CONTENTS

Review of performance of Bank Saderat of Guilan on components of service quality scale

Mohammadreza sheikholeslami, Odinaev furkat farkhodovich

Financial economics faculty department of financial management Candidate of economics science, Dushanbeh,Tajikistan
sheikholeslami@gmx.com

Abstract: One of the most important issues in today's competitive world for economic activists and service institutions, especially banks is to attract customer's satisfaction. Customer satisfaction is one of the essential factors in creating and maintaining successful long-term relations between suppliers of financial services and customers. Understanding the factors affecting the increase in customer satisfaction is of particular importance for banks. Customer satisfaction for the successful marketing is a decisive factor meaning the degree of proportionality between customer expectation about a service and actual performance of that service. "Institutions that acquire their customers are those that do their duty well. They know that being committed to attract customer satisfaction should be supported through a complete understanding of customer, competition, market, and the ability to create environments which need change and responding appropriately to these environments," says an expert in this field. Banks that they can keep their customers satisfied can also convert them into loyal customers. This paper aims to identify the factors affecting customer satisfaction and ranking them based on their importance for customers of Bank Saderat of Guilan.

[Mohammadreza sheikholeslami, Odinaev furkat farkhodovich. **Review of performance of Bank Saderat of Guilan on components of service quality scale.** *Life Sci J* 2012;9(3):2018-2022]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 291

Keywords: Satisfaction; Expectations; Performance; Quality of services

1. Introduction

With a deep looking into banks and financial institutions, in can be obviously said that customer is the capital and philosophy of existence of these institutions. In order to play their role as an economic leverage in society, banks need to attract customer satisfaction and their activities must be based on providing desirable services and satisfying the customers. Given the importance of customers in banks and financial institutions, competition for attracting more customers has become an important part of Bank management. In such circumstances, the objective of organizations is to provide services that are better in one or more dimensions compared with other competitors in order to attract customers and make them willing to pay good prices for each product (Ruth N. Bolton and James H. Drew.1991).

1.1. Customer satisfaction:

Although customer satisfaction and service quality have some commonalities, satisfaction has generally a broader satisfaction than service quality, because quality is focused on services. From this perspective, service quality is considered part of satisfaction. The relationship between these two factors is shown in the figure 1.

As the chart suggests, service quality indicates common perceptions of the five dimensions of services, while satisfaction is a broader concept and includes service quality, product quality, price, individual factors, and situational factors Nitin Seth, S.G. Deshmukh, Prem Vrat, (2005)).

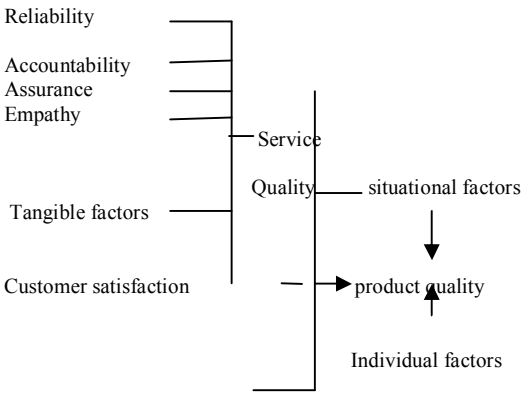


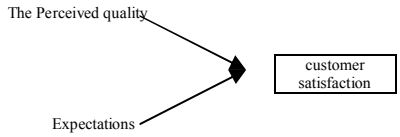
Figure 1: relationship between customer satisfaction and service quality

2.1. Advantages of customer satisfaction:

The presence of satisfied customers has many strategic advantages, especially in the financial services market (Estelami, Financial Services Marketing, page 234). Although any successful marketer is willing to provide the services that make the customers satisfied, this is not the only objective. Companies cannot ignore the other main objectives of trade such as achieving competitive profit and creating interests. Research results have shown that satisfied customers are less sensitive to prices than

dissatisfied. Customer satisfaction provides many benefits for companies and higher levels of customer satisfaction leads to greater customer loyalty.

It is not surprising that attracting the satisfaction of customers, due to its direct relationship with customer retention, market share and profit, has occupied the mind of managers (Vikas Mittal; Carly Frennea (2010)



Fiqure2: Formation of customer satisfaction model

3.1. Service quality scale:

Quality service scale has been derived from the conceptual model presented by Parasoraman and his colleagues in 1995. In this model, service quality is the resultant of comparison of expectations and perceptions of customers.

(Revised)Quintet	(1985) Basic dimensions
Tangible factors -1	Tangible factors-1
Accountability -2	Reliability-2
Accountability -3	Accountability-3
	assurance -4
	Competency -5
	communications-6
	Humility-7
	security-8
	Availability-9
	Understanding of customer-10

Fiqure 3: A summary of service quality scale proposed by Parasoraman and his colleagues

This scale was revised in 1991 and the revised version is consisted of three parts. The first and second parts include 22 questions apiece (5 dimensions) to assess customers' expectations and perceptions. The respondents are asked to express their ideas about this that what are their expectations of services and how they understand the services on a scale of 7 pieces from completely too completely disagree (The following chart). Finally, according to the difference between expectations and experiences of the respondents (performance), overall quality score of 22 above characteristics can be calculated. In the third part, the respondents are asked to determine the significance of each of the dimensions. Service quality scale is a tool to measure how the customers understand the quality of services. This model acts based on 5 dimensions (Tangible factors, reliability, accountability, assurance, and empathy) and a comparison between customer expectations of how services should be provided and their experience of how the services are provided.

Although service quality scale is widely used, it has been subjected to many criticisms. Many researchers such as Cronin & Taylor, Tiss, and Sterandvik & Jander state that measurement of perceptions merely cannot make for a better presentation of service quality. Additionally, both experience and performance should be taken into account. Hence, the usage service quality scale is not restricted to available services. However, one of the advantages of this tool is that its reliability and validity have been confirmed in a wide range of fields such as banks, hospitals, stores, etc. Although some revisions should be done in this tool to be used in some services, service quality scale is still considered the best choice for cross-sectional studies and modeling in industry (Zeithaml, Valarie A *et al*, 1999).

Parasoraman *et al* emphasized in their studies that service quality scale is a reliable valid scale service quality. They also stated that this tool is applicable in a wide range of service fields such as financial institutions, hotels, etc some of its components need to be revised or some components are needed to be added to it.

Completely agree / Completely disagree							Option (expectations)
1	2	3	4	5	6	7	A good bank has modern equipment
Completely agree / Completely disagree							Option (performance)
1	2	3	4	5	6	7	The bank A has modern equipment

Fiqure4: Quality scale chart

Although service quality scale is widely used, it has been subjected to many criticisms. Many researchers such as Cronin & Taylor, Tiss, and Sterandvik & Jander state that measurement of perceptions merely cannot make for a better presentation of service quality. Additionally, both experience and performance should be taken into account. Hence, the usage service quality scale is not restricted to available services. However, one of the advantages of this tool is that its reliability and validity have been confirmed in a wide range of fields such as banks, hospitals, stores, etc. Although some revisions should be done in this tool to be used in some services, service quality scale is still considered the best choice for cross-sectional studies and modeling in industry (Zeithaml, Valarie A *et al*, 1999). Parasoraman *et al* emphasized in their studies that service quality scale is a reliable valid scale service quality. They also stated that this tool is applicable in a wide range of service fields such as financial institutions, hotels, etc some of its components need to be revised or some components are needed to be added to it.

4.1. Parasoraman model:

Although previous efforts have a significant share in dividing service quality into process quality and output quality, these efforts in order to identify

factors determining the quality of services have been superficial.

5.1. Components of service quality scale:

This scale contains 5 dimensions including tangible factors, reliability, accountability, assurance, and empathy. Each of these dimensions has several components that is measured by a 7 rating scale (and occasionally 5 or 9 rating) from completely agree to completely disagree. Totally, these 5 dimensions have 22 components as follows:

1.5.1. Tangible factors:

1- New and advanced equipment
2- Employees with a neat appearance
3- Considerable physical facilities
4- Regular and ordered papers

2.5.1. Reliability:

5- Fulfillment of promises before the deadline
6- Showing the sincere willing to resolve the problems of customers
7- Exercising reforms in appropriate time
8- Providing services in fulfilled time
9- Providing correct reports

3.5.1. Accountability:

10- Employees inform the customers of the services they offer
11- Employees offer the services to customers in the shortest possible time
12- Employees are always willing to help customers
13- Employees are always ready to answer customers' questions

4.5.1. Assurance:

14- Employees' behaviors gradually create a feeling of trust in customers
15- Employees feel secure in their interactions with the organization
16- Employees always treat the customers politely
17- Employees have enough knowledge to answer the questions of customers

5.5.1. Empathy:

18- Individual attention to customers
19- Appropriate working hours for all customers
20- Employees pay a special attention to customers
21- Employees wish the best interests for the customers
22- Employees understand the special needs of customers

2. Material and Methods

100 customers of Bank Saderat of Guilan were randomly selected to fill the prepared questionnaire. Collected data were analyzed by SPSS software and relevant figures and tables were plotted using Excel software. According to this questionnaire, customer satisfaction was measured by 22 questions and the results are as follows. Firstly, customers' expectations were measured and then the performance perceived by customers was identified and measured. In the next stage, the importance of these factors from the view of customers was determined and then the importance of these factors from the view of employees was studied. Finally, the difference between the views of customers and employees was reviewed and analyzed.

3. Results

Table 1: Customers' Expectations of services provided by a good bank

Row	Index	7	6	5	4	3	2	1	Expectations
1	Beautiful and modern appearance outside and inside the bank branch	20	27	25	8	21	5	3	78.71
2	Clean and decent appearance of bank staff	16	31	71	11	12	7	6	75.09
3	Physical facilities within the bank branch	15	30	18	12	15	7	3	75.42
4	Regular documents and paper of bank branch	38	25	16	11	5	5	0	86.85
5	Announcement of the exact time the bank offers a special service	20	31	18	13	11	5	2	79.33
6	Willingness to help and guide customers by branch staff	20	30	21	19	10	0	0	82
7	Exercising reforms in the shortest time	16	40	30	9	5	0	0	85.14
8	Providing services by the bank at the promised time	26	28	18	16	8	3	1	82.37
9	Accuracy of bank services	45	35	15	5	0	0	0	94.71
10	Clear and honest speech of staff	10	40	25	14	9	2	0	80.71
11	Employees provide immediate services to customers	35	25	17	11	9	3	0	85.71
12	The staff are always willing to help customers	15	35	29	10	8	3	0	81.86
13	The staff are always ready to respond to customers	30	29	17	15	6	2	1	85
14	Employee's behavior creates a feeling of confidence in customers	22	30	20	10	10	6	2	83
15	Customers can feel secure in their interactions with the bank	11	53	19	12	5	0	0	85.14
16	Employees always treat customers with courtesy and respect	31	45	15	5	2	2	0	90.13
17	Bank employees have sufficient knowledge to respond and help customers	27	31	20	11	10	1	0	84.85
18	There is an individual attention to customers in the branch	21	34	15	19	5	5	1	81.57
19	Appropriate working hours for customers	9	32	25	16	11	7	2	76.56
20	Bank offers special services with regard to customers' position	14	39	24	12	7	2	2	81.42
21	Bank considers your interests	25	33	23	14	5	0	0	86
22	Employees understand the specific needs of customers	31	30	15	15	9	2	0	86.69

Table 2: Performance perceived by customers of the services provided by branches of Bank Saderat of Guilan

Row	Index	7	6	5	4	3	2	1	performance
1	Beautiful and modern appearance outside and inside the bank branch	11	35	30	19	5	0	0	81.57
2	Clean and decent appearance of bank staff	8	35	33	17	17	0	0	85.34
3	Physical facilities within the bank branch	8	31	28	19	11	3	0	76.52
4	Regular documents and paper of bank branch	27	33	20	5	11	3	1	83.38
5	Announcement of the exact time the bank offers a special service	3	30	30	14	12	8	3	72.14
6	Willingness to help and guide customers by branch staff	31	35	14	11	8	1	0	87.14
7	Exercising reforms in the shortest time	24	40	23	10	1	1	1	87.43
8	Providing services by the bank at the promised time	14	29	32	12	6	5	2	79
9	Accuracy of bank services	43	40	11	4	2	0	0	95.49
10	Clear and honest speech of staff	21	37	24	9	5	2	2	84.14
11	Employees provide immediate services to customers	18	25	21	11	11	8	6	74.71
12	The staff are always willing to help customers	20	41	17	11	11	0	0	84.42
13	The staff are always ready to respond to customers	28	33	18	14	5	2	0	86
14	Employee's behavior creates a feeling of confidence in customers	20	41	21	10	8	0	0	85.42
15	Customers can feel secure in their interactions with the bank	10	58	21	9	2	0	0	86.85
16	Employees always treat customers with courtesy and respect	30	48	14	5	1	1	1	91
17	Bank employees have sufficient knowledge to respond and help customers	28	36	20	11	5	0	0	87.71
18	There is an individual attention to customers in the branch	27	33	20	5	11	3	1	83.38
19	Appropriate working hours for customers	16	29	20	16	11	6	2	77.14
20	Bank offers special services with regard to customers' position	15	30	20	20	7	4	4	77.28
21	Bank considers your interests	22	30	29	14	3	1	1	84.28
22	Employees understand the specific needs of customers	33	33	17	10	5	2	0	87.71

Table 3: Customer expectations and bank performance and the degree of importance from the view of customers and bank clerk

Row	Index	expectations	performance	Importance from the view of customers	Importance from the view of staff
1	Beautiful and modern appearance outside and inside the bank branch	78.71	81.57	77.21	88.13
2	Clean and decent appearance of bank staff	75.09	85.34	80.16	85
3	Physical facilities within the bank branch	75.42	76.52	78.33	82.56
4	Regular documents and paper of bank branch	86.85	83.38	80.87	81.42
5	Announcement of the exact time the bank offers a special service	79.33	72.14	78.78	81.71
6	Willingness to help and guide customers by branch staff	82	87.14	81.86	79
7	Exercising reforms in the shortest time	85.14	87.43	84.14	83.27
8	Providing services by the bank at the promised time	82.37	79	81.65	80.78
9	Accuracy of bank services	94.71	95.49	94.21	96.33
10	Clear and honest speech of staff	80.71	84.14	81.50	81.76
11	Employees provide immediate services to customers	85.71	74.71	91.33	95.34
12	The staff are always willing to help customers	81.86	84.42	81.86	79.11
13	The staff are always ready to respond to customers	85	86	84.81	80.26
14	Employee's behavior creates a feeling of confidence in customers	83	85.42	82.07	82.68
15	Customers can feel secure in their interactions with the bank	85.14	86.85	84	87.37
16	Employees always treat customers with courtesy and respect	90.13	91	91	98.88
17	Bank employees have sufficient knowledge to respond and help customers	84.85	87.71	85.13	88
18	There is an individual attention to customers in the branch	81.57	83.38	82.13	81.42
19	Appropriate working hours for customers	76.56	77.14	78.54	77.14
20	Bank offers special services with regard to customers' position	81.42	77.28	85.39	76.14
21	Bank considers your interests	86	84.28	87.44	90.12
22	Employees understand the specific needs of customers	86.69	87.71	88.11	81.57

4. Discussions

One of the easiest ways to assess customer satisfaction is the estimation of customer expectations. So, the difference between customers' expectations and bank performance will be firstly analyzed at each components of service quality scale

1- Tangible factors: Among questions 1 to 4 relating to this section, bank performance was better than customer expectations in questions 1to 3 and only in question 4 the customer expectations were more than bank performance.
2- Reliability: Bank performance was better than customer expectations in questions 6 and 7, while customer expectations were more than bank performance in questions 5 and 8.
3- Accountability: In question 11, customer expectations were more than bank performance and bank performance was better than customer expectations in other questions.
4- Bank performance was also better than customer expectations in questions 14 to 17.
5- Empathy: In questions 19 and 22, bank performance was in a good condition, but customer expectations were more than bank performance in questions 20 and 21.
Altogether, it can be observed that bank performance was not in a acceptable condition in questions 4, 5, 8, 11, 20, and 21. It is noteworthy to say that time is a common factor in questions 5, 8, and 11.
Then, the highest expectations of customer were identified and compared with bank performance:
Accuracy of bank services (performance > expectations)
Employees always treat customers with courtesy and respect (performance > expectations)

1.4. Regular documents and paper of bank branch (performance < expectations)

The highest importance from the view of customers is as follows:
Accuracy of bank services (94.21%)
Employees always treat customers with courtesy and respect (91.33%)
Employees always offer immediate services to customers (91%)
The highest importance from the view of employees is as follows:
Employees always treat customers with courtesy and respect (98.88%)
Accuracy of bank services (96.33%)
Employees always offer immediate services to customers (95.34%)

It can be found with a simple review that two factors of accuracy and respect for customer, which are the most important factors from the view of customers and also include the highest expectations of customers from a successful bank, are regarded by bank clerks and good understanding of employees of the importance of this issue has led to an acceptable performance of banks. Despite the importance of service speed from the view of customers and employees, we still observe that the performance of bank is less than what expected. Although the customers believed that beautiful and modern appearance outside and inside the bank branch and physical facilities within the bank branch are less important compared with other factors, banks are still spending enormous costs on these things.
With an in-depth look at the data of the present study we find that Bank Saderat of Guilan, instead of spending budget on above-mentioned items and other less important factors from the view of customers, should exercise reforms in the process of providing services and in addition to increase the speed of service providing and reforming other factors related to service time, provide special services due to the specific requirements of customers. "You can choose any color you want as long as it is black"; this ridiculous look of Ford to customer satisfaction has no position in today's competitive world. Nowadays, profitability of companies and service and manufacturing institutions has a direct relationship with customer satisfaction. Hence, considering the interests of customers and attracting their satisfaction should be the top priority of banks.

Acknowledgements:

Authors are grateful to the Financial economics faculty department of financial management Candidate of economics science, Tajikistan for support to carry out this work.

Corresponding Author:
Mohammadreza sheikholeslami
E-mail: sheikholeslami@gmx.com

References

1. Nitin Seth, S.G. Deshmukh, Prem Vrat, (2005) "Service quality models: a review", International Journal of Quality & Reliability Management, Vol. 22 Iss: 9, pp.913 - 949.
2. Ruth N. Bolton and James H. Drew.1991. A Multistage Model of Customers' Assessments of Service Quality and Value. *Journal of Consumer Research* Vol. 17, No. 4, pp. 375-384.
3. Vikas Mittal; Carly Frennea (2010). "Customer Satisfaction: A Strategic Review and Guidelines for Managers." Marketing Science Institute: *MSI Fast Forward* (10-701).
4. Zeithaml, Valarie A., Leonard L. Berry and A. Parasuraman. 1999. The Behavioral Consequences of Service Quality. *Journal of Marketing* Vol. 60, No. 2 (Apr., 1996), pp. 31-46.

8/15/2012

A comparison of irrational beliefs between trained couples and normal couples

Seyyed Fateh Moradi

MA in clinical psychology, Islamic Azad University, Science and Research Branch of Tehran
fatehzhwan@yahoo.com

Abstract: Objective: This study is devoted to a comparison of irrational beliefs between trained couples and normal couples in Javanrood city, Iran. **Method:** the research was conducted between 15 trained couples & 15 normal couples (total 60 individuals). The first group was trained in the scope of identifying irrational beliefs (for 7 sessions), then both groups were evaluated by Irrational Beliefs Test (IBT). For data analysis independent-T test was applied. **Results:** findings showed that the irrational beliefs have significant differences between normal & trained couples. These differences have been more significant concerning: demand for approval (DA), high self-expectation (HSE), emotional irresponsibility (EI), problem avoiding (PA), hopelessness changes (HC), perfectionism (P) and the entire beliefs, so that irrational beliefs intrained couples showed a major decrease. **Conclusion:** training and identifying irrational beliefs have a significant effect on reducing irrational beliefs and also improving these beliefs.
[Seyyed Fateh Moradi. **A comparison of irrational beliefs between trained couples and normal couples.** *Life Sci J* 2012;9(3):2023-2026]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 292

Keywords: irrational beliefs, trained couples, normal couples.

Introduction

According to Ellis human behaviors and emotions arises from his beliefs, principles, and attitudes. He calls the negative part of these beliefs irrational beliefs [Safee Abadi, A, 2001; solati, K, 2000].These beliefs have impact upon cognitive coordination of individuals, and is related and depended to their cognitive systems [Feldman, P, 1998]. From this point of view people will be more easily captured by childish beliefs, and inappropriate environment also doubles its impact [Ellis, A, 2009; Ellis, A, 2004]. For couples, research has shown that the psychological profile of unsatisfied couples who had no desire to live together will get worried so that with more anxiety they blame themselves more [Salimi, S and et al, 2005; Cooper, 2001]. These indexes are also closely related with irrational beliefs' parameters; in such a way that what affects and challenges the marital relationship are not external events, but their irrational beliefs [Legha, F, 2010]. For these mental events of beliefs, more emphasis is put on feelings and obligations and results in anxiety and evil character in person, which will face people with more difficulties in life? Regarding that, these thoughts can affect people in various ways [Thomas and Luren, 2011]. It is significant and noteworthy. Major studies have also shown that there is significant difference between people's life with irrational beliefs and other groups 'clinical and ordinary issues [Timothy and et al., 2008; Janice and etal, 2007; Mandic and etal, 2010]. This process may also be reflected upon, that even studies that point to irrational attitudes varies according to region and culture [Jones and Chao 1997]. Studies have a lot of emphasis on teaching dysfunctional beliefs and changing attitude of individuals through Rational Emotional Behavior therapy (REBT) to reach more rational beliefs, to that extent that education has improved symptoms of depression and other mental disorders [Nel and Nel, 1982; Macaskill and Macaskill, 1996; Slomn and et al, 1998; Shwan and Dave, 2994; Faramarzi, 1997; Nikmanesh, 2000; Safaee, 2002; Fooladgar, 2000]. Some studies also showed that education has no impact on improving irrational beliefs [Terry and et al., 2007; Motlagh Moghemi, 2004]. Some other research has also conducted on marital satisfaction of couples regarding the beliefs; the results indicate a relationship between rational beliefs with marital satisfaction and irrational beliefs with marital dissatisfaction [Takhti, 2001; Jabbari, 2006; Sharaf, 1996; Derube is and et al., 1998]. Also somestudies reported that there is no relationship between problems of couples, including duration of marriage, and ... with irrational beliefs [Najafpour, 2000; Azkhosh, 2007]. Hence, more efforts should be done to the cognitive development of individuals and particularly couples, so reflection and recognition of these beliefs is constructive and useful in solving marital and dysfunctional cognitive problems of couples. Thus, the aim of this study is to investigate the impact of teaching irrational beliefs on the wife.

Methods

Statistical population and sample: statistical population of this research is couples who live in Javanrood city, among them two groups are selected and evaluated. 15 couples in the first group during seven sessions underwent training on irrational beliefs and cognitive system. Training was done using books, articles and handouts, and explained personally.

During the training there were three more couples for risk avoidance, and probable absence of them; and among them, 15 couples were regularly trained in seven sessions. The samples were matched in terms of age, education and financial status. Educational level of the couples ranged from diploma to bachelor and age ranged from 22 years to 38 years old. Their mean of age was 29.4. For data analysis the mean, standard deviation and T-independent were used.

Research instruments: Jones Irrational Beliefs Test (IBT) is consisted of 100 items as a Liker scale. It is graded from strongly agree to strongly disagree (5 levels).It measures ten scales; each scale has 10 questions that measure an irrational belief. The minimum score in this test is 100 and maximum is 500. The higher the score it is indicative of more irrational belief, and the less it is indicative of irrational belief. Jones [1968] has reported the reliability of this test as0.92 and totally 0.74. In Iran Taghipour [1998] has reported the reliability of this test as 0.71. Also in Dotapoush's research [Donaposh, 1998] its reliability is reported to be 0.82that shows a high dependability. Scales of the test are followings: Demand for Approval (DA), High self-expectation (HSE), Blame proneness (BP), Frustration reactive (FR), Emotional Irresponsibility (EI), Anxious over concern (PA), Problem avoiding (PA), Despondency (D), hopelessness changes, Perfectionism (P).

Results

As it can be seen, in most of the scales irrational beliefs scores were higher for untrained couples than the trained couples. So that for irrational beliefs Demand for Approval (DA), High self-expectation (HSE), Emotional Irresponsibility (EI), Problem avoiding (PA) and hopelessness changes, Perfectionism (P)and total irrational beliefs there is significant difference.

Table1: analysis of mean, variance and T-independent in 2 groups

Scales	**Trained couple**		**normal couples**		**Df = 58**	
Irrational beliefs	mean	variance	mean	Variance	t	Significance level
Demand for Approach (DA)	27.50	4.20	30.12	3.75	1.57	.001
High self expectation (HSE)	27.10	3.42	32.11	4.22	.897	-.02
Blame proneness (BP)	29.10	4.02	29.30	4.70	-1.27	.485
Frustration reactive (FR)	26.11	3.20	26.5	4.01	.069	.746
Emotional Irresponsibility (EI)	28.20	3.70	31.3	3.60	2.12	.004
Anxious over concern (PA)	26.20	5.40	29.25	4.2	1.01	.165
Problem avoiding (PA)	30.5	4.80	32.03	3.80	.059	.005
Despondency (D)	29.60	3.85	29.25	4.11	-.033	.872
Perfectionism (P)	27.86	4.27	31.89	4.78	1.01	.002
hopelessness changes	27.40	3.40	33.90	3.42	1.87	.003
Total	279.57	3.95	306.25	4.04	.815	.001

Conclusion

The aim of this study is to investigate irrational beliefs in trained and untrained couples. On this basis research findings showed that using irrational beliefs is different in these two groups, so that training and identifying irrational beliefs is effective in reducing the scores on these beliefs in the couples. Research findings were compatible with the findings in [Nel and Nel, 1982; Solmn and etal, 1998; Shawn and Dave, 2004; Safaee, 2002; Charalabos and et al., 2006] and some other minor parameters of other studies. Most of these studies have shown that instructing irrational beliefs and awareness toward them in some way is effective in reducing and modifying these beliefs. The most significant differences were observed in the beliefs of Demand for Approval (DA), High self-expectation (HSE), Emotional Irresponsibility (EI), Problem avoiding (PA) and hopelessness changes, Perfectionism (P), and total irrational belief that it may be an indicator of the effect of training of irrational beliefs and entering a new life. At one hand beliefs that have shown less difference may have existed longer time in people and somehow are institutionalized. Appropriate and continuing training may also affect the beliefs that may not lead to negative and gloomy feedbacks and assumptions. Since cognitive changes and individual's beliefs may be influenced by training [Robert and et al., 2001; Haoton and Sakloess2006; Davoodi, 2000]. Since support is needed from spouses and such supports give them positive and logical feedbacks [Brek, 1998]. These trainings can be a medium for such supports and may provide kind of emotional investment that even by existence of inefficient beliefs individuals can tolerate them [Pines, 1996; Salimi and et al., 2005]. But, since irrational beliefs have a contrariwise relation to the total positive love and marriage relationships, if needed training doesn't take place it can harmfully affect adaption [Stackert and Bursik, 2003; Addis and Bernard, 2000]. Then you

may not pay attention seriously to binding and required beliefs that are unrealistic and ineffective and cause confusion of thought. And there will be no program to amend or remove it, and results other major concerns. Because of life and cultural factors that affect irrational beliefs and exacerbate them are also acquired from parents. Sharp differences between the couples in terms of culture, race and region are important, therefore, it's not expected that these beliefs to be resolved with spontaneous backgrounds without the intervention of training programs [Jones and Chao, 1997; Derubeis and Beck, 1998; Azkhosh, 2007; Ellis, 2001and 2000; Datilio, 2000].Burns [1992] believes that irrational thoughts often occur among unsuccessful couples. Ellis Vision Care (ABC) is an appropriate and effective approach for training that can be used in this concern. Regarding the cultural conditions in Iran and some other countries, cultural differences and several other factors can be considered in the training pertinent to the beliefs' that their effectiveness occurs faster and more convenient. Like any other research, this study was not free of constraints, participation of the couples was faced with problems such as short absences, we had to call and strengthen the couples under training, and lack of a place to give recognition to the research, forced the researcher make use of private houses for gatherings and instruction. It is suggested that a possible generalization of the results should be done with caution, because as mentioned above cultural, age, economic and differences may be important concerns in changing the results. Also it's recommended that instruction of irrational beliefs for other groups and in educational, clinical and medical places to be done in various other forms. Counseling Clinical Centers may for various reasons focus their efforts on individual rather than group, but group training at the time, cost, and morale of the referents can be effective, so it is recommended it be performed with greater precision and at a group level.

References

1. Addis, J. & Bernard, M. (2000). Marital adjustment and irrational beliefs. Journal of rational-emotive and cognitive- behavior therapy, (20), 3-13.
2. Azkhosh, M., Askari, A. (2007). Measuring irrational beliefs in marital relationships: Standardization of relationship beliefs questionnaire. *Iranian psychologists quarterly, (4), 14, 137-153.*
3. Berk, L.E. (1998). Development through the lifespan. Boston: Allyn & Baco.
4. Brinz, D. (1992). Cognitive therapy. Translated by: M Gharche Daghi, Tehran, Alborz Publication.
5. Burgman, A.G; Nyland, J.E; Burns, L.R. (2007). Correlates with perfectionism and the utility of dual process model, personality and invidual differences, (43), 299-399.
6. Charalabos, P; Demosthenes B. P; Christos, P; Efi,T; Kostantinos, K; Christodoulos, S; Constantin, S. (2006). Association between plasma inflammatory markers and irrational beliefs; the ATTICA epidemiological study. Progress in Nero-Psychopharmacology and Biological Psychiatry, Vol: 30(8), 1496-1503.
7. Cooper, C.C. (2001). Men and divorce in gary R. Brooks in Gary R. Brooks & Glenn E. Good (editors), The New handbook of psychotherapy and Counseling with Comprehensive Guide to settings, problems, and treatment approcaches. Jossey Francisco, California. P:335-352.
8. Datilio, F.M. (2000). Cognitive-behavior strategies. In J. Carlson and L. Sperry (Eds.). Brief therapy with individuals and eouuples. (pp.33-70). Phoenia, AZ: Zeig. Turker and Theisen.
9. Davoodi, J. (1999). Comparison of the effects of individual and group counseling procedures with emphasis on the rational-affective method in reducing irrational beliefs among 14-18 years old male offenders in Tehran's upbringing and reform center. *MA Thesis, Tehran, Tarbiat Moallem university.*
10. Derubeis, R.J & Beck, A.T. (1998). Cognitive therapy. In K.S. Dobson (E.d), Handbook of cognitive- behavior therapies. newyork: *Guilford press, pp:273-306.*
11. Donapoush, A. (1998). Primary norm seeking of Jones Irrational beliefs test for male and female students 15-18 years old in public high schools in Yasouj city in the academic year 1996-1997. *M.A Thesis in Education Science, Tehran,Tarbiat Moallem University.*
12. Ellis, A. (2009). Ask Albert Ellis: straight answers and sound advice from American's best known psychologies. Translated: Ferozbakht, M, *Tehran, Rasa Publication.*
13. Ellis. A. (2004). Behavior Psychotherapy: Rational and Emotive International Encyclopedia of the Social & Behavioral Sciences. P:1072-1076.
14. Ellis, A. (2001). Overcoming destructive beliefs, feelings, and behaviors. *Amherst, NY: Prometheus Books.*
15. Ellis, A. (2000). How to control your anxiety before it controls you. *New York: Citadel prees.*
16. Esscat, M. (1997). Cognitive behavioral Therapy. Translated: Y, Dosti. *Sari city, Tosee Aloom publication.*
17. Faramarzi, M. (1997). An investigation of the impact of teaching logical thinking to mothers on reducing anxiety in young high school girls in district 17, Tehran, *advising and counseling discipline M.A Thesis Tarbiat Moallem university*.
18. Feldman, p (1998). The psychology of crime,v Cambrdige: *Cambridge university press.*
19. Fooladgar, F. (2000). The effect of group counseling with logical-affective approach on reducing depression among orphan boys in Imam Sadeq (AS) in Shahrekord city. *MA thesis in General Psychology, Islamic Azad University (Iran,Khorasgan Branch).*
20. Ghardi, F. (2002). Investigation irrational beliefs and mental health in Ma students in Baheshti UNI. *Tehran, MA Theses of University of Behesti.*

21. Haoton, K; Sakloess, K. (2006). Cognitive Behavioral Therapy. Translated: H, Gasemzadeh. *Tehran, Arjomand press.*
22. Jabbari, K. (2006). An investigation of Irrational beliefs relationship with marital satisfaction. *M.A Thesis in Clinical Psychology, Islamic Azad University, Roodehen branch.*
23. Janice L. DeLucia-Waack, Rebecca A. Gellman. (2007). The Efficacy of Using Music in Children of Divorce Groups: Impact on Anxiety, Depression, and irrational beliefs About Divorce. *Group Dynamics, Vol: 11(4), 272-282.*
24. Jones, R.G. (1968). A factorial measure of Ellis "irrational belief system", with personality and maladjustment correlates. Dissertation Abstracts international, 29, 4379 B—4380 B. (university microfilms. NO 69-6443).
25. Jones, A.C., & Chao, C.M. (1997). Racial, ethnic and cultural issuesin couples therapy. IN W.K. Hallford and H.J. Markman (Ed.) *clinical handbook of marriage and couples intervention (pp. 157-178) Chichester, U.K. Wiley.*
26. Legha, F. (2010). A comparison of irrational beliefs for choosing jobs between employed and unemployed graduates referring to work Department in Borazjan city. *M.A Thesis. Tehran, Allameh Tabatabai University*.
27. Macaskill, N.D; Macaskill, A. (1996). Rational Emotive therapy plus pharpnaco theraphy verus pharnaco theraphy alone in the treatment of high cognitive dysfunction depression, cognitive theraphy research, Vol: 20(6), 575-592.
28. Mandic, V; Perunicic, I; Lukic, B; Rakovic, I; Marjanovic, S; Lecic D; Tosevski. (2010). p01-60 – irrational beliefs of patients with major depressive disorder; *European Psychiatry, Vol: 25(1), 281.*
29. Motlagh Moghimi, F. (2004). An investigation of the effects of schooling with cognitive approach (indirect method) in reducing irrational beliefs (illogical) young girl 17-27 years old in Pishva Relief Committee in 2002. *M.A Thesis, Tehran, Islamic Azad University, Roodehen branch.*
30. Nel, C.M; Nel, A. (1982). A group technique for the management of depression. *South-African; journal of psychology. Vol:13(2); 45-50.*
31. Nikmanesh, z. (2000). An investigation of the role of group counseling with rational-affective approach, a behavior in favor of strengthening self-confidence in Al-Zahra university students. Tehran,*M.A Thesis, Al-Zahra university students*.
32. Nikseresht, M. (2001). Comparison of adventure seeking and Irrational beliefs in normal and offender teenagers (boys and girls) in Tehran. *MA thesis in Educational Psychology, , Al-Zahra University.*
33. Pines, A.M. (1996). Couple burn out: Causes and Cures. Routledge, London, Great Britain.
34. Robert, B; Breen, N,G; Kruedelbach, H,I; Walker. (2001) Cognitive Changes in Pathological Gamblers Following a 28-Day Inpatient Program Psychology of Addictive Behaviors, Vol: 15 (3), 246-248.
35. Safaee, M. (2002). Influences group cal counseling (REBT) on changes irrational beliefs in adolescents girls. Ma Theses, Rodehen University, Tehran.
36. SafeeAbadi, A. (2001). Theorists of consoling and psychotherapy. Tehran, Nashredaneshgahi Publication.
37. Salimi, S; Karami nia, B., Kirzamani, S., Khaghani Zadeh, M., Karami, Gh, Ramezani, A. (2005). Irrational beliefs relationship with life cycle in divorce seekers. *Family research quarterly, 1 (3), 209-220.*
38. Sharf, R. (1996). Theories of psychotherapy and counseling concepts and cases. *Brooks Cole publishing company.*
39. Shawn P; Dave, M. (2004). Treatment of adjustment disorder with anxiety: A September 11, 2001, case study with a 1-year follow-up.*Cognitive and Behavioral Practice, Vol: 11(3),331-336.*
40. Solati, K. (2000). Investigation irrational beliefs in suicidal and normal cases. *Journal of medical sciences, Iran,ShahreKurd University.1(2)52-60.*
41. Solmn, A; Hagan, D.A; Brody, C; krik, L; Friedman, D.G. (1998). Prining irrational beliefs in recoverd depression. *Journal of abnormal psychology vol:107(3)44-9.*
42. Stackert, R; & Bursik, A.C. (2003). Why am I unsatisfied? Adult attachment still, gendered irrational relation beliefs, and young adult romantic relationships: satisfaction. Personality and individual differences, (34), 1419-1429.
43. Taghipour, M. (1998). Investigation and comparison of irrational beliefs between psychosomatic patients and normal I (healthy) ndividuals, 20 to 50 years old in Tehran.M.AThesis*, Allameh Tabatabai University.*
44. Takhti, M. (2001). Investigation and comparison of Irrational beliefs and marital satisfaction among employed couples with the degrees below diploma, diploma, BA and higher. *MA thesis in advising and counseling, Tehrn, Al-Zahra University*.
45. Terry, A. DiLorenzo, Daniel David, Guy H. Montgomery. (2007). The interrelations between irrational cognitive processes and distress in stressful academic settings. Personality and Individual Differences, Vol:42(4),765-776.
46. Thomas, K; Lauren, B (2011). Nonconscious effects of peculiar beliefs on consumer psychology and choice, *Journal of Consumer Psychology. Vol: (21), 1; 101-111.*
47. Timothy, M; Danielle, P; Gina, A; Sharon, M. (2008). The irrational food beliefs scale: Development and validation. *Eathing Behaviors, Vol:(9), 25-40.*
48. Najafpour, A. (2000). Comparison of marital satisfaction between typical couples and couples that their spouses were grown up in Boarding Welfare Organization of Isfahan province. *M.A Thesis in general psychology, Iran, Islamic Azad University, Khorasgan Branch.*

08/08/2012

Multiobjective Cascade Control System Design with an application to Level Control in Liquid Level process

Agees Kumar C [1], Kesavan Nair N [2]

[1.] Department of EIE, Noorul Islam College of Engineering, Kumaracoil, TamilNadu 629 175, India
[2] Department of EEE, C.S.I Institute of Technology, Thovalai, TamilNadu 629 203, India
ageesofficials@gmail.com, kesavannaireeecsit@gmail.com

Abstract: This paper proposes a Multi-Objective Cascade Control approach to tune the various controllers employed in the cascade control loop. Most of the modern cascade loops require simultaneous tuning of primary and secondary controllers and hence the design task becomes complicated. Non-Dominated Sorting Genetic Algorithm (NSGA-II) and Non-Dominated Sorting Particle Swarm Optimization algorithm (NSPSO) based multiobjective approaches are employed in the design to fine tune the controller parameters of both primary and secondary loop. Inner loop comprises of flow process and the outer loop comprises of level process.The process considered in this paper is highly non-linear with varying time delay and provides a challenging test bed for most of the modern control problems.Experimental results confirm that a multi-objective, Paretobased NSPSO search gives a better performance for regulatory process when compared to NSGA-II. Finally, multiobjective optimization using NSPSO for the level process are compared with NSGA-II and the former exhibit good disturbance rejection capability which is a primary factor considered in cascade control.

[Agees Kumar C, Kesavan Nair N. **Multiobjective Cascade Control System Design with an application to Level Control in Liquid Level process.** *Life Sci J* 2012;9(3):2027-2032]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 293

Keywords: Multi-objective Controller, NSPSO, NSGA-II, Disturbance Rejection, Non-Dominated Sorting, Cascade Control

1. Introduction

Control Engineering problems are characterized by several multiple conflicting objectives, which have to be satisfied simultaneously which inturn yields Pareto-optimal solutions. Lot of Researchers have employed various techniques for cascade control of various processes.To improve overall control system performance, where multiple loops are involved cascade control becomes important. Cascade loops are employed where disturbances acting on the secondary process have major impact on the primary process. The system can lower the effect of the disturbances entering the secondary variable on the primary output.The task of regulating the level in an process control system is a challenging problem. Disturbances arising in the secondary loop further complicate the dynamics of the control problem. Due to these reasons, level control is viewed as an benchmark for control of highly non linear processes. Earlier level Control was performed using linear conventional controllers by employing cascade and feed forward controllers as proposed by Mcmillan. They suffer from the problems of robustness and load disturbances. Cascade control has two objectives. First is to suppress the effects of disturbances on the primary process output via the action of a secondary or inner control loop around a secondary process measurement. The second is to reduce the sensitivity of a primary process variable to gain variations of the part of the process in the inner control loop. A robust fuzzy cascade control strategy is used with minimum number of rules for any number of inputs [1]. In main steam temperature of a boiler cascade control is used which improves the static and dynamic performances [16]. Cascade schemes of PI torque and speed controllers are presented to enhance the objectives of speed control in the system [14].

Cascade control is used to reduce the effect of load disturbances to overcome the failure of traditional PID control [15]. Cascade control uses PID and Fuzzy control logic to improve the dynamic characteristics of level control in horizontal tank [10].Simple relay feedback test is applied to the outer loop of the cascade control to identify both loop parameters [5] .An improvement is achieved over an existing feedback cascade temperature control system using new hybrid control approach [9]. Using offline PID selection methods, cascade control methods has been designed and simulation done on Matlab/Simulink [18]. Cascade control is designed to ensure enhanced robustness by minimizing the mutual influence among loops [1].

Effect of hydro viscous drive speed regulating start depends on control strategy, present control has many problems, the problem is resolved by fuzzy PID cascade control system, the fuzzy PID cascade control was simulated by Matlab/Simulink [19]. Cascade control inner loop used for sliding control, outer loop uses PI control are designed and analyzed

for a boost converter [20]. Cascade control configuration used in Two degree of freedom design approach guarantees smooth control [11].

The multiobjective PID control problems are characterized in terms of Eigen value problem and it can be efficiently solved by the LMI toolbox in Matlab [3]. In cascade control arrangement, the inner loop consists of multivariable control of three compressors which gives high performance compared to SISO scheme [2]. NSPSO combines the operation of both NSGA-II and multiobjective PSO with a single particle swarm optimizer (PSO) and the obtained results are better than the two compared algorithms [12].

The multiobjective optimization problems are solved by evolutionary algorithm NSGA and its performance is compared with other algorithms [4]. A new multiobjective optimization algorithm is introduced to design optimal PID controller by tissue P systems to satisfy objectives synchronously [13].

By minimizing overshoot, settling time and by smoothening of output curve, the optimal fuzzy controller designed using GA [6]. GA are used in order to find the fittest solutions because of their ability to discover solutions quickly for complex searching and optimization problems [6]. A research on Multiobjective problems can be found on [7].

This study aims at designing a cascade control scheme for liquid level process based on multiobjective optimization technique. Multiobjective Optimization based on NSGA-II and NSPSO are presented. Comparative Analysis of NSGA-II and NSPSO are performed and simulation results are analysed.

2. Material and Methods

In Industries, Cascade control is employed in drum level boilers, distillation columns, evaporators and batch reactors. Cascade control is most advantageous on applications where the secondary closed loop can include the major disturbance and second order lag and the major lag is included in only the primary loop. The secondary loop should be established in an area where the major disturbance occurs. It is also important that the secondary variable respond to the disturbance.

Figure1 shows the block diagram of cascade control system employed.

The primary loop monitors the control variable and uses deviation from its set point to provide an output to secondary loop. The secondary loop receives its set point from primary loop and controls the reference variable accordingly. Multiobjective Evolutionary algorithms NSGA-II and NSPSO are used. The two objectives considered are Overshoot and Settling time.

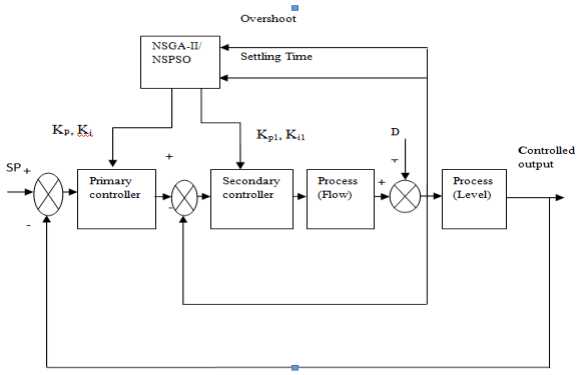


Fig.1 Multiobjective Cascade Control of Liquid Level Control System

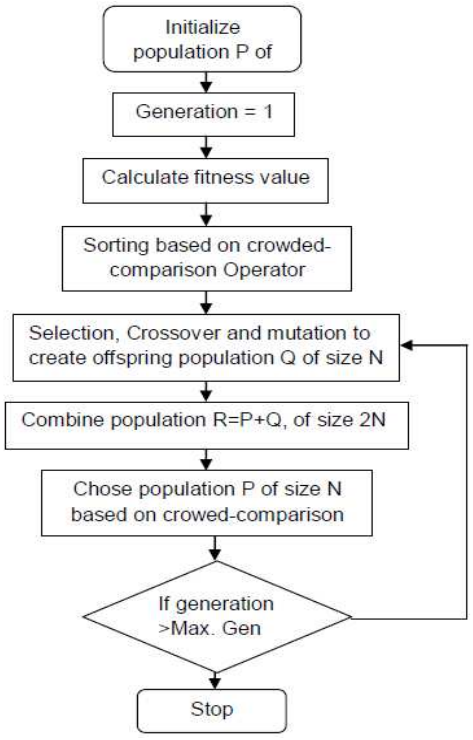


Fig. 2: Flow Chart of NSGA-II

Non Dominated Sorting Genetic Algorithm –II (NSGA – II): The primary reason for choosing EA is their ability to find multiple pareto-optimal solutions in a single run. The main criticism in NSGA was the high computational complexity of non-dominated sorting, lack of elitism and need for specifying the shared parameter. To overcome these, NSGA-II, a slight modification in NSGA approach is being used, which has a better sorting algorithm.

The population is initialized and sorted based on non-domination into each front. The first front being completely non-dominant set in the current population when compared to other higher

fronts. Each individual in each front are assigned a rank (fitness value) based on front in which they belong to. The crowding distance is calculated for each individual, which is based on how close an individual is to its neighbors. Large average crowding distance will result in better diversity in the population. An individual is selected in the rank is lesser than the other or if the crowding distance is greater than the other. The selected population generates offspring's from crossover and mutation operators. This algorithm is same as adopted in [12].

Multi-Objective Non Dominated Soting Particle Swarm Optimization (NSPSO): NSPSO is a modified form of PSO which is highly competitive with other evolutionary and multiobjective algorithms. In the entire population NSPSO compares the personal bests of all particles and their offspring's instead of comparison between single particle and its offspring. This approach yields more non-dominated solutions through dominant comparisons and sorts the entire population into different non-dominated levels as used in NSGA-II. This NSPSO based on PSO and NSGA-II thereby it combines the features of other algorithms such as crowding distance ranking, elitist strategy, and selection and mutation operations with single objective PSO as adopted in [12].

Step 1: Generate an initial population P (Population size = N) and velocity for each individual (agent or particle) in a feasible space; Set the maximum speed vi max (vi max = its upper bound minus lower bound) for a variable.
Step 2: Sort the population based on the non-domination and crowding distance ranking.
Step 3: Do rank-based selection operator (Carlos and Peter, Fleming, 1993).
Step 4: Assign each individual a fitness (or rank) equal to its non-domination level (minimization of fitness is assumed).
Step 5: Randomly choose one individual as gbest for N times from the nondominated solutions and modify each searching point using previous PSO formula and the gbest:

$$v_i(k+1)=k\,[v_i^k+c_i \text{ x rand ( ) x } (pbest_i-s_i^k) + c_2 \text{ x rand ( ) x } (g)]$$

$$K=\frac{2}{\left|2-\varphi-\sqrt{\varphi^2-4\varphi}\right|} \quad \text{where} \varphi = c_1+c_2, \varphi>4$$

$$Si^{-k+1}=s_i^k+v_i^{-k+1}$$

where, rand ( ) is a random number between (0, 1). The constriction factor approach can generate higher quality solutions than the conventional PSO approach. If current position outside the boundaries, then it takes the upper bound or lower bound and its velocity is generated randomly ($0 \le v_i^{k+1} \le v_i^{max}$) and multiplied by -1 so that it searches in the opposite direction.

Step 6: Do mutation operator (David, 1985).
Step 7: Combine the offspring and parent population to form extended population of size 2N.
Step 8: Sort the extended population based on nondomination and fill the new population of size N with individuals from the sorting fronts starting to the best.
Step 9: Modify the pbesti of each searching point: If current rank of the new individual (offspring) P_i^{K+1} is smaller than or equal to the previous one (parent) in R, replace the $pbest_i$ with current individual; otherwise keep the previous $pbest_i$.
Step 10: Perform steps (2-9) until the stopping criterion is met.

Mathematical Modeling of Flow and Level Process:

Transfer function of level process is given by

$$G_1(s)=\frac{1.03e^{-0.5s}}{49.5s+1}$$

Transfer function of flow process is given by

$$G_2(s)=\frac{17.6336}{s}$$

Objective Functions Employed in the Design

i. Overshoot:

$$f_1(K_p,K_i,K_d)=\max\left(\frac{1}{1+OS}\right)$$

where OS refers to Overshoot.

ii. Settling Time:

$$f_2(K_p,K_i,K_d)=\max\left(\frac{1}{1+Ts}\right)$$

where Ts refers to Settling Time.

3. Results

System Requirements:

PC : Intel Pentium, Dual core
Software used : MATLAB 7.10.0(R2010a)

Multi-objective optimal level control of cascade control system tuned using NSGA-II and NSPSO to be implemented in MATLAB/SIMULINK is shown in Fig.3.

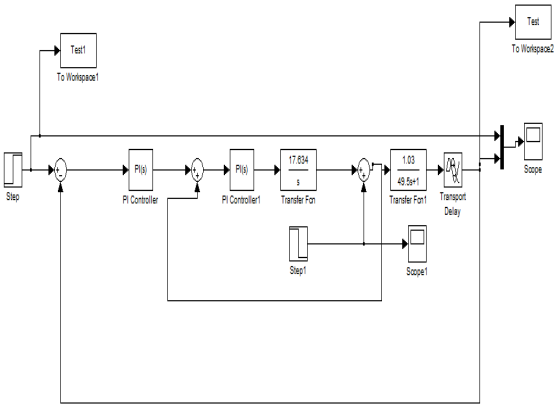


Fig. 3: Simulink Diagram of Cascade Control System

Basic Parameters employed in the system are listed in Table 1.

Table 1: Basic parameters of NSGA-II Algorithm

ALGORITHM PARAMETER	VALUE
Population, N	20
Generations, G	20
Pool size, N/2	10
Tour size	2
Crossover probability	0.9
Mutation probability	0.33

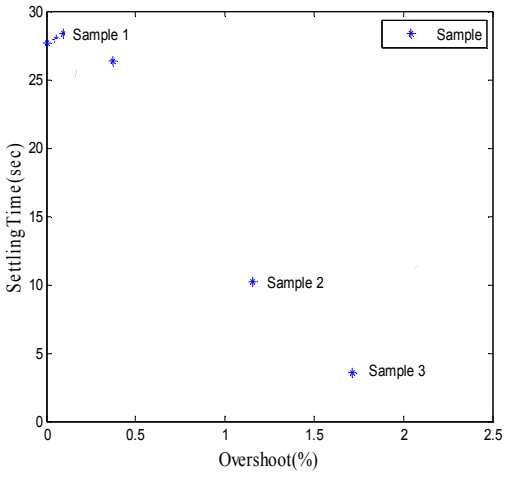


Fig.4: Pareto optimal front with Overshoot and Settling time as objectives with NSGA-II

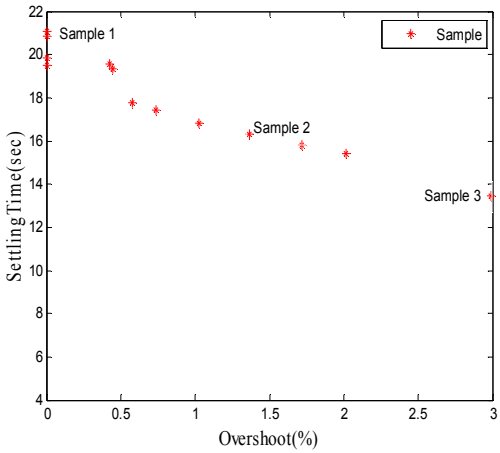


Fig.5: Pareto optimal front with Overshoot and Settling time as objectives with NSPSO

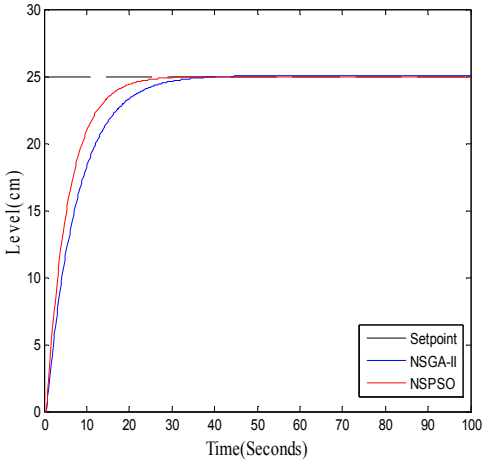


Fig. 6: Comparative Output level response with NSGA-II and NSPSO (Sample 1)

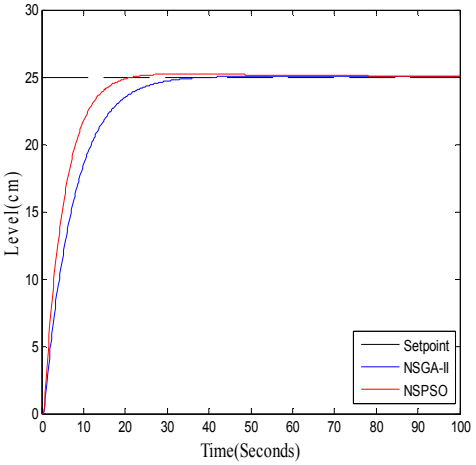


Fig. 7: Comparative Output level response with NSGA-II and NSPSO (Sample 2)

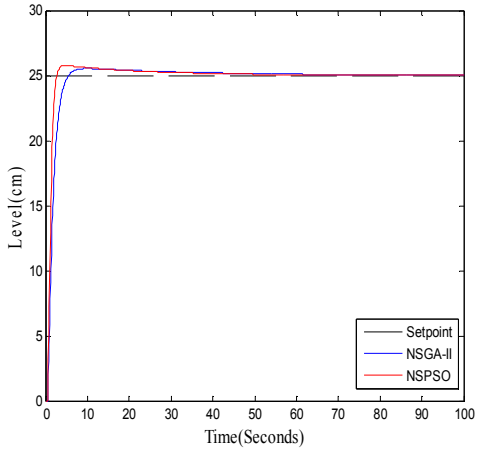


Fig. 8: Comparative Output level response with NSGA-II and NSPSO (Sample 3)

Basic Parameters employed in NSPSO are listed in Table 2.

Table 2: Basic parameters of NSPSO Algorithm

ALGORITHM PARAMETER	VALUE
Particle size, N	20
Generations, G	20
Pool size, N/2	10
Tour size	2
Crossover probability	0.9
Mutation probability	0.33

The Pareto optimal front with overshoot and settling time as objective functions obtained with NSGA-II and NSPSO are shown in Fig.4 and Fig.5. From the Pareto optimal front, samples are taken and from these samples, corresponding optimum Kp, Ki values are obtained as per the requirements of the user. Three samples are taken from the Pareto front for analysis.

Comparative analysis of output level responses in the absence of disturbances is shown in Fig. 6, 7and 8 for Samples 1, 2 and 3 respectively and the results are tabulated in Table 3.

From Table 3, it is inferred that NSPSO provides less overshoot, short settling time and quick disturbance rejection compared to NSGA-II.

Comparative analysis of output level responses in the presence of disturbances introduced at t = 70 seconds with intermediate values of overshoot and settling time is shown in Fig. 9 and the results are tabulated in Table 4.

Table 3: Performance comparisons of NSGA-II and NSPSO Algorithm in absence of disturbances

Set Point 25 cm	Algorithm	Over shoot (%)	Settling Time (sec)	Rise Time (sec)	Steady state error (%)	Dist. Rej Time	Peak Time (sec)	ISE	IAE	ITAE
Sample 1	NSGA-II	0.01	28.38	15.82	0.00	40.6	0.01	0.63	13.98	0.01
	NSPSO	0.00	21.41	10.16	0.00	30.6	0.00	0.51	11.95	0.00
Sample 2	NSGA-II	1.34	12.97	15.34	0.00	42	0.01	0.61	9.05	1.34
	NSPSO	1.23	10.67	10.16	0.00	22	0.00	0.56	8.27	1.23
Sample 3	NSGA-II	2.04	11.34	2.64	0.00	11.2	0.01	0.35	3.83	2.04
	NSPSO	1.98	10.56	1.30	0.00	5.86	0.00	1.08	9.54	1.98

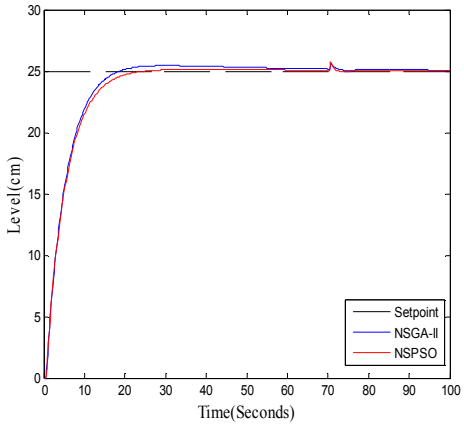


Fig. 9: Comparative Output level response with NSGA-II and NSPSO with disturbance.

Table 4: Performance comparisons of NSGA-II and NSPSO algorithm in the presence of disturbances

Set Point 25 cm	Algorithm	Over shoot (%)	Settling Time (sec)	Rise Time (sec)	Steady state error (%)	Dist. Rej Time	Peak Time (sec)	ISE	IAE	ITAE
Sample 1	NSGA-II	0.2	44	22.6	0.00	3.1	70.75	0.03	2.04	110.9
	NSPSO	0.0	22.53	11.6	0.00	2.1	40.34	0.01	1.68	64.58
Sample 2	NSGA-II	2.24	14.8	9.85	0.00	3.5	32.6	0.00	0.08	5.52
	NSPSO	1.75	13.23	8.74	0.00	2.5	20.3	0.00	0.05	3.78
Sample 3	NSGA-II	8.98	21.4	0.94	0.00	0.02	2.43	0.61	6.24	44.6
	NSPSO	3.45	5.76	1.13	0.00	0.01	1.97	0.01	1.20	30.4

From Table 4, it is inferred that NSPSO provides less overshoot, short settling time and quick disturbance rejection compared to NSGA-II.

4. Discussions

Multiobjective evolutionary algorithms NSGA-II and NSPSO are successfully implemented in cascade control loops with time domain specifications namely overshoot and settling time as objective functions. PI controllers are used for building the cascade controller in order to control the level in the cylindrical tank. Mathematical modeling of cylindrical tank for level and flow process is developed. Simulation results show that NSPSO gives accurate Pareto front values and good diversity as compared to NSGA-II. Simulation results of both the evolutionary algorithms NSGA-II and NSPSO are compared in absence and presence of disturbances. The comparative results prove that NSPSO provides better disturbance rejection and less overshoot than NSGA-II. Thus NSPSO outperforms NSGA-II in cascade control of level process.

Corresponding Author:
C.Agees Kumar
Assistant Professor,
Department of EIE,
Noorul Islam College of Engineering,
Kumaracoil 629 175, TamilNadu, India
E-mail: ageesofficials@gmail.com

References

1. Claudi Maffezzoni, Nicola Schiavani, Gianni Feretti "Robust design of cascade control", IEEE, 1990.
2. J.M.Maciejowski, I.French, T.Urquhart, "Cascade Control of a process plant using predictive and Multivariable control", Proceedings of the 30th conference on decision and control, Brighton, England, 1991.
3. Chung-Shi Tseng, Bor-Sen Chen, "Multiobjective PID control design in uncertain robotic systems using neural network elimination scheme", IEEE transactions on systems, MAN, and cybernetics-Part A:Systems and humans,VOL-31,No.6,Nov 2001.
4. Alexandre H.F.Dias, Joao A.de Vasconcelos, "Multiobjective Genetic Algorithms applied to solve optimization problems", IEEE transactions on Magnetics,VOL-38, No.2, March 2002.
5. Sihai Song, Lihua Xie, Wen-Jian Cai, "Auto-tuning of cascade control systems", Proceedings of the 4th world congress on Intelligent control and automation, June 2002.
6. Ginalber L.O.Serra, "A Multiobjective fuzzy PI controller based on Genetic Algorithm", IEEE, 2003.
7. Hailiang Zhao, Tsu-Tianlee, "Research on multiobjective optimization control for nonlinear unknown systems", The IEEE international conference on Fuzzy systems. 2003.
8. Khoa Duc Tran, "Elitist Non-Dominated Sorting GA-II as parameter-less multiobjective genetic algorithm", IEEE, 2005.
9. A.J.Modak, S.B.Deshpande, P.V.Dhawas, "New hybrid control approach for cascade temperature control in catalyst regeneration process", IEEE, 2006.
10. Satean Tunyasrirut, Santi Wangnipparnto, "Level control in horizontal tank by fuzzy PID controller", World academy of science, engineering and technology, 2007.
11. V.M.Alfaro, R.Vilanova, O.Arrieta, "Two-degree-of-freedom PI/PID tuning approach for smooth control on cascade control systems," Proceedings of the 47th IEEE conference on decision and control, Cancun, Mexico, Dec 2008.
12. Yang Liu, "A fast and elitist multiobjective particle swarm algorithm NSPSO", IEEE Xplore, Dec 2008.
13. Huang Liang, Wang Ning, Zhao Jin-Hui, "Multiobjective optimization for controller design", ACTA AUTOMATICA SINCA, VOL-34,No.4, April 2008.
14. Cheng-Yi Cheng, C.H.Hsu, Shiang-Hwua Yu, Cheng-Fu Yang, Hong-Hsin Huang, "Cascade PI controller designs for speed control of permanent magnet synchronous motor drive using direct torque approach", IEEE, Fourth international conference on innovative computing, information and control, 2009.
15. Raad Z.Homod, Khairul Salleh Mohamed Sahari, Haider A.F.Mohamed, Farrukh Nagi, "Hybrid PID-Cascade control for HVAC system",International journal of systems control VOL-1,2010.
16. Xue Wei, Mu Jingjing, Jia Hongyan, Ye Fei, "The main steam temperature cascade control of high order differential of feedback controller", IEEE, International conference on intelligent system design and engineering application, 2010.
17. Benedikt Alt, Christoph Hartung, Ferdinand Svaricek, "Robust fuzzy cascade control revised: application to the rotary inverted pendulum", IEEE, 19TH Mediterranean conference on control and automation, Corfu, Greece, June 2011.
18. Zhang Honglian, "Simulation of furnace cascade PID control system based on Matlab/Simulink", International conference on computer and management (CAMAN), 2011.
19. Meng Qing-Rui, Hou You-Fu, "A Fuzzy PID cascade control system of a hydro-viscous drive speed regulating start", IEEE, Third international conference on measuring technology and mechatronics automation, 2011.
20. Zengshi Chen, Wenzhong Gao, Jiangang Hu, Xiao Ye, "Closed loop analysis and cascade control of a nonminimum phase boost converter", IEEE Transactions on power electronics, VOL-26, No.4, April 2011.

8/17/2012

Management and prognosis of rare tumors in the sellar region

Dongbin Yang[1,2], Fuyou Guo[1], Hongwei Sun[1], Laijun Song[1]

[1.] Department of neurosurgery, the first affiliated hospital of Zhengzhou University, Zhengzhou, 450052, China.
[2.] Department of neurosurgery, the first hospital of Hebi, HeNan, 458000, China.
dongbinyang@126.com

Abstract: Six rare cases of sellar region treated by microsurgery from Jan 2000 to Jan 2010 were reviewed to study the management and prognosis of rare tumors in the sellar region. Subsequent treatments were according to the instruction of preoperative alpha fetal protein (AFP) and human chorionic gonadotropin (HCG) measurement as well as confirmed histopathological examination in all patients. Variety of histological types were observed in present series, which include leiomyosarcoma, malignant yolk sac tumor, mixed germ cell tumor, embryonal carcinoma, pilocytic astrocytoma and fungal pseudotumor, the contents of AFP and HCG were elevated to some extent in patients with malignant yolk sac tumor or mixed germ cell tumor or embryonal carcinoma, follow-up was conducted in all patients varied from 1 month to 3 years, patients with malignant yolk sac tumor and embryonal carcinoma as well as leiomyosarcoma died in 5 , 6, 10 months after operation. Conclusions: rare nongerminomatous malignant germ cell tumors were predominantly susceptible to the sellar region, Furthermore, High misdiagnose and poor prognosis occurred in present study, dynamic AFP and HCG detection play an important role in nongerminomatous malignant germ cell tumors located in sellar region, awareness of present rare lesions of sellar region is emphasized.

[Dongbin Yang, Fuyou Guo, Hongwei Sun, Laijun Song. **Management and prognosis of rare tumors in the sellar region**. *Life Sci J* 2012;9(3):2033-2037] (ISSN:1097-8135). http://www.lifesciencesite.com. 294

Keywords: sellar region; rare tumors; human chorionic gonadotropin; alpha fetal protein

1. Introduction

The sellar region is one which of spots tumor most often occurs in Neurosurgery. The common pathological change includes: Pituitary tumor, craniopharyngioma, saddle tubercle meningioma, reproduction cytoma, optic nerve glioma, hypothalamic hamartoma and so on. Halbauer DJ (2003) firstly reported 3 examples rare tumor involved in sellar region, that's the ganglion cell differentiation pituitary adenoma, intracranial granular cell tumor and germinoma in hypophysial fossa. Few of reports were seen about this after that. The author collects 6 examples of rare tumor involved in sellar region after the pathology confirmation from Jan 2000 to Jan 2010. All of these were misdiagnosed completely before operation. We review and analyze these examples in this study in order to improve recognition to the complication.

2. Material and Methods

A total of six cases with rare tumors involved in sellar region were identified. This group included four males and two females between the ages of 13 and 23 years (mean 23 years). The duration of initial manifestations until diagnosis ranges from 15 days and 2 years. The symptoms were the binocular vision carries on the drop, drink, the polyuria (1/6); headache, simple eye vision descender (2/6); headache, disgusting, vomiting, menstruation anomalous (1/6); left eyelid sagging, ocular movement barrier after pituitary tumor technique latter 14 years(1/6); headache, low heat , say a word unclearly (1/6). The fundus examination demonstration regards the nipple edge clearly to wither palely were seen in four cases; binocular field of vision nie side damage in two cases; oculomotor nerve paralysis in one cases. The diameter of tumors were 3~6.5cm(mean 4.5cm.).

CT scan showed: the saddle area equilibrium density, promiscuous density's perch venereal diseases change. The signal intensity of tumors in suprasellar region was iso- or hyperintense in T1-weighted imaging, whereas it was usually hyperintensity in T2 weighting imaging. On the enhanced images, there was contrast enhancement. Pouch changes, the necrosis signal were in three cases. The tumors downward cerebral ganglion, the fossa interpeduncularis, three ventricles of the brain direction grows, varying degree hydrocephalus were in two cases. And the mixing property reproduction cytoma presents the honeycomb-shaped enhancement; The sellar region smooth myosarcoma enhanced scanning demonstrated enhanced evenly. They were infiltrative growth obviously to cabernous sinus, and circumvolutioed the internal carotid artery. For lack of specificity of preoperative imaging , it is easily to misdiagnosed as Invasive pituitary adenoma. Inflammatory pseudotumor of fungal was homogeneous enhanced after enhancement, and extensive destruction of tumor invasion to the

sphenoid sinus, ethmoid sinus and the cavernous sinus and skull base and other parts of the former in the extensive growth. Preoperative tumor markers in 3 cases: embryonal carcinoma, yolk sac tumor and mixed germ cell tumor patients were AFP: 71.4ng/ml (0-20) and HCG: 189.70mIU/ml (0-5), AFP 105 ng / ml and HCG: 12.1 mIU / ml, AFP: 4.45ng/ml and HCG: 53.8mIU/ml.

Table 1： Rare sellar lesions detailed clinical information

	Age	Gender	Complaints	Preoperative diagnosis	Pathology	Follow-up
1	20y	male	Headache Decreased vision	goniolma	malignant yolk sac tumor	Died after operation 5 months later
2	13y	male	Decreased vision Diabetes insipidus	craniopharyngioma	mixed germ cell tumor	recurrence has not yet
3	54y	female	Decreased vision Right ptosis	hypophysoma	leiomyosarcoma	Died after operation 10 months later
4	16y	male	Decreased vision Headache	craniopharyngioma	pilocytic astrocytoma	good
5	15y	female	Headache Irregular menstruation	Invasive pituitary adenoma	Embryonal carcinoma	Died after operation 6 months later
6	21y	male	Low heat Headache Slurred speech	Meningioma	Inflammatory pseudotumor of fungal	SAH after operation 2 months later

All patients were treated by microsurgery. Pterion approach was utilized in three cases. Incision starting from the right side of the zygomatic arch before the tragus 1cm, The midline 1~2cm, the dura surrounding the sphenoid ridge arc of cut, coagulation butterfly cut to the top of sinus drainage draining veins, and then open the side of the cistern arachnoid slow release of CSF, and then application of section II, III gap block removal of lesions under the microscope. Subfrontal approach was utilized in two cases. The arc incision is in the right amount of hair over the center line. Then turn the pedicled bone flap about 6x7cm to the temporal bone of skull base side. When the tension of the dural is not high, the CSF from the lateral fissure is released. The frontal lobes are gently elevated. Focus is then removed from I gap. Lamina terminalis approach was utilized in one case. The incision is same to the subfrontal approach. For the I gap is small and the prefixed optic chiasm, after reveal the bilateral optic tract and lamina terminalis, then cut the lamina terminalis longitudinally shows soft taupe tumor in the third ventricle which blood supply amply. And the tumor was removed block under a microscope.

3. Results

6 patients in this group were all misdiagnosed before operation, 2 cases of imaging and clinical diagnosis of craniopharyngioma, the pathological were mixed germ cell tumor and pilocytic astrocytoma; two cases of imaging diagnosis pituitary tumor, 1 case of previous surgery and pathology confirmed the pathological smooth muscle sarcoma, and embryonal carcinoma; 1 patients with a preoperative diagnosis of germ cell tumors and experimental radiotherapy given week, review head CT and MRI showed hydrocephalus increased with increasing tumor After microsurgery, pathology of malignant yolk sac tumor. 1 case of consideration for the huge space-occupying meningioma saddle, the pathological diagnosis of fungal inflammatory pseudotumor (Table 1).

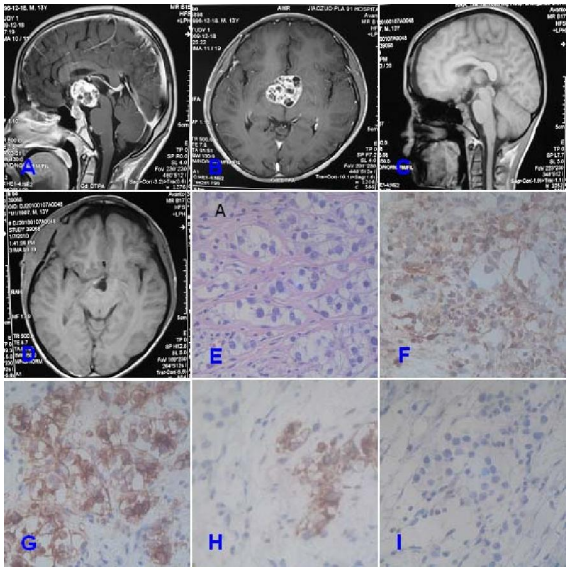


Fig 1: mixed germ cell tumor of the skull sagittal and axial MRI scan shows sellar lesions enhanced irregular enhancement, cyst containing necrotic tissue (FigA, B), review of surgery MRI showed total removal of the tumor (FigC , D), a large number of conventional HE staining of tumor cells (FigE), strongly positive immunohistochemical expression of HCG (Fig F), PLAP strong expression (Fig G), AFP weak expression (Fig H), GFAP-negative expression (Fig I) (Fig E-I x 400)

Total resection was performed in two patients under endoscopic, subtotal resection in four patients. The preoperative detection of AFP and HCG were in three patients. Embryonal carcinoma was significantly higher AFP and HCG, AFP only yolk sac tumor and mixed germ cell tumor patients only HCG significantly increased tumor markers, after reviewing the corresponding decline in varying degrees; immunohistochemistry showed embryonal carcinoma GFAP (-), EMA (-), S-100 (-), PLAP (+), AFP weak (+) , HCG (+); yolk sac tumor of AFP

(++), PLAP (++), Ki 67 (+), P53 (+) GFAP (-), EMA (-); mixed germ cell tumor of HCG (++), PLAP (++), AFP weak (++), GFAP (-) [Fig 1]. Leiomyosarcoma of the actin SMA (+), MSA (+), Vimention (+) [Fig 2]; fungal inflammatory pseudotumor of the acid-fast staining and silver staining were six ammonium (-), PAS staining showed a small number of fungal spores [Fig 3].

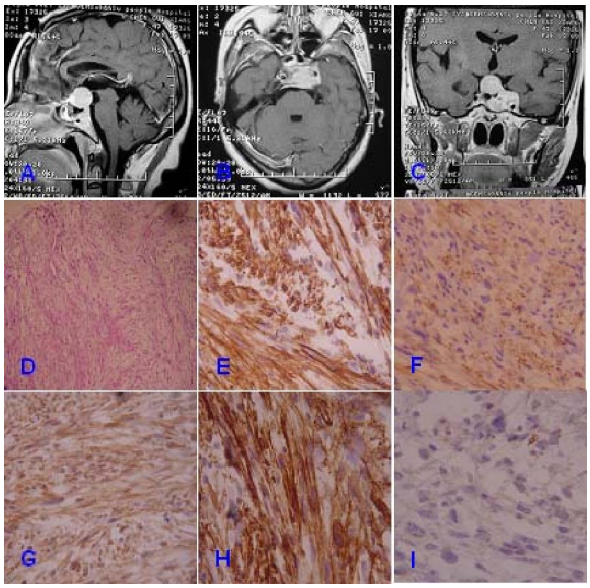


Fig 2: leiomyosarcoma in sellar region head MRI, axial and coronal significantly enhanced scan shows homogeneous enhancement sellar lesion, the direction of invasion to the cavernous sinus growth, particularly in the left cavernous sinus and carotid artery were surrounded tumor (Fig AC). A large number of conventional HE staining of tumor composed of spindle smooth muscle cells (Fig D), immunohistochemistry smooth muscle actin antibody SMA (Fig E), MSA strong expression (Fig F), Vimention was strong expression (Fig G), cerebral aneurysms SMA positive control of vascular smooth muscle (Fig H), SMA negative control (Fig I)

4 cases of postoperative diabetes insipidus, electrolyte disturbance in 3 cases, transient oculomotor nerve palsy in 1 case, malignant pathology after 2 weeks for comprehensive chemotherapy with Nemo Sting, DDP, etc. The course was from 4 to 6 months. And they were given radiotherapy after 1 month. The duration of follow-up ranged from 1 month to 3 years. The patients with malignant yolk sac tumor, embryonal carcinoma, and leiomyosarcoma respectively dead after May, June and October. The patients with inflammatory pseudotumor appeared SAH after 2 months. Then secondary MRA showed left side of the vertebral artery fusiform aneurysm, the current ventilator to maintain breathing in a critical condition. The patient with mixed germ cell tumor is still under close follow-up. No recurrence of this tumor was found. The patients with pilocytic astrocytoma were followed up for good now.

