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Comparative Study of Knowledge and Attitude toward Breastfeeding Practices among Egyptian and Saudi Mothers in Qassim Region

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Abstract: Background: Approximately 52 % of babies in Saudi Arabia are breast fed for more than 6 months compared with approximately 96% in Egypt. Few studies compared the infant feeding of these two neighboring countries despite the similarities in their social systems. **Objectives:** The present study aimed to compare knowledge and attitude toward breastfeeding practices among Egyptian and Saudi mothers in Qassim region. **Methods:** A descriptive explorative study was conducted. The data was collected from 340 mothers (191 Egyptian and 149 Saudi) including socio-demographic data, mother's knowledge and attitude towards breastfeeding and the practices. **Results:** exclusive BF was reported by 74.6% of Egyptian compared to 42.1% of Saudi mothers with highly statistically significant difference. The mean duration of breast feeding was 17.5 months among Egyptian compared to 8.1 months among Saudi mothers. Both Egyptian and Saudi mothers had a high level attitude toward BF practice. **Conclusion:** Research in infant feeding should be a health priority to improve the rate of breastfeeding and to minimize other inappropriate practices. Most of the Egyptian and Saudi participants who ceased breastfeeding attributed this to return to work; consequently governmental policies regarding longer leave for new mothers and child care centers inside large institutions should be considered. More effort is needed to support and encourage breast-feeding particularly in Saudi Arabia.

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1. Introduction

Breastfeeding has been accepted as the most vital intervention for reducing infant mortality and ensuring optimal growth and development of children (Ekambaram *et al.*, 2010). Various researches have proven that breastfeeding (BF) has enormous advantages not only to infants and mothers, but also to families and society; these include health, nutritional, immunologic, developmental, psychological, social, economic, and environmental benefits (Astin *et al.*, 2006). One and a half million deaths among infants could be avoided each year if all infants were breastfed exclusively during the first six months of life (Al-Akour *et al.*, 2010) and estimates predict that improved breast-feeding practices could save the lives of 1.5 million children per year (UNICEF, 2008).

Khassawneh *et al.* (2006) stated that to improve rates of full breastfeeding, specific information regarding the beliefs and practices that influence this outcome is needed⁵. In addition, one of the factors known to play a role is attitude towards infant feeding; moreover, mothers' breastfeeding attitudes are known to influence infant feeding choice (Laanterä *et al.*, 2010).

Measures of psychosocial variables such as knowledge, attitudes, beliefs and experiences can improve the prediction of feeding behavior, or be used as outcome measures relating to the behavior itself.

Such measures are important for both researchers and policy makers to provide services that meet the needs of breastfeeding mothers and to inform robust evidence-based practice for health professionals (Chambers *et al.*, 2007).

According to Zhou *et al.* (2010) education and promotion of breastfeeding have become a public health focus worldwide. Breastfeeding practices and attitudes are influenced by demographic, biophysical, social, cultural and psychological factors.

Recent studies in Saudi Arabia showed a decline in breastfeeding between the ages of 6 and 12 months and the introduction of bottle formula has been become more frequent at earlier infant ages. The majority of mothers start to breastfeed their infants but soon introduce a bottle feeding (El-Gilany, 2010), while in Egypt prolonged breastfeeding is encouraged, where Egyptian women, especially those from a rural background, depend on breastfeeding as the major source of infant feeding for a long time; usually for 2 years (Shaaban & Glasier, 2008).

The purpose of this study is to compare knowledge and attitude toward breastfeeding practices among Egyptian and Saudi mothers in Qassim region. In the hope of understanding the reasons behind the notable differences. The findings of the present study may participate in altering the breast-feeding

promotion strategies, particularly in Saudi women where the situation remains alarming.

2. Methods

Study design and participants

A descriptive explorative study was utilized to meet the aim of this study. A sample of 340 Egyptian and Saudi married women aged 18 years and above, living in Qassim region and had one child at least; aged to a maximum of 3 years to diminished the risk of recall bias were included in the current study. The total number of Egyptian mothers was 191 and the total number of Saudi women was 149. A 'snowball' technique was used to increase sample size, i.e. participants were requested to spread word of this study and distribute the questionnaires to their friends or relatives. Twenty-three of the study sample showed their unwillingness in participation in the study due to various reasons including illnesses and shortage of time. Also 12 of them were excluded due to incomplete data. The study was conducted in Qassim University, female section and other Nongovernmental Organizations (NGOs) in Qassim Region.

Tool of data collection

A self-administered questionnaire was used to collect data; the questionnaire consisted of four sections. **The first section** included data regarding participants' socio-demographic characteristics including: mother age (years), level of education, monthly family income, number of children and occupation. **The second section** included data concerning practice of breast feeding including: duration of breast feeding, source of information about breast feeding, problems and obstacles related to breast feeding, exclusive breast feeding for 6 months, type of feeding, cessation of breast feeding before 2 yrs, causes and duration of cessation of breast feeding. **The third section** included knowledge toward breast feeding practice, knowledge about breastfeeding practices including benefits, duration and exclusiveness of breastfeeding for six months, types of other feeding ...etc. The responses to the questions were "yes", "no" or "don't know" was obtained from the 20-item questionnaire. **The fourth section** concerning the data on attitude toward breast feeding practice, including beliefs, feelings, and intention to breastfeed, the questions used the 5-point Likert scale from strongly agree to strongly disagree. There were 15 items, either positive or negative. The scores were then calculated for the mean scores which were then categorized as follows: Mean scores < 2.5 = low level of attitude, Mean scores = 2.5 - 3.5 = moderate level of attitude and Mean scores > 3.5 = high level of attitude. The questionnaire was adopted from Hengsiri (2003) and modified by the researchers to accommodate with the cultural differences and translated into Arabic

language using back translation technique. The questionnaire was revised and validated by committee of 10 experts; also pilot study was carried out on 10 mothers, whom were not included later in the study sample to test clarity, simplicity and applicability of the study tool.

Data collection and analysis

A self-administered questionnaire was used to collect data from Egyptian and Saudi mothers. The study was conducted between October 2010 and April 2011. Data were analyzed using the Statistical Package for Social Sciences (SPSS) windows version 16. Chi-square was used to compare between two groups. A *p*-value of 0.05 or less was considered as statistically significant.

3. Results

A total of 340 women participated in the study. The mean age of Egyptian women was 38.84±4.52 years while the mean age of Saudi women was 34.01±6.5 years. Regarding education, table (1) showed that more than half of Egyptian and Saudi women (52.9%) and (58%) respectively had higher education, also it showed that nearly (80%) were worked and the majority of them had sufficient income, also nearly half of the participated women had one to two children (Table 1).

Table (2) illustrated that the majority of Egyptian and Saudi women (92.7%, 89.3%) respectively were practice breast feeding. As regards to the duration of breast feeding, Egyptian and Saudi women (67.8% & 20.3%) respectively their duration was more than 18 months, also the mean of duration among Egyptian was 17.51±7.74 months while among Saudi was 8.11±7.25 months with highly statistically significant difference ($P < 0.00$).

In accordance to exclusive BF the table showed that (74.6%) of Egyptian women and (42.1%) of Saudi women were practice BF exclusively with highly statistically significant difference. In relation to most common types of feeding of not exclusive BF was artificial feeding. Regarding to weaning, the results clarified that (48%, 69.2%) of Egyptian and Saudi women respectively were weaned their children before 2 years with highly statistically significant difference.

Figure (1) showed that, in accordance to the source of information about BF the highly percent was mother followed by physician and media for both groups. Figure (2) showed that the most common problems related to BF practice among Egyptian women were nipple fissure and sleeplessness (27.7% , 23.6%) respectively while increase number of BF and nipple fissure (16.8%, 15.4%) respectively were the most common problems among Saudi women.

