identified using the 16S rRNA gene. Besides, the antibacterial effect of some antibiotics as well as honey against these pathogens has been tested.

2. Materials and Methods
Sampling, bacterial isolation and enumeration

Water samples were taken from Musk lake, Jeddah, KSA, directly to sterilized bottles in December, 2011. Water samples were diluted up to 10^{-6} in sterile distilled water before cultivation on nutrient agar (NA), Difco, Detroit, USA, plates. Petri-dishes then incubated for 24h at 35 °C and the number of general viable bacteria was counted.

Morphological characterization

Pure cultures were examined for colony morphology. Cell morphology was tested using Gram and Malachite green stains.

Biochemical methods

Biochemical characterization was performed using API 20E kit (Biomereux, France). 0.1 ml of overnight cultures has been used to inoculate each well, containing freeze-dried test substrate. The inoculated strips were incubated at 35°C for 24 h. For the sensitivity test, the following antibiotics were purchased from Sigma co. and their concentrations are in μg/disc: Norfloxacin (10), Amikacin (30), Amoxycillin (30), Nalidixic acid (30) and Sulfamethoxazole (50).

Source and dilution of honey

The honey (Gannah Bee Honey, Al-Farouk apiaries co., Cairo, Egypt) used in this study was diluted in sterile nutrient broth (Difco, Detroit, USA) to different concentrations, 10-80% (v/v) in order to determine the minimum inhibitory and/or the most effective concentration of the used honey. 50 μl of each bacterial culture (approximate $O.D_{550}$= 0.9) was added to each dilution. Tubes were then incubated at 35 °C for 48h (Agbaje et al., 2006). Optical densities were detected at 550 nm using Genesys 10 UV spectrophotometer, Thermo Scientific, USA. Two sets of controls were employed; one was a row of positive tubes containing only the growth medium and each of the microorganism, while the other was a negative control which consists of a row of tubes containing different concentration of honey with no organism (Agbaje et al., 2006).

DNA extraction, purification and amplification

DNA was extracted from the bacterial cultures and purified using EZNA bacterial DNA extraction kit (Omega Biotek, USA). The 16S rDNA region was partially amplified (approx 1000 bp) by polymerase chain reaction (PCR) using the forward primer (FP) 5'-AGAGTTTGATCMTGGCTCAG-3' and the reverse one (RP) 5'-TACGGYTACCTTGTTACGACTT -3'. The PCR mixture consists of 30 pmol of each primer, 100 ng of DNA, 200 μM dNTPs, 1.5 mM magnesium (Mg) Cl2, 20 mM potassium chloride (KCl), 10 mM trishydrochloric acid (HCl), pH 8.3, and 2.5 U of Taq polymerase (Pharmacia Biotech, USA). The 50 μl PCR mixture containing tube was placed in the DNA thermocycler, Gene cycler TM BIO- RAD, USA. The PCR conditions were as follows: initial denaturation of DNA at 95°C for 3 min and then 30 cycles of three-step PCR amplifications consisting of denaturation at 94°C for 1 min, primer reannealing at 55°C for 1 min and extension at 72°C for 2 min. Samples were subjected to an additional extension at 72°C for 10 min at the end of the amplification cycles (Ausubel et al., 1999). The amplicons were finally purified using QIA quick PCR purification kit (QIAgen, USA).

Gel electrophoresis

Ten μl of PCR products, mixed with loading buffer, were loaded on a 2% w/v agarose gel and electrophoresed with 1X TEA (Tris EDTA Acetate) buffer. DNA was visualized by UV transillumination after staining with ethidium bromide (0.5 μg/ml). The molecular sizes of the amplified DNA fragments were estimated using DNA molecular weight marker X (Roche Applied Science, Germany).

DNA sequencing and data analysis

The purified PCR products have been sequenced using the BigDye Terminater cycle sequencing kit (Applied Biosystems, USA) in ABI Prism 3730 sequencer (Perkin Elmer, Applied Biosystem, USA). Sequences were deposited in the GenBank and the accession numbers were obtained. After obtaining the sequences, homology search was performed against DDBJ (DNA Data Base Japan), using Blast program to find the sequences producing significant alignment with the obtained ones. Similarity percentages among the sequences were obtained using Biology WorkBench software version 3.2.

3. Results

Water samples were taken from Musk lake, Jeddah, KSA, in December, 2011. The total bacterial viable count was 8×10^6. Orange and yellow colonies represented almost 60% of the total count. Therefore, two of these colonies were picked up as representatives for the environmental sample.

Table 1 represents the results of the morphological examination, biochemical tests performed using API system and sensitivity test using some antibiotics. Although both cultures have a lot of common characteristics, they are differing from each other with respect to colony and cell morphology.

Besides, *Staphylococcus aureus* ET (yellow colonies) is unique to hydrolyze arginine and urea. On the other hand, only *Exiguobacterium* ET (orange colonies) can utilize raffinose. The sensitivity test was also distinguishable for some extent, especially for nalidixic acid (30) and sulfamethoxazole (50). *S. aureus* ET was sensitive to nalidixic acid (30) and resistant to sulfamethoxazole (50), while *Exiguobacterium* ET was resistant to the first antibiotic and sensitive to the second one, respectively.