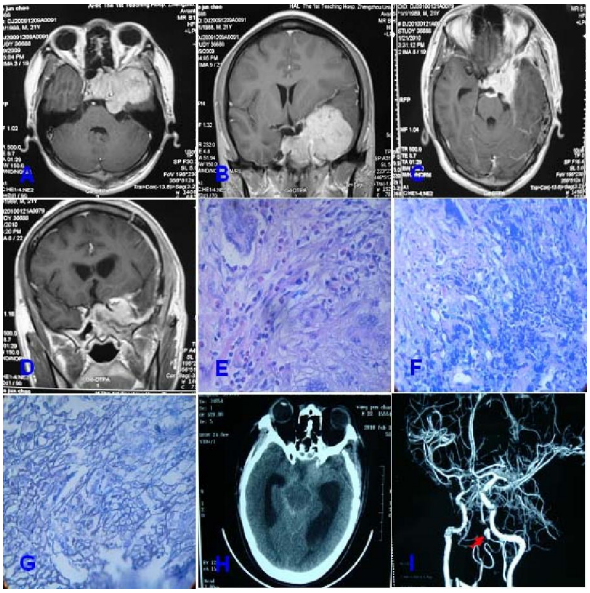


Fig 3: Inflammatory pseudotumor in sellar region Head axial and coronal MRI scan shows sellar enhanced lesions were irregular enhancement huge, invasion, sphenoid, ethmoid and move forward in the cavernous sinus and skull base growth (FigA, B), review of surgery MRI showed the tumor remnant (FigC, D), a large number of conventional HE staining lymphocytes, plasma cells and eosinophils (FigE), acid-fast staining negative (FigF), PAS staining showed a small number of fungal hyphae and spores (FigG), 2 months after the head CT showed SAH (FigH), MRA showed a left vertebral artery fusiform aneurysm (Fig I, arrow)

4. Discussions

Currently the most common pathological includes: sellar pituitary tumors and craniopharyngioma (Heaney AP. 2011), but other less rare disease reported in the literature (Arai A and Nishihara M,. 2010) especially the rare case of sellar region due to improper treatment can lead to catastrophic consequences, such as early hospital depending on the saddle area bit due to inadequate understanding of germ cell tumor surgery taken, after the hypothalamus due to severe reactions in children have died .in recent years by giving radiotherapy to be confirmed after the experimental radiotherapy can be given only to achieve the desired clinical effect. Instead, the group 1 patients for the imaging diagnosis of germ cell tumors, and to give experimental aggravated sexual symptoms after

radiotherapy to take surgery, postoperative pathology confirmed a rare malignant yolk sac tumor, preoperative AFP was significantly higher after 1 Review the week decreased tumor markers, AFP immunohistochemical staining further confirmed strong positive after 1 month after the head MRI showed tumor recurrence and reoperation, occurred after five months extensive metastasis within the spinal canal and upper cervical cord compression produces a large occipital herniation and death; followed by the group 1 patients with a preoperative diagnosis of recurrent hypophysoma, leiomyosarcoma confirmed pathologically

Leiomyosarcoma in sellar region is very rare foreign J. Niwa (1996) first reported by the after surgery and hypophysoma radiotherapy can be transformed into leiomyosarcoma, the disease so far reported a total lack of foreign literature in 10 cases, the current domestic not been reported. Leiomyosarcoma of the cases were due to a history of postoperative radiotherapy for hypophysoma, suggesting that the leiomyosarcoma may be caused by secondary cancer radiotherapy, the clinical manifestations of leiomyosarcoma saddle area, the lack of specificity of imaging features, preoperative misdiagnosis rate is extremely high by immunohistochemistry after SMA, MSA, Vimention were positive to confirm that most of the patients by surgical resection and postoperative radiotherapy supplemented by close follow-up of patients after 10 months was also died.

Malignant goniolmas such as yolk sac tumor, embryonal carcinoma and mixed germ cell tumors occur most often the site is the ovary, and the prognosis is good, 5-year survival rate of 96.7% (Lee KH and Lee IH,. 2009). Intracranial malignant goniolmas mainly occur in the pineal region can also occur in the sellar region, but the incidence rate is low. Wu Maochun (2006) , Beijing Tiantan Hospital were collected between 10 to 19 cases of intracranial high grade non-germ cell tumor goniolmas, only 3 cases of sellar regions, the site of yolk sac tumor is the occurrence of pineal region and the cerebellar hemispheres and lateral ventricles, the 1-year survival rate was 25.0%. While the yolk sac tumors was occurred in the sellar region in this case, although given standard postoperative radiotherapy and chemotherapy, the patient still died 5 months after operation. Huang Xiang(2008) reported that the Shanghai Huashan Hospital, 12 to 39 years were collected non-reproductive cell of cases of intracranial malignant goniolmas, 14 cases in pineal region, only 2 cases of yolk sac tumor.

Only 1 case of embryonal carcinoma was occurred in sellar region. Due to the incidence rate of the high grade non-germ cell tumor goniolmas is low in sellar region. And lack of information on preoperative imaging specificity, combined with lack of understanding of the disease, 3 cases of goniolmas in this group misdiagnosed before surgery. All through this group Preoperative tumor markers with immunohistochemistry after sellar region can be clearly malignant germ cell tumor subtypes, the AFP was significantly elevated, suggesting that the endodermal sinus (yolk sac tumor) or sinus within the embryo based mixed germ cell tumor; HCG was significantly higher for those who should be considered as choriocarcinoma or choriocarcinoma components with mixed germ cell tumor, the AFP and HCG were significantly increased in the same time, the first consider the embryonal carcinoma as embryonal carcinoma with syncytiotrophoblast and endodermal sinus component which allows for both AFP and HCG.

3 patients in this group 1 week after surgical resection of tumor markers review the corresponding decline in varying degrees, but the yolk sac tumor and embryonal carcinoma patients were reviewed after 1 month at the head MRI showed tumor recurrence, review the re-rise of tumor markers high, the full description of preoperative tumor markers AFP and HCG on the diagnosis, prognosis and recurrence of some significance.

Presently, because of yolk sac tumor and embryonal carcinoma high degree of malignancy, postoperative easy to relapse, although 2 patients in this group given standard radiotherapy and timely chemotherapy, patients were respectively died after 5 months and 6 months.1 case of mixed germ cell tumor is a good survival, suggesting that the tumor may be mainly composed of germ cell tumors. Recent studies have shown radiotherapy combined with cisplatin and bleomycin-based combination chemotherapy can effectively delay the highly malignant germ cell tumor survival time of patients (Cheon HC and Jung S,. 2006).

In addition to the height of this group of rare malignant goniolmas (yolk sac tumor, embryonal carcinoma and mixed germ cell tumor in 1 case), and pituitary adenoma into leiomyosarcoma after radiation therapy, the less can be malignant tumors, such as pilocytic astrocytoma, the pilocytic astrocytoma is most common in the cerebellum (Desai KI and Nadkami TD,. 2001) very little occurred in the saddle. This group after the cases were pathologically confirmed as received no follow-up radiation therapy, clinical results were satisfactory, follow-up no recurrence. In addition, 1 patient in this group there are the most specific granulomatous fungal disease that saddle area, preoperative imaging before the highly suspected clinoid meningiomas, pathological diagnosis of

fungal inflammatory pseudotumor, but tuberculosis or a fungal difficult to distinguish inflammatory pseudotumor, after confirmed by PAS staining fungal mold inflammatory pseudotumor.

Currently inflammatory lesions occurred only 0.5% of pituitary lesions in sellar region(Rao S and Rajkumar A, 2008) clinical very rare, as many as case reports of pituitary abscess, clinical is not very common, and the mycosis occurs in sellar region is rare, due to fungal Inflammatory pseudotumor occurs mostly in immunocompromised patients, this group of young patients without immune system dysfunction, or underlying diseases such as diabetes, so little consideration for the preoperative inflammatory pseudotumor, high rate of misdiagnosis. SAH occurred 2 months later, secondary search MRA shows left vertebral artery aneurysm, now in critical condition, breathing machine needed to maintain breathing. Patients with a history of fungal inflammatory pseudotumor, the author believe that the Department of vertebral artery aneurysms and more spread fungal diseases due to the great vessels of skull base Willis ring, therefore, in this case gives the biggest lesson is that once clear of intracranial fungal lesions exists, early MRA or DSA, and early treatment may prevent catastrophic SAH.

In short, this group of 6 cases of rare lesions of the sellar region of clinical data showed that the extremely complex pathology, preoperative misdiagnosis rate is high, especially for young people sellar lesions, a rare highly malignant germ cell tumor should be attention to the preoperative imaging studies should be strengthened, while tumor markers AFP and HCG test in order to determine the nature of the tumor and prognosis for the first time under the microscope during surgery for total resection of tumor and given standard radiotherapy and chemotherapy to improve clinical efficacy.

Acknowledgements:

Foundation item: The National Project of India. Authors are grateful to the Department of Science and Technology, Government of India for financial support to carry out this work.

Corresponding Author:

Dr. Song Naijun
Department of Botany
The first affiliated hospital of Zhengzhou University, Zhengzhou, 450052, China.
E-mail: laijunsong@126.com

1/26/2012

References

1. Halbauer DJ, Mészáros I, Dóczi T, et al. Rare sellar region tumors. Pathol Oncol Res 2003; 9: 134-137.
2. Heaney AP.Clinical review: Pituitary carcinoma: difficult diagnosis and treatment. J Clin Endocrinol Metab 2011; 96: 3649-3660.
3. Arai A, Nishihara M, Sasayama T, et al. Xanthogranuloma of the sellar region--case report. Neurol Med Chir (Tokyo) 2010; 50: 488-491.
4. J.Niwa, K.Hashi, T.Minase. Radiation Induced Intracranial Leiomyosarcoma: Its Hist opathological Features. Acta Neurochir 1996; 138:1470-1471.
5. Lee KH, Lee IH, Kim BG, et al. Clinicopathologic characteristics of malignant germ cell tumors in the ovaries of Korean women: a Korean Gynecologic Oncology Group Study. Int J Gynecol Cancer 2009; 19: 84-87.
6. Wu MC; Luo SQ; Jia Ge, et al. Intracranial nongerminomatous malignant germ cell tumors. Chinese Journal of Neurosurgery 2006; 22: 199-203.
7. Huang X, Zhang R, Zhou LF. Grading System for Diagnosis and Treatment of Intracranial Nongerminomatous Malignant Germ Cell Tumors. Zhonghua Yi Xue Za Zhi. 2008; 89: 2333-2336.
8. Cheon HC, Jung S, Moon KS, et al. Primary endodermal sinus tumor of the cerebellar hemisphere: a case report with review of the literature. J Neurooncol. 2006; 77:173-178.
9. Desai KI, Nadkami TD, Muzumdar DP, et al. Prognostic factors for cerebellar astrocytomas in children: a study of 102 cases. Pediatr Neurosurg 2001; 35: 311-317.
10. Rao S, Rajkumar A, Kuruvilla S, et al. Sellar Lesion: not always a pituitary adenoma. Indian J pathol Microbio 2008; 51: 269-270.

Identify and evaluate factors affecting non-oil exports using FGDM

[1]Sudabeh Morshedian Rafiee (Ph.D.) and [2]Zahra Houshmand Neghabi

[1]Assist. Prof. & Faculty Member, Department of Commercial Management, Islamshahr Branch, Islamic Azad University (IAU), Iran. E-mail: ikiu2011@gmail.com
[2]Faculty member, Department of Commercial Management, Islamshahr Branch, Islamic Azad University (IAU), Iran

Abstract: Economic development is one of the main objectives of every society in the world and economic growth is fundamental to economic development. Nowadays, the international trade policy is deemed as a key strategy in most countries, in such a manner that the process moves forward with a speed more than production growth of the goods and services rendered in developed countries. Non-oil export growth is one of the effective factors in the development of the country's economy. The aim of this study is to evaluating the effective factors on non-oil exports, using fuzzy multi criteria decision making. We contribute non-oil exports literature by identify and evaluate seven critical factors. The findings show that foreign direct investment, technology and quality of products are the most important factors that have significant impact on non-oil exports.

[Sudabeh Morshedian Rafiee (Ph.D.) and Zahra Houshmand Neghabi . **Identify and evaluate factors affecting non-oil exports using FGDM.** *Life Sci J* 2012;9(3):2038-2047]. (ISSN:1097-8135). http://www.lifesciencesite.com. 295

Key word: Economic growth, Non-oil exports, Fuzzy AHP, Fuzzy Delphi

1. Introduction

Economic development is one of the main objectives of every society in the world and economic growth is fundamental to economic development. There are many contributors to economic growth. Export is considered as one of the very important contributors among them.

Although there is no overall consensus that support the export led economic growth, there are some economists such as Kavoussi (1984), Ram (1985), and Salvatore and Hatcher (1991) that argue export benefit economic growth.

Nowadays, the international trade policy is deemed as a key strategy in most countries, in such a manner that the process moves forward with a speed more than production growth of the goods and services rendered in developed countries. Hence, one of the effective elements in line with the advancement of export development policies is the production of competitive products at the foreign markets.

Within the framework of trading strategies, reducing amount of imports and putting emphasis on the increase of domestic products and also in line with alternative policies for the imports which necessitate the admission of the supportive and tariff policies, and also the exports development policies as a supplementary strategy which seeks for elimination of trading obstacles and limitations, both of these two strategies are used for foreign exchange earnings which causes the improvement of international transactions and facilitates; one of the important economic objectives.

Non-oil export growth is one of the effective factors in the development of the country's economy. For example in developing countries, export of the agricultural products has been considered in order to supply the foreign currency as required for sectors of industry and consumption of the society (Naderi, 1992).

Non-oil goods are goods produced in rural production cooperatives, including agricultural products and crafts that can be exported abroad. Because the Iranian economy is among the oil-reliant economies, this reliance on oil revenue has gone so far that many economists consider it as the main cause of inflation and liquidity growth.

Non-oil exports are the rural production cooperatives' important performances which is effective to reduce the dependence on oil. After the first, second, and third development plans, the country has not completely achieved the anticipated objectives concerning the non-oil exports and they still have a little share of earnings than oil exports. Seriously revising production process in order to improve export's chain has become inevitable (Tavassoli, 2005). The aim of this research is to identify effective factor that have the most impact on

non-oil export growth. For this reason we use fuzzy AHP to evaluate the weight of these factors.

The organization of this paper is as follows. Section 2 discusses the literature review. In Section 3, we explain the process of the research, fuzzy Delphi and fuzzy AHP methods. Section 4 is data analysis and the paper ends with concluding remarks in section 5.

2. Literature review

According to Mehrizi (2000), the factors that make our failure in international markets are: clear information about the market of exported goods, having no attention on advantage of country in export, attention to quality standards, defining goals and coordination.

Farokhian et. al., (2010), presented the effective factors on increasing the export from the standpoints ofthe Iranian exporters under a model. They found that four main factors influenced exports which were: Individual factor (education, experience, export knowledge, public communications), economical factor (export markets, governmental subsidies, export pricing, export marketing), environmental factor (rules and regulations, culture, technology, informal communications, political factor) and product marginal factor (design and packaging, quality of products, guarantee and after-sell services, distribution canals, products' brands).

In 2001 Zargarzade designed marketing strategies for agricultural products exporters. According to his research long term interest of export merchants is affected by proper selection of exportable goods and target markets regarding variety of factors and the choosing of goods and target markets should take place by considering these factors.

according to Aghdaie and Zardeini (2012), Lack of attention to world economic situation and having no proper marketing plan are the causes of Persian carpet retardation but the thing which has made the most damage to Persian handmade carpet and puts its Export in Danger is its Identity in Foreign Markets which is hidden versus take carpets of competitors, for example, sometimes it happens when a buyer goes to a carpet shop in his or her country for buying a Persian handmade carpet, The seller inadvertently or intentionally introduced some carpet which are not really Persian carpet and have only Persian design and woven by Iran's competing countries such as Pakistan and India

In 2011, 448 large Brazilian companies had been considered to identify effective factors on export. The results showed that the external environment, firm characteristics and firm strategy have important effect on export (Carneiro et. al., 2011).
Samimi and Peikani (2002), in their study mentioned internal and external effective factors and non-oil export development obstacles, optimize production weakness, export organization weakness and … as non-oil export problems through a forecasting study pattern.

Ghazizade in 2003 has studied the effect of four variables including target market environment, national and internal environment of the company and mixed marketing elements.

Darvishkhani in 2004 has studied the role of packing, mixed marketing elements and hygienic and nutritious standards and proved their positive effects on increasing local and global selling from producer's perspective.

On the other hand there are a number of ways through which Trade flows and foreign direct investment (FDI) can be linked. Goldberg and Klein, (1998) asserted that FDI may encourage export promotion, import substitution, or greater trade in intermediate inputs which often exist between parent and affiliate producers.

The orientation of most investments by multinational firms is towards exports and this may most likely serve as a catalyst for the integration of the FDI host economy to a global production network in sectors in which it may formerly have had no industrial experience (OECD, 1998). Rodriguez Clare (1996); Calderón, Mortimore and Peres (1996) argue that the very nature of the activities of multinational enterprises in Mexico could encourage the expansion of its industrial exports.

These studies clearly indicate that FDI could be associated with export trade in goods, and the host country may enjoy an FDI led export growth. Goldberg and Klein (1998, 1999) do not find evidence to support a significant link between FDI and aggregate exports in Latin America. According to them, the trade-promoting effects of FDI appear to be weak or insignificant with regard to Latin American trade with the United States and Japan. Their results also failed to find a systematic linkage between sectorial trade and FDI in Latin America. So according to literature these factors are extracted (see table 1).

Table 1; factor affecting the non-oil exports

Row	Factors	Row	Factors
1	Education	10	Culture
2	Experience	11	Informal communications
3	Governmental subsidies	12	Technology
4	Public communications	13	Design and packaging
5	Exports' markets	14	Quality of products
6	Foreign direct investment	15	Guarantee and after-sell services
7	Export pricing	16	Distribution canals
8	Export marketing	17	Products' brands
9	Rules and regulations	18	export knowledge

2.1. Fuzzy logic

Fuzzy set theory first was introduced by Zadeh (1965) to map linguistic variables to numerical variables within decision making processes. Then the definition of fuzzy sets were manipulated to develop Fuzzy Multi-Factors Decision Making (FMCDM) methodology by Bellman and Zadeh (1970) to resolve the lack of precision in assigning importance weights of factors and the ratings of alternatives against evaluation factors.

A fuzzy set is characterized by a membership function, which assigns to each element a grade of membership within the interval $[0,1]$, indicating to what degree that element is a member of the set (Bevilacqua, Ciarapica, & Giacchetta, 2006). As a result, in fuzzy logic general linguistic terms such as ''bad'', ''good'' or ''fair'' could be used to capture specifically defined numerical intervals.

A tilde ''~'' will be placed above a symbol if the symbol represents a fuzzy set. A triangular fuzzy number (TFN) $\tilde{M}$ is shown in Fig. 1. A TFN is denoted simply as (l,m,u). The parameters l, m and u denote the smallest possible value, the most promising value and the largest possible value that describe a fuzzy event (Kahraman, Cebeci, & Ruan, 2004). When l = m = u, it is a non-fuzzy number by convention (Chan & Kumar, 2007).

Each TFN has linear representations on its left and right side such that its membership function can be defined as (Kahraman, Cebeci, & Ruan, 2004);

$$m_{\tilde{M}} = \begin{cases} 0, x < l \\ (x-l)/(m-l), l \le x \le m \\ (u-x)/(u-m), m \le x \le u \\ 0, x > u \end{cases} \quad (1)$$

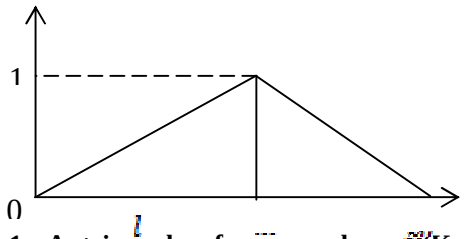


Fig 1: A triangular fuzzy number, $\tilde{M}$(Kahraman, Cebeci, & Ruan, 2004)

$\otimes$: multiply fuzzy numbers, e.g. assuming two triangular fuzzy numbers

$$\tilde{A} = (a_1, b_1, c_1), \tilde{B} = (a_2, b_2, c_2)$$

$$\tilde{A} \otimes \tilde{B} = (a_1 \times a_2, b_1 \times b_2, c_1 \times c_2) \quad (2)$$

$\oslash$: divide fuzzy numbers, e.g.: assuming two triangular fuzzy numbers

$$\tilde{A} = (a_1, b_1, c_1), \tilde{B} = (a_2, b_2, c_2)$$

$$\tilde{A} / \tilde{B} = (a_1 / a_2, b_1 / b_2, c_1 / c_2) \quad (3)$$

3. Methodology

This study proposes a process integrating fuzzy Delphi and fuzzy AHP methods to engage the challenge of factors selection and evaluation. Our experts are ten people of Industry, Mines and Trade organization with over 8 years of experience in this organization. Firstly we define factors that are extracted from literature. Then the fuzzy Delphi method effectively gathers information toward developing critical factors. In this problem, the relative importance of different decision factors involves a high degree of subjective judgment and individual preferences. The linguistic assessment of human feelings and judgments are vague and it is not reasonable to represent them in terms of precise numbers. It feels more confident to give interval judgments. Therefore triangular fuzzy numbers were used in this problem to decide the priority of one decision factors over another. The triangular fuzzy numbers were determined from reviewing literature (Kahraman, C.; Cebeci, U.; Ulukan, Z., 2003). In order to evaluate the weights of factors that were obtained by fuzzy Delphi method, fuzzy AHP was used.

3.1. Fuzzy Delphi method:

Murry et al. (1985) proposed the concept of integrating the traditional Delphi Method and the fuzzy theory to improve the vagueness of the Delphi Method. Membership degree is used to establish the membership function of each participant. Ishikawa et al. (1993) further introduced the fuzzy theory into the Delphi Method and developed max–min and fuzzy integration algorithms to predict the prevalence of computers in the future. In this study we used Fuzzy Delphi Method was proposed by Ishikawa et al. (1993), and it was derived from the traditional Delphi technique and fuzzy set theory.

Noorderhaben (1995) indicated that applying the Fuzzy Delphi Method to group decision can solve the fuzziness of common understanding of expert opinions. In this study we use eleven experts to extract the critical factors of Industry, Mines and Trade organization.

The FDM steps are as follows:

1) Collect opinions of decision group: Find the evaluation score of each alternate factor's significance given by each expert by using linguistic variables in questionnaires.
2) Set up triangular fuzzy numbers: Calculate the evaluation value of triangular fuzzy number of each alternate factor given by experts, find out the significance triangular fuzzy number of the alternate factor. This study used the geometric mean model of mean general model proposed by Klir and Yuan (1995) for FDM to find out the common understanding of group decision.

The computing formula is illustrated as follows:

Assuming the evaluation value of the significance of No. j element given by No. i expert of n experts is $\tilde{W} = (a_{ij}, b_{ij}, c_{ij}), \quad i = 1,2,...,n, \quad j = 1,2,...,m.$

Then the fuzzy weighting $\tilde{W}$ of No j Element is $\tilde{W}_{ij} = (a_{ij}, b_{ij}, c_{ij}), \ j = 1,2,...,m.$

Among which $a_j = \min\{a_{ij}\}, b_j = \frac{1}{n}\sum_{i=1}^{n} b_{ij}$, $c_j = \max\{c_{ij}\}$. (4)

3) Defuzzification : Use simple center of gravity method to defuzzify the fuzzy weight $\tilde{W}_{ij}$ of each alternate element to definite value Sj, the followings are obtained:

$$S_j = \frac{a_j + 4b_j + c_j}{6}, \qquad j = 1,2,...,m \quad (5)$$

4) Screen evaluation indexes: Finally proper factors can be screened out from numerous factors by setting the threshold a. The principle of screening is as follows:

If $Sj \geq a$, then No. j factor is the evaluation index.

If $Sj < a$, then delete No. j factor.

Table 2; Linguistic variables for importance of each factor

Absolutely appropriate	(9,10,10)
Appropriate	(7,9,10)
Slightly appropriate	(5,7,9)
Neutral	(3,5,7)
Slightly inappropriate	(1,3,5)
Inappropriate	(0,1,3)
Absolutely inappropriate	(0,0,1)

For the threshold value r, the 80/20 rule was adopted with r set as 0.8. This indicated that among the factors for selection, ''20% of the factors account for an 80% degree of importance of all the factors''. The selection factors were:

If MA $\geq$ r =0.8, this appraisal indicator is accepted.

If MA $<$ r = 0.8, this appraisal indicator is rejected.

3.2. Fuzzy Analytic Hierarchy Process

Laarhoven and Pedrycz (1983) proposed the Fuzzy Analytic Hierarchy Process in 1983, which was an application of the combination of Analytic Hierarchy Process (AHP) and Fuzzy Theory. The linguistic scale of traditional AHP method could express the fuzzy uncertainty when a decision maker is making a decision. Therefore, FAHP converts the opinions of experts from previous definite values to fuzzy numbers and membership functions, presents triangular fuzzy numbers in paired comparison of matrices to develop FAHP, thus the opinions of experts approach human thinking model, so as to achieve more reasonable evaluation factors.

Laarhoven and Pedrycz (1983) proposed the FAHP, which is to show that many concepts in the real world have fuzziness. Therefore, the opinions of decision makers are converted from previous definite values to fuzzy numbers and membership numbers in FAHP, so as to present in FAHP matrix.

Table 3; Linguistic variables for weight of each factor

Extremely strong	(9,9,9)
Intermediate	(7,8,9)
Very strong	(6,7,8)
Intermediate	(5,6,7)
Strong	(4,5,6)
Intermediate	(3,4,5)
Moderately strong	(2,3,4)
Intermediate	(1,2,3)
Equally strong	(1,1,1)

The steps of this study based on FAHP method are as follows:

1) Determine problems: Determine the current decision problems to be solved, so as to ensure future analyses correct; this study discussed the ‘‘evaluation factors for verification of supplier selection factors”.

2) Set up hierarchy architecture: Determine the evaluation factors having indexes to be the factors layer of FAHP, for the selection of evaluation factors, relevant factors and feasible schemes can be found out through reading literatures. This study screened the important factors conforming to target problems through FDM investigating experts’ opinions, to set up the hierarchy architecture.

3) Construct pairwise comparison matrices among all the elements/factors in the dimensions of the hierarchy system. Assign linguistic terms to the pairwise comparisons by asking which is the more important of each two dimensions, as following matrix $\tilde{A}$:

$$\tilde{A} = \begin{pmatrix} 1 & \tilde{a}_{21} & \Lambda & \tilde{a}_{21} \\ \tilde{a}_{21} & 1 & \Lambda & \tilde{a}_{21} \\ \tilde{a}_{21} & \tilde{a}_{21} & \Lambda & 1 \end{pmatrix} = \begin{pmatrix} 1 & \tilde{a}_{21} & \Lambda & \tilde{a}_{21} \\ \frac{1}{\tilde{a}_{21}} & 1 & \Lambda & \tilde{a}_{21} \\ \frac{1}{\tilde{a}_{21}} & \frac{1}{\tilde{a}_{21}} & \Lambda & 1 \end{pmatrix}$$

Where $\tilde{a}_{ij} \begin{cases} \tilde{9}^{-1}, \tilde{8}^{-1}, \tilde{7}^{-1}, \tilde{6}^{-1}, \tilde{5}^{-1}, \tilde{4}^{-1}, \tilde{3}^{-1}, \tilde{2}^{-1}, \tilde{1}^{-1}, \tilde{1}, \tilde{2}, \tilde{3}, \tilde{4}, \tilde{5}, \tilde{6}, \tilde{7}, \tilde{8}, \tilde{9} & i \neq j \\ \tilde{1} & i = j \end{cases}$

4) To use geometric mean technique to define the fuzzy geometric mean and fuzzy weights of each factor by Hsieh et al. (2004).

$$\tilde{r}_i = \left(\tilde{a}_{i1} \otimes \tilde{a}_{i2} \otimes .... \otimes \tilde{a}_{in}\right)^{\frac{1}{n}} \quad (6)$$

$$\tilde{w}_i = \tilde{r}_i \otimes \left(\tilde{r}_1 \oplus \tilde{r}_2 \oplus .... \oplus \tilde{r}_n\right)^{-1} \quad (7)$$

Where a_{ij} is fuzzy comparison value of dimension i to factor j, thus, $\tilde{r}_i$ is a geometric mean of fuzzy comparison value of factor i to each factor, $\tilde{w}_i$ is the fuzzy weight of the ith factor, can be indicated by a TFN, $\tilde{w}_i = \left(lw_i, mw_i, uw_i\right)$.

The lw_i, mw_i and uw_i stand for the lower, middle, and upper values of the fuzzy weight of the ith dimension.

4. Data analysis

Stage one: reviewing relevant literature of non-oil export and proposing important factors: 18 factors for non-oil export based on relevant literature are proposed.

Stage two: Screen important factors by fuzzy Delphi Method: First a DM interview table is setup and second interview was done with ten experts from Industry, Mines and Trade organization.

Seven factors were extracted from this stage (see table 8).

Table 4; the extracted factors by FDM

Row	Factor
1	F1: Governmental subsidies
2	F2: Foreign direct investment
3	F3:Rules and regulations
4	F4:Technology
5	F5:Quality of products
6	F6: Distribution canals
7	F7: Export knowledge

Stage three: The weights of evaluation factors

We adopt fuzzy AHP method to evaluate the weights of different factors affecting non-oil export. Following the construction of fuzzy AHP model, it is extremely important that experts fill the judgment matrix.

According to the committee with ten representatives about the relative important of factors, the pairwise comparison matrices of factors will be obtained. We apply the fuzzy numbers defined in Table 4. We transfer the linguistic scales to the corresponding fuzzy numbers. Computing the elements of synthetic pairwise comparison matrix by using the geometric mean method suggested by Buckley (1985) that is:

$$\tilde{a}_{12} = (\tilde{a}_{ij}^{1} \otimes \tilde{a}_{ij}^{2} \otimes \ldots \otimes \tilde{a}_{ij}^{10})^{\frac{1}{10}}$$

For example

$$\tilde{a}_{12} = ((0.167, 0.2, 0.25) \otimes (0.125, 0.143, 0.167) \otimes (0.25, 0.33, 0.5) \otimes (4, 5, 6) \otimes (0.33, 0.5, 1) \otimes$$

$$(0.167, 0.2, 0.25) \otimes (0.167, 0.2, 0.25) \otimes (0.25, 0.33, 0.5) \otimes (1, 2, 3) \otimes (0.25, 0.33, 0.5))^{\frac{1}{10}} = (0.32, 0.43, 0.6)$$

It can be obtained the other matrix elements by the same computational procedure, therefore, the synthetic pairwise comparison matrices of the five representatives will be constructed as follows matrix **A**:

Table 5; Fuzzy comparison matrix for the relative importance of factors

	F1			F2			F3			F4			F5			F6			F7		
F1	1	1	1	0.32	0.43	0.60	1.05	1.35	1.78	0.47	0.62	0.81	0.56	0.73	1.03	2.73	3.55	4.43	2.08	2.79	3.60
F2	1.67	2.33	3.10	1	1	1	2.42	3.31	4.14	1.08	1.42	1.81	1.24	1.66	2.26	4.02	4.89	5.95	3.27	4.05	4.89
F3	0.56	0.74	0.96	0.25	0.31	0.43	1	1	1	0.35	0.43	0.56	0.48	0.62	0.82	2.17	2.67	3.19	1.18	1.59	2.11
F4	1.23	1.61	2.12	0.55	0.70	0.93	1.79	2.31	2.84	1	1	1	0.99	1.34	1.80	3.73	4.59	5.64	2.51	3.36	4.25
F5	0.97	1.37	1.77	0.44	0.60	0.81	1.22	1.61	2.07	0.56	0.75	1.01	1	1	1	3.57	4.33	5.16	2.22	3.03	3.90
F6	0.23	0.28	0.37	0.17	0.20	0.25	0.31	0.37	0.46	0.18	0.22	0.27	0.19	0.23	0.28	1	1	1	0.4	0.5	0.68
F7	0.28	0.36	0.48	0.20	0.25	0.31	0.44	0.60	0.81	0.23	0.29	0.38	0.26	0.33	0.45	1.47	1.99	2.52	1	1	1

To calculate the fuzzy weights of factors, the computational procedures are displayed as following parts:

$$\tilde{r}_1 = (\tilde{a}_{11} \otimes \tilde{a}_{12} \otimes \tilde{a}_{13} \otimes \tilde{a}_{14} \otimes \tilde{a}_{15} \otimes \tilde{a}_{16} \otimes \tilde{a}_{17})^{\frac{1}{7}}$$

$$\tilde{r}_1 = \big((1,1,1) \otimes (0.32,0.43,0.6) \otimes (1.05,1.35,1.78) \otimes (0.47,0.62,0.81) \otimes (0.56,0.73,1.03) \otimes (2.73,3.55,4.43) \otimes (2.08,2.79,3.6)\big)^{\frac{1}{7}} = (0.908,1.145,1.462)$$

Table 6; The fuzzy comparison value of each factor among other factors

$\tilde{r}_i$	lr_i	mr_i	ur_i
$\tilde{r}_1$	0.909	1.145	1.462
$\tilde{r}_2$	1.838	2.319	2.851
$\tilde{r}_3$	0.670	0.826	1.035
$\tilde{r}_4$	1.413	1.768	2.189
$\tilde{r}_5$	1.126	1.443	1.796
$\tilde{r}_6$	0.287	0.342	0.416
$\tilde{r}_7$	0.417	0.518	0.655

For the weight of each factor, they can be done as follows:

$$\tilde{W}_i = \tilde{r}_i \otimes (\tilde{r}_1 \oplus \tilde{r}_2 \oplus \tilde{r}_3 \oplus \tilde{r}_4 \oplus \tilde{r}_5 \oplus \tilde{r}_6 \oplus \tilde{r}_7)^{-1}$$

$$\tilde{W}_1 = \tilde{r}_1 \otimes (\tilde{r}_1 \oplus \tilde{r}_2 \oplus \tilde{r}_3 \oplus \tilde{r}_4 \oplus \tilde{r}_5 \oplus \tilde{r}_6 \oplus \tilde{r}_7)^{-1}$$

$$\tilde{W}_1 = (0.908, 1.145, 1.462) \otimes (1/(1.462 + 2.851 + 1.034 + 2.189 + 1.796 + 0.415 + 0.655),$$
$$1/(1.145 + 2.319 + 0.826 + 1.767 + 1.443 + 0.341 + 0.518),$$
$$= 1/(0.908 + 1.837 + 0.669 + 1.413 + 1.125 + 0.287 + 0.416) = (0.087, 0.137, 0.219)$$

We also can calculate the remaining $\tilde{W}_j$, there are:

Table 7; the weights and rank of factors

$\tilde{W}_j$	a	b	c	Rank	Factor
$\tilde{W}_1$	0.087	0.137	0.220	4	governmental subsidies
$\tilde{W}_2$	0.177	0.277	0.428	1	Foreign direct investment
$\tilde{W}_3$	0.064	0.099	0.155	5	Rules and regulations
$\tilde{W}_4$	0.136	0.211	0.329	2	Technology
$\tilde{W}_5$	0.108	0.173	0.270	3	Quality of products
$\tilde{W}_6$	0.028	0.041	0.062	7	distribution canals
$\tilde{W}_7$	0.040	0.062	0.098	6	export knowledge

5. Conclusion

The role of exports in economic performance of developing countries has become one of the more intensively studied topics in recent years. The major impetus for most studies on this relationship is the export-led economic growth which interestingly represents a dominant explanation in this context. The performance of non-oil export sector, as pointed out earlier, has however been relatively impressive in recent times.

We developed non-oil export literature by extracting and evaluating critical factors that affecting non-oil export. By this work seven factors were extracted that are; governmental subsidies, foreign direct investment, rules and regulations, technology, quality of products, distribution canals and export knowledge. As it is consider in table X foreign direct investment, technology and quality of products are the most important factor that have significant impact on non-oil export. Furthermore list of factors that have impact on non-oil export were extracted from non-oil export literature.

This study used MADM method for the first time in order to extract and evaluate factors affecting non-oil export. In general, selection problems as well as any human decision making are vague and uncertain, and so fuzzy set theory helps to convert DM preferences and experiences into meaningful results by applying linguistic values to measure each factor. In this paper, a multi-criteria group decision making model has been used based on fuzzy set theory to efficiently deal with the

ambiguity of the decision making problems in practical cases to evaluate the factors.

Reference

Aghdaie, S. F. A. and Zardeini, H. Z. (2012), "**A SWOT Analysis of Persian Handmade Carpet Exports**" International Journal of Business and Management Vol. 7, No. 2, pp 243-251.

Bellman, R., and Zadeh, L. (1970). **Decision-making in a fuzzy environment management**. Science, 17, 141–164.

Calderón, A.; Mortimore, M. and Peres (1996): "**Foreign Investment as a Source of International Competitiveness**". In Dunning J.H. and Narula (eds.) Catalyst for Economic Restructuring. Routledge, 241-279.

Carneiro, J.; Rocha, A. and Silva, J. F. (2011), "**Determinants of export performance: a study of large Brazilian manufacturing firms**". BAR, Braz. Adm. Rev. vol.8, n.2, pp. 107-132.

Darvish Khani, M. (2003), "**studying the effect of marketing management on Sohan export local and global sales increase**", thesis, economy and official university of Isfahan, p35.

Farokhian, S., Sadeghi, T., andEsmail, HRK, (2010). "**The Effective Factors on Increasing the Export from the Iranian Exporters' Standpoints**". Asian Journal of Business Management Studies 1 (2): 26-29, 2010.

GhaziZade, Mostafa (1382); "**study and determination of effective factors in export company success in Iran in Middle east and representation of strategies for non-oil export increase**", thesis, Tehran university. P98.

Goldberg, S. and Klein, W. (1998): "**Foreign Direct Investment, Trade, and Real Exchange Rate Linkages in Developing Countries**". In Reuven Glick (ed.) Managing Capital Flows and Exchange Rates: Lessons from the Pacific Basin. Cambridge University Press.

Goldberg, S. and Klein, W. (1999): "**International Trade and Factor Mobility: an Empirical Investigation**", NBER Working Paper No. 7196.

Hsieh, T.-Y., Lu, S.-T., & Tzeng, G.-H. (2004). "**Fuzzy MCDM approach for planning and design tenders selection in public office buildings**". International Journal of Project Management, 22, 573–584.

Ishikawa, A., Amagasa, M., Shiga, T., Tomizawa, G., Tatsuta, R., & Mieno, H. (1993). "**The max–min Delphi method and fuzzy Delphi method via fuzzy integration**". Fuzzy Sets and Systems, 55, 241–253.

Kahraman, C., Cebeci, U., & Ruan, D. (2004). **"Multi-attribute comparison of catering service companies using fuzzy AHP: The case of Turkey"**. International Journal of Production Economics, 87, 171–184.

Kahraman, C.; Cebeci, U.; Ulukan, Z. (2003). **"Multi-criteria supplier selection using fuzzy AHP"**. Logistics Information Management, 16, 382–394.

Kavoussi, R. M., (1984), **"Export Expansion and Economic Growth: Further Empirical Evidence"**, Journal of Development Economics, vol.14, pp. 241-250.

Klir, G., & Yuan, B. (1995). **"Fuzzy sets and fuzzy logic – Theory and application"**. New Jersey: Prentice-Hall Inc.

Laarhoven, P., & Pedrycz, W. (1983). **"A fuzzy extension of Sati's priority theory"**. Fuzzy Sets and System, 11, 229–241.

Murry, T., Pipino, L., & Gigch, J. (1985). **"A pilot study of fuzzy set modification of Delphi"**. Human Systems Management, 5, 76–80.

Naderi, A. (1992), **"comparative advantage and export development in Iran"**, Institute of Business and Research
Studies, Tehran: 1992, p. 273

Noorderhaben, N. (1995). **"Strategic decision making"**. UK: Addison-Wesley.

OECD (1998), "**Foreign Direct Investment and Economic Development: Lessons from Six Emerging Economies"**. Paris.

Ram, R., (1985), **"Exports and Economic Growth: Some Additional Evidence"**, Ecnomic Development and Cultural Change, vol 33, pp. 415-423.

Rodriguez-Clare, A. (1996): **"Multinationals, Linkages, and Economic Development"**. The American Economic Review 86, 852-873.

Salvatore, D., and T. Hatcher, (1991), **"Inward Oriented and Outward Oriented Trade Strategies"**. The Journal of Development Studies, vol. 27, pp. 7-25.

Samimi J., ahmad and Katrin Peykani (2002); **"the role of export credit on non-oil export development"**, commerce research magazine, commerce research and study institute, magazine no24,p p61-75.

Tavassoli A (2005). **"Role of rural production cooperatives in agricultural development"**. Dissertation of science and research branch, Islamic Azad University.

Zadeh, L. A. (1965). Fuzzy sets. Information and Control, 8, 338–353.

9/23/2012

An AFLP Male-Specific Marker Detected in 15 Iranian Sheep and Goats Populations

Sayed Ziaeddin Mirhoseini [1], Nejat Badbarin [1], Akbar Khaleghzadegan [1]

[1.] Department of Animal Science, University of Guilan, Guilan, Rasht, Iran
szmirhoseini@gmail.com; mirhosin@guilan.ac.ir

Abstract: This study employed the Amplified Fragment Length Polymorphism (AFLP) approach to searching for sex-specific DNA markers in the genome of the Iranian sheep (*Ovis aries*) and goats (*Capra hircus*) populations. Among AFLP primers used to determining sex specific markers, one of them, E42/T32, produce a 100 bp DNA fragment in sheep populations and a 147 bp DNA fragment in goats populations founded only on tested males. This sex specific band in the PCR gel products was represented in males but none was found in females when the population's genomic DNA samples were amplified with these two primers by PCR. This marker frequency among male sheep and goats were 0 to 92.3% and 80 to 100% respectively. The size of the marker was 100 bp and 147 bp in sheep and goats populations respectively.
[Mirhoseini SZ, Badbarin N, Khaleghzadegan A. **An AFLP Male-Specific Marker Detected in 15 Iranian Sheep and Goats Populations**. *Life Sci J* 2012;9(3):2048-2052] (ISSN:1097-8135). http://www.lifesciencesite.com. 296

Keywords: Iranian sheep and goats populations; Male specific marker; AFLP

1. Introduction

Sex determination using genomic DNA from several origins is often an important tool in routine genotyping. Embryo sexing before freezing or transfer into recipient ewes and goats is of considerable economic advantage. Embryo transfer will be more efficient if it could be possible to know the sex of embryos before transfer or freezing. Since reliability is the most important concern in sex determination, PCR is one suitable method that meets this criterion and is rapid, inexpensive and highly sensitive (Dervishi et al., 2008). In addition, sex determination of domestic animal meat has received great attention in recent years. An example exists in beef, where male beef is designated to be of higher quality than cow or heifer meat, and therefore yields higher prices. To avoid unfair competition and to assure consumers of accurate labeling, it is necessary to develop reliable methods for determining the gender of the meats (Bai et al., 2010). Appa Rao et al (1995) developed a method for accurate identification of male and female raw meats in cattle, buffalo, sheep and goat using the PCR technique.

AFLP technique first described by Vos et al (1995), is one of the most powerful DNA fingerprinting technique that could be used for genome studying with any source and complexity. This method was used for studying many investigations including evolution, classification, assessing genetic diversity, construction QTL maps, studying family relationship, assessing population genetic parameters and conservation of genetic pools in animals (Mueller et al., 1999). The AFLP technique provides an efficient marker system for revealing polymorphic loci and for linkage map construction. An AFLP approach in combination with a sex type pool strategy resulted in a quick analysis for identifying sex-linked loci in rainbow trout. Similar approaches may be useful for molecular mapping of markers linked to genes of interest in other species (Felip et al., 2005).

There are 27 native sheep and 3 wild sheep populations in Iran (Valdez et al., 1978). Goats are the most adaptable and widely spread ungulate livestock spices in the world. They have played a central role in the Neolithic agricultural revolution and spread of human civilization (Marsan et al., 2002). In most farms in Iran, sheep and goats keep together in the same herd, so in this study we use AFLP technique to find sex specific markers for both sheep and goat species together.

Sex identification at immature stages may be done using molecular genetic markers [e.g. goat (Shi et al., 2008), sheep (Dervishi et al., 2008), pig (Horng and Huang, 2003), yak (Bai et al., 2010), Giant tiger shrimp (Khamnamtong et al., 2006), Giant Catfish (Sriphairoj et al., 2007), rainbow trout (Felip et al., 2005)]. Among which the AFLP approach has been especially widely used due to the simplicity and reproducibility of the procedure (Griffiths and Orr, 1999). Therefore, AFLP was used in this study aiming at identifying markers specific to each sex of the Iranian sheep and goats populations.

2. Material and Methods

Blood samples were taken from 10 sheep populations including Torki-Ghashghaei, Lori-Bakhtiari, Makuei, Grey-Shiraz, Zandi, Naeini, Moghani, Kalakui, Shal, Taleshi, and 6 goat populations including Naeini, Torki, Abadeh, Khalkhali, Taleshi and Ghazvini (Table 3). Each sample was taken from local populations randomly.

Genomic DNA was isolated from whole blood by the modified salting out method. The concentration of extracted DNA was estimated using a knowing standard DNA (λ DNA) on agarose gels. The procedure adopted for the production and detection of *Eco*RI/*Taq*I AFLP markers was that described in Ajmone-Marsan *et al.* (1997). Table 1 shows *Eco*RI and *Taq*I adapters and used primers were used in the study. Individual goats were assayed with seven highly polymorphic AFLP primer pairs, selected from a 24 combinations previously tested on five individuals (Table 2).

The 25 µl PCR preamplification reaction mixtures were added to 2 µl of diluted 1:5 H_2O and ligated DNA. The preamplification reaction mixture contains 14.8 µl H_2O, 50ng/µl T01 or T02, 50 ng/µl E01, 0.2 mM dNTPs, 10X PCR buffer, 2mM $Mgcl_2$ and 5 u/µl Taq DNA polymerase. After the preamplification reaction, the reaction mixture was diluted 1:5 with double distilled H_2O. The 10 µl PCR selective amplification mixture contained 1 µl of product from the diluted preamplification reaction, 25 ng/µl of both selective primers, 5 u/µl of Taq DNA polymerase, 0.2 mM of dNTPs, 2mM of $Mgcl_2$ and 1x PCR buffer. All amplification reactions were performed as touch down reaction condition. The touch-down PCR conditions were as follows: (30 s at 94°C and 30 s at 65°C followed by 12 cycles with an annealing temperature decreased by 0.7°C per cycle, and 60 s at 72°C), 23 cycles of normal PCR (30 s at 94°C, 30 s at 56°C, and 60 s at 72°C). The products were analyzed on a 6% polyacrylamide sequencing gel in TBE buffer and silver staining method. For DNA visualization, a silver staining protocol with some modification was used.

3. Results

An AFLP approach was used for identification of sex-linked markers in 366 Iranian sheep and 237 goats populations (Table 3). Seven primer combinations assayed revealed 235 and 299 AFLP bands on sheep and goats population respectively. The length of the bands in AFLP fingerprinting was between 50 bp and 600 bp. Several main bands of AFLP fingerprinting of every animal were found. There were some minor bands in many lanes of the individuals tested. This indicated that genetic material from the same population of animals was similar, although not entirely homogenous. In the sheep populations 121 polymorphism bands and in the goat's population 78 polymorphism bands was found, and 114 and 221 bands were homogenous in sheep and goats respectively.

We did not find any bands that were abundant in females but not in males. While one primer pair (E42/T32) gave a 100 and 147 bp fragment in sheep and goats populations respectively that was highly abundant in male samples but not in females, after screening DNA samples (Fig. 1 and 2) we refer to these band as male marker. This band was not found in any female, and its frequency among male sheep and goats were 0 to 92.3% and 80 to 100% respectively (Table 3). These bands also were polymorph among sheep and goats populations with ranged from 100 to 147 base pair (Fig. 1 and 2).

4. Discussions

During the last few decades, based on PCR technique, several different methodologies were developed for determining the gender of domestic animal. Appa Rao et al (1995) developed a simple and reliable method for accurate identification of male and female raw meats in cattle, buffalo, sheep and goat using PCR technique. The PCR assay was conducted on genomic DNA extracted from raw muscle tissue of male and female animals. The method has been found to be accurate, reliable and quick. AFLP analysis is an effective technique for characterizing the chromosomal location of a gene of interest for the ultimate goal of isolation of the gene because a large number of loci can be scored in each primer combination used (Vos et al., 1995).

The result showed that E42/T32 primers could be used for a PCR based sexing technique in these populations. These PCR primers amplified more reliable marker that can be used in sex identification. During the screen for sex-specific markers, no population specific markers were identified. It could be because of any selection within these populations and uncontrolled crossbreeding.

Genetic sex in mammals is normally determined by presence of either XX or XY chromosomes, since genes determining sex are normally in the correct chromosomal locations. Sry (sex determining region on the Y chromosome) is the gene that encodes the testis determining factor in mammals. Thus, in mammalian sex determination, it is expected that XY animals, having the Sry gene, will develop testes and that XX animals, having no Sry gene, will develop ovaries (Baker, 2006). We suppose that this sex specific marker is located on the holandric part of Y chromosome. This band was polymorph in all males of sheep populations but it was homogenous in three goat populations including Torki, Abade and Khalkhali. The location of the sex locus on the Y-chromosome of these populations needs to be further analyzed. A larger number of molecular markers mapping on both sides of this gene may finally help to elucidate this feature on the Y-chromosome.

Table 1. Adapters and primer used in AFLP analysis

	Name	Sequence
Adapter *TaqI*	*Taq* top strand	5'-GACGATGAGTCCTGAC
	Taq bottom strand	5'-CGGTCAGGACTCAT
Adapter *EcoRI*	*Eco* top strand	5'-CTCGTAGACTGCGTACC
	Eco bottom strand	5'-AATTGGTACGCAGTCTAC
Pre amplification primer *EcoRI*	E01	5'-GAC TGC GTA CCA ATT CA
Pre amplification primer *TaqI*	T01	5'-GAT GAG TCC TGA CCG AA
	T02	5'-GAT GAG TCC TGA CCG AC
Selective primer *EcoRI*	E31	5´-GAC TGC GTA CCA ATT C AAA
	E32	5´-GAC TGC GTA CCA ATT C AAC
	E35	5´-GAC TGC GTA CCA ATT C ACA
	E42	5´-GAC TGC GTA CCA ATT C AGT
	E43	5´-GAC TGC GTA CCA ATT C ATA
	E45	5´-GAC TGC GTA CCA ATT C ATG
Selective primer *TaqI*	T32	5´-GAT GAG TCC TGA CCG A AAC
	T33	5´-GAT GAG TCC TGA CCG A AAG
	T48	5´-GAT GAG TCC TGA CCG A CAC
	T50	5´-GAT GAG TCC TGA CCG A CAT

Table 2. Seven primers used for selective amplification

	Pre amplification with E01/T01		Pre amplification with E01/T02	
Primers	T32	T33	T48	T50
E31	E31/T32	E31/T33	**E31/T48**	E31/T50
E32	E32/T32	E32/T33	E32/T48	E32/T50
E35	E35/T32	**E35/T33**	**E35/T48**	E35/T50
E42	**E42/T32**	E42/T33	E42/T48	E42/T50
E43	**E43/T32**	**E43/T33**	E43/T48	E43/T50
E45	**E45/T32**	E45/T33	E45/T48	E45/T50

Table 3. Number of males and females, males, males with specific band and Polymorphism percent of the male specific marker is listed as a proportion of individuals having that marker.

populations		Number of males and females	Number of males	Number of males with specific band	Polymorphism percent (%)
Sheep's breed	Torki-Ghashghaei	15	13	12	92
	Lori-Bakhtiari	40	5	4	80
	Makuei	40	13	7	54
	Grey-Shiraz	15	13	6	46
	Zandi	40	10	3	30
	Naeini	40	20	5	25
	Moghani	40	12	2	17
	Kalakui	51	20	2	10
	Shal	45	13	1	8
	Taleshi	40	10	0	0
Goat's breed	Naeini	42	10	9	90
	Torki	43	12	12	100
	Abadeh	39	16	16	100
	Khalkhali	41	11	11	100
	Taleshi	32	11	10	91
	Ghazvini	40	10	8	80
Total		603	199	108	54

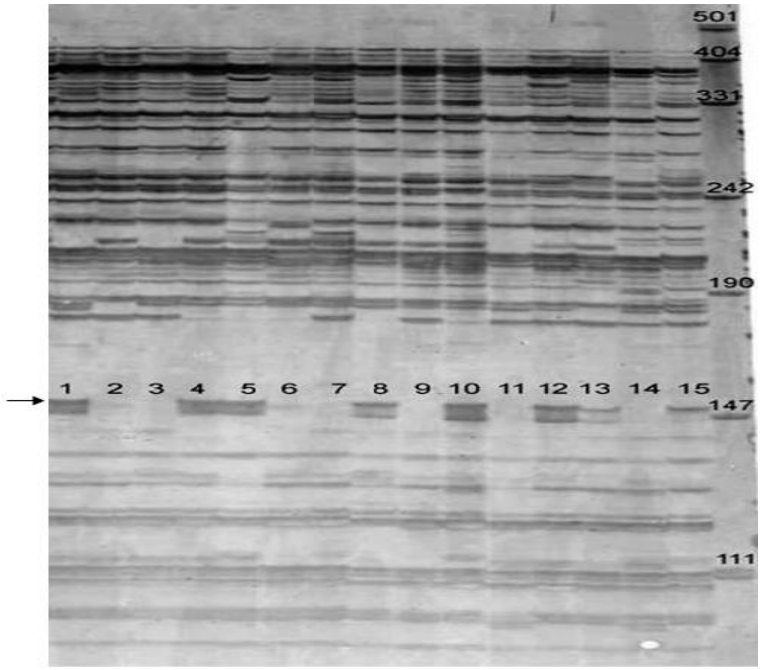


Figure 1. A polyacrylamide gel electrophoresis showing AFLP products of male specific marker in Naeini goat population using E42/T32 primer, with Ladder 1Kb in right. Lanes 1, 4, 5, 8, 10, 12, 13 and 15 are male's samples and Lanes 2, 3, 6, 7, 9, 11 and 14 are female's samples. An arrowhead indicates a candidate sex-specific AFLP marker.

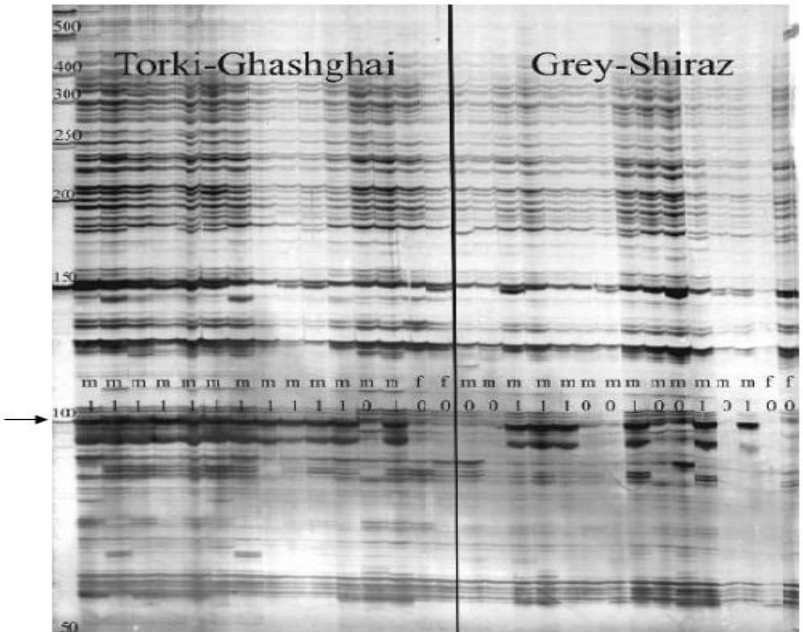


Figure 2. A polyacrylamide gel electrophoresis showing AFLP products of male specific marker in Torki-ghashghaei and Grey-Shiraz sheep populations using E42/T32 primer, with Ladder 1Kb in right. Lanes indicated with m are male's samples and Lanes indicated with f are female's samples. An arrowhead indicates a candidate sex-specific AFLP marker.

It would be interesting to investigate further the structure of the male-specific sequence in these populations. Testing a larger number of individuals is important in order to estimate more correctly the sex specificity of our marker. Screening a larger number of markers may be required to find a more tightly linked one. If sex specific AFLP markers will be verified with more individuals, we plan to convert them to SCAR markers (Sequence Characterized Amplified Regions) to increase the reliability and speed of the testing. In conclusion, novel male specific DNA sequences in Iranian sheep and goats populations could be obtained using a AFLP fingerprinting. The sex of these populations could also be easily and effectively determined using the PCR technique.

Acknowledgements:

This work was supported by Agricultural Biotechnology Research Institute of North of Iran.

Corresponding Author:
Dr. Seyed Ziaeddin Mirhoseini
Department of Animal Science
University of Guilan. Guilan,
Rasht, Iran
E-mail:szmirhoseini@gmail.com;
mirhosin@guilan.-ac.ir

References

1. Dervishi E, Martinez-Royo A, Snchez P, Alabart JL, Cocero MJ, Folch J. Reliability of sex determination in ovine embryos using amelogenin gene (AMEL). Heriogenology. 2008. 70:241-247.
2. Bai WL, Yin RH, Zhao SJ, Li C, Ma ZJ, Yin RL, Luo GB, Zhao ZH. A PCR assay for sex determination of yak (*Bos grunniens*) meat by amplification of the male-specific SRY gene. Food Control. 2010. 21:726-731.
3. Appa Rao KBC, Kesava Rao V, Kowale BN, Totey SM. Sex-specific identification of raw meat from cattle, buffalo, sheep and goat. Meat Science. 1995. 39:123-126.
4. Vos P, Hogers R, Bleeker M, Reijans M, Lee T, Hornes M, Frijters A, Peleman J, Kuiper M, Zabeau M. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 1995. 23:4407-4414.
5. Mueller UG, Wolfenbarger LL. AFLP genotyping and fingerprinting. Tree. 1999. 14:389-394.
6. Felip A, Young WP, Wheeler PA, Thorgaard GH. An AFLP-based approach for the identification of sex-linked markers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 2005. 247:35-43.
7. Valdez R, Nadler CF, Bunch TD. Evolution of wild sheep in Iran. Evolution. 1978. 32:56-72.
8. Marsan PA, Negrini R, Milanesi E, Crepaldi P, Cicogna M, Zagdsuren Y, Ertugrul O, Luikart G, Taberlet P. Geographic structure in goat diversity as revealed by AFLP molecular markers. 7th World Congress on Genetics Applied to Livestock Production, 2002. Montpellier, France.
9. Shi L, Yue W, Ren Y, Lei F, Zhao J. Sex determination in goat by amplification of the HMG box using duplex PCR. Animal Reproduction Science. 2008. 105:398-403.
10. Horng YM, Huang MC. Male-specific DNA sequences in pigs. Theriogenology. 2003. 59:841-848.
11. Khamnamtong B, Thumrungtanakit S, Klinbunga S, Aoki T, Hirono I, Menasveta P. Identification of Sex-specific Expression Markers in the Giant Tiger Shrimp (*Penaeus monodon*). Journal of Biochemistry and Molecular Biology. 2006. 39:37-45.
12. Sriphairoj K, Kamonrat W, Na-Nakorn U. Genetic aspect in broodstock management of the critically endangered Mekong giant catfish, Pangasianodon gigas in Thailand. Aquaculture. 2007. 264:36-46.
13. Griffiths R, Orr K. The use of amplified fragment length polymorphism (AFLP) in the isolation of sex-specific markers. Molecular Ecology. 1999. 8:671-674.
14. Ajmone Marsan P, Valentini A, Cassandro M, Vecchiotti Antaldi G, Bertoni G, Kuiper M. AFLP™ markers for DNA fingerprinting in cattle. Animal Genetics. 1997. 28: 418-426.
15. Baker JA. Genetics, genomics, and molecular biology of sex determination in small animals. Theriogenology. 2006. 66:1655-1658.

1/26/2012

Hybrid Performance of Fuel Cell and Wind Turbine in Islanding Operation Mode

Mohammad H. Moradi[1], Eidy Hadadi[2*]
[1.] Assistant professor within Bu Ali SinaUniversity, Hamedan, Iran
Mh_moradi@yahoo.co.uk
[2.] Master student within Bu Ali SinaUniversity, Hamedan, Iran
power89hadadi@yahoo.com

Abstract: Microgrid connection to network causes various conditions which microgrid can deliver power by fix voltage under any disturbance. But microgrid operation in islanding mode, there's needs to the voltage and frequency controller. In this paper, the microgrid that includes fuel cell and wind turbine is studied in islanding operation mode and to control voltage and frequency, a hybrid controller is introduced. Frequency control is done by inverter. Voltage control is done by designing an AC buse and PI controller. The designed controller causes that wind turbine deliver 1pu and fuel cell that has more power generation cost. Simulation results showed that efficient performance of the controller in its task being robust against load change, set point change and wind speed change.
[Mohammad H. Moradi, Eidy Hadadi. **Hybrid Performance of Fuel Cell and Wind Turbine in Islanding Operation Mode**. *Life Sci J* 2012; 9(3):2053-2060]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 297

Keywords: Microgrid, islanding operation mode, Fuel cell and wind turbine and Power and voltage control

1) Introduction

Environmental concerns and increase of energy consumption has offered new opportunities for public use of renewable energy sources. The transmissions and distribution networks have become weak and old and require much investment to be renovated and expanded. These facts together with the need for high quality power have attracted comments to new methods of electrical energy production [1, 2]. Microgrid is one of the suitable options for electricity supply. Microgrids are LV networks which include Distributed Energy Resources (DER) such as micro turbines, solar cells and power storages such as power batteries controllable loads and a powerful control system. Microgrid advantages can be cited as follows:

1- The need for additional suppliers felt due to the rapid growth of load and fossil fuels reduction.
2- Establish of new power generating sources will reduce environmental pollution and global warming.
3- Distributed Generation (DG) sources make it easy to combined heat and power (CHP) which increases its efficiency by reducing losses.
4- These resources are suitable for consumers with low capacity.
5- DG Resource, can back up and thus improves power quality and network reliability due to both possible performances, Islanding and Grid Connected [3-5].

Control of some variables is necessary to improve the microgrid performance in connection to grid and islanding operation mode. These variables are: 1) voltage 2) frequency 3) power.

Microgrid studied in this paper includes solid oxide fuel cell (SOFC), wind turbine, batteries, power electronics converter, and the load and voltage and power controller. Hybrid operation of fuel cell and wind turbine is discussed in references [1-3]. Connection between microgrid and load or network could be done through an AC bus or DC. When microgrid is connected to grid, voltage and frequency control is done. So, when microgrid is in islanding operation mode, designing of voltage and frequency controller is necessary [8].

Frequency stability in microgrid is very sensitive. So power control is a main problem. When microgrid is in islanding operation mode and peak load, a disturbance, such as new load, leads to disturbance in frequency. It makes two problems: first, a small unbalance in power causes large unbalance in frequency and second, this problem occurs very fast. First problem is solvable by using of power storage. Second problem is solved by using the inverter. So, the best choice is using of both power storage and inverter. In many references, different methods are used to fix voltage and frequency. In [11]-[16], some methods are introduced to hybrid operation of DG sources. In [13], hybrid system is introduced which includes fuel cell and photovoltaic and an electrolyzer to produce hydrogen and a battery as power storage. In [14], hybrid system is introduced which includes fuel cell and electrolyzer and diesel generator. Hybrid system including PV, WT and FC is introduced in [15], which tracks maximum power point (MPPT). But a complex fuzzy logic controller is used in it. In [16], a PI is used to regulate voltage of DC/DC converter. In [17], frequency of system is

controlled by load control; and by using of a reactive power compensator, voltage of system is fixed in islanding operation mode.

In this paper, voltage, power and frequency controller are introduced to have advantage of other controller mentioned above. In this paper, a control method introduced to control power and fix voltage in reference value. This method reduces cost of control and also fixes frequency in 50Hz. This controller by designing AC buse removes the need of converting AC output to DC output. Performance of this controller is verified by: 1) load change 2) active and reactive set point change 3) wind speed change. Simulation results showed efficient performance of the controller in its task to control power and voltage in islanding operation mode. This article has five sections: Section 2 deals with the introduction of studied microgrid network. The results of performed simulations are presented in Section 3. In Section 4, the general discussion is about the preparation process of this paper. In Section 7, finally, conclusions represented.

2) System Description

Microgrids are small-scale, LV CHP supply networks designed to supply electrical and heat loads for a small community, such as a housing estate or a suburban locality, or an academic or public community such as a university or school, a commercial area, an industrial site, a trading estate or a municipal region [2, 3]. The schema of a sample Microgrid represented in Figure 1. In this paper, power generators are fuel cell and wind turbine. Fuel cell has DC output and connects to AC buse through a voltage source inverter (VSI). Voltage regulator, by measuring load voltage and d&q component voltage, generates pulses for inverter and makes an AC buse that is necessary for islanding operation mode. On the other hand, wind turbine has an AC output. In this study, AC voltage is converted to DC by using of a voltage rectifier, and then it is connected to AC buse through a voltage source inverter. Power controller receives measured active and reactive power and generates pulse for inverter. In result, it has control on power generated by wind turbine. Capacitance is used on the inner of both inverters. This capacitance is used to improve power quality and reject disturbance. The inductor has is used to connect inverter to main grid. Each of these Microgrid subsystems described separately below:

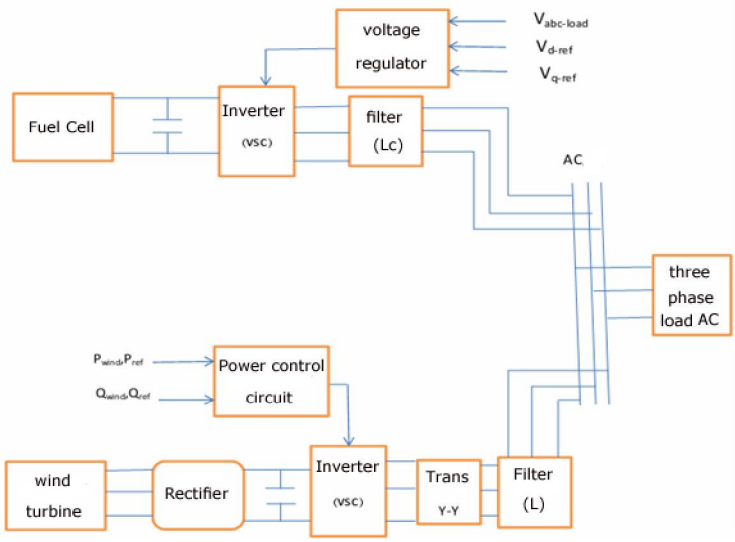


Figure 1.Overview of the described Microgrid

2-1) Fuel Cell

Fuel cell is a technology to generate electricity without producing pollution which has taken the place of conventional methods of energy production. Fuel cell generates electrical energy with an electrochemical reaction directly [1]. Based on reactions that occur at the anode and cathode cell, fuel cell during generate electricity, produces water and thus prevents pollution [1]. According to reference [1], the chemical reactions that occur at the anode and the cathode are as follows:

Anode reaction:

$$H_2 \Rightarrow 2H^+ + 2e^- \quad (1)$$

The cathode reaction:

$$\frac{1}{2}O_2 + 2H^+ + 2e^- \Rightarrow H_2O \quad (2)$$

Figure 2 showing the general Schematic of reaction in the fuel cell.

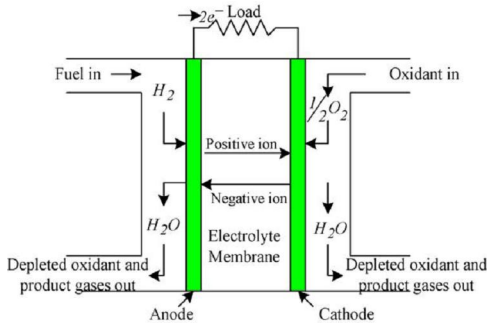


Figure 2)The overall schematic of fuel cell reaction

When the fuel cell is connected to the load and current is drawn from it, Terminal voltage and the efficiency will decrease. Among all reasons, voltage would loss polarization due to internal connections.

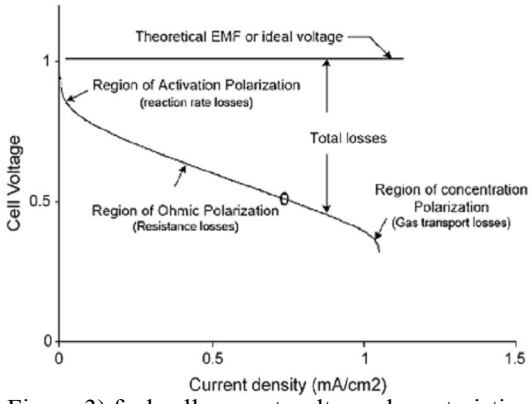


Figure 3) fuel cell current-voltage characteristic

Figure 3: shows the fuel cell voltage as a function of the current [1].

2-2) Wind Turbines

The basic diagram of the wind energy conversion system to be analyzed on this paper is illustrated in Fig. 1. The system is composed by a wind rotor which transforms the kinetic energy from the wind with U wind speed in mechanical torque in the shaft. The shaft drives directly the PMSG, which generates power with variable-frequency and alternate current.

2-2-1) Wind Rotor

The fundamental dynamics of VSWT can be expressed by this simple mathematical model:

$$(1)J\frac{d\omega_m}{dt} = T_a - T_f - T_c$$

Where J is the moment of inertia (rotor inertia plus generator inertia), ω_m is the mechanical angular speed, T_a is the aerodynamic torque; T_f is the friction torque (rotor fiction plus generator friction); and Te is the electrical load torque from wind turbine. Aerodynamic torque T_a is determined by:

$$(2)T_a = \frac{1}{2}C_T(\lambda,\beta)\rho A\frac{D}{2}u_{wind}^2$$

Where C_T is the rotor torque coefficient; ρ is the air density; A is the rotor swept area; D is the rotor diameter; and u_{wind} is the wind speed. Torque coefficient is a non-linear function of the tip-speed ratio λ and blades pitch angle β. This relation can be found by computational simulations or experimentally. Tip-speed ratio is expressed by:

$$(3)\lambda = \frac{\omega_m.D/2}{u_{wind}}$$

For a fixed-pitch blade rotor, C_T is a function of λ only The CT (λ) curve to be used on this work is illustrated on Fig. 3. Friction torque is determined by:

$$(4)T_f = B\omega_m$$

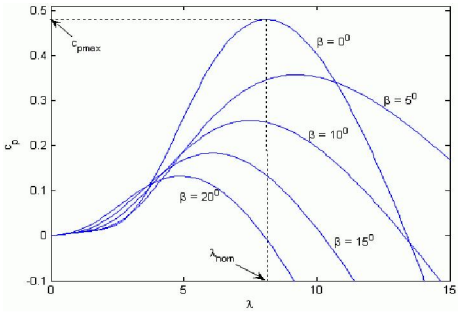


Figure 4) $C_T(\lambda)$ used in this paper

2-2-2) PMSG

Dynamic modeling of PMSG can be described in d-q reference system [7]:

$$(5)v_q = -(R + pL_q)i_q - \omega_e L_d i_d + \omega_e \lambda_m$$

$$(6)v_d = -(R + pL_d)i_d + \omega_e L_q i_q$$

The expression for the electromagnetic torque can be described as:

$$(7)T_e = \left(\frac{3}{2}\right)\left(\frac{P}{2}\right)\left[(L_d - L_q)i_q i_d - \lambda_m i_q\right]$$

Where P is the number of poles, the relation between electrical angular speed ω_e

And mechanical angular speed ω_m is expressed by:

$$(8)\omega_e = \frac{P}{2}\omega_m$$

2-3) Power Control Circuit

Figure 4 clearly describes the power controller used in article. Power Controller input is active and reactive power, and its output is the "d" and "q" components of reference current for hysteresis control. The hysteretic controller output is also inverter gate pulse and ultimately leading to deliver active and reactive power to the network equal to the reference values [10].

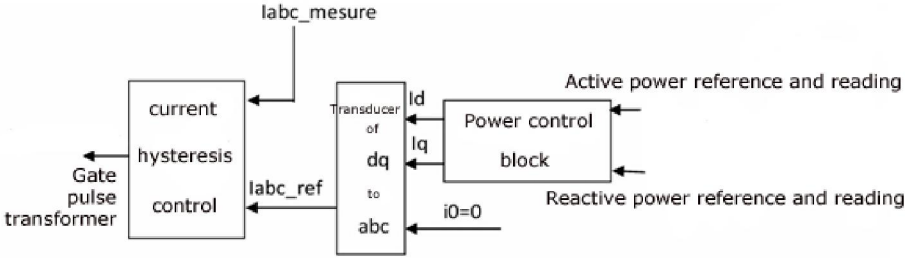


Figure 5) the overall diagram of a Power Control for Wind Turbine

2-3-1) Current Hysteresis Control

The diagram of current hysteresis control that simulated in simulink, illustrate in figure 5.

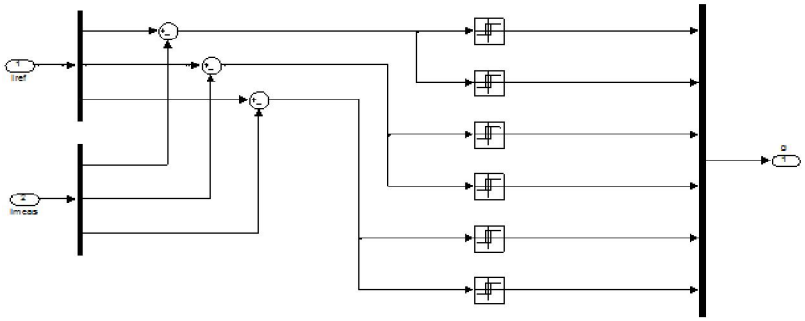


Figure 6) the diagram of current hysteresis control that simulated in simulink

In hysteresis controllers, the feedback from output current is compared with references value and switches state is changed. It led to current error be in allowed domain. The diagram of current hysteresis control for $i_{a,ref}$, $i_{b,ref}$, $i_{c,ref}$, illustrate in figure

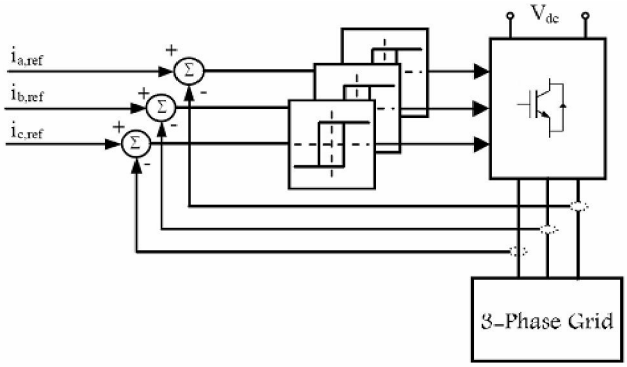


Figure 7) 3phase current hysteresis control

2-4) Voltage Regulation Circuit

In islanding operation mode, the voltage regulator design is necessary for delivery power under fix voltage. The overall diagram of the voltage regulator studied in this paper is illustrated in figure 10. In the Figure 7, it can be seen that voltage regulator receives load voltage and converts it to d&q components and then crosses these value and reference value from PI controller, and so, it fixes load voltage in reverence value

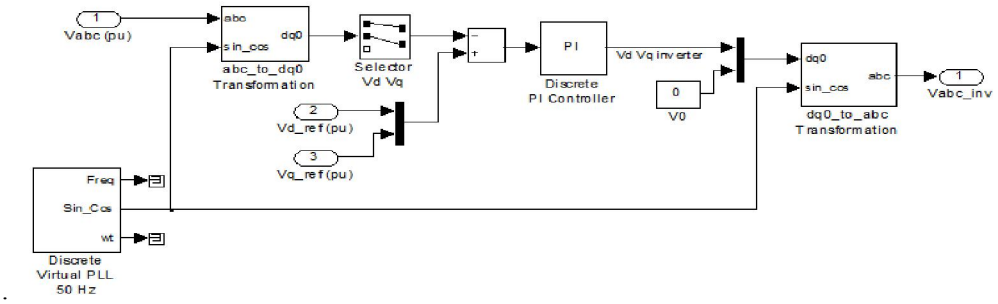


Figure 8) Voltage regulator diagram in simulink

3) Simulation and Results

In this section in order to verify the voltage and power controller performance**,** studied system (Figure 1) was simulated using MATLAB software (Figure 8). For this microgrid, three different operation modes are used:

1) Step change in connected to grid load
2) Step change in active and reactive power value
3) Change in wind speed

For these operation modes, these values measured and verified. These values are: load current and voltage, active and reactive power of fuel cell and wind turbine. In this paper, base power is 25kw.