The present findings illustrated that the most common reasons of cessation of breastfeeding among

Egyptian and Saudi women (23.7%, 27.8%) respectively were “mother’s return to work” followed by “insufficient milk” (22%, 31.6%) respectively (Figure 3).

The majority of both groups were knowledgeable regarding advantages, time of initiation and nutrition of mothers with a highly statistically significant difference. As regards to the preparation of breast, the results revealed that the lowest percent of satisfactory knowledge among Egyptian and Saudi women were “BF demand” (25.7%, 20.1%) and “misconception” (33%, 22.8%) respectively. Also it was found that the

mean score of total knowledge among Egyptian and Saudi women was 14.73 ± 1.94 and 11.93 ± 4.88 respectively with highly significant difference ($P < 0.000$) (Table 3). Table (4) showed that the highest percent of Egyptian and Saudi women had high level attitude toward BF practice (73.3%, 62.4%) respectively and (26.7%, 36.2%) had moderate level attitude toward BF practice. Also it showed that mean score of total attitude among Egyptian and Saudi women was 3.68 ± 0.3 and 3.57 ± 0.5 respectively with statistically significant difference ($P < 0.000$).

Table (1): Number & percent distribution of women according to their socio-demographic data

Parameters	Egyptian		Saudi	
	No	%	No	%
Age (years)				
< 25	0	0	4	2.7
25-34	27	14.1	88	59.1
35- 44	143	74.9	41	27.5
≥ 45	21	11	16	10.7
Mean and standard deviation of age	38.84 \pm 4.52		34.01 \pm 6.51	
Education				
Read and write	2	1	5	3.4
Secondary	88	46.1	57	38.3
Higher	101	52.9	87	58.4
Occupation				
Housewife	39	20.4	27	18.4
Employed	152	79.6	120	81.6
Income				
Sufficient	175	91.6	126	84.6
Insufficient	16	8.4	23	15.4
Child numbers				
1-2	93	48.7	81	54.4
3-4	92	48.2	42	28.2
≥ 5	6	3.1	26	17.4

Table (2): Number & percent distribution of mothers according to their practice of breast feeding

Parameters	Egyptian		Saudi		P value
	No=191	%	No=149	%	
Practice of Breast Feeding (BF)					
Yes	177	92.7	133	89.3	0.2
No	14	7.3	16	10.7	NS
Duration of BF(177-133)					
< 6 months	4	2.3	62	46.6	
6-	15	8.5	28	21.1	0.000
12-	38	21.5	16	12	
≥ 18	120	67.8	27	20.3	
Mean and SD of BF duration	17.51 \pm 7.74		8.11 \pm 7.25		0.000
Exclusive BF for 6 months (177-133)					
Yes	132	74.6	55	41.4	0.000
No	45	25.4	78	58.6	
*Types of feeding					
Artificial	40	22.6	56	42.1	0.000
Herbs and Water	5	2.8	21	15.8	0.000
Nutrition	14	7.9	27	20.3	0.003
Cessation of breastfeeding before 2 yrs (177-133)					
Yes	85	48	92	69.2	
No	92	52	41	30.8	0.000

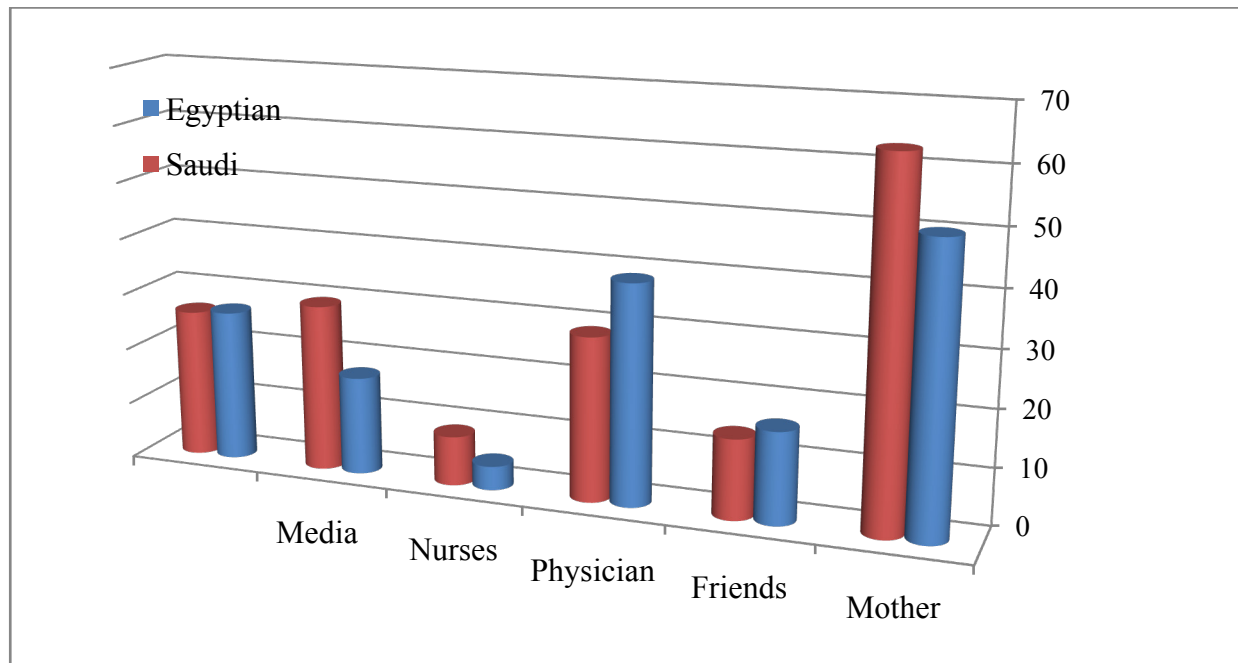
*items not mutually exclusive

Table (3): Number & percent distribution of mothers according to their satisfactory knowledge regarding breast feeding practice

Parameters	Egyptian		Saudi		P value
	No	%	No	%	
Advantage	191	100	128	85.9	0.000
Time of initiation	191	100	124	83.2	0.000
Preparation of breast					
During pregnancy	114	59.7	51	34.2	0.000
Before feeding	157	82.2	100	67.1	0.001
Duration of exclusive BF	87	45.5	77	51.5	0.2
BF demand	49	25.7	30	20.1	0.2
Nutrition of mother	189	99	130	87.2	0.000
Misconception	63	33	34	22.8	0.03
Mean score of total knowledge	14.73±1.94		11.93±4.88		0.000

Table (4): Number & percent distribution of women according to their level of attitude regarding breast feeding practice

Parameters	Egyptian		Saudi		P value
	No	%	No	%	
Low level attitude	0.0	0.0	2	1.3	0.01 S
Moderate level attitude	51	26.7	54	36.2	
High level attitude	140	73.3	93	62.4	
Mean score of total attitude	3.68±0.3		3.57±0.5		