TABLE 1. The morphological and biochemical tests of the two bacterial isolates.

Tests	*Staphylococcus aureus* ET	*Exiguobacterium* ET
Conventional methods:		
Colony color	Yellow	Orange
Gram reaction	Positive	Positive
Cell shape	Cocci	Rods
Spore formation	None	None
Catalase production	Positive	Positive
API System:		
Utilization of:		
D-Glucose	Positive	Positive
D-Fructose	Positive	Positive
D-Mannose	Positive	Positive
Maltose	Positive	Positive
Lactose	Positive	Positive
Mannitol	Positive	Positive
Raffinose	Negative	Positive
Trehalose	Positive	Positive
Sucrose	Positive	Positive
Pyruvate	Positive	Positive
Melibiose	Negative	Negative
Xylose	Negative	Negative
Xylitol	Negative	Negative
α-Methyl-D-glucoside	Negative	Negative
N-acetyl-glucosamine	Negative	Negative
Nitrate reduction	Positive	Positive
Production of:		
Arginine hydrolase	Positive	Negative
Urease	Positive	Negative
Alkaline phosphatase	Positive	Positive
Antibiotic tolerance (μg/disc):		
Norfloxacin (10)	Resistant	Resistant
Amikacin (30)	Sensitive	Sensitive
Amoxycillin (30)	Sensitive	Sensitive
Nalidixic acid (30)	Sensitive	Resistant
Sulfamethoxazole (50)	Resistant	Sensitive

As can be shown in Table 2, honey has been proved to be an effective antibacterial agent, especially after dilution to a certain limit (60-80%). The ODs for both strains were dramatically decreased upon using diluted honey (not less than 60%). It can be said that the minimum inhibitory concentration for honey against both species is 60%, while the most effective one is 70%. Interestingly, neat honey (100%) was not so effective (ODs were decreased to 0.28 and o.32 for *S. aureus* and *Exiguobacterium*, respectively) as much as the diluted ones, 60 and 70%, where the ODs decreased to 0.05-0.03 and 0.055-0.023 for *S. aureus* and *Exiguobacterium*, respectively.

TABLE 2. Sensitivity of the two isolates against the antibacterial effect of different concentrations of honey.

Honey concentration % (v/v)	Optical Density of bacterial cultures at 550 nm	
	S. aureus ET	*Exiguobacterium* ET
0	1.2	1.13
40	0.53	0.49
50	0.35	0.24
60	0.05	0.055
70	0.03	0.023
80	0.033	0.029
100	0.28	0.32

The 16S rDNA universal primers, FP and RP, have been used to amplify and sequence around 1000 bp of the 16S rRNA genes from the newly isolated bacteria, *Staphylococcus aureus* ET and *Exiguobacterium* ET (Figure 1). The homology search of the obtained DNA sequences revealed that the yellow colonies are belonging to *Staphylococcus aureus* with a similarity percentage of 99% and the orange ones to *Exiguobacterium sp* with 98% similarity. The sequences were deposited in the GenBank and accession numbers are given in Table 3.
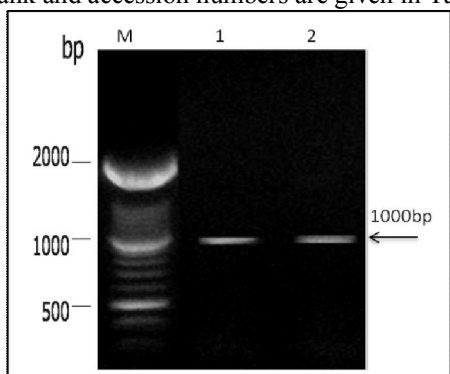

Figure 1. Gel electrophoresis of approx. 1000 bp of the 16S rRNA gene. M, DNA molecular weight marker X; 1, *Staphylococcus aureus* ET; and 2, *Exiguobacterium* ET.

TABLE 3. Accession numbers of the newly isolated bacteria.

Bacteria	Accession numbers
Staphylococcus aureus ET	JX163859
Exiguobacterium ET	JX163860

Discussion

As expected from a highly polluted wastewater lake, high bacterial viable count (8×10^6 CFU/ml) was found in Musk lake, Jeddah, KSA, in December, 2011. However, two unique colonies, orange and yellow, were picked up as representatives for the taken environmental sample. The orange one is for the genus *Exiguobacterium* which was firstly found by Collins et al., 1983 to accommodate Gram positive and non-spore

forming rods. This genus was found before by some researchers in polluted water bodies (Kim et al., 2005; Karami et al., 2011). Besides, genus *Staphylococcus* (yellow colonies) forms Gram –positive cocci that posses catalase- positive activity (Woo et al., 2001). Pathogens belonging to this genus have been recently isolated from fishery products (Vazquez-Sanchez et al., 2012), polluted water (Motsepe and Warwick, 2000) and sea water (Goodwin and Pobuda, 2009). Moreover, some studies have shown that *Staphylococcus aureus* count can be greater than that of coliforms and in some cases *S. aureus* is present in the absence of coliforms (Yoshe-Purer and Golderman, 1984). This finding is in parallel to the current results where the orange and yellow colonies were predominant in the total bacterial count. This suggests that staphylococci may be a suitable indicator in evaluating water quality (Yoshe-Purer and Golderman, 1984).