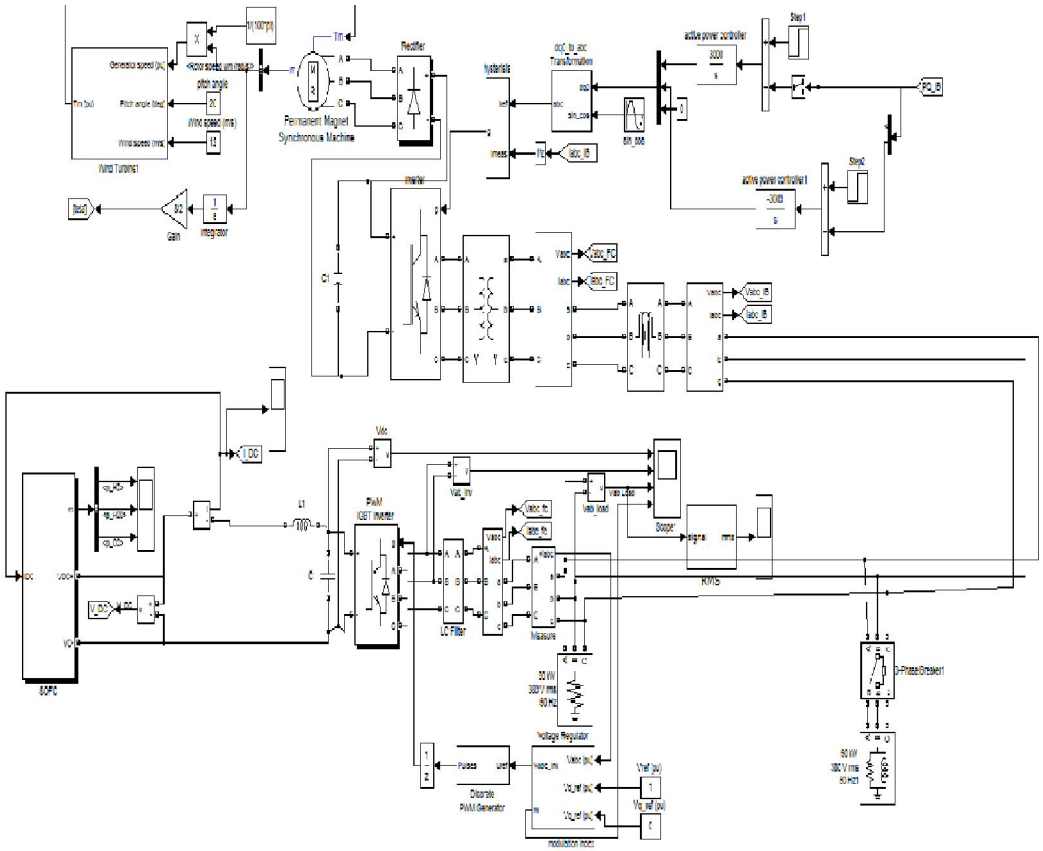


Figure 9) studied microgrid simulated in simulink

3-1) Step change in connected to grid load

The load that is connected to the microgrid studied is a 50kw resistive load whit 50Hz frequency. In this section, in order to verify the voltage and power controller, a RL load, in 1 second, and a RC load, in 1.5 second are added to microgrid (resistive load is 5w, capacitance load is 1kvar and L load is 1 kvar). Simulation results are shown in Figures 9 - 14. These Figures show that all wave forms get its steady state value in 0.3 sec, and this is because of dynamic of fuel cell used in this paper.

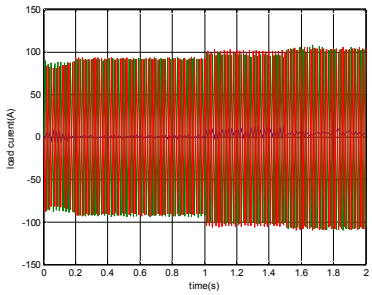


Figure 10) load current under load change

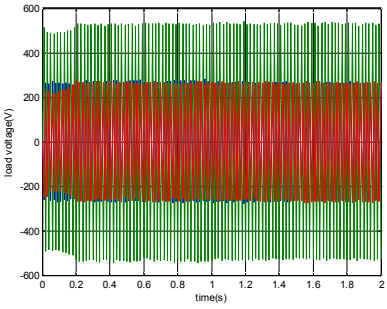


Figure 11) load voltage under load change

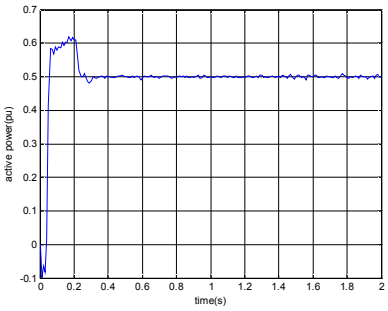


Figure 12) active power generatedby winde turbine under load change

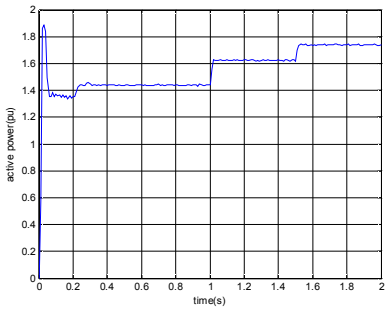


Figure 13) active power generated by fuel cell under load change

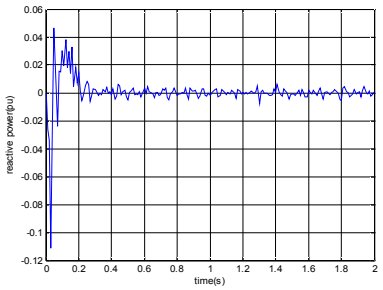


Figure 14) reactive power generated by winde turbine under load change

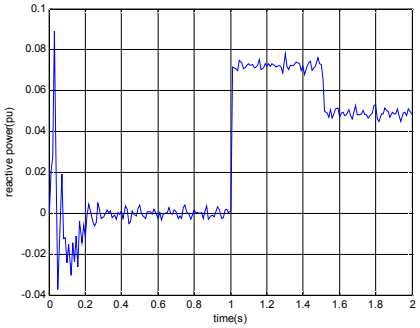


Figure 15) reactive power generated by fuel cell under load change

For this load change, wind turbine active power set in 0.5 pu and its reactive power set in zero. Figures 9 and 10 show that under load change, load current is changed and load voltage is fixed by voltage controller. Figures11-14 shows the active power of fuel cell and wind turbine under this change. It is visible from these Figures that power controller, under this change, can track set point.

3-2) Step change in active and reactive power value

In this section, active and reactive power set points are changed in order to verify the tow controller performance. For active power set point goes from 0.4 pu to 0.7 pu in 1 sec and for reactive power set point goes from 0 to 0.2 pu in 1.5 sec. Results are shown in Figures 15 - 19. Figure 15 shows that the voltage controller is robust against this change in set point, and it, under fix voltage, delivers power. Figures 16 and 17 show that power controller is able to track set point changes.

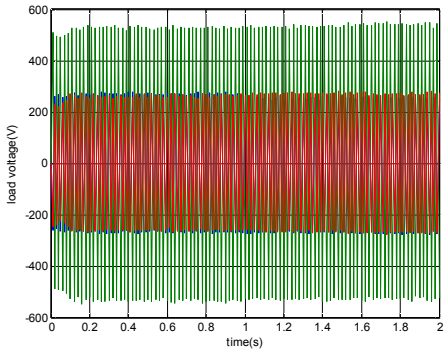


Figure 16) load voltage under change in set point

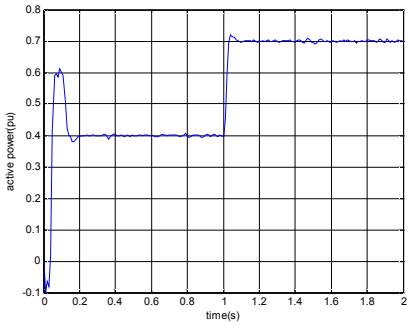


Figure 17) active power generated by wind turbine under change in set point

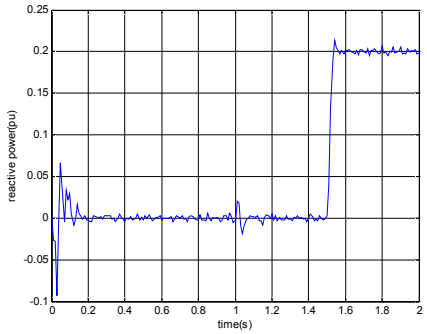


Figure 18) reactive power generated by wind turbine under change in set point

3-3) Change in wind speed

For two before state, wind speed for wind turbine is fixed in 20m/sec. In this section, figure18 proposed as wind speed inner to wind turbine. Results are shown in figures 19-21.

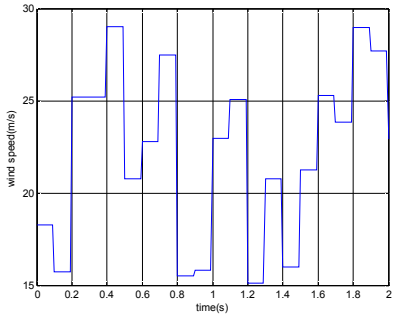


Figure 19) wind speed (m/sec)

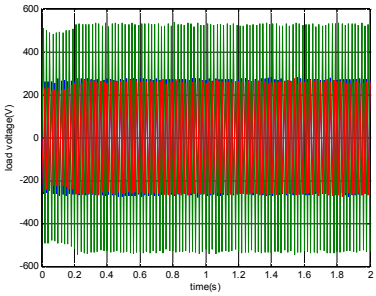


Figure 20) load voltage under changes in wind speed

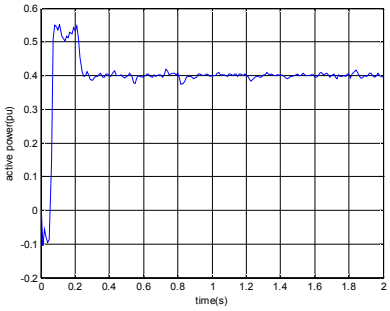


Figure 21) active power generated by wind turbine under changes in wind speed

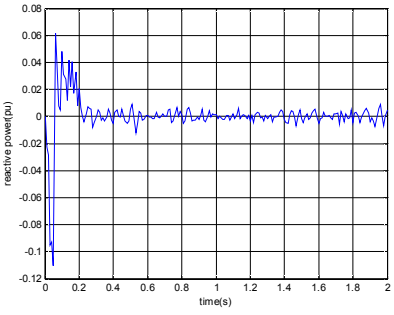


Figure 22) reactive power generated by wind turbine under changes in wind speed

Figure 19 shows that voltage controller delivers power to load by variable wind speed and under fix voltage. Figure 20 and 21 show that power controller, under these conditions, follows the set point.

Discussion

In this paper, a new strategy to operation of mircrogrid including fuel cell and wind turbine is introduced. Simulation results show that:

Load step change: voltage and power introduced in this paper are robust against load step change; and microgrid, under fix voltage, delivers power to load.

Following set point: by step change in amounts of active and reactive power, power controller follows these changes and voltage controller fixes voltage.

Variable wind speed: by variable wind speed for wind turbine, power controller follows set point and load voltage is fixed.

Simplicity is the other advantage of these two controllers, and it is because of using of PI controller in its configurations. Frequency is fixed by inverter. Cost reduction of controller is the other advantage of controller.

4) Conclusion

Islanding mode operation of microgrid needs voltage and frequency controller. In this paper, a new method is introduced to fix load voltage; and frequency is fixed by inverter. Power controller in wind turbine side causes that we are able to control the active and reactive power, which is generated by wind turbine. Simulation results show that these two controllers are resistive against load step change, load change and variable wind speed.

Acknowledgment:

This research is supported by Iran Renewable Energy Organization (Steering Committee of the fuel cell).

References:

[1] Harald Miland, Ronny Glöckner, Phil Taylor , Rolf Jarle Aaberg , Georg Hagen,"Load control of a wind-hydrogen stand-alone power system", International Journal of Hydrogen Energy 31 (2006) 1215 – 1235

[2] M. Y. El-Sharkh, Member, IEEE , A. Rahman, Life Senior Member, IEEE, M. S. Alam , Senior Member, IEEE , A. A. Sakla, Senior Member, IEEE , P. C. Byrne, Member, IEEE , and T. Thomas , Member, IEEE,"Analysis of Active and Reactive Power Control of aStand-Alone PEM Fuel Cell Power Plant", IEEE TRANSACTIONS ON POWER SYSTEMS, VOL. 19, NO. 4, NOVEMBER 2004

[3] M.T. Iqbal," Simulation of a small wind fuel cell hybrid energy system", Renewable Energy 28 (2003) 511–522

[4] Francisco Jurado"Power supply quality improvement with a SOFC plant by neural-network-based control"Journal of Power Sources 117 (2003) 75–83.

[5] A.M. El-Tamaly, P.N. Enjeti, H.H. El-Tamaly "An improved approach to reduce harmonics in the utility interface of wind, photovoltaic and fuel cell power systems" in: Proceedings of the 2000 IEEE Applied Power Electronics Conference and Exposition, vol. 2, 2000, pp. 1059–1065.

[6] Y. Zhu, K. Tomsovic" Development of models for analyzing the loadfollowing performance of microturbines and fuel cell" Electr. Power Syst. Res. 62 (1) (2002) 1–11.

[7] Obara Shin'ya. " Equipment plan of compound interconnection micro-grid composed from diesel power plants and solid polymer membrane-type fuel cell" Int J Hydrogen Energy January 2008;33(1):179–88.

[8] W. Choi, P. Enjeti, J.W. Howze, G. Juong" An experimental evaluation of the effects of ripple current generated by the power conditioning stage on a proton exchange membrane fuel cell stack"J. Mater. Eng. Perform. 13 (June (3)) (2004) 257–264.

[9] P.A. Dahono, et al"Analysis and Minimization of ripple components of input current and voltage of PWM inverters" IEEE Trans. Ind. Electron. 32 (July/August (4)) (1996) 945–950.

[10] K. Masoud and G. Ledwich,"Asperts of grid interfacing: currentand voltage controllers" Proceedingof AUPEC 99, PP258-263

[11] K. Rajashekara, Hybrid fuel-cell strategies for clean power generation, IEEETrans. Ind. Appl. 41 (May/June (3)) (2005) 682–689.

[12] K. Jin, X. Ruan, M. Yang, M. Xu, A hybrid fuel cell power system, IEEE Trans. Ind.Electron. 56 (April (4)) (2009) 1212–1222.

[13] S. Islam, R. Belmans, Grid independent photovoltaic fuel-cell hybrid system:design and control strategy, KIEE Int. Trans. Electric. Machinery Energy Convers.Syst. 5-B (4) (2005) 399–404.

[14] T. Senjyu, T. Nakaji, K. Uezato, T. Funabashi, A hybrid power system using alter-native energy facilities in isolated island, IEEE Trans. Energy Conver. 20 (June(2)) (2005) 406–414.

[15] T.F. El-Shatter, M.N. Eskander, M.T. El-Hagry, Energy flow and managementof a hybrid wind/PV/fuel cell generation system, Energy Convers. Manage. 47(9–10) (2006) 264–1280.

[16] D. Das, R. Esmaili, L. Xu, D. Nichols, An optimal design of a grid connectedhybrid wind/photovoltaic/fuel cell system for distributed energy production,in: Proceedings of the 31st Annual Conference of the IEEE Ind. Electr. Society,IECON'05, Raliegh, NC, USA, November 6–10, 2005, pp. 1223–1228.

[17]Harald Miland, Ronny Glöckner, Phil Taylor, Rolf Jarle Aaberg, Georg Hagen"Load control of a wind-hydrogen stand-alone power system",International Journal of Hydrogen Energy 31 (2006) 1215 – 1235.

08/02/2012

Redefining the Border line of the Neka River's Watershed with Comparing ASTER, SRTM, Digital Topography DEM, and Topographic Map by GIS and Remote Sensing Techniques

Hassan Ahmadi[1*], Dr. Arun Das[2], Mehdi Pourtaheri[3], Dr. Chooghi Bairam Komaki[4], Houshang Khairy[5]

[1.] Assistant Professor, Department of Studies in Geography, Tarbiat Modares University, Tehran, Iran.
[*]Corresponding Author E-mail: ahamdineka@yahoo.com
[2.] Associate Professor, Department of Studies in Geography, University of Mysore, Mysore, India. E-mail: arundas109@hotmail.com
[3.] Assistant Professor, Department of Studies in Geography, Tarbiat Modares University, Tehran, Iran.
[4.] Assistant Professor, Agricultural Sciences & Natural Resources University of Gorgan, Gorgan City, Golestan Province, Iran. E-mail: bkomaki@gmail.com
[5.] Ph.D. Scholar Department of Studies in Earth Science University of Mysore India. E-mail: khairy1700@yahoo.com

Abstract: The accurate and precise calculation of the area for land features has a key role in the estimating the change detection of land uses and the classification of geomorphologic units as well as in the evaluating of land use. In particular, the delineation of borders between watersheds is a base in hydrologic analysis. Recent advances in spatial tools of GIS environment and the availability of various remotely-sensed data make the reliable determining of topographical boundaries possible. So an integrated approach of data analysis and modeling can accomplish the task of delineation. The main aim in this research is to evaluate the delineation method of watershed boundary by using four different digital elevation models (DEM) including ASTER, SRTM, Digital Topography, and Topographic maps. In order to determine a true reference of boundary of watershed, sample data were also obtained by field survey and using GPS. The comparison reference points and the results of these data showed the average distance difference between reference boundary and the result of ASTER data was 43 meters. However the average distance between GPS reference and the other data was high; the difference between the reference data and SRTM was 307m, and for Digital Topographic map, it was 269m. The average distance between Topographic map and the GPS points differed 304 meters as well. For the statistical analysis of comparison, the coordinates of 230 points were determined; the paired comparisons were also performed to measure the coefficient of determination, R^2, as well as the analysis of variance (ANOVA) in SPSS. As a result, the R^2 values for the ASTER data with the Digital Topography and Topographic map were 0.0157 and 0.171, respectively. The results showed that there were statistically significant differences in distances among the four means of the selected models. Therefore, considering other three methods, the ASTER DEM is the most suitable applicable data to delineate the borders of watersheds, especially in rugged terrains. In addition, the calculated flow directions of stream based on ASTER are close to natural tributaries as well as real positions of streams.

[Hassan Ahmadi, Dr. Arun Das, Mehdi Pourtaheri, Dr. Chooghi Bairam Komaki, Houshang Khairy. **Redefining the Border line of the Neka River's Watershed with Comparing ASTER, SRTM, Digital Topography DEM, and Topographic Map by GIS and Remote Sensing Techniques.** *Life Sci J* 2012; 9(3):2061-]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 298

Keywords: Watershed Boundary, GPS, GIS, Remote Sensing, DEM.

1. Introduction

The sustainable land-use system in the mountainous regions has vital importance in government and community levels. So that, in the developing countries, the hydrologic study of mountain watersheds is considered seriously. In fact, the accurate and authentic knowledge about the natural phenomena of watersheds is necessary to construct a real model of the process, especially in simulation models and evaluation systems (Corresponding & De Jong, 2005).

In hydrology studies, the demarcation and delineation of boundaries between watersheds is a challenge to estimate the planimetric area of watershed. However, only few researches about the delineation of watershed have been done, particularly at small scales, such as the report of the American Society of Agriculture and Biological Research (Pryde, 2007) in Illamanga subwatershed in North America.

2. Previous Research

2.1 Digital Elevation Data

Digital elevation data are available from various sources including the spaceborne earth observation sensors, eventually through Google Earth images, which have reduced the complexity of the authenticity of the elevation data. Additionally, high-

resolution digital elevation data are provided by the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER), which have the capability of taking along-track stereo images. Despite this fact, in some places such as less developed regions or in rugged terrains with steep slopes, the accurate data of digital elevation are not accessible (Kaab, 2002, 2005; Wang & Qiu, 2006). Digital elevation model (DEM), or digital terrain model (DTM) in remote sensing, is a continuous elevation surface as a grid (Podobnikar, 2009). Since the generation of DTM in the 20th century, many different techniques have been developed (Gesch, et al., 2002; Hirano, Welch, & Lang, 2003; MAUNE, 2007; Miller & Laflamme, 1958; Muskett, et al., 2009). The recent developments in remote sensing have also made better topographic observations; accordingly, topographic measurements have been reliable (Homer, et al., 2007) . At small scales, spaceborne systems (with coarse Ground Sampling Distance - GSD) such as shuttle radar topographic mission (SRTM) collected 80% of altitude of the earth's landmass with the spatial resolution of 30m or 90m (Rabus, Eineder, Roth, & Bamler, 2003). At medium scales, radar interferometric techniques (medium to high resolution) had been applied to generate global DTMs (Heipke, et al., 2007; Madsen, Zebker, & Martin, 1993). For large scales and more local usage, airborne laser scanning like Light Detection and Ranging (Linder) and aerial photogrammetric techniques have been applied to create DTMs with high spatial resolutions(Hudak, Lefsky, Cohen, & Berterretche, 2002; Li, Andersen, & McGaughey, 2008; Næsset, 2002)

DTM has versatile usages from forestry to water resources including watershed management, flood hazard mapping, and even to timber harvest or fire management in forest. The elevation of terrain is a basic input for environmental, forestry, topographic and hydrologic models; therefore, the accuracy of elevation models is critical to modeling environment (Andersen, Mcgaughey, & Reutebuch, 2008; Kellndorfer, et al., 2004). Hence, there are many standards for topographic mapping such as the National Standard for Spatial Data Accuracy (Zandbergen, 2008) and the National Digital Elevation Program (Gesch, et al., 2002).

2.2 The Accuracy Assessment of SRTM DTM

The measured signals of the Shuttle Radar Topography Mission (SRTM) are the reflected radar of elevation, and mainly their attributes are related to the structure of terrain as well as the electromagnetic behaviors of scattering environment t(Bhang, Schwartz, & Braun, 2007). The land cover of the terrain has a major influence over the signals. In particular, the existence of vegetation increases the complexity in scattering medium, as the wavelengths of C-band could not reach the earth under vegetation cover (Braun & Fotopoulos, 2007; Carabajal & Harding, 2005). Considering performance evaluations, the SRTM project team have endeavored to decrease the absolute vertical error, approximately 5m (Brown Jr, Sarabandi, & Pierce, 2005; Rosen, et al., 2001). An analysis by using GPS and NED data to evaluate the accuracy of the SRTM data in southeastern Michigan showed that the absolute and relative height errors are less than GPS ground control point targets (Braun & Fotopoulos, 2007). Further study indicates the accuracy of SRTM DGPS data is acceptable even for barren land surface. And the DTM data derived from IFSAR are dependent on terrain. According to SRTM project team, the absolute horizontal circular accuracy of SRTM is less than 20m, while the absolute vertical accuracy and the relative vertical accuracy are less than 16m and 10m, respectively (Kellndorfer, et al., 2004).

2.3 The Accuracy Assessment of ASTER DTM

Before the launch of ASTER, the team project applied four study fields to evaluate the accuracy of elevation (Hirano, et al., 2003). Even, using the stereo pair of ASTER images on personal computers, DEMs are calculated with 30 to 150-meter spatial resolutions; and the quality of these data are also satisfactory with a RMSE (a root-mean-square error) of ±7 and ±15m. These results are also confirmed by the United States Geological Survey (USGS) at EROS Data Center (EDC) with a RMSE of ± 8.6m. The US and Japan ASTER Project group studied the extraction of elevation validity by means of correlation techniques and estimated the accuracy of new ASTER Global DEM. This result was little greater than the 20-meter accuracy at a 95% confidence level prior to GDEM production. In comparison to the angle of horizontal measurement the vertical accuracy from NED data was 2-to-3 meters as the RMSE. As per the study, when more than 13,000 GPS points were chosen the RMSE dropped to 9.35 meters. The vertical error was below the estimated ASTER GDEM vertical error of 20 meters at a 95% confidence level. However the major shortcoming of the ASTER GDEM version 1 is, it cannot be applied for inland water bodies, because the elevation of inland lakes are not accurate and most the lakes existence are not indicated in ASTER GDEM. The vertical accuracy of this ASTER DEM was checked against 40 DGPS survey points and 12 points digitized from the USGS 1:24,000-scale topographic quadrangles, yielding an RMSE of ±8.6 m. This generally corresponds with other validation results reported by EDC (DAAC, 2001; Hirano, et al.,

2003) The recent exploitable advantages of the automatic generation of digital elevation models are useful in the modeling of watershed, especially in the delineation of basins as well as the deriving of their boundaries. The Advanced Space borne Thermal Emission and Reflection Radiometer (ASTER), onboard Terra spacecraft of NASA's Earth Observing System (EOS), has 14 bands in the spectrums of the visible and near-infrared (VNIR), short wave infrared (SWIR), and thermal infrared (TIR). They provide images in high-quality spatial resolutions including 15-meter resolution in VNIR bands, 30-meter resolution in SWIR bands, and 90-meter resolution in SWIR bands. The ASTER Digital Elevation Model (DEM) is obtained through bands such as 3N (Nadir Viewing) and 3B (backward viewing) captured from Visible near Infrared (VNIR) Sensor. In VNIR two telescopes have been assemble to generate stereoscopic data. The Band – 3 stereo pair have a spectral range of 0.78 and 0.86 microns with a base – to – height ratio of 0.6 and an angle of intersection at 27.7^0. There is a time lag of approximately one minute between the acquisition of the nadir and backward images(Grohmann, Smith, & Riccomini, 2010; Mo, Liu, Lin, & Guo, 2009).

2.4 Accuracy Assessment of Google Image

Google Earth data are significantly productive in the study of land-cover and land-use change, and its potential is not well harnessed (Sheppard & Cizek, 2009). Google Earth provides high spatial resolution of 2.5 meters for 20 % of the earth's surface. They are exploitable to extract land features as well as to study the effects of human activities in environment.

To characterize the horizontal positional accuracy of the high-resolution Google Earth archive, the locations of 436 control points in the GE imagery to their equivalent positions in the Landsat dataset was used, so that, it has the positional accuracy of 50 meters as the root mean squared error (RMSE). In an ideal assessment of spatial accuracy, it would determine the position of these Sensors 2008, 976 control points through a global ground-based campaign using global positioning satellites (GPS).done for below cities such as Sao Paolo, Brazil, San Salvador, El Salvador, Chonan, South Korea, and Anqing, China(Potere, 2008)

3. The characteristics of Study Area

Neka watershed is located in the northern part of Iran. The Neka River basin is one of the largest watersheds in the Iranian province of Mazandaran. The Neka River is flowing down the northern flank of the Alborz Mountains towards the Caspian Sea, and it goes through Neka City and divides this city into two parts - eastern and western parts. The Neka watershed is covered with Quaternary formations. Climate is temperate with mild winter and hot summer. The study area is used as rain-fed agriculture and rangeland of cattle-grazing. The geographical location of the Southern Neka basin is shown in Figure 1.

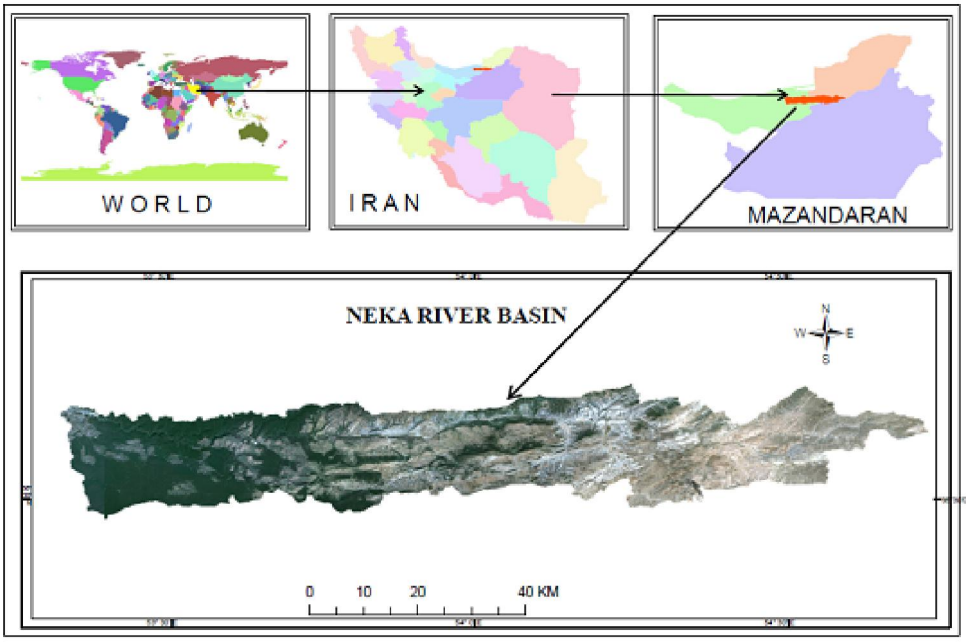


Figure 1: The location of the Neka River basin

4. Materials and methods

4.1. Database

The accurate delineation of watershed's boundary and the comparisons of delineation methods need various data sources, available through scientific database such as ASTER and STRM projects. Table 1 shows all the data applied in this project.

Table 1: The list of database

Data	Date	Spatial Resolution/Scale	Source
SRTM (DEM)	2002	90 m	USGS
ASTER (DEM)	2009	29 m	ASTER G DEM
Digital Topographical Map	2004	1:25,000 scale	Iran Geographical Organization
Topographical Map	1965	1:50,000 scale	Iranian Geographical Organization
PAN IRS	2004	5.5 m	Indian Remote Sensing
Google Image	2010	1.5 m	Google Earth (SPOT)

The topographical map at a 1:50,000 scales, which were published by the Iran Geographical Organization in 1965, were scanned and geo-referenced in GIS environment. For this study area, 11 topographical maps were used to draw the manual border line of the watershed of Neka.

The ASTER DEM data for the year 2009 were downloaded from GDEM-ASTER website and re-sampled to the spatial resolution of 29m. In addition, SRTM data (DEM) of USGS with 90-meter resolution (2002) were also downloaded. The digital topographical map, published by Iranian geographical organization in 2004 at scale 1:25,000 (Ahmadi & Nusrath, 2010), was applied to obtain the boundary of the considered watershed in the €study area.

The product of Google images were downloaded through Google Web Service (2009).

The IRS panchromatic data on the first of June, 2004 were geo-referenced with 50 GPS points, and the RMSE (the Root Mean Square Error) was less than 0.5 pixels, image Geo referenced to the UTM projection based on the WGS84 datum. The re-sampling was done at 5.5m.

4.2. Methodology

In order to delineate the boundary of watershed, four kinds of data were utilized such as ASTER, digital topography DEM, SRTM DEM, and Topographic map (manual). The topographic map from the Iranian Geographical Organization was used to digitize the boundary of the Neka river watershed (Figure 2). The total area of this watershed was about 1,887.62 square kilometers.

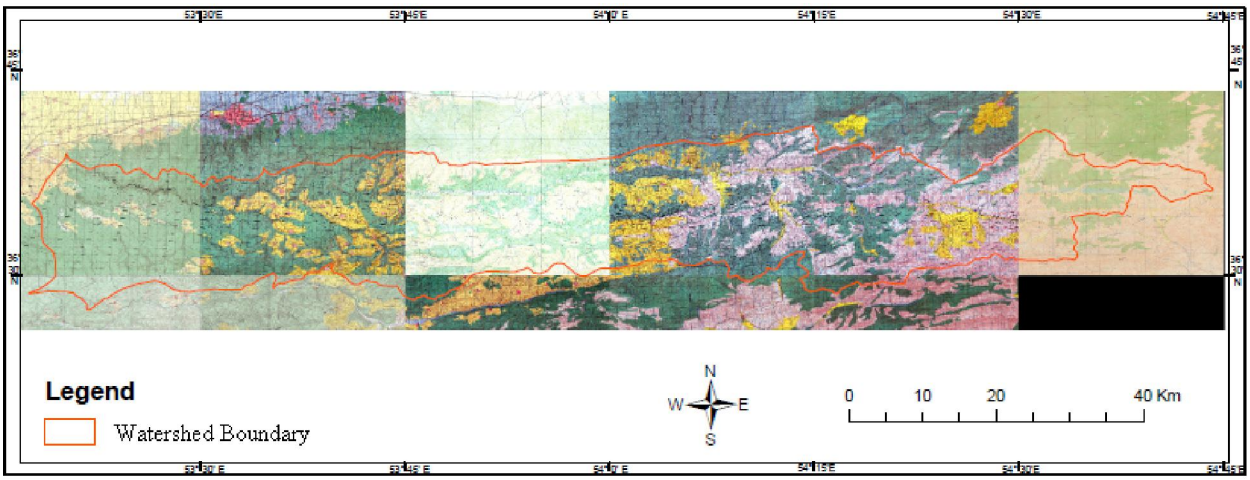


Figure 2: The catalog of watershed border line (red-colored line) based on the topographic map

Digital topography DEM, Aster DEM and SRTM DEM were analyzed using spatial hydrology tools of ArcGIS. The available techniques in GIS environment include filling sinks; determining flow direction, finding flow accumulation, and identifying the watershed outlet are applied. The final output of

this process is generally in a raster format, so it is converted to a polygon map.

5. Results

The four obtained boundaries of the considered watershed were compared statistically. Regression analyses were applied to compare each of the DEM-based watershed boundaries with the 230 GPS points, which had 120-meter intervals. For the regression analyses, a Cartesian coordinate system was used to determine the similarity of the pair values of the boundaries. Additionally, the one way analysis of variance (ANOVA) was conducted to determine the discrepancy between the GPS point and the watershed boundary line.

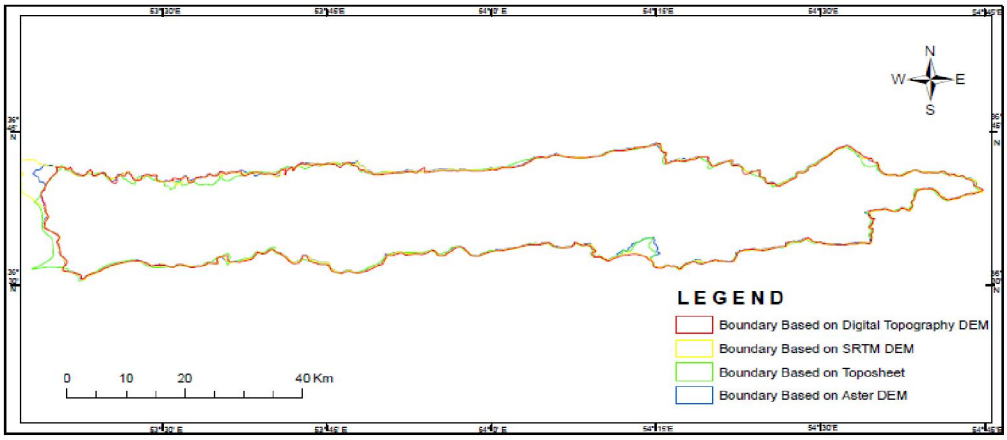


Figure 3: The comparison of the Neka River watershed resulted from ASTER, SRTM, Digital Topographical DEM and Topographic map

Figure 3 shows the dissimilarity between the ground GPS points and the delineated ASTER, SRTM, Topographic map (manual) and Digital topographical map boundaries. The area of the watershed delineated from a topographic map (manual) is 1,887.62 sq. km, while the amounts of area from ASTER, the digital topography DEM, SRTM, and are 1,901, 1,906.72, and 1,934.31 sq. km, respectively. As a result, there are differences among the designed boundaries. For land evaluation research, instead of checking the error in the total area of the watershed, the exact water- dividing points are required. Eminently, finding error in the area was discarded and instead the distance error between the GPS point and other boundary line has been calculated.

Table.2: Descriptive statistics of the difference in distance between limits

	SRTM	Aster	Digital	Topograp
Mean	304.2	43.41	269.87	307.33
Standard Error	42.08	13.22	32.47	32.12
Median	111.6	23.44	129.33	171.75
Mode	68.93	8.60	129.36	73.23
Standard	639.5	200.8	493.52	488.13
Sample	409,0	40,35	243,562.81	238,267.1
Kurtosis	10.37	217.2	14.98	15.73
Skewness	3.33	14.54	3.82	3.78
Range	3155.	3037.	3190.11	3403.47
Minimum	1.54	0.01	1.77	1.35
Maximum	3,156.	3,037	3,191.88	3,404.82
Sum	70,29	10,02	62,339.95	70,992.57
Count	231.0	231.0	231.00	231.00
Confidence	82.91	26.04	63.98	63.28

The ArcGIS tool, that measures the straight-line distance from each GPS point cell to the closest boundary line source, were used to get the statistical descriptions of the differences in the distance and to compare between four DEM-based boundaries to find out which boundary is closer to the exact ground data, as shown in Table 2. The calculation of error was made between ground GPS points and the boundary line derived from ASTER DEM, Top sheet hand boundary, Digital Topography DEM and SRTM DEM using analysis tools in ArcGIS. As per the analysis the ASTER DEM boundary line has a mean variation of 43 meters distance from the GPS point which is less than the other three boundary lines while this for SRTM is 304m, for Topographic map is 307m and for Digital topography DEM is 269m.

5.1. Visual cross examination of four boundary line with Google Image and PAN IRS

Visually the four boundary lines, overlaid on Google image, are overlapping on each other on steep slopes, but on gentle slopes the boundary lines are deviating. Among the four boundary lines, the Aster DEM boundary line is exactly cutting across the water divided line, as shown in Figure 4. Similarly, when the boundary lines were overlaid on PAN IRS, it is confirmed that the boundary lines merge on steep slopes, but on gentle slopes, they deviate each other, as shown in Figures 5. In this case, Aster DEM boundary line is cutting exactly on the water divided point, either on steep slope or gentle slope.

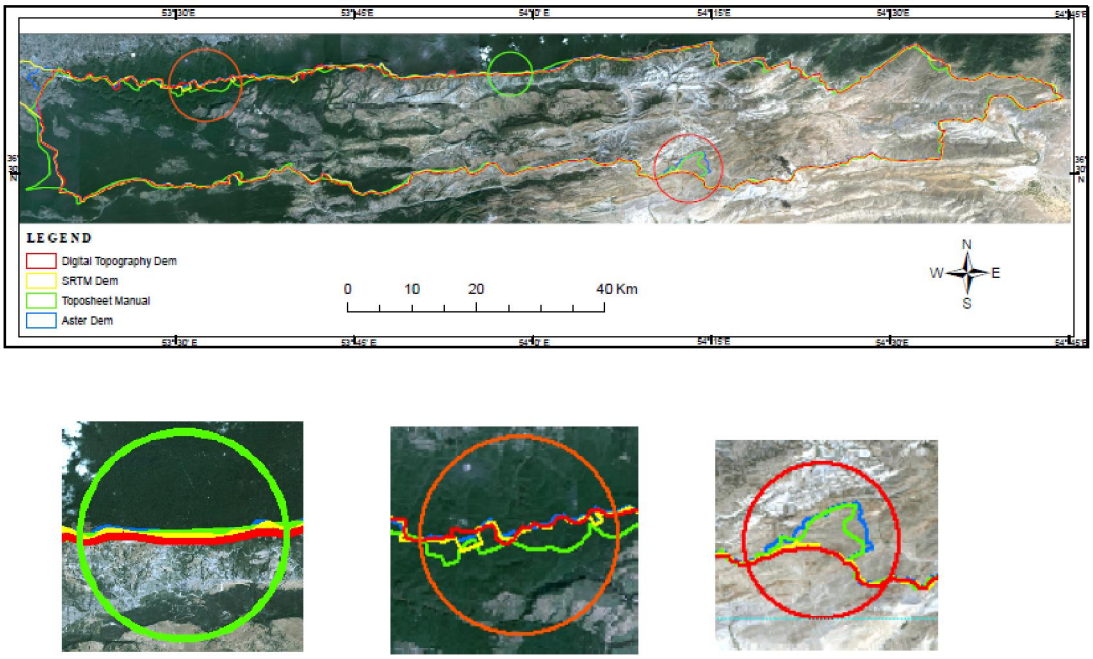


Figure 4: Visual Cross-section of four boundary line with Google Image

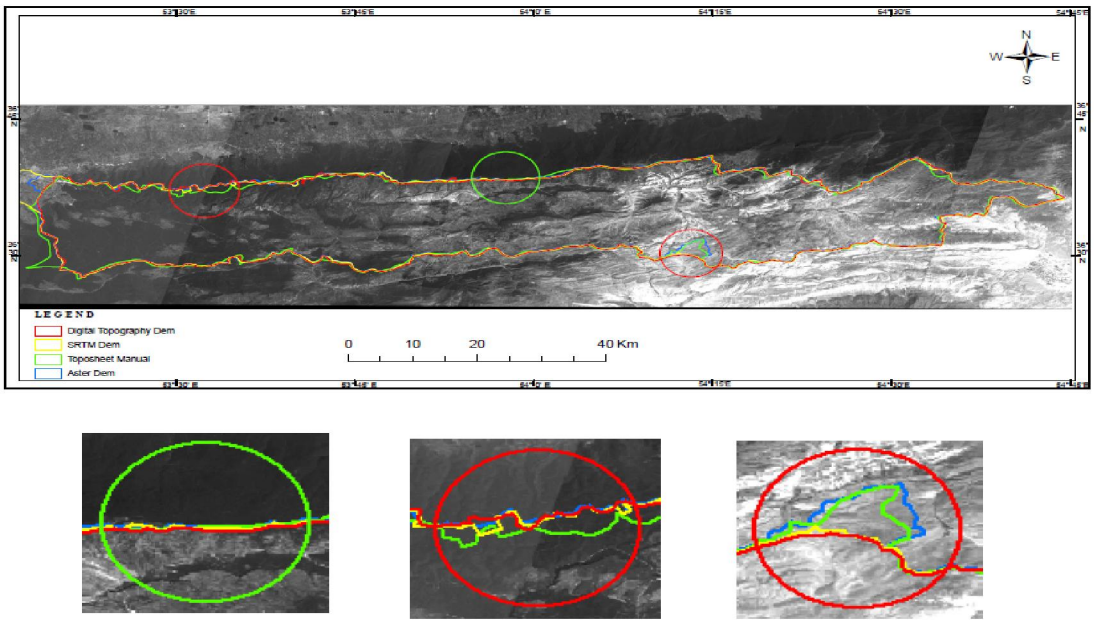


Figure 5: Visual Cross-section of four boundary lines with PAN IRS

6. Conclusions

The methodology of this project describes the evaluation of delineation techniques of a watershed based on digital elevation models. As a result, the obtained accuracy for the delineation of watershed is dependent upon the quality of digital elevation models. The ASTER DEM is a suitable demarcation model to delineate the boundary of watershed in the places with rugged and steep slopes.

According to the observation on Google map, the demarcated boundary of watershed based on ASTER data has showed less errors comparing with the other three boundary lines. Additionally, the comparison with IRS PAN Data has also proved the same result.

ASTER products are reasonable having low price and good resolution. Comparing to this, as the SRTM data have higher spatial resolution, the vertical accuracy of SRTM data is higher than others. Their disadvantages also are the vegetation influence; in other words, radar could not reach the true terrain's surfaces. As a result, the delineated boundary of watershed based on ASTER digital elevation models has a complementary role over the other demarcation models.

The overall methodology adapted in this research has evaluated the delineation of the watershed boundary comparing with each other and proved that, ASTER is the best source of data for the delineation.

Based on the above testing and comparisons, it also imply the fact that the future researcher can straight away use the ASTER data for any hydrology and land use system and land evaluation studies.

References

Ahmadi, H., & Nusrath, A. (2010). Vegetation Change Detection of Neka River in Iran by Using Remote-sensing and GIS. *Journal of Geography and Geology, 2* (1), P58.

Andersen, H. E., Mcgaughey, R., & Reutebuch, S. (2008). Assessing the influence of flight parameters, interferometric processing, slope and canopy density on the accuracy of X‐band IFSAR‐derived forest canopy height models. *International Journal of Remote Sensing, 29* (5), 1495-1510.

Bhang, K. J., Schwartz, F. W., & Braun, A. (2007). Verification of the vertical error in C-band SRTM DEM using ICESat and Landsat-7, Otter Tail County, MN. *Geoscience and Remote Sensing, IEEE Transactions on, 45* (1), 36-44.

Braun, A., & Fotopoulos, G. (2007). Assessment of SRTM, ICESat, and survey control monument elevations in Canada. *Photogrammetric Engineering and Remote Sensing, 73* (12), 1333.

Brown Jr, C. G., Sarabandi, K., & Pierce, L. E. (2005). Validation of the shuttle radar topography mission height data. *Geoscience and Remote Sensing, IEEE Transactions on, 43* (8), 1707-1715.

Carabajal, C. C., & Harding, D. J. (2005). ICESat validation of SRTM C-band digital elevation models. *Geophysical Research Letters, 32* (22), L22S01.

Corresponding, D. K., & De Jong, K. (2005). Dynamic environmental modelling in GIS: 2. Modelling error propagation. *International Journal of Geographical Information Science, 19* (6), 623-637.

DAAC, E. (2001). ASTER DEM data product. *URL:< http://edcdaac. usgs. gov/aster/ast14dem. html*.

Gesch, D., Oimoen, M., Greenlee, S., Nelson, C., Steuck, M., & Tyler, D. (2002). The national elevation dataset. *PE & RS-Photogrammetric Engineering & Remote Sensing, 68* (1), 5-11.

Grohmann, C. H., Smith, M. J., & Riccomini, C. (2010). Multiscale analysis of topographic surface roughness in the Midland Valley, Scotland. *Geoscience and Remote Sensing, IEEE Transactions on* (99), 1-14.

Heipke, C., Oberst, J., Albertz, J., Attwenger, M., Dorninger, P., Dorrer, E., Ewe, M., Gehrke, S., Gwinner, K., & Hirschmüller, H. (2007). Evaluating planetary digital terrain models--The HRSC DTM test. *Planetary and Space Science, 55* (14), 2173-2191.

Hirano, A., Welch, R., & Lang, H. (2003). Mapping from ASTER stereo image data: DEM validation and accuracy assessment. *ISPRS Journal of Photogrammetry and Remote Sensing, 57* (5-6), 356-370.

Homer, C., Dewitz, J., Fry, J., Coan, M., Hossain, N., Larson, C., Herold, N., McKerrow, A., VanDriel, J. N., & Wickham, J. (2007). Completion of the 2001 National Land Cover Database for the Counterminous United States. *Photogrammetric Engineering and Remote Sensing, 73* (4), 337.

Hudak, A. T., Lefsky, M. A., Cohen, W. B., & Berterretche, M. (2002). Integration of lidar and Landsat ETM+ data for estimating and mapping forest canopy height. *Remote Sensing of Environment, 82* (2-3), 397-416.

Kaab, A. (2002). Monitoring high-mountain terrain deformation from repeated air-and spaceborne optical data: examples using

digital aerial imagery and ASTER data. *ISPRS Journal of Photogrammetry and Remote Sensing, 57* (1-2), 39-52.

Kaab, A. (2005). Combination of SRTM3 and repeat ASTER data for deriving alpine glacier flow velocities in the Bhutan Himalaya. *Remote Sensing of Environment, 94* (4), 463-474.

Kellndorfer, J., Walker, W., Pierce, L., Dobson, C., Fites, J. A., Hunsaker, C., Vona, J., & Clutter, M. (2004). Vegetation height estimation from shuttle radar topography mission and national elevation datasets. *Remote Sensing of Environment, 93* (3), 339-358.

Li, Y., Andersen, H. E., & McGaughey, R. (2008). A comparison of statistical methods for estimating forest biomass from light detection and ranging data. *Western Journal of Applied Forestry, 23* (4), 223-231.

Madsen, S. N., Zebker, H. A., & Martin, J. (1993). Topographic mapping using radar interferometry: Processing techniques. *Geoscience and Remote Sensing, IEEE Transactions on, 31* (1), 246-256.

MAUNE, D. F. (2007). Digital elevation model technologies & applications: the DEM users manual (with DVD-ROM).

Miller, C. L., & Laflamme, R. A. (1958). *The Digital Terrain Model-: Theory & Application*. MIT Photogrammetry Laboratory.

Mo, X., Liu, S., Lin, Z., & Guo, R. (2009). Regional crop yield, water consumption and water use efficiency and their responses to climate change in the North China Plain. *Agriculture, Ecosystems & Environment, 134* (1), 67-78.

Muskett, R. R., Lingle, C. S., Sauber, J. M., Post, A. S., Tangborn, W. V., Rabus, B. T., & Echelmeyer, K. A. (2009). Airborne and spaceborne DEM-and laser altimetry-derived surface elevation and volume changes of the Bering Glacier system, Alaska, USA, and Yukon, Canada, 19722006. *Journal of Glaciology, 55* (190), 316-326.

Næsset, E. (2002). Predicting forest stand characteristics with airborne scanning laser using a practical two-stage procedure and field data. *Remote Sensing of Environment, 80* (1), 88-99.

Podobnikar, T. (2009). Methods for visual quality assessment of a digital terrain model. *SAPI EN. S. Surveys and Perspectives Integrating Environment and Society* (2.2).

Potere, D. (2008). Horizontal positional accuracy of Google Earth's high-resolution imagery Archive. *Sensors, 8* (12), 7973-7981.

Pryde, J. K. (2007). Comparison of watershed boundaries derived from SRTM and ASTER digital elevation datasets and from a digitized topographic map. *Systems Engineering, 300* (07).

Rabus, B., Eineder, M., Roth, A., & Bamler, R. (2003). The shuttle radar topography mission--a new class of digital elevation models acquired by spaceborne radar. *ISPRS Journal of Photogrammetry and Remote Sensing, 57* (4), 241-262.

Rosen, P. A., Hensley, S., Gurrola, E., Rogez, F., Chan, S., Martin, J., & Rodriguez, E. (2001). SRTM C-band topographic data: quality assessments and calibration activities. In (Vol. 2, pp. 739-741 vol. 732). IEEE.

Sheppard, S. R. J., & Cizek, P. (2009). The ethics of Google Earth: Crossing thresholds from spatial data to landscape visualisation. *Journal of environmental management, 90* (6), 2102-2117.

Wang, B., & Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza, 16* (5), 299-363.

Zandbergen, P. A. (2008). Positional Accuracy of Spatial Data: Non‐Normal Distributions and a Critique of the National Standard for Spatial Data Accuracy. *Transactions in GIS, 12* (1), 103-130.

8/2/2012

Student's psychological factors and science performance: does gender matter for Iranian Students

Maryam Sahranavard[1], *Siti Aishah Hassan[2], Habibah Elias[1], Maria Chong Abdullah[1]

[1.] Department of Foundations of Education, Faculty of Educational Studies, University Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia
[2.] Department of Counselor Education & Counseling Psychology, Faculty of Educational Studies, University Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia
Sahra1102004@yahoo.com
* siti_aishahh@putra.upm.edu.my

Abstract: This study examined whether, self-efficacy, science self-efficacy, general self-concept, science self-concept, self-esteem, anxiety, and science anxiety can be considered as predictors for science performance. Also, this study explored the moderating effects of gender on the link between student's psychological factors with science performance among guidance school students. The participants in the study were 680 guidance school students, (317 male and 363 female, in the age 14 years old) at Tehran and Shahriar City, the province of Tehran, Iran. Five valid and reliable instruments were used to assess Self-concept Attribute Attitude Scale, State-Trait Anxiety Inventory, Coopersmith Self-Esteem Inventory, General Self-Efficacy, and Science Self-Efficacy Questionnaire and students' science performance which measured by the report school test. Descriptive statistics, multiple and hierarchical regression analysis were used to analyses the data. The result demonstrated that science performance be influenced by general self-concept and science self-concept. In addition, the moderating effect of gender on the relationship of general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety, and science anxiety with science performance was not established.
[Sahranavard. M, Hassan. S.A., Elias. H. Abdullah, M.C. **University Putra Malaysia (UPM), 43400, UPM Serdang, Selangor Darul Ehsan, Malaysia**. *Life Sci J* 2012;9(3):2069-2075] (ISSN:1097-8135).
http://www.lifesciencesite.com.299

Keywords: self-concept, self-efficacy, self-esteem, anxiety, science performance, gender

1. Introduction

Educational psychologists and theorists have long believed that students who believe in their abilities tend to perform successfully (Bandura, 1993). Literature on *self-efficacy*, which is define as the capabilities to accomplish a given task, seems to be a major predictor of academic achievement among the Western society but may not necessarily so for different cultural background (Shen, 2002). Of related positive psychological factors, self-concept and self-esteem also vary from culture to culture. Self-concept refers to the global understanding a sentient being has of him or herself. It is also more general than self-esteem, which is the purely evaluative element of the self-concept (Fleming & Courtney, 1984). In addition, negative psychological factor such as anxiety may also influence the student performance (Milford, 2011). To date, very limited numbers of research investigate these psychological factors among Iranian students. Therefore, this study aimed to fill the gap and provide empirical data from Iranian perspective on the influence of psychological factors and science performance. Gender is another factor that may have effect the relationship between students' psychological factor and science performance. Previous studies on the relationship of gender and science achievement show that boys in general tend to perform better than girls(Comber & Keeves, 1973). More recent study indicate that find no interaction between grade level and gender (Senler & Sungur, 2009). Therefore, this study also aims to investigate the role of gender in moderating the relationship between students' psychological factor and science performance among 8th grade Iranian students.

Science self-efficacy, science self-concept, science anxiety and gender

Science self-efficacy is the belief in one's own capability to do science, in terms of organizing and executing the skills and knowledge needed to manage science content and processes (Miller, 2006). Science self-concept is a term used to describe one's perception of self in relation to achievement in science (Byrne & Shavelson, 1987) and one's confidence in science (Campbell, 1992). Science anxiety in students is a debilitating fear of learning science—but with the emotion processed on a cognitive level, and lastly, science anxiety manifests itself primarily during examinations, but is distinct from an apprehension towards examinations in general, since students who exhibit science anxiety often react normally in their non-science subjects (Mallow, 1994).

Therefore, Naderi, et al., (2009) indicate that there is no relationship between self-esteem and

academic achievement (Sig=.074, P>0.05). and also, Meanwhile, Milford (2011) at the country level, the relationship was negative between self-concept and academic achievement in science (i.e., countries with higher science self-concept tend to achieve lower on scientific literacy), while science self-efficacy and science self-concept positively influenced science achievement. Mokshein (2002) meanwhile indicates that the effects of self-concept in science and gender are significantly related to achievement. Senler and Sungur (2009) find no interaction between grade level and gender, with F (3,496) = 0.37, P> .05, or significant grade level and gender, with F (3,496) = 76.39, P< .0001, η2 = .32 and F (3,496) = 5.74, P = .001, η2 = .03 respectively. Naderi, et al., (2009) asserts that although self-esteem indicates a strong significant relationship on academic achievement when gender is controlled (Chi-Square =14.173, Sig=.007, P<0.01, there is no relationship between self-esteem and academic achievement (Sig=.074, P>0.05). In other words, a significant difference between gender and self-esteem was observed (Sig=.001, P<0.01). The findings of study's Ghaderi et al., (2009) revealed that anxiety and Stress level of Indian students are significantly higher than those of Iranian students. Furthermore, gender differences are not found significant. Other studies (such as Bacharach, Baumeister, & Furr, 2003; Dimitrov, 1999; Von Secker, 2004) indicate that gender is another factor which influences science achievement in boys to generally perform better than girls in science. There is no significant difference in term of the level of self-esteem between the two genders (Fathi, 2006a). Mirabi(2004) indicates that there are no statistically significant differences in "feelings of self-esteem, nor self-concept" between male and female students. Fathi (2006a) indicates that There is no significant difference in term of the level of self-esteem between the two genders. Whereas, the finding of Baran and Maskan's study (2011) indicate that the male students' academic self-concept total score means and their mean scores in science were equal female students' mean scores (P<.05). There is a statistically significant relationship between gender and a person's self-esteem for his or her physical abilities (Longmire, 2008). The students with low self-esteem score the teachers low, and girls from the groups with high and middle self-esteem have a higher evaluation of the teachers than boys (Qi & Zhang, 2010).

2. Objective

The objective of the present study are to investigate if gender has any moderating effect upon the relationship between the students' psychological factors (general self-concept, science self-concept, science self-efficacy, self-efficacy, self-esteem, science anxiety, and anxiety) on science performance among Iranian 8th grade students.

3. Hypothesis

There is a significant moderating effect of gender on the relationship between students' psychological factors and science performance.

4. Material and Methods

4.1. Sample

The sample for this study is selected from the total population of Eighth Grade students in lower secondary schools from large community schools in Tehran city as urban and Shahriar as suburban and the rural areas of Shahriar, during the academic year of 2010/2011. For the present study, stratified sampling was used, and therefore the sample of this study involves two centrally-located school districts among 21 districts of Tehran with 120 male and 160 female students, and also Shahriar lower secondary schools with 202 male and 198 female students.

4.2. Procedure

Data was collected by means of structured questionnaires and the questionnaires were applied in class. Based on verbal agreements of the training lecturers and participants, the questionnaire forms were distributed to the 680 participants and were asked to complete the questionnaires simultaneously at the start of a core lecture and return them to their lecturer Immediately after completing them. All completed questionnaires were passed on to the researchers.

4.3. Measures

All participants responded to Iranian translation of the instruments in this study which is listed below. They were translated into Persian and then the questionnaires were verified by the panel of lecturers and researchers to check the format, arrangement, appropriateness of the content and the language used in the instruments (Asghar-Nezhad, Karimi Klwadapanahi, & HeydariI, 2004; Fathi-Ashtiani, Ejei, Khodapanahi, & Tarkhorani, 2007; Fathi, 2006b; Hayati & Ostadian, 2008; Khodarahimi, 2010).

4.3.1. Self-concept Attribute Attitude Scale (SaaS);

The SaaS instrument was developed by Campbell (1991). The response format is a five-point Likert scale. The first version of SaaS was developed by factor analyzing the data from 1300 high achieving high school students, with exploratory and confirmatory factor analyses determined for each

sample. These factors were extracted by using the Principal Component Analyses with varimax iterations. The three factors that were produced from the factor analyses are math self-concept, science self-concept, and general self-concept. In the present study, only general self-concept and science self-concept were used which include 6 and 14 items relating to general self-concept, For example, I take a positive attitude toward myself and science self-concept, for example, I have a lot of self-concept in science. A major contribution to the validity of the self-concept scales comes from the extensive factor analyses used in the development of the SaaS. Most items had factor loadings in excess of .60 (Campbell, 1991). Alpha reliability values were calculated for general self-concept of 0.85 and a science self-concept of 0.89 were used, (Carmines & Zeller, 1979). In this study, the reliability coefficient for each subscale ranged between 0.87 for science self-concept and 0.61 for general self-concept.

4.3.2. State-Trait Anxiety Inventory (STAI);

The STAI developed by Spielberger (1970a) contains self-report scales for measuring both state and trait anxiety. The S-Anxiety Scale (STAI Form Y-1) used in this study consists of twenty statements designed to evaluate how a respondent feels at that particular time, for example, I feel calm in science. The T-anxiety (STAI Form Y-2) refers to the relatively stable-individual differences in anxiety proneness, i.e., the tendency of an individual to perceive stressful situations as a threat, and to then respond to these situations with a heightened S-anxiety reaction (O'Neil & Spielberger, 1979) and used in this study consists of twenty statements, for example, I feel pleasant. The S-Anxiety Scale required the respondent to determine how he or she feels at a particular moment in time. Evidence bearing on the construct validity of the state scales was derived from a sample of 977 undergraduate students at Florida State University with a median r of .73 for females and .60 for males (Spielberger, Gorsuch, & Lushene, 1983). Caldwell (1988) obtained an alpha coefficient of 0.94 for the S-Anxiety. T-Anxiety scores (Dreger, 1978; Katkin, 1978). In this study, the reliability coefficient for each subscale ranged between 0.88 for S-Anxiety and 0.85 for T-Anxiety.

4.3.3. Coopersmith Self-Esteem Inventory (CSEI);

The CSEI measures general self-esteem. Coopersmith's (1967) own inductive work examined CSEI scores as they related to other personality constructs. The present study has used the Adult Form of the CSEI, which is adapted from the School Short Form for children. The CSEI-A is a 58-item questionnaire completed by respondents by way of answering a five-point Likert scale. As Coopersmith (1967) claims, the questionnaire is designed to measure "the evaluation a person makes and customarily maintains with regard to him or herself". The CSEI has been the subject of many validity research studies (Taylor & Reitz, 1968). For example, I spend a lot of time daydreaming. A study by Kokenes (1978) confirmed the construct validity of the subscales used to measure of self-esteem that were proposed by Coopersmith. Test retest reliability for the CSEI was originally reported by Coopersmith to be 0.88 for a sample of 50 children in grade V and 0.70 for a sample of 56 children, 12 years old (Azar & Vasudeva, 2006). In this study, the Cronbach's coefficient alpha for CSEI was 0.86.

4.3.4. General Self-Efficacy (GSE);

General Self-Efficacy (GSE) developed by Sherer et al. (1982) is designed to gauge self-efficacy in clinical, educational, and organizational settings (Chen, Gully, & Eden, 2001). The measure contains items assessing GSE and social self-efficacy, but only GSE items be considered in the present study. As Sherer et al. (1982) claim, these items tap a "general set of expectations that the individual carries into new situations." The GSE Scale contains is 17-items, for example, When I make plans, I am certain I can make them, while the response format is a five-point Likert scale. The sum of item scores reflects general self-efficacy, meaning that the higher the total score, the more self-efficacious the respondent. Convergent validity has been established in studies comparing the general self-efficacy scale and similar clinical measures (Sherer, *et al.*, 1982). Reliability, measured with Chronbach's alpha, was found to be .86 for General Self- Efficacy (Sherer, *et al.*, 1982). In this study, the Cronbach's coefficient alpha for CSE was 0.79.

4.3.5. Science Self-Efficacy Questionnaire (SSEQ);

The SSEQ was developed by Smist (1993) to assess students' self-efficacy in science by measuring beliefs about competence in school science tasks (Smist, 1993). The SSEQ-A is a 27-item questionnaire completed by respondents by way of answering a five-point Likert scale. The SSEQ was developed to assess students' self-efficacy in science by measuring students' own beliefs about their competence to perform or complete science-related tasks. This questionnaire includes physics, chemistry, biology, and laboratory. The researcher has used science totally. In the present study, only science self-efficacy was included which includes nine items related to science, for example, I can use a computer in science class. In this study, the Cronbach's coefficient alpha for SSEQ was 0.70.

5. Results

Data was analyzed by using statistical package for social sciences (SPSS 18.0). Besides, descriptive statistics, multiple regression analysis were also used in this study.

5.1. Descriptive statistics;

A perusal of table 1 reveals that the largest mean scores on self-esteem is 188.77 with the SD of 25.32 and the smallest mean scores on science score is 15.94 with the SD of 3.12.

5.2. Multiple Regression Analysis (MRA);

MRA was computed to assess the strength of relationship between dependent and independent variables. MRA provides an opportunity with little ambiguity to assess the importance of each of the predictors to the overall relationship. The results of regression analysis for the dependent variable (science performance) are presented in table 2. It is clear from the results that the regression analysis accepted the variables (general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety).

5.3. Hierarchical Multiple Regression (HMR);

Hierarchical Multiple Regression was employed in examining the effects of moderator variable (gender) on the relationships between the independent (general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, and anxiety) and dependent (science performance) variables.

HMR involves two steps. First, it is needed to form two regression equations, one includes the first-order only and a second model includes the first-order effects as well as a product term including the moderator variable (Bennett, 2000). In this research, the product term is gender. The following are the two equations formed that derived from the regression procedure by entering independent variables and product term block by block in order to create two models.

Table 3 shows that for model 1, R = .139, R2 Model 2 incorporates the product term into the prediction equation. As shown in table 3, the addition of the product term resulted in an R2 change of .005, F change (7,664) = .485, 'Sig. F' change = .846 with a P >.05. This result does support presence of a moderating effect. In other words, the moderating effect of gender explains .9% of variance in science performance above and beyond the variance explained by general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety. The result suggests that the gender is not important moderating the relationships of general self-concept, science self-concept, self-efficacy, science self-efficacy, self-esteem, anxiety with science performance.

6. Discussion

The results of the study found students' psychological factors were significantly in science self-concept and self-concept, only, there are not correlated with the other variables. This study also supports that gender is not significant moderate for the relationship between students' psychological factors with science performance.

This finding is in line with (Campbell, 1991; Coopersmith, 1970; Sherer, 1982; Smist, 1993; Spielberger, Gorsuch, & Lushene, 1970b), Also between students' psychological factors and science scores, the finding of this study provides evidence to the claims of the previous researchers (Bacharach, et al., 2003; Beaton, et al., 1996; Chang, 2008; Dimitrov, 1999; Erickson & Farkas, 1991; Fathi-Ashtiani, et al., 2007; Fathi, 2006a; Kabiri & Gharbi, 2009; Kiamanesh, 2004; Martin, et al., 2000; Mettas, Karmiotis, & Christoforou, 2006; Preckel, Goetz, Pekrun, & Kleine, 2008; Rasi, 2002; Senler & Sungur, 2009; Von Secker, 2004).

7. Conclusion

The results of the present study indicated that the independent variables of science self-concept and self-concept have positive correlation with the science score so that these variables (science self-concept and self-concept) together have determined 32 percent of the variance of science scores. Consequently, the role of these variables and concerning them gains so much importance in the instruction of the science. The investigation of semi partial correlation coefficients achieved from the analysis, which is the indicator of the allocated ratio of each variable, shows that the variable of science self-concept has determined 5% of explained variance (33%) and self-concept has determined 10% of explained variance that shows that the self-concept is more important than science self-concept. As a result, it can be stated that 17% of the explained variance is due to the impact of both variables together. According to the relationship between science self-concept and self-concept, it seems that totally self-concept variable has more important role in the instruction of the science and practitioners of the education should have a close eye to this variable.

In addition, the investigation of the moderating role of gender in the relation between dependent variable of science score and independent variables indicated that at least in the cultural structure of Iran, gender has no moderating role. Of course, as the literature indicated, gender doesn't have the

moderating role. So, it can be mentioned that the existence of the moderating role for gender follows the cultural structure and is not necessarily consistent everywhere.

Table 1. Descriptive Statistics of the Independent & Dependent Variables

	N	Maximum	Minimum	Mean	Std. Deviation
Science score	680	6.00	20.00	15.94	3.12
Science self-concept	680	14.00	70.00	47.97	10.81
Self-concept	680	6.00	30.00	20.73	4.43
Science Anxiety	680	20.00	80.00	44.02	11.25
Anxiety	680	20.00	80.00	45.43	10.54
Self-esteem	680	94.00	274.00	188.77	25.32
Self-efficacy	680	17.00	85.00	58.61	10.11
Science Self-efficacy	680	9.00	45.00	28.42	6.67

Table 2. Result of Multiple Regression Analysis

Variables	Summary of Regression	Un-Std Coefficient B	Un-Std Coefficient Std. Error	Std. Coefficient Beta	t	Sig. Value
(constant)		7.161	5.520		1.297	.199
Science self-concept		.093	.043	.328	2.158	.035*
Self-concept		.257	.085	.403	3.015	.004*
Science Anxiety		.001	.042	.003	.019	.985
Anxiety		.015	.047	.059	.325	.746
Self-esteem		-.026	.022	-.241	-1.167	.248
Self-efficacy		.019	.043	.064	.431	.668
Science Self-efficacy		.062	.072	.117	.869	.388
Multiple R	.572					
R Square	.328					
Adjusted R Square	.25					
F-Statistics	4.245*					

Note. Predictor: students' psychological factors. Dependent Variable: Total science performance, * p < .05.

Table 3. Result of HMR Analysis for the Moderated Effect of Gender on the Relationship between gender and students' psychological factors

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.139	.019	.007	3.11648	.019	1.641	8	671	.110
2	.155	.024	.002	3.12488	.005	.485	7	664	.846

Note. Predictors step 1: students' psychological factors; step 2: students' psychological factors, Students Gender, * p < .05.

Acknowledgments:

We would like to thank all the administration officers at all participating schools for giving the research team information about their students in the schools. We also appreciate the contribution of lower secondary schools students by participating in this research, thus allowing us to collect the necessary data for the study.

Corresponding Authors:
Siti Aishah Hassan
Maryam Sahranavard
Faculty of Educational Studies
Universiti Putra Malaysia (UPM)
43400 UPM Serdang, Selangor, Malaysia.
E-mail: sahra1102004@yahoo.com, siti_aishahh@putra.upm.edu.my

References

Asghar-Nezhad, T., Karimi Klwadapanahi, M., & HeydariI, M. (2004). The relationship between general self-efficacy, locus of control and academic achievement *Journal of Psychology*.

Azar, I. A. S., & Vasudeva, P. (2006). Relationship between Quality of Life, Hardiness, Self-efficacy and Self-esteem amongst Employed and Unemployed Married Women in Zabol. *Iranian Journal of Psychiatry, 1*(3).

Bacharach, V. R., Baumeister, A. A., & Furr, R. M. (2003). Racial and gender science achievement gaps in secondary education. *The Journal of genetic psychology, 164*(1), 115-126.

Bandura, A. (1993). Perceived self-efficacy in cognitive development and functioning. *Educational Psychologist, 28*(2), 117-148.

Baran, M., & Maskan, A. K. (2011). A Study of Relationships between Academic Self Concepts, Some Selected Variables and Physics Course Achievement. *International Journal of Education, 3*(1).

Beaton, A. E., Mullis, I. V. S., Martin, M. O., Gonzales, E. J., Kelly, D. L., & Smith, T. A. (1996). *Mathematics achievement in the middle school years: IEA's Third International Mathematics and Science Study (TIMSS)*: TIMSS International Study Center, Boston College, Chestnut Hill, MA.

Bennett, J. A. (2000). Focus on Research Methods Mediator and Moderator Variables in Nursing Research: Conceptual and Statistical Differences. *Research in Nursing & Health, 200 23*, 415-420.

Byrne, B. M., & Shavelson, R. J. (1987). Adolescent self-concept: Testing the assumption of equivalent structure across gender. *American Educational Research Journal, 24*(3), 365.

Caldwell, L. M. (1988). *Preferences for information and self-care, stress, and coping with outpatient surgery: A descriptive correlational study*. Unpublished Dissertation Abstracts International., Boston University.

Campbell, A. M. (1992). Chromosomal insertion sites for phages and plasmids. *Journal of bacteriology, 174*(23), 7495.

Campbell, J. R. (1991). The roots of gender inequity in technical areas. *Journal of Research in Science Teaching, 28*, 251-262.

Carmines, E. G., & Zeller, R. A. (1979). *Reliability and validity assessment CA: Sage Publications*.

Chang, Y. (2008). *Gender Differences in Science Achievement, Science Self-concept, and Science Values*, Proceedings of the IRC-2008 TIMSS, Taiwan.

Chen, G., Gully, S. M., & Eden, D. (2001). Validation of a new General Self-Efficacy Scale. *Organizational Research Methods, 4*, 62-83.

Comber, L. C., & Keeves, J. P. (1973). Science Education in Nineteen Countries: An Empirical Study. International Studies in Evaluation. *1*.

Coopersmith, S. (1967). *The antecedents of self-esteem*: WH Freeman San Francisco.

Coopersmith, S. (1970). Self-esteem Inventories (Palo Alto, CA, Consulting Psychologists Press).

Dimitrov, D. M. (1999). Gender differences in science achievement: Differential effect of ability, response format, and strands of learning outcomes. *School science and mathematics, 99*(8), 445-450.

Dreger, R. M. (1978). *Review, state-trait anxiety inventory*. New Jersey: The Gryphon Press.

Erickson, G., & Farkas, S. (1991). Prior experience and gender differences in science achievement. *Alberta journal of educational research, 37*(3), 225-239.

Fathi-Ashtiani, A., Ejei, J., Khodapanahi, M. K., & Tarkhorani, H. (2007). Relationship Between Self-Concept, Self-esteem, Anxiety, Depression and Academic Achievement in Adolescents. *Journal of Applied Sciences, 7*, 995-1000.

Fathi, H. (2006a). A Study On The Relationship Between Self-Esteem And Academic Achievement Of Iranian Students [BF697. 5. S46 H615 2006H615 2006 ff rbrb].

Fathi, H. (2006b). *A Study On The Relationship Between Self-Esteem And Academic Achievement Of Iranian Students [BF697. 5. S46 H615 2006H615 2006 ff rbrb]*. Universiti Sains Malaysia Penang, Published Master of thesis.

Fleming, J. S., & Courtney, B. E. (1984). The dimensionality of self-esteem: II. Hierarchical facet model for revised measurement scales. *Journal of personality and social psychology, 46*(2), 404-421.

Ghaderi, A. R., Venkatesh Kumar, G., & Kumar, S. (2009). Depression, Anxiety and Stress among the Indian and Iranian Students. *Journal of the Indian Academy of Applied Psychology, 35*(1), 33-37.

Hayati, A. M., & Ostadian, M. (2008). The Relationship between Self-esteem and Listening Comprehension of EFL Students.

Kabiri, M., & Gharbi, E. S. (2009). Comparing high with low science performance students in some variables of fourth grader in Iran.

Katkin, E. S. (1978). *Review, state-trait anxiety inventory*. New Jersey: The Gryphon Press.

Khodarahimi, S. (2010). General self-efficacy and worry in an Iranian adolescents and youths samples. .

Kiamanesh, A. (2004). The evaluation of the Third phase of the global education and participatory learning project (second grade of guidance school): achievements and visions. *Research on issues of education*.

Kokenes, B. (1978). A factor analytic study of the Coopersmith Self-Esteem Inventory. *Adolescence*.

Longmire, B. M. (2008). *Exploring the relations among racial identity, perceptions of family socioeconomic status, and self-esteem for emerging adults*. New York University.

Mallow, J. (1994). Gender-related science anxiety: A first binational study. *Journal of Education and Technology, 3*, 227-238.

Martin, M. O., Mullis, I. V. S., Gonzalez, E. J., Gregory, K. D., Smith, T. A., Chrostowski, S. J., et al. (2000). *TIMSS 1999 international science report: Findings from IEA's repeat of the Third International Mathematics and Science Study at the eighth grade*: International Study Center, Lynch School of Education, Boston College, Chestnut Hill, MA.

Mettas, A., Karmiotis, I., & Christoforou, P. (2006). Relationship between students' self-beliefs and attitudes on science achievements in cyprus: Findings from the third international mathematics and science study (TIMSS). *Eurasia Journal of Mathematics, Science and Technology Education, 2*(1), 41-52.

Milford, T. (2011). *An Investigation of International Science Achievement Using the OECD's PISA 2006 Data Set*. University of Victoria.

Miller, M. D. (2006). *Science self-efficacy in tenth grade Hispanic female high school students*. University of Central Florida Orlando, Florida.