**Figure (1): Source of information about breast feeding among Egyptian and Saudi mothers**

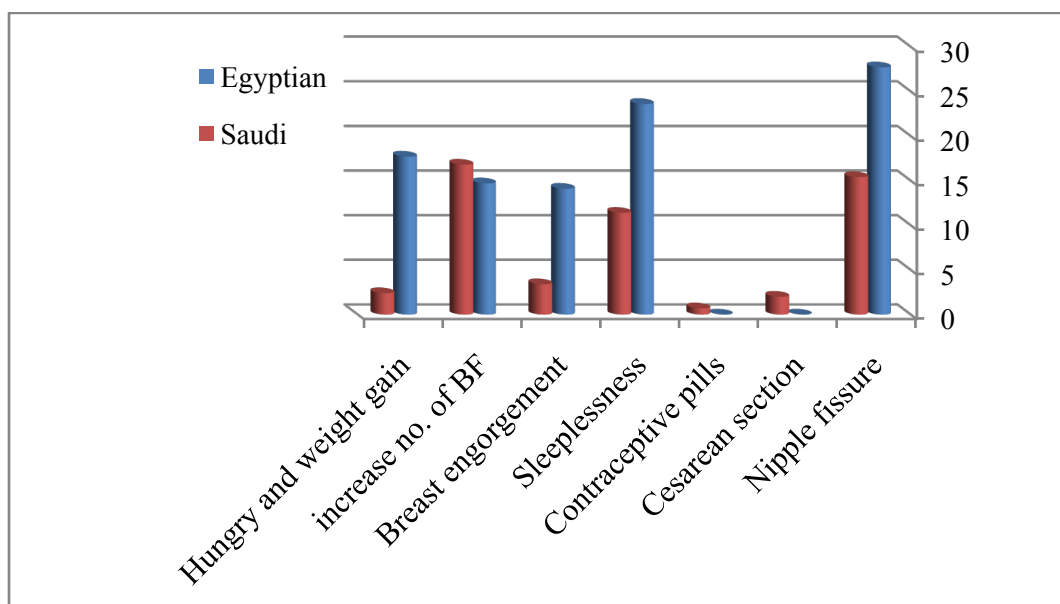


Figure (2) Problems and obstacles in BF among Egyptian and Saudi mothers

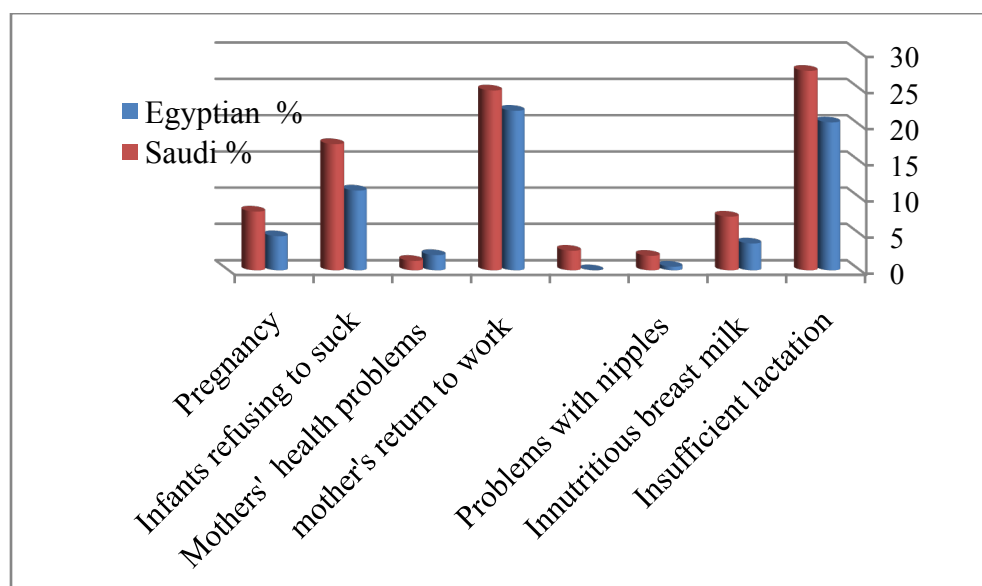


Figure (3) Reasons given for breast-feeding cessation before 2 years among Egyptian and Saudi mothers

4. Discussion

Encouraging women to breast feed presents a major challenge to health care professionals. Despite attempts to increase the number of women choosing to breast feed, rates of initiation and continuation in many countries remain less than optimal (Swanson and Power, 2005). In terms of demographic characteristics, the two groups were homogeneous and well matched, rendering the overall comparisons between the two groups straightforward (Table 1).

The majority of participants in this study were knowledgeable regarding the advantages of

breastfeeding (85.9% & 100%), nutrition of mother (87.2% & 99%), and their knowledge was inadequate in areas of duration of exclusive BF (51.5% & 45.5%), BF demand (20.1% & 25.7%) Misconception (22.8% & 33%) for Saudi and Egyptian mothers respectively. Those findings are in agreement with the recent study which conducted in India by Ekambaram *et al.* (2010).

Despite many studies showing the benefits of breastfeeding for infants and mothers, the practice of breastfeeding, especially exclusively, is still far below the standard recommendation Adtina *et al.* (2006). In Saudi Arabia, there is a significant downward trend in

breastfeeding and upward trends in both bottle and mixed feeding rates with increasing infant's age (El-Gilany, 2010). The present findings concurred with that, however about half of the Saudi mothers (46.6%) breastfeed their infants for less than six months compared to only 2.3% of Egyptian mothers (Table 2).

In accordance with the previous studies (El-Mouzan *et al.*, 2009 Egypt Demographic and Health Survey, 2010), the breast-feeding duration was significantly longer for Egyptian mothers compared with Saudi mothers. The mean duration among Egyptian was 17.5 months while among Saudi was 8.1 months with highly statistically significant difference. This may reflect a difference in the socio-economic and cultural status.

The cross-country comparison, as expected, revealed many between-country differences which correspond to the results of previous international studies (Suhonen *et al.*, 2008 and Papastavrou *et al.*, 2012). It is possible to speculate that these differences may be attributed to organizational factors, different healthcare systems, different aspects of education and training and cultural differences (Watson *et al.*, 2003).

Exclusive breastfeeding (EBF) for the first six months of an infant's life is a cost effective intervention in saving children's lives and it is recommended by the World Health Organization (WHO, 2009). The prevalence of exclusive breastfeeding up to infant age of 6 months was 74.6 % for Egyptian mothers compared to 41.4% for Saudi mothers. Only 20.3% of Saudi mothers continued breastfeeding for equal or more than 18 months compared to 67.8% of Egyptian mothers. This difference can be attributed to many possible factors, but the foremost reason is undoubtedly the different approach of each country to breast-feeding promotion.

In present study it was seen that only 59.7 % of the Egyptian and 34.2% of Saudi mothers were knowledgeable regarding the preparation of breast required during pregnancy. Support and counseling should be available routinely during antenatal care, to inform mothers to prepare her breast during pregnancy and before feeding, which help them to initiate breastfeeding; and in the postnatal period to ensure that breastfeeding is fully established (Table 3).

As all mothers believed that breast milk was beneficial for the infant, they had a positive attitude to breast feeding. The present study showed that the most of the both groups (73.3% of Egyptian and 62.4% of Saudi mothers) had a high level of attitude toward breastfeeding (Table 4).

Examination of the reasons given for breast-feeding cessation shows that both Egyptian and Saudi mothers experience difficulties with the establishment of breast feeding, but these difficulties are far less likely to result in cessation for Egyptian mothers. For Saudi mothers, insufficient lactation (31.6%) and

Mother's return to work (27.8%) accounted for most the cases of breast-feeding cessation. In Egypt, early breast-feeding cessation was due to return to work (23.7%), followed by insufficient lactation (22%). The reasons given for breast-feeding cessation by the two groups are supported by the literature (Figure 3).

Our findings are indicated that 92.7 % of Egyptian mothers and 89.3% of Saudi mothers are practice the breastfeeding for their infants after delivery to some point of time. In spite of this, 57.9 % of Saudi mothers introduce Herbs and/or artificial feeding to their babies early during the first six months compared to only 25.4% of Egyptian mothers. Those findings are in quite consistent with report of another study carried out by El-Mouzan *et al.* in 2009 in Saudi Arabia. El-Mouzan *et al.* reported high prevalence of breastfeeding initiation at birth; which indicates the willingness of Saudi mothers to breastfeed. However, early introduction of complementary feedings reduced the period of exclusive breastfeeding.

Conclusion

There is a need for upgrading knowledge for Saudi mothers regarding breastfeeding. Research in infant feeding should be a health priority to improve the rate of breastfeeding and to minimize other inappropriate practices. Most of the Egyptian and Saudi participants who ceased breastfeeding attributed this to return to work; consequently governmental policies regarding longer leave for new mothers and child care centers inside large institutions should be considered.