Although API system was successful to characterize the two species under test (*Exiguobacterium sp.* and *S. aureus*) giving results similar to great extent to those of Woo et al., 2001 and Kim et al., 2005, it was not so effective in differentiation between them. Only 3 tests were discriminative, arginine hydrolysis, urease production and raffinose utilization. This reflects the need to use more than one tool for discrimination among the environmental isolates. Sensitivity test using 5 different antibiotics showed that the infections by the pathogens under test can be healed using Amikacin (30) and Amoxycillin (30). However, differentiation between the two species is obvious using Nalidixic acid (30) and Sulfamethoxazole (50).

Honey has been shown convincingly to have a potent antibacterial activity. The holy Quran intimates honey as the healing for diseases. It occupied a prominent place in traditional medicine and its antimicrobial effect has been reported by a number of workers (Armon, 1981; Haffeejee and Mosa, 1985; Reynold, 1989; Hamid and Saeed, 1991). Interestingly, dilutions from 60 to 80% of honey inhibited the growth of the 2 pathogens to a very great extent (ODs were decreased to 0.03 and 0.029 for *Staphylococcus aureus* ET and *Exiguobacterium* ET, respectively). On the

other hand, undiluted honey showed less activity against both pathogens (ODs were not less than 0.28) despite its high osmotic pressure. This is may be due to hydrogen peroxide produced by the enzymatic activity of glucose oxidase in honey when diluted (Cooper et al., 1999). The current results of the diluted honey are in parallel with cooper et al., 1999 studies in Manuka and Pasteur honeys. They have found that the antibacterial activity of honey doesn't depend wholly on its high osmolarity (due to supersaturation with carbohydrates). It also depends on other factors such as the release of hydrogen peroxide, the presence of inhibine (Obaseiki-Ebror et al., 1983), an antibacterial substance in honey which can be reduced by sunlight, and acidity (Hamid and Saeed, 1991), pH 3.2-4.5. Therefore, in this research the minimum inhibitory concentration of honey is 60%, but the most effective ones are 70 and 80%. These results confirm the effectiveness of the diluted honey against Gram positive bacteria especially the toxic pathogen, *Staphylococcus aureus*, as indicated before by Cooper et al., 1999 and Agbaje et al., 2006.

Small rRNA gene sequencing, particularly the 16S rRNA sequencing in bacteria has led to advances on multiple fronts in microbiology. First, construction of phylogenetic tree for classification (Olsen et al., 1992; Olsen and Woese, 1993; Thompson et al., 1994). Second, it revolutionizes the classification of microorganisms and makes the classification of non cultivable microorganisms possible (Relman et al., 1990; Relman et al., 1992). Third, it helps to elucidate the relation of unknown bacterial species to known ones (Woo et al., 2001). In the current study, partial sequencing of the 16S rRNA gene (approx. 1000 bp) has successfully identified the two different Gram positive isolates, *Exiguobacterium* ET (98% similsrity to *Exiguobacterium sp.* in the database) and *Staphylococcus aureus* ET (99% similarity to *S. aureus* in the database). The use of that gene makes the turnaround time short. Besides, the 16S rRNA gene hypervariant region of the 5′ end, which has been sequenced in the current study, has been proved to be an efficient molecular chronometer which can be used effectively to distinguish between closely related species (Goto et al., 2002; Mohamed and ElSersy, 2009; ElSersy and Mohamed, 2011). However, many species belonging to S. *aureus* and other staphylococci have been successfully identified in previous studies using that gene (Golledge and Gordon, 1989; Olsen et al., 1992; Woo et al., 2001). Besides, Karami et al., 2011 have stated that sequencing of the 16S rRNA gene is a powerful tool for identification of many *Exiguobacterium* spp.

Acknowledgment

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Virulence of *Candida albicans*, *Trichomonas vaginalis* and *Chlamydia trachomatis* Vaginal Pathogens in Sexually Transmitted Diseases