Mirabi, R. (2004). *the relationship and compares the differences between self-esteem and self-concept with achievement among students of third grade in secondary schools, conducting the Global Education Program in comparison with peered schools, not conducting the Global Education Program*. University of Tarbiat Moallem, Tehran.

Mokshein, S. E. (2002). Factors significantly related to science achievement of Malaysian middle school students an analysis of TIMSS 1999 data. *Doctoral dissertation, University of Iowa, (UMI No. 3073388)*.

Naderi, H. (2009). Relationship Between Intelligence, Creativity, Selfesteem and Academic Achievement among Iranian Undergraduate Students in Malaysian Universities.

O'Neil, H. F., & Spielberger, C. D. (1979). *Cognitive and affective learning strategies*: Academic Pr.

Preckel, F., Goetz, T., Pekrun, R., & Kleine, M. (2008). Gender Differences in Gifted and Average-Ability Students: Comparing Girls' and Boys' Achievement, Self-Concept, Interest, and Motivation in Mathematics. *Gifted Child Quarterly, 52*(2), 146.

Qi, G., & Zhang, W. (2010). A Study of Teaching Evaluation Based on Network: PE Majors' Evaluation of the Theory Course of College Teachers. *Asian Social Science, 6*(9), P100.

Rasi, M. (2002). *investigation of relationship between attitude towards religions and self-esteem and self-efficacy among second grade high school iranian students in Tabriz city*. Tabriz University, Tabriz, Iran.

Senler, B., & Sungur, S. (2009). Parental influences on students' self-concept, task value beliefs, and achievement in science. *Spanish journal of psychology, 12*(1), 106.

Shen, S. N. A. S. (2002). The meshless local Petrov-Galerkin (MLPG) method: a simple & less-costly alternative to the finite element and boundary element methods.

Sherer, M. (1982). The Self-efficacy Scale: Construction and validation. *Psychological reports*.

Sherer, M., Maddux, J. E., Mercandante, B., Prentice-Dunn, S., Jacobs, B., & Rogers, R. (1982). The Self-Efficacy Scale: Construction and validation. *Psychological Reports, 51*, 663-671.

Smist, J. M. (1993). General Chemistry and Self-Efficacy.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. (1983). Manual for the state-trait anxiety inventory.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970a). *State-trait anxiety inventory-STAI manual: Calif: Consulting Psychologists Press*: Inc.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970b). *State-trait anxiety inventory-STAI manual: Calif:Consulting Psychologists Press*: Inc.

Taylor, J., & Reitz, W. (1968). The three faces of self-esteem. *Research Bulletin #80. London, Ontario: University of Western Ontario*.

Von Secker, C. (2004). Science achievement in social contexts: Analysis from national assessment of educational progress. *The Journal of Educational Research, 98*(2), 67-78.

8/21/2012

Studying the Activity of Alkaline Phosphatase, Digestive Proteases and Some Carbohydrate Enzymes in the Mid-Gut of the Third Instar Larvae of *Gasterophilus intestinalis* and Comparing Some of Them with Pupae

Ahmed S. El-Ebiarie

Zoology and Entomology Dept., Faculty of Science, Helwan Univ., Ain Helwan, Cairo, Egypt
aselebiarie@hotmail.com

Abstract: Proteinases contained in the mid-gut of the early third instar of ***Gasterophilus intestinalis*** have been tentatively identified by midgut hydrolysis of synthetic substrates**.** Trypsin was identified by maximal hydrolysis of benzoyl-DL-arginine-p-nitroanilide (BApNA) at pH 8 and chymotrypsin by maximal hydrolysis of benzoyl-L-tyrosine ethyl ester (BTEE) at pH 9.Carboxypeptidase A and B were identified by their maximal hydrolysis of hippuryl-DL- phenyllactic acid and hippuryl-L-arginine at pH 9 and 8 respectively. Aminopeptidase was identified by maximal hydrolysis of leucine-p-nitroanilide at pH 9. The activity of alkaline phosphatase and some carbohydrate enzymes (invertase, amylase and trehalase) were determined in the midgut of 3rd instar larvae and pupae of *Gasterophilus intestinalis.* The activity of alkaline phosphatase as well as the trend of amylase and trehalase activity were higher in the larval stage than that of pupa. There were no significant changes in invertase activity between the larvae and pupae. The results are discussed in view of the utilization of metabolites during metamorphosis.

[Ahmed S. El-Ebiarie. **Studying the Activity of Alkaline Phosphatase, Digestive Proteases and Some Carbohydrate Enzymes in the Mid-Gut of the Third Instar Larvae of *Gasterophilus intestinalis* and Comparing Some of Them with Pupae**. *Life Sci J* 2012; 9(3):2076-2085]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 300

Key Words*:* *Gasterophilus intestinalis*, alkaline phosphatase, digestive proteases, carbohydrate enzymes, larvae, pupae

1. Introduction:

The larvae of the botfly ***Gasterophilus intestinalis*** (De Geer) infect the alimentary tract of horses and donkies in Egypt and they are completely endoparasitic. The veterinary importance of ***G. intestinalis*** has largely dealt with the damage, which the larvae produce, in gastric tissue of the horse. Gastric disturbances include ulceration (Shefstad, 1978, Pandey *et al*., 1980) subserosal abscess formation (Waddell, 1972; Shefstad, 1978), and nodule or papilla formation (Ashizawa *et al.*, 1972; Pandey *et al.*, 1980).

Roy (1937) showed that the larvae of ***G. intestinalis*** contain amylase, proteinase and lipase in their mid-gut. Tatchell (1958) suggested that larvae of this insect contain maltase, invertase, dipeptidaes and polypeptidase in the mid-gut and amylase, maltase and invertase in the salivary glands, while the haemolymph contain lipase, amylase and an anticoagulant.

Proteinases are divided into subclasses on the basis of catalytic mechanism and they are serine proteinases with a serine and a histidine in the active site, cysteine proteinases possess a cysteine in the active site, aspartic proteinase with an acidic amino acid residue and metalloproteinases with an essential metal involved in the catalytic mechanism. Exopeptidases include enzymes, which hydrolyse single amino acids from the N-terminus (aminopeptidases) or from the C-terminus (carboxypeptidases) of the peptide chain, (Terra *et al*., 1996).

Digestive serine proteinases, including chymotrypsin and trypsin, and the aspartate proteinase pepsine were first studied in insects since these were the principle digestive enzyme in vertebrates and reviews (Houseman and Downe, 1983), pepsin-like enzymes in some Diptera (Greenberg and Paretsky, 1955; Sinha, 1975; Pendola and Greenberg, 1975) and trypsin-like enzymes with maximal activity at pH values greater than 9 are frequently reported in Lepidoptera (Applebaum, 1985).

The aim of the present work was to characterize the major protease activities present in the mid-gut of the early third larval instar of ***G. intestinalis.*** Also to study the activity of alkaline phoshatase and some carbohydrases in both larvae and pupae that are important in understanding protein digestion, as part of the overall nutritional process and utilization of metabolities in this insect.

2. Material and Methods

Collection of insect larvae and pupae:

Third larval instar of ***Gasterophilus*** was collected from the stomach of freshly slaughtered donkies and horses in the Zoo, Giza, Egypt. They

were identified according to Zumpt (1965).Pupae were obtained by incubating late third instars larvae at 30 °C for 10 days.

Preparation of mid-gut homogenate:

The selected larvae were immobilized by placing on ice and dissected in insect saline solution.

The alimentary canals were separated and 0.23 gm of mid-gut was homogenized in 10 ml cold distilled water and was centrifuged at 8000 g for 10 minutes at 4 °C. Then supernatant was used for carrying the following experiments.

Determination of total protein:

The total protein was estimated according to the method specified by Lowry *et al*. (1951). The intensity of the colour was measured using Jenway 6100 spectrophotometer.

Buffers used:

0.2 M phosphate-citrate buffer at pH 4, 5, 6 and 7, 0.05M and 0.01M Tris-HCl at pH 8 and pH 9, 1M sodium acetate-hydrochloric acid at pH 2, 0.2M glycine-HCl buffer at pH 3

Determination of optimal activity of the following enzymes:

All substrate and chemicals were purchased from Sigma chemical company.

- **Trypsin:** The substrate N – benzoyl – DL – arginine – P- nitroanilide Hcl (BApNA) was prepared by dissolving 4.34 mg in 1 ml dimethyle sulphoxide Dimethyl sulphoxide (DMSO). The activity of trypsin was measured according to a modified method of Erlanger *et al*. (1961).The change in activity was measured at 410 nm (using Jenway 6100 spectrophotometer).

- **Chymotrypsin:** The substrate N – benzoyl – L – Tyrosine ethylester (BTEE) was prepared by dissolving 0.1567 gm in 50 ml of 50% methanol. The activity of chymotrypsin was determined according to the modified method of Hummel (1959).

- **Amino peptidase**: The substrate Leucine – p – nitroanilide (LpNA) was prepared by dissolving 4 mg in 0.1 ml DMSO. Activity of Leucine – aminopeptidase (LAP) was determined according to modified method of Houseman *et al*. (1985).

  The Change in activity of trypsin, chymotrypsin and aminopeptidase was measured at 410 nm (using Jenway 6100 spectrophotometer).

- **Carboxypeptidase:** A and B both substrate Hippuryl – DL – phenyl Lactic acid (HpLA) and Hippuryl – L – phenyl alanine (HA) were prepared by dissolving 0.01 gm of each substrate in 20 ml of 0.15 M NaCl. The activity of carboxyeptidase A and B was measured according to modified methods of Folk *et al*. (1960), and Gooding and Rolseth (1976).The change in activity was measured at 254 nm (using Shimadzu spectrophotometer).

- **Acidic protease:** Acid –denatured hemoglobin was prepared by dissolving 2 gm of hemoglobin in 100 ml distilled water and mixing it with 100 ml of 0.06M Hcl.
  Bovine serum albumin (BSA) was prepared by dissolving 2 gm in 100 ml distilled water.

  The activity of acidic proteases was measured according to modified method of Francisco *et al*. (1991).
  The change in activity was measured at 280 nm (using Shimadzu spectrophotometer).

- **Alkalinephosphatase**: Colorimeteric determination of alkalinephosphatase activity using phosphate alkaline-kit (ref, 61511) measured spectrophotometrically at an absorbance of 550nm.

- **Invertase and amylase**: Invertase and amylase activities were determined according to the method of Ishaaya and Swiriski (1970), Ishaaya *et al*. (1971) using 3-5 dintrosalicylic acid reagent for determining the free aldehylic groups of glucose formed after carbohydrate digestion, this reaction based on the reduction of dinitrosalysilic acid by the aldehydic groups of glucose units in a basic medium. A reduce dinitrosalysilic acid is measured spectrophotometrically at an absorbance of 550 nm.

  The reaction medium for invertase contained 0.2 ml 4% sucrose, 0.1ml 0.2M acetate buffer (pH 5.5) and 0.1ml 0.2% enzyme solution. The reaction medium for amylase contained 0.1ml 2% starch, 0.1ml 0.2M phosphate buffer (pH 6) and 0.2 ml 0.2% enzyme solution.

- **Trehalase:** Trehalase activity was determined by a procedure similar to that of invertase and amylase using the dintrosalicylic acid reagent for determining the glucose formed after trehalase digestion, the reaction medium contained 0.2ml 3% trehalase, 0.1ml 0.2M acetate buffer (pH 5.5) and 0.1ml 0.2% enzyme extract. Dintrosalicylic acid reagent was prepared by the procedure similar to that of Ishaaya and Swiriski (1976). One gm of 3, 5 dintrosalicylic acid was dissolved in 20ml of 2N NaOH and 50 ml of distilled water with the aid of magnetic stirrer. Thirty grams of K-Na tartarate was added, and magnetic stirring was continued until a clear solution was obtained. Distilled water was then added to bring the final

volume to 100 ml of the reagent, when stored in the dark, is stable at least for three months.

Pupae:

In case of pupae, whole body (4 gm) was homogenized in cold distilled water and centrifuged at 5000 rpm, and the detection processes were performed as in larvae.

Statistical analysis:

All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL USA). Data were expressed as mean ± standard error of 6 replicates in each experiment. Mean values of continuous variables were compared using t-test or analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1955). Correlations between variables were calculated by Pearson's method. The significance level was set at $P < 0.05$ or less.

3. Results

Enzyme activity assays:

The results of enzyme activity assays showed clearly that the mid-gut of the early third larval instar of ***G. intestinalis*** is capable of digesting proteins.

Figs (1- 5) show that the effect of different pH value on the activity of trypsin, chymotrypsin, leucine- aminopeptidase, carboxypeptidase A and carboxypeptidase B was not significant. The highest enzyme activity of trypsin and chymotrypsin was at pH 8 and pH 9, respectively (Figs 1, 2) and that of leucine-aminopeptidase was at pH 9 (Fig 3). Carboxypeptidase B activity was low at pH 6 and pH 7, but it increased at pH 8 and pH 9 with highest activity was at pH 8 (Fig. 4). Figure (5) shows that the highest pH activity of carboxypeptidase A was at pH 9. The results also revealed a positive significant correlation ($r = 0.87$, $P < 0.05$) between leucine-aminopeptidase and chymotrypsin (Fig 6) and also a positive significant correlation ($r = 0.83$, $P < 0.05$) between trypsin and carboxypeptidase B (Fig. 7). Maximum hydrolysis of haemoglobin by acidic protease was at pH 4 (Fig. 8), while maximum hydrolysis of BSA was at pH 3 (Fig. 9).

Activity of alkaline phosphatase for *Gasterophilus* was high during third instar larvae and significantly decreased ($P < 0.01$) during pupal stages (Table 1).The results for carbohydrate enzymes (invertase, amylase and trehalse) varied among the larvae and pupae of *Gasterophilus.* Invertase activity has not significant change between larvae and pupae of *Gasterophilus* ($P < 0.2$) (Table 2 Fig. 11).The trend of amylase activity shows decreasing in pupal stages than third larval stages. This decrease was not significant in *Gasterophilus* ($P < 0.066$, Table 1).Trehalase activity in *Gasterophilus* was high in third instar larvae and significantly decreased in pupal stages ($P < 0.004$, Table 1)

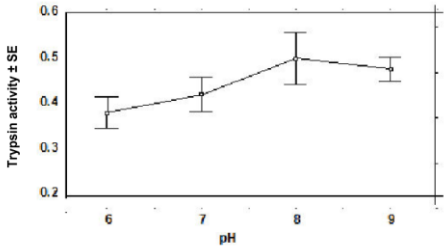


Fig. (1): Trypsin-like activity at different pH values using BApNA in homogenates of mid-gut of early third larval instars of ***G. intestinalis.***
Activity of enzyme expressed as O.D. / 10 min / 0.001 mg protein

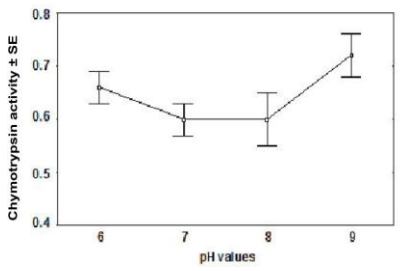


Fig. (2): Chymotrypsin-like activity at different pH values using BTEE in homogenates of midgut of early third larval instars of ***G. intestinalis***.
Enzyme expressed as O.D. / 10 min / 0.003 mg protein

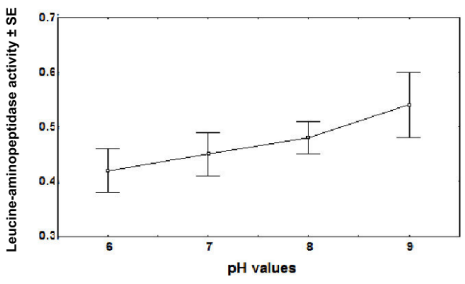


Fig. (3): Leucine-aminopeptidase activity at different pH values using LpNA in homogenates of mid-gut of early third larval instar of ***G. intestinalis***
Activity of enzyme expressed as O.D. / 10 min / 0.0002 mg protein

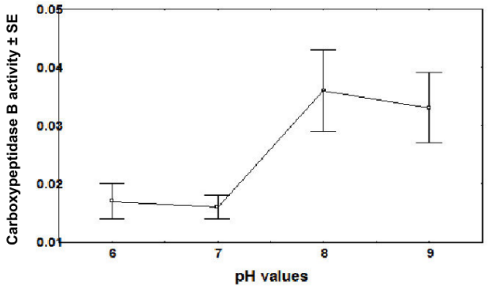


Fig. (4): Carboxypeptidase B activity at different pH values using HA in homogenates of mid-gut of early third larval instars of ***G. intestinalis***.
Activity of enzyme expressed as O.D. / 5 min / 0.0013 mg protein

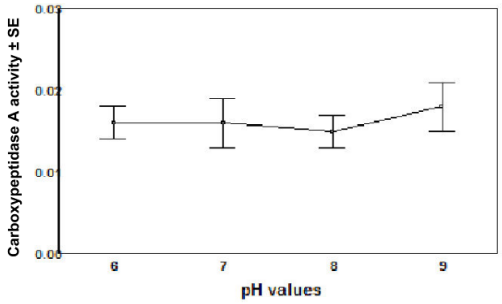


Fig. (5): Carboxypeptidase A activity at different pH values using HpLA in homogenates of mid-gut of early third larval instar of ***G. intestinalis***.

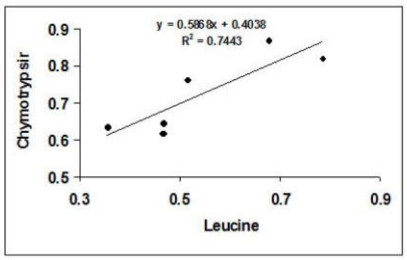


Fig. (6): Correlation in homogenates of mid-gut of early third larval instars of ***G. intestinalis*** between the activity of chymotrypsin and leucine–aminopeptidase at pH 9. Positive significant correlation can be observed ($r = 0.87$, $P < 0.05$).

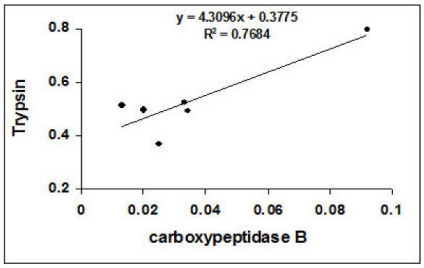


Fig. (7): Correlation between the activity of trypsin and carboxypeptidase B enzymes at pH 8 in homogenates of mid-gut of early third larval instars of ***G. intestinalis***. Positive significant correlation can be observed (r =

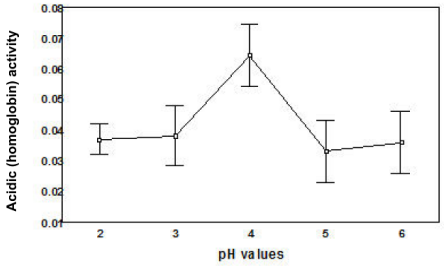


Fig. (8): Acidic protease activity at different pH values using acid– denatured haemoglobin in homogenates of mid-gut of early third larval instars of ***G. intestinalis***. Optimum pH at 4.
Activity of enzyme expressed as O.D. / 30 min / 0.003 mg protein

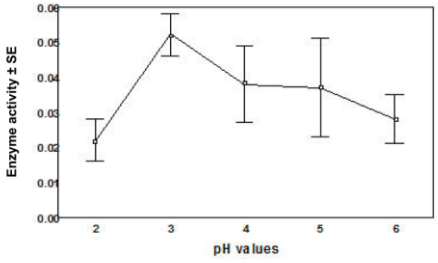


Fig. (9): Acidic protease activity at different pH values using BSA in homogenates of mid-gut of early third larval instars of ***G. intestinalis*** optimum pH at 3.
Activity of enzyme expressed as O.D. / 30 min / 0.003 mg protein

Table (1): Activity of alkaline phosphatase, invertase, amylase and Trehalase of 3rd instar larvae and pupae of ***Gasterophilus intestinalis***

Stage	Alk. Phos. activity* (mean±SD)	Invertase activity** (mean±SD)	Amylase activity** (mean±SD)	Trehalase activity** (mean±SD)
Larvae	**16.7 ± 1.15**	**0.13 ± 0.006**	**0.21 ± 0.008**	**0.15 ± 0.007**
Pupae	**6.5 ± 0.25**	**0.13 ± 0.006**	**0.17 ± 0.006**	**0.13 ± 0.002**

*Enzyme activity expressed as O.D./min/gm protein
**mg glucose/min/gm protein

4. Discussion

The mid-gut of the early third larval instars of ***G. intestinalis*** showed activity of some proteolytic enzymes at different pH values. It was clear from the results of the present work that trypsin, chymotrypsin, leucine- aminopeptidase, carboxypeptidase A and carboxypeptidase B showed great activity in the alkaline pH range mainly around pH 8 and 9.

The highest activity of trypsin in the mid-gut of the early third larval instars of ***G. intestinalis*** was at pH 8. This pH value matches those values (pH 7.8–10) of other insects recorded by different authors *e.g.* ***Pterostichus melanarius*** (Gooding and Rolseth, 1976), ***Tenebrio molitor*** (Levinsky *et al.*, 1977), ***Vespa crabo*** (Jany *et al.*, 1978), ***Attagenus megatoma*** (Baker, 1981b) ***Hypoderma lineatum*** (Tong *et al.*, 1981), ***Bombyx mori*** (Sasaki and Suzuki, 1982), ***Aedes***

aegypti (Graf and Briegel, 1985), ***Costelytra zealandica*** (Christeller *et al.,* 1989), ***Locusta migratoria*** (Sakal *et al.,* 1989), ***Muscat domestica*** (Lemos and Terra , 1992), ***Thrombi domestica*** (Zinkler and Polzer, 1992), ***Choristoneura fumiferana*** (Milne and Kaplan, 1993), ***Nauphoeta cinerea*** (Elpidina *et al*., 2001) and ***Mamestra configurata*** (Hegedus *et al* ., 2003) .

Trypsin is a serine proteinase. Lehninger (1970) stated that trypsin catalyzes the hydrolysis of peptide bonds in which the carbonyl function is donated by a basic amino acid residue like lysine or arginine. Trypsin – specific substrate (N- benzoyl –DL – arginine – p – nitroanilide) has a bond that is susceptible to trypsin hydrolysis because the carbonyl function is contributed by the basic residue arginine. This may indicate that the trypsin- like nature of the enzyme in the early third larval instar of ***G. intestinalis*** is responsible for the basic proteolytic activity in the mid- gut.

Digestive trypsin-like activity has been reported in most insect species. Important exceptions are Hemiptera species and species belonging to the series Cucujiformia of Coleoptera. The optimum pH of trypsin in most insects are always alkaline (mostly between 8 and 9), irrespective of the pH prevailing in mid-guts from which the trypsins were isolated. Nevertheless, trypsin isolated from Lepidopteran insects have higher optimum PH' corresponding to the higher pH values found in their mid-guts (Terra *et al.,* 1996). Also cleavage specificity against polypeptides was studied in trypsins from several insects (Terra and Ferreira, 1994). Results showed that specificities of these enzymes are similar (but not identical) to that of vertebrate trypsins. Nevertheless, some properties of insect trypsins contrast with those of vertebrate trypsins. Insect trypsins are not activated or stabilized by calcium ions (Levinsky *et al*., 1977; Jany *et al.,* 1978; Lemos and Terra, 1992), in most cases they are unstable in acid pH (Sakal *et al.,* 1989) and have different sensitivities to natural trypsin inhibitors (Purcell *et al*., 1992).

Barillas – Mury *et al.,* (1991) sequenced what seems to be the precursor of mid-gut trypsin in ***Aedes aegypti*** .The sequence shows significant differences from the vertebrate trypsin precursors in the region of the activation peptide. Similar results were found with a putative trypsinogen from ***Simulium vittatum*** (Ramos *et al.,* 1993). These differences suggest that the processing of precursors of insect trypsins may be different from that of vertebrates. In ***Erinnyis ello*** (Santos *et al.,* 1986) and in ***Musca domestica*** (Lemos and Terra, 1992; Jordao *et al.,* 1996b), trypsin is synthesized in the mid-gut cells in an active form, but is associated with the membranes of vesicles. These vesicles then migrate to the cell apex and trypsin precursors are processed to a soluble form before being secreted.

Secretory granules isolated from the opaque zone cells from ***Stomoxys calcitrans*** adults contain trypsin precursor, which is also different from that found in vertebrates. (Moffat and Lehane, 1990).

Determination of the chymotrypsin esterase- like activity in the early third larval instar of *G. intestinalis* indicates a maximum activity at pH 9. This pH value is similar to recorded values (pH 8 – 10) in other insects *e.g.* ***Pieris brassicae*** (Lecadet and Dedonder, 1966), ***Vespa orientalis*** (Jany and Pfleiderer, 1974), ***Glossina morsitans*** (Gooding and Rolseth, 1976), ***Locusta migratoria*** (Sakal *et al*., 1988), females of ***Anopheles*** (Horler and Briegel, 1995), ***Nauphoeta cinerea*** (Elpidina *et al.,* 2001) and ***Mamestra configurata*** (Hegedus *et al.,* 2003).

Chymotrypsin is a serine proteinase. Lehninger (1970), reported that chymotrypsin catalyzes the hydrolysis of peptide bonds in which the carbonyl function is contributed by an aromatic amino-acid residue like phenylalanine, tyrosine or tryptophane This also may indicate that a chymotryptic-like enzyme is also responsible for the basic proteolytic activity in ***G. intestinalis*** mid-gut.

It seems that the distribution of chymotrypsin-like enzymes among insect taxa is similar to that of trypsin (Applebaum, 1985). The optimum pH of chymotrypsin in most insects is in the range (8 – 9), irrespective of the pH prevailing in the mid-guts from which the chymotrypsins were isolated (Terra *et al.,* 1996). The sequences of the chymotrypsin-like proteinases were determined from ***Vespa orientalis*** and ***Lucilia cuprina*** and are similar to vertebrate chymotrypsins (Jany *et al.,* 1983; Casu *et al*., 1994). Also, insect chymotrypsins act on glucagon and B-chain of oxidized insulin in a manner similar to vertebrate chymotrypsins. However, some properties of insect chymotrypsins contrast to those of vertebrate chymotrypsins, such as their instability at acid pH and their strong inhibition by soyabean trypsin inhibitor. (Terra *et al*., 1996).

Maximum activity of leucine-aminopeptidase in the early third larval instar of ***G. intestinalis*** was at pH 9. This pH is more or less similar to pH values (7.2 – 8.5) in other insects *e.g.* ***Glossina morsitans*** (Gooding and Rolseth, 1976, Cheeseman and Gooding, 1985), ***Attagenus megatoma*** (Baker and Woo, 1981), ***Rhodinus prolixus*** (Houseman and Downe, 1981; Ferreira *et al.,* 1988), ***Trinervitermes trinervoides*** (Van der Westhuizen *et al.,* 1981), ***Rhynchosciara americana*** (Ferreira and Terra, 1984, 1985, 1986a, b; Klinkowstron *et al.,* 1994), ***Costelytra zealandica*** (Christeller *et al.,* 1989), ***Pheropsophus aequinoctiolis*** (Ferreira and Terra, 1989), *Teleogryllus commodus* (Christeller *et al*., 1990), ***Anopheles stephensi***

(Billingsley , 1990b) ***Spodoptera littoralis*** (Lee and Anstee, 1995). However, Leucine–aminopeptidase activity from ***Acanthoscelides obtectus*** (Osuala *et al.,* 1994) was maximum between pH ranges of 5.5 – 8.

In the early third larval instar of ***G. intestinalis*** carboxypeptidase A showed maximum peak at pH 9 while carboxypeptidase B showed maximum activity at pH 8. The specificity for trypsin hydrolysis of peptide bonds on the carboxyl side of basic L-aminoacids such as arginine or lysine means that the products, a carboxyl terminal of arginine or lysine is generated, which is the preferred substrate for carboxypeptidase B. This may indicate that these two enzymes may act in a sequential manner. This may be true in case of the early third larval instar of ***G. intestinalis***, as results revealed a positive significant correlation between trypsin and carboxypeptidase B.

Also the same may be true for chymotrypsin, which hydrolyze peptide bonds that give amino acids (as phenylalanine, tyrosine or tryptophane), these amino acids are preferred as a substrate to carboxypeptidase A. This also may be true in case of the early third larval instar of ***G. intestinalis***, as the results showed that both enzymes work optimally at the same pH value.

The optimum activity of carboxypeptidase A is near to the optimum pH (8-8.5) found in other insects *e.g.* ***Teleogryllus commodus*** (Christeller *et al*., 1990), ***Attagenus megatoma*** (Baker, 1981 a), and ***Costelytra zealandica*** (Christeller *et al.,* 1989). Also the optimum activity of carboxypeptidase B is more or less similar to the optimum activity (pH 7.8) of ***Glossina morsitans*** (Gooding and Rolseth, 1976).

Briegel and Lea, (1975) suggested that trypsin is the major primary hydrolytic protease in the mosquito mid-gut and is responsible for the initial breakdown of proteins and peptides in the mosquito mid-gut.

Billingsley (1990 a) stated that three aminopeptidases are responsible for the post-tryptic digestion of peptides throughout the mid-gut in ***Anopheles stephensi***. Billingsley and Hecker (1991) suggested that although trypsin is responsible for primary proteolytic events in the mid-gut, secondary digestion of peptides is brought about by aminopeptidases and carboxypeptidases.

The mid-gut of the early third larval instar of ***G. intestinalis*** also possessed proteinases that have optimum pH below 5. Terra. *et al.,* (1996) reported that aspartic proteinases have an optimum pH below 5, due to the involvement of a carboxyl residue in catalysis. The first report of aspartic proteinase in insects was made by Greenberg and Paretsky (1955), where they found a strong proteolytic activity at pH (2.5 –3.0) in homogenates of whole bodies of ***Musca domestica***. They hypothesized that this activity may be due to a pepsin-like enzyme. Lemos and Terra (1991) were able to demonstrate that the enzyme is cathepsin–D- like. Sequence studies have shown that pepsin may have evolved from the same archetypical gene as cathepsin D in vertebrates. A similar evolutionary trend seems to have occurred in Cyclorrhaphous Diptera, which apparently use cathepsin D as a digestive enzyme in the acid zone of their mid-guts.

The aspartic proteinase in the early third larval instar of ***G. intestinalis*** hydrolyzes haemoglobin maximally at pH 4.

The optimum pH at which haemoglobin was hydrolyzed in the early third larval instar of ***G. intestinalis***, was near to those pH values (3 – 4.5) reported in ***Rhodinus prolixus*** (Terra *et al.,* 1988), ***Leptinotarsa decemlineata*** (Thie and Houseman, 1990), Musca ***domestica*** (Lemos and Terra, 1991) *Aedes aegypti* (Cho *et al.,* 1991), ***Parasarcophaga surcoufi*** (Dorrah *et al.,* 2000) ***Callosobruchus maculatus*** (Silva and Xavier – Fiho, 1991).

The present study identifies an aspartic proteinase but does not distinguish between cathepsin D and pepsin. Cathepsin D (as an aspartic proteinase) can be differentiated from pepsin by the fact that bovine serum albumin is hydrolysed at 10 % or less of the rate for haemoglobin, whereas pepsin hydrolyzes both substrates equally (Barrett, 1977).

Since that the aspartic proteinase in the mid-gut of the early third larval instar of ***G. intestinalis*** hydrolyses haemoglobin maximally at pH 4, and that the hydrolysis of bovine serum albumin is 59.4 % that of haemoglobin, (at optimum pH of hemoglobin hydrolysis), therefore the characters of the acidic proteinase in ***G. intestinalis*** may be that of cathepsin D (further investigation is needed to confirm this result).

Phospatases are involved in the process of digestion and cellular replacement (Srivastava and Saxena 1967, Dadd 1970).Alkaline phosphatase may act as hydrolase during the final stages of digestion (Chung and Low, 1975). Furthermore, it has been suggested that alkaline phosphatase isozymes are involved with nutrient absorption and membrane transport (Eguchi 1995). On the same time alkaline phosphatase is one of the enzymes with relatively broad specificity, capable of acting on a number of different structurally related substrates as well as hydrolyzes many different esters of phosphoric acid (Lehninger, 1993).

The present study revealed that the alkaline phosphatase activity in the mid-gut of larvae is higher than that of pupae. Sridhara and Bhat (1963) in their studies of the variation in the alkaline phosphatase activities of the silkworm ***Bombyx mori*** in all stages of life cycle reported a steady increase in the enzyme activity. Also, Srivastava and Saxena (1967) found alkaline phosphatase to be widely distributed in the gut, salivary glands and Malpigian tubules of both

nymph and adults of ***Periplaneta Americana***. They reported that the presence alkaline phosphatase activity at those sites indicates a role in active transportation. Moreover, Beadle (1971) found that alkaline phosphatase was associated with those mid-gut cells containing lipid droplets in ***Carausius morasus*** and he reported that alkaline phosphatase probably plays a role in lipid absorption in insects. Likewise, Nath and Butler (1973) and Barker and Alexander (1958) compared the alkaline phosphatase in larval stage of two insect species, the black carpet beetle and house fly. The larval longevity of black carpet beetle requires 9 months while that of house fly requires 6 days. The maximum alkaline phosphatase activity possessed towards the end of the larval stages and species with shorter larval duration show a peak at a much earlier age. Those findings could also explain the increase of alkaline phosphatase activity in the mature larvae of ***Gasterophilus*** than pupae, as ***Gasterophilus*** larvae remain attached for nine months in the stomach of donkies and then pupate in sand.

The increase of the alkaline phosphatase activity in the mid-gut of *Gasterophilus* third instar larvae could indicate its important in transport of materials like glucose, fatty acid etc… across the mid-gut walls because during the third instars, the larvae consume their food voraciously and need mechanisms for the speedy transport of the metabolites across the mid-gut walls, to be stored for the adult tissue development.

The activity of amylase, trehalse was higher in the mid-gut of third instar larvae than that of pupal stages. The value of invertase activity was almost the same for all third instar larvae and pupae. Most of the dipterous insects e.g. blow flies are known to rely mainly on carbohydrates as a metabolic fuel (Sacktor, 1965).

Trehalose sugar plays an important role in carbohydrate metabolism insects. These disaccharides are the main sugar reserve in insect haemolymph being a specific substrate for trehalase enzyme to hydrolyze to glucose for internal energy supply (Wyatt 1967 and Takesue *et al*. 1989).

The larvae feed on blood rich in conjugated disaccharides during their attachment of the hosts. The high activities of the investigated enzymes result from the adaptability of the larvae to the diet. This explanation is supported by the observations of other authors (Frouz *et al*. 2002 and Taha 2003).

The amylase and trehalase activities in the third instar larvae could therefore used as additional parameters for assessing the availability of di and polysaccharides as nutrients and adaptability of these larvae to their hosts.

The assumption, which is generally applicable, that a correlation can be drawn between the presence of a high concentration of a specific substrate in the natural milieu of an insect and the presence of the corresponding digestive enzyme is true in this case. The assumption has been confirmed by Applebum *et al*. 1961 who stated that the high starch content of stored grains indicates important role of amylase in the digestive enzyme of ***Tenbrio molitor***. In view of the biochemical tests for the presence of these enzymes in the mid-gut of the larvae, it was found that dissaharides and polyssacharides present in the diet are able to support larval life and it was established that ***Gasterophilus*** larvae could be dependent on carbohydrates as a major source of energy. The high activities of these enzymes in the larvae than pupae indicate that the larvae are metabolically more active than pupae.

Corresponding author
Ahmed S. El-Ebiarie
Zoology and Entomology Dept., Faculty of Science, Helwan Univ., Ain Helwan, Cairo, Egypt
aselebiarie@hotmail.com

References

Applebaum, S.W. (1985). Biochemistry of digestion. In: Comprehensive Insect physiology, Biochemistry and Pharmacology (Edited. by Ker Kut G. A. and Gilbert L, T), (4): 279 – 312. Pergamon press, New York

Applebaum, S.W., Jankovic, M., and Brik, Y. (1961). Studies on the midgut amylase activity of Tenebrio *molitor* L, larvae. J, Insect Physiol. 7:100-108

Ashizawa, H., Nosaka D., Tateyama S. and Murakami, T., (1972). Pathological findings on stomach and duodenum of horse parasitic with *Gasterophilus* larvae (In Japanese, with English abstract). Bull. Fac. Agric. Migazaki Univ., 19: 437-448.

Baker, J.E. (1981b) Resolution and partial characterization of the digestive proteinases From larvae of the black carpet beetle, in Current Topics in Insect Endocrinology and Nutrition (eds G. Bhaskaran, S. Friedman and J.G. Rodriguez), Plenum Press, New York, pp. 283 – 315.

Baker, J.E. and Woo, S.M. (1981). Properties and specificities of a digestive aminopeptidase from larvae of *Attagenus megatoma* (Coleoptera: Dermestidae). Comp. Biochem. Physiol., 69B:189-93.

Baker, J.E., (1981a). Isolation and properties of digestive carboxypeptidases from mid-gut of larvae of the black carpet beetle Attagenus megatoma. Insect Biochem. 11: 588 – 91.

Barillas-Mury, C., Graf, R, Hagedorn, H.H. and Wells, M.A. (1991). cDNA and deduced amino acid sequence of a blood meal-induced trypsin from the mosquito, *Aedes aegypti*. Insect Biochem. 21: 825-31.

Barker, R.J. and Alexander, B.H. (1958). Acid and alkaline phosphatase in house flies of different ages. Ann, Entomol. Soc. Am., 51: 256-257.

Barrett, A.J. (1977). Cathepsin B and other thiol proteinases, pp .181– 208 .In A. J. Barrett (ed.) Proteinases in Mammalian Cells and Tissues. Research Monographs in Cell and Tissue Physiology, Vol. 2. North Holland, Amsterdam.

Beadle, D.J. (1971). The localization of alkaline phosphatase in the midgut epithelium of Carausius morasus. Hitochemie, 27: 370-372.

Billingsley, P. F. and Hecker, H. (1991). Blood digestion in the mosquito, Anopheles stephensi liston (Diptera: Culicidae). Activity and distribution of trypsin, Aminopeptidase, and Glucosidase in the mid-gut. J. Med. Entomol. 28 (6): 865 – 871

Billingsley, P. F. (1990 a). The mid-gut ultrastructure of haematophagous insects. Annu. Rev. Entomol. 35: 219 – 248

Billingsley, P. F. (1990 b). Blood digestion in the mosquito, *Anopheles stephensi liston* (Diptera: Culicidae). Partial characterization and post – feeding activity of mid-gut aminopeptidases. Arch. Insect Biochem. Physiol. 15: 149 – 163

Briegel, H., Lea, A. O. (1975) Relation –ship between protein and proteolytic activity in the mid-gut of mosquitoes J. Insect. Physiol. 21: 1597 – 1604

Casu, R.E., Pearson, R.D., Sarmey, J.M. *et al*, (1994) Excretory/ secretory chymotrypsim from *Lucilia cuprina*: Purification, enzymatic specificity and amino acid sequence deduced from mRNA. Insect. Mol. Biol., 3: 201-11.

Cheeseman, M.T. and Gooding, R.H.(1985). Proteolytic enzymes from testseflies, *Glossina morsitans* and *Glossina palpalis* (Diptera: Glossinidae). Insect Biochem, 15:677-80.

Cho, W., Dhadialla, T. S. and Raikhel, A. S. (1991) Purification and characterization of lysosomal aspartic protease with cathepsin activity from the mosquito. Insect Biochem. 221: 165 –176.

Christeller, J.T., Laing, W.A., Shaw, B.D. and Burgess, E.P.J. (1990). Characterization and partial purification of the digestive proteases of the black field cricket, *Teleogryllus commodus* (Walker): elastase is a major component. Insect Biochem. 20: 157-64.

Christeller, J.T., Shaw, B.D, Gardiner, S.E. and Dymock, J. (1989). Partial purification and characterization of the major mid-gut proteases of grass grub larvae *(Costelytra zealandica).* Coleoptera: Scarabaeidae. Insect Biochem. 19: 221-31.

Chung, W.W.K. and Low, K.W. (1975). Ultrastructural and functional of differentiation of the midgut of the sugar cane beetle, *Protaetia acuminate* (F.) (Coleoptera: Cetoniidae). Int. J. Insect Morphol. Embryol. 4: 349-361.

Dadd, R.H. (1970). Arthropod nutrition. In Florkin M. and Scheer, B.T., editors. Chemical Zoology. Vol. 5. Academic Press New York, 35-95.

Dorrah, M.A., Yousef, H. and Bassal, T.T.M. (2000). Partial characterization of acidic proteinase in the mid-gut of *Parasarcophaga surcoufi* larvae (Diptera: Sarcophagidae). J. Egypt. Soc. Parasitol. 30(2): 643-625.

Duncan, D.B. (1955). Multiple range and multiple F-tests. Biometrics. 11: 1 – 41.

Eguchi, M. (1995). Alkaline phosphatase isozymes in insects and comparison with mammalian enzyme. Comp. Biochem. Physiol., IIIB: 151-162.

Elpidina, EN, Vinokurov Ks; Gromenko, VA; Rudenskaya, YA; Dunaevsky, YE; Zhuzhikov, DP. (2001). Compartmentalization of proteinases and amylases in *Nauphoeta cinerea* mid-gut. Arch. Insect Biochem. Physiol., 48 (4): 206-16.

Erlanger, B.F.N., Kokowsky and W. Coheh, (1961). The preparation of two nechromogenic substrates of trypsin. Arch. Biochem. Biophys. 95:271-278.

Ferreira, C. and Terra, W. R. (1989). Spatial organization of digestion secretory mechanisms and digestive enzyme properties in *Pheropsophus aequinoctialis* (Coleoptera: carabidae). Insect Biochem. 19 383 – 91.

Ferreira, C. and Terra, W.R. (1984). Soluble aminopeptidases from cytosol and luminal contents of *Rhynchosciara americana* mid-gut caeca. Properties and phenanthroline inhibition. Insect Biochem. 14:145-50.

Ferreira, C. and Terra, W.R. (1985). Minor aminopeptidases purified from the plasma membrane of mid-gut caeca cells of an insect (*Rhynchosciara americano*) larva. Insect Biochem. 15: 619-25.

Ferreira, C. and Terra, W.R.(1986b). Substrate specificity and binding loci for inhibitors in an aminopeptidase purified from the plasma membrane of midgut cells of an insect (*Rhynchosciara americana*). Larva. Arch. Biochem. Biophys. 244: 478-85.

Ferreira, C., and Terra, W.R. (1986a). The detergent form of the major aminopeptidase from the plasma membrane of mid-gut caeca cells of *Rhynchociara americana* (Diptera) Larva. Comp. Biochem. Physiol., 84B: 373-6.

Ferreira, C., Ribeiro, A.F., Garcia, E.S. and Terra, W.R. (1988). Digestive enzymes trapped between and associated with the double plasma membranes of *Rhodinus prolixus* posterior mid-gut cells. Insect Biochem. 18: 521-30.

Folk, J.E., K.A. PEIZ, W.R. Carroll and J.A. Glander (1960). Carboxypeptidase B. IV. Purification and characterization of porcine enzyme. J. Biol. Chem., 235: 2272-2277.

Francisco J. A. Lemos and Walter R. Terra (1991). Properties and intracellular distributionof a cathepsin D-like proteinase active at the acid-region of *Musca domestica* mid-gut. Insect Biochem. 21, (5): 457 – 465

Frouz, J., Elhottova, D., Sustr, V., Kristufek, V. and Hubert, J. (2002). Preliminary data about compartmentalization of the gut of the sarcophagus dipteran larvae Penthetria holosercae (Bibionidae). Eur. J. Soil Biol. 38: 1, 47-51.

Gooding, R. H. and Rolseth, B, M. (1976). Digestive process of haematophagous insects XI. Partial

purification and some properties of six proteolytic enzymes from the tsetse fly *Glossina morsitans morsitans*. West wood (Diptera: Glossinidae). Can. J. Zool. 54: 1956 – 1959.

Graf, R. and Briegel, H. (1985). Isolation of trypsin isozymes from the mosquito *Aedes aegypti* (L). Insect Biochem. 15: 611-1

Greenberg, B. and Paretsky, D. (1955). Proteolytic enzymes in the house fly, *Musca domestica* (L). Ann. Entomol. Soc. Am., 48: 46-50.

Hegedus, D; Baldwin, D; O'Grady, M; Braun, L; Gleddic, S; Sharpe, A; Lydiate, D; Erlandson, M. (2003). Mid-gut proteases from *Mamestra configurata* (Lepidoptera: Noctuidae) Larvae: Characterization, cDNA cloning, and expressed sequence analysis Arc. Insect Biochem. Physiol., 53(1): 30-47.

Horler, E. and Briegel, H. (1995). Proteolytic enzymes of female *Anopheles:* biphasic synthesis, regulation and multiple feeding. Arch. Insect Biochem. Physiol., 28: 189 – 205.

Houseman, J. G. and A. E. R., Downe, (1981). Exoproteinase activity in the posterior mid-gut of *Rhodinus prolixus* stal (Hemiptera = Reduviidae) Insect Biochem. 11: 579 – 82

Houseman, J. G. and A. E. R., Downe, (1983). Cathepsin D-like activity in the posterior mid-gut of Hemipteran insects. Comp. Biochem. Physiol. 75B: 509-512.

Houseman, J.G, A.E.R. Downe, and P.G. Morrison (1985). Similarities in digestive proteinase production in *Rhodinus prolixus* (Hemiptera: Reduviidae) and *Stomoxys calcitrans* (Diptera: Muscidae). Insect Biochem. 15(4): 471-474.

Hummel, B.C.W., (1959). A modified spectrophotometric determination of trypsin and chymotrypsin. Can. J. Biochem. 37: 1393-1399.

Jany, K.D. and Pfleiderer, G. (1974). Purification and some physicalproperties of a chymotrypsin-like protease of the larva of the hornet, *Vespa orientalis*. Eur. J. Biochem., 42: 419-28.

Jany, K.D. Bekelaer, K., Pfleiderer, G. and Ishay, J. (1983). Amino acid sequence of an insect chymotrypsin from the larvae of the hornet, *Vespa orientalis*. Biochem. Biophys. Res. Comm., 110,1-7.

Jany, K.D., Haug, H. and Ishay, J. (1978). Trypsin-like endopeptidases from the mid-guts of the larvae from the hornets of *Vespa orientalis* and *Vespa crabro*. Insect Biochem. 8, 221-30.

Jordao, B.P., Terra, W.R., Ribeiro, A.F. *ET at*. (1996b). Trypsin secretion in *Musca domestica* larval mid-guts. A biochemical and immunocy to chemical study. Insect Biochem. Mol. Biol. 26, 337-346……

Klinkowstrom, A.M., Terra, W.R. and Ferreira, C. (1994). Aminopeptidase A from *Rhynchosciara americana* (Diptera) larva midguts. Properties and mid-gut distribution. Arch. Insect Biochem. Physiol., 27:301-15.

Lecadet, M.M. and Dedonder, R. (1966). Les proteases de *Pieris brassicae*. I. Purification ET proprietes. Bull. Soc. Chem. Biol., 48: 631-60.

Lee, M. J. and Anstee, J. H. (1995). Characterization of mid – gut exopeptidases activity from larval *Spodoptera littoralis*. Insect Biochem. Molecular. Biol. 25 (1): 63 –71.

Lehninger, A.L. (1993). Biochemistry, Kalyani Publishers, p. 218

Lehninger, A.L., (1970). Biochemistry, worth publishers, New York. 833 pp.

Lemos, F.J.A. and Terra, W.R. (1991). Properties and intracellular distribution of a cathepsin D-like proteinase active at the acid region of *Musca domestica* mid-gut. Insect Biochem. 21: 457-65.

Lemos, F.J.A. and Terra, W.R. (1992). Soluble and membrane-bound forms of trypsin-like enzymes in *Musca domestica* larval mid-guts. Insect Biochem. Mol. Bid. 22: 613-19.

Levinsky, H, Birk, Y. and Applebaum, S.W.(1977). Isolation and characterization of a new trypsin-like enzyme from *Tenebrio molitor* L. larvae. Int. J. Pept. Protein Res., 10: 252-64

Lowry, O.H., Rosebraugh, N. J., Farr, A. L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J. Bio. Chem. 176 – 275.

Milne, R., and Kaplan, H. (1993). Purification and characterization of a trypsin-like digestive enzyme from spruce budworm *(Choristoneura fumiferana)* responsible for the activation of γ -endotoxin from *Bacillus thuringiensis*. Insect Biochem. Mol. Biol., 23: 663-73.

Moffatt, M.R. and Lehane, M.J. (1990). Trypsin is stored as an inactive zymogen in the mid-gut of *Stomoxys calcitrans*. Insect Biochem. 20: 719-723.

Nath, J. and Butler, L. (1973). Alkaline phosphatase during development of the black carpet beetle. Ann. Entomol. Soc. Am., 66: 280-284.

Osuala, C.I. Donner, R. L. and Nielsen, S. S. (1994). Partial purification and characterization of an aminopeptidase from the bean weevil larvae *Acanthoscelides obtecus* say (Coleoptera: Brichidae). Comp. Biochem Physiol., 107 B: 241 – 248.

Pandey, V.S., Ouhelli, H. and Elkhalfane, A., (1980). Observations on the epizootiology of *Gasterophilus intestinalis* and *G. nasalis* in horses in Morocco Vet. Parasitol. 7: 347 – 356.

Pendola S. and Greenberg B. (1975). Substrate specific analysis of preolytic enzymes in the larval mid-gut of *Calliphora vacinia*. Ann. ENT. Soc. Am. 68: 341-345.

Purcell, J.P., Greenplate, J.T. and Sammons, R.D. (1992). Examination of mid-gut luminal proteinase activities in six economically important insects. Insect Biochem. Mol. Biol., 22: 41-7.

Ramos, A., Mayoral, A. and Jacobs-Lorena, M: (1993). Gut-specific genes from the black-fly *Simulium vittatum* encoding trypsin-like and carboxypeptidase-like proteins. Insect Mol. Biol., 1: 149-63.

Roy, D. N. (1937). The physiology of digestion in larvae of *Gasterophilus equi* Parasitology, 29: 150-62

Sacktor, B. (1965). Energetics and respiratory metabolism of muscular contraction. In physiology of insect. Vol. 2, Rockstein, M. (ed). Academic Press, New York, 483-580.

Sakal, E., Applebaum, S. W. and Birk, Y. (1989) Purification and characterization of trypsin from the digestive tract of Locusta migratoria Int. J. Pept Protein Res, 34: 498 – 505.

Sakal, E., Applebaum, S.W. and Birk, Y. (1988). Purification and characterization of *Locusta migratoria* chymotrypsin. Int. J. Pep. Protein Res., 32: 590 - 8.

Santos, C.D., Ribeiro, A.F. and Terra, W.R. (1986). Differential centrifugation, calcium precipitation and ultrasonic disruption of mid-gut cells of *Erinnyis ello* caterpillars. Purification of cell microvilli and inferences concerning secretory mechanisms. Can. J. Zool. 64: 490-40.

Sasaki, T. and Suzuki, Y. (1982). Alkaline proteases in digestive juice of the silkworm, *Bombyx mori*. Biochem. Biophys. Acta, 703: 1-10.

Shefstad D.Y. (1978). Scanning electron microscopy of *Gasterophilus intestinalis* lesions of the equine stomach. J. Am. Vet. Med. Assoc., 172: 310-313.

Silva, C.P. and Xavier-Filho, J. (1991). Comparison between the levels of aspartic and cysteine proteinases of the larval mid-gut of *Callosobruchus maculatus* (F) and *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae). Comp. Biochem. Physiol., 99B: 529-33.

Sinha M. (1975). Pepsin-like activity in the mid-gut of *Sarcophaga ruficornis* and Musca *domestica*. Appl. ENT. Zool. 10: 313.

Sridhara, S. and Bhat, J.V. (1963). Alkaline and acid phosphatase of the silkworm, *Bombyx mori* L. J. Insect Physiol., 9: 693-701.

Srivastava, J.P. and Saxena, S.C. (1967). On the alkaline acid phosphatase in the alimentary tract of *Periplaneta amricana* L. Appl. Entomol. Zool., 2:85-92.

Taha, N.M. (2003). Physiological aspects of the midgut of *Gasterophilus* spp. (Diptera: Gasterophilidae). MSc thesis, Cairo University, Cairo, Egypt.

Takesue, Y., Yokota, K., Miyajima, S,, Taguchi, R. and Ikezawa, H. (1989). Membrane anchors of alkaline phosphatase and trehalase associated with plasma membrane of larval midgut epithelial cells of silkworm, Bombyx mori. J. Biochem., 105: 998-1001;

Tatchell, R. J. (1958). The physiology of digestion in the larvae of the horse botfly, *Gasterophilus intestinalis* (De Geer). Parasitology, 48: 448 – 54.

Terra, W.R. and Ferreira, C. (1994). Insect digestive enzymes: properties, compartmentalization and function. Comp. Biochem. Physiol., 109B: 1-62.

Terra, W.R., Ferreira, C. and Garcia, E.S. (1988). Origin, distribution, properties and functions of the major *Rhodinus prolixus* mid-gut hydrolases. Insect Biochem. 18: 423-34.

Terra, W.R., Ferreira, C., Jordao B.P., and Dillon R.J. (1996). (Chapter 6: Digestive enzymes) In: Biology of the Insect Mid-gut. Edited by. Lehane M.J. and. Billingsley P. F

Thie, N. M. R. and Houseman, J .G. (1990). Identification of cathepsin B, D and H in the larval mid-gut of Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: chrysomelidae) Insect Biochem. 20: 313 – 318

Tong, N.T., Imoff, J.M., Lecroisey, A. and Keil, B. (1981). Hypodemin A, a trypsin-like neutral proteinase from the insect *Hypoderma lineatum*, Biochem. Biophys. Acta, 658: 209-19.

Van der Westhuizen, M.C., Hewitt, P.H. and du Toit, P.J. (1981). Aminopeptidase from the harvester termite *Trinervitermes trinervoides* (Sjosted): distribution, purification, physical and chemical properlies. Insect Biochem. 11: 311-21.

Waddell, A. H, (1972). The pathogenicity of *Gasterophilus intestinalis* larvae in the stomach of the horse. Aust. Vet. J., 48: 332 – 335

Wyatt, G.R. (1967). The biochemistry of sugars and polysaccharides in insects. Adv. Insect Physiol., 4: 287-360.

Zinkler, D. and Polzer, M. (1992). Identification and characterization of digestive proteinases from the Pirebrat. *Thermobia domestica*. Comp. Biochem. Physiol., 103B: 669-73

Zumpt, T. F., (1965). Myiasis in man and Animals in the old World. Butterworths, London.

8/25/2012

Molecular Characterization of Serine Proteases from both First and Third Larval Instars Of *Chrysomya megacephala*

Ahmed S. El-Ebiarie and Nancy Taha

Zoology and Entomology Dept., Faculty of Science, Helwan Univ., Ain Helwan, Cairo, Egypt
aselebiarie@hotmail.com

Abstract: The analysis of excretory/secretory products from third larval instar of *C. megacephala* using SDS-gel electrophoresis produces a band at 16KDa, band between 16KDa and 23KDa, and a broad band between 23 and 45KDa. The PCR product produced bands at 573 bp for trypsin of both first and third instars of *C. megacephala* and 715 bp for chymotrypsin of both instars. Comparisons with other dipterans trypsin and chymotrypsin showed that all the *C. megacephala* sequences have identity with other dipterans sequences.
[Ahmed S. El-Ebiarie, Nancy Taha. **Molecular Characterization of Serine Proteases from both First and Third Larval Instars Of *Chrysomya Megacephala***. *Life Sci J* 2012; 9(3):2086-2093]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 301

Key Words: *Chrysomya megacephala*, serine proteases, excretory/secretory, larvae

1. Introduction:

Blowflies are distributed worldwide and cause medical problems and losses to the animal industry (Zumpt, 1965; Greenberg, 1971, 1973; Kuhlhorn, 1983; Ghandour, 1988). Norris (1965) gave a comprehensive review on the bionomics of blowflies. Among blowflies, the oriental latrine fly *Chrysomya megacephala* (Fabricius) is one of the most common blowflies in Egypt (Gabre, 1994) and its range is expanding (Greenberg, 1988; Wells, 1991; Tomberlin *et al*., 2001). Greenberg (1971, 1973) reported that this species is among the most dangerous dipteran vectors of enteric pathogens. In Malaysia, *C. megacephala* is the dominant vector of helminthes parasite eggs (Sulaiman *et al*., 1988, 1989). On the positive side, *C. megacephala* is an important pollinator of mango in Australia (Anderson *et al*., 1982), and in Taiwan, farmers increase *C. megacephala* population to increase pollination of mango (Hu *et al*., 1995).

Proteolytic enzymes are a major component of the digestive process of parasites and are presumed to be released to interact with host tissues (Rhoads and Fetterer, 1997). Parasitic and microbial organisms utilize the digestive actions of proteases on proteins of cells, tissues and organs for the purposes of invasion and migration in host tissues.

The serine proteases are the dominant class of proteolytic enzymes in many insect species (Applebaum, 1985; Terra and Ferreira, 1994; Terra *et al.*, 1996). The parasitic insects of mammals have used the serine proteases in establishing suitable environments upon or within their hosts (Bowles *et al*., 1988; Sandeman *et al.*, 1990). Commonly, the same group of proteases is used for nutrient digestion within and without the gut (Casu *et al.*, 1994, Bowles VM *et al*., 1990).

These proteases are thought to be potential targets for vaccines or pesticides that disrupt establishment of larval stages on or within the host, and interfere with the digestion of food in the gut of larval and adult stages (Tellam and Bowles, 1997).

Serine protease (SPs) constitutes one of the largest families' enzymes in the animal kingdom. They play important roles in dietary protein digestion, blood clotting, immune responses, signal transduction, hormone activation, inflammation, and development [Herrero *et al*., 2005; O'Connell *et al* 2006]. SPs are characterized by an a.,ctive site termed the catalytictriad, which contains H, D, and S amino acids. The serine residue at the active site participates in the formation of a transient acylenzyme intermediate between the substrate and the protease (Rawlings and Barrett, 1994). Trypsin and chymotrypsin are among the best-characterized serine proteinases in the insect's digestive system (Sudeshna *et al.*, 2000).

Numerous clinical reports have been published that describe the outstanding effects of maggot therapy, most notably on debridement, cleansing, disinfection and healing of indolent wounds, many of which have previously failed to respond to conventional treatment (Baer, 1931, Weil *et al*., 1933; Thomas *et al*., 1996; Johnson *et al*., 1999; Thomas and Jones, 2000; Graninger *et al*., 2002; Sherman, 2003; Sherman *et al*., 2004). Current day maggot therapy, with its multi-action approach to wound cleansing and healing, is highly successful**.** Enzymes can be produced from any living organism, either by extracting them from their cells or by recovering them from cell exudates**.** The molecules involved in the beneficial effects of maggots are believed to be contained in their excretions/secretions (ES).

Antibiotic resistance has become a global public-health problem, thus it is imperative that new antibiotics continue to be developed.

Antimicrobial peptides (AMPs) are an evolutionarily conserved component of the innate immune response, which is the principal defense system for the majority of living organisms, and are found among all classes of life ranging from prokaryotes to humans (Herrero *et al*. ,2005; O'Connell *et al*., 2006). They represent a new family of antibiotics that have stimulated research and clinical interest (Rawlings and Barrett 1994).

In the present study the excretory/secretory products of third larval instar of *C. megacephala* were analyzed by using gel electrophoresis. The cDNA was completely sequenced encoding a trypsin-like and a chymotrypsin-like proteinase and their respective genomic DNA from first and third larval instars of *C. megacephala*. In addition, the predicted protein sequences used for extensive phylogenetic comparison with Dipteran trypsins and chymotrypsins. The aim of this study is to ensure the production of serine proteases especially trypsin and chymotrypsin by larvae of *C.megacephala* which may lead us in the near future to extract and use these proteolytic enzymes in maggot therapy.

2- Material and Methods

2.1. Rearing of insect

The laboratory colony of *C. megacephala* used in this study was established in the Department of Entomology, Faculty of Science, Helwan University**.** *C. megacephala* was reared according to the method of Gaber *et al*., 2005.

Adults from the stock colony of *C. megacephala* were kept in cages (38 × 38 × 56 cm) at 25 ± 3 °C, 14 hrs photoperiod and 60–70% R.H. The cages were made with a wooden floor, a glass roof, and wire gauze on three of the sides. The fourth side was wooden with a circular hole fitted with a cloth sleeve to facilitate daily feeding, cleaning of the cage, and removal of eggs. Adults were supplied daily with granular sucrose, water, and beef meat (beef hereafter).Water was supplied by dipping a piece of cotton as a wick in a bottle filled with water, and the beef was provided in a Petri- dish. Egg batches were removed daily and transferred to a fresh piece of beef placed in a rearing enamel bowl (35 cm in diameter) covered with muslin secured with a rubber band. At the prepupal stage, dry autoclaved sawdust was added to the bowl as a medium for pupation. Pupae were sieved from the sawdust and transferred to adult cages described above for adult emergence.

2.2. Collection of larval secretions

Native excretions/secretions (nES) were collected by incubating third-instar larvae of *C.megacephala* in a small quantity (100 larvae per 1ml) of sterile Milli-Q ultrapure water (Millipore UK Ltd., Hertfordshire, UK) for 1 h at 30 °C in darkness. The sterile liquid was siphoned from the containers and centrifuged at 10,000 × *g* for 5 min to remove particulate material, after which the supernatant was retained for testing.

2.3. Using sodium dodecyl sulphate-polyacrylamide gel electrophoresis

The individual components of ES products were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). ES products were concentrated by ultra- filtration followed by precipitation in 80 % (v/v) ice-cold acetone. The precipitate was redissolved in reducing sample buffer and boiled for 5 minutes prior to electrophoresis on 12 % polyacrylamide gel. Proteins bands were detected by staining with 0.25 % Coomassie brilliant blue R250 in 25 % ethanol, 10% acetic acid followed by distaining in the same solvent without the Coomassie stain. Proteinase components were examined after separation on substrate gels.

2.4. Polymerase chain reaction (PCR)

Degenerate oligonucleotide primers were synthesized using a Gene Assembler Plus oligonucleotide synthesizer (Pharmacia). Design of the forward PCR primers was based on the amino-terminal amino acid sequences of purified *Chrysomya bezziana* trypsin (F1) or chymotrypsin (F2). The sequences of the reverse primers were based on conserved amino acids surrounding the active site residue Ser-195 (Elvin *et al*., 1993). In order to limit primer redundancy, three reverse primers, termed R1, R2 and R3 were used (Elvin *et al*., 1993, 1994).

The sequences of the primers are as follows:

F1 (trypsin): 5-AT (TCA) GGNAA (TC) TT (TC) CCNTGGCA (AG) GT-3:

F2 (chymotrypsin): 5-CCNGGNCA (AG) TT (TC) CCNTA (TC) CA (AG) G-3;

R1:-5-A(GA)NGGNCCNCCNGA(AG)TCNCC-3;

R2: 5-A(GA)NGGNCCNCC(AG)CT(AG)TCNCC-3;

R3: 5-A(GA)NGGNCCNCCNGT(AG)TCNCC-3.

R1 and R2 reverse primers were used to amplify trypsin cDNAs, whilst all three reverse primers were used to amplify chymotrypsin cDNAs.cDNA was prepared from mRNA isolated from homogenates of first and third instar larvae of *Chrysomya megacephala.*

Briefly, total RNA was extracted from larvae using TRIZOL (Life Technologies, Grand Is., and

NY). MRNA was purified from total RNA by affinity chromatography on an oligodT affinity column (Invitrogen, Groningen, the Netherlands) and double stranded cDNA was synthesized using a riboclone cDNA synthesis kit (Promega, Madison, WI). PCRs were conducted in 100 _l volumes containing 3 mM MgCl2, 0.25 mM of each dNTP, 100 pmol of each primer, 2.5 unit *Taq* DNA polymerase (Qiagen, Hilden, Germany), and 5 ng DNA, through 40 thermocycles (2 min, 94 °C; 1 min, 50°C and 2 min, 72 °C).

2.4- Sequence and phylogenetic analyses

Sequences were carried out in the RNA sequencing facility at Jenagen Labs Corporation, Germany. Searches of databases were carried out using BLAST programs at the National Center for Biotechnology (http://www.ncbi.nlm.nih.gov/).

3-Results

i. Total protein

The total protein measured from native excretory/secretory product of 100 larvae per I ml distilled water was 0.9 mg.

Ii.Gel electrophoresis

The analysis of native excretory/secretory (100 larvae/1ml or 0.9mg protein) products from third larval instar of *Chrysomya megacephala* produces a single band at 16KDa,a band between 16KDa and 23KDa and a broad band between 23KDa and 45KDa (Fig. 1).

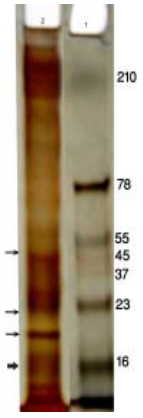


Fig. 1: Electrophoretic pattern using SDS-gel electrophoresis. Lane (1) representing marker and lane (2) representing native excretory/secretory product produced from third larval instars of *Chrysomya megacephala*

iii. RT-PCR product

The F1 forward primer and R1 or R2 reverse primers were used to amplify *Chrysomya megacephala* trypsin cDNAs, and the F2 forward primer and all three reverse primers were used to amplify *Chrysomya megacephala* chymotrypsin cDNAs. The amplified product resulting from PCR using cDNAs isolated from both first and third larval instars of *Chrysomya megacephala* produce bands between 500bp and 715bp (Fig. 2). For trypsin of both instars the amplified PCR product is at 573bp while for chymotrypsin of both instars the amplified PCR product is at 715bp (Fig. 2, Table 1).

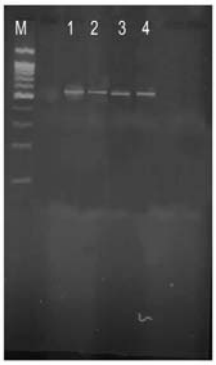


Fig. 2: Electrophoretic pattern showing rt-pcr product.
Lane (M) representing marker,
Lane (1) representing trypsin of first larval instar
Lane (2) representing trypsin of third larval instar
Lane (3) representing chymotrypsin of first larval instar Lane (4) representing chymotrypsin of third larval instar

Table 1: Showing molecular weight bands for lanes 1-4 against 100bp ladder

Molecular weight	Lane 1	Lane 2	Lane 3	Lane 4
900				
800				
			715	715
700				
600				
	573	573		
500				
400				
300				
200				
100				

BLAST Results

An overview of the database sequences aligned to the query sequence of the studied species using BLAST showed that sequence of chymotrypsin from both first and third larval instars of *Chrysomya megacephala* has 84% identity with *Drosophila melanogaster* 211000022280419

(gb|AABU01002389.1) (Celniker *et al*., 2007), *Drosophila ananassae* strain TSC#14024-0371.13 (gb|AAPP01004053.1|) (Clark *et al*.,2007), *Drosophila melanogaster* mitochondrion (ref|NC_001709.1|) (Lewis *et al*., 1995), *Drosophila grimshawi* strain TSC#15287-2541.00 Ctg01_3398 (gb|AAPT01003399.1|) (Clark *et al*.,2007), *Drosophila mojavensis* strain TSC#15081-1352.22 Ctg01_3824 (gb|AAPU01003823.1|) (Clark *et al*.,2007), *Drosophila virilis* strain TSC#15010-1051.87 Ctg01_17602 (gb|AANI01017563.1|) (Clark *et al*.,2007),83% identity with *Drosophila mojavensis* strain TSC#15081-1352.22 Ctg01_1947 (gb|AAPU01001946.1|) (Clark *et al*.,2007), *Drosophila virilis* strain TSC#15010-1051.87 Ctg01_7082 (gb|AANI01007064.1|) (Clark *et al*.,2007), *Drosophila mojavensis* strain TSC#15081-1352.22 Ctg01_5844 (gb|AAPU01005843.1|) (Clark *et al*.,2007), *Drosophila virilis* strain TSC#15010-1051.87 Ctg01_4771 (gb|AANI01004754.1|) (Clark *et al*.,2007) , *Drosophila mojavensis* strain TSC#15081-1352.22 Ctg01_3366 (gb|AAPU01003365.1|) (Clark *et al*.,2007), *Drosophila ananassae* strain TSC#14024-0371.13 Ctg01_16291 (gb|AAPP01016269.1|)(Clark *et al*.,2007), 85% identity with *Drosophila willistoni* strain TSC#14030-0811.24 1099000011229 (gb|AAQB01011230.1|) (Clark *et al*.,2007), 82% identity with *Drosophila virilis* strain TSC#15010-1051.87 Ctg01_18064 (gb|AANI01018023.1|) (Clark *et al*.,2007), *Drosophila mojavensis* strain TSC#15081-1352.22 Ctg01_4387 (gb|AAPU01004386.1|) (Clark *et al*.,2007), 86% identity with *Drosophila willistoni* strain TSC#14030-0811.24 1099000002653 (gb|AAQB01002654.1|) (Clark *et al*.,2007), 80% identity with *Drosophila virilis* strain TSC#15010-1051.87 Ctg01_17027 (gb|AANI01016988.1|) (Clark *et al*.,2007),87% identity with *Drosophila willistoni* strain TSC#14030-0811.24 1099000006770 (gb|AAQB01006771.1|) (Clark *et al*.,2007), 74% identity with *Nasonia vitripennis* genomic contig, reference assembly (based on Nvit_1.0 SCAFFOLD916)(ref|NW_001820656.1|NviUn_WGA916_1), 75% identity with Nasonia vitripennis genomic contig, reference assembly (based on Nvit_1.0 SCAFFOLD423) (ref|NW_001818170.1|NviUn_WGA423_1), 73% identity with Nasonia vitripennis genomic contig, reference assembly (based on Nvit_1.0 SCAFFOLD2404) (=ref|NW_001816131.1|NviUn_WGA2404_1) Table 2).

The sequence of trypsin from both instars of *C. megacephala* has100% identity with *Chrysomya bezziana* clone CbSp22 serine protease K4.1/F1R2 mRNA, partial cds (AF302489.1) (Murshani *et al*.,2001),99% identity with *Chrysomya bezziana* clone CbSp20 serine protease K6.1/F1R1 mRNA, partial cds (AF302487.1) (Murshani *et al*.,2001),99% identity with *Chrysomya bezziana* clone CbSp13 serine protease K6.2/F1R2 mRNA, partial cds (AF302480.1) (Murshani *et al*.,2001),98% identity with *Chrysomya bezziana* clone CbSp14 serine protease K4.1/F1R2 mRNA, partial cds (AF302481.1) (Muharsini *et al*.,2001),95% identity with *Chrysomya bezziana* clone CbSp15 serine protease K17/F1R2 mRNA, partial (AF302482.1) (Murshani *et al*.,2001) and *Chrysomya bezziana* clone CbSp1 serine protease K14/F1R2 mRNA, partial cds (AF302468.1) (Murshani *et al*.,2001),94% identity with *Chrysomya bezziana* clone CbSp10 serine protease K10/F1R1 mRNA, partial cds (AF302477.1) (Murshani *et al*.,2001),93% identity with *Chrysomya bezziana* clone CbSp12 serine protease K8/F1R1 mRNA, partial cds (AF302479.1) (Murshani *et al*.,2001),93% identity with *Chrysomya bezziana* clone CbSp19 serine protease K16/F1R2 mRNA, partial cds (AF302486.1) (Murshani *et al*.,2001),91% identity with *Chrysomya bezziana* clone CbSp21 serine protease K2.2/F1R2 mRNA, partial cds (AF302488.1) (Murshani *et al*.,2001) (Table 3,).

Table 2 Sequences producing significant alignments using blast between chymotrypsin of both first and third larval instars of *C.megacephala* and other Dipterans sequences.

Accession	Description	Max. score	Total score	E value	Max. ident.
gb|AABU01002389.1|	Drosophila melanogaster 211000022280419, w... 0.0	710	710	0.0	84%
gb|AAPP01004053.1|	Drosophila ananassae strain TSC#14024-0371...0.0	704	704	0.0	84%
ref|NC_001709.1|	Drosophila melanogaster mitochondrion, compl...0.0	693	693	0.0	84%
gb|AAPT01003399.1|	Drosophila grimshawi strain TSC#15287-2541... 0.0	689	689	0.0	84%
gb|AAPU01003823.1|	Drosophila mojavensis strain TSC#15081-135...0.0	678	678	0.0	83%
gb|AAPU01001946.1|	Drosophila mojavensis strain TSC#15081-135... 0.0	673	673	0.0	84%
gb|AANI01017563.1|	Drosophila virilis strain TSC#15010-1051.8...0.0	673	673	0.0	83%
gb|AANI01007064.1|	Drosophila virilis strain TSC#15010-1051.8...0.0	667	667	0.0	85%
gb|AAQB01011230.1|	Drosophila willistoni strain TSC#14030-081... 0.0	665	665	0.0	83%
gb|AAPU01005843.1|	Drosophila mojavensis strain TSC#15081-135... 0.0	662	662	0.0	83%
gb|AANI01004754.1|	Drosophila virilis strain TSC#15010-1051.8...0.0	656	656	0.0	82%
gb|AANI01018023.1|	Drosophila virilis strain TSC#15010-1051.8...7e-171	608	608	0.0	83%

Accession	Description	Max. score	Total score	E value	Max. ident.
gb|AAPU01003365.1|	Drosophila mojavensis strain TSC#15081-135...2e-146	527	527	0.0	86%
gb|AAQB01002654.1|	Drosophila willistoni strain TSC#14030-081... 2e-136	494	494	0.0	85%
gb|AAPU01004386.1|	Drosophila mojavensis strain TSC#15081-135...1e-114	422	422	0.0	80%
gb|AANI01016988.1|	Drosophila virilis strain TSC#15010-1051.8...4e-108	399	399	0.0	80%
gb|AAPP01016269.1|	Drosophila ananassae strain TSC#14024-0371...2e-106	394	394	0.0	83%
gb|AAQB01006771.1|	Drosophila willistoni strain TSC#14030-081...5e-93	350	350	0.0	87%
ref|NW_001820656.1|NviUn_WGA916_1	Nasonia vitripennis genomic...5e-63	250	250	0.0	74%
ref|NW_001818170.1|NviUn_WGA423_1	Nasonia vitripennis genomic...1e-49	206	206	0.0	75%
ref|NW_001816131.1|NviUn_WGA2404_1	Nasonia vitripennis genomi...8e-41	176	176	0.0	73%

Table 3: Sequences producing significant alignments using blast between trypsin of both first and third larval instars of *C. megacephala* and other Dipterans sequences

Accession	Description	Max score	Total score	E value	Max ident	Links
AF302489.1	Chrysomya bezziana clone CbSp22 serine protease K4.1/F1R2 mRNA, partial cds	970	970	0.0	100%	
AF302487.1	Chrysomya bezziana clone CbSp20 serine protease K6.1/F1R1 mRNA, partial cds	929	929	0.0	99%	
AF302480.1	Chrysomya bezziana clone CbSp13 serine protease K6.2/F1R2 mRNA, partial cds	929	929	0.0	99%	
AF302481.1	Chrysomya bezziana clone CbSp14 serine protease K4.1/F1R2 mRNA, partial cds	926	926	0.0	98%	
AF302482.1	Chrysomya bezziana clone CbSp15 serine protease K17/F1R2 mRNA, partial cds	826	826	0.0	95%	
AF302468.1	Chrysomya bezziana clone CbSp1 serine protease K14/F1R2 mRNA, partial cds	824	824	0.0	95%	
AF302477.1	Chrysomya bezziana clone CbSp10 serine protease K10/F1R1 mRNA, partial cds	769	769	0.0	94%	
AF302479.1	Chrysomya bezziana clone CbSp12 serine protease K8/F1R1 mRNA, partial cds	767	767	0.0	93%	
AF302486.1	Chrysomya bezziana clone CbSp19 serine protease K16/F1R2 mRNA, partial cds	760	760	0.0	93%	
AF302488.1	Chrysomya bezziana clone CbSp21 serine protease K2.2/F1R2 mRNA, partial cds	719	719	0.0	91%	

4-Disscussion:

The analysis of native excretory/secretory products from early third instar of *Chrysomya megacephala* produces a single band at 16 KDa, a band between 16 KDa and 23 KDa and a broad band between 23 KDa and 45 KDa. Insect trypsins have been characterized and purified from species of Coleoptera, Orthoptera, Lepidoptera and Diptera. Most insect trypsins are 20–30 kDa as determined by SDS-PAGE **(Terra and Ferreira, 1994)**. These insect trypsins are most active at alkaline pH, are not activated by calcium ions, and are sensitive to natural trypsin inhibitors **(Terra and Ferreira, 1994).** Recently, workers in Nottingham, UK, demonstrated *in vitro* a range of enzymes secreted by *Phaneicia sericata* larvae **(Chambers *et al.*, 2003)**. Four proteolytic enzymes, comprising two serine proteases, a metalloproteinase and an aspartyl proteinase, were detected, with molecular weights ranging from 20 to 40 kDa, with activity across a wide pH range.