Ethical considerations

The study was approved by the Ethics Committee of Scientific Research (ECSR), Qassim University. Eligible Nongovernmental Organizations (NGOs) were given an information letter explaining the aims of the study assuring them of anonymity of the collected data and written approval were obtained from the director of each NGO. Written informed consent was obtained from all participants who were advised that they could withdraw from the study without having to provide justification. The confidentiality of the data and the privacy of mothers were respected at all times.

Limitations of the study

Study had some limitations. First, these findings were generated from one region of Saudi Arabia, and may not be generalisable to other region or cities. Second, some obstacles faced the researchers during carrying out the study; the most obvious was the dropouts of 55 mothers from the study sample, where they were excluded due to incomplete data and refused to participate in the study.

Declaration of Conflicting Interests

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Effect of High Percentage of Sodium Chloride (NaCl) on the Behavior of Reinforced Concrete BeamsS. Abd El- salam m. of ASCE¹, H. Shehab Eldin², E.A. El-Shamy³ and Sh. M.M. Shawky⁴

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Abstract: In this paper an experimental investigation is carried out to study the effect of high percentages of sodium chloride (NaCl) on the structural behavior of R.C. beams. The experimental study contained thirteen of R.C. beams with fixed steel reinforcement and a common concrete mix by adding different high percentages of salts by weight of cement (S/C) to the mixing water of concrete mix. The beams were tested up to failure, and the influence of variable factors on the structural deformations such as, failure loads, strains, cracking behavior and modes of failure were reported. Also, the mass' loss of steel reinforcement bars was studied.

[S. Abd El- salam , H. Shehab Eldin, E.A. El-Shamy and Sh. M.M. Shawky. **Effect of High Percentage of Sodium Chloride (NaCl) on the Behavior of Reinforced Concrete Beams.** *Life Sci J* 2012; 9(3):315-320]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 43

Keywords: Experimental method, corrosion, sodium chloride, R.C. structures.

1. Introduction

The problem of deterioration of concrete structures due to corrosion of steel reinforcement has received worldwide of attention. Even though current codes of practice provide recommendations and precautions to avoid corrosion, evidence of corrosion of steel in concrete continues to be reported in the field situations (Leema *et al.*, 2009). Chloride may be presented in the concrete from several sources. Soluble chlorides may be introduced in the fresh concrete by the use of aggregates contaminated by chlorides, saline water when used as mixing water. In addition, some cement may contain amounts of chlorides. Chlorides may be also inter the hardened concrete from the surrounding environments, such as sea-water, soil and industrial zones (Mohamed *et al.*, 1995). The chloride ions in concrete are considered to be the major cause of corrosion (Mustafa *et al.*, 2007). Generally after casting concrete, a passivity film is formed surrounding the steel bars and protects them from corrosion initiation. Chloride ions depassivate the protective film, and the embedded steel bars in concrete are no longer protected against corrosion in the presence of moisture and oxygen. The corrosion products are expansive in nature and effectively cause a tensile stresses around the reinforcing steel.

Once sufficient corrosion has occurred, splitting cracks typically develop and loss of bond is observed (Transportation, 2006). Moreover, the cross section of reinforcing bar is decreasing, thus reducing the load-carrying capacity of the concrete member.

Thus, in general the use of steel in salty concrete is not recommended and this is probably the most serious disadvantage if the material is to be used for structural applications. The development of reliable methods for predicting chloride affect into concrete is very important to determine the service life of a reinforced concrete structure. A number of studies have

been carried out to understand the effect of different percentage of chloride ions as 0.1% to 3.5% of weight of concrete mix (Mohamed *et al.*, 1995, Atia *et al.*, 1996, , Abdel Salam *et al.*, 1998 & 2000, , Tarek *et al.*, 2001, Jon *et al.*, 2001, Li *et al.*, 2001 & 2002, T.R.B., 2006, , Mustafa *et al.*, 2007 and Leema *et al.*, 2009). Other researchers studied the behavior of reinforced concrete structure at percentage of sodium chloride up to 5% of weight of concrete mix (Abdel Salam *et al.*, 1998 & 2000, Nhan *et al.*, 2006 and Kutarba, 2007).

The main objective of this study was to quantify how high percentage of (NaCl) on the concrete mix of R.C beams by loading influences on the R.C beam behavior.

2. Experimental program

The test program consisted of three groups of reinforced concrete rectangular beams with fixed reinforcement and concrete constituents when exposed to four percentages of sodium chloride (NaCl) by weight of cement(S/C). In addition different corrosion time periods were considered. Details of the tested beams and concrete mix were given in Tables 1 &2.

The beams were tested to failure and the influence of variable factors on the structural deformation, initial cracked-load, steel and concrete strains and mode of failure were reported.

Details and testing of beams

Each rectangular beam was 100 mm * 250 mm in cross section and 1600 mm long. The internal longitudinal high grade steel reinforcement consisted of 2 ϕ 12 mm at the bottom and 2 ϕ 10 mm bars at the top of the beam as shown in Figure 1. Three different batches of concrete mixtures with different percentage of sodium chloride (NaCl) by weight of cement(S/C) of 0%, 1%, 5% and 10% were used for the construction of the three groups of beams as shown in Table 1. After 28 wet and dry days, the specimens were left in natural

weather out side the laboratory without any curing. Groups (A, B & C) of concrete beams were tested to failure after three time periods of 6, 12 & 18 months, respectively.

Structural testing

Once beams reached the target ageing (6, 12 or 18 months) they were tested to failure. Beams were test with load applied symmetrically at two points at mid-span through a spreader beam load application points near the center of the specimen were spaced 500 mm apart load was measured with load cell placed between the spreader beam and hydraulic cylinder.

Measurements

Displacement was measured at mid-span of the beam and underlying load section as shown in Photo 1.

Concrete strain was measured using electrical strain-gage located at the compressive face at mid-span between the load points. Electrical strain gage was pasted at mid-span on the longitudinal main steel on one side of the beam face. The test setup and its instrumentation placement are shown in Figure 1 & Photo 1. After verification of data collection, the load was increased monotonically until failure, loading was suspended at 2 KN intervals to mark and measure cracks. For each group of spacemen, percentage of steel mass-loss was calculated by weighting 20 cm long of each steel reinforcement bar after corrosion and divided it by the weight of the same part of steel bar of control spacemen at (S/C)=0% .

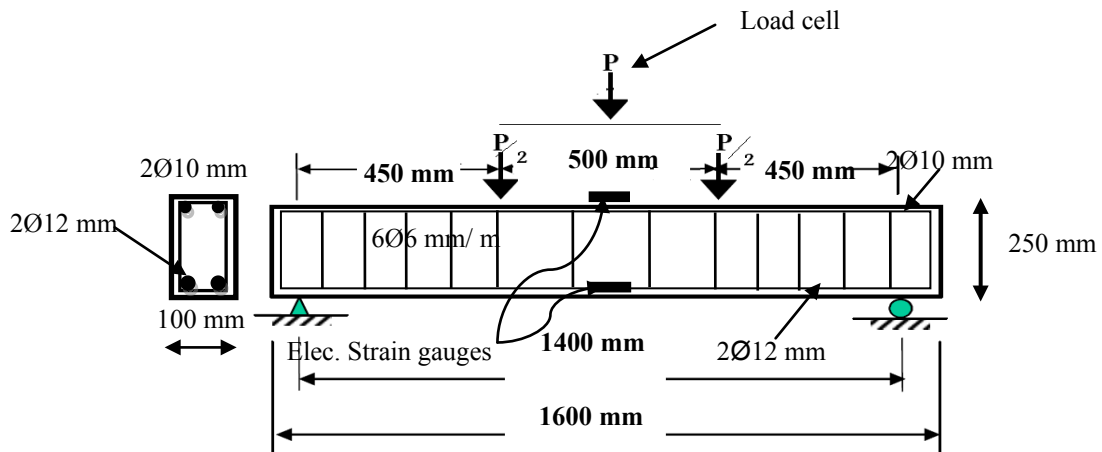


Figure (1): Set up details.