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Abstract: The effects of four environmental stresses of heat, drought, wounding and cell culture, on the transcriptional reactivation of seven long terminal repeat retrotransposons (LTRs) of barley (*Hordeum vulgare*) genome, were investigated. These LTRs included two *Copia*-type (*Bare* and *Maximus*); and five *Gypsy*-type (*Erika, Jeli, Sabrina, Sukkula*1 and *Sukkula*3) LTRs. RT-qPCR analyses revealed that *Erika*1 LTR was highly reactivated under heat, drought, and wounding with 28.1, 9.9 and 9.4 fold increments, respectively. *Sabrina* LTR was 6.2 fold reactivated under cell culture. *Bare*1 LTR was reactivated by drought (4.1 fold) and cell culture (3.4 fold). Transcription activity of *Jeli* LTR was increased by 3.4 fold under cell culture. *Sukkula*1, *Sukkula*3 and *Maximus* LTRs were slightly reactivated under drought, wounding and cell culture. These changes in the reactivation pattern of LTRs provide fingerprints for tracking the molecular changes occurred in barley genome upon exposure to environmental stresses, which might result in gain or loss of yield. Such sensitive LTR expression profiles underline one of the important role of LTR genetics in agriculture.
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1. Introduction

Sexually transmitted infections (STIs) are infections that are spread primarily through person-to-person sexual contact. There are more than 30 different sexually transmissible bacteria, viruses and parasites (Slotnick *et al.,* 1963 and Faro., 1991). An extensive and diverse spectrum of pathogenic and non-pathogenic organisms may be observed in vaginal microflora. In spite of the wide-ranging literature on the microbiology of normal and abnormal flora of the vagina, many questions have still not been completely answered (Fleury., 1981and Hammill *et al.,* 1989). Various infectious processes in the vagina are the result of disequilibrium of this flora[1], such as that occurring during pregnancy(McCue *et al.,* 1979 and *et al.,* 1991). The most common STDs infections are caused by *Candida sp., Gardnerella vaginalis, Trichomonas vaginalis* and *Chlamydia trachomatis* (Bauer *et al.,* 1991and . Bosch, 1992[)]

Most STDs affect both men and women, but in many cases the health problems they cause can be more severe for women (Schneider *et al.,* 1987 and Murta *et al.,* 1997). If a pregnant woman has an STD, it can cause serious health problems for the baby (Moore *et al.,* 1982).Sexually transmitted diseases (STDs), also called venereal diseases, are caused by sexual contact or contact with infected objects such as toilet seats. Sexually transmitted

diseases have become a serious health problem (Kinghorn, 1978).Young women are at greater risk than older women for reproductive and health complications caused by STDs (Carne, 1990 and Strand *et al.,* 1993)

Because Sexually transmitted diseases are prevalent in the world, our study advise a sexually active adult to have regular sexual check-ups to identify and diagnose STDs. There are several methods to test for sexually transmitted diseases (or sexually transmitted infections) such as physical examination and laboratory diagnosis when symptoms are visible.

2. Materials and Methods
Specimens

Clinical specimens include urine, vaginal fluid, urethral discharge, prostate secretions, or urethral scrapings semen, and endocervical smear. Vaginal specimens for culture may be either self collected with a swab, or collected by the clinician with a swab or plastic loop for the diagnosis of bacterial, fungal and parasitic infections (Voog *et al.,* 1995).

Diagnosis (Suzanne *et al.,* 2003, Yin *et al.,* 2008 and Martínez 2009:
- Physical Exam to look for symptoms of disease
- Microscopic examination of vaginal secretions
- Culture of male urine or urethral swab
- Polymerase chain reaction (PCR)

Microscopic examination

Identification of the organism on microscopic examination of clinical specimens was done using Wet mount preparation for diagnosis of trichomoniasis in women. Vaginal secretions are obtained from the lateral walls using a swab or plastic loop; in men, any urethral discharge, prostate secretions, or urethral scrapings may be used. The secretions are then suspended in 0.85% normal saline and examined under the microscope.

Culture on Diamond's modified medium was used for diagnosing *T vaginalis* infect In men, microscopy of urine is insensitive, although occasionally organisms are visible in a first-voided morning specimen or a centrifuged specimen. Cultures of urine and urethral swabs are more sensitive.

Polymerase chain reaction (PCR) Anke and Birgit (2008)

Because of the insensitivity of wet mount preparation and the problems with culture methods, nucleic acid amplification methods, such as polymerase chain reaction (PCR), have become increasingly attractive for diagnosing of STIs. PCR has the advantage of requiring only DNA, from either viable or non-viable organisms, and in concentrations as low as one organism per PCR reaction. Microbial samples were treated with enzymes that amplified specific region of the microbe. After amplification, the number of DNA fragments were quantified. PCR were carried out in a total volume of 25 μl consisting of 1x Eurogentec Master Mix, 5 mM $MgCl_2$, Amprease, 115 nM primers and 100 nm probe and 2.5 μl of template DNA. The X^2 test was used for statistical analysis with the significance level set at less than <0.05(Platz-Christensen *et al.,* 1994).

Blood, Urine or Cell Samples

The testing and diagnosis of chlamydia, *Trichomonas vaginalis,* require blood, urine or cell samples to be taken and analyzed.

Chlamydia

Chlamydia can be detected by a urine test or swab sample from the urethra, the cervix, rectum, throat or eye. A visible symptom of chlamydia is inflamed cervix cells or a discharge.