In the present study the amplified product resulting from PCR using cDNA isolated from whole first and third instars of *Chrysomya megacephala* formed a band from about 500 bp to 715 bp. Chymotrypsin isolated from whole first and third instars of *Chrysomya megacephala* showed band at 715 bp while trypsin showed band at 573 bp. At the molecular level, an analysis of a cDNA library showed 40 clones of 115–810 bp representing putative digestion-associated proteins **(Pedra *et al*., 2003).** 12 clones to trypsin (533–810 bp) and 22 clones to chymotrypsin (115–806 bp) two other clones of 691 and 710 bp were classified to belong to different serine proteinases **(Kollien *et al*.,2004).**

Chymotrypsin of both first and third instars of *C.megacephala* (Cmchy) shows a 73-75% identity with chymotrypsins of *Nasonia* species and 80-87% with chymotrypsins of *Drosophila* species. Trypsin of both instars of *C.megacephala* (Cmtry) shows 91-100% identity with trypsins of *C.bezziana*.

As serine proteinases have different functions, gene families of trypsin and chymotrypsin have been described in many insects. In the noctuid moth *Mamestra configurata* and the lesser grain borer *Rhyzopertha dominica* eight and three genes encoding trypsin were identified, respectively **(Hegedus *et al*., 2003).**

Sequences of seven trypsin-like genes of a blood-sucking crustacean, the salmon louse *Lepeophtheirus salmonis*, show high similarities in their nucleotides and corresponding amino acid residues, differences ranging from one amino acid residue between trypsin types 1 *and 4 to 18 amino acid residues between types 2 and 7* ***(Johnson et al.*, 2002)**. A blood-sucking Diptera, *Haematobia irritans exigua,* possesses at least four genes encoding trypsin **(Elvin *et al*., 1993)**. In the malaria vector *Anopheles gambiae*, seven different genes encoding trypsins are clustered within 11 kb and two genes encoding chymotrypsin within 6 kb **(Vizioli *et al*., 2001)**. In the gut of female *Aedes aegypti* one constitutively transcribed early trypsin is augmented by two blood meal-induced late trypsins **(Barillas-Mury *et al*., 1995).**

Considering chymotrypsin, at least three and eight genes are expressed in larvae of the Lepidoptera *Helicoverpa zea*, *Agrotis ipsilon* and *Mamestra configurata*, respectively **(Hegedus *et al*., 2003).**

These numbers of trypsin and chymotrypsin encoding genes represent only a minimum number, since more than twenty genes encoding trypsin were identified during genome analysis of the fruit fly *Drosophila melanogaster* **(Wang *et al*., 1999).**

Graf and Briegel (1985) identified at least 20 different trypsin isozymes in midgut homogenates prepared from blood-fed female mosquito *(Aedes aegypti).*

In bloodsucking insects, such as mosquito or stable fly, *(Stomoxys calcitrans)* the vast majority (75%-85%) of proteolytic activity involved in blood meal digestion is due to serine proteases, representing both trypsin- and chymotrypsin-like activity **(Briegel and Lea 1975)**. **Muharsini *et al*., (2001)** using PCR analysis and subsequent cloning and sequencing identified 22 different sequences falling into two trypsin and four chymotrypsin families from larvae of *Chrysomya bezziana.*

Corresponding author
Ahmed S. El-Ebiarie
Zoology and Entomology Dept., Faculty of Science, Helwan Univ., Ain Helwan, Cairo, Egypt
aselebiarie@hotmail.com

References:

Anderson, DL., Sedgley, M., Short, JRT, Allwood, AJ, (1982). Insect pollination of mango in northern Australia *Mangifera indica*, includes *Apis mellifera*. Aust. J. Agr. Res. 33 (3), 541–548.

Applebaum, SW, (1985). Biochemistry of digestion. In: Kerkut, G.A., Gilbert, L.I. (Eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 4. Pergamon Press, New York, pp.279–311.

Baer WS, (1931). The treatment of chronic osteomyelitis with the maggot (larva of the blow fly) *J Bone Joint Surg.*; 13: 438–75

Barillas-Mury CV, Noriega FG, Wells MA, (1995). Early trypsin activity is part of the signal transduction system that activates transcription of the late trypsin gene in the midgut of the mosquito, *Aedes aegypti*. Insect Biochem Mol Biol., 25:241–246.

Bowles VM, Carnegie PR, Sandeman RM, (1988). Characterization of proteolytic and collagenolytic enzymes from the larvae of Lucilia cuprina, the sheep blow fly. Aust J Biol Res;41: 269 - 78.

Bowles VM, Feehan JP, Sandeman RM, (1990). Sheep plasma inhibitors influencing protease activity and growth of *Lucilia cuprina* larvae in vitro. Int J Parasitol; 20: 169-74.

Briegel H, Lea AO, (1975). Relationship between protein and proteolytic activity in the midgut of mosquitoes. J Insect Physiol., 21 : 1597-1604.

Casu RE, Jarmey JM, Elvin CM, Eisemann CH, (1994). Isolation of a trypsin-like serine protease gene family from the sheep blow fly *L. cuprina*. Insect Mol Biol; 3:159-70.

Celniker SE, Wheeler DA, Kronmiller B, and CarlsonJW, (2007). Finishing a whole-genome shotgun: release 3 of the *Drosophila melnogaster*

euchromatic genome sequence .*Genome Biol*. 3 (12), 25-28.

Chambers L, Woodrow S, Brown A P, HarrisP D, Phillips D, Hall M, Church JC, and Pritchard D I, (2003). Degradation of extracellular matrix components by defined proteinases from the greenbottle larva Lucilia sericata used for the clinical debridement of non-healing wounds. Br. J.Dermatol. 148:14-23.

Clark AG, Eisen MB, Smith DR, and Bergma CM, (2007). Evolution of genes and genomes on the Drosophila phylogeny.*Nature*, 450 (7167), 203-218.

Elvin CM, Whan V, Riddles PW (1993) A family of serine protease genes expressed in adult buffalo fly (*Haematobia irritans exigua*) Mol Gen Genet., 240:132–139

Elvin, CM, Vuocolo T, Smith WJM, Eisemann CH, RiddlesPW, (1994). An estimate of the number of serine protease genes expressed in sheep blowfly larvae (*Lucilia cuprina*). Insect Mol. Biol. 3, 105–115.

Gabre Refaat M, Fatma K Adham, Hsin Chi, (2005). Life table of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). *Acta Oecologica* 27 , 179–183.

Gabre R M, (1994). Studies on the bionomics and medical importance of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) in Egypt. Master thesis, Cairo University, Cairo, Egypt.

Ghandour A M , (1988). Health hazards in humans and animals caused by imported livestock diseases in SaudiArabia. Fauna of Saudi Arabia. Pro. Entomologia, Basle, Switzerland. 9, 468–477.

Graf R, Briegel H (1985). Isolation of trypsin isozymes from the mosquito *Aedes aegypti*. Insect Biochem. 15: 611-618.

Graninger M, Grassberger M, Galehr E, Huemer F, Gruschina E, Minar E, (2002). Biosurgical debridement facilitates healing of chronic skin ulcers *Arch Int Med*; 162: 1906–7

Greenberg B, (1971). Flies and Disease, volume I: Ecology, classification, and Biotic Association. Princeton University Press, Princeton, New Jersey.

Greenberg, B., 1973. Flies and Disease, volume II: Biology and Disease Transmission. Princeton University Press, Princeton, New Jersey.

Greenberg B, (1988). *Chrysomya megacephala* (F.) Diptera: Calliphoridae) collectd in North America and notes on *Chrysomya* species present in the NewWorld. J. Med. Entomol. 25, 199–200.

Hegedus D, Baldwin D, O'Grady M, Braun L, Gleddie S, Sharpe A, Lydiate D, Erlandson M, (2003). Midgut proteases from *Mamestra configurata* (Lepidoptera: Noctuidae) larvae: characterization, cDNA cloning, and expressed sequence tag analysis. Arch Insect Biochem Physiol., 53:30–47.

Herrero S, Combes E, Oers MMV, Vlak JM, RAde Maagd, eekwilder J, (2005). Identification and recombinant expression of a novel chymotrypsin from *Spodoptera exigua*. Insect Biochem Mol Biol; 35:1073–82.

Hu T, Len CH, Lee BS, (1995). The laboratory rearing and radiation effects of gamma ray on the pupae of *Chrysomya megacephala* (Fabricius). Chin. J. Entomol. 15, 103–111.

Johnson S, (1999). Larval therapy in the treatment of wounds: case history *Br J Community Nurs*; 4: 293–5

Johnson SC, Ewart KV, Osborne JA, Delage D, Ross NW, Murray HM (2002). Molecular cloning of trypsin cDNAs and trypsin gene expression in the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitol Res., 88:789–796

Kuhlhorn F, (1983).Verbreitung der Toxoplasmose. Katzenkot und Dipteren.Tierarztl. Prax. 11 (3), 385–392.

Lewis DL, Farr CL and Kaguni LS, (1995). Drosophila melanogaster mitochondrial DNA: completion of the nucleotide sequence and evolutionary comparisons. *Insect Mol. Biol*. 4 (4), 263-278.

Muharsini S, Sukarsih G, Partoutomo S, Hamilton S, Willadsen P and Wijffels G, (2001). Identification and characterization of the excreted/secreted serine proteases of larvae of the Old World Screwworm Fly, *Chrysomya bezziana*. *Int. J. Parasitol.* 30, 705–714.

Norris KR, (1965). The bionomics of blowflies. Annu. Rev. Entomol. 10, 47–68.

O'Connell AR, Lee BW, Stenson-Cox C, (2006). Caspase-dependant activation of chymotrypsin-like proteases mediates nuclear events during Jurkat T cell apoptosis. Biochem Biophys Res Commun; 345: 608–6.

Pedra JHF, Brandt A, Li H-M, Westerman R, Romero-Severson J, Pollack RJ, Murdock LL, Pittendrigh BR (2003). Transcriptome identification of putative genes involved in protein catabolism and innate immune response in human body louse (Pediculicidae: *Pediculus humanus*). Insect Biochem Mol Biol 33:1135–1143.

Rawlings ND, Barrett AJ, (1994). Families of serine peptidases. Methods Enzymol; 244: 19–61.

Rhoads ML, Fetterer R H, (1997). Extracellular matrix: a tool for defining the extracorporeal

function of parasite proteases. Parasitol. Today 13, 119–122.

Sandeman RM, Feehan JP, Chandler RA, Bowles VM, (1990). Tryptic and chymotryptic proteases released by larvae of the blow fly, *Lucilia cuprina*. Int J Parasitol; 20:1019-23.

Sherman RA and Shimoda KJ, (2004). Presurgical maggot debridement of soft tissue wounds is associated with decreased rates of postoperative infection *Clin Infect Dis*; 39: 1067–70

Sherman RA, (2003). Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy *Diabetes Care*; 26: 446–51

Sudeshna Mazumdar-Leighton, Roxanne M, (2001). Broadway Identification of six chymotrypsin cDNAs from larval midguts of *Helicoverpa zea* and *Agrotis ipsilon* feeding on the soybean (Kunitz) trypsin inhibitor. Insect Biochemistry and Molecular Biology 31: 633–644 www.elsevier.com/locate/ibmb

Sulaiman S, Sohadi AR, Jeffery J, (1989). Human helminth parasite burdens on cyclorrhaphan flies (Diptera) trapped at an aboriginal settlement in Malaysia. Bull. Ent. Res. 79 (4), 625–629.

Sulaiman S, Sohadi A R ,Yunus H, Iberahim R, (1988). The role of some cyclorrhaphan flies as carriers of human helminths in Malaysia. Med.Vet. Entomol. 2 (1), 1–6.

Tellam RL, Bowles VM, (1997). Control of blow fly strike in sheep: current strategies and future prospects. Int J Parasitol; 27:261±73.

Terra WR, Ferreira C, (1994). Insect digestive enzymes: properties, compartmentalization and function. Comp. Biochem. Physiol. 109, 1–62.

Terra WR, Ferreira C, Jordao B P, Dillon RJ, (1996). Digestive enzymes. In: Bilingsley, P., Lehane, M. (Eds.) Biology of the Insect Midgut. Chapman and Hall, London, pp. 159–194.

Thomas S and Jones M, (2000). Maggots can benefit patients with MRSA *Pract Nurse*; 20: 101–4

Thomas S, Jones M, Shutler S, Jones S, (1996). Using larvae in modern wound management *J Wound Care*; 5: 60–9

Tomberlin JK, Reeves WK, Sheppard DC, (2001). First record of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) in Georgia, USA. Fla. Entomol. 84 (2), 300–301.

Vizioli J, Catteruccia F, della Torre A, Reckmann I, Müller HM, (2001). Blood digestion in the malaria mosquito *Anopheles gambiae*. Eur J Biochem 268:4027–4035.

Wang S, Magoulas C, Hickey D, (1999). Concerted evolution within a trypsin gene cluster in *Drosophila*. Mol Biol Evol 16:1117–1124.

Weil GC, Simon RJ, Sweadner WR. A biological bacteriological and clinical study of larval or maggot therapy in the treatment of acute and chronic pyogenic infections *Am J Surg* 1933; 19: 36–48

Wells JD, (1991). *Chrysomya megacephala* (Diptera: Calliphoridae) has reached the continental United States: review of its biology, pest status, and spread around the world. J. Med. Entomol. 28 (3), 471–473.

Zumpt F, (1965). Myiasis in Man and Animal in the OldWorld; a Text Book for Physicians, Veterinarians, and Zoologists. Butterworths, London.

8/20/2012

Investigation of the relationship between real option method and escalation of commitment in capital budgeting

Farshid Ahmadi Farsani

[1.] Department of Accounting, Azad University of farsan, Iran
Ahmadi.farshid2@gmail.com

Abstract: The major goal of the current study is to investigate whether using the real option method along with the discounted cash flow techniques can reduce the decision-makers' escalation of commitment(EC hereafter) or their desire to keep up their commitment to a failed project. . The sample size was 50. Data were gathered via the use of questionnaries. The instrument's validity and reliability were confirmed based on the opinions of a panel of experts and Cranbach's Alpha coefficient respectivety. The results revealed that using the real option method in capital budgeting can affect the users' behavior and decisions and lead to better decision-making in the long-term projects.
[Farshid Ahmadi Farsani. **Investigation of the relationship between real option method and escalation of.** *Life Sci J* 2012;9(3): 2094-2099]. (ISSN: 1097-8135). http://www.lifesciencesite.com.302

Keywords: Investment projects evaluation, Real option method, Discounted cash flow, Capital budgeting, Escalation of commitment

Introduction

The aim of this paper is to investigate whether the decision-makers who use the real option method together with other discounting methods such as net present value in capital budgeting are less likely to show escalation of commitment (EC hereafter) towards a failed project than those who solely use the conventional and discounting methods. EC occurs when despite receiving negative feedback from a project, the decision-makers raise their commitment to that project to make sure it won't be abandoned (TeachT,2003). The situations in which people show EC to a failed project depend on many conditions and processes. One of such situations in which people have the potential of commitment rise is capital budgeting, because in capital budgeting there are two factors which facilitate EC; that is, uncertainty and accountability for measures taken within the project.

Real option method measures the value of the project, taking into consideration all current option available to the management, such as the abandonment, relinquishment or continuance of the project and the expected cash flow for each option. This method is applied where there is uncertainty regarding an action. It is used to assess the management flexibility in case of uncertainty. Coperl and Staw and Teach believe the use of real option method leads to better decision-making (Copeland and Antikarov, 2001; Staw, 1976; Teach, 2003).

These arguments are based on the fact that the use of this method improves the quality of the management's available information. Coy, however, argues as there is no specified date for exercising the option of abandoning the project, the managers might abandon the project before or after its optimal time and this, in turn, may rather worsen than improve the problem of EC (Coy, 1999).

Little research has been conducted on the EC in capital budgeting. Moreover, the results have not made it clear whether using real option method in a failed project has any effect on EC. No experimental research within Iran's economic, cultural and social context has directly considered this question. Besides, the use of the managers who are practically involved in capital budgeting has not been given proper consideration. This research intends to investigate these matters using an experimental method.

Theoretical bases and hypotheses development:

Theoretical bases

Capital budgeting: Capital budgeting is among the strategic decisions in the management of a company. Such decisions are of utmost importance because they remain in effect for several periods of time and consume a great portion of the company's funds. Making such decisions requires careful consideration and analysis.

Escalation of Commitment:

EC in a failed project occurs when despite receiving negative feedback on the last decision, the decision-maker raises his commitment to the project (Staw, 1976). EC, regardless of whether the decision yields positive or negative results, refers to the the activities done without the consideration of the decisions taken at the decision-making stage.

Many accounting studies have focused on EC in the field of capital budgeting and have investigated the affective factors in the reduction of EC. Researchers have studied a number of the potential

factors influencing EC. These factors can be classified into three groups:

- Personal justificatory hypotheses stating that decision-makers escalate their commitment for that stage of activity which justifies their initial choice
- Prospect theory that indicates the decision-makers who receive negative feedback and consider themselves to be in a counterproductive system are motivated to exhibit riskier dynamic behavior; and
- Other factors including sunk costs, the extent to which the project has been completed and evident and real desire to avoid wasting capital and time (Staw, 1976)

It has generally been agreed that EC is created by several factors not merely one particular factor (Ross, 1995).

Real option method:

The first use of the term "real option" dates back to thirty years ago and refers to the application of the techniques of evaluating the options in real investment scenarios. The analyses regarding the real option evaluation basically involve considering the potential decisions which might be taken during the implementation of a project and the best possible reaction of the management to each of these decisions. To gain the final value using the real option method, the manager needs to estimate the net present value of different options such as relinquishment or implementation of the project and then, considering the weighted mean of the possible results of each option, he should determine the final value of the project with respect to the probability of the occurrence of each option. The final value of a project which has been calculated using real option method is always either larger than or as large as the final value determined by net present value. The difference between the obtained values using these two methods reflects the flexibility value at the management's disposal (Bargh and Ferguson, 2000).

literature Review

Wolf and Conlon conducted a study to find out whether the method and indices of evaluation can reduce EC. Results showed that only along with other factors, rather than alone, do evaluation methods show a significant effect on EC (Conlon and Wolf, 1980).

Ross, Coy, Coperland and Antikarov studied separately the use of real option methods with discounting and conventional methods. The results of these researches showed the use of real option method together with discounting and conventional methods leads to better decision-making. They argued that using the real option method improves the quality of the information at the managers' disposal (Copeland and Antikarov, 2001; Coy, 1999; Ross, 1995).

Newton *et al.* (2000) discussed the advantages of the use of real option method and concluded that using the real option method along with other methods has some merits. Bargh and Ferguson (2000) showed the construct accessibility has a significant effect on the decision-makers' understanding, behavior and their use of received information. Moon discovered in his research that to decide about the EC, the decision-makers consider the factors related to the future of the project (the completion rate) and also those related to the past (sunk costs); so that the higher the sunk costs, that is at the final stages of the project, the greater the need felt for EC. Besides, the higher the project's completion rate, the greater the need for continuing and completing the project (Henry, 2001).

A research, titled "investment option, base rates and discounted cash flow techniques" was carried out by Dastgir in England. Results showed that using the real option method together with other conventional and discounting methods offers an appropriate tool for decision-making (Dastgir, 2004).

Xin and Mittal found out in their empirical research that the closer the project is to the end, the greater the need to finish the project and the less great the need for information for completing the project are and, vice versa, the earlier stage the project is at, the greater the need for information for completing the project is. Therefore, at the middle stages of the project, the decisions about EC are the most sensitive and the more risky the decision, the less the desire for EC (Xin and Mittal, 2007).

Research questions and hypotheses:

Research questions are as follows:

- Does the real option method affect the EC in capital budgeting?
- How does the use of real options in capital budgeting affect the managers and decision-makers' behavior?
- Does there exist any relation between partial judgment and the acceptance of EC in capital budgeting using real option method?

In response to the above question the following hypotheses were put foreward:

Hypothesis 1:

when the real option method is applied explicitly to the evaluation stage of the project, it is less likely that the decision-makers show EC to the

failed project than when only discounting and conventional methods are used.

Hypothesis 2:

gaining an understanding of the construct accessibility of possibility of abandoning the project early changes the relation between the capital bugeting technique used by a decision-maker and his EC towards a failed project.

Experimental method

Research method:

This is an experimental research in which there are two groups: experimental and control.

Population and Sample:

In current research after identifying the total number of thefinancial managers in 2012, a sample of 50 financial managers of contracting companies were randomly selected as the representatives offinancial managers. Moreover, all the participants were graduates in accounting and management. They had an average work experience of 10 years in capital budgeting.

Measurement tools:

The required data in this study were collected through a questionnaire comprised of three general sections. The first section presented a general picture of the research topic and also general information about the participants including their age, sex, major of study and the latest academic degree and experience.

The second section consisted of two different parts. The former involved a brief explanation on the applied capital budgeting methods (real option or net present value), how to perform calculations and how to make decisions according to these methods. In the latter part, the manner of calculation in the methods and decision-making in capital budgeting were elaborated and exemplified. The third section was constituted of three parts. In the first part the hypothetical project was explained to the participants and then they were asked to comment whether they would accept or reject the project giving a rating from 0-100. In the second part, a hypothetical problem during the implementation of the project was posed to participants and they were asked to decide whether to continue or abandon the project and then asked to comment on a scale of 0-100. In the third part the participants were required to answer 9 six- choice questions (0-5) about the hypothetical project.

After developing the questionnaire and testing its validity, both the experimental and control groups were provided with the information about the questionnaire. In the questionnaire the capital budgeting method and the time were manipulated in two stages as independent variables. At the first stage both the experimental and control groups (each containing 50 participants) were told that the company in question was using the net present value method in the project. Both groups were given 90 minutes to answer the questions. The second stage was conducted with just the experimental group and the control group left. The experimental group were told that the company was using the real option method in the project evaluation. Time, the second independent variable, was measured at two stages. First, at the stage of the acceptance of the project and then at the stage of the recommendation to continue the project after the problem is posed.

The questionnaire had two dependent variables. The first was the Recommendation to Continue the Project and the second was the acceptance of the project which both were answered on a 101-point scale of 0 (completely disagree) to 100 (completely agree). The second dependent variable was the relation between the score of the recommendation to continue the project and EC. The higher the mentioned score, the higher the EC. EC is often measured by money. In this study, however, it is measured by recommendation.

The two questionnaires were the same in terms of the information given to the participants but were different in the applied methods.

Validity and reliability of the questionnaire:

In order to evaluate the reliability of the research tools, Cronbach's alfa coefficient method was used. The calculated Chronbach's alfa Coefficient in this research was %90which is indicative of the enough reliability of the research tools. Following the preliminary development of the measurement tools, during the assessment, in order to evaluate the validity of the questionnaire, it was commented on by the experts and commentators. At the stage of the assessment of the measurement tools, the questionnaire was once more distributed among a number of experts and commentators in order to use their suggestions for improving the questionnaire. Thus the content validity of the questionnaire seems to have been met.

Moreover, given that in this research it is predicted that the people who use the real option method with other methods show less EC in comparison with those who do not use this method, the confirmation of the this hypothesis will be indicative of the construct validity of the measurement tools.

Statistical methods of the research:

The statistical methods used in this research were descriptive and inferential statistics including mean, variance, t test and one-way analysis of

variance. In order to make a comparison between the means of the scores of the two groups in the research hypotheses, t test was used. It is worth mentioning that in the hypotheses of this research the significance level was $\alpha = \%5$.

RESULTS

Test of Hypothesis 1:

The first hypothesis proposed when the real option method is applied explicitly to the evaluation stage of the project, it is less likely that the decision-makers show EC to the failed project than when only discounting and conventional methods are used. To test this hypothesis we use descriptive and inferential statistics.

Table 1 shows the results of the descriptive and inferential statistics of the first hypothesis.

In Table 1 the mean of the participants' given scores to the second question of the second part of the questionnaire was analyzed. This question asked the participants to determine the probability of the continuation of the project. To answer this question a 101-point difference spectrum (0-100) had been used so that 100 indicated "completely" agree and 0 denoted "completely disagree". 50 members of the control group and 71 of the experimental group answered the mentioned question. The mean scores for the control and experimental groups were 68 and 34, respectively. t statistic was -3.15and the error level stood at 0.03. Table 2 has a more detailed look at the second question and presents the number and percentage of the participants who recommended the continuation of the project. According to Table 2, 36 members of the control group and 7 of the experimental group had recommended continuing the project. Regarding the fact that lower number of the participants in the experimental group recommended the continuation of the project in relative to the control group, it might be concluded that the real option method is more precise than the conventional and discounting methods.

With respect to Table 1 and Table 2, because the significance level of this question is 0.03, it can be claimed with a confidence level of over %95 that the people who apply the real option method at the evaluation stage of the long-term projects exhibit lower EC in the event of the project failure compared with those who only use the conventional and discounting methods. The reason may be that net present value method considers only the expected value of the future cash flows and, unlike the real option method, does not take into regard the option of abandoning the project before its completion. The above hypothesis is supported by the current theories in this field.

Table 1: The mean of the given scores to the 2nd question by the participants:

	Capital budgeting Method	
	Real option	Net present
Participants	25	50
The mean of scores	33	71
t-statistics	-3/15	
Error level	**0/03**	
Test result	Pass	

Table 2: The percentage and number of participants who recommended continuing the project

	Capital budgeting method	
	Real option	Net present value
Total number	25	50
Number	N=7	N=36
percentage	28%	72%

Test of Hypothesis 2:

The second hypothesis put forth that: "gaining an understanding of the possibility of abandoning the project, changes the relation between the capital budgeting technique used by a decision-maker and his EC towards a failed project."

To test the second hypothesis, a path analysis diagram drawn by Amos software was used. This diagram measures the direct effect of the capital budgeting methods (recommendation to continue the project) and also their indirect effects (recommendation to continue the project) on EC, through through the effect of the capital budgeting (Capbud) on the construct accessibility of possibility of abandoning the project only (CAPA) and EC or the Recommendation to Continue the Project (RCP). Figure 1 presents the results of testing the second hypothesis through path analysis.

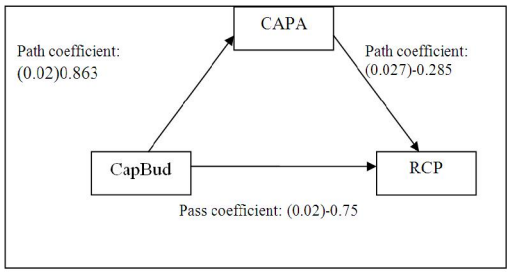


Fig. 1: Path analysis diagram

In Fig. 1 the numbers inside the parentheses are the error levels and the numbers outside are path coefficients. In the path analysis diagram the effect of the path coefficient of the capital budgeting method on the recommendation to continue the project is directly meaningful and this shows that the participants who

used the real option method are less likely to recommend continuing the project compared to those who used the conventional and discounting methods.

Questions 5 and 6 of the third part and question 2 of the second part of the questionnaire are contained in Table 3. (The mean scores of questions 5 and 6 are on the scale of -5-5 and that of question 2 is on the scale of 0-100).

Table 3: Questions 2,5 and 6 of the third part and question 2 of the second part of the questionnaire

---

Question 2 (the second part): As the responsible manager mark the questionnaire to determine the probability of continuing the project

---

The mean of the experimental group	34
The mean of the control group	72
Error 0.030	

Question 5 (the third part): I had considered the possibility of the failure of the project before accepting it

---

The mean of the experimental group	1.7
The mean of the control group	-0/1
Error 0.056	

Question 6 (the third part): I had considered the possibility of the project failure before recommending continuing the project

---

The mean of the experimental group	1.5
The mean of the control group	0.4
Error 0.010	

The effect of the variable of capital budgeting methods on the Construct Accessibility of Possibility of Abandoning the project (CAPA) is 0.863 which is meaningful at the confidence level of over %99. The effect of the variable of CAPA on the variable of the Recommendation to Continue the Project (RCP) is -0.285 which is meaningful with the confidence level of more than %95. Therefore the indirect effect of the variable of the capital budgeting methods on the variable of the RCP is (0.863, -0.285)-0.159.

These effects are completely in line with the third hypothesis. This means there is a meaningful positive relation between the capital budgeting methods and the construct accessibility of possibility of abandoning the project and there is, on the other hand, a meaningful negative relation between the construct accessibility of possibility of abandoning the project and the recommendation to continue the project; so that, there is a probability of recommending continuing the project and, in fact, the reduction in EC on the part of those who use real option method because they consider the earlier abandonment of the project. Thus the second hypothesis is confirmed.

CONCLUSION

This research uses an experimental method to investigate whether considering real options in capital budgeting has any effect on EC in a failed project. The results showed the people who use the real option method at the stage of initial evaluation are less likely to decide to continue the project in case the project fails than those who use solely net present value method. The results also showed that the reduction in EC in case of using real option method is because of the increased construct accessibility of possibility of abandoning the project. In other words, the principal factor in the reduction of EC is this point that the project can be abandonment before the completion so that the sunk costs can be somewhat recovered.

Considering the established higher efficiency of the real option method than other conventional and discounting methods in this research, it is suggested that those who are somehow responsible for the acceptance and implementation of the projects use this method along with other methods. The central bank, for instance, can notify other banks to do so in order to make better decisions about granting loans and credits. Moreover, with respect to the importance

of this issue, it is also suggested a chapter with the title of "real option method" be included in the course of capital budgeting in financial management lessons at undergraduate level.

This research faced some limitations which must be considered in the generalization of the results. While the experimental studies in social sciences have yielded useful results in different fields, it must be noted that these results are obtained in the conditions of laboratory environment and, therefore, drawing conclusion from these results should be done with enough care and consideration.

References

1- Bargh, J.A. and M.J. Ferguson, 2000. Beyond behaviorism: On the automaticity of higher mental processes. Psychological Bull., 126: 925-945.
2- Conlon, E. and G. Wolf, 1980. The moderating effects of strategy, visibility and involvement on allocation behavior-An extension of Staw's escalation paradigm. Organiz. Behav. Human Perform., 26: 172-192.
3- Copeland, T.E. and V. Antikarov, 2001. Real options: A practitioner's guide. New York: Texere, 40: 100-110.
4- Coy, P., (1999). Exploiting uncertainty: The "real-options" revolution in decision-making. Business Week Online, pp: 70-78.
5- Dastgir, M., 2004. Investment option of base rates and the techniques of discounted cash flows. The examinations of accounting and audit.
6- Henry, M., 2001. Looking forward and looking back: integrating completion and sunk cost effects within an escalation of commitment progress design. J. Applied Psychol., 86: 104-113.
7- Newton, D., D. Paxson and M. Widdicks, 2000. Real R and D Options. Int. J. Manage. Rev., 5: 113-120.
8- Ross, S., 1995. Uses, abuses and alternatives to the net-present- value rule. Financ. Manage., 24: 96-102
9- Staw, B.M., 1976. Knee-deep in the big muddy: A study of escalating commitment to a chosen course of action. Organiz. Behav. Human Perform, 160: 16-44.
10- Teach, E., 2003. Will real options take root? Why companies have been slow to adopt the valuation technique. CFO Magazine, pp: 34-450.
11- Xin, H. and V. Mittal, 2007. The effects of decision risk and projects stage on escalation of commitment. Organiz. Behav. Human Decision Processes, 103: 225-237.

8/20/2012

Impacts of Different Exercise Intensities on Hematopoietic Stem Cells and Certain Physiological Parameters on Handball Players and Non-Athletes

Mohamed Nader Shalaby [1], Jin Yu Liu[2], Mohamed Saad[3] and Hossam Elaraby[4]

[1]Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China and Department of Sports Science, Faculty of Physical Education, Port Said University, Egypt
[2]Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China,
[3]Faculty of Physical Education, Suez Canal University
[4]Faculty of Physical Education Port said University ,Egypt
dr.m.nader@hotmail.com

Abstract: Exercise is one of the most powerful non-pharmacological strategies, which can affect nearly all cells and organs in the body. Changes in the behavior of adult stem cells have been shown to occur in response to exercise. **Hypothesis:** Exercise may act on regenerative potential of tissues by altering the ability to generate new stem cells and differentiated cell that are able to carry out tissue specific functions. The purpose of this study was to Impacts of different exercise intensities on hematopoietic stem cells and certain physiological parameters on handball players and non-athletes.Twenty healthy male handball players aged (19-23yrs.) were recruited for this study. Healthy, low active males and BMI matched participants (n=10) aged 21-23yrs.were recruited as controls. Aerobic and anaerobic exercises were performed on a cycle ergometer. The testing wasa modification of the Astrand Rhyming protocol for Vo2max. Pulserate estimation, RBCs, WBCs, HB and Hematocrit were estimated using a coulter counter. Lactate by Accusport, CD34+ stem cells were determined by flow cytometry. Results indicated:Vo2 max was higher during aerobic compared to anaerobic exercise. Lactate concentration decreased in aerobic compared to anaerobic exercise bouts. H, RBCs,WBCs, and Hematocrit increased after both types of exercise bouts. The increase in CD34+ stem cells during anaerobic exercise bouts was greater than it was during aerobic exercise bouts. It is concluded that these findings deserve further study.

[Mohammed Nader Shalaby, Jin Yu Liu, Mohamed Saad and Hossam Elaraby. **Impacts of Different Exercise Intensities on Hematopoietic Stem Cells and Certain Physiological Parameters on Handball Players and Non-Athletes.** *Life Sci J* 2012;9(3):2100-2105]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 303

Key words: Aerobic and anaerobic exercise bouts, CD^{34+}stem cells, physiological parameters.

1. Introduction:

Exercise is one of the most powerful non-pharmacological strategies, which is able to affect nearly all cells and organs in the body. In this context, a new research avenue focusing on the action of exercise on adult stem cells has emerged during the last decade. Changes in the behavior of adult stem cells from different regions including skeletal muscle and the cardiovascular system have been shown to occur in response to exercise. Jogging is an endurance exercise. In contrast, resistance exercise, such as weight lifting, involves short periods of contractile activity against high resistance. On the other hand, sprint exercise consists of short periods of maximal contractile activity against low resistance. For example, a competitive 50m swim is a sprint exercise (Mougios, 2006).He also suggested an alternative way to describe exercise type using the terms aerobic and anaerobic. Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes that do not require oxygen.

Although exercise is considered a physiological stimulus for cell release by the bone marrow (Brenner *et al.,* 1998), surprisingly few data are available on circulating hematopoietic precursors in athletes. Erythrocyte production was studied relative to athlete's anemia (Szygula, 1990) and to assess the effect of intermittent hypoxic exposure on exercise performance (Baily and Davies, 1997). Conversely, little is known about the effect of exercise on myeloid precursors. For many years, it was reported that colony forming cells in peripheral blood increased after a short and intense exercise bout in normal subjects (Harrett *et al.,* 1978), but a detailed characterization of hematopoietic precursors in well trained subjects was never obtained. (Bonsignore *et al.,* 2002).

The rationale to study myeloid precursors in athletes is that intense and prolonged exercise increases white blood cell (WBC) and neutrophil counts (Nieman, 1997 and Brenner *et al.,* 1998)

Stem cells are not specialized and incomplete division was no similarity of any specialized cell. But are able to form an adult cell is divided after several divisions in appropriate circumstances, and the importance of these cells comes from being unable to form any kind of specialized cells after grow and develop into cells is required(Laufs *et al.,* 2004).

Thus, stem cells, in turn, depend on the so-called "old fetal" of the body. These are stem cells that have the ability to become anything. Then, there are the stem cells "college ability" that can become one of several types of tissue. There are also adult stem cells that can proliferate to create a special texture to the body such as the liver, bone marrow, or skin, etc. Thus, with each step toward adulthood, the successes achieved by the stem cells are narrower, leading to specialization. During adulthood, do not regenerate liver cells, but other liver cells, skin cells, generate another. Impacts of different exercise intensities on hematopoietic stem cells and certain physiological parameters on handball players and non-athletes. However, recent research indicates that the amount of cells can be manipulated to return back and enable to produce various tissues, such as conversion of bone cells to produce muscle tissue. There are two forms of stem cells: embryonic and adult stem cells. (Rehman *et al.,* 2004;Barrett *et al.,* 2010).

In healthy, moderately trained subjects, an acute bout of moderate to high intensity endurance exercise has been shown to increase EPC number, EPC migration, and colony forming units. (Laufs *et al.,* 2005).

The Aim of this study

Is to reveal the effect of physical activity on the support and enhance the natural behavior of stem cells in the body:

Impacts of different exercise intensities on hematopoietic stem cells and certain physiological parameters on handball players and non-athletes.

2. Material and Methods

Participants:

Twenty healthy male Handball players, aged 19-23yrs., with a training history of 3-10yrs. were recruited for this study. Handball players are required o participate in low to intense exercise bouts more than 3 days/week. Healthy, low active male and BMI-matched participants (n=10) aged 21-23yrs. were recruited as controls. Control subjects could not be participating in or have had a recent history of regular low to intense exercise. Participants were screened and asked to fill out health and physical activity history questionnaires.

All participants were nonsmokers, non-diabetic and free of cardiovascular, lung, and liver disease. Participants did not take any medications that could affect EPC number or function. These included statins, angiotensin II receptor antagonists, ACE inhibitors; peroxisome proliferators activated receptor (PPARα) agonist and EPO.

Testing procedures

Written informed consent was obtained for all participants, and the study was approved by the University of Suez Canal institutional review board. All participants engaged in a preliminary screening visit to evaluate resting blood pressure and fasting blood chemistry profile, and to rule out the presence of cardiovascular disease and assess and obtain samples of blood for analyses and BMI testing.

They were given a weight data log and instructed to weigh themselves in the morning and evening and record their weight in the log. All participants refrained from caffeine, vitamins, and any medications 48 hours prior to testing. Participants were provided with a log book to record their food intake for the three days prior to testing.

Aerobic and anaerobic tests were performed on a cycle ergometer under the supervision of a physician. Heart rate and blood pressure were monitored continuously throughout the tests. The testing was a modification of Astrand Rhyming protocol, until the subject exhaustion.

Maximal oxygen consumption (VO_{2max}) is the maximal rate at which the body can consume oxygen during exercise (Davis *et al.,*1976). The test of maximal oxygen consumption is an example of both low and high intensity exercise (50 watt increment, 3 min stage protocol in aerobic exercise 25 watts each as for anaerobic exercise 100 watt increment, 30 second stage protocol by adding 50 watts each). The incremental exercise is used by bicycle ergometer against increasing intensities until volitional fatigue. The Astrand Rhyming nomogram for estimating Vo_{2max} to use the nomogram for cycle ergometry exercise, a line is drawn connecting the gender specific heart rate to the specific workload (kg/min). When this straight line intersects the diagonal Vo_{2max}, the line represents the Vo_{2max} value.

The predicted VO_{2max} value is obtained by connecting the point on the VO_{2max} scale with the corresponding point on the pulse rate scale.

RBCs, WBCs, HB and Hematocrit values were estimated using a coulter counter.

The human erythrocyte, which is the mature unit of the red blood corpuscle, is a circular, elastic non-nucleated, biconcave disc, whose primary function is to transport hemoglobin.

Hemoglobin is a protein made up of 200 to 300 million nearly spherical molecules in each red blood cell, having a molecular weight of 64.458 based on the chemical structures of its alpha and beta chains.

Hematocrit (the packed cell volume) is the percentage of the total volume of whole blood that is occupied by packed red blood cells when a known volume of whole blood is centrifuged at a constant speed for a constant period.

White blood corpuscles (leukocytes) include all white cells of the blood: lymphocyte, monocyte neutrophil and basophil and eosinophil.(Guyton and Hakk, 2006).

All blood cells were counted using a coulter counter, with which numerical values may be easily read.

Lactate analyses were performed using Accusport before and after the tests by venipunture:

Circulating progenitor cell number:

CD^{34+} (HPc, hematopoietic progenitor cell number was determined by flow cytometry. For this assay. 0.5 ml of blood was collected into an EDTA-coated tube.

Mononuclear cells were separated via density centrifugation. Cells were washed and counted with a hem cytometer.

Mononuclear cell were immunostained with monoclonal antibodies against human CD^{34+} for each group of analyses, and one set of control tubes for machine calibration was generated. Flow cytometry was performed in the specialized laboratory.

The forward- side-scatter plot was used to identify the lymphocyte gate. 100.000 events per sample were acquired. Total cell count was averaged.

The following principle, clinical applications, precautions and methodology were as follows:

IOTest CD34-PE:

The use of the fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD^{34+} antigen present in human biological samples using flow cytometry.

Principle

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes.

Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis, and the leucocytes, which are unaffected by this process, are analyzed by flow cytometry.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed to distinguish the positive lystained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

Note: In all cases, keep the preparations between 2 and 8°C and protected from light.

Height and weight were recorded, and body mass index, BMI (kg/m2), was calculated for all subjects.A BMI score less than 20 is considered underweight, 20 to 24.9 is considered desirable, 25 is considered overweight, and greater than 30 is considered obese.

Statistical Analyses

Student's t-tests were used to test for significant differences between Handball players and control groups and between aerobic and anaerobic groups. When data did not meet the assumption of normality, nonparametric Mann Whitney u-test (Wilcoxon rank sum test) was used to compare differences between groups. In these cases, for descriptive data, the median (lowest value-highest value) are displayed. Differences among groups were tested using a analysis of variance (ANOVA). For parameters with non-normal distributions, nonparametric Spearman correlation coefficients were used. An *F*-test was used to test 3 groups. An α level of 0.05 was used to indicate statistical significance.

3. Results

Subjects characteristics:

Twenty Handball players and 10 low active control males participated in the study. Groups were matched for age, weight, and height (Table 1). Also, for BMI, non-significant changes in basic characteristics were examined to compare Handball player and control males. Pulse rate and VO_{2max} showed significant changes (Table 1), i.e., expected Handball players had lower pulse rates compared to controls. Physical activity questionnaire data revealed that Handball player exercised an average of 5±0.5 days a week for 5±0.2 years.

Control group participants did not exercise regularly, nor did they have a recent history of physical activity.

Table (1): Basic characteristics

Variable	Handball players n=20			Control n=10			Sig.
Age (yr.)	21.6	±	1.83	20.6	±	0.89	NS
Height (cm)	163	±	2.18	168.1	±	1.12	NS
Weight (kg)	73	±	2.16	69	±	1.5	NS
BMI	21	±	1.3	25	±	2.5	NS
Pulse rate (count/m)	68	±	2.3	74	±	2.1	S
VO_{2max}(ml/kg)	52	±	1.8	40	±	1.9	S
Lactate (mmol/L)	1.1	±	0.02	1.5	±	0.05	NS

Values are means ±SE P<0.05 BMI = body mass index

Table (2): CD^{34+} in aerobic and anaerobic exercise bouts and control

	CD^{34+}						ANOVA	
	Range			Mean	±	SD	F	*P*-value
GI (anaerobic exercise bout) n=10	230	-	379	290.5	±	54.8	29.35	0.001
GII (aerobic exercise bout) n=10	138	-	213	192	±	32.9		
Control n=10	120	-	190	159	±	22.4		
Tukey's test								
GI (anaerobic) VS GII (aerobic)	GI (anaerobic) VS control			GII (aerobic) VS control				
0.001	0.001			0.999				

Hematopoietic stem cells:

Data for CD^{34+} number. There were significant differences between athletes after anaerobic exercise bout compared to aerobic and the control (Table 2).

Table (3): Revealed data of CD^{34+} (SC) and lactate after exercise bout aerobic and anaerobic

Variable	Aerobic exercise bout			Anaerobic exercise bout		
CD^{34+} (HPc) cells	168	±	20.4	292.1	±	63.1
Lactate (mmol/L)	4.2	±	0.7	7.2	±	0.9

Table (3) Data for CD^{34+} number. There were significant differences between Handball player after aerobic and anaerobic exercise bouts(Table 3).

Lactate increase significantly after anaerobic bouts; values are means +SE $P<0.05$.

Table (4): Hematological values of RBCs, WBCs, Hematocrit (PCV) and hemoglobin in control and athletes at rest.

Variable	Control			Athletes			Sig
RBCs (million/mm3)	5.9	±	0.9	4.1	±	0.6	NS
WBCs (thousands/ mm3)	4.3	±	0.6	6.3	±	0.9	NS
HB (g/dL)	11.4	±	0.6	15.2	±	0.9	NS
Hematocrit (%)	41	±	3.1	44.2	±	3.1	NS

$P< 0.05$ mean ± SE.

Table (4) Revealed NS change in case of participants of control and athletes groups at rest in hematological values.

Table (5): Hematological values of RBCs, WBCs, HB, and Hematocrit (PCV) in aerobic and anaerobic exercise bout

Variable	Aerobic			Anaerobic			Sig
RBCs (million/mm3)	5.8	±	0.5	6.1	±	0.2	S
WBCs (thousands/ mm3)	5.3	±	0.7	7.2	±	0.6	S
HB (g/dL)	14.2	±	0.9	16.1	±	0.9	S
Hematocrit (%)	43	±	1.4	46	±	1.6	S

Table (5): Revealed a significant change in participants after aerobic and anaerobic bouts of exercise in hematological values $P< 0.05$.

Table (6): The variation in VO_{2max} for participants healthy sedentary, aerobic and anaerobic exercise bouts.

Participants	VO_{2max} (ml/kg/min)		
Healthy sedentary (ml/kg/min)	40	±	2.3
Aerobic exercise (ml/kg/min)	58	±	2.6
Anaerobic exercise (ml/kg/min)	53	±	2.3

The results are expressed as mean ± SE ($P<0.05$).

Table (6) VO_{2max} (ml/kg/min) results indicated an increased value between the healthy sedentary participants and after both aerobic and anaerobic exercise bouts.

4. Discussion:

The data presented indicates that lactate concentrations (Table 1) were in the normal range with no significant changes in both groups (control and handball player).

The concentration of lactate was higher following anaerobic exercise than it was following aerobic exercise (Table 3). Such an increase in lactate may be a result of a greater decrease in oxygen during intense anaerobic exercise.

In the case of intense exercise, which can be defined as any intensity that exceeds an individual's capacity to maintain a steady state condition, ATP regeneration must be met by creatine phosphate hydrolysis and by glycolysis, terminating in the production of lactate and the eventual development of acidosis. Intense exercise can be performed in many ways, such as the intense exercise of sprint, swimming, cycling, or in incremental exercise (Robergs and Roberts, 1997), lactate and protons leave the muscle fiber by a similar mechanism of incremental exercise. Roth and Brooks (1989) presented the kinetics of a lactate transporter and have shown that it is a saturated transport process. It is believed that protons leave the muscle in combination with the lactate transporter (MCTs) via facilitated transport (Stanley *et al.,* 1985),which accounts for similar changes in blood lactate and acidosis during intense exercise.

During prolonged exercise, muscles and blood lactate concentration peak a few minutes after the start of exercise of moderate to low intensity and drop slightly as exercise continues. After the end of exercise, both gradually return to baseline values (Fitts, 2004).

Vo_{2max}. values range from those of persons with extremely low capacities, such as chronically ill individuals (< 20 ml/ kg/min), to those of well-trained and elite endurance athletes (> 80ml/kg/ min.(Robergs and Roberts, 1997). They also added that the factors that combine to influence Vo_{2max} are a high proportion of slow twitch motor units, high central and peripheral cardiovascular capacities, and the quality and duration of training. Having slower twitch muscle fibers increases the oxidative capacity of the muscle (Jacobs, 1983).He stated that muscle motor unit proportions are genetically determined, and therefore a person's ability to respond to endurance training and increase to Vo_{2max} has important genetic constraints. This opinion is in accordance with the results in Table 6, i.e.,Vo_{2max} was higher in those who participated in aerobic exercise bouts compared to the controls and those who participated in anaerobic exercise bouts. An increase in mitochondrial volume would also provide skeletal muscle with the ability to increase maximal oxygen consumption. However, cardiovascular adaptation is also involved in increasing Vo_{2max} after training, and muscle adaptations should not be viewed as the sole determinant of Vo_{2max}. Different training strategies can influence the values of Vo_{2max}, and it appears that the type and quality of training are also important. The extent of improvement in Vo_{2max} depends on the value of Vo_{2max} before training. (Robergs and Roberts, 1997).

The hem concentration may be the main cause of the increase blood parameters of RBCs, WBCs, HB, and Hematocrit (Tables 4,5) after the aerobic and anaerobic exercise bout, and the increased blood parameters could be caused by the stress induced by physical activities (Montain and Coyle, 1992).

The results in Tables (2, 3) indicate that CD^{34+} increased after exercise bouts. The increased hematopoietic stem cells CD^{34+} revealed positive results, especially the anaerobic bout for those handball players who were subjected to stress more than those who were subjected to aerobic bouts.

Previous studies have shown that an acute bout of exercise increases the number of bone marrow derived endothelial cells in the blood (Shaffer *et al.,* 2006 and Vancraenenbroeck *et al.,* 2008 and Amany and Mohamed, 2011).

This is consistent with our data, as aerobic and anaerobic exercise bouts resulted in an increase in CD^{34+}(SC)(Table 3). On the other hand, $CD34^{+}$ (SC) tended to increase following anaerobic exercise bouts.

The number of circulating EPSs likely represents abalance between liberating of EPCs from the bone marrow and incorporation at the level of the vessel or differentiation. Laufs *et al.*(2005) demonstrated that CD^{34+}/KDr^{+} increased after 30 minutes of high intensity running in healthy participants, but returned to resting levels within 24 hours following exercise. It can be speculated that in healthy regularly exercising individuals, by 24 hours following exercise. Also, it was reported that human subjects undergoing exhaustive dynamic exercise had high EPC counts in the peripheral blood (Rehman *et al.,*2004 and Laufs *et al.,*2005).

Giuseffe *et al.* (2005) reported an increase in CD^{34+}stem cells and reticulocytes after supramaximal exercise, and they added that it was unlikely that this increase depended upon changes in blood or plasma volume, because these were much smaller than the changes in cell counts. Either been incorporated for endothelial repair, neovascularization or have undergone differentiation.

Ewa and Pawet, (2007) reported that a decrease in the blood supply to a body organ or tissue, caused by constrictor or obstruction of the blood vessels, is a common cause of ischemia. This process is probably responsible for the use of EPCs in postnatal vascular growth and remodeling. In the study performed by Adams *et al.* (2004), patients with stable CAD were subjected to the single-exercise stress test to compare

peripheral blood EPC counts before and after the experiment. It was found that the peripheral blood EPC count increased significantly in ischemic patients within 24-48 hours after exercise. They observed that an increase in EPC levels was accompanied by an elevation of VEGF concentration in the plasma of these patients. These results confirmed that VEGF is a significant factor responsible for EPC mobilization from bone marrow to peripheral blood (Adams *et al.,*2004)Corresponding author.

Conclusion

It may be concluded that:

- Vo2 max increased in case of aerobic exercise bout compared to anaerobic one due to the longer period of cycling.
- Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobic one due to the higher intensity expressed in anaerobic bout leading to decrease oxygen.
- HB, RBCs, WBCs and Hematocrit value were increased after aerobic and anaerobic exercise bout.
- CD^{34+} HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout.

Acknowledgments

Funding was provided by the China National Science Foundation (30930026) and Science and Technology Development of Ji Lin Province (200905180).

Corresponding author
Mohammed Nader Mohammed Shalaby

Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China and Department of Sports Science, Faculty of Physical Education, Port Said University, Egypt

Phone number: +201000400900

E-mail :dr.m.nader@hotmail.com

References

Adams V, Lenk K and Linke A (2004): Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise induced ischemia arterioscler. Thrombvasc Biol, 24: 684-690.

Amany A and Mohamed S (2011): Effect of concurrent training on CD^{34+} /CD^{45+} stem cells, $VO_{2\ max}$, certain physical variables and record level of 1500m running 52 Ischper- SD World congress, Cairo, Egypt.

Bailey D and Davies B (1997): Physiological implications of attitude training for endurance performance at sea level a review. Br J Sports Med., 31: 183-190.

Barrett,K,Barman.S, Boitano, S (2010):Ganong Review of Medical physiology 3rded .McGraw Hill Lange.

Bonsignore M, Giuseppe M and James C (2002): Circulating hematopoietic progenitor cells in runners. J Appl Physiol, 93: 1691-1697.

Brenner I, Shek P and Zamecnik J (1998): Stress hormones and the immunological responses to heat and exercise. Int J Sports Med, 19: 130-143.

Davis J, Vodak P, Wilmore J, et al. (1976): Anoierobic threshold and animal aerobic power for three modes of exercise. I Appl Physiol, 41: 544-550.

Ewa M and Pawet J (2007): Endothelial progenitor cells as a new agent contributing to vascular repair. Arch Immunol Ther Experim, 55:247-259.

Fitts R (2004): Mechanisms of muscular fatigue. In poortmnas JR principles of exercise. Biochemistry (Karger, Basel) pp. 279-300.

Giuseffe, M. ,Daniele, Z,and Alessandra, S(2005): Supamaximalexercise mobilizes hematopoietic progenitors and reticulocytes in athletes. Am J Physiol Reguling Tegr Comp Physiol 289:1496-1503

Guyton ,A and Hall, J.(2006): Texbookof medical physiology Elsevier Saunders.USA.

Harrett A, Longhurst P and Watson J (1978): Mobilization of CFU-C by exercise and ACTH induced stress in man. Exp Hematol., 6: 590-599.

Jacobs I (1983): Lactate in human skeletal muscle after 10 and 30 seconds of supramaximal exercise J Appl. Physiology 55: 365 – 367.

Laufs U, Urhausen A, Werner N (2005): Running exercise of different duration and intensity. Eur J Cardiovasc Prev Rehabil., 12: 407-414.

Laufs U, Werner.N, Link,A (2004): physical training increases endothelial progenitor cells, inhibitsneointima formation and enhances angiogenesis. Circulation 109:220-226

Montain ,S and Coyle, E(1992): Influence of graded dehydration on hyperthermia and cardio vascular drift during exercise. J Appl. Physiol 73:1340-1350

Mougios V (2006): Exercise biochemistry. Human kinetics, USA.

Nieman D (1997): Immune response to heavy exertion. J Appl Physiol, 82: 1385-1394.

Rehman J, Parvathanenin, Karlsson G, et al. (2004): Exercise acutely increases circulating endothelial progenitor cells monocyte/ macrophage derived

Robergs R and Roberts S (1997): Exercise physiology. Mosby – year book inc. USA.

Roth,Dand Brooks.G**(1989):**Facilitated lactate transport across muscle membranes. Med Sci Sports Exerc 21:535, abstract

Shaffer R, Greene S and Arshi A (2006): Effect of acute exercise on endothelial progenitor cells in patients with peripheral arterial disease vascular. Medicine 11: 219-226.

Stanley W, Gertz E, Wisneski J, et al. (1985): Systematic lactate kinetics during graded exercise in man. Am J Physiol, 249: 595-602.

Szygula Z (1990): Erythrocytic system under the influence of physical exercise and training. Sports Med., 10: 181-197.

Van craenenbroeck,E,Veints,C,and Conroad, V.(2008):A maximal exercise bout increases the number of circulating CD^{34+}/KDR^{+} endothelial progenitor cells in healthy subjects. J Appl. Physoil., 01210**.**

8/20/2012

Serum levels of soluble Endoglin, soluble FMS-like Tyrosine kinase-1, and Uterine Artery Doppler in Pre-eclamptic patients

Elattar N.[1], Swelam E[1]., El Anwar A.[2]

Clinical Pathology Department[1], Gynecology &Obstetric Department[2], Faculty of Medicine, Zagazig University
enasswelam19_8@yahoo.com

Abstract: Background: Pre-eclampsia, a pregnancy-specific disorder, contributes substantially morbidity and mortality of both mother and newborn. An increasing number of biochemical agents were evaluated as markers for predicting pre-eclampsia, much effort has been put into assessing noval potential markers and their combination with other screening methods such as Doppler sonography. The aim of the study was to assess the serum levels of soluble Endoglin (sEng) , soluble FMS like tyrosine kinase-1 (sFlt-1), and Uterine Artery Doppler in pre-eclampsia to evaluate their clinical utility in diagnosis, and assessment of severity of the disease. **Patients and Methods:** The study was conducted on 35 pre-eclamptic patients and 20 healthy pregnant control subjects. All individuals were subjected to clinical examination and estimation of sEng and sFlt-1 by enzyme linked immunosorbent assay and estimation of ALT, Platelet count and urinary proteins, Uterine Artery Doppler measured as pulsatility Index (PI). **Results:** revealed highly significant increase in sEng and sflt-1,PI and systolic and diastolic blood pressure (SBP and DBP), proteins in urine, and ALT in patients than control(p<0.001), and in severe preeclampsia than in mild preeclampsia (p<0.001),. sEng and sFlt-1 were significantly increased in pre-term pre-eclampsia than in term per-eclampsia and in patients with abnormal Doppler than in normal Doppler(p<0.001). PI was not significantly increased in pre-term than term pre-eclampsia (p>0.05). sEng is positively correlated to sFlt-1(r =0.94) and both of them are positively correlated to SBP, DBP, PI, urinary proteins, and negatively correlated with gestational age and platelet count. Receiver operating characteristic (ROC) curve analysis was applied to evaluate the diagnostic utility of sEng, sFlt-1,and PI for discriminating the early onset from late onset pre-eclampsia , the best diagnostic cut off levels for sEng and sFlt-1 were >15 ng/ml, >900 pg/ml respectively. Both had a diagnostic sensitivity of 100%, specificity 78.9%,accuracy 88.6%, positive predictive value(PPV) 80% and negative predictive value(NPV) 100%, the area under the curve 0.97, 0.99 respectively . While the cut off levels for P1>1.42 , had a diagnostic sensitivity of 68% specificity, 58%, accuracy 62%, PPV 58% and NPV 58%. Also (ROC) curve analysis was applied to evaluate the diagnostic utility of sEng, sFlt-1,and PI for discriminating the mild from severe pre-eclampsia they had a diagnostic sensitivity 89%, 83% specificity 65%, 70% ,accuracy for both 77%, PPV, 72%, 75% and NPV 84%, 80% respectively .As regard PI had a diagnostic sensitivity of 100%, specificity 94%, accuracy 97%, PPV 95% and NPV 100%. **Conclusion:** The results of this study indicate that s Eng, and sFlt-1 are efficient in prediction of early onset pre-eclampsia and can discriminate between severe and mild pre-eclampsia, and both with pulsatility index of Doppler give better prediction of pre-eclampsia. This results will help in finding a new strategy for early management and so reduction of associated complication of pre-eclampsia.

[Elattar N., Swelam E., El Anwar A. **Serum levels of soluble Endoglin, soluble FMS-like Tyrosine kinase-1, and Uterine Artery Doppler in Pre-eclamptic patients**. *Life Sci J* 2012;9(3):2106-2113]. (ISSN: 1097-8135).
http://www.lifesciencesite.com. 304

Key words: Pre-eclampsia - s Eng -sFlt-1 - pulsatility index .

1-Introduction

Pre-eclampsia is a multi-system pregnancy specific hypertensive syndrome which is characterized by hypertension and proteinuria after 20 weeks of gestation that causes substantial maternal and fetal morbidity and mortality. The lack of an effective test for identification for women at risk of developing pre-eclampsia remains a contributing factor for the high morbidity of the disease. In most developing countries, where the incidence of the disease is high, women present late with complications.[1]

Although pre-eclampsia is called the disease of theories, the overwhelming evidence points to endothelial dysfunction as the central mechanism in the pathogenesis of the maternal syndrome in pre-eclampsia. The causes of this endothelial dysfunction remain elusive. However, poor placentation has been proposed as a major factor.[2]

Ischemic placenta secretes soluble factors into the maternal vasculature which have been implicated in inducing the endothelial dysfunction and the clinical features of pre-eclampsia. Excess secretion of naturally occurring anti-angiogenic molecule of placental origin referred to as soluble endoglin (sEng) and soluble Fms-like tyrosine kinase 1(sFlt-1) may contribute to the pathogensis of pre-eclampsia.[3]

Soluble endoglin acts by antagonizing an angiogenic and vasodilator molecule know as

transforming growth factor beta-1 (TGF-β1*)* which is important not only in angiogenesis but also in keeping the lining of the blood vessels healthy. As a result the cells lining of the blood vessels begin to sicken and die, the blood pressure increases and the blood vessels leak protein into the tissues and urine[3].Soluble endoglin is elevated not only during clinical pre-eclampsia but also 2-3 months before onset of clinical symptoms. It was also suggested that sEng correlates with disease severity and falls after delivery. Therefore, this anti-angiongenic protein in the maternal blood is a subject research as potential diagnostic and screening test for pre-eclampsia.[4]

Soluble fms-like tyrosine kinase 1 (sFlt1) is truncated form of the Flt-1 receptor [vascular endothelial growth factor receptor-1(VEGFR1)] including the extracellular ligand-binding domain, but not the trans membrane and intracellular domains; it is secreted (hence named "soluble") and antagonizes VEGF and placental growth factor (PIGF) in the circulation by binding and presenting their interaction with their endothelial receptors.[5] Concentrations of sFlt-1 is increased in women with established pre-eclampsia and begin to increase steeply about five weeks before the onset of clinical disease.[6]

The antiangiogenic state which occur in pre-eclmpsia may be reflected by changes in the impedance to flow in the maternal circulation.[7] Uterine artery Doppler is a non-invasive assessment of utero-placental circulation, and its clinical value in high risk pregnant women has been encouraging.[8]

The aim of the study was to assess the serum levels of soluble Endoglin (sEng) ,soluble FMS like tyrosine kinase-1 (sflt-1), and role of Uterine Artery Doppler in pre-eclampsia to evaluate their clinical utility in diagnosis, and assessment of severity of the disease.

2. Subjects and Methods:

SUBJECTS :

This study has been carried out in the outpatients clinics and inpatients departments of Obstetrics & Gynecology and Clinical Pathology departments, Zagazig University Hospitals. It included 35 pre-eclamptic patients in addition to 20 healthy pregnant control subjects. All participants gave their consent to participate in this study.

Patient Group (group I):

Thirty five pre-eclamptic patients. Their ages ranged between 18 to 40 years. Patients were diagnosed according to the diagnostic criteria outlined by **The American College of Obstetrics and Gynecology (AGOG) practice bulletin (2002)[9]:**Blood pressure: systolic blood pressure (SBP) of 140 mmHg or greater or diastolic blood pressure (DBP) of 90 mmHg or greater on 2 occasions at least 6 hours apart, Proteinuria: at least 300 mg in 24-hours urine collection.

Patients were classified according to onset of Pre-eclampsia: Early- onset or preterm pre-eclamptic group: This group included 16 pregnant females who developed pre-eclampsia before 32 weeks of gestation, and late- onset or term pre-eclamptic group: This group included 19 pregnant females who developed pre-eclampsia after 32 weeks of gestation.

The pre-eclamptic patients were re-classified according to severity **(AGOG, 2002)**[9] into: Mild pre-eclamptic group: This group included 17 pre-eclamptic females. They had SBP <160 mmHg , DBP<110 mmHg. 24 hrs urinary protein <3gm/day, ALT<40 IU/L and platelet count >100 x 10^3 /uL ,and severe pre-eclamptic group: This group included 18 pre-eclamptic females. They had SBP $\geq$ 160 mmHg or DBP $\geq$ 110 mmHg or 24 hrs urinary protein $\geq$ 3 gm/day or ALT $\geq$ 40 IU/L or platelet count $\leq$ 100 x 10^3 /uL

Pre-eclamptic patients were re-classified with Doppler velocimetry according to absence and presence of persistent diastolic notch **(Cnossen *et al.*, 2008)**[10] into: Normal uterine artery Doppler velocimetry group: It includes 17 pre-eclamptic patients, among them there are 5 preterm and 12 term pre-eclamptic patients, and abnormal uterine artery Doppler velocimetry group: It includes 18 pre-eclamptic patients having persistent diastolic notch, among them there are 11 preterm and 7 term pre-eclamptic patients.

Control group (group II): It included Twenty apparently healthy pregnant females (neither have hypertension nor diabetes or renal diseases) matched in age with patients group, their ages ranged between 20-35 years.

All individuals included in this study were subjected to the following: Full history taking and clinical examination, assessment of blood pressure, Laboratory estimation of fasting serum glucose , urea and creatinine, ALT, platelet count,24 hours urinary protein, serum soluble endoglin (sEng), serum soluble Fms-like tyrosine kinase-1 (sFlt-1),and uterine artery Doppler assessment. . Individuals with repeated upper urinary tract infections, chronic hypertension, diabetes mellitus or pre-existing renal disease were excluded.

SAMPLES: Five milliliters of venous blood were collected and divided into an EDTA tube for platelet count and a plain test tube for serum separation. , 24-hours urine sample was collected from each subject in a clean container, examined immediately (**National Committee for Clinical Laboratory Standards, 2001).**[11]

Analytical Methods*:*

-Serum glucose, Serum urea and creatinine ,and Serum Alanine Aminotransferase (ALT), were carried out on

ADVIA 1650 auto-analyzer (Siemens Medical Solutions Diagnostic, USA).

-Platelet count was done using cell coulter Sysmex KX21 N (Japan).

-Urine Analysis for 24hrs Protein: by turbidimetric assay using trichloracetic acid (TCA) according to (**Shahanigan *et al.*, 1984).** [12]

-Serum sEng and sFlt-1**:** were assayed by a sandwich enzyme-linked immunosorbent assay (ELISA) using reagents provided by Quantikine R&D International (R&D International, Inc., 614 NC Kinely Place N. E Minneapolis, MN 55413. USA). This assay employed the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for antigen has been pre-coated on a micro plate. Standards & samples were pipetted into the wells and any antigen present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for antigen was added to the wells. Following a wash to remove an unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of antigen bound in the initial step. The color development was stopped and the intensity of the color was measured by ELISA reader.

B-Uterine Doppler artery:

Pulsed wave and color Doppler ultrasound examination of both uterine arteries was performed on both pre-eclamptic patients and controls using (volusion 730 PRO V),by sitting in semi-recumbent position , recording uterine artery waveforms which was made at the point where the uterine and external iliac arteries appeared to have crossed each others as detected by color flow Doppler then three consecutive pulsed waveforms were recorded and pulsatility index (PI) was measured and diastolic notch if present. Uterine artery Doppler velocimetry was defined as abnormal if either the mean PI was above the 90th percentile for gestational age and/or early diastolic notch was found **(Cnossen *et al.*, 2008).**

C- Statistical methods:

This was done on a personal computer using software SPSS (version 15) (SPSS Inc. Chicago, IL, USA). Descriptive statistics**:** Data were summarized using the arithmetic mean, the standard deviation, median and range for numerical variables. Mann–Whitney *U* test (MW) as non-parametric test for assessing whether two independent samples of observations have equally large values , and finally correlation was done to evaluate correlation between variables using Spearman rank correlation coefficient "r". *P* value of <0.05 indicates a significant results. Receiver Operating Characteristic (ROC) curves analysis was carried out to demonstrate the diagnostic performance of sEng, sFlt-1 and PI as indicators of early versus late onset pre-eclampsia. In addition ROC curve was constructed to evaluate the efficiency of sEng, sFlt-1 and PI for discriminating severe pre-eclampsia patients from mild patients.

3. Results:

Statistical comparison of mean ±SD of some studied parameters among cases and controls revealed a highly significant increase was found regarding SBP, DBP, PI and soluble Flt-1 (t=10.78, 9.04,3.47, 13.44 ;p<0.001 respectively). and non-significant difference regarding gestational age, Statistical comparison of median using Mann Whitney U test(MW) of some studied parameters among cases and controls revealed a highly significant increase was found regarding protein in urine, ALT, and sEng in cases (M.W.= 22.9, 12.4, 22.8 ;p<0.001 respectively) and non-significant difference regarding platelet count (Table1).

Statistical comparison between preterm, term pre-eclampsia regarding soluble endoglin ,and soluble Flt-1, revealed a highly statistically significant difference(t=8.29,7.89,p<0.001)respectively, while PI had no significant different between them. (Table 2).

The descriptive data of the different parameters among mild and severe pre-eclamptic groups. showed a significant increase regarding SBP,DBP, urinary protein, ALT, sEng and sFlt-1 ,except platelet count significant decrease in severe cases (t=0.46, 7.01, 7.23, 2.3, 5.14, 5.79, 3.16; *P*<0.001 respectively). While GA was non-significant. Also PI revealed a significant increase in pulsatility index in severe cases (MW 4.5;*P*<0.001) (Table 3).

Statistical comparison between normal and abnormal Doppler regarding sEng and sFlt-1 revealed a highly statistically significance increase in both s.Eng,sFlt-1 in abnormal Doppler group (t=5.74, 5.79 ;p < 0.001) respectively (Table 4).

Correlation study between sEng , and sFlt-1 level in pre-eclamptic patients ,and correlation between them and other parameters .Regarding sEng it revealed a highly significant positive correlation with SBP, DBP, urinary protein, ALT and PI ,sFlt-1.(r = 0.67,0.66,0.69, 0.3,0.49, 0.94 ;p<0.001) respectively, and high significant negative correlation with gestational age ,and platelet count(t=-0.63,-0.4; *P*<0.001), also as regard sFlt-1 it revealed a highly significant positive correlation with SBP, DBP, urinary protein, PI, and sEng (t=0.65,0.64,0.73, 0.67,0.94 respectively). and high significant negative correlation with gestational age ,and platelet count (t=-0.70,-0.45 *P*<0.001) while ALT non-significant correlate with sFlt-1 (Table5).

Receiver operating characteristic (ROC) curve analysis (Fig 1) was applied to evaluate the diagnostic utility of serum s.Eng ,sFlt-1,and PI for discriminating the early from late pre-eclampsia ,the best diagnostic cut off levels for sEng and sFlt-1 were >15 ng/ml,>900 pg/ml respectively. Both had a diagnostic sensitivity of

100%. Specificity 78.9%, accuracy 88.6%, positive predictive value 80%, and negative predictive value 100%,the area under the curve were 0.97,0.99 respectively.

While the cut off levels for PI >1.42 and had a diagnostic sensitivity68%, specificity 58%,accuracy 62%,PPV 58% and NPV58%,the area under the curve was 0.72.

Also, Receiver operating characteristic (ROC) curve analysis (Fig 2) was applied to evaluate the diagnostic utility of serum sEng, sFlt-1,and PI for discriminating the mild from severe pre-eclampsia. sEng, and sFlt-1 had a diagnostic sensitivity 89%, 83%, specificity 65%, 70%, accuracy for both 77%, PPV 72%, 75%, and NPV 84%,80%, the area under the curve 0.90,0.89, respectively.

As regard PI had a diagnostic sensitivity of 100%, specificity 94%, accuracy 97%, PPV 95%, and NPV 100%, the area under the curve was 0.94 .

Table (1): Clinical and laboratory data among the studied groups.

	Cases (group I) N = 35	**Control (group II) N = 20**	**Test of significance**	***P***
- GA (weeks) $\bar{X}$±SD Range	32.4±3.4 23-36	32.7±2.9 28-36	0.22	>0.05 N.S
-SBP (mmHg) X±SD Range	153.1±13.4 140-190	106.0±5.1 100-110	10.78	<0.001 H.S
- DBP (mmHg) X±SD Range	102.6±10.0 90-120	73±4.2 70-80	9.04	<0.001 .H.S
-Soluble Flt-1 (pg/ml) X±SD Range	917.5±127.98 700-1169	322.8±103.8 137-450	13.44	<0.001 H.S
- Pulsatility index (PI) X±SD Range	1.62±1.0 (0.4-5)	0.52±0.14 (0.4-0.8)	3.47	<0.001 H.S
- Urinary TP(gm/24h) Median range	3.1 0.46-10.9	0.05 0.05-0.06	MW 22.9	<0.001 H.S
- ALT (IU/L)Median range	19 10-68	11 10-15	MW 12.4	<0.001 H.S
- Plt (x 10^3/cmm) Median range	198 99-350	221 197-260	MW 1.86	>0.05 N.S
-Soluble endoglin (ng/ml) Median range	15.5 7.5-34	4.2 1.3-6.5	MW 22.8	<0.001 H.S

P >0.05 Non-significan; P<0.001Highly-significant; M.W:Mann-WhitneyUtest GA: Gestational age; SBP: systolic blood pressure; DBP: Diastolic blood pressure; ALT: Alanine aminotransferase; Urinary TP: urinary protein Plt: Platelet count

Table (2): Soluble endoglin ng/ml and, soluble Flt-1 pg/ml,and pulsatility index according to gestational weeks in pre-eclampic patients.

	Preterm group N=16	Term group N=19	T	*P*
Soluble endoglin (ng/ml) $\bar{X}$±SD (Range)	26.3±6.56 (15.4-34)	12.15±3.2 (7.5-17)	8.29	<0.001 HS
Soluble Flt-1 (pg/ml) $\bar{X}$±SD (Range)	1028.6±66.8 (940-1169)	823.8±83.6 (700-950)	7.89	<0.001 HS
Pulsatility index(PI) $\bar{X}$±SD (Range)	1.87±0.82 (0.8-3)	1.41±1.09 (0.4-5)	1.39	>0.05 NS

P<0.001 Highly significant; *P* >0.05 Non-significant

Table (3): Studied parameters in Mild and severe pre-eclampsia.

	Mild group N = 17	Severe group N = 18	Test of significance	*P*
GA(weeks)	33.2±2.86	31.7±3.7	1.39	>0.05
Blood pressure - Systolic (mmHg) - Diastolic (mmHg)	 142.9±5.8 94.7±4.8	 162.8±11.3 110±7.7	 0.46 7.01	 <0.001 <0.001
TP (gm/24h)	1.46±0.9	5.2±2.1	7.23	<0.001
ALT (IU/L)	19.2±9.2	29.7±16.2	2.3	<0.001
Platelet (x10^3/cmm)	227.9±55.9	166.1±59.1	3.16	<0.01
S. endoglin (ng/ml)	12.4±4.1	24.5±7.7	5.14	<0.001
S.flt-1 (pg/ml)	825.3±96.9	1004.5±86	5.79	<0.001
PI*	0.8(0.4-5)	2.1(1.8-3)	4.5 MW	<0.001

GA: Gestational age; MW :Mann -Whitney-U test; * PI: Uterine artery pulsatility index median & range;
P >0.05 Non-significant *P* <0.01 significant; *P*<0.001 highly significant

Table (4): Statistical comparison between normal, abnormal uterine Doppler artery regarding soluble endoglin ng/ml and soluble Flt-1pg/ml in Pre-eclamptic patients.