Photo (1): Test setup

3. Results and Discussion

The main objective of this series of testes was to establish the effect of different high percentages of sodium chloride(S/C) and ageing time periods on the structural behavior of R.C. beams. From the data obtained during the experimental program, results are presented here.

Figures 2&3 show that both percentage of salt(S/C) and time period had a significant effect on the load capacity of tested beams; which decreases about 40% than in case of (S/C)=0% as the (S/C) and the time period increase specially at (S/C)=10% and time period =18 months as shown in Figure 3; on the other hand, first cracked load (p_{cr}) and yield load (p_y) showed a continuous increase as the (S/C) increases up to 10% in group (A) of specimens, may attributed to formation crystals which fills the concrete pores leading to a reduction in the porosity of the concrete and hence increasing the compressive strength of the concrete.

Typical load deflection curves for tested beam are shown in Figures 4&5 for different ageing and percentage of salt(S/C). It can be seen that the deflection was always increases with increasing the time periods in average equal to 25% from group to other duo to a continuous reduction in the stiffness and the flexural rigidity of tested beams with ageing as shown in Figure 4. However, Figure 5 shows strength of tested beams increases up to 10% at (S/C)=1%& 5%

than in their strength at (S/C)=0%, respectively. And the beams strength decreases about 20% at (S/C)=10% due to the reduction of steal section, pitting corrosion and effect of corrosion on the mechanical properties of steel bars. As well as degradation of the steel- concrete bond with ageing.

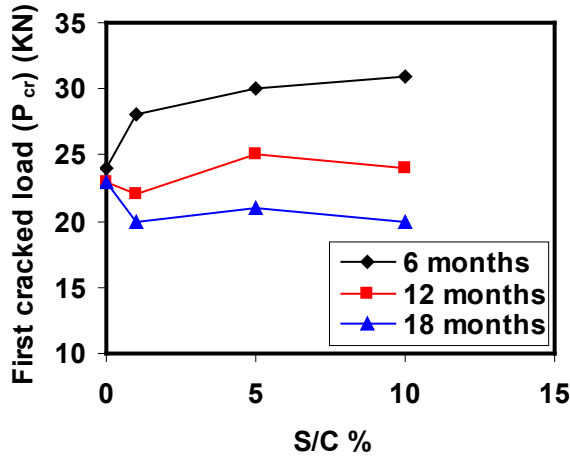
Figures 6 shows the load-strain curves of concrete and steel reinforcement for group C. It is indicated that the rate of strain increases for steel, through the whole range of loading, was higher than that for concrete, although varying degrees. This may be attributed to the interaction between the load level, the reduction of the effective bond strength and the crake propagation. Group C of tested beams showed extensive cracking, large deflection, neutral axis movement towards the compression edge and plenty of warning prior to flexural followed by compression failure. While the beams with (S/C)=10% showed greater neutral axis depth at failure because of wider cracking before and after loading and greater deflection as shown in photo 2. The effect of percentage of salt (S/C) on percentage of steel mass-loss due to corrosion for each group of specimens is shown in Figure 7. In general, it is indicated that, the percentage of steel mass-loss increases as the percentage of salt (S/C) and the time period increases especially at (S/C)=10% at 18 months time period.

Table (1): Details of the test program

Group No.	Specimen No.	% NaCl of Cement Weight(s/c)	Corrosion time period
Control	C. B. 1	0	28 days
A	C. B. 2	0	6 months
	X. B. 1	1	
	X. B. 4	5	
	X. B. 7	10	
B	C. B. 3	0	12 months
	X. B. 2	1	
	X. B. 5	5	
	X. B. 8	10	
C	C. B. 4	0	18 months
	X. B. 3	1	
	X. B. 6	5	
	X. B. 9	10	

Table (2): Concrete Mix.

Composition	kg/m ³ of concrete
Coarse aggregate (20 mm)	1250
Washed and dried fine aggregate	625
Cement	350
Water	162.5



Figure(2): Effect of percentage of S/C on first cracked-load at different time periods.

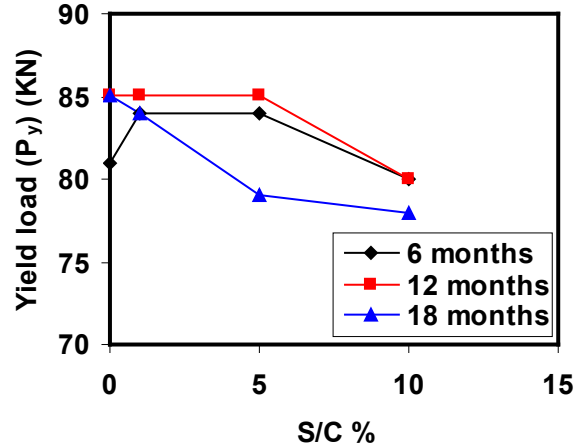


Figure (3): Effect of percentage of S/C on Yield- load at different time periods.

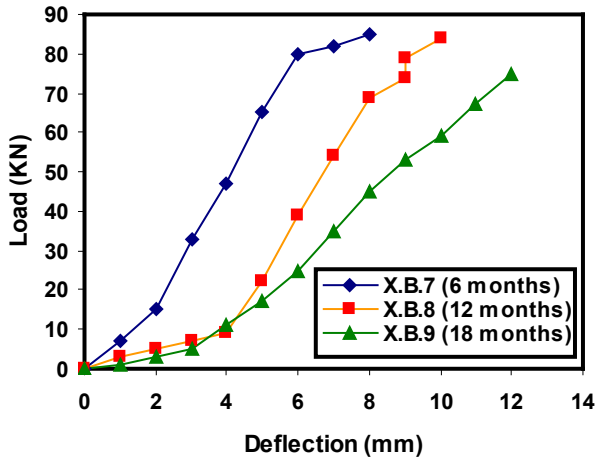


Figure (4): Effect of corrosion time period on load-Deflection relation at mid-span at s/c=10%.

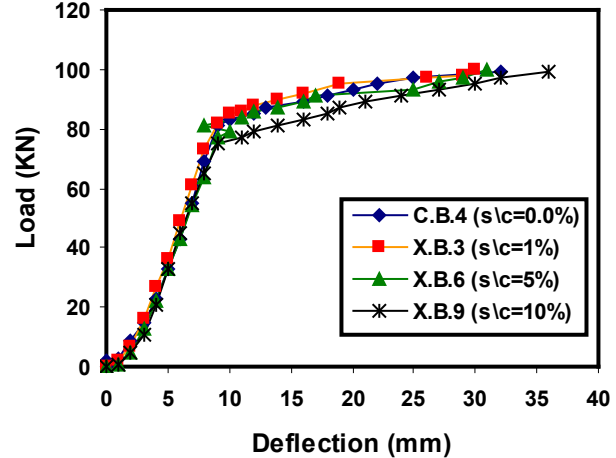


Figure (5): Effect of percentage of salt (s/c) on load-deflection relation at load-section at 18 months time period.