Trichomoniasis

Trichomoniasis is caused by the parasite *Tricohomonas vaginalis.* Trichomoniasis, or trich, can be detected in a physical examination when an unusual discharge is apparent or during a routine cervical smear test. Another method to test for this STD is through a urine sample or a cell sample from the vagina, genital area or urethra. For all of the STDs listed

Hyaluronidase, chondroitin sulphatase and proteinase assay (Baeten *et al.,* 2001).

Hyaluronidase, chondroitin sulphatase and proteinase secretion were tested using Sabouraud glucose agar (Difco, USA). An aqueous solution of 2 mg/ml human umbilical cord sodium hyaluronate (Sigma Chemical Co., USA) was sterilized by Seitz filter (0.02 μm), 4mg/ml bovine trachea chondroitin sulphate type A (Sigma) and 5% of bovine albumin fraction V (Sigma) were sterilized by Millipore filtration (0.02 μm). The substrates were added to cooled media to give final concentrations 400 mg/ml, bovine albumin fraction V was added to give final concentrations of 1%. The final pH was 5.6. Plates were point inoculated and incubated for 3-4 days at 37ºC. The plates were flooded with 2 N acetic acid for 10 min. The enzyme activity was visualized by the presence of clear zone around the colonies and determined using the method described by Price *et al.* (1982). Hyaluronidase and chondroitin sulphatase activity (Hy, Ch) was measured in terms of the ratio of the diameter of the colony to the total diameter of the colony plus clear zone around it. When the value of Hy or Ch is below 1 it indicates that the isolate is releasing the enzyme into the medium. Sabouraud glucose agar containing 1% BSA was used for detection of proteinase.

Epithelial cells(Banno *et al.,* 1985)

The urine samples were obtained in the morning from healthy women and centrifuged at 350 rpm for 10 min to harvest the epithelial cells. The sediment was washed twice in phosphate buffered saline (PBS) pH 7.2 and the number of cells were estimated microscopy with a counting chamber, then standardized to 10^5 cell/ml in PBS buffer.

Adhesion assay (Douglas, 1986)

A mixture of equal volumes of epithelial cells (10^5 cell/ml) and *Candida albicans* (10^8 cell/ml) was incubated in plastic tubes on a rotator at 37 ºC for 2 hrs. The epithelial-yeast mixture was passed through polycarbonate filters (12 μm pore size) to remove non adhering yeast. Adhesion was evaluated spectrophotometer by measuring the optical density of epithelial cells with adherent yeasts.

3.Results

After isolation, purification and identification of microorganisms from urine, vaginal fluid, urethral discharge, prostate secretions, or urethral scrapings semen, and endocervical smear from 50 control group and 50 patients group of women and men, aerobic Gram positive cocci such as *Staph. aureus, Staph. epidermidis*, *Streptococci spp* and *Sarcinaspp*, Gram negative cocci as *proteus spp.* and Gram negative rods as *E. coli*, anaerobic bacteria such as *Bacteroids distasonis* and *lactobacilli spp,* yeast such as *Candida albicans* were isolated from control and patients women and men groups. However, *Trichomon vaginalis* and *chlamydia* were

isolated only from patient groups and as shown in   Table 1.

Table 1: Infection by various microorganisms in patients with signs and symptoms in relation to control group

Microorganisms	Women						Men					
	Control		Patients		X^2	P	Control		Patients		X^2	P
	No	%	No	%			N	%	No	%		
Staph aureus	4	8	7	14	0.9	NS	2	4	2	4	0.0	NS
S. epidermidis	2	4	12	24	8.3	<0.05	3	6	10	20	4.3	<0.05
Streptococci spp	3	6	5	10	0.5	NS	5	10	5	10	0.0	NS
Sarcinaspp	1	2	1	2	0.0	NS	1	2	1	2	0.0	NS
E. coli	-	-	4	8	5	<0.05	-	-	4	8	5	<0.05
Proteus spp	1	2	-	-	1.0	NS		2	-	-	1.0	NS
Bacteroids spp	2	4	8	16	4.0	<0.05	2	4	7	14	4.0	<0.05
Lactobacilli spp	20	40	24	48	0.7	NS	20	40	23	46	0.4	NS
Candida sp	3	6	25	50	0.0	NS	4	8	4	8	0.0	NS
Trichomonas	-	-	12	24	8.3	<0.05	-	-	4	8	5	<0.05
Clamydia	-	-	4	8	5	<0.05	-	-	4	8	5	<0.05

Table 2 shows features of common Infectious vaginitis. Yeast symptoms of itching and burning often overlap with other conditions such as allergic or irritant vulvitis, then the exact signs and symptoms of yeast vulvovaginitis were diagnosed by cultures and microscopic examinations.

Table 2. Typical Features of Common Infectious Vaginitis

	Vulvovaginal candidiasis
Symptoms	
vulvar irritation	++
Dysuria	-
patients –	++
Odor	+ -
Signs	
Labial erythema –Satellite lesions	+
Discharge	+ -
Consistency	Crudy
Color	White
Microscopy	Normal
Epithelial cells	Variable
PMNs/epithelial cell	Gm +ve
Bacteria Gram-	Yeast and Pseudohyphae
Pathogens	
Bimanual examination	
Vaginal tenderness	+
Rugal hypertrophy	+
Adnexal tenderness	-

++ = usually present and may be severe; + = usually present; ± = variably present; – = not usually present and presence should raise concern for other diagnosis; PMNs = polymorphonuclear leukocytes.