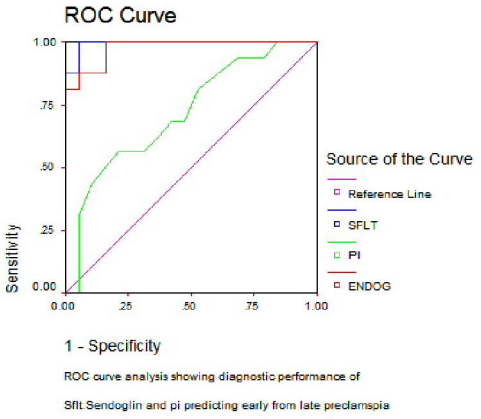
	Normal doppler N=17	Abnormal doppler N=18	t	*P*
Soluble endoglin (ng/ml) $\bar{X}$±SD (Range)	12.4 ±4.1 (7.5-21)	25.2±8.8 (16.4-34)	5.74	<0.001 HS
Soluble Flt-1 (pg/ml) $\bar{X}$±SD (Range)	825.29±96.91(700-965)	1004.5±86.05(870-1169)	5.79	<0.001 HS

P<0.001 Highly significant

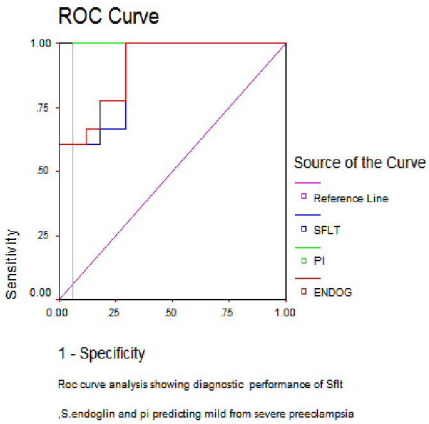
Table (5): Correlation between s- endoglin ng/ml, sflt-1pg/ml in pre-eclamptic patients and correlation between them and other parameters.

	S.Eng	S Flt-1
Gestational age(weeks)	r = -0.63**	r = -0.70**
Systolic Blood pressure(mmHg)	r = 0.67**	r = 0.65**
Diastolic Blood pressure(mmHg)	r = 0.66**	r = 0.64**
TP (urinary total protein)(g/24 h)	r = 0.69**	r = 0.73**
ALT IU/L	r = 0.3*	r = 0.25
Platelet (Plt)(x10^3/cm)	r = -0.4**	r = -0.45**
Pulsatility index (PI)	r = 0.49**	r = 0.67**
S-flt-1 Pg/ml	r = 0.94**	
S Eng ng/ml		r = 0.94**

P>0.05 non significant * *P* <0.05 significant ** *P* <0.001highly significant



AUC of: sEng=0.97 , sFlt-1=0.99 , PI=0.72



AUC of: sEng=0.90, sFlt-1=0.89, PI=0.94

4. Discussion

Pre-eclampsia is a pregnancy specific syndrome and a leading cause of maternal and fetal morbidity and mortality worldwide. It affects about 2-5% of western countries and complicates up to 10% of pregnancies in the developing countries. [13]

Levine *et al.*[1] reported that blood levels of placental derived anti angiogenic proteins might eventually be involved in the endothelial dysfunction which is the hall mark pathological finding in Pre-eclampsia.

The aim of this study was to assess levels of s.Eng and s.flt-1, a circulating placental-derived anti-angiogenic proteins, combined with uterine artery Doppler velocimetry in a group of pre-eclamptic pregnant females to evaluates their clinical utility in diagnosis and assessment of severity of the diseases.

The results revealed highly significantly increased in s.Eng,s.flt-1,PI, SBP, DBP ,proteins in urine, , and ALT in patients than control, and in severe pre-eclampsia than in mild pre-eclampsia. These results are similar to **Eremina *et al.***[14],and **Levin *et al.***[3] who believed that excess levels of sflt-1 lead to endothelial dysfunctions, Hypertension and proteinuria this effect explained also by **Luttun** and **Carmeliet**[5] that it was due to inhibition of VEGF (vascular endothelial growth factor) a factor important not only in blood pressure regulation, but also in maintaining the integrity of glomerular filtration barrier.

These results was also similar to **Masuyama *et al.***[15]. They found that the more severe the condition is, the higher the levels of sEng and s Flt-1 in sera of pre-eclamptic females. They concluded that the relationship of sEng and sFlt-1 to the severity of pre-

eclampsia is possible a casual one in which these markers might be playing a role in the pathogenesis of pre-eclampsia. **Venkatesha *et al.*[16]** reported that exogenous sEng and sFlt-1 administration in pregnant rats lead to severe pre-eclampsia including HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) and restriction of fetal growth.

In the present study pre-term pre-eclampsia has highly significant increase sEng and sflt-1 than term pre-eclampsia while PI was not significantly different. Similar results are reported by **Hirashima *et al.*[17], Levine *et al.*[3]** measured serum levels of sEng and sFlt-1 in pre- eclampsia in the second trimester and found that levels of sEng rose earlier and more steeply in women in whom pre-eclampia developed and were highest in preterm (early onset pre-eclampsia). **Lesili *et al.*[18]** reported that the prediction of term pre-eclampsia is poor with Doppler. **Whitley *et al.*[19]** reported significant association between abnormal uterine artery Doppler with pre-term but not with term pre-eclampsia.

The results showed that not all cases of pre-eclampsia had abnormal Doppler, this agree with **Zahumensky[20].** Also the results revealed that pre-eclamptic patients with abnormal uterine Doppler have higher sEng, and sFlt-1 concentrations than patients with normal Doppler. This is consistent with finding of **Stepan *et al.*[21]** who reported that in patients with pre-eclampsia subgroups with abnormal Doppler findings have higher sEng and sFlt-1 levels in comparison with the group with normal uterine perfusion.

In studying the correlation of sEng and sFlt-1 and other laboratory findings: sEng is positively correlated to sFlt-1 and both of them are positively correlated to SBP,DBP, PI and urinary proteins but AlT with sEng only and negatively correlated to gestational age and platelet count.

As regards positive correlation of s Eng and s Flt-1 with urinary protein go hand in hand with **Venkatesha *et al.*[16]** who demonstrated that sEng act by antagonizing the angiogenic molecule transforming growth factor beta-1 (TGF-B1) which is important both in mediating NO-dependent vasodilation and in keeping lining of blood vessels healthy. So excess secretion of s. Eng lead to intense vasoconstriction, with resultant hypertension, and leakage of proteins into tissues and urine. These data clearly indicate that s.Eng is a major cause of the maternal manifestation of pre -eclampsia.

Also **Buhimschi *et al.*[22]** reported that podocyte and mesengial cell destruction as well as loss of glomerular basal membrane integrity (glomerular endotheliosis) in women with pre-eclampsia resulted from increased exposure to sFlt-1. **Chaiworapongsa *et al.*[7]** who found a significant relationship between sEng and sFlt-1 and Doppler abnormalities in the uterine circulation these findings also consistent with **Gilbert *et al.*[23]**, who demonstrated that reducing uterine perfusion pressure by clamping the aorta above the iliac bi-furcation in pregnant rats led to increase the serum concentration and placental expression of anti angiogenic proteins.

Assessment of the diagnostic performance of sEng and sFlt-1 in early pre-eclampsia patients versus late pre-eclampsia patients using Receiver operating characteristic (ROC) curve analysis revealed that the best diagnostic cut off levels for sEng and sFlt-1 was >15 ng/ml,and >900 pg/ml respectively ,both had a diagnostic sensitivity of 100%. Specificity 78.9%, accuracy 88.6%, positive predictive value 80%, and negative predictive value 100% respectively. Similar results were obtained by **Abdel Fattah *et al.*[24]**this results in agreement with **Chen *et al.*[25]** who reported that serum sFlt-1 levels >350 pg/ml in pateints of early pre-eclampsia. , **Savvidow *et al.*[26]** found that women who developed early onset pre -eclampsia often had sEng levels more than 10.2 ng/ml. **Woolcock *et al.*[27]** explained these findings by the fact that the early onset pre-eclampsia is more associated with placental ischemia than late onset pre-eclampsia, leading to more pronounced alternation in sFlt-1.While **Stepan *et al.*[21]** reported that the increase of circulatory sEng is detectable approximately 2-3 months before the clinical manifestation of preeclampsia, it is known that sFlt-1 increase is described as detectable 5 weeks before onset of disease, so sEng could be an earlier marker for pre eclampsia.

As regard pulstality index cut off levels >1.42 and had a diagnostic sensitivity68%, specificity 58%,accuracy 62%,PPV 58% and NPV58%. That go hand in hand with **Yu *et al.*[28]** who reported that Doppler screening for predicting pre-eclampsia yield sensitivity up to 60% and PPV up to 40%,also **Thangaratinan *et al.*[29]** found that PI have a sensitivity of 60%,and so on **Lapaire *et al.*[30]** demonstrated that abnormal uterine artery Doppler velocimetry was independent risk factors for the occurrence of pre-eclampsia. Assessment of the diagnostic performance of sEng and sFlt-1 in severe pre-eclampsia patients versus mild pre-eclampsia patients using Receiver operating characteristic (ROC) curve analysis revealed that sEng, and sFlt-1 had a diagnostic sensitivity 89%, 83%, specificity 65%, 70%, respectively, while the PI had a diagnostic sensitivity of 100%, specificity 94%,that is mean that all the three items were efficient to discriminate severe pre-eclampsia from mild cases. In consistent with our finding **Buhiomshi *et al.*[22] and Venkalesha *et al.*[16]** who established this strong association between sEng and sFlt-1 and severity of pre-eclampsia .

Also Masuyama *et al.*[15] reported that the relationship of sFlt-1 and sEng to the severity of pre-

eclampsia is possibly a causal one of the severity,and also reported that sEng level >22.0 ng/ml in this cases, and **Alexandre *et al.*[31]** recorded that serum s Flt levels were often > 800 pg/ml in severe pre –eclampsia.

As regard the results of PI its sensitivity 100%,specificity 94% is better than sFlt-1,sEng in severe cases .In agreement with this results **Plasenica *et al.*[32]** who reported a sensitivity 82% of PI in pre – eclampsia at 11-13 weeks. While **Leslie *et al.*[18]** reported that single screening modality by uterine Doppler may not adequately predict all presentation. So that **Stepan *et al.*[21]** said that concurrent measurement of uterine perfusion and anti angiogenic factors sEng, sFlt-1 allows a highly efficient prediction of early onset pre-eclampsia ,Although anti angiogenic factors don't improve the good sensitivity of Doppler sonography they substantially improve the specificity.

Conclusion: The results of this study indicate that s Eng, and sFlt-1 are efficient in prediction of early onset pre-eclampsia and can discriminate between severe and mild pre-eclampsia, and both with pulsatility index of Doppler give better prediction of pre-eclampsia. This results will help in finding a new strategy for early management and so reduction of associated complication of pre-eclampsia.

5.References:

1-Levine, R.J.; Lam, C.; Qian, C.; Yu, F.; Maynard, S.E. (2007): Soluble Endoglin and Other Circulating Antiangiogenic factors in pre-eclampsia. Obstet& Gynecological Survey.62(2):82-83.

2-Sharon Maynard; Franklin, H.; Epstein, S. and Ananth Karumanchi (2008): Pre-eclampsia and angiogenic imbalance. Annual Review of Medicine; (59):61-78.

3-Levine, R.J.; Lam, C.; Qian, C.; Yu, K.F.; Maynard, S.E.; Thadhani, R. and Karumanchi, S.A. (2006): Soluble endoglin and other circulating anti-angiogenic factors in pre- eclampsia. N. Engl. J. Med.; 355:992-1005.

4-Stepan, H.; Kramer, Thomas, Faber, Renaldo, A. and Walther, T. (2007): Maternal Plasma Concentrations of Soluble Endoglin in Pregnancies with intrauterine Growth Restriction.The Endocrine Society ; 92(7): 2831-2834.

5-Luttun, A. and Carmeliet, P. (2003): Soluble VEGF receptor Flt-1:The elusive pre-eclampsia factor discovered?. J. Clin. Investig.; 111(5):600-602.

6-Levine, R.J.; Maynard. S.E. and Qian, C. (2004): Circulating angiogenic factors and the risk of pre-eclampsia.N. Engl. J. Med.;350:672-683.

7-Chaiworapongsa, T.; Romero, R. and Kusanovic, J. (2010): Plasma soluble endoglin concentration in pre-eclampsia is associated with an increased impedance to flow in the maternal and fetal circulations. Ultrasound in Obstetrics and Gynecology; 35 (2):155-162.

8-Harrington, K.; Fayyad, A.; Thakur, V. and Aquilina, J. (2004): The value of uterine artery Doppler in the prediction of uteroplacental complications in multiparous women. Ultrasound Obstet Gynecol; 23:50-55.

9-American collage of obstetric and gynecology (ACOG) practice bulletin (2002): Diagnosis and management of pre-eclampsia and eclampsia. Obstet. Gynecol; 99:159-67.

10-Cnossen, J.S.; Morris, R.K. and ter Riet, G. (2008): Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bio variable meta-analysis.CMAJ;178:701-11.

11-National Committee for Clinical Laboratory Standards (2001): Routine Urine analysis and Collection, Transportation, and Preservation of Urine Specimens: Approved Guideline G.P. 16-A2. 2nd ed., Wayne, P.A., National Committee for Clinical Laboratory Standards.

12-Shahanigan, S.; Brown, P.I. and Ash, K.O. (1984): Turbidimetric measurement of total urinary proteins: a revised method. Am J. Clin. Pathol.; 81:651-654.

13-Grill S., Rusterholz C., Zanetti- Dallenbach R.(2009): Potential markers of pre-eclampsia -a review. Rprod Biol Endocrinol; (1):70-84.

14-Erman V. ,Sood M.,and Haigh J. (2003): Glomerular specific alternation of VEGF-A expression lead to distinct congenital and acquired renal disease.J.Clin.Invest.;III:707-717.

.**15-Masuyama, H.; Nakatsukasa, H.; Takamoto, N. and Hiramatsu, Y. (2007):** Correlaton between soluble Endoglin, Vascular Endothelial Growth Factor Receptor-1, and Adipocytokines in pre-eclampsia. J. Clin. Endocrino. Metab.; 92:2672-2679.

16-Venkatesha, S.; Toporsian, M. and Lam, C. (2006): Soluble endoglin contributes to the pathogenesis of pre-eclampsia. Nat. Med.; 12:642-649.

17-Hirashima, C.; Ohkuchi, A. and Arai, F. (2005): Establishing reference values for both total soluble fms-like tyrosine kinase 1 and free placental rowth factor in pregnant women. Hypertens. Res.;28:727-732.

18-Leslie K., Thilaganathan B. , Papageorghiou A.,(2011): Early prediction and prevention of pre-eclampsia. Clinical Obstet.Gynecol 25;343-354.

19-WhitleyG.S.,DashP.R.,AylinL.J., (2007) : Increased apoptosis in first trimester extravillous trophoblasts from pregnancies at higher risk of

developing pre-eclampsia.Am.J.Pathol.Hypertension ;53:399-403.

20-Zahumensky J. (2009): Doppler flowmertry in pre-eclampsia. Bratist Lek Listy; 110(7):432-435.

21-Stepan Holger, Giepel Annegret, Kramer Thomas (2008): Circulatory soluble endoglin and its predictive value for pre-eclampsia in second-trimester pregnancies with abnormal uterine perfusion. Am J Obstet Gynecol.;198 (2):175.e 1-6.

22-BuhimschiC.S.,NorwitzE.R.,FunaiE .(2005): Urinary angiogenic factors cluster hypertensive disorders and identify women with severe pre-eclampsia. Am.J. Obstet. Gynecol;192:734-741.

23-Gilbert, J.S.; Gilbert, S.A. and Arany, M. (2009): Hypertension produced by placental ischemia in pregnant rat sis associated with increased sEng expression .Hypertension, 53:399-0403.

24-Abdel Fattah H.I.,Farag D.H.,Saleh S.A.R.,AbdelmageedA.I.,SaabA.R.,Shahin R.S.,and Fahmy A.A.,(2009): Study of serum levels of soluble Fms-like tyrosin kinase -1 and soluble endoglin in pregnant women with pre-eclampsia .Egyptian J. of Laboratory Medicine XX; March ;49-59.

25-Chen Q.,Zhao Y.,Zou I.,and Wang Z.H.,(2007): Predictive value of soluble VEGF receptor -1 in pre-eclampsia in second trimester J.Clin.Obstet. Gynecol.; 42 (3): 161-164.

26-Savvido.M.D.,NooriM.,Anderson J.M .(2008): Maternal endothelial function and serum concentration of placental Growth factor, soluble endoglin in women with abnormal placentation. Ultrasound Obstet. Gynecol.;32(7):871-876 .

27-Woolcock J.,Hennessy A.,Xu B. Thornton C.,Tooher J.,Makvis A.,and Ogle R.(2008): Soluble Flt-1 as a daignostic marker of pre-eclampsia .Aust NZJ Obstet Gynecole;48(1):64-70.

28-Yu C.K, Papageorghiou A., Parra M .(2003): Randomized control trial using low dose aspirin in the prevention of pre-eclampsia in women with abnormal uterine artery Doppler at 23 weeks gestations .Ultrsound. Obestet .Gynecol.22;233-239.

29-Thangaratiran S., Langenveld J., Ben w., Khalid S.,Kahan M.(2011): Prediction and primary prevention of pre-eclampsia. Clinical Obstet.Gynecol;25;419-433.

30-Lapaire o., Shennan A., Stepan H., (2010): The pre-eclampsia biomarkers soluble Fms-like tyrosine kinase -1 and placental growth factor: current knowledge, clinical implications and future application. Journal of obeste. Gynecol and Reproductive Biology 151;122-129.

31-Alexandre H., Nadia B., Guillaume L. (2004): Maternal serum sFlt-1 concentration is an early and reliable predictive marker of preeclampsia. J.Clin. Chemistry;50:1702-3

32-PlasenciaW., MaizN., BoninoS.(2007): Uterine artery Doppler at 11+0 to 13+6 weeks in the prediction of pre-eclampsia .Ultrasound Obstet.Gynecol;30:742-749.

8/20/2012

Reduced VEGF Signaling in Corpus Cavernosum of Rat with Alloxan Induced Type I Diabetes Mellitus

Xiangfei He [1], Monong Li [2], Fengfu Guo [1], Donghua Xie [3]

[1.] Department of Urology, Linyi People's Hospital, Linyi, Shandong, China
[2.] Department of Urology, Qingdao Municipal Hospital, Qingdao, Shandong, China
[3.] Department of Urology, University of Illinois College of Medicine, Chicago, USA
hexiangfei2000@yahoo.com.cn

Abstract: Aim: Vascular endothelial growth factor (VEGF) is an angiogenic growth factor that plays a critical role in controlling survival and apoptosis. It is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase. PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a major negative regulator of the PI3K/Akt signaling pathway. We sought to investigate the changes in VEGF signaling in type I diabetes mellitus (DM) induced erectile dysfunction (ED) in rats. Material/Methods: In total, 16 were divided into 2 groups (n=8/group). Eight of these animals (Group 1) had no treatment. The remaining 8 of them (Group 2) were injected with alloxan (100mg/kg body weight) to induce DM. All rats were sacrificed 8 weeks after alloxan induced DM in Group 2. Corporal tissues were harvested and studied for level of VEGF and cyclic guanosine monophosphate (cGMP) by enzyme immunoassay assay (ELISA); Levels of VEGF receptor (VEGFR)-1, VEGFR-2, Akt, phosphorylated Akt, eNOS, and phosphorylated eNOS were assessed by western blot analysis. Results: VEGF, VEGFR-1, VEGFR-2, Akt phosphorylation, eNOS phosphorylation, and cGMP were significantly decreased in the corporal tissue of diabetic rats. Conclusions: The accompanying decrease in cGMP may be a result of VEGF signaling dysregulation and this may have an effect on erectile function.

[He X, Li M, Guo F, Xie D. **Reduced VEGF Signaling in Corpus Cavernosum of Rat with Alloxan Induced Type I Diabetes Mellitus.** *Life Sci J* 2012;9(3):2114-2117].(ISSN: 1097-8135). http://www.lifesciencesite.com. 305

Keywords: penis; impotence; angiogenic growth factors; diabetes mellitus

1. Introduction

Diabetes mellitus (DM) is one of the major risk factors for erectile dysfunction. It has been estimated that 50%–75% of diabetic men have some degree of erectile dysfunction and the incidence of erectile dysfunction is higher in diabetic men than in age-matched nondiabetic men [1-2]. Diabetic rat model provide excellent opportunities to explore potential mechanisms and therapeutic approaches for human erectile dysfunction (ED) [3-11]. The purpose of our current study is to test for changes in VEGF (vascular endothelial growth factor) signaling in corporal tissue of the diabetic rat.

One of the downstream effects of VEGF includes VEGF receptor (VEGFR) dimerization and autophosporylation, the phosphorylation and activation of Akt and endothelial nitric oxide synthase (eNOS), which has been shown to mediate VEGF-induced penile erection by further mediating activation of Guanosine 3', 5' -cyclic monophosphate (cGMP) [12, 13]. Therapeutic angiogenesis using vascular growth factors has recently been demonstrated to be feasible in animal models with diabetic ED [8-10]. However, the expression profile of VEGF signaling in corporal tissue of diabetic rat has not been studied. We sought to determine the potential changes.

2. Material and Methods

Animal model.

In total, 16 male Sprague-Dawley rats weighing 200–250g were obtained from Shanghai Slac Laboratory Animal Co Ltd (Shanghai, China). Eight of these animals were selected randomly and treated with vehicle (0.1 mol/L citrate-phosphate buffer, pH 4.5) as normal controls (Group 1). The remainders were injected with alloxan (100mg/kg body weight) to induce DM (Group 2). All rats were kept in a temperature-controlled, air-conditioned animal house with a 12-h light-dark cycle and were given free access to food and water. Seventy-two hours after the rats were injected with alloxan, the blood glucose level in each rat was monitored at regular intervals throughout the study and immediately before sacrifice. Of the 8 Alloxan-induced rats, those having blood glucose levels higher than 300 mg/dL (16.6 mmol/L) were selected for the study. Procedures were performed according to the recommendations of the institutional animal care committee.

Tissue procurement, histological section preparation, protein isolation.

At study termination all rats were deeply anesthetized with ketamine and xylazine and penectomy was performed with careful dissection of the corpora cavernosa from the tunica albuginea before sacrifice, as previously described. Tissue was cryoprotected and frozen sections (5μm) were prepared. Protein lysates were prepared and concentrations determined by Bradford assay, as previously described (14, 15).

Measurement of VEGF protein.

VEGF was determined using a solid-state ELISA system with a Quantikine VEGF ELISA Kit (R&D Systems China Co. Ltd.), as previously described (14). At the final step, the optical density of samples and standards were measured. The amount of the VEGF protein in each sample was calculated based on a standard curve.

Assessments of VEGFR-1, VEGFR-2, Akt, p-Akt, eNOS and p-eNOS.

Western analysis for VEGFR-1, VEGFR-2, Akt, phosphorylated Akt (p-Akt), eNOS, and phosphorylated eNOS (p-eNOS) expression was performed using methods previously described (15). Alpha-Tubulin was used as a protein loading control. The intensity for each band was quantified using NIH image software based on pixel values. The p-Akt or p-eNOS density was calculated relative to total Akt or eNOS giving the p-eNOS/eNOS fraction. The primary antibodies were purchased from Beijing GBI Biotechnology Co. Ltd (Beijing, China); all secondary antibodies were purchased from Beijing Chief-East Tech Co. Ltd (Beijing, China). The working concentration for all the first antibodies was 1:1000, and for all the secondary antibodies the concentration was 1:5000.

Quantitative Determination of cGMP Nucleotides.

Determination of the cyclic nucleotide concentration was done by using a commercial kit: Parameter cyclic GMP Assay kit (R&D Systems China Co. Ltd.). This method is based on the ELISA, a competitive immunoassay for the quantitative determination of the relevant nucleotide in samples. At the final step, the optical density of samples and standards were measured. The amount of the nucleotide in each sample was calculated based on a standard curve.

Statistical analysis.

Results are expressed as the mean ± SD. The mean values were compared using Student's t test. P<0.05 was considered statistically significant.

3. Results

General Data

Of 8 alloxan-induced rats, all had blood glucose higher than 300 mg/dL with significantly increased food and water intakes, hyperuresis, and weight loss compared with the rats in the control group. Body weight and blood glucose level of the rats are shown in Table 1.

TABLE 1. Results of Body Weight, Blood Glucose, VEGF, and cGMP (Group 2 vs. Group 1)

	Initial Body Weight (g)	*8 Weeks Body Weight (g)*	*Initial Blood Glucose (mg/dL)*	*8 Weeks Blood Glucose (mg/dL)*	*VEGF (pg/mg)*	*cGMP* (pmol/mg)
Group 1	225.88	380.88	75.75±	76.25±	413.67	0.01853
	±16.88	±21.87	6.61	6.48	±84.53	±0.00445
Group 2	225.13	189.38	377.75±	494.50±	292.61	0.00947
	±16.50	±16.76	31.23	80.07	±11.28	±0.00450
p value	p=NS	p<0.001	p<0.001	p<0.001	p<0.05	P<0.01

Table 1. 8 weeks: 8 weeks after inducing diabetes in Group 2; VEGF, vascular endothelial growth factor; cGMP, cyclic guanosine monophosphate

Diabetes mellitus was associated with decreased vasoreactivities in corporal tissue

The levels of VEGF protein was significant different between 2 groups (See Table 1, Figure 1).

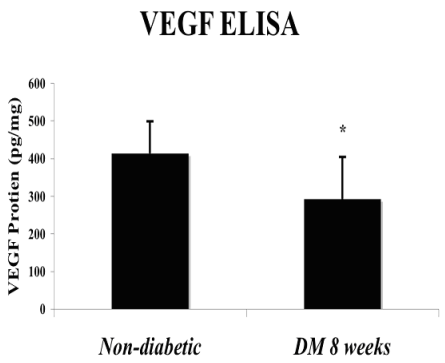


Figure 1. VEGF ELISA. There was significant decrease VEGF expression in diabetic rat. VEGF= vascular endothelial growth factor; DM=diabetes mellitus; p<0.05*

Diet Induced diabetes mellitus is associated with a reduction in VEGF downstream protein expression.

There was significant difference in VEGFR-1 (180 KD) or VEGFR-2 (200 KD) protein expression between 2 groups as detected by western blot. With respect to VEGFR-1 or VEGFR-2 expression, a 1.54-fold or 1.43-fold decrease was observed (Group 2 vs. Group 1; p<0.05 each) (See Figure 2). There was no difference in total Akt (60 KD) and total eNOS (135 KD) protein expression between 2 groups. However, the level of Akt phosphorylation at Ser 473 or eNOS phosphorylation at Ser 1177 was significantly

decreased. With respect to phospho-Akt or phospho-eNOS expression, a 1.75-fold or 1.72-fold decrease was observed (Group 2 vs. Group 1; $p<0.05$ each) (See Figure 2).

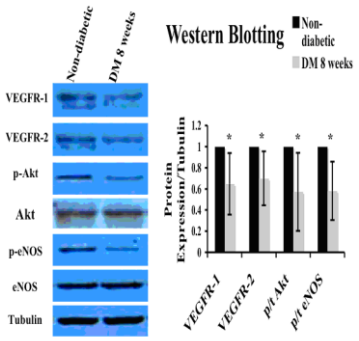


Figure 2. Western blotting for VEGFR-1, VEGFR-2, Akt and eNOS expression. There was significant decrease in Akt and eNOS phosphorylation. VEGF= vascular endothelial growth factor receptor; DM= diabetes mellitus; eNOS= endothelial nitric oxide synthase; p/t = phosphorylated/total; Tubulin =α-tubulin; $p<0.05$*

Diabetes mellitus is associated with abnormalities in cGMP expression

The levels of cGMP was significant different between 2 groups (See Table 1, Figure 3).

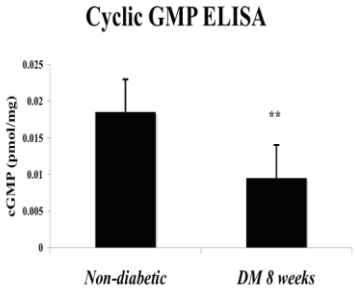


Figure 3. Cyclic GMP ELISA. There was significant decrease cGMP expression in diabetic rat. cGMP, cyclic guanosine monophosphate; DM=diabetes mellitus; $p<0.01$**

4. Discussions

Diabetes mellitus is a major cause of erectile dysfunction. The purpose of this study was to determine whether diabetes mellitus would result in any abnormalities in VEGF signaling in corporal tissues that is relevant to erectile dysfunction. We were able to demonstrate changes in Akt and eNOS phosphrylation, and cGMP level. Many of these changes are known to be part of pathophysiology of ED in human.

VEGF binds to one of three VEGF receptors that lead to receptor dimeration and autophosphorylation. This activates the enzyme phoshitidylinosityl 3 kinase (PI3K). PI3K in turn converts 4, 5-inositylphophate (PI) to 3, 4, 5 PI and this phosphorylates and thereby activates the protein kinase Akt. One of the downstream effects of Akt activation includes the phosphorylation and activation of eNOS [13].

Decreased VEGF or VEGF receptor protein expression in corporal tissue was shown in rats with diabetes or arteriogenic erectile dysfunction in limited number of studies [5, 16, 17]; however, it is not yet well clarified. This study has shown us clear decrease in the protein levels of VEGF and its receptors in rats with 8 weeks diabetes. Also, there follows a decrease in Akt and eNOS activation. However, we found the changes of Akt and eNOS were at phosphorylation which is similar to another study on Type I diabetic rats [7] but different from 3 studies on Type Ⅱ diabetic rats or Type I diabetic rats where they found that changes were at total Akt or total eNOS [5, 6, 17]. So different types or durations of diabetes may have different effects on Akt and eNOS changes.

Nitric oxide (NO) is the principal mediator of arterial dilation and smooth muscle relaxation within the sinusoids, which bring about tumescence of penile erectile tissue [1]. The NO-dependent signal transduction system contains several molecular targets available for pharmacologic manipulation to treat ED. The most prominent target identified thus far is phosphodiesterase 5 (PDE5), which enzymatically converts the intracellular second messenger molecule cGMP to its inactive form [18].

Endothelial NOS is an important source to generate NO and plays an important role in modulating intracorporal blood flow to maintain tumescence [3, 4, 7, 12]. In this study, decreased VEGF led to decreased Akt then eNOS phosphorylation and finally decreased cGMP expression. This observation advances our understanding of the mechanisms of diabetes mellitus induced ED and provides the theoretical basis for the application of VEGF gene therapy in vasculogenic ED.

Corresponding Author:
Xiangfei He, MD
Department of Urology
Linyi people's hospital
27 Jiefang RD, Linyi, Shandong, China
E-mail: hexiangfei2000@yahoo.com.cn
Contribute equally for all authors

References

1. Lue TF. Erectile dysfunction. N Engl J Med, 2000; 342:1802-1813
2. Siu SC, Lo SK, Wong KW, Ip KM, Wong YS. Prevalence of and risk factors for erectile dysfunction in Hong Kong diabetic patients. Diabet Med 2001; 18:732-738

3. Musicki B, Burnett AL. eNOS function and dysfunction in the penis. Exp Biol Med (Maywood) 2006; 231:154-165
4. Musicki B, Burnett AL. Endothelial dysfunction in diabetic erectile dysfunction. Int J Impot Res 2007; 19:129-138
5. Jesmin S, Sakuma I, Salah-Eldin A, Nonomura K, Hattori Y, Kitabatake A. Diminished penile expression of vascular endothelial growth factor and its receptors at the insulin-resistant stage of a type II diabetic rat model: a possible cause for erectile dysfunction in diabetes. J Mol Endocrinol 2003; 31:401-418
6. Vernet D, Cai L, Garban H, Babbitt ML, Murray FT, Rajfer J, Gonzalez-Cadavid NF. Reduction of penile nitric oxide synthase in diabetic BB/WORdp (type I) and BBZ/WORdp (type II) rats with erectile dysfunction. Endocrinology 1995; 136:5709-5717
7. Musicki B, Kramer MF, Becker RE, Burnett AL. Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. Proc Natl Acad Sci U S A. 2005; 102:11870-11875.
8. Dall'Era JE, Meacham RB, Mills JN, Koul S, Carlsen SN, Myers JB, Koul HK. Vascular endothelial growth factor (VEGF) gene therapy using a nonviral gene delivery system improves erectile function in a diabetic rat model. Int J Impot Res. 2008; 20:307-314
9. Shirai M, Yamanaka M, Shiina H, Igawa M, Kawakami T, Ishii N, Lue TF, Fujime M, Dahiya R. Vascular endothelial growth factor restores erectile function through modulation of the insulin-like growth factor system and sex hormone receptors in diabetic rat. Biochem Biophys Res Commun. 2006; 341:755-762.
10. Yamanaka M, Shirai M, Shiina H, Tanaka Y, Enokida H, Tsujimura A, Matsumiya K, Okuyama A, Dahiya R. Vascular endothelial growth factor restores erectile function through inhibition of apoptosis in diabetic rat penile crura. J Urol. 2005; 173:318-323.
11. Yang R, Wang J, Chen Y, Sun Z, Wang R, Dai Y. Effect of caffeine on erectile function via up-regulating cavernous cyclic guanosine monophosphate in diabetic rats. J Androl. 2008; 29:586-591.
12. Musicki B, Palese MA, Crone JK, Burnett AL. Phosphorylated endothelial nitric oxide synthase mediates vascular endothelial growth factor-induced penile erection. Biol Reprod. 2004; 70: 282-289
13. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signaling - in control of vascular function. Nat Rev Mol Cell Biol. 2006; 7:359-371
14. Xie D, Hazarika S, Andrich AJ, Padgett ME, Kontos CD, Donatucci CF, Annex BH. High cholesterol feeding in C57/Blc6 mice alters expression within the VEGF receptor-ligand family in corporal tissue. J Sex Med. 2008; 5:1137-1148.
15. Xie D, Odronic SI, Wu F, Pippen AM, Donatucci CF, Annex BH. A mouse model of hypercholesterolemia-induced erectile dysfunction. J Sex Med. 2007; 4:898-907.
16. De Young L, Bella A, Howard J, Brock G. Arteriogenic erectile dysfunction alters protein expression within the cavernosal tissue in an animal model. J Sex Med. 2005; 2:199-206.
17. Liu G, Sun X, Dai Y, Zheng F, Wang D, Huang Y, Bian J, Deng C. Chronic administration of sildenafil modified the impaired VEGF system and improved the erectile function in rats with diabetic erectile dysfunction. J Sex Med. 2010;7:3868-3878.
18. Burnett AL. Phosphodiesterase 5 mechanism and therapeutic applications. Am J Cardiol 2005; 96: 29M-31M

8/20/2012

The construct validation of Iranian student's Reading Comprehension Tests through Multitrait-Multimethod Procedure

Sohrab Rezaei

Assistand Professor Allameh Tabatabaei University
Sohrab_rezaei@yahoo.com

Abstract: Construct validity is a process of investigating what a test measures, in which one validates a test not against a criterion or another test, but against a theory. There is a specialized construct validation procedure called Multitrait-Multimethod procedure. This study has aimed at determining the impact of testing method on Iranian EFL learner's reading comprehension using multitrait-multimethod procedure. In other words, this study has been an attempt to determine whether the learners who take multiple choice cloze tests on vocabulary and grammar would score significantly higher than those who take multiple choice paraphrase and multiple choice comprehension tests on vocabulary and grammar or vice versa. A population of 100 grade 4 male undergraduate students majoring English translation in Karaj Azad university have been selected as subjects. Following administration of a TOFEL test 35 students have been selected as final subjects to be tested. The research procedure has included three methods namely comprehension, cloze and paraphrase, each of which contained two traits of vocabulary and grammar. A battery of statistical analysis namely correlational analysis, multivariant analysis of variances and factor analysis has been employed to investigate the results. The findings have revealed that there has been a strong correlation among methods. They have also revealed that the method, skill and the interaction between them have a high impact on the mean scores of different tests. The findings have also indicated that it has been due to the teachers and test practitioners to make use of different methods in their testing methodologies.

[Sohrab Rezaei. **The construct validation of Iranian student's Reading Comprehension Tests through Multitrait-Multimethod Procedure**. *Life Sci J* 2012;9(3):2118-2126] (ISSN:1097-8135).
http://www.lifesciencesite.com. 306

Key words: validity, testing, construct validation, multitrait-multimethod, comprehension, cloze, reading, paraphrase.

Introduction

Validity is one of the important concepts in testing. A valid test is one that actually tests what the designer of the test intends to test. Regarding the term "valid test" in relation to testing, there are a number of cautions to be born in mind. Validity deals with the results of a test not with the instrument itself. In this respect, according to Gronlund (1976), we speak of the validity of the test results, or more specifically, of the interpretation to be made from the results. Validity is a matter of degree. Therefore, we should avoid thinking of a test results as valid or not valid. Validity is best considered in terms of categories that specify degrees, such as a high validity, moderate validity, and law validity. Validity is not general but specific. Accordingly, if a test of vocabulary measures vocabulary and nothing else, it is a valid test of vocabulary. In this regard Ingram (1977), states:" when a test measures that which is supposed to measure, and nothing else, it is valid." (P.18)

One of the most important ways of evaluating validity is construct validity. Construct validity is a process of investigating what a test measures. Through which one validates a test not against a criterion or another test, but against a theory. There is a specialized construct validation procedure called the multitrait- multimethod convergent- divergent procedure. The procedure was first described by Campbell and Fiske (1959) and was first recommended for use in the evaluation of language proficiency measures by Stevenson (1974). It is based on the assumption that a test score is a function both of the trait the test measures and of the method by which it is measured. In order to measure the relative contributions of trait and method, it is necessary, for statistical reasons, that two or more traits each be measured by two or more distinct methods. It is for this reason that the procedure is called multitrait-multimethod (**MTMM**) procedure.

Campbell and Fiske (1959) argue that the demonstration of construct validity requires both convergent validity and discriminant validity, that is, multiple of the same construct should be substantially correlated with each other, but les correlated with indicators of other constructs. They propose collecting measures of more than one trait, each of which is assessed by more than one method. Convergent validity is inferred from agreement between measures of the same trait assessed by different methods. Discriminant or divergent validity refers to the distinctiveness of the different traits, and is inferred from the relative lack of correlation between different

traits. Support for these characteristics is based upon inspection or analysis of a multitrait- multmethod (**MTMM)** matrix. **MTMM** matrices have been analyzed by a variety of different procedures. The most frequently employed procedures have been the original Campbell- Fiske Criteria, and an **ANOVA** model. More recently, applications of confirmatory factor analysis have been applied to **MTMM** matrices. Though the approach has been described under a variety of different labels: restricted factor analysis (Brounch & Wolins, 1970), confirmatory factor analysis (Kenny, 1976, Werts, Joreskog, Linn, 1972), path analysis (Schmitt, 1978; Schmitt et al., 1977), and exploratory factor analysis (Lomax and Algina, 1979).

Review of Related Literature

Validity is a frequently misunderstood concept. It is often erroneously believed that a test is simply valid or not valid as if validity were a property of the test itself. In fact, as Cronbach (1971) has pointed out, one does not validate a test. One validates "an interpretation of data arising from a specified procedure. The elements affecting validity include, among others, the test itself, the setting in which the test is administered, the characteristics of the inferences intended to be drawn from the test. The general purpose of the validation procedure is to investigate the extent to which inferences can properly be drawn from performance. The process of collecting evidence of the extent to which such inferences are warranted is called validation.

Although validity has traditionally been discussed in terms of different types, psychometricians have increasingly come to view it as a single, unitary concept. Messik (1988) proposes a unified framework of validity that brings consideration of value implications and social consequences into the validity framework. (P 20). Messik describes validity as an internal evaluative judgment of the degree to which empirical evidence and theoretical rationales support the adequacy and appropriateness of inferences and actions based on test scores. (P13). One of the first characteristics of a test that we examine is its content. If we cannot examine an actual copy of the test we would generally like to see a table of specification and example items, or at least a listing of the content areas covered, and the number of, or relative importance of each area. The consideration of test content is an important part of both test development and test use. Demonstrating that a test is relevant to and covers a given area of content or ability is therefore a necessary part of validation. There are two aspects of this part of validation; content relevance and content coverage. (Messick, 1988). Henning (1987) refers to response validity to describe the extend to which examinees responded in the manner expected by the test developers. If examinees respond in a haphazard or non-reflective manner, their obtained scores may not represent their actual ability. Also if instructions are unclear and the test format is unfamiliar to the students, their response may not reflect true ability and the test may be said to lack response validity. This type of validity investigates the correspondence between the scores obtained from the newly developed test and the scores obtained from some independent outside criteria (Farhady et al, 1994). There are two types of criterion validity; concurrent and predictive. In concurrent validity the criterion behavior may be concurrent with, or occur nearly simultaneously with administration of the test (Backman, 1990). Information on concurrent criterion relatedness is undoubtedly the most commonly used in language testing. Such information typically takes one of the two forms:(1) examining differences in test performance among groups or individuals at different levels of language ability or (2) examining correlations among various measures of a given ability. This type of validity is used to determine how well test scores predict some future behavior. Examining predictive utility is often problematic because, the criterion behavior that we want to is often a complex one that may depend upon a large number of factors in addition to language ability.

Construct validity

Cronbach and Meeld (1965), define a construct as a postulated attribute of people assumed to be reflected in test performance. Thus construct can be viewed as definition of abilities that permit us to state specific hypotheses about how these abilities are or are not related to other abilities, and observed behavior. The notion of construct validity was formerly originated in 1954 with the publication of technical recommendations of a committee of the American Psychological Association. While construct validity is empirical in nature because it involves the gathering of data and the testing of hypotheses, unlike concurrent and predictive validity, it does not have any one particular validity coefficient associated with it. The purpose of construct validation is to provide evidence that underlying theoretical construct begins with a psychological construct that is part of a formal theory. Henning (1987) argues that we have a very great difficulty in establishing construct validity. It is the fact that the construct itself cannot be measured directly. Backman (1990) argues that construct validity concerns the extend to which performance on test is consistent with predictions that we make on the basis of a theory of abilities or constructs. Messik (1980) sees construct validation as a unifying concept that investigates criterion and content considerations into a common framework for testing rational hypotheses about theoretically relevant relationships.

There are different ways through which one can establish the construct validity of a given test. One of the versions is the multitrait –multimethod (MT_MM), convergent divergent validity developed by Campbell and Fiske (1959) and its modified form used by a number of experts. They proposed the use of multimethod matrix in which inter-correlations among several traits each measured by several methods are appraised for evidence of discriminant and convergent validity. Four informal criteria have been suggested by Campbell and Fiske for the purpose of evaluating the MT_MM matrix. First the correlations between similar traits measured by different methods. Second, the convergent validities should be higher than the correlations between different traits measured by different methods. Third, the convergent validities should be higher than the correlations between different traits measured by the same method. Finally, a similar pattern of trait intercorrelations should be apparent in the hetrotrait-monomethod sub matrices and hetrotrait- hetromethod sub matrices. This criterion was questioned by many scholars for example, Jackson (1967), Conger (1971), Tucker (1976) and Seidman (1974). Alwin (1974), Joreskog (1989), Kalleberg and Kluegel (1975), and Werts and Linn (1990), among others, have proposed a path analysis approach for evaluating MT_MM matrices. Jackson (1969) proposed multimethod factor analysis which involves replacing the monomethod blocks of the matrix with identity matrices, thus removing method variance and basing estimated scores on the portion of variance common to more than one method of measurement. Marsh and Hocevar (1983) proposed confirmatory factor analysis as superior method to use in the analysis of multitrait – multimethod data.

Jackson and Singer (1996) got to the conclusion claiming the correlation between males and females upon four different traits are higher in the hetro-method blocks for bearing the same name as compared with the values for the heterotrait-heteromethod correlations. Backman and Palmer (1985) in their study on construct validity of speaking and reading ability concluded that the effect of test method on test score is strong and meaningful. Farr and Jongsma (1993) in their study examined convergent and discriminate validity of three components of a set of integrated reading/writing assessments. The three factors assessed by the test included Response to Reading, Management of content and command of Language. Tepper et al (1992), tried to establish the discriminant and convergent validity of the Problem Solving Style Questionnaire (PSSQ).In this investigation they tried to assess the validity of the PSSQ's two subscales (abstractness/ concreteness and action/ reflection). Findings of the research provided further support for construct validity of new scales. Hierarchically nested confirmatory factor analysis demonstrated the superiority of a model representing the latent variables abstractness/ concreteness and reflection /action over a one trait model (providing evidence of discriminant validity) and a null model (providing evidence of convergent validity).

Statement of the problem

Regardless of the recent researches and findings in the field of teaching reading skill to foreign /second language learners, many teachers are still teaching via different methods. Following the methods, teachers try to use different kinds of testing methods to measure the students reading comprehension ability.

The present study has aimed at determining the impact of testing procedures on reading comprehension ability of the Iranian EFL learners. In other words, this study has been an attempt to determine whether the learners who take a multiple choice cloze test would score significantly higher on reading comprehension than those who take multiple choice comprehension tests, or vice versa.

Nowadays most teachers have a great tendency to use the cloze test technique to check learner's ability and progress on reading comprehension. Therefore, if cloze technique is theoretically a good method to test reading comprehension why do teachers and other examiners use other methods such as paraphrase and multiple choice items in practice?

Concerning the problems, mentioned above, the researchers set forth the following objectives in designing this study:

1- To investigate and compare the impact of cloze test vs. paraphrase tests and Comprehension tests on reading comprehension ability of the subjects under study to find out if there is any correlation among them.

2- To investigate and compare the impact of cloze test vs. paraphrase test procedure on reading comprehension ability of the subjects under study and find out if there is significant correlation between them.

3- To investigate and compare the impact of paraphrase tests vs. comprehension tests on reading comprehension ability of the subjects under study and to fine out if there is any significant correlation between them.

4- To investigate and compare the impact of cloze test vs. comprehension test on reading comprehension ability of the subjects under study to find out any significant correlation between them.

Research Questions

To come up with logical answers, the following research questions have been asked in order to

determine the construct validity of reading comprehension tests through traits of vocabulary and Grammar and methods of multiple choices cloze, **MC** paraphrase and **MC** comprehension called multitrait-multimethod (**MTMM**) procedure.

- IS there any significant correlation among the **MC** cloze, **MC** paraphrase and **MC** comprehension tests on Vocabulary?
- IS there any significant correlation among the **MC** cloze, **MC** paraphrase, and **MC** comprehension tests on grammar?
- IS there any significant correlation among the **MC** cloze, **MC** paraphrase, and **MC** comprehension tests on vocabulary and grammar?
- IS there any underlying construct for the **MC** cloze, **MC** paraphrase and **MC** comprehension tests on Vocabulary and grammar?

Significance of the Study:

Language testing is central to language teaching. It provides goals for language teaching, and it monitors, for both teachers and learners' success in reaching those goals. Its influence on teaching is strong. It provides methodology for experiment and investigation in language teaching and language learning/ acquisition.

The findings of this research would have both theoretical and practical implications for the field of language testing. Obviously, through these findings one positive step would be set forward to make decision upon education careers of testing English as a foreign/ second language. This study has direct relevance to developing test methods as well as moderating methodology and text- books used in language instruction. In other words, the result of such a research would have been of great interest to most language testing practitioners interested in improving the quality of their testing methodologies. Materials developers and curriculum designers may also find the findings of this study useful for instructional goals.

To be more specific, in most language testing situations especially in the case of our country, cloze test technique and multiple choice items have been given more weight in testing reading comprehension ability of learners. More over, nowadays there have been a lot of tests with different methodologies, constructed for beginners and advanced levels, available in the market, whereas the validity of the tests and their methodologies have rarely been verified, and a lot of money and time is spent on producing them. Therefore, investigation in this regard is highly warranted.

Limitations of the study:

1- In this study only two traits (i.e. Vocabulary and grammar) have been tested through three different procedures (ie. **MC** cloze, **MC** paraphrase and **MC** comprehension).

2- Due to the nature of the study, there has been a need to have different kinds of tests, and hence there has been no single test for the purpose of the study, the researchers have had to use different valid tests to collect data.

Method and procedure

To pave the way for the application of this study based on the questions mentioned, several steps have been required in the acts of subject selection, preparation and selection of the needed tests and application of the selected tests to the subjects as well as the interpretation of the results.

Subjects:

A sample of one hundred male students from Karaj Islamic Azad University has been chosen randomly. The subjects were grade 4 under-graduate students majoring in English translation. To ensure the homogeneity of the subjects a TOFEL test was administered. The subjects have been ranked according to the score through the application of the t-Test.

Finally thirty five students who clustered around the mean score have been selected for the study. The rationale behind choosing the subjects from this level and field of study was that the nature of given tests needed subjects who should have been familiar with different kinds of testing procedures namely cloze, comprehension and paraphrase.

Procedure:

After selecting the required subjects, another test has been administered to measure the relative contribution of traits and methods. Two traits of "grammar & vocabulary" have been tested through different methods, namely, "MC cloze, MC comprehension, MC paraphrase" .This test consisted of 15 items for each trait and method. The overall framework of traits and methods treated is shown in the table 1 below:

Table 1. The overall framework of traits and methods.

Method Trait	Mc Cloze	Mc Compreh	Mc Paraph	Total
Grammar	15	15	15	45
Vocabulary	15	15	15	45
				90

Since there was no single valid test for the study. The researchers have necessarily

used Michigan and TOFEL tests as valid sources for the procedure.

Statistical procedure

In order to investigate the research questions, a battery of statistical analyses: namely descriptive statistics, correlation analysis, multivariate analysis of variances and factor analysis have been carried out. The major findings of the study have been presented under the above mentioned topics.

1- Descriptive statistics

Table 2 has represented the mean, standard deviation, and variance for the different instruments employed in this study.

Table 2. Descriptive Statistician

Tests	Mean	Standard Deviation	Variance
Paraphrase Voc.	9.28	2.12	4.50
Paraphrase gram	8.27	2.44	5.95
Comprehension Voc	8.34	2.48	6.17
Comprehension gram	7.08	3.21	10.31
Cloze Voc	10.85	1.97	3.98
Cloze gram	8.28	2.56	6.56

As it has been indicated in Table 2, the highest mean observed, 10.85, belongs to the cloze vocabulary. After cloze vocabulary the highest mean score belongs to paraphrase vocabulary with 9.28 and at the third place, there comes comprehension vocabulary with the mean score of 8.34. The lowest mean observed, 7.08, belongs to the Comprehension grammar tests respectively. Cloze grammar and paraphrase grammar have nearly the same mean scores.

The comprehension grammar tests enjoy the highest standard deviation i.e.3.21 and cloze vocabulary tests enjoy lowest standard deviations with 1.97 respectively. The same fact is observed about the variance with 10.31 for comprehension grammar tests and 3.98 for cloze vocabulary tests.

This table has revealed that there has been a meaningful relationship between the results observed for mean, standard deviation and variance in each group of tests.

2- Correlation analysis;

The correlations existing between the tests have been measured through the Pearson product correlation coefficients. Table 3 displays the correlation coefficients between the tests.

Table 3. Correlation Analysis

	paraph. Voc	Paraph. Gram	comp voc	comp gram	cloze voc	cloze gram
paraphrase voc.	1					
paraphrase gram.	0.14	1				
Comprehension voc	0000 0.40	0.26	1			
Comprehension gram	0.13	0.34	0000 0.36	1		
cloze voc	0.10	0.32	0000 0.55	0000 0.53	1	
cloze gram	0.25	0.34	0000 0.52	0.24	0000 0.36	1
P < .52	Significant at .05 level of significance					

As table 3 has represented, eight correlation coefficients, out of fifteen calculated coefficients, are significant at. 05 level of significance. The highest observed correlation coefficient, 0.55, exists between the cloze vocabulary and the comprehension vocabulary tests. The second high correlation coefficients, i.e. 0.53, exists between cloze vocabulary and comprehension grammar tests and at the third place there is correlation coefficients between cloze grammar and comprehension vocabulary tests with 0.52. The fourth correlation coefficients exists between comprehension vocabulary and paraphrase vocabulary tests with 0.40 level of significance. The correlation coefficients

between cloze grammar and cloze vocabulary tests has been the same as comprehension grammar and comprehension vocabulary tests with 0.36. This fact is seen about the correlation between comprehension grammar and paraphrase grammar and cloze grammar and paraphrase grammar tests with 0.34 level of significance. For the low correlation coefficients, the lowest coefficients observed, 0.10 is that of the cloze vocabulary and the paraphrase vocabulary tests. Here is an interesting point which indicates a close correlation coefficients between paraphrase vocabulary tests with paraphrase grammar and comprehension grammar tests.

3- Analysis of Variance

An analysis of variance (ANOVA) has been employed to investigate the possible difference and interaction between the variables. There has been six tests in this study that could have been grouped under two within subject factors, namely method with three levels, paraphrase, comprehension and cloze, and trait with two levels, vocabulary and grammar.

Prior to the analysis of variance, the Mauchly Sphercity test was employed to determine the homogeneity of the variance of the means. The amount of statistic calculated, .95, with a probability level of .42, has revealed that the mean enjoys enough homogeneity to be analyzed through the ANOVA.

Table 4 shows the F ratio calculated for the method, skill and method by skill factors.

Table 4 .ANOVA Table

Source of variations	SS	DF	MS	F	P
Within cells	273.48	68	4.02	15.48*	.000
Method	124.52	2	62.26		
Within cells	147.78	34	4.32	27.70*	.000
Skill	120.39	1	120.39		
Within cells	299.42	68	4.40	3.62*	.03
Method by skill	31.91	2	15.96		
P ≥ .05 * Significant at .05 level of significance					

The significant F ratios has indicated that the method, skill and the interaction between them have had a high impact on the means of the different tests.

Following the ANOVA, a post hoc Comparison of means through the Scheffe test was used. Table 5 has represented the results of Scheffe tests.

Table 5. Scheffee Tests

Comparison	X1	X2	Tobs	Tcrit	Signific
paraph x comp	7.71	8.92	3.57	2	*
paraph Voc x Par gram	9.28	8.57	1.41	2.02	
paraph voc x Voc	9.28	8.34	1.87	"	
paraph voc x gram	9.28	7.08	4.38	"	*
paraph voc x cloz voc	9.28	10.85	3.13	"	*
paraph voc x cloz gram	9.28	8.28	1.99	"	
Par gram x com voc	8.57	8.34	.45	"	
Par gram x com gram	8/57	7.08	2.97	"	*
Par gram x cloz voc	8.57	10.85	4.54	"	*
Par gram x cloz gram	8.57	8.28	.57	"	
Com voc x com gram	8.34	7.08	2.51	"	*
Com voc x cloz voc	8.34	10.85	5	"	*
Com voc x cloz gram	8.34	8.28	.12	"	
Com gram cloz voc	7.08	10.85	7.51	"	*
Com gram cloz gram	7.08	8.28	2.39	"	*
Cloz voc x cloz gram	10.85	8.28	5.12	"	*
Voc x gram	9.49	7.97	5.28	1.96	*
Voc x cloz	9.49	9.56	.34	1.96	
Gram x cloze	7.97	9.56	5.52	1.96	*

As table 5 has shown, twelve comparisons out of the nineteen made comparisons display significant difference between the means.

4- Factor Analysis

To probe the underlying constructs of the implemented tests a factor analysis through varimax rotation has been run. As shown in table 6, the cloze vocabulary, comprehension grammar, and the paraphrase grammar tests load on the first factor. Their loadings have been .81, .79 and .61 respectively. The paraphrase vocabulary, comprehension vocabulary and cloze grammar tests have loaded on the second factor; with loadings equal to.86, .68 and .59. The correlation between the two factors has been .59.

Table 6. Factor Analysis through Varimax Rotation.

	Factor 1	Factor 2
cloze voc	.81	
Comp gram	.79	
paraph gram	.61	
Paraph voc		.86
Comp voc		.68
Cloze gram		.59
R factor 1,2 = 59		

Data Analysis for question I

The correlation coefficients between the three tests of vocabulary have been as follows:

Cloze with paraphrase. 10

Cloze with comprehension. 55*

Comprehension with paraphrase.40*

The t- transformation of the above coefficients have been; .57, 3.8 and 2.5. Since the critical value of t at 33 degrees of freedom has been 2.02, it could be claimed that the last two correlation coefficients have statistically been significant while the correlation coefficients between the cloze and comprehension, and comprehension and paraphrase tests on vocabulary has not statistically been meaningful.

Data Analysis for question II

In order to probe the second question, the correlation coefficients between the three tests on grammar have been calculated as follows:

Cloze with paraphrase .34*

Cloze with comprehension .24*

Comprehension with paraphrase .34*

The t- equivalent of the above coefficients have been 2.08, 1.42 and 2.08 when compared with the critical value of t at 35 degrees of freedom, it could be concluded that the first and last coefficients have statistically been significant.

Two out of the three calculated coefficients have been meaningful, while the correlation between the cloze and comprehension tests hasn't been significant.

Data Analysis for question III

The correlation coefficients between the vocabulary and grammar test (skills or traits), through the three methods have been:

Vocabulary paraphrase with grammar paraphrase .14

Vocabulary comprehension with grammar comprehension .36*

Vocabulary cloze with grammar cloze, 36.

The t- transformation of these coefficients have been; .81, 2.21 and 2.21. The critical value of t at 33 degrees of freedom has been 2.02. Thus it can be concluded that the correlation between the paraphrase vocabulary and grammar tests was the only non-significant coefficient.

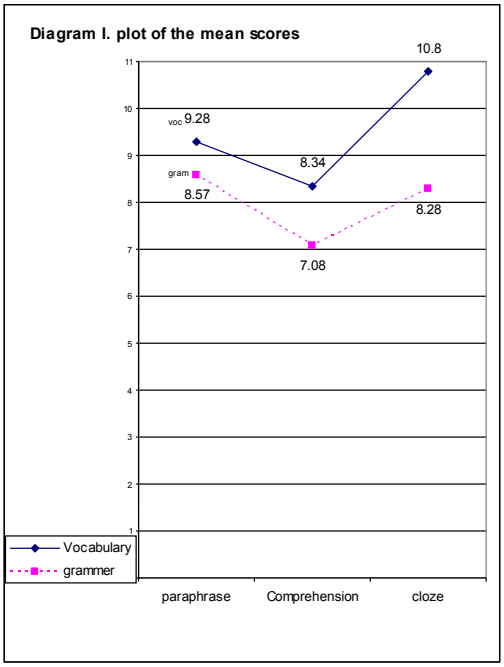
Data Analysis for question IV

To probe the possible differences between the tests, an analysis of variance (ANOVA) has been carried out. The F ratios for the method, trait and the interaction between them (Method by trait) have been 15.48, 27.70 and 3.62 which were all significant at .05 level of significance. In other words, the method, trait and the interaction between them has had a significant impact on the student's performance on the tests.

A close investigation of the results has revealed the fact that the overall mean score of the vocabulary test has been higher than the mean score for grammar. In both traits, the mean scores of the paraphrase method have been higher than those of the comprehension method, after which the mean scores increase for the cloze method.

A factor analysis through varimax rotation has been employed to answer the fourth question. The results have shown that the cloze vocabulary, comprehension grammar and paraphrase grammar tests have loaded on the first, and the paraphrase vocabulary, comprehension vocabulary and cloze grammar tests have loaded on the second factor.

Diagram 1 has represented the pattern of the means plotted against the method and trait factors.



Conclusion

These findings have been consistent with findings of Handel's (1987) study which suggested that two commonly used measures of college outcome yield scores that are reasonably high indices of convergent validity. The highest correlation observed between the three tests of college outcome. The findings of the study have shown that distinct traits within a given method are moderately inter-correlated.

Correlations of the three factors as scored by different scorers have been studied by a multitrait-multimethod procedure. The results provided strong support for both convergent and discriminant validity of three factors. Having in mind the mentioned questions and problems the researchers used a multitrait – multimethod matrix.

A hetro-method – monotrait analysis has been conducted to find answers for the first two developed questions. The findings has shown that there has been a strong correlation among methods applied. A heterotrait- heteromethod analysis has been used to answer the third developed question, and the result has shown that there has been a strong correlation among methods and traits.

Finally, the researchers have found that there has been an underlying construct for traits (grammar and vocabulary) and methods (MC cloze, MC paraphrase, and MC comprehension), in the conducted experiment.

Pedagogical Implications

Language testing is central to language teaching. It provides goals for language teaching and monitors for both language teachers and learners success in reaching those goals. It has a very strong influence on methodology.

Language testing is a complicated issue and much of these complications come from problems of description and measurement which are particularly acute in linguistic and psychological investigations.

This study has clearly shown that performance on language tests is influenced by at least two independent factors: the effect of test method and the effect of the traits being measured. As it was shown in the process of study there has been general agreement among the six tests in ranking the subjects across traits and methods.

Since language teaching and language testing have backwash and wash back effect on each other, it is due to the teachers and test practitioners to make use of different methods in their testing methodologies. It is also helpful to the material developers to benefit from the findings of this study when designing materials for teaching and testing.

Suggestions for Further Research

The research discussed here only has begun to scratch the surface. Many unanswered questions remain, and many of the answers proposed, will no doubt need to be replicated and refined if not in fact discarded.

Given the importance of testing in language teaching and the significant role of the testing methods in reading comprehension, strategies of testing methods used for this purpose will be a fruitful area for further research.

The sample population chosen as subjects for this study was only male students. One can broaden the scope of research to a large extend to include female participants in different universities.

The same investigation may give more insights to language testing instruction if it is carried out with the lower intermediate and advanced level of language learners.

The same procedure can be applied for more traits and methods as well as different language skills like listening comprehension. It can also be applied to other fields of science like physics and chemistry.

Corresponding Author:
Sohrab Rezaei
Assistand Professor Allameh Tabatabaei University
Tel:+982122195144
Mobile:+989363224553
E-mail:Sohrab_rezaei@yahoo.com

BIBLIOGRAPHY

1. Baker David (1989) language testing. A critical survey and practical Guide. University of Bahrain.
2. Bennett Tepper et al (1993) Discriminant and Convergent Validity of the problem Solving Style Questionnaire Educational and Psychological Measurement.V 53,PP 434-444
3. Bresna Han, Mary J, et al.(2005), A Multitrait-Multimethod validity assessment of self construal in Japan, Korea and the U.S. Human Communication Research; V 31, N 1, PP 33-59.
4. Campbell, D.T, and Fiske, D.W. (1959) convergent and discriminant validation by the multitrait – multimethod matrix. Psychological Bulletin.
5. Campbell, D.T, and O'Conneil, E, J. (1967). Method factors in multitrait – multimethod matrices. Multiplicative rather than additive. Multivariat Behavioral Research. V2, PP 409-426.
6. Chang Lie, (1993), Using confirmatory factor analysis of MT-MM data to assess the psychometrical equivalence of 4-point and 6-point Likert-type scales.
7. Darwin D.Hendel (1991), Evidence of Convergent and Discriminant Validity in three Measures of College Outcome. PP 351-357.
8. Davies Alan (1990) Principles of language testing. Basil Blackwell publisher, Cambridge, USA.
9. Douglas N. Jackson. (1975) Multimethod Factor Analysis: A Reformulation Multivariate Behavioral Research. PP 259– 276.
10. Farhady. H, Jafarpoor, A, and Birjandi P. (1994) Language skills Testing from theory to practice, SAMT.
11. Farr, Roger and Jongsma, Eugene (1993) the Convergent, Discriminant Validity of integrated reading, writing assessment. Journal of Research and Development in Education.
12. Henning Grant (1987) A Guide to Language Testing Development Evaluation Research, Newbury House publishers University of California, Los Angles.
13. Harris P.David (1990), Testing English as a Second language MC Graw Hill book Company.
14. Herbert W.Marsh & Dennis Hocevar, (1983) Confirmatory Factor Analysis of Multitrail-Multimethod Matrices.20, 231-248.
15. -Joseph Levin (1989). Multiple Group Factor Analysis of Multitrait - Multimethod Matrices. Multivariate Behavioral, Research, 23,469-487.
16. Joseph Stevens and Patricia, S .Clauser, (2005). Multitrait-Multimethod comparison of selected and constructed response, Assessment of language achievement. University of New Mexico.
17. Kavanaough, M.J.A.C Mackinney and L Wolins (1991) Issues in managerial performance: Multitrait – Multimethod analysis of Ratings. Psychological Bulletin 75, 34-49.
18. Kellabery, A.L. and R. Kluegel (1975) Analysis of the Multigrain.
19. Multimethod Matrix. Some Limitations and an Alternative. Journal of Applied Psychology 60:1-9.
20. Lorena Loisa, (2007). Validating a standard-based classroom assessment of English proficiency. Journal of Language Testing, V, 24, N. 4, PP 489-515.
21. Lowe – NK Ryanwenger – NM, (1992) Beyond Campbell and Fiske- Assessment of convergent and Discriminant validity, Research in Nursing & Health, 15, 67-75.
22. Lomax, R.G. and J.Algina (1979). Comparison of two procedures for analyzing Multitrait- Multimethod matrices. Journal of Educational Measurements 16,177-186.
23. Madsen S. Harolds, (1983) Techniques in testing. Oxford, England.
24. Muis Krista R. (2007). Using MT-MM analysis to examine conceptual similarities of three self regulated learning inventories. British Journal of Educational Psychology. V. 77, PP.177-195.
25. Oller, John W.Jr, (1979) language tests at school. A pragmatic Approach. Longman group limited, London.
26. Plamer, s Adrian and Groot, M.J. peter. The construct validation of test of communicative competence.
27. Pike G. and Banta T. (1989), Using construct validity to Evaluate Assessment Instruments. Research Report.
28. Robert J. Vandenberg & Vida Scarpello (1991) Multitrait- Multimethod Validation of the satisfaction with my supervisor scale; Educational and Psychological Measurement 51,203-212.
29. Ray, M.L, Heeler, R.M. (1975) Analysis techniques for exploratory use the multitrait multimethod matrix Educational and Psychological Measurement, 35,255-265.
30. Roger Farr Evgene jonsma (1993), the convergent, Discriminant validity of Integrated Reading/Writing assessment. Journal of Research and Development in Education. 26,83-90.
31. Schmitt, N, Coyle, B.W. & Saari, B.B. (1997) A review and critique of analyses of multitrait – multimethod matrices. Multivariate Behavioral Research, 12,447-478.
32. Schmitt, N, et al, (1977), A review and critique of analysis of multitrait – multimethod Matrices. Multirariate Behavioral Research, 12:442-778.
33. Stephen L. Goling & Edward Seidman (1974) Analysis of Multitrait- Multimethod Matrices: A two step principle components procedure. Multivariate Behavioral Research, 9,479-496.
34. Susanna Martha Brutsh (1980) convergent, discriminant validation of proficiency in oral and written production of French. Paper presented at the TEOSL.
35. Vinary Kanetkar, Martin G. Evans (2001). Confirmatory factor analysis of MT-MM matrices. University of Toronto.
36. WWW.Socialresearchmethods.net.
37. WWW.sagehpub.com.
38. WWW.uoregon.edu.
39. WWW.ingentaconnect.com.
40. WWW.wikipedia.org.

1/26/2012

On the solution of a functional integral equation of Fredholm type with degenerate kerenel

Abdallah A. Badr

Department of mathematics, Faculty of Science Alexandria University, Egypt
badrzoo@yahoo.co.com

Abstract: Here, the existence and the uniqueness of the solution of a class of a nonlinear integral equation with discontinuous kernel are discussed and proved. A degenerate kernel method is used, as a numerical method, to obtain a class of a system of a nonlinear algebraic equations. Many important theorems related to the existence and uniqueness of the produced algebraic system are derived. Finally, numerical examples are discussed and the error estimate, in each case, is calculated.
[Abdallah A. Badr. **On the solution of a functional integral equation of Fredholm type with degenerate kerenel**. *Life Sci J* 2012;9(3):2127-2130] (ISSN:1097-8135). http://www.lifesciencesite.com. 307

Keywords: Nonlinear integral equation (NIE), nonlinear algebraic system (NAS), degenerate kernel method, Hammerstein integral equation.

1. Introduction

Integral equations of various types and kinds play an important role in many branches of linear and nonlinear functionals analysis and their applications in the life science, mathematical physics, engineering and contact problems in the theory of elasticity (see [1-3]). Therefore many different methods and numerical treatments are established to obtain the solution of the NIE. For these methods see Brunner et al. [4], Kaneko and Xu [5], Kilbas and Saigo [6], Dariusz [7], Abdou et al. [8,9], and Diogo and Lima [10]. This paper is concerned with finding a numerical solution of the following functional integral equation

$$\mu\,\phi(x)-\lambda f(x,\int_0^1 k(x,y)\gamma(y,\phi(y))dy)=g(x) \qquad (1)$$

where f, k, γ and g are known continuous functions while ϕ is unknown function, μ is a constant determine the kind of the IE and λ is a constant, may be complex, and has many physical meanings. The importance of Eq.(1) comes from it's special cases, for example when $f(x,u(x))=u(x)$, we have

$$\mu\phi(x)-\lambda\int_0^1 k(x,y)\gamma(y,\phi(y))dy=g(x).$$

This equation is called a Hammerstein integral equations.

In this work, the existence and uniqueness solution of Eq.(1), under certain conditions, are discussed and proved. Also, we present the degenerate kernel method and we consider the problem of the existence and uniqueness of the solution of the new NAS associated with the degenerate kernel. Also, the convergence problem of the numerical solution is also considered. Many examples are presented and the error estimate, in each case, is computed.

2. The existence and uniqueness solution

In order to guarantee the existence of a unique solution to Eq.(1), we will assume throughout this work the following conditions:

(i) The kernel $k(x,y)$ and the given function $g(x)$ are in the class $C([0,1]\times[0,1])$ and satisfies, in general the condition

$$\{\int_0^1\int_0^1 k^2(x,y)dxdy\}^{1/2}\le A,$$

$$\|\,g(x)\,\|_{L_2[0,1]}=\{\int_0^1 g^2(x)dx\}^{1/2}=B.$$

(ii) The two continuous functions $f(x,u(x))$ and $\gamma(x,v(x))$, where $x\in[0,1]$, and $u,v\in(-\infty,\infty)$ satisfy the condition

$$\{\int_0^1 |\,f^2(x,u(x))\,|^2\,dx\}^{1/2}\le C_1\,\|\,u\,\|,$$

$$\{\int_0^1 |\,\gamma(x,v(x))\,|^2\,dx\}^{1/2}\le C_2\,\|\,v\,\|$$

(C_1, C_2 are constants).

(iii) The two functions $f(x,u(x))$ and $\gamma(x,v(x))$ satisfy Lipschitz condition for the second argument

$$|\,f(x,u_1(x))-f(x,u_2(x))\,|\le D_1\,|\,u_1(x)-u_2(x)\,|,$$

$$|\,\gamma(x,v_1(x))-\gamma(x,v_2(x))\,|\le D_2\,|\,v_1(x)-v_2(x)\,|$$

(D_1 and D_2 are constants).

Theorem (1): Under the following condition

$$|\,\lambda\,|<\frac{|\,\mu\,|}{AD_1D_2}$$

the NIE (1) has a unique solution in $L_2[0,1]$ where the radius of convergence is given by

$$\rho = \frac{B}{(|\mu| - |\lambda| AD_1D_2)}.$$

This can be proved by a direct application to the Banach contraction principal. To obtain a higher order convergence rate, we need to assume higher order smoothness conditions on the kernel $k(x,y)$.

3. Degenerate kernel method

Suppose that $k_n(x,y)$ is an approximation of the kernel $k(x,y)$ and that it is of the degenerate form

$$k_n(x,y) = \sum_{i=1}^{n} B_i(x)C_i(y) \qquad (2)$$

where $\{B_i(x)\}$ and $\{C_i(y)\}$ are assumed to be a linearly independent set of functions in $L_2[0,1]$. Also, we assume

$$\{\int_0^1\int_0^1 |k(x,y) - k_n(x,y)|^2 \, dxdy\}^{1/2} \to 0, \; n \to \infty. \qquad (3)$$

Hence, the expected solution of the NIE associated with the degenerate kernels $k_n(x,y)$ which converges to the exact solution of Eq.(1) is of the form

$$\mu\phi_n(x) - \lambda f(x, \int_0^1 k_n(x,y)\gamma(y,\phi_n(y))dy) = g(x). \qquad (4)$$

To obtain the solution of this equation, $\phi_n(x)$, we use (2), in this equation to get

$$\mu\phi_n(x) = g(x) + \lambda f(x, \sum_{i=1}^{n}\alpha_{i,n}B_i(x)), \qquad (5)$$

where

$$\alpha_{i,n} = \int_0^1 C_i(y)\gamma(y,\phi_n(y))dy, \quad 1 \le i \le n. \qquad (6)$$

Once the constants $\alpha_{i,n}$ have been determined, the approximate solutions of (5) are obtained.

Substituting (5) into (6), we have

$$\alpha_{j,n} = \int_0^1 C_j(y)\gamma[y, \frac{g(y)}{\mu} + \frac{\lambda}{\mu} f(y, \sum_{i=1}^{n}\alpha_{i,n}B_i(y))]dy. \qquad (7)$$

Define

$$H_j(\alpha_1,\alpha_2,,\alpha_n) = \int_0^1 C_j(y)\gamma[y, \frac{g(y)}{(\mu)} + \frac{\lambda}{\mu} f(y, \sum_{i=1}^{n}\alpha_{i,n}B_i(y))]dy \qquad (8)$$

Then, the formula (7) represents a NAS, which can be written in a vector notation as

$$\alpha = F(\alpha) \qquad (9)$$

where $\alpha^T = (\alpha_1, \alpha_2, , \alpha_n)$ and $F^T(\alpha) = (F_1(\alpha), F_2(\alpha), ..., F_n(\alpha))$.

In other words, the numerical solution of the NIE (1) reduces to an optimization problem in which an unknown scalar vector α is to be found such that $\alpha - F(\alpha)$ is minimized.

4.Nonlinear algebraic system

Now, we shall show that, under some mild assumptions, the unique solution of the NAS (9) corresponds to the unique solution of Eq.(5) for each $n, n = 1,2,3,..$

To prove that the NIE (5) has a unique solution in $L_2[0,1]$, we write Eq.(5) in the integral operator form

$$(\overline{W}_n\phi)(x) = \frac{g(x)}{\mu} + (W_n\phi)(x), \quad (\mu \ne 0), \qquad (10)$$

(2) where

$$(W_n\phi)(x) = \frac{\lambda}{\mu} f(x, \int_0^1 k_n(x,y)\gamma(y,\phi(y))dy). \qquad (11)$$

Also, in view of conditions (i) and (iii) there exists an integer N such that for each $n > N$, and after neglecting a very small constant, we have

$$\{\int_0^1\int_0^1 |k_n(x,y)|^2 \, dx\, dy\}^{1/2} \le A. \qquad (12)$$

Theorem (2): Under the conditions (ii), (iii) and (12), the NIE (5) has a unique solution.

The proof of this theorem depends on the following two lemmas.

Lemma 1: Under the conditions (12) and (ii) the operator $\overline{W}_n$ defined by (10) maps the space $L_2[0,1]$ into itself.

Proof: In view of the formulas (11) and (10), we get

$$\| \overline{W}_n\phi \|_{L_2[0,1]} \le \frac{1}{|\mu|} \| g(x) \| + |\frac{\lambda}{\mu}| \| f(x, \int_0^1 k_n(x,y)\gamma(y,\phi(y))dy) \|. \qquad (13)$$

Applying Cauchy-Schwarz inequality, then using the conditions (12) and (ii), the above inequality can be adapted to

$$\| \overline{W}_n\phi \|_{L_2[0,1]} \le \frac{B}{|\mu|} + \sigma_1 \| \phi \|, \quad (\sigma_1 = \frac{\lambda}{|\mu|} CC_1C_2 > 1). \qquad (14)$$

The last inequality shows that, the operator $\overline{W}_n$ maps the ball S_{ρ_1} into itself where

$$\rho_1 = \frac{B|\mu|}{(|\mu| - |\lambda| CC_1C_2)}. \qquad (15)$$

Moreover, the inequality (14) involves the boundness of the operator W and $\overline{W}$ given by Eq.(11) and of Eq.(10) respectively.

lemma 2 : Under the conditions (12), (ii) and (iii) the operator $\overline{W}_n$ is continuous in the space $L_2[0,1]$.