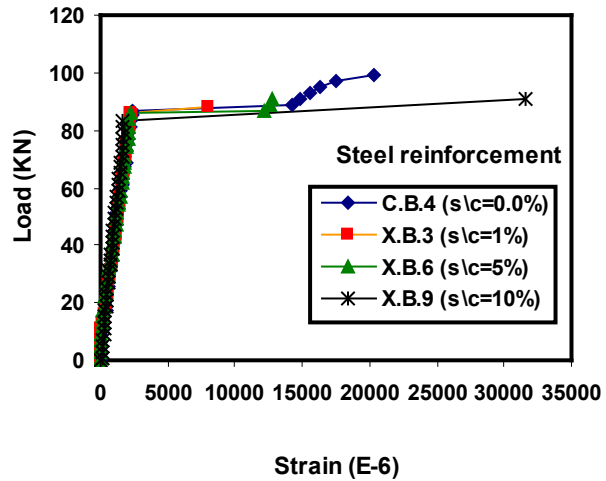
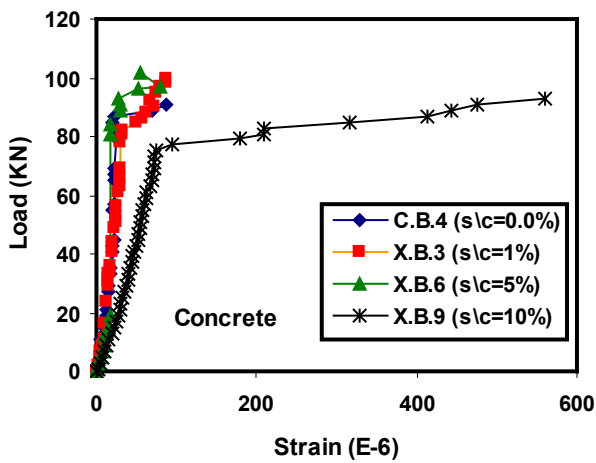


Figure (6): Effect of percentage of salt (s/c) on load-strain relation on concrete and steel reinforcement at 18 months time periods.

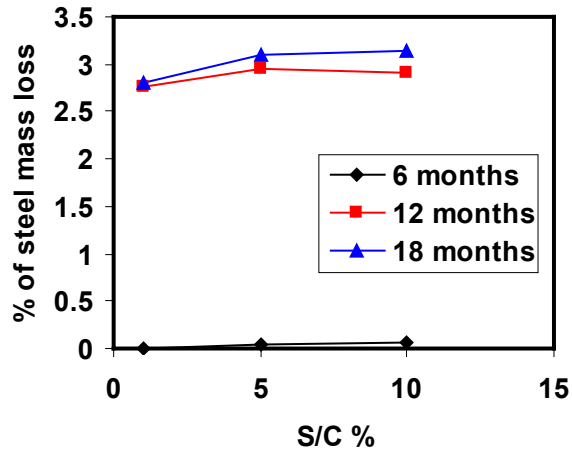


Figure (7): Effect of percentage of (s/c) on the percentage of steel mass loss.



Photo 2. Effect of salt-percentage (s/c) on failure-mode of tested beams at 18 months age.

Conclusion

Based on the test results and discussion presented in this paper, the following conclusions are drawn on the structural behavior of R.C. beams with high percent of sodium chloride (NaCl).

Flexural rigidity of beams with different percentage of sodium chloride at 6months age showed marginal increases compared with (S/C)=0% control beam ,however tested beams with (S/C)=1%and 5% at 18 months age maintaining their stiffness. With increased age, the corrosion is gradual and slow until the stability of steel reinforcement affected, both loss of stiffness and strength develop rapidly at an accelerated rate especially at (S/C)=10%.Cracking behavior of beams at (s/c)=1% at age equal 18 months tend to be concentrated in the central part (pure bending zone) of the beam and they tended to form at closer space, on the other hand, the cracks of beams at (S/C)= 5% and 10% of sodium chloride were more and distributed over the central part and followed with inclined cracks outside the pure bending. The 10% percent of sodium chloride showed a significant effect on the loss of structural integrity and stiffness of tested beam. The percentage loss is about 30% at 18 months age.

Acknowledgement

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Corresponding author**5. References**

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A Smart Algorithm for QoS Support in Ad hoc Networks

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Abstract: Real-time traffic support is one of the interesting Quality of Service (QoS) issues in Ad hoc networks. The wireless ad hoc networks are severely affected by bandwidth, so, supporting QoS in these networks face problems. In this paper, we have proposed a fully distributed MAC algorithm to support the QoS in ad hoc networks. This algorithm provides delay fairness for real-time flows and services these flows considering current QoS of them. This algorithm could be used in emergency and hospital environments. By simulation, we have shown that our mechanism achieves delay fairness, and functions adequately to support real-time traffic in practical environments where real-time traffic and non-real-time traffic coexist in an identical wireless Ad hoc networks.

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Keywords: Ad hoc, Quality of Service, Differential Service, Back-off mechanism.

1. Introduction

An ad-hoc network is a local area network (LAN) that is built spontaneously as devices connect. Instead of relying on a base station to coordinate the flow of messages to each node in the network, the individual network nodes forward packets to and from each other. Considering the comfortably establishing of ad hoc networks, the use of this type of network is increasing day to day. So, real-time applications will be popular in these networks. Therefore, supporting real-time traffic in wireless LANs (Local Area Network) is an interesting QoS issue. To support real-time traffic in wireless LANs, several algorithms have been studied [1, 2, 3]. All of them use Point Coordination Function (PCF) of IEEE802.11 [4] to support real-time traffic. These mechanisms can provide bounded delay service, but, considering that they could not be used in distributed mode, so, they could not be used in ad hoc mode. On the other hand, for supporting real-time traffic in ad hoc mode, a distributed control mechanism is required. To give the finite transmission opportunities to flows having various features, distributed control mechanisms provide fairness for flows. Take into account that real-time applications are sensitive to delay rather than throughput, we believe that fairness provided for real-time flows should be delay fairness.

For supporting real-time traffic in ad hoc networks, many distributed control mechanisms have been proposed [5, 6, 7]. Although these mechanisms provide higher-priority service for real-time traffic by differentiating real-time traffic from non-real-time traffic they can provide neither delay fairness. In this paper, a fully distributed MAC mechanism is proposed for supporting the QoS of the flows in ad hoc networks that provides delay. To achieve delay

fairness, we introduce a back-off algorithm that controls back-off time based on waiting-time, the time a frame has experienced since it was entered to the link interface. This algorithm is preferred to others because it classifies the flows in the network without overhead and any control packet.

The rest of this paper is organized as following. Section 2 describes the DCF method of IEEE 802.11, Section 3 concerns with the QoS frameworks, algorithms and works done to classify the flows and support QoS of flows. In Section 4, by simulation, we confirm that our mechanism functions adequately for supporting real-time traffic in wireless Ad hoc networks, and Section 5 is associated with conclusion.

2. IEEE802.11 Standard-DCF

IEEE802.11 standard describes MAC layer and physical layer specifications for IEEE802.11 wireless LAN [4]. Two access control methods are defined in the MAC layer specifications. One is DCF (Distributed Coordination Function), and the other is PCF (Point Coordination Function). In the following we describe the DCF method (PCF method usable only in infrastructure mode of IEEE 802.11).

Distributed Coordination Function (DCF) is the fundamental MAC technique of the IEEE 802.11 based WLAN standard. DCF employs a Carrier Sense Multiple Access with Collision Avoidance (CSMA/CA) with Binary Exponential Back-off (BEB) algorithm. In DCF, if a station has frames to be transmitted, a station creates back-off time of the frame, which is used to resolve a contention. Back-off time is calculated using a back-off algorithm. In BEB, back-off time is calculated using the following expressions:

$$CW = (CW_{\min} + 1)2^{RC} - 1 \quad (1)$$

$$CW = \min(CW, CW_{\max}) \quad (2)$$

$$B = \lfloor CW * rand() \rfloor * Slot_Time \quad (3)$$

Where CW is contention window, RC is retransmission count of a frame, which RC=0 when the frame is to be transmitted at the first time and RC=n when the frame is to be retransmitted at the n-th time, CW_{\min} is the minimum value of CW, which is equal to CW when RC=0, CW_{\max} is the maximum value of CW, min(a,b) is the function that returns the smaller number of a and b, rand() is a function that returns a value chose n randomly from the interval from 0 to 1, B is back-off time. If the station, for a time interval greater than a DIFS, determines the medium in idle state, decrements the Back-off time. When the back-off time reaches to 0, the station transmits a frame. After transmitting the frame, if the node receives the ACK for the transmitted frame, the station resets RC and repeats the above procedures for the next frame. If the node does not receive the ACK, it repeats the above procedures for the same frame with incremented RC.