Table 3 shows a group of women who had positive cultures for *candida albicans* (some women have positive cultures but no symptoms) signs of vulvar swelling, cracked skin fissures, a reddened vulva or sores from scratching and a thick, curdy vaginal discharge were infrequent. When the women had their symptoms and findings categorized by whether the culture was positive or negative and whether the microscopic wet-prep was positive or negative, the frequencies in the different groups were interesting.

Table 3: Symptoms and Signs by Test Results

Symptom/ Sign	Culture negative wet-mount negative (presumably not infected)	Culture positive wet-mount negative (uncertain infection)	Culture positive wet-mount positive (presumably has infection)
Symptoms			
Chief complaint of vulvar itching or burning	8%	13%	38%
Vulvar swelling	10%	22%	25%
White discharge	63%	62%	68%
Yellow discharge	23%	26%	22%
Examination findings			
Vulvar swelling/edema	2%	11%	22%
Vulvar redness	21%	32%	72%
Thick curdy discharge (exam)	1%	3%	28%

Morphology of *Candida albicans* strains isolated from vagina of asymptomatic and symptomatic patients:

The results demonstrated non germinative non virulent strains of *Candida albicans* with typical yeast-like form similar to the morphology in their respective in vitro cultures isolated from the vagina of asymptomatic patients (Figure 1).
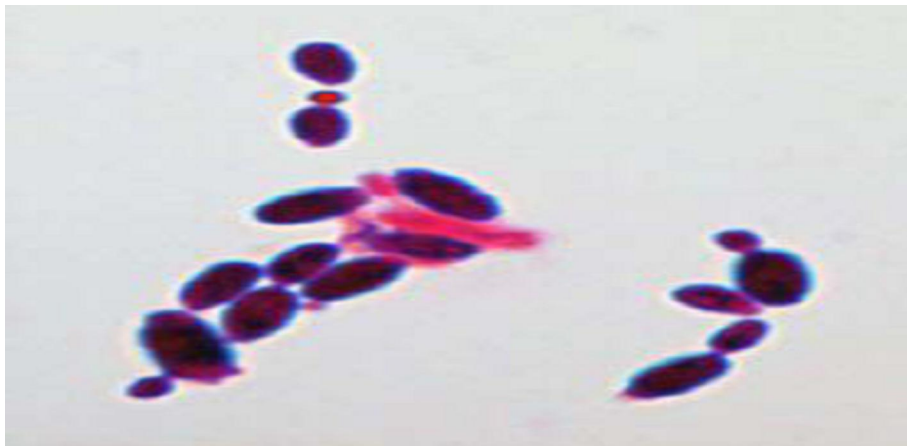

Figure 1: Non virulent strains of *Candida albicans* yeast

The morphology of *Candida albicans* strains isolated from the vagina of symptomatic patients showed long filaments tube of pseudomycelial and germ tube forming cells (Table 4) .
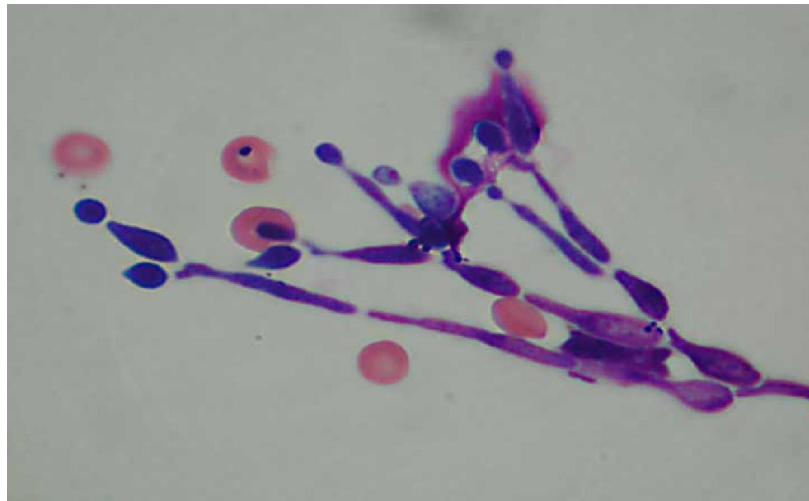
Figure 2: Virulent strains of hyphae and germ tube forming *Candida albicans*