Proof : For two functions ϕ_1 and ϕ_2 in $L_2[0,1]$, we have

$$\| \overline{W}_n\phi_1 - \overline{W}_n\phi_2 \| \leq |\frac{\lambda}{\mu}| . \| f(x, \int_0^1 k_n(x,y)\gamma(y,\phi_1(y))dy) - f(x, \int_0^1 k_n(x,y)\gamma(y,\phi_2(y))dy) \| .$$

Applying Cauchy-Schwarz inequality, and with the aid of conditions (12) and (iii), we get

$$\| \overline{W}_n\phi_1 - \overline{W}_n\phi_2 \| \leq |\frac{\lambda}{\mu}| CD_1D_2 \| \phi_1 - \phi_2 \| . \quad (16)$$

This inequality shows that, the operator $\overline{W}$ is continuous in the space $L_2[0,1]$. Moreover under the condition $|\lambda| < \frac{|\mu|}{CD_1D_2}$, the operator $\overline{W}_n$ is a contractive in the space $L_2[0,1]$. Then by Banach fixed point theorem, the operator $\overline{W}_n$ has a unique fixed point which is, of course, the unique solution of Eq. (5) .

Now, we go to prove that the NAS of Eq.(9) has a unique solution. And this solution is the same solution of Eq. (5).

Theorem (3) : Under the condition

$$G = B_1D_1\{\sum_{i=1}^n \int_0^1 |B_i(x)|^2 dx\}^{1/2} \{\sum_{i=1}^n \int_0^1 |C_i(x)|^2 dx\}^{1/2} < 1. \quad (17)$$

The NAS (9) have a unique solution $\alpha^* = (\alpha_{1n}^*, \alpha_{2n}^*, .., \alpha_{nn}^*)$ and

$$\mu\phi_n(x) = g(x) + \lambda f(x, \sum_{i=1}^n \alpha_{in}^* B_i(x)) \quad (18)$$

is the unique solution of Eq. (5).

Proof: Define the discrete ℓ_2 norm by $\| \alpha \|_{\ell_2} = \{\sum_{i=1}^n |\alpha_i|^2\}^{1/2}$ for $\alpha = (\alpha_1, \alpha_2, ., \alpha_n)^T \in \ell_2(n)$.

Then for $\alpha^{(1)} = (\alpha_1^{(1)}, \alpha_2^{(1)}, , \alpha_n^{(1)})$ and $\alpha^{(2)} = (\alpha_1^{(2)}, \alpha_2^{(2)}, , \alpha_n^{(2)})$, using Eq.(8), we have

$$\| H(\alpha^{(1)}) - H(\alpha^{(2)}) \|_{\ell_2} = \| \int_0^1 C_i(y)\{\gamma[y, g(y) + f(y, \sum_{i=1}^n \alpha_{in}^{(1)} B_i(y)] - \gamma[y, g(y) + f(y, \sum_{i=1}^n \alpha_{in}^{(2)} B_i(y)]\} dy \|_{\ell_2} .$$

Using the condition (iii) on γ, we follow

$$\| H(\alpha^{(1)}) - H(\alpha^{(2)}) \|_{ell_2} \leq (\sum_{i=1}^n \int_0^1 |C_i(x)|^2 dx)^{1/2} B_1 \| \int_0^1 \{f(y, \sum_{i=1}^n \alpha_{in}^{(1)} B_i(y)) - f(y, \sum_{i=1}^n \alpha_{in}^{(2)} B_i(y) dy \|_{\ell_2} .$$

Finally, we have

$$\| H(\alpha^{(1)} - H(\alpha^2) \| \leq B_1D_1 (\sum_{i=1}^n \int_0^1 |C_i(x)|^2 dx)^{1/2} (\sum_{i=1}^n \int_0^1 |B_i(x)|^2 dx)^{1/2} \| \alpha^{(1)} - \alpha^{(2)} \|_{\ell_2} .$$

Using the condition (17), H is a contraction operator in $\ell_2(n)$. There fore H has a unique fixed point α^*, i.e $\alpha^* = H(\alpha^*)$. For this α^*, it is obvious that $\phi_n(x)$ defined by (18) is a solution of (9), and by Theorem (2), $\phi_n(x)$ is the unique solution of (5).

5.The convergence

In this section, we study the rate of convergence of the the approximate solution $\phi_n(x)$ to the solution of Eq.(1), $\phi(x)$.

Theorem (4) : If condition (3) holds and if

$$\| k - k_n \|_{L_2[0,1]} = \{\int_0^1 \int_0^1 |k(x,y) - k_n(x,y)|^2 dxdy\}^{1/2} \quad (19)$$

then we have

$$\| \phi - \phi_n \|_{L_2} \leq \frac{|\lambda| D_1C_2 \| \phi \|}{|\mu| - |\lambda| CD_1D_2} \| k - k_n \| . \quad (20)$$

Proof : We can write

$$\| \phi - \phi_n \| \leq |\frac{\lambda}{\mu}| \{\int_0^1 | f(x, \int_0^1 k(x,y)\gamma(y,\phi(y))dy) - f(x, \int_0^1 k_n(x,y)\gamma(y,\phi_n(y))dy |^2 dx\}^{1/2} . \quad (21)$$

Using condition (iv) and the inequality properties, we obtain

$$\| \phi - \phi_n \| \leq |$$

$$\frac{\lambda}{\mu} | D_1 \{ [\int_0^1 \int_0^1 | k(x,y) - k_n(x,y) |^2 \, dxdy$$

$$\int_0^1 | \gamma(y, \phi(y)) |^2 \, dy]^{1/2}$$

$$+ [\int_0^1 \int_0^1 | k_n(x,y) |^2 \, dxdy$$

$$\int_0^1 | \gamma(y, \phi(y)) - \gamma(y_1 \phi_n(y)) |^2 \, dy]^{1/2} \}.$$

Using the conditions (12), (iii) and (iv) and with the aid of Eq. (19), we have

$$\| \phi - \phi_n \| \leq \frac{| \lambda | D_1 C_2 \| \phi \|}{| \mu | - | \lambda | C D_1 D_2} \| k - k_n \|.$$

6. Examples

Example 1:

Consider the integral equation

$$\phi(x) - \{\int_0^1 x^2 y \phi^2(y) dy\}^{\frac{1}{2}} = \frac{x}{2}.$$

Assume $B_1(x) = x^2, C_1(y) = y.$ Hence Eq.(5) gives an approximate solution of the above equation in the form

$$\phi^* = \frac{x}{2} + x\sqrt{\alpha}.$$

Using Eq.(6), the parameter α is given by

$$\alpha = \int_0^1 y[\frac{y}{2} + y\sqrt{\alpha}]^2 dy.$$

Solving this equation, we have $\alpha = \frac{1}{4}$ or $\alpha = \frac{1}{36}$ and hence the approximate solution is $\phi^*(x) = x$ or $\phi^*(x) = \frac{2x}{3}$.

Example 2:

Consider the integral equation

$$\phi(x) - \{\int_0^1 (1 + xy) \phi^2(y) dy\}^2 = \frac{5x}{6} - \frac{x^2}{16} - \frac{1}{9}.$$

Assume

$B_1(x) = 1, B_2(x) = x, C_1(y) = 1, C_1(y) = 1, C_2(y) = y$. Hence, Eq. (5) gives

$$\phi^*(x) = \frac{5x}{6} - \frac{x^2}{16} - \frac{1}{9} + (\alpha_1 + \alpha_2 x)^2.$$

Using Eq.(6), the parameters α_1, α_2 are given by

$$\alpha_1 = \int_0^1 \{g(x) + [\alpha_1 + \alpha_2 * B_2(x)]^2\}^2 dx,$$

$$\alpha_2 = \int_0^1 x\{g(x) + (\alpha_1 + \alpha_2 * B_2(x))^2\}^2 dx.$$

Solving these equations, we obtain $\{\alpha_1 = 0.2206977763, \alpha_2 = 0.1518207037\}$ or $\{\alpha_1 = 0.3390085493, \alpha_2 = 0.2128606384\}$.

7. Discussions

We see from this paper that the numerical solution of the functional integral equation, Eq. (1) reduces to an optimization problem, Eq.(9). Once we obtain the solution of this optimization problem, an approximate solution to the functional integral is obtained. In practice, searching for this optimal solution is not an easy question. In example one, we found two solutions in which one of these two solutions gives us the exact solution. In example 2, we obtained a system of two algebraic equations each is of order four. One of this four solutions gives us a very good approximate to the exact . Among of the these solution, we select the solution in which $\alpha - F(\alpha)$ is minimized.

Corresponding Author:
Dr. Abdallah A. Badr
Department of Mathematicsy
Faculty of Science, Alexandria University
Alexandria, Egypt
E-mail: badrzoo@yahoo.com

References

1. Kilbas A. A., M. Saigo. On Solution of nonlinear Abel-Volterra integral equation, J. Math. Analy. Appl. 1999; 229:41-56.
2. Abdou M.A., Badr A.A.. On a method for solving an integral equation in the displacement contact problem, J.Appl. Math. Comp. 127, (2002) 65-78.
3. Abdou M.A. On the solution of linear and nonlinear integral equation, Appl. Math. Comput. 2003; 46:857-871.
4. Brunner H., Crisci M. R., Russo E., Recchio A. A family of methods for Abel integral equations of the second kind, J. Comp. Appl. Math. 1991; 34:211 - 219 .
5. Kaneko H., Xu Yu. Degenerate kernel method for Hammerstein equations, Math. Comput. 1991; 56(195):141-148.
6. Bugajeweski D. On BV-Solutions of some nonlinear integral equations, integer. Equ. Oper, theory 2003; 46: 387 - 398.
7. Abdou M. A., El-Sayed W., Deebs E. A Solution of a nonlinear integral equation, J. Appl. Math. Comp. 2005; 160: 1-14.
8. M. A. Abdou, M. M. El-Borai and M. M. El-Kojok, Toeplitz matrix method and nonlinear integral equation of Hammerstein type, J. Comp. Appl. Math. 2009; 223: 765-776.
9. Diogo T., Lima P. Super convergence of collocation methods for a class of weakly singular Volterra integral equations J.comp. Appl. Math. 2008; 218: 307 - 316.

Study of Cardiac Valvular Calcification in ESRD Patients on Regular Hemodialysis (A Single Center Study)

Abdel-Bassit El Shaarawy[1], Mona Hosny[1], Manar Raafat[2] and Nelly Gendy[2]

[1]Internal Medicine Department, Faculty of Medicine, Ain Shams University
[2]Internal Medicine Department, Theodor Bilharze Institute.
elhamed_3@yahoo.com

Abstract: Cardiac valve calcification is common among patients with chronic kidney disease (CKD). Risk factors include alterations in calcium and phosphorus metabolism, elevated calcium phosphorus product and persistent elevations in plasma parathyroid hormone (PTH). Echocardiography is a simple and inexpensive method for detection of valvular calcifications as suggested by KDIGO guidelines. 60 Patients on regular HD constituted group A (36 males and 24 females) and 25 healthy volunteers constituted group B. Group A was subdivided into: Group I: 21 patients with no valvular calcification, group 2: 26 patients with aortic valve calcification and group 3: 13 patients with aortic and mitral valve calcification. For all, the following was done: clinical examination, serum Ca, serum P, serum albumin, serum creatinine, BUN and PTH level in blood. M-mode echo cardiography was done for all. Age, duration of dialysis and duration of 1ry kidney disease was higher in group 2 and 3 compared to group 1 (p = 0.0001). Calcium was higher in group 2 than group 1 (P = 0.09) and group 3 (p = 0.004) than group I phosphorus was higher in group 2 and 3 than group 1 (P = 0.001). P was higher in group 3 than group 2 (p = 0.0001). Ca x P was higher in group 2 and 3 than group 1 (P = 0.0001), in group 3 than group 2 (p = 0.01) PTH was higher in group 1 than group 2 (P = 0.06). Cardiac dysfunction by echocardiography was least in group 1, increasing in group 2 and being highest in group 3. It was found that calcified valve groups has taken higher doses of Calcium and Vitamin D3. We have to take care on prescribing Ca and vitamin D3 to ESRD patients on regular HD.
[Abdel-Bassit El Shaarawy, Mona Hosny, Manar Raafat and Nelly Gendy. **Study of Cardiac Valvular Calcification in Esrd Patients on Regular Hemodialysis (A Single Center Study).** *Life Sci J* 2012; 9(3):2131-2146]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 308

Key Words: Valvular Calcification- Ca- P- PTH –Ca x P- Hemodialysis.

1. Introduction

Patients with end-stage renal disease (ESRD) are frequently burdened with calcific valvular heart disease ***(Adragoa et al., 2004).*** Valvular involvement in ESRD is most commonly manifested as mitral annular calcification and aortic valve calcification. Both mitral and aortic valve calcification (MAC) occur more frequently and at younger age in those with ESRD than in those with normal renal function ***(Ernesto et al., 2003).***

KDIGO provided a new definition of CKD-MBD: a systemic disorder of mineral and bone metabolism due to CKD, manifested by abnormalities of calcium, phosphorus, PTH or vitamin D metabolism, abnormalities in bone turnover, vascular or other soft-tissue calcification. The prevalence and severity of extraosseous calcification, increase as kidney function decreases ***(Uhlig et al., 2010).***

KDIGO recommended that an echocardiogram can be used to detect the presence or absence of valvular calcification, as reasonable alternative to computed tomography based imaging in patients with CKD stages 3-5 ***(Moe et al., 2009).***

Aim of the Work

To determine the frequency of valvular calcifications in hemodialysis patients using echocardiography and its relation to demographic and laboratory data.

2. Patient and Methods:

The study was carried on 60 patients (group A) with ESRD on regular hemodialysis [24 females (40%) and 36 males (60%) mean age 49 years] and 25 healthy control subjects (group B), [11 females (44%) and 14 males (56%), mean age 49.32 years]. The study was conducted in hem dialysis unit of Theodor Bilharz research institute between July 2011 and January 2012. Group A patients started hemodialysis for more than six months prior to the study. Patients were dialyzed for 4 hours three times weekly using high flux filters and acetate solution as dialysate. All patients had arteriovenous fistula. We excluded from the study patients having rheumatic heart disease, congenital heart disease or rheumatic fever history.

Group A was divided into 3 subgroups according to the presence or absence of valvular calcification:

Group 1: Patients with no valvular calcification.
Group 2: Patients with aortic valve calcification only.
Group 3: Patients with both aortic and mitral valve calcification in ESRD.

For all patients and controls the following was done:

- Full medical history and clinical examination.
- Laboratory investigations including:

- Serum creatinine (mg/dL): according to the method of ***Yatzidis (1974).***
- Blood urea (mg/dl): according to the method of ***Tobacco et al.(1979).***
- Serum calcium (mg/dl): according to the method of ***Renoe et al. (1980).***
- Serum phosphorus (mg/dl): according to the method of ***Farrel (1987).***
- Serum albumin (mg/dl): according to the method of ***Doumas et al. (1971).***
- Parathyroid hormone level (ng/ml): according to the method of ***Endras et al. (1989).***
- For purposes of the study we calculated the value of calcium phosphorus product (CaxP (mg^2/dl^2).

3. Echocardiography:

According to the standard protocol, a professional skilled cardiologist had performed echocardiographic examination to all patients and control subjects included in the study.

M^- mode, two dimensional echocardiography and Doppler ultrasound studies (pulsed, continuous wave and colour flow imaging) were performed using a high resolution (ALT 5000 HDI) scanning device.

We measured aortic valve dimension (AO in mm), valve calcification in ESRD, Right atrium dimension (RA in mm), End-diastolic dimensions (EDD in mm), End-systolic dimensions (ESD in mm), shortening fraction (FS in %), ejection fraction (EF in %), interventricular septum dimensions (IVST d in mm), and posterior wall dimensions in diastole (PWTd in mm).

4. Statistical methods:

Statistical package of social science (SPSS) version 15.0 was used for analysis of data. Data was summarized as mean and standard deviation, T-test was used for analysis of two quantitative data and non parametric test (Mann-Whitney U) was used when data was not symmetrically distributed. Also Pearson linear correlation test was used in study valve calcification in ESRD.

***P*-value**

* *P*-value was considered significant if < 0.05 (S).
* *P* -value was considered borderline significance if < 0.1 (BS).
* *P* -value was considered highly significant if < 0.01 (HS).
* *P* -value was considered non-significant if ≥ 0.1 (NS).

Table (1): Frequency distribution of demographic data of group A

Variables	N	%
Sex		
Male	36	60
Female	24	40
Primary kidney disease:		
Unknown	13	21.7
Diabetes mellitus	12	20
Hypertension	27	45
Chronic glomerulonephritis	8	13.3
Vascular insults:		
Non	29	48.3
Peripheral vascular insult	19	31.7
Ischemic heart disease	8	13.3
Stroke	4	6.7
Aortic calcification		
Negative	21	35
Positive	39	65
Mitral calcification		
Negative	47	78.3
Positive	13	21.7
Aortic regurge		
AR	18	10.8
Aortic stenosis	0	0
Mitral stenosis	0	0

Table (2): Comparison of age between group A and group B

Variables	Group A		Group B		*P*-value
	Mean	SD	Mean	SD	
Age (yrs)	25.00	65.00	49.32	10.68	0.9 (NS)

*** Unpaired t-test**

Table (3): Comparison of laboratory data between group A and group B

Variables	Group A		Group B		*P*-value
	Mean	SD	Mean	SD	
Urea (mg/dl)	40.00	5.00	29.24	5.03	0.0001* (HS)
Creatinine (mg/dl)	0.80	0.130	1.03	0.16	0.0001* (HS)
Albumin (g/day)	3.59	0.21	4.11	0.45	0.0001* (HS)
Ca (mg/dl)	8.47	1.07	9.22	0.47	0.0001* (HS)
P (mg/dl)	6.26	1.80	3.43	0.56	0.0001* (HS)
Ca x P (mg^2/dl^2)	52.47	14.46	31.56	5.29	0.0001* (HS)
PTH (mg/dl)	476.86	46.60	34.88	8.6	0.0001* (HS)

* Unpaired t-test.

Table (4): Comparison of laboratory data between group A and group B

Variables	Group A		Group B		*P*-value
	Mean	SD	Mean	SD	
AO (mm)	21.00	4.2	29.52	4.41	0.9(NS)
RA (mm)	27.00	6.4	35.76	3.79	0.005*(HS)
EDD (mm)	35.00	68.00	50.55	7.17	0.8(NS)
ESD (mm)	20.00	43.00	30.77	5.21	0.007*(HS)
FS (%)	29.00	54.00	39.00	5.48	0.1(NS)
EF (%)	55.00	84.00	68.72	6.91	0.0001*(HS)
IVSTd (mm)	7.00	1.2	8.94	1.25	0.0001*(HS)
PWTd (mm)	7.000	1.2	8.92	1.35	0.000*(HS)

* Unpaired t-test.

Group 1 constituted 35% of all patients of group A and it comprised 9 females (42.8%) and 12 males (57.15%), group 2 constituted 43.33% of all patients of group A and it comprised 12 females (46.15%) and 14 males (53.85%). Group 3 constituted 21.66% of all patients of group A and it comprised 3 females (23.06%) and 10 males (76.94%) .

Table (5): Results of valvular calcification and valvular lesions in HD patients

	Valvular calcification	No valvular calcification
Number (n %)	39 (65%)	21 (35%)
Isolated mitral valve calcification	--	--
Isolated aortic valve calcification	26 (66.66%)	--
Mitral and aortic valve calcification	13 (33.33%)	--
Mitral regurge	17 (43.5%)	--
Mitral stenosis	--	--
Aortic regurge	18 (46.15%)	--
Aortic stenosis	--	--

Data are (n %)

Table (6): Comparison between hemodialysis patients without valvular calcification (group 1) and with valvular calcification (group 2 and 3).

Variables	Group 1 (n = 21)	Group 2 & 3 (n = 39)	*P*-value
Age (years)	38.62±5.54	54.54±8.80	0.0001
Duration of hemodialysis (years)	2.43±1.35	9.15±2.83	0.0001
Laboratory data			
Urea (mg%)	132.24±49.99	111.15±31.24	0.06
Creatinine (mg%)	9.08±3.35	9.58±5.67	0.7
Albumin (g%)	3.61±0.16	3.58±0.23	0.5
Ca (mg%)	8.20±1.00	8.62±1.09	0.2
P (mg%)	4.62±0.99	7.14±1.5	0.0001
Cax P (mg^2/dl^2)	37.51±7.37	60.52±10.28	0.001
PTH (mg%)	596.47±535.82	412.45±363.03	0.1
Echocardiographic data			
AO (mm)	29.19±4.30	30.33±4.5	0.3
LA (mm)	37.86±5.99	40.38±5.76	0.1
EDD (mm)	50.83±7.28	49.44±9.54	0.6
ESD (mm)	33.60±6.96	36.99±9	0.1
FS(%)	39.57±9.50	34.59±6.92	0.02
EF (%)	65.24±9.28	57.51±8.97	0.003
IVSTd (mm)	11.05±2.16	11.67±2.07	0.3
PWTd (mm)	10.95±2.20	11.83±1.53	0.08
Vascular disease			
Peripheral vascular disease	5 (23.8%)	14 (35.9%)	0.04
Ischemic heart disease	1 (4.8%)	7 (17.9%)	0.04
Stroke	0 (0%)	4 (10.3%)	0.04
No vascular insults	15 (71.4%)	14 (35.9%)	0.04

Drugs Calcium (mg/day) Alfacalcidol (µg/wk)	 1857.14±654.65 2.93±0.33	 3384.62±891.93 2.31±0.76	 0.0001 0.001

Table (7): Valvular calcification according to the etiology of renal disease

Etiology of renal disease	N = 39
Hypertension	20 (51%)
Diabetes	10 (25.6%)
Chronic glomerulonephritis	4 (10.25%)
Unknown etiology	5 (12.8%)

Table (8): Comparison between group 1 and group 2 as regards demographic data.

Variables	**Group 1**		**Group 2**		***P*-value**
	Mean	**SD**	**Mean**	**SD**	
Age (yrs)	38.62	5.54	53.73	9.46	0.0001* (HS)
Duration of primary kidney disease (yrs)	5.54	2.11	16.14	7.66	0.0001* (HS)
Duration of hemodialysis (yrs)	2.43	1.35	8.50	2.61	0.0001* (HS)

* Unpaired t-test.

Table (9): Comparison between group 1 and group 2 as regards laboratory data

Variables	**Group 1**		**Group 2**		***P*-value**
	Mean	**SD**	**Mean**	**SD**	
Urea (mg/dl)	132.24	49.99	107.88	26.88	0.04* (S)
Creatinine (mg/dl)	9.08	2.35	9.89	2.74	0.6 (NS)
Albumin (g/day)	3.61	0.16	3.59	0.24	0.7 (NS)
Ca (mg/dl)	8.20	1.00	8.96	0.90	0.009* (HS)
P (mg/dl)	4.62	1.00	6.48	1.22	0.0001* (HS)
Ca x P (mg^2/dl^2)	37.51	7.37	57.66	10.28	0.0001* (HS)
PTH (mg/dl)	596.47	135.82	362.12	85.23	0.06 (BS)

* Unpaired t-test.

Table (10): Comparison between group 1 and group 2 as regards echocardiographic parameters

Variables	**Group 1**		**Group 2**		***P*-value**
	Mean	**SD**	**Mean**	**SD**	
AO (mm)	29.19	4.30	29.96	4.89	0.6 (NS)
RA (mm)	37.86	5.99	40.19	6.49	0.2 (NS)
EDD (mm)	50.83	7.28	48.16	9.85	0.3 (NS)
ESD (mm)	33.60	6.96	35.19	8.04	0.5 (NS)
FS (%)	39.57	9.50	35.38	7.68	0.1 (NS)
EF (%)	65.24	9.28	58.19	9.88	0.02* (S)
IVSTd (mm)	11.05	2.16	11.88	2.29	0.2 (NS)
PWTd (mm)	10.95	2.20	11.89	1.72	0.1 (NS)

* Unpaired t-test.

Table (11): Comparison between group 1 and group 2 as regards drug doses.

Variables	**Group 1**		**Group 2**		***P*-value**
	Mean	**SD**	**Mean**	**SD**	
Calcium (mg/dl)	1857.14	454.65	3173.08	782.52	0.04* (S)
Alphacalciferol (µg)	2.93	0.33	2.42	0.74	0.006* (HS)

*** Unpaired t-test**

Table (12): Comparison between group 1 and group 3 as regards demographic data.

Variables	Group 1		Group 3		*P*-value
	Mean	SD	Mean	SD	
Age (yrs)	38.62	5.54	56.15	7.39	0.0001* (HS)
Duration of primary kidney disease (yrs)	5.54	2.11	19.42	8.68	0.0001* (HS)
Duration of hemodialysis (yrs)	2.43	1.35	10.46	2.90	0.0001* (HS)

* Unpaired t-test.

Table (13): Comparison between group 1 and group 3 as regards laboratory data

Variables	Group 1		Group 3		*P*-value
	Mean	SD	Mean	SD	
Urea (mg/dl)	132.24	29.99	117.69	38.93	0.4 (NS)
Creatinine (mg/dl)	9.08	2.35	8.96	2.56	0.9 (NS)
Albumin (g/day)	3.61	0.16	3.56	0.21	0.4 (NS)
Ca (mg/dl)	8.20	1.00	7.94	1.15	0.5 (NS)
P (mg/dl)	4.62	1.00	8.45	1.13	0.0001* (HS)
Cax P (mg^2/dl^2)	37.51	7.736	66.25	7.80	0.0001* (HS)
PTH (mg/dl)	596.47	53.58	513.10	48.102	0.7 (NS)

* Unpaired t-test.

Table (14): Comparison between group 1 and group 3 as regards ecvhocardiographic parameters

Variables	Group 1		Group 3		*P*-value
	Mean	SD	Mean	SD	
AO (mm)	29.19	4.30	31.08	3.68	0.2 (NS)
RA (mm)	37.86	5.99	40.77	4.13	0.1 (NS)
EDD (mm)	50.83	7.28	52.00	8.68	0.7 (NS)
ESD (mm)	33.60	6.96	40.58	10.03	0.02* (S)
FS (%)	39.57	9.50	33.00	4.95	0.03* (S)
EF (%)	65.24	9.28	56.15	6.95	0.005* (HS)
IVSTd (mm)	11.05	2.16	11.23	1.54	0.8 (NS)
PWTd (mm)	10.95	2.20	11.69	1.11	0.3 (NS)

* Unpaired t-test.

Table (15): Comparison between group 1 and group 3 as regards drug doses

Variables	Group 1		Group 3		*P*-value
	Mean	SD	Mean	SD	
Calcium (mg)	1857.14	454.65	3807.69	678.31	0.0001* (HS)
Alphacalciferol (µg)	2.93	0.33	2.08	0.76	0.0001* (HS)

* Unpaired t-test.

Table (16): Comparison between group 2 and group 3 as regards demographic data

Variables	Group 2		Group 3		*P*-value
	Mean	SD	Mean	SD	
Age (yrs)	53.73	9.46	56.15	7.39	0.4 (NS)
Duration of primary kidney disease (yrs)	16.14	7.66	19.42	8.68	0.3 (NS)
Duration of hemodialysis (yrs)	8.50	2.61	10.46	2.90	0.04* (S)

* Unpaired t-test.

Table (17): Comparison between group 2 and group 3 as regards laboratory data

Variables	Group 2		Group 3		*P*-value
	Mean	SD	Mean	SD	
Urea (mg/dl)	107.88	26.88	117.69	38.93	0.4 (NS)
Creatinine (mg/dl)	9.89	2.74	8.96	2.56	0.6 (NS)
Albumin (g/day)	3.59	0.24	3.56	0.21	0.7 (NS)
Ca (mg/dl)	8.96	0.90	7.94	1.15	0.004* (HS)
P (mg/dl)	6.48	1.22	8.45	1.13	0.0001* (HS)
Cax P (mg^2/dl^2)	57.66	10.28	66.25	7.80	0.01* (HS)
PTH (mg/dl)	362.12	85.23	513.10	48.102	0.2 (NS)

* Unpaired t-test.

Table (18): Comparison between group 2 and group 3 as regards echocardiographic parameters

Variables	Group A		Group B		*P*-value
	Mean	SD	Mean	SD	
AO (mm)	29.96	4.89	31.08	3.68	0.5 (NS)
RA (mm)	40.19	6.49	40.77	4.13	0.8 (NS)
EDD (mm)	48.16	9.85	52.00	8.68	0.2 (NS)
ESD (mm)	35.19	8.04	40.58	10.03	0.1 (NS)
FS (%)	35.38	7.68	33.00	4.95	0.3 (NS)
EF (%)	58.19	9.88	56.15	6.95	0.5 (NS)
IVST (mm)	11.88	2.29	11.23	1.54	0.4 (NS)
PW (mm)	11.89	1.72	11.69	1.11	0.7 (NS)

* Unpaired t-test.

Table (19): Comparison between group 2 and group 3 as regards drug doses

Variables	Group 2		Group 3		*P*-value
	Mean	SD	Mean	SD	
Calcium (mg)	3173.08	±782.52	3807.69	±778.31	0.03* (S)
Alphacalciferol (µg)	2.42	±0.74	2.08	±0.76	0.1 (NS)

* Unpaired t-test.

Table (20): Correlation between Ca, P, Cax P, PTH and demographic data of patients within group 1.

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Age (yrs)	r p	0.5 0.04* (S)	-0.4 0.06 (BS)	-0.2 0.5 (NS)	-0.2 0.3 (NS)
Duration of primary kidney disease (yrs)	r p	0.4 0.2 (NS)	-0.256 0.4 (NS)	-0.1 0.7 (NS)	0.2 0.5 (NS)
Duration of hemodialysis (yrs)	r p	0.5 0.01* (HS)	-0.1 0.6 (NS)	0.2 0.4 (NS)	0.2 0.4 (NS)

Pearson Linear Correlation test

Table (21): Correlation between Ca, P CaxP, PTH, and laboratory data of patients within group 1

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Ca (mg/dl)	r p		-0.4 0.05* (S)	0.1 0.6 (NS)	0.2 0.3 (NS)
P (mg/dl)	r p	-0.4 0.05* (S)		0.8 0.0001* (HS)	-0.2 0.4 (NS)
Cax P (mg^2/dl^2)	r p	0.1 0.6 (NS)	0.8 0.0001* (HS)		- 0.07 0.8 (NS)

PTH (mg/dl)	r p	0.2 0.3 (NS)	-0.2 0.4 (NS)	-0.07 0.8 (NS)	
Urea (mg/dl)	r p	-0.5 0.02* (S)	-0.09 0.7 (NS)	-0.4 0.09 (BS)	0.3 0.2 (NS)
Creatinine (mg/dl)	r p	-0.5 0.01* (HS)	0.3 0.2 (NS)	0.005 1.0 (NS)	0.02 0.9 (NS)
Albumin (g/day)	r p	0.03 0.9 (NS)	0.1 0.6 (NS)	0.120 0.6 (NS)	-0.2 0.3 (NS)

Pearson Linear Correlation test

Table (22): Correlation between Ca, P, CaxP, PTH and echocardiographic parameters within group 1

Variables	Correlation coefficient	Ca	P	CaxP	PTH
AO (mm)	r p	0.1 0.6 (NS)	0.3 0.2 (NS)	0.4 0.06 (BS)	0.1 0.6 (NS)
RA (mm)	r p	0.1 0.7 (NS)	0.04 0.9 (NS)	0.1 0.6 (NS)	-0.3 0.2 (NS)
EDD (mm)	r p	0.02 0.9 (NS)	-0.2 0.3 (NS)	-0.3 0.2 (NS)	0.3 0.3 (NS)
ESD (mm)	r p	0.2 0.5 (NS)	0.2 0.5 (NS)	0.3 0.2 (NS)	0.08 0.7 (NS)
FS (%)	r p	-0.2 0.3 (NS)	0.2 0.5 (NS)	0.03 0.9 (NS)	-0.2 0.4 (NS)
EF (%)	r p	0.2 0.5 (NS)	0.2 0.4 (NS)	0.3 0.2 (NS)	-0.09 0.7 (NS)
IVSTd (mm)	r p	0.2 0.3 (NS)	0.1 0.6 (NS)	0.3 0.2 (NS)	-0.09 0.7 (NS)

Pearson Linear Correlation test

Table (23): Correlation between Ca, P, CaxP, PTH and drug doses within group 1 patients

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Calcium (mg)	r p	0.03 0.9 (NS)	-0.2 0.5 (NS)	-0.2 0.5 (NS)	-0.3 0.3 (NS)
Alphacalciferol (µg)	r p	-0.2 0.4 (NS)	0.2 0.4 (NS)	0.09 0.7 (NS)	0.08 0.7 (NS)

Pearson Linear Correlation test

Table (24): Correlation between Ca, P, CaxP, PTH and demographic data within group 2

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Age (yrs)	r p	-0.09 0.6 (NS)	0.3 0.2 (NS)	0.2 0.2 (NS)	0.07 0.7 (NS)
Duration of primary kidney disease (yrs)	r p	-0.2 0.4 (NS)	-0.02 0.9 (NS)	-0.1 0.6 (NS)	0.2 0.3 (NS)
Duration of hemodialysis (yrs)	r p	-0.5 0.01 (HS)	0.2 0.3 (NS)	-0.03 0.9 (NS)	-0.004 0.9 (NS)

Pearson Linear Correlation test

Table (25): Correlation between Ca, P, CaxP, PTH and laboratory data within group 2

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Ca (mg/dl)	r p	- -	-0.4 0.06 (BS)	0.2 0.3 (NS)	-0.4 0.9 (NS)
P (mg/dl)	r	-0.4	-	0.8	-0.05

	p	0.06 (BS)	-	0.0001* (HS)	0.8 (NS)
Cax P (mg^2/dl^2)	r p	0.2 0.3 (NS)	0.8 0.0001* (HS)	- -	-0.05 0.8 (NS)
PTH (mg/dl)	r p	-0.04 0.9 (NS)	-0.05 0.8 (NS)	-0.05 0.8 (NS)	- -
Urea (mg/dl)	r p	-0.2 0.2 (NS)	0.1 0.6 (NS)	-0.008 0.6 (NS)	-0.2 0.3 (NS)
Creatinine (mg/dl)	r p	0.05 0.8 (NS)	0.1 0.6 (NS)	0.2 0.4 (NS)	0.05 0.8 (NS)
Albumin (g/day)	r p	0.3 0.2 (NS)	0.04 0.8 (NS)	0.2 0.3 (NS)	0.08 0.7 (NS)

Pearson Linear Correlation test

Table (26): Correlation between Ca, P, CaxP, PTH and echocardiographic parameters within group 2

Variables	Correlation coefficient	Ca	P	CaxP	PTH
AO (mm)	r p	-0.07 0.7 (NS)	0.2 0.3 (NS)	0.2 0.4 (NS)	0.04 0.9 (NS)
RA (mm)	r p	0.2 0.3 (NS)	-0.3 0.1 (NS)	-0.2 0.2 (NS)	0.3 0.1 (NS)
EDD (mm)	r p	0.02 0.9 (NS)	-0.2 0.4 (NS)	-0.2 0.4 (NS)	0.07 0.7 (NS)
ESD (mm)	r p	-0.06 0.8 (NS)	-0.02 0.9 (NS)	-0.07 0.7 (NS)	-0.02 0.9 (NS)
FS (%)	r p	0.01 0.9 (NS)	0.4 0.03* (S)	0.5 0.01* (HS)	0.05 0.8 (NS)
EF (%)	r p	-0.4 0.06 (BS)	0.08 0.7 (NS)	-0.1 0.5 (NS)	-0.4 0.02 (S)
IVSTd (mm)	r p	0.04 0.8 (NS)	0.02 0.9 (NS)	0.05 0.7 (NS)	-0.01 0.9 (NS)
PWTd (mm)	r p	0.1 0.6 (NS)	0.08 0.7 (NS)	0.2 0.4 (NS)	0.06 0.8 (NS)

Pearson Linear Correlation test

Table (27): Correlation between Ca, P, Cax P, PTH and drug doses within group 2

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Calcium (mg)	r p	0.4 0.04 (S)	-0.4 0.06 (BS)	-0.2 0.5 (NS)	-0.08 0.7 (NS)
Alphacalciferol (µg)	r p	-0.4 0.03 (S)	0.3 0.1 (NS)	0.1 0.6 (NS)	0.2 0.3 (NS)

Pearson Linear Correlation test

Table (28): Correlation between Ca, P, CaxP, PTH and demographic data within group 3

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Age (yrs)	r p	0.09 0.7 (NS)	-0.4 0.2 (NS)	-0.3 0.4 (NS)	-0.6 0.05* (S)
Duration of primary kidney disease (yrs)	r p	-0.1 0.7 (NS)	-0.3 0.4 (NS)	-0.5 0.1 (NS)	0.01 0.9 (NS)
Duration of hemodialysis (yrs)	r p	0.3 0.4 (NS)	-0.4 0.2 (NS)	-0.1 0.7 (NS)	0.07 0.8 (NS)

Pearson Linear Correlation test

Table (29):Correlation between Ca, P, CaxP, PTH and laboratory data within group 3

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Ca (mg/dl)	r p	- -	-0.7 0.01* (HS)	0.5 0.06 (BS)	-0.3 0.2 (NS)
P (mg/dl)	r p	-0.7 0.01* (HS)	- -	0.3 0.4 (NS)	0.3 0.4 (NS)
Cax P (mg^2/dl^2)	r p	0.5 0.06 (BS)	0.3 0.4 (NS)	- -	-0.1 0.7 (NS)
PTH (mg/dl)	r p	-0.3 0.2 (NS)	0.3 0.4 (NS)	-0.1 0.7 (NS)	- -
Urea (mg/dl)	r p	-0.1 0.6 (NS)	0.03 0.9 (NS)	-0.1 0.6 (NS)	0.7 0.01* (HS)
Creatinine (mg/dl)	r p	-0.6 0.03* (S)	0.1 0.7 (NS)	-0.7 0.01* (HS)	0.1 0.7 (NS)
Albumin (g/day)	r p	-0.2 0.6 (NS)	-0.06 0.8 (NS)	-0.4 0.2 (NS)	-0.4 0.2 (NS)

Pearson Linear Correlation test

Table (30):Correlation between Ca, P, CaxP, PTH and echocardiographic data within group 3

Variables	Correlation coefficient	Ca	P	CaxP	PTH
AO (mm)	r p	-0.09 0.8 (NS)	0.04 0.9 (NS)	-0.07 0.8 (NS)	0.4 0.2 (NS)
RA (mm)	r p	0.4 0.2 (NS)	-0.2 0.5 (NS)	0.3 0.4 (NS)	0.2 0.5 (NS)
EDD (mm)	r p	0.2 0.6 (NS)	0.2 0.6 (NS)	0.4 0.2 (NS)	0.3 0.3 (NS)
ESD (mm)	r p	2 0.5 (NS)	-0.4 0.2 (NS)	-0.2 0.6 (NS)	-0.1 0.8 9NS)
FS (%)	r p	0.01 0.9 (NS)	-0.01 0.9 (NS)	-0.04 0.9 (NS)	0.04 0.9 (NS)
EF (%)	r p	0.08 0.8 (NS)	0.1 0.6 (NS)	0.2 0.5 (NS)	-0.2 0.4 (NS)
IVSTd (mm)	r p	-0.5 0.1 (NS)	-0.03 0.9 (NS)	-0.6 0.03* (S)	09 0.7 (NS)
PWTd (mm)	r p	-0.04 0.9 (NS)	-0.5 0.08 (BS)	-0.6 0.03* (S)	-0.07 0.8 (NS)

Pearson Linear Correlation test

Table (31):Correlation between Ca, P, CaxP, PTH and drug doses within group 3

Variables	Correlation coefficient	Ca	P	CaxP	PTH
Calcium (mg)	r p	-0.3 0.2 (NS)	0.6 0.05 (S)	0.2 0.5 (NS)	0.2 0.6 (NS)
Alphacalciferol (µg)	r p	-0.3 0.3 (NS)	3 0.4 (NS)	-0.06 0.9 (NS)	0.7 0.007* (HS)

Pearson Linear Correlation test

Table (32): Predictors of valvular calcification in multiple linear regression analysis in hemodialysis patients.

Variables	B*	95% confidence interval	*P*-value
Constant	-0.1	-0.7-0.5	0.7
Duration of hemodialysis (yrs)	0.01	0.02-0.07	0.0001
CaxP (mg^2/dl^2)	0.01	0.004-0.02	0.002
EF (%)	-0.001	-0.02 – 0.004	0.003
Age of patients (years)	0.01	0.002-0.02	0.01

R2 = 0.78, SE (standard error) = 0.22 *B (standard regression coefficient)

A multiple stepwise linear regression analysis was performed to determine the independent factors of valvular calcification in hemodialysis patients. Variables significantly associated with valvular calcification and other known to have effect on valvular calcification were introduced in the model. After adjustment for parameters found significant in the univariate analysis, the independent predictors of valvular calcification were duration of hemodialysis, CaxP product, age and reduced EF%.

We didn't find any significant correlation between Ca, P, CaxP, PTH and age of healthy controls within group B.

We didn't find any significant correlation between Ca, P, CaxP, PTH and laboratory data within group B.

We didn't find significant correlations between Ca, P, CaxP, PTH and echocardiographic parameters within group B.

4. Discussion

Lesions of cardiovascular calcification are also seen in dialysis patients at a much younger age than in the general population. Even adults less than 30 years of age on dialysis have a high incidence of coronary artery calcification and these lesions progress at a relatively rapid rate ***(Isidro & William, 2002).***

Calcification of the mitral and aortic valves. Valvular calcification is a marker of systemic cardiovascular disease in CKD-stage 5 patients ***(Raggi et al., 2011).***

Echocardiography is the gold standard for assessment of cardiac valve morphology and function. It is non invasive and relatively inexpensive. Calcification of the cardiac valves is found in dialysis patients with a prevalence four to five times higher than in the general population ***(Straumann et al., 1992; Mazzaferro et al., 1993 and Ribeiro et al., 1998).***

In our present study on 60 patients on regular HD, of age (49.32±10.68) years, the frequency of valvular calcification was 39 patients, 26 patients (43.33%) with aortic calcification and 13 patients (21.66%) with both aortic and mitral calcification. None of the patients had mitral calcification only. Also non of our patients had pulmonary or tricuspid valve pathology. The presence of valvular calcifications in ESRD patients is remarkably high in all published series. ***Braum et al. (1996)***, initially studied 49 maintenance HD patients, and identified calcification of mitral valve in 59% of case and aortic valve in 55%. ***Ribeiro et al. (1998)*** found among 92 patients whose mean age was 60 years, 44% had mitral valve calcifications on echocardiography, and 52% had aortic valve calcifications. Nearly 60% of these patients demonstrated calcifications of both valves. In other studies, mitral valve calcifications were reported in 45%, and aortic valve calcifications in 34% of patients, while both valves were affected in 21% of the patients studied ***(Raggi et al., 2002).*** Valvular thickening and sclerosis, is a frequent finding in HD patients. Among such patients, aortic and mitral valve sclerosis occur in 55 to 69 and 40 to 60 percent of individuals, respectively ***(Stinebaugh et al., 1995 and Straumann et al., 1992)***.

In the Jackson cohort of the atherosclerotic risk in communities study ***(Fox et al., 2004)***, the Framingham offspring study ***(Fox et al., 2006)***, and the Multi-ethnic study of atherosclerosis (MESA) ***(Ix et al., 2007)*** cross sectional associations were seen between CKD and mitral annular calcification. In addition, the cardiovascular health study (CHS) showed a high prevalence of CKD in adults with combined mitral annular calcification, aortic annular calcification, and aortic valvular stenosis ***(Barash et al., 2006).***

Non of our control group had aortic or mitral valve calcification. In our study patients with no calcification (group 1)had a relatively short hemodialysis duration (mean±SD = 2.43±1.35) years, compared to patients with calcified aortic valve (group 2) who had a duration on HD (8.5±2.61) years with a highly statistically significant difference between both (p = 0.0001). Patients with calcified aortic and mitral valve (group 3) had a hemodialysis duration of (10.46±2.90) years with a highly significant statistical difference between group 3 and group 1 (p = 0.0001) and a statistically significant difference between group 3 and group 2 (p = 0.04). This means that hemodialysis duration is implicated in the occurrence of cardiac calcification and also implicated in the extent of calcification.

Patients with valvular calcification receiving hemodialysis, had longer hemodialysis duration ***(Volkov et al., 2009 and Strozecki et al., 2005)***, echocardiographically examined 65 HD patients ages (49±12). Years, with duration of HD therapy (38±32) months. Valvular calcification were found in 32 of 65 patients (49%), mitral valve calcifications in 10, aortic valve calcifications in 9, and both valves calcifications in 13 patients. In patients with valvular calcification, duration of HD therapy was longer. Also ***Ribeiro et al. (1998)***, study suggests that patients with valvular calcification were on long-term renal replacement therapy.

In our study, age of patients with no calcification was (38.62±5.54) years, while age of patients with calcified aortic valve was (853.73±9.46) years. Age of patients with calcified aortic and mitral valve was (56.15±7.39) years. Patients with calcified aortic valve showed a statistically higher age when compared to patients

with no calcification (p = 0.0001). The same result was found on comparing parents with calcified aortic and mitral valve and patients with no calcification (*p* = 0.001). While patients with calcified aortic valve and patients with calcified aortic and mitral calcification didn't show significant difference as regards age (*p* = 0.4). This agrees with studies that suggest that valvular calcification is common in old age patients, group B and group A didn't show significant difference as regards age (*p*= 0.9).

Ribeiro et al. (1998) reported that mitral valve calcification was associated with age. ***Ikee et al. (2010)*** suggested that mitral valve calcification was associated with increased age.

Ribeiro et al. (1998) reported that aortic valve calcification was correlated with age. ***Ikee et al. (2010)***, detected heart valve calcification using two dimensional echocardiography in patients treated with HD three times a week. In 112 patients, (77 men and 35 women, age (67±10) years, duration of HD (95±67) months, aortic and mitral valvular calcification were observed in 84 (75.0%) and 58 (51.7%) patients, respectively aortic valve calcification was associated with age.

Aortic valve calcification is found in 25 to 55 percent of HD patients occurring 10 to 20 years earlier than in the general population ***(Nasri et al., 2004)***

Mitral annular calcification occurs earlier in patients with chronic renal failure than those without renal dysfunction ***(Forman et al., 1984)***.

In our study, 12 patients (20%) of group A were diabetic, 10 patients had valvular calcification.

Rufino et al. (2003), had a cross sectional observational study, patients with valvular calcification were more commonly diabetic.

In our study, 27 patients (4%) of group A, were hypertensive, 20 patients had valvular calcifications, all of them had a history of predialysis hypertension.

In our study we found 20 patients with aortic calcification who had predialysis hypertension, also patients with aortic insufficiency who suffered from diabetes were 4 patients while patients with aortic insufficiency who suffered from hypertension were 9 out of a total of 15 patients with aortic insufficiency.

In ***Ribeiro et al. (1998)*** study patients with valvular calcification had longer duration of predialysis arterial hypertension. Previous studies have reported a history of hypertension prior to starting dialysis as a predictor of valvular calcifications ***(Braun et al., 1996; Ribeiro et al., 1998 and Yoshida et al., 1999)***. ***Braun et al. (1996)***, studied 49 chronic HD patients aged 28 to 74 years that were compared to 102 non-dialysis patients aged 32 to 73 years. They assessed calcification of mitral and aortic valves. The mitral valve was calcified in 59% of dialysis patients, while aortic valve was calcified in55%. They drew attention to hypertension as an important risk factor in this process.

Huting et al. (1994) observed that mitral valvular calcifications was associated with the severity of predialysis hypertension.

Ribeiro et al. (1998) study suggests no correlation was found between valvular calcifications and arterial hypertension, but he reported that aortic valvular calcification was related with duration of hypertension and with the longer duration of predialysis arterial hypertension.

Aortic insufficiency following aortic valve calcification may be due to the progression of the complications of hypertension ***(Kahnooj et al., 2010)***.

Urea level was significantly higher in patients with no calcification as compared to patients with calcified aortic valve (*p* =0.04), this is goes with what was expected that uremia is one the risk factors for calcification.

Low serum albumin is not specific for valvular calcification groups (2 and 3), but also it does affect (group 1) without calcification, seeming to be a characteristic of ESRD and HD.

In contrast to our study, ***Ikee et al. (2010)*** stated that aortic valve calcification was associated with lower serum albumin. Ca, P, and CaxP were highly significantly lower in group A than group B (*p* = 0.0001) but PTH was much higher in group A (*p* = 0.0001), Ca, P, CaxP, were significantly lower in group 1 as compared to group 2 (with aortic calcification) (*p* = 0.009, 0.0001, 0.0001) respectively. PTH was significantly higher in group 1 than group 2 (*p* = 0.06), which is against what was expected.

P and C ax P product were higher in patients with calcified aortic and mitral valve as compared to patients with no calcification (*p* = 0.001), which points to the role played by P and CaxP product in calcification. At same time, PTH didn't show significant difference between the two groups, which means that it has a less important role in valvular calcification (being insignificantly higher in group 1, *p* = 0.7).

Hyperphosphatemia may directly cause or exacerbate other aspects of CKD-MBD, including secondary hyperparathyroidism, decreased serum calcitriol levels, abnormal bone remodeling and soft-tissue calcification ***(Katrin et al., 2010)***.

Ca is significantly higher in patients with calcified aortic valve than in patients with calcified aortic and mitral valve (p = 0.004). P and CaxP product were much higher in patients with calcified aortic and mitral valve than in patients with aortic calcification, again incriminating them of the more extensive calcification of the heart valves.

PTH was insignificantly higher in patients with calcified aortic and mitral valve than in patients with calcified aortic valve (p = 0.2).

Persistent elevations in serum calcium and phosphorus levels can be aggravated by the large doses of vitamin D sterols used often to treat secondary hyperparathyroidism ***(Raggi et al., 2011)***.

Tarras et al. (2006) studied 90 patients on maintenance HD for more than 12 months, 36 (40%) presented with valvular calcifications and showed higher levels of serum calcium (92.00±7.54 versus 89.27±6.86 mg/L, p = 0.04), phosphorus (69.70±18.33 versus 44.90±12.43 mg/L), ($p < 0.001$), CaxP product (6164.97±1797.64 versus 4024.70±1066.40 $mg^2/L^{2)}$, ($P < 0.0001$) as compared with patients without valvular calcifications.

Volkov et al. (2009), reported that patients with valvular calcification had high serum calcium level. This was not the case in our study as regards serum calcium.

Strozeeki et al. (2005) study showed that no statistical significant differences were found with respect to Ca, P, PTH and mean CaxP product in patients with or without valvular calcifications, but the incidence of CaxP product above 4.43 $mmol^2/L^2$ was higher in valvular calcifications compared with those without valvular calcifications.

Aortic valve calcification was associated with higher serum calcium. Mitral valve calcification was not associated with high serum calcium as stated by ***Ikee et al.(2010).***

Torun et al. (2005), found that significantly higher phosphate level was observed in patients with valvular calcifications (5.1±1.4 versus 4.5±1.4 mg/dL, $p = 0.04$). ***Rufino et al. (2003)*** had a cross sectional observational study of a cohort of 52 stable patients on maintenance HD for more than 12 months. 20 patients (38.4%) presented with valvular calcifications and showed higher levels of serum phosphorus compared to patients without valvular calcifications.

In the presence of low calcium, high phosphours, vitamin D deficiency and uremia parathyroid cells leave quiescence ***(Cozzolino et al., 2009)***.

Small decreases in serum Ca (++) and more prolonged increases in phosphate stimulate the parathyroid gland to secrete parathyroid hormone ***(Rochelle et al., 2010)***.

Calcium can drive the initial steps in hydroxyapatite formation and small changes in calcium concentration have profound effects on calcification of aorta ***(O'Neill et al., 2011)***.

Torun et al. (2005), found that CaxP product was high in the study population (48.6±16.2 versus 39.8±11.8, $p = 0.01$). ***Rufino et al. (2003)*** illustrated that a CaxP product level > 43 mg^2/dL^2 was the optimal value in terms of sensitivity and specificity for predicting the presence of valvular calcification in their patients population.

Multiple stepwise regression analysis selected CaxP as one of the most predictive parameters for mitral calcification ***(Ribeiro et al., 1998)***.

Kahnooj et al. (2010), findings showed that the optimal cut-off point for the CaxP. Product measurement for predicting the severity of aortic valve calcification was 42 mg^2/dL^2. This discrimination level was different in other studies. ***Movilli et al. (2005),*** obtained a break point of 55 mg^2/dL^2 for an optimal CaxP product discrimination value.

Volkov et al. (2009), stated that patients with valvular calcification had high blood parathyroid hormone level while ***Ribeiro et al. (1998)*** found no correlation between valvular calcification and parathyroid hormone level. Relatively low PTH concentrations have been associated with a high prevalence of soft tissue and vascular calcification among patients receiving dialysis ***(Raggi et al., 2011)***.

Patients with no calcification showed a higher significant doses of calcium and alphacalcidiol than patients with calfied aortic valve ($p = 0.04$, 0.006) respectively, this is against what was expected. Ca doses was much higher in patients with calcified aortic and mitral valve than patients with no calcification ($p = 0.0001$) but alphacalcidiol was significantly lower in patients with calcified aortic and mitral valve and patients with no calcification ($p = 0.0001$).

Patients with calcified aortic and mitral valve had a higher significant doses of calcium compared to patients with calcified aortic valve (p=0.03), but alphacalcidiol was non-significantly higher in patients with calcified aortic valve than in patients with calcified aortic and mitral valve ($P = 0.1$). Does this point to calcium drug as a calcification progression factor whenever calcification occurs. On the contrary does alfacalciferol protect patients with and without calcification from progression of calcification by its well known anti-inflammatory effect over body tissues.

Effects of vitamin D such as modulation of an inflammatory response may have a preventive effect ***(Razzaque, 2011)***.

Increasing evidence suggests that calcium supplementation may enhance soft tissue calcification and cardiovascular disease in CKD-MBD ***(Peacock, 2010)***. The thrombotic or ulcerative changes that occur within heavily calcified valves when exposed to the intracardiac lumen can result in an erupted mass of calcium entering circulation.

Numerous studies have shown that in animal models of uremia, vascular calcification appears to be accelerated by the administration of vitamin D ***(Mizobuchi et al., 2007)***.

Tarras et al. (2006) studied 90 patients on maintenance HD for more than 12 months, 36 patients (60%) presented with valvular calcification patients with valvular calcification required higher doses of alfacalcidiol for treating secondary hyperparathyroidism (0.43±0.60 versus 0.11±0.46 μ/day, $p < 0.0001$).

This was not the case in our study.

On making correlations within group 1(patients with no calcification), we found a positive correlation between age and calcium (p = 0.04) which could explain calcification of elderly valves in ESRD patients on regular HD. We found borderline significant negative correlation between phosphorus and age (P = 0.06). Also calcium had a highly significant positive correlation with duration of dialysis . Calcium and phosphorus had a negative correlation which is well known. A positive correlation was found between P and CaxP which supports our study opinion of incriminating both in the process of extraosseous calcification in ESRD on HD, we found a negative correlation of calcium with creatinine and urea which is well known.

In group 2 (aortic valve calcification), we found a negative highly significant correlation between serum calcium and duration of dialysis which is against incriminating serum calcium in valvular calcification process in ESRD on HD.

Serum ionized calcium concentration is maintained in a very limited range thanks to parathyroid hormone (PTH) and the active vitamin D metabolite calcitriol. A decrease in ionized calcium level inactivates the calcium sensoring receptor (CaSR), a membranous protein located principally in the parathyroid glands and the kidney, thus stimulating PTH secretion ***(Courbebaisse and Souberbielle, 2011)***.

Serum Ca and P in group 2(with aortic valve calcification) had a borderline negative correlation which is well known, P and CaxP product had a highly significant positive correlation ($p = 0.0001$) again supporting the opinion of their incrimination in vavlular calcification process.

In group 3(aortic and mitral valve calcification), parathyroid hormone had a negative correlation with age ($p = 05$), which supports that it doesn't play much a role in valvular calcification in elderly.

We also found highly significant negative correlation of serum Ca and P, which is well known. Against what was expected, we found a positive borderline correlation between serum calcium and CaxP product. Also against what was expected a non significant positive correlation was found between serum P and CaxP product. Does serum Ca predict CaxP product level whenever valvular calcification is more extensive or prolonged? This does need further research. We found in group 3(aortic and mitral valve calcification), a highly significant correlation between urea and parathyroid hormone. There was a negative highly significant correlation between CaxP product and creatinine. Also an inverse significant correlation between serum calcium and creatinine level which is well known.

In group 2 (aortic calcification), there was a positive significant correlation between serum calcium level and calcium dose which is predicted and an inverse borderline significant correlation between serum P and calcium dose. We found an inverse correlation between serum calcium level and alfacalcidiol dose which may mean that calcium is consumed in valve and vascular calcification all over the body.

In group 3(aortic and mitral calcification), there was an indirect significant correlation between serum P and calcium dose, which could incriminate the calcium as a drug encouraging increases in CaxP product and tissue calcification in ESRD on HD with extensive valve calcification.

In our study, patients having aortic calcification were 39, patients having mitral calcification were 13, 5 patienst had aortic regurge while 5 patients had mitral regurge. The rest of our patients had no valvular insufficiency.

In our study, aortic calcification was noted in 65%dialysis patients. The incidence of valvular insufficiency was 3.9% in patients with aortic calcification versus 0.63% in patients without calcification.

Valvular calcification may be associated with aortic regurgitation ***(Voklov et al., 2009)***. ***Ribeiro et al. (1998),*** found that aortic calcification was noted in 52% of dialysis patients as compared to 4% of controls. The prevalence of valvular insufficiency was 22% in patients with aortic calcification versus 6% in patients without calcification.

In our study none of the patients had stenosis of mitral nor aortic valves. Valvular calcification may be associated with stenosis of mitral and aortic valves ***(Volkov et al., 2009)***.

Myocardial dysfunction is common just prior to the onset of dialysis ***(Zoccali et al., 2006 and McCullough et al., 2004)***.

Ejection fraction was significantly higher in patients with no calcification compared to patients with aortic valve calcification ($p = 0.02$) and this is expected.

ESD was significantly higher in group 3(aortic and mitral valve calcification), compared to group 1(

no calcification) which means some degree of enlargement of ventricle in group 3 (p = 0.02). Also in group 3, ejection fraction and shortening fraction are significantly lower than group 1 (p = 0.03 and 0.005) and this shows the relation between calcification and cardiac dysfunction.

There was no much difference in echocardiographic parameters between group 2(aortic calcification) and group 3(aortic and mitral calcification) and this means that once calcification happends, changes of echo parameters starts.

Cardiovascular calcification is associated with cardiovascular disease and mortality among patients receiving dialysis, and the disorder progresses rapidly once established ***(Raggi et al., 2011)***.

In our study, 3% of patients had left ventricular hypertrophy. ***Turkmen et al. (2008)***, studied a total of 82 consecutive patients (52 male, mean age 48±12 years) undergoing chronic HD treatment for > 1 year subjected to echocardiography, left ventricular hypertrophy was detected in 59 (72%) the study patients, ***Strozecki et al. (2005)*** found that valvualr calcification coexisted with left ventricular hypertrophy.

Left ventricular hypertrophy (LVM) is the consequence of combined effects of chronic hemodynamic overload and non hemodynamic biochemical and neurohumoral factors characteristic of uremia ***(Nasri et al., 2004)***.

Left ventricular hypertrophy is the most frequent cardiac alteration in ESRD ***(London et al., 2008)***.

LVH has been found in as many as 30 to 45% of patients with CKD. (**Mizobuchi *et al.*, 2007**).

In our present study, left atrial enlargement was found in (7.8%) the patients.

Kocinaj et al. (2009), analyzed data from 123 patients who were on regular HD, by means of traditional transthoracic echocardiographic examination. The most presented age group was 60 to 90 years old, with predominance of females (56.1%). He found dilated left atrium in 26.02% of study patients. In group 1, we found in our study a direct positive correlation between CaxP and aortic valve dimension (p = 0.06), which was expected.

Group 2(aortic calcification), calcium had an inverse correlation with ejection fraction which was against our expectations (p = 0.06) also phosphorus had a positive correlation with shortening fraction, (p = 0.03) which was against our expectations.

In group 3, with mitral and aortic calcification, CaxP product was inversely correlated to interventricular septal dimension (p = 0.03). Increase in CaxP product can be associated with decrease in interventricular septal dimension in case of ventricular enlargement, Which may be due to use of high flux dialysers.

Increased LV wall stress (from pressure and volume overload) fuels changes in the composition and function of the myocardium ***(Bethesda, 2004)***.

Pronounced interstitial fibrosis is a dominant feature of CKD associated structural myocardial remodeling ***(Ahmed et al., 2007).***

P and CaxP product both had an inverse correlation with posterior wall dimension (p = 0.08, p = 0.03) respectively, this needs more research by cardiologists as both P and CaxP are incriminated in tissue calcification in ESRD patients on HD.

The extensive intramyocardial fibrosis in ESRD patients with elevated parathyroid hormone could be responsible for attenuation of the hypertrophic response to pressure overload and the development of high stress cardiomyopathy and cardiac failure ***(London et al., 2002)***.

Conclusion:

We had a very high incidence of valvular calcification (65%) as compared to other studies we have to revise our policy towards HD treatment using calcium supplement and α-cholecalciferol treatment. Also, we have to revise our policy towards the high flux dialysers now. Phosphorus and CaxP product should be controlled as much as possible.

Corresponding author
Mona Hosny
Internal Medicine Department, Faculty of Medicine, Ain Shams University
elhamed_3@yahoo.com

Rerfrences

1. Adragao T (2009). Non invasive evaluation of vascular calcification in CKD patients. KDIGO CKD-MBD work group. Kidney Int; 76 (suppl 113): S1-S130.
2. Adragao T, Pires A, Lucas C, et al. (2004). A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. Nephrol Dial Transplant; 19(6):1480-1488.
3. Ahmed A, Rich MW, Sanders PW, *et al.* (2007). Chronic kidney disease associated mortality in diastolic versus systolic heart failure: a propensity matched study. Am J Cardiol; 99(3):393-398.
4. Barasch E, Gottdiener JS, Marino Larsen EK, Chaves PH, Newman AB (2006). Cardiovascular morbidity and mortality in community-dwelling elderly individuals with calcification of the fibrous skeleton of the base of the heart and aortosclerosis (The Cardiovascular Health Study). Am J Cardiol; 97(9):1281-1286.
5. Bethesda (2004). US renal data system. USRDS 2004 Annual Data report. National Institutes of

Health, National Institute of Diabetes and Digestive and Kidney Diseases.
6. Braun J, Oldendorf M, Moshage W, *et al.* (1996). Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. Am J Kidney Dis; 27(3):394-401.
7. Courbebaisse M, Souberbielle JC (2011). Phosphocalcic metabolism: regulation and explorations]. Nephrol Ther; 7(2):118-138.
8. Cozzolino M, Ciceri P, Volpi EM, Olivi L, Messa PG (2009). Pathophysiology of calcium and phosphate metabolism impairment in chronic kidney disease. Blood Purif; 27(4):338-344.
9. Doumas B, Watson W and Biggs M (1971). Albumin standards and the measurement of serum albumin with bromocresyl green. Clin Chim Acta; 31:87-96.
10. Endras DB, Villanueva R, Sharp CF Jr, Singer FR (1989). Measurement of parathyroid hormone. Endocrina Metab Clin North Am 18(3): 611-29.
11. Ernesto U, Ahmed W, Alpert MA (2003). Valvular and perivalvular abnormalities in end-stage renal disease. Am J Med Sci; 325(4):237-242.
12. Farrel EC (1987). Phosphorus. In: Pesce AJ, Kaplan LA, eds. Methods in clinical chemistry, St Louis: CV Mosby: 1038-1042.
13. Forman MB, Virmani R, Robertson RM, Stone WJ (1984). Mitral annular calcification in chronic renal failure. Chest; 85(3):367-371
14. Fox CS, Larson MG, Vasan RS, *et al.* (2006). Cross-sectional association of kidney function with valvular and annular calcification: the Framingham heart study. J Am Soc Nephrol; 17(2):521-527
15. Fox E, Harkins D, Taylor H, *et al.* (2004). Epidemiology of mitral annular calcification and its predictive value for coronary events in African Americans: the Jackson Cohort of the Atherosclerotic Risk in Communities Study. Am Heart J; 148(6):979-984
16. Huting J (1994). Mitral valve calcification as an index of left ventricular dysfunction in patients with end-stage renal disease on peritoneal dialysis. Chest; 105(2):383-388.
17. Ikee R, Honda K, Ishioka K, *et al.* (2010). Differences in associated factors between aortic and mitral valve calcification in hemodialysis. Hypertens Res; 33(6):622-626.
18. Isidro B and William G (2002). Cardiovascular clacification in endstage Renal disease.Nephrol. Dial Transplant; 17(2): 336-339.
19. Ix JH, Shlipak MG, Katz R, *et al.* (2007). Kidney function and aortic valve and mitral annular calcification in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Kidney Dis; 50(3):412-420.
20. Kahnooj M, Masoomi M, Naderinasab A, *et al.* (2010). Relationship between calcium-phosphorus product and severity of valvular heart insufficiency in patients undergoing chronic hemodialysis. The Journal of Tehran University Heart Center; 5(2): 78-82.
21. Katrin U, Jeffrey S, Bryan K, *et al.* (2010). KDOQI US commentary on the 2009 KDIGO clinical practice Guideline for the diagnosis, Evaluation, and Treatment of CKD-mineral and bone disorder (CKD-MBD). American Journal of Kidney Diseases; 55: 5.
22. Kocinaj D, Gashi M, *et al.* (2009). Echocardiographic assessment of left atrial size in patients with end stage renal disease. Cardiovasc J Afr 20(3): 183-6.
23. London GM (2002). Left ventricular alterations and end-stage renal disease. Nephrol Dial Transplant; 17 Suppl 1:29-36.
24. London GM, Marchais SJ, Guerin AP, *et al.* (2008). Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. J Am Soc Nephrol; 19(9):1827-1835.
25. Mazzaferro S, Coen G, Bandini S, *et al.* (1993). Role of ageing, chronic renal failure and dialysis in the calcification of mitral annulus. Nephrol Dial Transplant; 8(4):335-340.
26. McCullough PA, Sandberg KR, Dumler F, Yanez JE (2004). Determinants of coronary vascular calcification in patients with chronic kidney disease and end-stage renal disease: a systematic review. J Nephrol; 17(2):205-215
27. Mizobuchi M, Finch JL, Martin DR, Slatopolsky E (2007). Differential effects of vitamin D receptor activators on vascular calcification in uremic rats. Kidney Int; 72(6):709-715.
28. Moe M, Drueke T, Block A *et al.* (2009). KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease- mineral and bone disorder (CKD-MBD). Kidney International; 76(suppl 113): s22-s49.
29. Moran A, Katz R, Jenny NS, *et al.* (2008). Left ventricular hypertrophy in mild and moderate reduction in kidney function determined using cardiac magnetic resonance imaging and cystatin C: the multi-ethnic study of atherosclerosis (MESA). Am J Kidney Dis; 52(5):839-848.
30. Movilli E, Feliciani A, Camerini C *et al.* (2005). A high calcium-phosphate product is associated with high C-reactive protein concentrations in

hemodialysis patients. Nephron Clin Pract; 10: 161-167.
31. Nasri H, Baradaran A, Naderi AS (2004). Close association between parathyroid hormone and left ventricular function and structure in end-stage renal failure patients under maintenance hemodialysis. Acta Med Austriaca; 31(3):67-72
32. O'Neill WC, Lomashvili KA, Malluche HH, Faugere MC, Riser BL (2011). Treatment with pyrophosphate inhibits uremic vascular calcification. Kidney Int; 79(5):512-517.
33. Peacock M (2010). Calcium metabolism in health and disease. Clin J Am Soc Nephrol; 5 (1):S23-30.
34. Raggi P, Boulay A, Chasan-Taber S, *et al.* (2002). Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? J Am Coll Cardiol; 39(4):695-701
35. Raggi P, Chertow GM, Torres PU, *et al.* (2011). The ADVANCE study: a randomized study to evaluate the effects of cinacalcet plus low-dose vitamin D on vascular calcification in patients on hemodialysis. Nephrol Dial Transplant; 26(4):1327-1339.
36. Razzaque MS (2011). The dualistic role of vitamin D in vascular calcifications. Kidney Int; 79(7):708-714.
37. Renoe BW, McDonald JM, Ladenson JH (1980). The effects of stasis with and without exercise on free calcium, various cations and related parameters. Clin Chim Acta 103: 91-100.
38. Ribeiro S, Ramos A, Brandao A, *et al.* (1998). Cardiac valve calcification in haemodialysis patients: role of calcium-phosphate metabolism. Nephrol Dial Transplant; 13(8):2037-2040.
39. Rochelle C, Corretti M and Henrich W (2010). Valvular Heart Disease in patients with End-Stage Renal disease. J Am Soc Nephrol; 20: 388-396.
40. Rufino M, Garcia S, Jimenez A, *et al.* (2003). Heart valve calcification and calcium x phosphorus product in hemodialysis patients: analysis of optimum values for its prevention. Kidney Int 85:S115-118
41. Silver J, Levi R (2005). Regulation of PTH synthesis and secretion relevant to the management of secondary hyperparathyroidism in chronic kidney disease. Kidney Int 95:S8-12
42. Stinebaugh J, Lavie CJ, Milani RV, Cassidy MM, Figueroa JE (1995). Doppler echocardiographic assessment of valvular heart disease in patients requiring hemodialysis for end-stage renal disease. South Med J; 88(1):65-71
43. Straumann E, Meyer B, Misteli M, Blumberg A, Jenzer HR (1992). Aortic and mitral valve disease in patients with end stage renal failure on long-term haemodialysis. Br Heart J; 67(3):236-239.
44. Strozecki P, Odrowaz-Sypniewska G, Manitius J (2005). Cardiac valve calcifications and left ventricular hypertrophy in hemodialysis patients. Ren Fail; 27(6):733-738.
45. Tarrass F, Benjelloun M, Zamd M, *et al.* (2006). Heart valve calcifications in patients with end-stage renal disease: analysis for risk factors. Nephrology (Carlton); 11(6):494-496.
46. Tobacco A, Meiattini F, Moda E, *et al.* (1979). Simplified enzymic/colorimetric serum urea nitrogen determination. Clin Chim 25: 336-7.
47. Torun D, Sezer S, Baltali M, *et al.* (2005). Association of cardiac valve calcification and inflammation in patients on hemodialysis. Ren Fail; 27(2):221-226.
48. Turknen F, Emre A, Ozdemir A, Sevinc C, Erisken E, Yesilcimen K (2008): Relationship between aortic valve sclerosis and left ventricular hypertrophy in chronic hemodialysis patients. Int Urol Nephrol 40 (2): 497-502.
49. Uhlig K, Berns JS, Kestenbaum B, *et al.* (2010). KDOQI US commentary on the 2009 KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation, and Treatment of CKD-Mineral and Bone Disorder (CKD-MBD). Am J Kidney Dis; 55(5):773-799.
50. Volkov MM, Smirnov AV, Dobronravov VA, *et al.* (2009). Heart valve calcification in patients with chronic kidney disease. Klin Med (Mosk); 87(6):31-35
51. Yatzidis H (1974). New method for direct determination of true creatinine. Clinical Chemistry; 20(9): 1131-1134.
52. Yoshida M, Takamatsu J, Yoshida S, *et al.* (1999). Scores of coronary calcification determined by electron beam computed tomography are closely related to the extent of diabetes-specific complications. Horm Metab Res; 31(10):558-563
53. Zoccali C, Benedetto FA, Tripepi G, *et al.* (2006). Left ventricular systolic function monitoring in asymptomatic dialysis patients: a prospective cohort study. J Am Soc Nephrol; 17(5):1460-1465.

Rotation and Size Tolerant Feature Set for Static Off-line Signature Identification Technique

[1]Muhammad Afzal, [1]Syed Ahsan, [1]Tauqir Ahmad, [1]M. Faisal Hayat, [1]Shahzad H. Asif, [1]Khadim Asif, [2]Talab Hussain

[1]Department of Computer Science and Engineering, University of Engineering and Technology, Lahore, Pakistan.
[2]Centre of High Energy Physics, University of the Punjab, Lahore, Pakistan.
shmafzal@yahoo.com

Abstract: Use of automated signature verification systems is getting popular due to frequent use of signatures for personal identity in banks etc. Dynamic signature identification techniques have been explored and implemented by a number of researchers for signatures that are input to the system through digitizer gadgets. Nevertheless identification of static handwritten signatures is lacking researchers' attention. A novel technique of handwritten off-line static signature identification is presented in this paper which tolerates fault in signature like variation in size and rotation of the signature. The technique uses discriminative features of signatures based of geometric properties represented by pixel density of annular regions of normalized binary image of signatures. We used 2 to 5 signature samples of thirty authors to train our system which was then evaluated with test signature samples. Test samples were made varying in size and rotation. The false recognition rate of our system was 35%, 22%, 20% and 15% for training sample count to be 2, 3, 4 and 5, respectively. Our system tolerated appreciable variation in size and rotation of test signature samples.

[Muhammad Afzal, Syed Ahsan, auqir Ahmad, M. Faisal Hayat, Shahzad H. Asif, Khadim Asif, Talab Hussain. **Rotation and Size Tolerant Feature Set for Static Off-line Signature Identification Technique.** *Life Sci J* 2012;9(3):2147-2151] (ISSN:1097-8135). **http://www.lifesciencesite.com**. 309

Keywords: Signature identification, pattern matching, biometrics, discriminative feature

1. Introduction

Frequent use of handwritten signatures in daily life has ensued active research in the field of signature recognition. Many systems are operative to handle transactions through automatic verification of signatures [1]. Most of the research focuses on on-line signature recognition. This paper presents work on off-line static signature recognition. A technique to extract discriminative features from static image of signature is proposed that imparts tolerance to the signature recognition system against variation in size, rotation and pen type used for system training signature samples and testing signature sample. Our system withstood these variations and exhibited accuracy up to 85%. The following section describes background knowledge of image processing and pattern matching involved followed by proposed technique in section 3. Section 4 explains experiment setup to evaluate the proposed technique where results are discussed in section 5. Conclusions then follow in section 6.

2. Background Knowledge

Characterizing a person with his or her handwritten signatures falls under a large title called Biometrics. The biometric identification of a person does not require password and PIN--personal identification numbers which may be forgotten, lost or stolen. Thus authentication with biometric based systems is receiving greater interest. Hence real-time identification using face, iris, and fingerprint biometric matching is getting popular day by day. Other biometric systems make use of retinal scan, speech, signatures, and hand geometry. A typical biometric system consists of five integrated components:

- A **sensor** device to collect the data and convert it to digital form.
- **Signal processing algorithms** that control quality of activities that develop the biometric template.
- A **database component** that stores information of biometric templates to be compared to for identification.
- A **pattern matching algorithm** that compares the new biometric template with one or more templates kept in the database.
- A **decision process** uses the results from the matching component to make a system-level decision.

Off-line signature recognition systems first digitize the signature image captured through a scanner or a camera and store for later processing. The images thus obtained are called static images.

Features are extracted from the stored images. Off-line signature recognition systems are useful in the situations where only hard copies of the signatures are available on the documents to be authenticated [1].

On-line signature recognition systems capture information when the signature is being signed with a special pen on an electronic surface such as a digitizer combined with a liquid crystal display [2]. The information about the signature thus captured consists of the two dimensional coordinates, the pen pressure, the pen angle and direction.