3. QoS Framework

3.1. Find_Fix_Routers for real-time traffics

When a node wants to send a real-time flow, it must, first of all, call for Find_Fix_Router process in order to find a valid path. By a valid path, it is meant a path which is composed of fixed nodes and/or lea mobile nodes and provides for the QoS of the desirable flow.

Find_Fix_Router process based on the modified AODV routing protocol. The modified protocol reflect the selection of stationary routes for real-time traffics. When a source node initiates route discovery for real-time traffic with strict quality requirements, only the fixed routers respond to the control packets by either forwarding the RREQ, or unicasting a RREP. The mobile nodes do not respond to these packets, unless they are the destination.

Find_Fix_Router also enables effective admission control when the network utilization is saturated. This requires accurate estimation of channel utilization and prediction of flow quality, i.e., throughput or transmission delay. The proposed QoS approach is based on model-based resource estimation mechanism, called MBRP[17]. By modeling the node back-off behavior of the MAC protocol and analyzing the channel utilization, MBRP provides both per-flow and aggregated system wide throughput and delay [16].

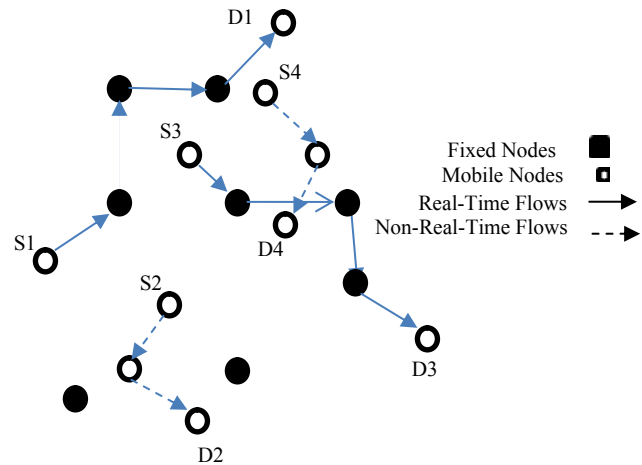


Figure 1: Different paths for different flows

3.2. Distributed MAC for Real-time Flows

In Ad hoc networks, the priority scheduling algorithms are based on IEEE 802.11 [8]. Currently, there are some algorithms that differentiate between flows through assigning various minimum contention window sizes (CW_{\min}), Arbitrary Inter Frame Spacing (AIFS), or back-off ratios, but, these differentiations are static. To achieve service differentiation, as well as to adapt to the current usage of network, we combine the collision rate and current QoS of flows with the exponential back-off mechanism in IEEE802.11. To do it, classifies flows to Real-Time flows and Best-effort flows. Upon receiving the first packet from the flow related to one of those classes, each node, locally builds a queue for that flow. Then it inserts this packet and subsequent packets related to that flow in this queue. It is noted that contrary to real-time flows where a separate queue is built in every node for each flow, only a queue is built for all best-effort flows in every node. Figure 2 shows the queues built in each node to manage various flows. We will use these queues in managing flows and calculating contention window of them.

3.3. Mechanisms for Supporting QoS of Flows

A distributed MAC mechanism supporting real-time traffic is desired to coexistence with a MAC mechanism for non-real-time traffic. Because real-time traffic and non-real-time traffic coexists in a practical wireless LAN, a MAC mechanism of wireless LANs is required to support both real-time traffic and non-real-time traffic. Now, DCF of IEEE802.11 is the most widely used wireless LAN protocol and is the standard for non-real-time traffic. Therefore, a MAC mechanism for real-time traffic is required to coexist with a MAC mechanism for non-

real-time traffic such as DCF.

To meet this purpose, we propose a new MAC mechanism. DCF cannot support delay fairness, because BEB (Binary Exponential Back-off), the back-off algorithm have been used in DCF, decides back-off time of a frame regardless of how long the frame is kept waiting to be transmitted. Then, to achieve delay fairness, we introduce “waiting-time” defined as the time a frame has experienced since it was entered to the link, and we give smaller back-off time to a frame having larger waiting-time.

The Real-time flows, such as conversational audio/video conferencing, require that packets arrive at the destination within a certain delay bound ("Request-Time). The Best-effort flows, such as file transfer, can adapt to changes in bandwidth and delay. Due to the different requirements of flows, each types of flow have its own contention window adaptation rule, as follows:

A) Best Effort Flows:

Best effort flows are tolerant to changes in service levels and do not have any hard requirements about bandwidth or packet delay. The purpose of updating the back-off size of best effort flows is to prevent best effort flows from congesting the network and degrading the service level of real-time flows and this is done by controlling the network congestion.

$$CW^{(n+1)} = CW^{(n)} \times (1 + \gamma(f - F^{(n)})) \quad (4)$$

In header of real-time packets we have added two fields entitled "Create-Time" and "Waiting-Time". The Create-Time field has been shown how many time elapsed as the birth time of a packet. Also, the waiting-time field has been shown the elapsed time of a packet in a node. In proposed algorithm, we have tried service to packets that have the largest waiting-time. On the other hand, if the value of "Create-Time" will be greater than the value of "Request-Time", then this packet will be rejected, because the time to live of this packet is finished. The "Request-Time" shows the acceptable value for service of real-time packets. A Real-Time flow adopts the back-off algorithm by the following expressions:

$$CW = (CW_{\min} + 1)2^{RC} - 1 \quad (5)$$

$$CW = \min(CW, CW_{\max}) \quad (6)$$

$$B = \lfloor CW * rand() \rfloor \quad (7)$$

$$B = \lfloor B * K / t \rfloor \quad (8)$$

$$B = \max(B, B_{\min}) \quad (9)$$

$$B = \min(B, B_{\max}) \quad (10)$$

Where t is waiting-time, K is a constant value of which is the same in any station.

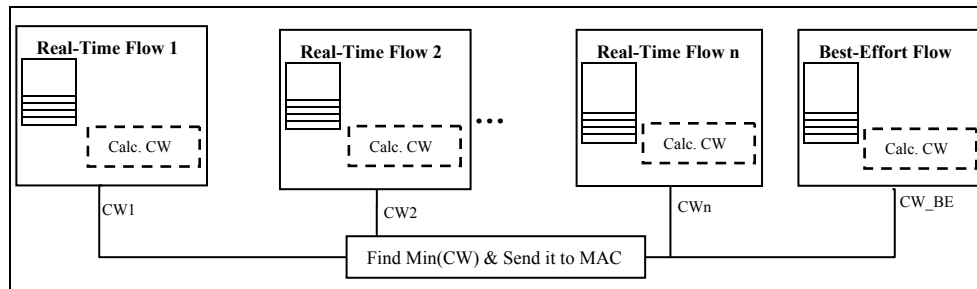


Figure 2: Queues in each node (Network Layer)

When the average idle channel time F is smaller than the threshold value f , the network is considered congested and the contention window size of the best effort traffic is increased to avoid decreasing the service level of real-time traffic. On the other hand, if the network is lightly loaded so that the idle channel time is larger than f , the contention window size of best effort traffic is decreased so that the idle bandwidth can be utilized.

B) Real-Time Flows:

This back-off calculation algorithm is composed of 2 steps. In the first step, the BEB procedure decides B according to the expressions (5), (6) and (7). (5), (6) and (7) are equivalent to (1), (2) and (3), respectively. We utilize the contention resolution functionality of BEB in this step. In the second step, B is adjusted according to the expressions (8), (9) and (10). This step decides B to be inversely proportional to t, whereby a frame of larger waiting time has higher transmission priority.