Table 4 morphological characteristics of selected isolates of *C. albicans*

Asymptomatic Strain	Serotype	Morphology			Symptomatic Strain	serotype	Morphology		
		C	H	G			C	H	G
C1					C1				
C2	A	+	-	-	C2	A	-	+	+
C3	A	+	-	-	C3	A	-	+	+
C4	A	+	-	-	C4	A	-	+	+
C5					C5				
C6	A	+	-	-	C6	A	-	+	+
C7	A	+	-	-	C7	A	-	+	+
C8	A	+	-	-	C8	A	-	+	+
C9					C9				
C10	A	+	-	-	C10	A	-	+	+
C11	A	+	-	-	C11	A	+	+	+
C12	A	+	-	-	C12	A	+	+	+
C13					C13				
C14	A	+	-	-	C14				
C15	A	+	-	-	C15	A	-	+	+
C16	A	+	-	-	C16	A	-	+	+
C17	A	+	-	-	C17	A	-	+	+
C18					C18	A	-	+	+
C19					C19				
C20	A	+	-	-	C20	A	-	+	+
C21	B	+	-	-	C21	A	-	+	+
C22	A	+	-	-	C22	A	-	+	+
C23					C23				
C24	A	+	-	-	C24	A	-	+	+
C25	C	+	-	-	C25	A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		C	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+
	A	+	-	-		A	+	+	+

C, *Candida albicans* yeast   H, hyphae   G, Germ tube.

Hyaluronidase, chondroitin sulphatase and proteinase activity

The comparative study of the enzyme activity revealed that all tested *Candida albicans* isolates from asymptomatic carriers were proteinase, hyaluronidase and chondroitin sulphatase non-producer (Pr $^-$, Hy $^-$, Ch $^-$) except 4 isolates were weak chondroitin producer and 6 isolates were hyaluronidase producer (Table 5). All tested *Candida albicans* isolates from patients were proteinase, hyaluronidase and chondroitin producerexcept *C19* was hyaluronidase non producer (Pr $^+$, Hy $^-$, Ch$^-$) and *C21* was chondroitin sulphatase non-producer (Pr $^+$, Hy$^+$, Ch$^-$) . However, *Trichomonas vaginalis* and *Chlamydia trachomatis* strains were isolated only from patients showed the highest hyaluronidase, chondroitin sulphatase and proteinase enzyme activity (Table 6).

Table 5 Hyaluronidase, chondroitin sulphatase and proteinase production by vaginal *Candida albicans* isolates from asymptomatic carriers.

Isolate	Hy	Ch	Pr	Isolate	Hy	Ch	Pr
C1	1.00	1.00	1.00	C14	0.89	0.88	1.00
C2	1.00	1.00	1.00	C15	0.99	1.00	1.00
C3	1.00	1.00	1.00	C16	0.95	1.00	1.00
C4	1.00	1.00	1.00	C17	1.00	1.00	1.00
C5	1.00	1.00	1.00	C18	1.00	1.00	1.00
C6	1.00	1.00	1.00	C19	1.00	1.00	1.00
C7	1.00	1.00	1.00	C20	1.00	1.00	1.00
C8	1.00	1.00	1.00	C21	1.00	1.00	
C9				C22			
	1.00	1.00	1.00		1.00	1.00	1.00
C10	1.00	1.00	1.00	C23	1.00	1.00	1.00
C11	0.98	0.95	1.00	C24	1.00	1.00	1.00
C12	0.96	0.91	1.00	C25		1.00	
C13	0.93	0.89	1.00		1.00		1.00

Value (relative enzyme activity) lower than 1.0 indicates enzyme activity

Adhesion assay

Figure 3 showed adhesion of three strains of *Candida albicans* (yeast like cells, filamentous cells and germ tube forming cells). Germ tube forming *Candida albicans* strain revealed the highest percentage of adherence to epithelial cells (highest optical density). In comparison to germ tube forming *Candida albicans* strain, filamentous *Candida albicans* strain showed 60% adhesion. Avirulent yeast like *Candida albicans* strain reported 10 % adhesion. However <u>*Chlamydia*</u> and *Trichomon vaginalis* showed the highest percentage of adherence to epithelial cells (highest optical density) as in Germ tube forming *Candida albicans*.

4. Discussion

Sexually transmitted diseases (STDs) are infections generally acquired by sexual contact. The organisms that cause sexually transmitted diseases may pass from person to person in blood, semen, or vaginal and other bodily fluids (Menday, 2002) . Some of these infections can also be transmitted non sexually, such as from mother to infant during pregnancy or childbirth, or through blood transfusions or shared needles (Donders and Spitz, 2000)

Most STDs affect both men and women, but in many cases the health problems they cause can be more severe for women. If (STI) are not untreated, they can increase the risk of acquiring another STD such as HIV. This happens because an STD can stimulate an immune response in the genital area or cause sores, either of which might make HIV transmission more likely. Some untreated STDs can also lead to infertility and urinary tract infection in men, but often causes no symptoms in men(Schwebke and Morgan, 1997)

Trichomonus vaginalis, *Chlamydia trachomatis* and *Candida albicans* are the most commonly reported organisms cause sexually transmitted diseases. symptoms range from mild irritation to severe inflammation(Schwebke et al, 1999). Thrush, also known as candidiasis, is a yeast infection caused by the Candida species of fungus. Thrush is not technically a sexually transmitted infection, as Candida is a common yeast that is found on the skin and genitals of most people, even those who have not had sex. Candida is usually suppressed by the immune system and the natural bacteria found in the body, but there are many things that can upset the balance and allow Candida to grow. Thrush occurs a

lot less frequently in men (Hill, 1965 and Goldenberg, 2000)

Germ tube and hyphal development of Candida albicans is likely to play a role in pathogenesis of acute Candidal vaginitis.   A widely accepted suggestion holds that the yeast form of this fungus colonize the healthy vagina asyptomatically, whereas hyphal or pseudohyphal development is associated with the onset of disease. Mechanism by which hyphal growth can favor fungal infection, such as the increase of fungal adherence to vaginal epithelial cells, have been also postulated(Goldenberg et al., 2002).