The captured images of signatures are preprocessed to remove noise and normalize them like centering the signature in the stored images. After that selected features are extracted from the normalized signature images that discriminatively represent the personalized signatures. Different pattern recognition techniques [3] have been used to tackle signature recognition task using the stored extracted features of signatures. These techniques include minimum distance classifiers [4], dynamic programming [5], neural networks [6] and Hidden Markov Models [7, 8]. The accuracy of a biometric system is described in terms of 'false accept rate' (FAR) to relate how many imposters are accepted, and 'false rejection rate' (FRR) to describe how many authorized users are rejected by the system.

Signatures are essentially patterns of line and curve strokes that describe them. These descriptors or features that characterize a signature pattern are processed to model it and later identify or verify it. Features are of two types: the internal and external features. The internal features relate to the interior pixel data of segments of the object image. The external features represent the pixel data of the boundary lines of the segments. Together the features of the image represent the structure aspects of the object image under investigation. Object having common features are assigned an identity class by some processes on the pattern images from image processing. The classes of the patterns are stored in the database for future reference. Any new pattern countered can be recognized to belong to an existing class or a new class to be introduced to the database.

The features of a pattern bitmap include the size of the pattern, the slope of different segments of the pattern etc. These pattern features may be numerical or symbolic, or both. A set of quantitative features extracted computationally is represented feature vector**.** Pattern matching techniques based on decision functions determine the class of the pattern. The number of decision functions in any pattern recognition system depends upon the number of pattern classes. For example, if there are *K* number of classes then there should be *K* decision functions [9]. Before pattern recognition discriminative feature sets of signature must be extracted through signature image processing.

2.1. Preprocessing of Signatures Images

Noise removal is the first step followed by signature centering in the image rectangle. A two dimensional digital image **a**(m,n) of size *M* x *N* is a matrix of values of amplitude **a** in a discrete space**,** defined at the +ive integral values of coordinates m, n where *M* is the number of rows and *N* is the number of columns. The elements of the matrix represent the picture elements of the digital image In case of binary image there will be only two intensity levels; 0 for black and 1 for white pixels, respectively [1-11]. Noise removal uses adjacency properties of pixels defined by neighborhood. Any two pixels having the same grey level in the binary image are connected to form regions and boundaries if they are neighbors to each other.

The processing of image for feature extraction is performed normally in two domains; spatial domain and frequency domain [9]. The term spatial domain refers to the aggregate of pixels properties of the image. Those methods which directly operate upon these pixels are called spatial domain methods. These methods are represented by the transfer functions as below:

$$g(x, y) = T\,[f(x, y)]$$

where f (x, y) is the input image and g (x, y) is the output image. This output image may be a set of features or attributes of the image. This set of attributes may be used for reference as well as testing information of the image. In frequency domain a digital image is usually defined by the values of 'Fourier transform' and its frequency variables [9]. An image is decomposed into its sine and cosine components after the Fourier transform. Every point in the frequency domain represents a particular frequency present in the spatial domain.

2.2. Pattern Recognition Approaches

The scanned images of the signatures are processed to create white background to extract general features [10, 11] like gray shade, texture, shape or context of the image. Further image processing algorithms use this information [10-11] to build more efficient representations for the signatures. Clustering concept is used to find patterns of the targeted classes in the form of vectors of real

numbers. Minimum Distance Classification is frequently used in signature recognition system. Multi-dimensional distance is computed between test signature and each registered signatures to identify the minimum distance bearing signature class from the database.

An artificial neural network (ANN) has been used in signature recognition. Capabilities, like self-organization, fault-tolerance and adaptive learning are key advantages of neural networks. However,back-propagation algorithm based ANNs fall to local minima during training. Other issues include architecture selection, feature representation, learning speed, modularity and scaling.

3. Proposed Technique for Discriminative Feature Extraction

Our system for recognition of static off-line signature is based on geometric feature set that impart tolerance to size and rotation variation present in test signatures to the system. After getting the signature centered image, a rectangular outline is drawn around the signature image so that the center of the rectangle coincides with that of the signature image. Because signatures of a person have approximately similar geometry about their centers, so the useful features of the signatures are extracted by using this similarity characteristic. The geometry of the signatures remains approximately similar under different circumstances like; signing with different pens at different times, signing in different sizes, writing at different orientations, etc. This invariant attribute of a handwritten signature is used to implement this signature recognition system. In our signature recognition system, the pixel data is extracted from the annular regions of the signature images. These annular regions are selected in such a way that they have equal width as well as coincident with the center of the signature images. The width of the annular regions is determined according a suitable scale found after finding the extreme values of x and y coordinates in the signature region. The width of the annular regions varies from image to image because of the different sizes and orientations of the signature images. The number of annular regions for any signature image from the same or different authors is fixed. After the pixel data are extracted from the annular regions, these are normalized by dividing them with the total pixel data of the signature. The normalized data thus obtained represent the pixel density in the annular regions. This process of getting normalized data is repeated a number of times for the signatures of each author and thus average normalized data are obtained and saved. The same process of feature extraction is applied at the two stages of the signature recognition system; training stage as well as recognition stage. This signature recognition system was implemented by using Microsoft Visual C++ .NET frame work.

4. Experiment Setup

We test performance of our system for a dataset of 30 signature authors. Each author signed five signatures for system training which were scanned and saved as monochrome bitmap images. One of the bitmaps of signatures is shown in the Figure 1. The background of the bitmap is not free of noise.

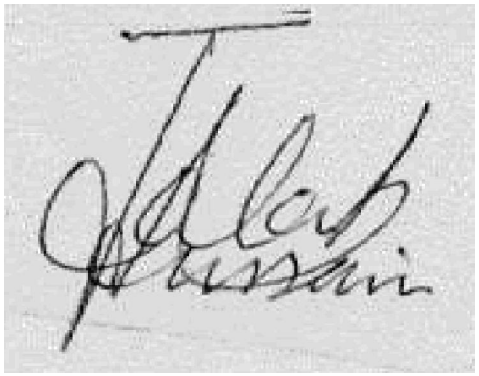


Figure 1: Scanned signature image with noise in the background

Noise is removed from the background of the bitmap to change it to white color as shown in Figure 2.

Figure 2: Signature outline extracted after noise removal

After finding the center the image was divided into 22 equal width annular regions. Pixels were counted in each annulus to compute normalized pixel densities for each annulus. Average values of the densities were stored for the number of signature samples used for system training. The same steps were repeated for each test signature sample at recognition time using minimum distance classifier.

5. Results and Discussion

Signatures from thirty authors were collected for training as well as recognition purposes. Each author was requested to provide five genuine signatures to train the system. The system was trained by using two, three, four and five genuine signatures of each writer. After training the signature recognition system, its performance was evaluated by observing the recognition of test signatures. Table 1 shows % false recognition rate (FRR) of the system while using two, three, four and five training signatures.

Table 1 show that error decreases as the number of training samples is increased. Other prominent attributes of this signature recognition system are elaborated as follows:

Table 1: Performance of signature recognition system

Training Samples	False Recognition Rate (%)
2	35
3	22
4	20
5	15

5.1. Rotation Invariance

A writer may sign at different angles at different times. The results obtained for signatures rotated at slightly different angles were found to be satisfactory. As the range of the orientation angles increases the results of signature recognition start becoming inconsistent. The sample signatures with different orientations used for training and testing are shown in Figure 3.

Figure 3: Rotation Variation in Signatures used in Training and Testing

5.2. Size Invariance

Our proposed feature extraction technique does not depend upon the sizes of the signatures used for training and testing and hence our signature recognition system does not degrade. The examples of bitmap samples of different sizes used for training and testing are shown in Figure 3.

,

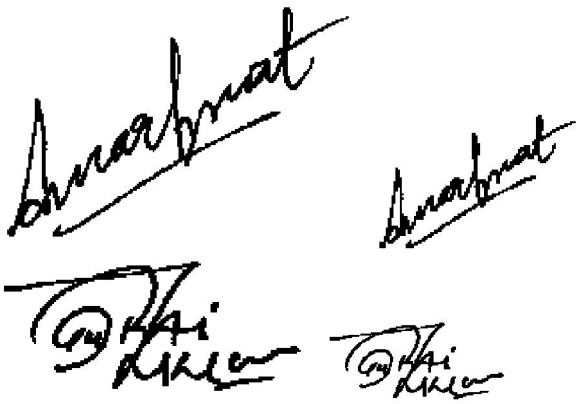


Figure 4: Size Variation in Signatures used in Training and Testing

5.3. Independent of writing pens

The results of our signature recognition system for signatures obtained from different writing pens are invariant of pen type used. We tested our system for signatures made through an ink pen or a ball point to be consistent.

6. Conclusions

A study of recognition of off-line static signature is presented in this paper. A technique of extracting discriminative feature set to impart a quality of the recognition system to tolerate variation in size and rotation of test sample of signature. The proposed technique used discriminative features of signatures based of geometric properties. The scanned signature images are first normalized by removing noise and centering the signature in the images in preprocessing step. Feature vectors are then extracted as normalized pixel density of 22 annular regions of equal width. We used 2 to 5 signature samples of thirty authors to train the system which was then evaluated with test signature samples for recognition rate. Test samples were made to vary in size and rotation. Our system tolerated appreciable variation in size and rotation of test signature samples. The false recognition rate of our system was 35%, 22%, 20% and 15% for training sample count to be 2, 3, 4 and 5, respectively.

Acknowledgment

This research work has been completed with the support of the University of Engineering and Technology, Lahore, Pakistan.

7. References

[1] Sabourin R., Genest G., and Preteux F., "Off-Line Signature Verification by Local Granulometric Size Distributions", IEEE Transactions on Pattern Analysis and Machine Intelligence, Vol. 19, No. 9, (1997), pp. 976-988.

[2] Plamondon R., "The Design of an On-Line Signature Verification System: From Theory to Practice", International Journal on Pattern Recognition and Artificial Intelligence, Vol. 8, (1994), pp.795-811.

[3] Fierrez-Aguilar J., Alonso-Hermira N., Moreno-Marquez G., and J. Garcia O., "An Off-line Signature Verification System Based on Fusion of Local and Global Information", Biometrics Research Lab., ATVS, Universidad Politecnica de Madrid, Spain.

[4] Lee L., Lizarraga, G., "An off-line method for human signature verification." In: Proc. of the Intl. Conf. on Pattern Recognition, ICPR. (1996) pp.195-198.

[5] Guo, K., Doermann, D., Rosenfeld, A., "Local correspondence for detecting random forgeries.", In: Proc. of ICDAR. (1997), pp.319-323.

[6] Huang, K., Yan, H., "Off-line signature verification based on geometric feature xtraction and neural network classification". Pattern Recognition Vol. 30 No.1 (1997) pp.9-17.

[7] Justino, E., Bortolozzi, F., Sabourin, R., "Off-line signature verification using HMM for random, simple and skilled forgeries." In: Proc. of ICDAR. (2001), pp.1031-1034.

[8] Muramatsu, D., Matsumoto, T.,"An HMM signature verifier incorporating signature trajectories.", In: Proc. of ICDAR. (2003) 438442.

[9] Gonzalez R., and Woods R., "Object Recognition in Digital Image Processing", 2nd Edition, (2004), pp. 693–750.

[10] Jesson J., "The Neural Approach to Pattern Recognition", Ubiquity, Vol. 5, No. 7, (2004),pp.14 - 20.

[11] Serge B., Jitendra M., Jan P., "Shape matching and Object recognition using shape Contexts", IEEE transactions on pattern analysis and machine intelligence, Vol. 24, No. 24, April 2002.

08/8/2012

Need for Software Design Methodology for Remote Sensing Applications

Tauqir Ahmad, Muhammad Afzal, Faisal Hayat, H.M. Shehzad Asif, Syed Ahsan, Yasir Saleem

Department of Computer Science & Engineering
University of Engineering and Technology, Lahore (Pakistan)
tauqir_ahmad@hotmail.com

Abstract: Remote Sensing (RS) is being widely used in many critical applications. It includes spatial information that is significant to the impression of communication, coordination, command and control in military operations. The heterogeneous and evolutionary nature of RS data adds complexity to the development process. Due to the complexity of remote sensing applications development, some systematic approach should be adopted. Software development methodology facilitates to subdivide a project to reduce the overall complexity. In this paper we have identified different characteristics of remote sensing data/applications and compared it with existing conventional software development methodologies and argue that a software design methodology suitable for this particular domain is needed.

[Tauqir Ahmad, Muhammad Afzal, Faisal Hayat, H.M. Shehzad Asif, Syed Ahsan, Yasir Saleem. **Need for Software Design Methodology for Remote Sensing Applications.** Life Sci J 2012;9(3):2152-2156] (ISSN:1097-8135). http://www.lifesciencesite.com. 310

Keywords: Remote Sensing, Software Design Methodology, Conventional methodologies, Software

1. Introduction

Software development community is expanding with the advancement of information technology field. Information retrieval is done by using web applications, having reliability as an important factor. A methodology is a combination of tools and techniques providing guidance to an information system development of large scale (Shah, 2008). To address the specific organizational needs and to enhance the functional capabilities for its business growth, an efficient information system is to be developed. The development of such an information system can be done with proper planning, analysis, design and implementation (Shalom and Haan, 2006). This process is known as a System Development Life Cycle (SDLC). A purposeful framework is to be provided by the methodologies to apply some techniques and resources well in time during the software development process to measure the standards of the development process. Methodology may provide a structural framework for the acquisition of knowledge. The challenge of developing a good information system is the main issue in computer science. Due to this reason, the development of a Software Engineering Methodology is the subject of extensive research.

RSAs are used in resource management, archaeology, epidemiology, anthropology, human health condition and international relations (Ahmad and Shah, 2010; Ronald, et al., 1998). Agriculture gets its benefits from RSAs through cultural wastelands identification, monitoring of temporal_behavior of vegetation (Srinivasa, et al., 2003). RSAs play an important role in disaster management. They offer and interpret fire fuels mapped data, analyzing fire effects to monitor fire danger (Ahmad and Shah, 2010; Emilio, et al., 2003). In the same way help to design mitigation and contingency strategies for electrical outages, volcanic eruption, tornadoes, tsunami, earth quacks and hurricanes (Haddi, et al., 2003). RS data is also decisive in timely and intelligent military operation decisions (Ahmad and Shah, 2010). The precise spatial_information is vital to the notion of command and control, communication and dexterity in armed forces operations (Ahmad and Shah, 2010; Stanley, et al., 2004).

2. Related Work

Shah has proposed a framework for prototype-based software development methodologies (Shah, 2001). He proposed an Object-Oriented Design Methodology (OODM) by modifying a classical waterfall model (Shah, 2003). Ahsan and Shah have proposed a software development methodology by modifying waterfall model for one of the scientific and evolutionary domain i.e. Bioinformatics. Agile SDM's have generated a lot of interest in SE community owning to their alleged suitability for

evolutionary, iterative and volatile domains such as bioinformatics and WBA's (Ahsan and Shah, 2008). However, it is not essential that a firm Agile Method (AM) suits all settings or individuals. In a comprehensive comparison of AM's, it is pointed out that little importance has been placed on analyzing the situations where AM's are more suitable than others. On the basis of their results, a new domain of applications is identified in the characteristics specifications of the domain for which AMD's are more suitable (Ahsan and Shah, 2008). The applications such as Computer Aided Construction (CAC) and the WA's belong to this set of applications or domain (Shah et al., 2009). One special characteristic of the objects of this class of applications is that these normally change their structure (methods and instance-variables), (state/data values, or both). (Shah, 2003) also added some other characteristics which are prevalent to bioinformatics and some other scientific domains, thus broadening the scope of the applications in compliance to these characteristics.

Wirfs-Brock et al and Shah suggested modification to the Spiral model and the Waterfall model to make them suitable frameworks for the object-oriented development methodologies and the prototype-based development methodologies, respectively. The strictly *static order* working of the phases was replaced by *iterative cycles* and *back cycles.* An iterative cycle meant for incorporating additional knowledge into an already developed system resulted in the processing of both design and development phases. For this reason the two phases were merged into a single development phase. A *back-cycle* is represented as an incorporating process for the revision to an under-development system. Due to these modifications, the modified life cycle model was able to consider, acquire and incorporate both the meta-data knowledge (functional requirements) and data knowledge (data instances) of the system, which were acquired at the start or during the system development of evolutionary domains.

3. Proposed Work

In this paper we have identified peculiar characteristics of remote sensing data/applications and are listed below:

- Distributed Temporally
- Spatially Categorized
- Critical Response_Time
- Temporal in nature: volatile
- Have validity constraints
- Evolutionary
- Uncertain, imprecise
- Heterogeneous
- Voluminous
- Broad Context
- Policy dependant
- Integration with society perspective
- Harmonic information

3.1 Limitations of Existing Agile Techniques

In our opinion, existing agile SD techniques despite of being used for the development of computer applications (CA) relating to evolutionary domain, have some limitations because, they are not supported by a suitable framework/methodology consisting of the characteristics of the evolutionary domain (ED), e.g. i) Explorative/iterative nature ii) Difficulty in specifying functional requirements iii) Emergent requirements. The above characteristics necessitate an iterative analysis process. Temporal aspect can be added to trace back the history of changes. To the best of our knowledge, the existing Agile SDM's such as XP, Scrum, Crystal methodologies, FDD and the RUP do not have an iterative analysis process/mechanism to store temporal information of a system. Hence, a methodology is needed having an iterative analysis process/mechanism for storing temporal information.

Table 3.1 gives some of the existing frameworks/methodologies, supporting remote sensing characteristics.

From Table 3.1, there are some frameworks and methodologies which support evolutionary type of data. But no single framework or methodology support the majority of these types of data, so there is a need for a methodology which enables to capture the evolutionary data types. Ahsan has proposed a methodology for bioinformatics which belongs to a scientific and evolutionary domain. Table 3.2 gives a comparison between the bioinformatics data characteristics and of remote sensing.

From Table 3.2, it is suggested that there is a need for domain specific methodology for remote sensing applications like bioinformatics as proposed by Ahsan and Shah (Ahsan and Shah, 2008).

4. Conclusions and Future Directions

We have identified the main characteristics of remote sensing applications and compared these characteristics not only with the conventional domains but also with a scientific and evolutionary domain such as Bioinformatics. Table 3.1 depicts that no single software development framework/methodology is available to handle all characteristics if RS data. Hence, a hybrid approach must be adopted to cater all such type of data.

Table 3.1: Frameworks/methodologies supporting remote sensing data/applications

Remote sensing Data characteristics	Methodology Factors	Methodology(s)	Framework
Distributed-Temporally and Spatially Categorized	Tight Control	SADT, JSD, Yourdon	Water Fall
	Measurable Progress	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental, RAD
	Resources needs to be conserved	SADT, JSD, Yourdon, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental, RAD, Spiral
	Online Requiring Extensive User Dialog	Shlaer & Mellor	Prototype, Incremental, Spiral
	Future Scalability of Design is Critical	Booch, coad & Yourdon	Water Fall, Incremental, Spiral
	Risk Avoidance is at high Priority	Booch, Shlaer & Mellor	Spiral
	High Degree of Accuracy	Booch, Shlaer & Mellor	Spiral
	Might benefit from mix Methodologies	Booch, Shlaer & Mellor	Spiral
	Dramatic savings in Time	XP, Scrum , Crystal	RAD
Critical Response Time	Active user Involvement	Fusion, XP	Prototype, RAD
	Interactive	Yourdon, Coad&Yourdon, Shlaer & Mellor, Fusion	Prototype, Incremental, Spiral, RAD
	Tight fit Between User Requirements and System Specifications	SADT, JSD, XP, Scrum	Water Fall, RAD
	Dramatic Saving in Time	Fusion, XP, Crystal, Scrum	Prototype, RAD
	Highly customized	Fusion, Booch, XP	Spiral, Prototype, RAD
	High Degree of Accuracy	Booch, Shlaer & Mellor	Spiral
	Highly Skilled and experienced Manager required	Fusion, Booch, Shlaer & Mellor, XP,	Prototype, Spiral, RAD
	Clear Objectives	SADT, JSD, Yourdon, Booch, Coad & Yourdon	Water Fall, Incremental, Spiral
Temporal in Nature: Volatile	Flexible Control	Fusion, Booch, Coad & Yourdon, Shlaer & Mellor, XP, Scrum	Prototype, Spiral, RAD, Incremental
	Requirements may Change Significantly	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
	Experienced Project Manager	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
	Risk Avoidance is a high Priority	Fusion, Booch	Spiral
	A High Degree of Accuracy	Fusion, Booch	Spiral
	Progress of System Development is Measurable	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor,XP	Water Fall, Incremental, RAD
	Documentation Required	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental, Spiral
	Project has Clear Objectives and Solutions	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental, Spiral
	Requirements are Stable	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental
Have a Validity Constraints	User is Involved Throughout the System Development	Fusion, XP	Prototype, RAD
	Pressure Exists for Immediate Implementation of Something	Fusion, XP	Prototype, RAD
	Low Project Risk	Fusion, Booch	Spiral
	A high Degree of Accuracy	Booch, Shlaer & Mellor	Spiral
	Experienced Team Members	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
Evolutionary	Possible Project Division	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Prototype, Incremental, Spiral
	Documentation not needed	Fusion, Booch, XP, Scrum	Prototype RAD
	Flexible Sequence Control	Fusin, Booch, Coad & Yourdon, XP	Prototype, Spiral, RA Incremental
	Requirements may Change Significantly	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, Spiral, RAD,
	Experienced Team Members	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
	Encourage Innovation and Flexible Design	Fusion, Booch, Shlaer & Mellor, Coad & Yourdon	Prototype, Incremental, RAD
Uncertain, Imprecise	Project Objectives are Unclear	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, RAD

C

	Functional Requirements may change Frequently and Significantly	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, Spiral, RAD
	Online System Requiring Extensive User Dialog	Yourdon, Coad & Yourdon, Shlaer & Mellor, Fusion	Prototype, Incremental, Spiral
	Flexible Control	Fusin, Booch, Coad & Yourdon, XP	Prototype, Spiral, RAD, Incremental
	Project Manager is Highly Skilled	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
	Rapidly Change ability to the System Design as Demanded by users	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, Spiral, RAD
Heterogeneous	Unstable Team Composition is and predictable to Fluctuate	SADT, JSD, Yourdon, Booch, Shlaer & Mellor	Water Fall, Incremental
	Encourage Innovation and Flexible Design	Fusion, Booch, Shlaer & Mellor, Coad & Yourdon	Prototype, Incremental, RAD
	Project Decomposition	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Prototype, Incremental, Spiral
	Flexible Control	Fusin, Booch, Coad & Yourdon, XP	Prototype, Spiral, RAD, Incremental
	Mixture of other Development Methodologies	Booch, Shlaer & Mellor	Spiral
	Requirements of the System are Unknown or Uncertain	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, Spiral, RAD
Voluminous	Project Division	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Prototype, Incremental, Spiral
	Measurable Progress	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Incremental, RAD
	Difficult to Respond Changes	SADT, JSD, Yourdon, Booch, Coad & Yourdon, Shlaer & Mellor	Water Fall, Spiral
	Project is Large, Expensive and Complicated	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Prototype, Incremental, Spiral
	Mainframe or Transaction Oriented Batch Systems	SADT, Yourdon, JSD	Water Fall
	Avoid to Solve Wrong Problems	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Incremental, Spiral
	Technical Requirements (e.g. Response Time, Throughput) are Reasonable.	XP, Scrum, Crystal	RAD
	Continual Evolution of the Project Requirements	Fusion, Booch, Shlaer & Mellor, Scrum	Prototype, Spiral, RAD
	Reduce Project Risk by Breaking Project into Smaller Segments	Booch, Shlaer & Mellor	Spiral
	Encourage Innovations and Flexible Changes	Fusion, Booch, Shlaer & Mellor, Coad & Yourdon	Prototype, Incremental, RAD
	Team Members are Fully Experienced	Fusion, Booch, Shlaer & Mellor	Prototype, Spiral, RAD
	Very Large Infrastructure Projects	SADT,Yourdon, JSD, Fusion, Shlaer & Mellor, Booch, Coad & Yourdon	Water Fall, Prototype, Incremental, Spiral

Table 3.2: Difference between biological and remote sensing data characteristics

Factors/Criteria	Biological Data	Remote Sensing Data
Volatility of Data	Volatility Results from new findings and environment	Volatility results from need and application
Source of Change	Evolution is Research based (experiments in the Wet-lab.)	Evolution is Observation and Need based
Source of Uncertainty	Uncertainty results from different experimental Procedures	Uncertainty results from equipment used
Interaction with Society	Need not be integrated (indirectly) with society perspectives	Need to be integrated (directly) with society perspectives
Data Generation Source	Primary Data generation sources are centralized	Primary data sources are Distributed
Location of data	It is generated in Lab.	It is generated through Geospatially (out of the Lab.)

Generation	environment	
Decision/Use	It is not used for real time decisions	It is used for real time decisions
Cause of Imprecision	Imprecision results from faulty or non-standardize Wet-lab. equipment	Imprecision results from faulty or non-standardize Wet-lab. Equipment in addition to Environmental /Weather conditions
Granularity	Extreme level of Granularity	Moderate level of granularity
Observations	New observations are unprecedented	New Observations are precedential up-to some extent
Interval of change	Discrete	Continuous
Interval of Data Generation	Discrete	Continuous

To best of our knowledge no software development methodology is reported in literature for remote sensing applications. So, there is need to have a methodology for remote sensing applications development. We are actively working on proposing a methodology for remote sensing applications. The automation of such methodology and its working on some case studies will be carried out in future.

5. References

1. Ahmad, T. and Shah, A. (2009). A Provenance-Enabled Replication Framework for Remote Sensing Data Dissemination and Management. Pakistan Journal of Science, Vol., 61(3), PP (167-174), 2009.
2. Ahmad, T. and Shah, A. (2010). Remote Sensing Data Dissemination and Management: Potential of Replication and Provenance Techniques. The Journal of American Science, Vol., 6(7), PP (188-198), 2010.
3. Emilio,C., Pilar,M., and Chris,J. (2003). Innovative concepts and methods in fire danger estimation. 4th International Workshop on Remote Sensing and GIS Applications to Forest Fire Management, Ghent – Belgium, 5-7 June, 2003.
4. Haddi, A. A. El, Lee, V., Banks, J. and Sustman, P. (2003). Using file replication for business continuance and content distribution. Retrieved from **(**www.constantdata.com**).**
5. Ho, A.T.S. (2002). Detecting clouds and cloud shadows in multispectral satellite images for tropical areas. Sixth International Conference on Image Processing and its Applications, Vol., 2, PP (848-851).
6. Shah, A., (2003). OODM: Object-oriented design methodology for development of web applications. Information Modeling for Internet Applications, Chap. X, Idea Group Publishing, USA, PP (189-229).
7. Shah, A. and Ahsan, S. (2008). A framework for agile methodologies for development of bioinformatics. The Journal of American Science, Vol. 4(1), PP (20-32).
8. Shah, A., Ahsan, A. and Jaffer, A. (2009). Temporal Object-Oriented System (TOS) for modeling biological data. Journal of American Science, Vol. 5(3), PP (63-73).
9. Shah, A. (2001). A Framework for the Prototype-Based Software Development Methodologies, Computer & Information Sciences, Vol. 13, PP (105-125), J.King Saud University, Saudi Arabia.
10. Shalom Cohen & Uzi De Haan. (2006). A software development life cycle model for improved stake holder's communication. International Journal of Computers, Communication and Control, Vol. 5(1), PP (20-41).
11. Srinivasa, S.R., Murthey, K., Adiga,S. and Emmineedu, E. (2003). Performance Index for Watershed development. Journal of India, Geophysics, Union, Vol. 1(4), PP (239-247).
12. Stanley, D. Brunn, Susan, L. Cutter, James, W. Harrington, (2004). Geography and Technology. Published by Springer,USA, Chap,18,PP(401-430).

8/8/2012

Tomato pomace as a protein supplement for growing Markhoz goat

[1]Farzad. Abdollahzadeh, [2]*Rahim Abdulkarimi

[1,2]Islamic Azad University, Boukan Branch, Boukan, Iran
E-mail: Rahim.abdulkarimi@yahoo.com

Abstract: This study was conducted to investigate the effect of dried tomato pomace (DTP) on the growth performance, nutrient digestibility and mohair production of Markhoz goats. Twenty four Markhoz male goat kids (BW = 18.6 ± 0.7 kg) were assigned randomly to 1 of 4 treatments and were fed with different levels of DTP (10, 20 and 30% DTP) for 94 days. The first group was fed a basal diet without DTP and considered as control, while the other three groups fed the basal diet after substituting part of the diet with DTP at 10, 20 and 30%, respectively. There was no significant ($P<0.05$) difference between experimental groups in live body weight, weight gains and feed intake. Differences between digestibility of DM, OM and NDF were not significant, while there was significant difference ($P<0.05$) in the CP digestibility between experimental diets and the diet contained 20% DTP had the highest values compared to other treatments. The inclusion of DTP in Markhoz goats' diet was associated with a higher ($P<0.05$) greasy fiber, fiber diameter and Barbe length than control diet. Results showed that DTP can be utilized efficiently and safely in the diets of Markhoz male goat kids to level up of 20% without any adverse effect on the growth performance, diet digestibility and mohair production.
[Farzad abdollahzadeh, Rahim Abdulmalaki. **Tomato pomace as a protein supplement for growing Markhoz goat**. *Life Sci J* 2012;9(3):2157-2161] (ISSN:1097-8135). http://www.lifesciencesite.com. 311

Key words: Dried tomato pomace, Protein supplement, Markhoz goat.

1. Introduction

Shortage of inexpensive feed resources often imposes major constrains on the promotion of animal production(Abdollahzadeh et al, 2010). Conventional feeds are the common known standards for supplying protein and energy needs of livestock. However, there are many suitable alternatives that may be considered to meet nutritional requirements while reducing the feeding cost. The tomato processing serves as an excellent example. The total waste produced from tomatoes from world production was estimated roughly to be 3.70 million ton per year (FAO, 1991). Tomato pomace (TP) is a mixture of tomato skin, pulp and crushed seeds that remain after the processing of tomato for juice, paste and or ketchup (Nobakht and Safamehr, 2007). This by product that remains from squeezing of tomato is rich in protein (21.7%), energy and crude fiber (Abdollahzadeh et al, 2010). Bordowski and Geisman (1980) reported that tomato seeds protein contained approximately 13% more lysine than soy protein, which would allow it to be used in fortifying low lysine feeds. Elloitt et al., (1981) demonstrated that TP is a good source of protein but may be limited in energy due to the high fiber content. Markhoz goat (Iranian Angora) with population at 25000 heads in 1996 is the only single coat goat producing shiny fine fibers in Iran (Bahmani et al., 2011). Fleece taken from Markhoz goat is called mohair. Mohair fiber is pure protein thus, Angora goats has a high protein requirement due to their rapid hair growth compared to other ruminant species. Sahlu, et al., (1992) reported that Angora goats require substantial amounts of dietary protein to produce mohair, in addition to meeting their nutrient requirements for maintenance, pregnancy, and lactation. Considering that the use of tomato processing by-products could provide extra income and at the same time reduce the waste disposal problem, the aim of the present research was to investigate the growth performance, digestibility and mohair production of Markhoz goat kids fed on different levels of dried tomato pomace (DTP).

2. Materials and Methods

The present study was designed to evaluate the effect of different levels of DTP (0, 10, 20, and 30%) of complete diets as a non-conventional ingredient on growth performance, digestibility and mohair production of Markhoz male goat kids. The feeding trial was carried out for 14 weeks (adaptation: 14 d and experimental period: 80 d).

2.1Tomato pomace preparation

Fresh experimental TP was collected from main factories in Urmia, Iran. The TP consisted mainly of skins, seeds and hard tissues of the whole tomatoes. The TP was subjected to the sun-drying until complete removal of moisture (less than10%). Then samples were taken for proximate chemical analyses.

2.2 Experimental diets

The experimental diets were prepared by thoroughly mixed the ingredients which composed of barley grain, soybean meal, wheat bran, chopped alfalfa hay, molasses and DTP. Four experimental diets were formulated to meet the nutrient requirements of growing kids according to AFRC (1998) guidelines to be isonitrogenic (CP, 16/00 % DM basis)and were offered twice daily in equal portions at 08:00 and 20:00 h to provide approximately 10% feed refusal as fed basis. The first group was fed a basal diet (0% DTP) and considered as control, while the other three groups were fed the basal diet after substituting part of the diet with DTP at 10, 20 and 30% respectively. Each diet was mixed completely and feed sorting by goats was minimal. Ingredients and composition of diets used in the study are given in Table1.

2.3 Animals, housing and feeding

Twenty four of Markhoz male goat kids of averaged 18.6 ± 0.7kg BW (4 months of age) were divided into four groups, of six each. Each treatment was assigned to one of four dietary treatments: 0, 10, 20 and 30% 0f DTP. Before initiation of the experiment, animals were allowed to adapt to treatment diets for 2 wk and were de-wormed with an effective anathematic and vaccinated against enterotoxaemia and foot and mouth disease. The goats were weighed before feeding in the morning at 14-d intervals throughout the experimental period and sheared before the beginning and at the end of the trials. They were housed and fed in individual metal-mesh cages and were adapted to human handling and the experimental setting. The floor area in each cage was 90*90 cm, and they were raised 85 cm from the floor. All the experimental animals were fed on the treatment diets ad-libitum. Fresh and clean water was available all time of experiment.

2.4 Digestibility study

Daily feed intake was monitored on individual goats and any refusals were collected, weighed and sampled for later analysis. The daily fecal matter excreted from each animal was collected during the collection period (last 6 days of the experiment) to plastic bags then weighed, sampled, mixed, dried at 60°C, ground and stored to be analyzed for different nutrients. From the analysis of the diets and fecal matter excreted, the digestion coefficient of dry matter and other nutrients were calculated according to the Maynard, (1979).

2.5 Measurements and laboratory analysis

At the beginning (d 0) and end (d 90) of the trial all goats were sheared. At the latter clipping, grease mohair weights were recorded and a sample from the mid-side area (10 × 10-cm) of each fleece was meticulously sheared. The samples were bagged separately in moisture proof- plastic bags and taken to the Wool Laboratory for yield, staple length, and proportions of Medullated and Kemp characterized. The sub samples were prepared for measurement with the projection microscope technique in accordance with ASTM, (1991) short – section procedure to determine fiber diameter, as well was paralleled in fibro liner component of Almeter 100 (Peyer Texlab FDA 200 Siegfried Peyer Ltd. Ch-8832 Wollerau – Switzerland), to determine the Simi rigid Hautuer (fiberpercent/number) and Barbe (fiber percent/ weight) length. Subsamples of diets, refusal and feces were ground through a 1.0 mm screen then analyzed for DM, CP (N x 6.251, ash, NDF, and ADF as described. Crude protein was calculated from Kjeldahl N values as total N x 6.25 (AOAC, 2000). Ether extract was determined using Soxhlet extraction procedure with anhydrous diethyl ether as the non-polar solvent. NDF and ADF were analyzed according to Van Soest et al. (1991). Ash was analyzed by ashing at 550°C, 6 h in a furnace. Calcium and phosphorus were measured by using an auto analyzer spectrophotometer (Unico, model S 2100 SUV, serial number 2165168, Japan).

2.6 Statistical analysis

Twenty four male goat kids arranged in balanced completely randomized design were used to evaluate the effects of feeding DTP on the growth performance, nutrient digestibility and mohair production. The collected data were subjected to statistical analysis using the Duncan procedure of SAS, (1998) (SAS Inst. Inc., Cary, NC). Level of significance was α=0.05, and the Duncan's multiple test was used to compare differences between treatments. The model used for this analysis was:

$$\hat{Y}ij = \mu + Ti + \sum ij$$

Where Y is the dependent variable; μ is the overall mean; T is the DTP effect level (i= 10, 20 and 30% of diet) and Σ is the random residual error term on the third day of treatment, atretic follicles, and decreased oviduct weight.

Table 1. Ingredients and nutrient composition of experimental diets (DM basis)

	Diets (DTP levels)‡			
	Control	**1**	**2**	**3**
	0%	10%	20%	30%
Ingredients				
Alfalfa hay	39.00	37.60	31.14	30.00
DTP‡	00	10.00	20.00	30.00
Soy bean meal	11.50	6.00	5.00	2.20
Barley grain	31.95	20.00	27.00	26.75
Wheat bran	11.60	20.8	10.50	5.00
Molasses	5.00	5.00	5.00	5.00
Calcium carbonate	0.450	0.440	0.450	0.450
Premix†	0.50	0.50	0.50	0.50
Nutrient content		**(% based DM)**		
DM	78.0	78.3	78.1	79.4
CP	16.00	16.00	16.00	16.00
NDF	35.4	35.2	35.1	36.3
ADF	21.4	23.1	24.3	24.00
Calcium	0.6	0.61	0.58	0.57
Phosphorus	0.4	0.39	0.43	0.42

‡Diets; control = (0% DTP); 1= (10% DTP); 2= (20% DTP); 3=(30%DTP); DTP, dried tomato pomace; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. †Premix supplied (on a concentrate DM basis): Each 3 kg contain: vitamin A, 12.000.000 IU; vitamin D, 2.500.000 IU; vitamin E, 10.000 mg; vitamin K3, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 5000 mg; vitamin B6, 1500 mg; niacin, 30.000 mg; biotin, 50 mg; folic acid, 1000 mg; pantothenic acid, 10.000 mg; Mn, 60.000 mg; Zn, 50.000 mg; Fe, 30.000 mg; Cu, 5.000 mg; Se, 100 mg; Co, 100 mg; Mn, 250.000 mg; CaCo3, up to 3kg.

3. Results and Discussion

3.1 Live body weight and weight gain

Mean values of live body weight and weight gain are presented in Table 2. Results showed that difference between experimental treatments in the live body weight and weight gain was not significant ($P<0.05$). Goats fed the diet contained 20%DTP recorded the highest average body weight (29.12kg) and daily gains (116.8 g), while goats fed 30% DTP diet showed the lowest values of live body weight (26.1kg) and daily gains (81.1g). These results were accordance with Fondevila et al. (1994) and Ibrahem and Alwash, (1983) who reported that diets with up to 50% dried tomato pomace (used to replace alfalfa hay or barley straw) did not affect average daily gain of lambs. On contrary, Weiss et al., (1997) showed that TP did not affect live body weight, when fed to lactating dairy cows.

3.2 Feed intake and nutrient digestibility

The apparent digestibility of diet nutrients and mean daily DM intake are presented in Tables 2 and 3. The results (Table3) showed that some nutrients digestibility tended to be increased when TP substituted in diets. Digestible CP content of diets increased significantly compared to control diet and CP digestibility values of 10% DTP and 30% DTP treatments were similar, but CP digestibility of 20% DTP treatment was highest. Differences between TP levels were not significant ($P<0.05$). These findings are in accordance with those reported by Tahmasbi et al. (2002), who reported that increasing TP level in corn silage increased CP digestibility of the silage. Fondevila et al., (1994), concluded that supplementation of barley-based diets with TP at a rate of 200 g/kg ration DM promote similar N retentions and growth performances to soybean protein in young lambs up to 28 kg BW. Table 2 showed that, no significant differences were observed in DM intake but, goats fed the diet contained 30% DTP level consumed the lowest amount of feed (564.0g), while goats fed the diet contained 10% DTP consumed the highest amount of feed (590.3g). These results were agreed with that of others, Ibrahem and Alwash, (1983); Fondevila et al. (1994) and Denek and Can, (2006) (growth studies with lamb) Belibasakis, (1990); Belibasakis and Ambatzidiz (1995) and Weiss et al., (1997) (with lactating dairy cow). Present results is contrast with our previous findings (Abdollahzadeh et al., 2010), as reported that DM intake

linearly increased (P<0.05) when TP and AP (apple pomace) was fed together (with ratio of 50:50 on DM basis) and used in dairy cow diet.

Table 3. Nutrient digestibility of goats fed diets differing in ratio of DTP.

	Diets (DTP Levels) ‡					
	Control 0%	**1** 10%	**2** 20%	**3** 30%		
Items					**S.E.M**	**P value**
DM	65.24	65.09	66.04	64.4	0.6	>0.05
OM	67.30	67.36	67.41	67.01	0.5	>0.05
CP	6o.19[b]	62.2[ab]	63.24[a]	62.2[ab]	0.14	<0.05
NDF	57.02	59.01	58.60	56.4	0.27	>0.05

‡Diets; control = (0% DTP); 1= (10% DTP); 2= (20% DTP); 3=(30%DTP) S.E.M = standard error of means

Table (4): Mean values of fleece characteristics of goats fed different levels of DTP.

	Diets (DTP Levels) ‡					
	Control 0%	**1** 10%	**2** 20%	**3** 30%		
Items					**S.E.M**	**P value**
Greasy fibers (g)	453[ab]	451[b]	493[a]	470[ab]	0.34	<0.05
Clean fiber, %	77.30	76.8	79.68	80.14[b]	0.5	>0.05
Fiber diameter (µm)	87.7[b]	105.1[ab]	116.8[a]	81.1[b]	0.14	<0.05
Staple length, cm	4.32	5.03	5.86	5.64	0.27	>0.05
B Length	31.77[b]	35.61[ab]	45.01[ab]	45.95[a]	0.23	<0.05
H Length	34.60	34.43	36.87	36.76	0.45	>0.05
Med fiber,%	2.17	3.6	3.67	2.87	0.16	>0.05
Kemp fiber,%	1.70	3.22	4.41	4.67	0.33	>0.05
True fiber, %	96.13	93.18	91.92	92.45	0.24	>0.05

‡Diets; control = (0% DTP); 1= (10% DTP); 2= (20% DTP); 3=(30%DTP) S.E.M = standard error of means

Table (2): Mean values for performance of growing goats fed different levels of DTP

	Diets (DTP Levels) ‡					
	Control 0%	**1** 10%	**2** 20%	**3** 30%		
Items					**S.E.M**	**P value**
Initial body weight(kg)	18.5	18.9	18.6	18.8	0.14	>0.05
Final body weight (kg)	27.30	28.36	29.12	26.1	0.5	>0.05
Daily weight gain (g)	97.7	105.1	116.8	81.1	0.14	>0.05
Daily feed intake (g)	570.3	590.3	586.0	564.0	0.37	>0.05
Feed conversion	5.83	5.61	5.01	6.95	0.23	0.14

‡Diets; control = (0% DTP); 1= (10% DTP); 2= (20% DTP); 3=(30%DTP) S.E.M = standard error of means

3.3 Mohair production, quantity and quality traits

Influence of diets differing in ratio of DTP on the quantity and quality of fiber produced by Markhoz goat kids are presented in Table 4. Goats fed DTP containing diets produced higher amount of greasy fiber, fiber diameter and Barbe length than control diet but, differences between amount of med and kemp fiber, clean fiber, staple length, Hautuer length and true fiber were not significant. The kind or quality of protein consumed by Markhoz goat, owing to their rapid hair growth is very important. The higher ($P < 0.05$) produced mohair by goats fed DTP containing diets compared to control diet may have reflected enhance of microbial growth and thereby ruminal fermentation as well, provide a reasonable high quality source of amino acids by DTP to the intestines. Production efficiency would potentially be improved if a large proportion of feed proteins could get through the rumen without being degraded. Huston,et al., (1993) reported that, fishmeal are effective protein source for goats and may be of greater value than oilseed byproduct meals in stimulating mohair growth because of their comparatively low ruminal protein degradation.

Corresponding Author:
Rahim Abdulkarimi
Islamic Azad University, Boukan Branch, Boukan, Iran .
E-mail: Rahim.abdulkarimi@yahoo.com

References

1. Abdollahzadeh, F., Pirmohammadi, R., Farhoomand, P ., Fatehi, F & Farhang. Pazhoh, F. (2010). The effect of ensiled mixed tomato and apple pomace on Holstein dairy cow. *Italian Journal of Animal Science,* 9, 212-216.
2. AFRC, (1998). The Nutrition of Goats. CAB International, New York, NY, pp. 41–51.
3. A,O,A.C.(2000). Official Methods of Analysis, 17th ed., Association of Official Analytical Chemists, Gaithersburg, MD, USA.
4. ASTM, (1991). Annual Book of ASTM Standards. Sect. 7. Vol. 07.01. ASTM. Philadelphia, PA.
5. Bahmani, H. R., Tahmoorespur, M., Aslaminejad, A.A., Abbasi, M.A & Ebnabbasi, R. (2011). Assessment of Demographic, Geographical and Genetic Risks in Markhoz Goat Population. *Journal of Animal and Veterinary Advances*, 10, 162-168.
6. Belibasakis, N.G & Ambatzidiz, P. (1995). The effect of ensiled wet tomato pomace on milk production, milk composition and blood components of dairy cows. *Animal Feed Science and Technology*, 60, 399- 402.
7. Belibasakis, N.G. (1990). The effects of dried tomato pomace on milk yield and its composition and on some blood plasma biochemical components in the cow. *World Review of Animal Production,* 25, 39-42.
8. Bordowski, I & Geisman, J.R. (1980). Protein content and amino acid composition of protein of seeds from tomatoes at various stages of ripeness. Journal of Food Science, 45, 228-235.
9. Denek, N & Can, A. (2006). Feeding value of wet tomato pomace ensiled with wheat straw and wheat grain for Awassi sheep. *Small Ruminant Research*, 65, 260–265.
10. Elloitt, J., Mulvihill, E., Dumcan, C., Forsythe, R & Kritchevsky, D. (1981). Effect of tomato pomace and mixed vegetable pomace on serum and liver cholesterol in rats. *Journal of Nutrition,* 111, 2203-2211.
11. FAO, (1991). Production year book, vol.44. Food and agricultural Organization of the United Nation, Rome.
12. Fondevila, M., Guada, J.A., Gasa, J & Castrillo, C. (1994). Tomato pomace as a protein supplement for growing lambs. *Small Ruminant Research*, 13,117-126.
13. Huston, J.E., Taylor, C.A., Lupton, C.J & Brooks, T.D. (1993). Effects of supplementation on intake, growth rate, and fleece production by female Angora kid goats grazing rangeland. *Journal of Animal Science*, 71, 3124-3130.
14. Ibrahem, H.M & Alwash A.H. (1983). The effect of different ratios of tomato pomace and alfalfa hay in the ration on the digestibility and performances of awassi lambs. *World Review of Animal Production,* 19, 31- 37.
15. Maynard, L.A. (1979). Animal Nutrition. 7th ed. McGraw-Hill Book Company, Inc. New York, London.
16. Nobakht, A and Safamehr, A.R. (2007). The effect of inclusion different levels of dried tomato pomace in laying hens diets on performance and plasma and egg yolk cholesterol content. *Journal of Animal and Veterinary Advances*, 6, 1101-1106.
17. Sahlu, T., Fernandez, J. M., Lu, C. D and Manning, R. 1992. Dietary protein level and ruminal degradability for mohair production in Angora goats. *Journal of Animal Science*, 70, 1526-1533.
18. SAS. (1998). The SAS system for windows 6.03. SAS Institute Inc, Cary, North Carolina.
19. Tahmasbi, R., Nasiri, H., Naserian, A and Saremi, B, (2002). Effect of different levels of mixed corn plant and tomato pomace on milk production and composition inHolstein dairy cows. *Journal of Animal Science,* 80 (Suppl. 1), 299-303.
20. Van Soest, P.J., Robertson, J.B and Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science,* 74, 3583-3597.
21. Weiss, W.P., Frobose, D.I and Koch, M.E., 1997.Wet tomato pomace ensiled with corn plants for dairy cows. *Journal of Dairy Science*, 80, 2896-2900.

8/15/2012

The Effects of Task Reasoning Demand and Dyadic versus Individual Task Conditions on Learner Affective Factors in ESL Classrooms

Lilliati Ismail[1], Arshad Abd. Samad[1] , Wong Bee Eng [2], Nooreen Noordin[1]

[1] Faculty of Educational Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Faculty of Modern Languages and Communication Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
lilliati75@yahoo.com

Abstract: In light of previous findings that classroom instructional materials contribute towards high anxiety, stress and lack of confidence among learners in Malaysian ESL (English as a Second Language) classrooms, the study aimed to investigate the effects of pedagogical practices on learner affective factors. In particular, the study investigated the main and interaction effects of two levels of Task Reasoning Demand (TRD) and individual versus dyadic Task Conditions (TC) on learner affective factors. The participants were 76 Form six students in a public secondary school in Selangor, Malaysia. Using a quasi-experimental research design incorporating a repeated-measures Latin-square design, participants were randomly assigned to one of four groups. Each group was exposed to all four combinations of the two levels of TRD and two levels of TC, but in different orders. Data were gathered using an affective questionnaire administered after each session. The data collected were analysed using descriptive statistics and repeated-measures MANOVA. Some of the main findings were that both TRD and TC had significant main effects on learner affective factors. The study suggests that educators should consider the effects of TRD and TC on learner affective factors when designing or selecting tasks for classroom use.
[Lilliati Ismail, Arshad Abd. Samad, Wong Bee Eng, Nooreen Noordin. **The Effects of Task Reasoning Demand and Dyadic versus Individual Task Conditions on Learner Affective Factors in ESL Classrooms**. *Life Sci J* 2012;9(3):2162-2168] (ISSN:1097-8135). http://www.lifesciencesite.com. 312

Keywords: ESL, Task reasoning demand, task condition, affective factors

1. Introduction

In Malaysia, learner affective factors including anxiety, lack of confidence and stress have been found to contribute to learner inhibitions in using the English language. Normazidah Che Musa, Koo and Hazita Azman (2012) in a study using document analysis procedures to review and synthesise research papers that examine English language learning in Malaysia from 2001 to 2011 found that one of the factors for low English attainment among Malaysian learners is their unwillingness and high anxiety to use English to communicate. Other than the anxiety and stress originating from the learners themselves, school instructional practices were found to cause language anxiety among learners (Rosemala Ismail, 2008; Noor Hashimah Abdul Aziz, 2007). This finding appears to suggest the need to investigate ways in which pedagogical practices could help reduce learner anxiety, stress and perceptions of difficulty, thus encouraging learners to communicate in English.

2. Task Reasoning Demand (TRD) and Dyadic versus Individual Task Conditions (TC)

In light of the findings in Malaysian classrooms, the researchers believe there is a need in using instructional materials and conditions that could help reduce stress and anxiety among learners. Two variables of interest to the researchers were task reasoning demand (TRD) and dyadic versus individual task conditions (TC). Studies show that TRD and TC could significantly affect learner affective variables. In a study involving 44 Japanese EFL learners organised into dyads, Robinson (2001) used two versions of a map task; a relatively low complexity task and a relatively high complexity task. A brief questionnaire was administered to the learners after the performance of each task to gauge learners' perceptions of affective variables. In the study, Robinson (2001) found that cognitive demands of tasks and ratings of their difficulty were related. Learners rated the complex version more difficult overall, and more stressful, while they rated their ability to perform the task lower than for the simple version.

Gilabert, Baron and Llanes (2009) also conducted a study to gauge learner perceptions of affective variables based on tasks of different complexity levels. In Gilabert et. al's (2009) study, 54 EFL learners were organised into dyads and carried out three different task-types. A repeated-measures Latin-square design was used with learners performing the six tasks in six different sequences. To measure learners' perception of task complexity, repeated-measures ANOVAs of the affective variables (difficulty, stress, confidence, interest and motivation) were carried out. Like the current study, Gilabert et. al (2009) used Robinson's (2001) affective variable questionnaire for learners to rate

their perceptions of difficulty, stress, confidence, interest and motivation levels when carrying out each task. Results showed that learners graded the three complex versions of the tasks as more difficult. Pairwise comparisons for stress and confidence showed that significant differences were found between the simple and complex versions of the narrative task. The authors concluded that, overall, more complex tasks were perceived as more difficult without the learners finding them less interesting or motivating.

In the case of the current study, two consensus tasks were used. The low complexity task was a revised version of the dictogloss task, while the high complexity task was an opinion-gap task. The topics of the two tasks were matched. However, the provision of content support in the dictogloss task made it less demanding than the opinion-gap task. During the dictogloss task, a short paragraph was read out twice to learners, and they were allowed to jot down notes during the second reading. Thus, the learners already had a general idea of the content of the text, and they had their written notes to aid them during the text-reconstruction stage. They were then required to reconstruct the text through discussions in dyads (dyadic TC) or on their own (individual TC). However, during the opinion-gap task, only a topic with two points for arguments was given to the learners. Thus, they had to access their schemata, select relevant information, build on the knowledge through interaction with other learners, express preference and feelings, argue and reason, justify their arguments, and provide cause-effect relationships during the dyadic TC and form arguments in their own minds during the individual TC. Thus, in terms of reasoning demands, the dictogloss task was considered relatively less demanding (-TRD) compared to the opinion-gap task (+TRD).

In terms of task condition, Dörnyei's (2002) study on the motivational basis of language learning tasks with dyadic and individual learning conditions indicate that dyadic TC would have a positive effect on learner affective variables compared to individual task conditions. In his study involving 44 learners, an oral argumentative task and a self-report questionnaire that focused on attitudinal and motivational issues were administered. The correlation coefficient results showed that the dyads reported considerably higher coefficients compared to the individual students. The variables that were tested included self-confidence and L2 use anxiety.

Understanding the effects of task types and individual versus dyadic task conditions on learner affective factors could assist teachers in selecting, designing and administering tasks for optimum effects on learner confidence and motivation. Though a few studies (e.g., Robinson, 2001; Gilabert, 2005) have investigated the effects of task reasoning demand on learner affective factors, they did not study the effects of participation conditions on those affective factors. There were also studies (e.g., Michel, Kuiken, and Vedder, 2007; Pica & Doughty, 1998; Gass and Varonis, 1985) that investigated the effects of participation conditions on L2 learning, but they did not investigate the effects of these conditions on learner affective factors. Thus, the current study attempts to fill in the gap in the research by investigating the main and interaction effects of task reasoning demand and individual versus dyadic task conditions on learner affective factors.

3. Research Question and Hypothesis

The study aimed to investigate the main and interaction effects of two levels of Task Reasoning Demand (TRD) and individual versus dyadic Task Conditions (TC) on learner affective factors. In particular, this study sought to answer the following research question:

What are the main and interaction effects of Task Reasoning Demand (TRD) and dyadic versus individual Task Conditions (TC) on learner perceptions of affective variables?

The following research hypothesis was formulated for the study:

The +TRD and individual TC will result in higher difficulty and stress levels compared to the –TRD task and dyadic TC.

4. Research Methodology

As the experimental conditions could be re-ordered, a counterbalancing and Latin-square analysis was used. In a Latin-square design, an equal number of subjects receive each treatment in each position. Treatments are assigned at random within rows and columns, with each treatment once per row and once per column, in order to control for variation in two directions; condition and sequence (Steel and Torrie, 1980). In the current research the conditions in the tasks were as follows;

a) + Task Reasoning Demand (+TRD), individual (ind.)
b) – Task Reasoning Demand (-TRD), individual (ind.)
c) + Task Reasoning Demand (+TRD), dyad
d) – Task Reasoning Demand (-TRD), dyad

Students from four intact classes were randomly assigned to one of four groups. Table 1 shows the order of task administration in the four groups. Using a Latin-square design, each group is exposed to all combinations of TRD and dyadic versus individual TC, but in a different order. This helps reduce the chances that changes or outcomes are due to the learners' perception of the tasks

(subject characteristic threat). Also, putting the treatments in different orders in the groups helps reduce the threat that change is a result of task-sequencing (Fraenkel and Wallen, 2008). Each of the four sessions involved the use of three tasks at a 1 to 3 day interval.

Table 1: Order of administration of tasks

Group	Session*			
	1	2	3	4
Group 1 N= 20	+TRD, dyad	-TRD, ind.	+TRD, ind.	-TRD, dyad
Group 2 N= 20	-TRD, ind.	+TRD, ind.	-TRD, dyad	+TRD, dyad
Group 3 N= 18	+TRD, dyad	-TRD, ind.	-TRD, dyad	+TRD, ind.
Group 4 N= 18	-TRD, dyad	+TRD, dyad	+TRD, ind.	-TRD, ind.

*Each session involved the use of three tasks at a 1 to 3 day interval, TRD=Task Reasoning Demand, ind=individual

5. Interaction-based Research

The current research is an interaction-based research which Mackey and Gass (2005, p.65) define as "research in which the focus is learners' conversational interactions with others and the developmental benefits of such interactions". They further explain that the most common way of gathering data is by engaging learners in various carefully planned tasks.

The two tasks used were consensus tasks. Consensus tasks involve pairs or groups of learners who must come to an agreement on a certain issue. Mackey and Gass (2005) classify a dictogloss task as a consensus task, as learners would have to work together in a pair or in a group to reconstruct a text while maintaining its original meaning. The opinion-gap task used in the current research is also a consensus task, as learners have to discuss the issues given and come to a shared decision.

Students were grouped in dyads because each student would have a greater chance of producing language in a pair than they would in a group. Self-selected dyads were allowed to encourage the learners to feel comfortable in completing the tasks. To ensure that the learners were always on task, the researcher with the help of the class' English language teacher monitored the learners closely. After each session, a questionnaire was administered to the learners to gauge the learners' perceptions of the tasks' difficulty levels.

6. Participants

The current study was carried out in a secondary school in Selangor, Malaysia. This school was chosen as it had a sufficient number of Form 6 students (N = 76) to conduct a quasi-experimental study with four groups and two independent variables. Form 6 is equivalent to pre-university and the students were all 18 years old. Table 2 summarises the basic profile of the learners.

Table 2: Learner characteristics

Gender	Male	32
	Female	44
L1 Background	Malay	17
	Chinese	48
	Tamil	11

7. Instrumentation and scoring

This study employed the usage of teaching materials (for the dictogloss and opinion-gap tasks) and a brief questionnaire.

7.1 Tasks

The researcher developed and adapted the dictogloss and opinion-gap tasks for use in the four classes.

7.1.1 The dictogloss Task (-TRD)

The dictogloss or passage reconstruction task (Nabei, 1996; Wajnryb, 1990) consists of a series of stages. The lessons started with pre-task activities to ease learners into the topic. Then, learners listened to a short, dense passage during which they were instructed to listen only and not write anything down. Next, they listened to passage a second time and were allowed to jot down notes, but not complete sentences. Then, working in pairs, learners pooled their notes together and attempted to reconstruct their own written version of the passage. It was during this reconstruction phase that learners produced LREs as they searched for correct words or forms they needed in order to convey their intended meaning and write a cohesive text. During the sessions involving learners working as individuals, instead of pooling notes with a partner and reconstructing the text together, learners worked on it individually. Finally, the instructor collected all the texts produced in dyads for purpose of data analysis, and affective questionnaires were administered to gauge learners' overall perceptions of the task difficulty level during dyadic and individual TCs.

7.1.2 The Opinion-gap Task (+TRD)

An opinion-gap task would require students to give their opinions based on given issues in pairs. The topics for the opinion-gap tasks were matched as closely as possible to the topics for the dictogloss tasks. The three main topics were school life, social issues, and environmental issues. These topics were chosen as they correspond to the topics in their syllabus. The topics were then broken down into a question each for students to discuss. For example, under the topic "school life", the question given was "what is the best way to improve the school canteen

and why?" Two options were also given "(a) improve on the quality of food served (b) reduce the price of food sold". In each pair, student A had to argue in support of option A, while student B had to argue in support of point B. Students discussed their opinions in pairs and wrote the outcome of their discussion in one paragraph of approximately 100 words (similar to the number of words in the dictogloss text). During the sessions involving learners working as individuals, instead of dealing with the task with a partner, learners worked on it individually. Finally, the instructor collected all the texts produced in dyads or individually for the purpose of data analysis, and affective questionnaires were administered to gauge learners' overall perceptions of the task difficulty level.

8. Questionnaire

A post-task difficulty questionnaire adapted from Robinson (2001) was administered to the learners after each task to assess "overall perceptions of the difficulty of cognitively complex, and cognitively simpler task versions" (Robinson, 2007, p. 196). Following Robinson (2001), questionnaire used a 9-point likert scale. Students were asked whether they thought the task was difficult, whether they felt stressed performing the task, whether they were confident they were able to do the task well, whether they thought the task was interesting, and whether they wanted to do more tasks similar to the given task. The brief questionnaire administered after each lesson was worded as follows:

I thought this task was **hard**	1 2 3 4 5 6 7 8 9	I thought this task was **easy**
I felt **stressed** doing this task	1 2 3 4 5 6 7 8 9	I felt **relaxed** doing this task
I did **not** do this task **well**	1 2 3 4 5 6 7 8 9	I did this task **well**
This task was **not interesting**	1 2 3 4 5 6 7 8 9	This task was **interesting**
I **don't want** to do **more** tasks like this	1 2 3 4 5 6 7 8 9	I **want to do** more tasks like this

Learners were asked to circle each item at the end of each lesson. They had to circle only one number for each item with the numbers ranging from 1 to 9. Each learner had to circle the number that best represents the degree to which they agree with either one of the statements on the two ends of the range of numbers.

9. Data analysis

Research Question: What are the main and interaction effects of Task Reasoning Demand (TRD) and dyadic versus individual Task Conditions (TC) on learner perceptions of affective variables?

Table 3 shows the descriptive statistics of the learners' perceptions based on the affective variables. The variables were (i) the level of difficulty learners experience while completing each task in the dyadic or individual TC, (ii) the learners' ratings of stress caused by the task and the dyadic or individual TC, (iii) their perceived ability in completing the tasks well during individual or dyadic TC, (iv) their interest in the task type as they performed the task in dyadic or individual TC, and (v) their motivation to attempt similar tasks in the same dyadic or individual TC. Table 3 shows the descriptive statistics obtained from the questionnaires on affective factors. In the table, the affective variables are coded as difficulty, stress, ability, interest, and motivation respectively.

Table 3: Descriptive statistics of the effects of TRD and dyadic vs. individual TC on affective perceptions

	Difficulty		Stress		Ability		Interest		Motivation	
	M	SD	M	SD	M	SD	M	SD	M	SD
+TRD										
Dyad	3.26	1.36	3.83	1.41	4.28	1.29	4.45	1.25	4.84	1.37
Ind.	2.77	1.98	3.54	1.29	3.93	1.30	1.25	1.25	4.01	1.32
-TRD										
Dyad	5.07	1.82	5.24	1.63	5.56	1.60	5.34	1.50	5.70	1.41
Ind.	4.78	1.70	4.77	1.40	5.10	1.28	5.25	1.23	5.43	1.07

TRD= Reasoning Demand, Ind..= individual, M= Mean, SD= Standard Deviation

Table 3 shows that the highest mean in the learners' perceptions of task difficulty was achieved in the dyadic TC with low task reasoning demand (M = 5.07). This indicates that learners found that doing a task of relatively low reasoning demand in dyads was the least difficult. The lowest mean for difficulty was found in the high reasoning demand task done individually (M = 2.77). This indicates that learners found the high reasoning demand task done individually to be the most difficult. The stress scores indicate that learners found the high reasoning demand task done individually to be the most stressful (M = 3.54), followed by the high reasoning demand task done in dyads (M = 3.83), and the low reasoning demand task done individually (M = 4.77). The least stressful task was found to be the low reasoning demand task done in dyads (M = 5.24). Learners' perceptions of their abilities to complete the tasks well also show that they were least confident of their abilities when doing the high reasoning demand task individually (M = 3.93) and the most confident when they were doing the low reasoning demand task in dyads (M = 5.56). Learners also showed the lowest interest in the high reasoning

demand task done individually (M = 1.25) and the highest interest in the low reasoning demand task done in dyads (M = 5.10).

Finally, learners showed the lowest motivation to do the high reasoning demand task individually (M = 4.01) and the highest motivation to do low reasoning demand tasks in dyads (M = 5.70). Results from the descriptive statistics appear to show a match in learner perceptions of task difficulty based on affective variables and high and low task complexity levels with the +TRD task resulting in greater difficulty and stress levels, and lower confidence in ability, interest and motivation levels. Also, learners have greater difficulty and stress levels, and lower confidence in ability, interest and motivation levels when engaged in tasks individually rather than in dyads. It is also interesting to note that the lowest mean score recorded in the "high reasoning demand task done individually" category (M = 1.25) was for interest in the tasks. This indicates very low interest in individually engaging in tasks that the learners found the most difficult and stressful. Meanwhile, the highest mean score was recorded in the "low reasoning demand task done in dyads" category (M = 5.70). This mean score indicates moderate motivation levels to perform more of these tasks which the learners also found to be moderately difficult (M = 5.07), moderately stressful (M = 5.24), moderately interesting and pose moderate challenge to their abilities (M = 5.56).

In order to determine the main and interaction effects of +/- TRD and dyadic versus individual TC on affective variables, a repeated-measures MANOVA was run. There were no between-subject effects to be measured as all learners were exposed to both levels of TRD and TC. Instead, the within-subject effects of the two variables were measured for their effects on affective variable scores using repeated-measures MANOVA. As mentioned in the previous chapter, MANOVA is often preferred over a series of ANOVAs if there are possible relationships among the variables to be tested. In the case of the current research, previous research (e.g., Gilabert, 2009, Robinson, 2001) has shown that the five affective variables could have relationships among them as a result of the effects of TRD and TC. Prior to running MANOVA, the Bartlett's test of sphericity was used to determine whether it was justified to apply a MANOVA test rather than a series of ANOVAs. Results of the test are shown in table 4.

Results of the Bartlett's test of sphericity indicate that Task Reasoning Demand, dyadic versus individual Task Conditions and the interaction between Task Reasoning Demand and Task Condition do have significant effects on the dependent variables ($p < .05$). Thus, it would be appropriate to proceed with MANOVA. The table below shows the main and interaction effects of TRD and dyadic and individual TCs on the affective variables using repeated-measures MANOVA with the Wilks' Lambda test.

Table 4: Bartlett's Test of Sphericity

Effect	Likelihood Ratio	Approx. Chi-square	df	Sig.
Within-subjects				
TRD	.000	339.96	14	.00
TC	.000	325.49	14	.00
TRD*TC	.000	329.70	14	.00

TRD= Task Reasoning Demand, TC= Task Condition

Table 5: Impact of TRD and TC on affective variables (repeated-measures MANOVA, Wilks' Lambda test)

Source	F	df	Error df	*p*
TRD	24.48	5	71	.00
TC	3.36	5	71	.01
TRD *TC	4.58	5	71	.06

TRD= Task Reasoning Demand, TC= Task Condition

According to the Wilks' Lambda test of the repeated-measures MANOVA, Task Reasoning Demand had a significant effect on affective factors (p =.00). The same goes for dyadic and individual Task Conditions (p =.01). However, the interaction between Task Reasoning Demand and dyadic and individual Task Conditions was not significant (p =.06).

Though the multivariate test informs us of the significance of at least one mean pairing within the +/- TRD and dyadic versus individual TC, it is unclear for which affective factor the observed mean difference is significant. In order to determine the significance of the differences, pair-wise comparisons were then run to identify the specific mean difference between dyadic and individual Task Conditions and +/-TRD for each of the affective variable. Results are shown in tables 6 and 7.

Table 6: Pairwise comparisons between dyadic and individual TC based on affective factors

Factor	(I) TC	(J) TC	Mean Difference (I-J)	*p*
Difficulty	Dyad	Individual	.39	.04
Stress	Dyad	Individual	.38	.02
Ability	Dyad	Individual	.41	.02
Interest	Dyad	Individual	.31	.05
Motivation	Dyad	Individual	.55	.00

*p< .05, TC= Task Condition

Table 6 shows that dyadic and individual Task Conditions had significant effects on all five affective variables ($p \leq .05$). The largest mean difference was for motivation level (mean difference= .55, p= .00). The positive result indicates that there were significantly higher levels of motivation when learners dealt with tasks in dyads rather than individually. Meanwhile, the smallest mean difference was for learners' interest in dealing with the tasks (mean difference= .31, p= .05). This would indicate that either being in dyads or working individually would have the least effect on the learners' interest levels. The positive mean difference for all five variables would indicate that dealing in tasks in dyads rather than individually had more positive effects on affective variables.

Next, pairwise comparisons were run to identify the specific mean difference between tasks with high and low TRD for each affective variable. Results are shown in table 7.

Table 7: Pairwise comparisons between +TRD and -TRD based on affective factors

Factor	(I) TRD	(J) TRD	Mean Difference (I-J)	p
Difficulty	+TRD	-TRD	- 1.91	.00
Stress	+TRD	-TRD	- 1.32	.00
Ability	+TRD	-TRD	- 1.22	.00
Interest	+TRD	-TRD	- 1.10	.00
Motivation	+TRD	-TRD	- 1.14	.00

TRD= Task Reasoning Demand

Table 7 shows that TRD levels had significant effects on all five affective variables ($p \leq$ .05). The largest mean difference was for difficulty level (mean difference= -1.91, p= .00). The negative mean difference indicates that learners perceived the +TRD task as significantly more difficult than the –TRD task. Meanwhile, the smallest mean difference was for learners' interest in dealing with the tasks (mean difference= -1.10, p= .00). This would indicate that TRD level would least affect the learners' interest levels as opposed to the other four affective variables. The negative mean difference for all five variables would indicate that the +TRD task rather than the –TRD task had more negative effects on affective variables. In other words, learners perceived lower levels of motivation, interest, and ability, as well as higher levels of stress and difficulty when dealing with +TRD task as opposed to –TRD task.

10. Hypothesis testing: The +TRD and individual TC will result in higher difficulty and stress levels compared to the –TRD task and dyadic TC.

This section discusses the research findings in relation to the research hypothesis. This hypothesis is answered by analysing the main effects of TRD on selected affective variables (i.e., perceived difficulty, stress, ability, interest and motivation), the main effects of TC on the affective variables and the interaction effects of TRD and TC on the affective variables.

This research hypothesis is heavily influenced by the Cognition Hypothesis put forth by Robinson (2007). The Cognition Hypothesis claims that "the more cognitively complex tasks will be perceived by all learners to be more difficult than less complex counterparts" (Robinson, 2007, p. 196). This claim was supported by the results of the current study. Results indicated that learner perceptions of task difficulty levels appear to correlate with the researcher's distinction of the opinion gap task and dictogloss task in relation to task complexity levels. This is reflected by the significantly higher rates of difficulty, stress, perceived ability to complete the task, interest and motivation for the +TRD task (opinion-gap) compared to the –TRD task (dictogloss). This is perhaps why Skehan and Foster (2001) used task complexity interchangeably with task difficulty to refer to the amount of attention a task demands from participants. Though Robinson (2007) makes the distinction between task difficulty (i.e., influenced by learner factors) and task complexity (i.e., influenced by task inherent factors) and cautions against assuming a fixed relationship between the two, he too agrees that task difficulty and task complexity would correlate as more complex tasks result in higher stress levels and perceived difficulty across learners.

As predicted in the research hypothesis, the individual TC was also found to result in significantly higher difficulty and stress levels compared to the dyadic TC. In addition, the individual TC also resulted in significantly lower confidence levels and motivation levels compared to the dyadic TC. These results point to the beneficial effects of engaging in tasks in dyads as opposed to working on tasks individually.

In sum, the current study appears to corroborate the findings of Robinson (2001) and Gilabert et. al's (2009) studies with regards to the effects of task complexity on affective variables. Though differentials among learners in terms of inherent ability could affect perceptions of task difficulty levels (Robinson, 2001), the results indicate that the more complex tasks would result in greater perceptions of overall difficulty and stress, and lower ratings of confidence in ability. This study also found that TC can have an effect on affective variables. In particular, the dyadic TC was found to have more positive effects on learner affective factors compared to the individual TC, as predicted by the hypothesis.

This would further consolidate the benefits of engaging learners in interactive tasks as opposed to monologic or individual tasks.

11. Conclusions

One of the findings of the study is that engaging in interaction to complete tasks could have positive effects on learner affective variables. The findings show that the dyadic task condition could have greater benefits on motivation levels, stress levels, confidence levels, interest and perceived ability to complete tasks compared to the individual task condition. Dörnyei (2002) contends that motivation is co-constructed or encouraged by task participation. Dörnyei's (2002) assertion, along with the findings of the current study, provides evidence for teachers to use dyadic interaction in the L2 classroom to enhance motivation and other affective variables. Also, though several studies (e.g., Michel et al., 2007; Pica & Doughty, 1998; Gass and Varonis, 1985) have investigated the effects of task conditions on L2 learning, they do not investigate the effects of these task conditions on learner affective factors. Thus, the current study provides valuable insights by providing some evidence of the beneficial effects of dialogic tasks on learner affective factors in the L2 classroom.

Results of the current study provided rather substantial support for Robinson's Cognition Hypothesis. In line with results from previous research (e.g., Robinson, 2001; Gilabert, 2005), the findings of the current research indicated that the more complex task resulted in significantly higher ratings for perceptions of overall difficulty and stress, and lower ratings for confidence to perform well in the task. Also, the results showed that engaging in interaction in dyads as opposed to dealing with the tasks individually had positive effects on affective factors.

Results of the study would then have pedagogical implications on teaching practices, task selection, and task implementation. The study indicated that interactive tasks could have positive effects on learner affective factors as opposed to individual tasks. Also, task complexity levels would have differential effects on learner affective factors. Thus, task complexity must be taken into consideration when selecting, designing or adapting tasks for use in the ESL classroom.

8/16/2012

References

1. Dörnyei, Z. (2002). The motivational basis of language learning tasks. In Peter Robinson (Ed.) *Individual differences in second language acquisition* (pp. 137-158). Amsterdam: John Benjamins.
2. Fraenkel, J.R., and Wallen, N.E (2008). *How to design and evaluate research in education* (7th ed.) New York: McGraw-Hill.
3. Gass, S., & Varonis, E. (1985). Task variation and nonnative/nonnative negotiation of meaning. In S. Gass & C. Madden (eds.) *Input in Second Language Acquisition* (pp. 149-161). Rowley, MA: Newbury House.
4. Gilabert, R. (2005). *Task complexity and L2 narrative oral production*. Unpublished Doctoral. dissertation, The University of Barcelona, Spain.
5. Gilabert, R., Baron, J. and Llanes, M. (2009). Manipulating cognitive complexity across task types and its impact on learners' interaction during oral performance. *IRAL*. 47: 3676-395.
6. Huberty, C. J, and Morris, J. D. (1989). Multivariate analysis versus multiple univariate analyses. *Psychological Bulletin*. *105*: 302-308.
7. Mackey, A., and Gass, S. M. (2005). *Second language research: Methodology and design.* Mahwah, NJ: Lawrence Erlbaum Associates.
8. Michel, M. C, Kuiken, F., and Vedder, I. (2007). The influence of complexity in monologic versus dialogic tasks in Dutch L2. *IRAL*. 45: 241-259.
9. Nabei, T. (1996). Dictogloss: Is it an effective language learning task? *Working Papers in Educational Linguistics*. *12*(1): 59-74.
10. Noor Hashimah Abdul Aziz. (2007). ESL students' perspectives on language anxiety. Unpublished Ph.D. thesis, Universiti Putra Malaysia: Serdang.
11. Pica, T. and Doughty, C. (1998). Variation in classroom interaction as a function of participation pattern and task. In J. Fine (Ed.) *Second language discourse*. (pp. 41- 55). Norwood, NJ: Ablex.
12. Robinson, P. (2001). Task complexity, task difficulty, and task production: Exploring interactions in a componential framework. *Applied Linguistics*. 22: 27-57.
13. Robinson, P. (2007). Task complexity, theory of mind, and intentional reasoning: Effects on L2 speech production, interaction, uptake and perceptions of task difficulty. *IRAL. 45:* 193-213.
14. Rosemala Ismail. (2008). Factors affecting less proficient ESL learners' use of strategies for language and content area learning. Unpublished PhD. thesis. Faculty of Modern Languages, Universiti Putra Malaysia Serdang.
15. Steel, R.G.D. and Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill.
16. Wajnryb, R (1990). *Grammar dictation.* Oxford: Oxford University Press.