Production of the enzyme aspartyl proteinases is also advocated as a characteristic of the most vaginopathic organisms (Bernardis et al., 1990)

Its apparent relevance as a vaginopathic factor for Trichomoniasi, Chlamydia and Candida albicans can be possibly explained by the virulence factors such as secretion of hyaluronidase, chondroitin sulphatase and proteinase enzymes and adhesion ability (King et al., 1980 and Lederman et al., 2008) . Proteinase secretion ofTrichomoniasi, Chlamydia and Candida is likely to play a role in human vaginitis. Hyaluronidase, chondroitin sulphatase and proteinase can affect the permeability of epithelium at the intercellular spaces by attacking the intercellular cementing substances of tissue. As a consequence of this destruction, microbial invasion of the tissue may occur (Rex et al., 2000 and Spinelli, 2010)

Table 6 Hyaluronidase, chondroitin sulphatase and proteinase production byvaginal *Candida albicans,Trichomonasvaginalis*and *Chlamydia trachomatis* isolates from patients

Isolate	Hy	Ch	Pr	Isolate	Hy	Ch	Pr
C1	0.52	0.65	0.71	C14	0.93	0.87	0.73
C2	0.55	0.59	0.61	C15	0.59	0.65	0.67
C3	0.61	0.77	0.79	C16	0.87	0.82	0.88
C4	0.76	0.80	0.83	C17	0.57	0.64	0.69
C5	0.68	0.74	0.68	C18	0.89	0.87	0.89
C6	0.88	0.75	0.72	C19	1.00	0.79	0.93
C7	0.72	0.69	0.59	C20	0.90	0.84	0.87
C8	0.79	0.72	0.78	C21	0.75	1.00	0.69
C9	0.64	0.81	0.80	C22	0.87	0.77	0.75
C10	0.69	0.65	0.55	C23	0.66	0.86	0.74
C11	0.84	0.98	0.82	C24	0.95	0.98	0.81
C12	0.84	0.79	0.68	C25	0.85	0.88	0.79
C13	0.69	0.73	00.54				
Trichomonas							
Tr1	0.54	0.55	0.46	Tr12			
Tr2	0.61	0.68	0.52	Tr13	0.62	0.75	0.50
Tr3	0.72	0.72	0.55	Tr14	0.75	0.68	0.62
Tr4	0.55	0.75	0.66	Tr15	0.68	0.58	0.75
Tr5	0.62	0.59	0.55	Tr16	0.58	0.55	0.68
Tr6	0.75	0.78	0.62	Tr17	0.55	0.61	0.58
Tr7	0.68	0.80	0.75	Tr18	0.61	0.72	0.55
Tr8	0.58	0.66	0.68	Tr1	0.72	0.58	0.61
Tr9	0.55	0.55	0.58	Tr20	0.55	0.55	0.57
Tr10	0.61	0.82	0.55		0.45	0.61	0.58
Tr11	0.72	0.55	0.66				
Chlamydia							
Ch1	0.70	0.66	0.52	Ch9	0.55	0.62	0.45
Ch2	0.62	0.55	0.55	Ch10	0.62	0.75	0.60
Ch3	0.66	0.62	0.66	Ch11	0.75	0.68	0.55
Ch4	0.69	0.75	0.55	Ch12	0.68	0.58	0.68
Ch5	0.71	0.68	0.62	Ch13	0.58	0.55	0.58
Ch6	0.56	0.58	0.75	Ch14	0.56	0.66	0.55
Ch7	0.48	0.55	0.68	Ch15	0.66	0.64	0.61
Ch8	0.64	0.66	0.58	Ch16	0.70	0.58	0.57

Value (relative enzyme activity) lower than 1.0 indicates enzyme activity
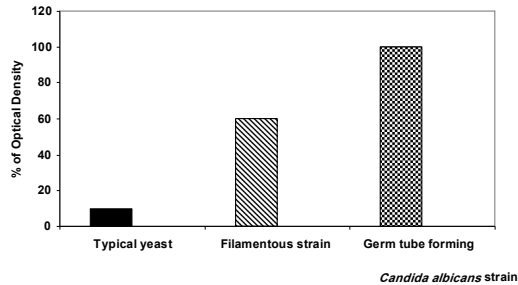
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Figure 3: Adhesion of *Candida albicans* to epithelial cells

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