Expressions (9) and (10) limit B in the range from B_{min} to B_{max} .

The pseudo-code of Receive and Send functions are as follows:

Receive Sensitive Packet (P as packet)

```
{
  If (Create_Time(p)>ReT(p)) then
    Reject (P)
  Static Waiting_Time=0;
  Waiting_Time++;
}
```

Send Sensitive Packet (P as packet)

```
{
  Create_Time(p) = Create_Time
  (p)+Waiting_Time(p);
}
```

The pseudo-code of back-off computation is as follows:

Back-off_Time()

```
{
  If (TypeOf(Flow)='Real-Time') then
     $CW = (CW_{min} + 1)2^{RC} - 1$ 
     $CW = \min(CW, CW_{max})$ 
     $Back-off = \lfloor CW * rand() \rfloor$ 
     $Back-off = \lfloor Back-off * K / t \rfloor$ 
     $Back-off = \max(Back-off, Back-off_{min})$ 
     $Back-off = \min(Back-off, Back-off_{max})$ 
  Else If (TypeOf(Flow)='non-Real-Time') then
     $CW^{(n+1)} = CW^{(n)} \times (1 + \gamma(f - F^{(n)}))$ 
     $Back-off = Rand[0, (2^r + R_{col}) * CW * Slot\_Time]$ 
}
```

Where R_{col} denotes the collision rate between a station's two successful frame transmissions and r is a positive number.

By the Back-off-Time(), all flows dynamically manage their contention parameters to meet their own QoS needs. A real-time flow that did not get its required QoS in the past due to competition from other flows try to lower back-off and so, give media as soon as possible. A best effort flow, on the other hand, increases its contention window size when the network is considered busy and hence releases the channel to the real-time flows (Eq.(4)). More importantly with attention to flow's current status, traffics with same class will have different back-off

value when collisions occur. Specifically, after a collision occurs, low priority traffic will back-off for longer, and subsequently high priority traffic will have a better chance of accessing the channel. Contrary to [9], [10], in our proposed algorithm, no piggy-backed schedule information and neighborhood scheduling tables are needed. Therefore, there is no control message overhead imposed by our proposed algorithm.

In next section, the simulation of the proposed algorithm is proved.

4. Simulation

In this section we evaluate the proposed MAC mechanism by simulation. The simulation is done in ns-2 and network size is 1000m*1000m. In the simulation environment n senders transmit frames destined for an identical receiver at constant bit rate. The configurations are shown in Table 1.

In this paper, we use fairness index [11] to evaluate accuracy of delay fairness and differentiation. Fairness index is defined by the:

$$fairness\ index = \frac{(\sum_{i=1}^n d_i / w_i)^2}{n \sum_{i=1}^n (d_i / w_i)^2}$$

Where n is the number of senders, d_i is the delay of sender i, and w_i is the weight of sender i. Fairness index is greater than 0, and less than or equal to 1. It approaches to 1 as $d_1 / w_1, d_2 / w_2, \dots, d_n / w_n$ are becoming the same.

Table I: Configuration

Sender	CW_{min}	CW_{max}	K	B_{min}
1-n	31	1023	0.0005	1
B_{max}	W	IFS	Queue length	Frame Size
1023	1	DIFS	16 KBytes	1024 byte

We evaluate the proposed MAC mainly by comparing the simulation results of the proposed MAC with those of DCF. In the configuration (Table I), n senders all implement the proposed MAC mechanism and have the parameters in the table. For the purpose of the comparison of the proposed MAC and DCF, we have all the senders implement DCF too. In the case, they have the parameters in the table except K, B_{min} , B_{max} .

Figure 3 shows simulation results in the case where the n sender all implement the proposed MAC or in the case where the n senders all implement DCF.

The lines of "min" present delays of the sender having the smallest delay of the n senders, the lines of "max" present delays of the sender having the largest delay of the n senders, and the lines of "ave" present the average values of the delays of the n senders. Both in the proposed mechanism and in DCF, if $n \leq 6$, all the senders experience little delay because the medium load is light. On the other hand, if $n \geq 7$, the average delay of the n senders increases as n grows larger. Additionally, there are two features when $n \geq 7$. One is that the average delay in the proposed mechanism is smaller than that in DCF. The other is that the difference between the maximum delay and the minimum delay in the proposed mechanism is much smaller than that in DCF. In other words, the proposed MAC is a better mechanism to achieve low delay and delay fairness than DCF. This advantage is derived from our original procedure (8), where larger waiting-time leads to smaller back-off time.

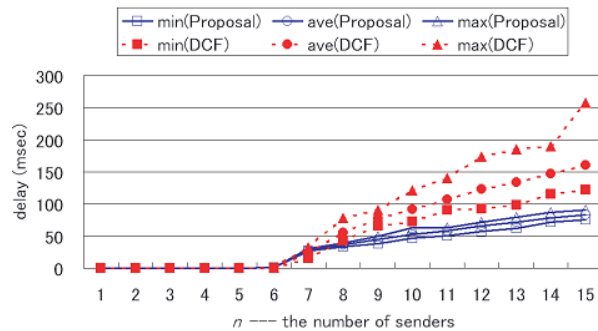


Figure 3: Delay of Flows

5. Discussions

In this paper we introduce a new distributed MAC protocol that supports QoS of flows in ad hoc networks, which this protocol is simple, fully distributed and use no control packets. An important benefit of this protocol is that it does not need resource reservation and therefore, it does not have the problems related to the use of in-bound and out-bound signals to reserve and free the resources, and the network bandwidth is not occupied by reserving and freeing the resources.

In the future, we will investigate the effect of different values t, f, r on the throughput and delay related to different classes.

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Hypoxia and pyruvate/uridine have synergic effect on induction of stemness factors in human esophageal cancer cells

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Abstract: The present study was to examine our hypothesis that hypoxia and pyruvate/and uridine may have synergic effect on the induction of stemness factors in cancer. Esophageal cancer cell lines KYSE450 and KYSE70 were cultivated under different oxygen tensions with/without pyruvate and uridine addition in medium. In comparison to the cells cultivated in 20% O₂ tension, the cells cultivated in 7% O₂ and 1% O₂ showed higher levels of Oct3/4 and SOX2, which were in parallel with increasing HIF-1 α , HIF-2 α . A stronger induction of these gene expression could be seen in either pyruvate or uridine treatment under hypoxic condition. The strongest induction of the expression of these genes was repeatedly shown under hypoxia with both chemicals. Although the expressions of stemness factors Oct3/4 and SOX2 were higher in hypoxia than that in normoxia, the cells colony formation ability was reduced in hypoxia. However, addition of pyruvate and uridine in the medium, the cells in hypoxia not only showed highest levels of the stemness genes expression, but also high colony formation capability with highest number of colonies. We conclude that hypoxia and pyruvate/uridine synergistically induce the expression of stemness genes and increase the colony formation capability of esophageal cancer cells *in vitro*, indicating that esophageal cancer cells stemness can be upregulated *in vitro* for potential cancer stem cell targeting studies.

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Keywords: Hypoxia; HIF; esophageal cancer; Oct3/4; SOX2; pyruvate; uridine

1. Introduction

Several solid tumors including esophageal cancer are considered to contain a small subset of stem-like cells called cancer stem cells, which have the capacity to initiate new tumor. The cancer stem cells particularly have been demonstrated to escape from the radiotherapy and chemotherapy and are able to form metastatic tumor in other organs (Crea et al., 2009; Maitland and Collins, 2008). Investigations on how tumour cells change their stemness are currently demanded.

Low oxygen levels (hypoxia) have been shown to upregulate stemness factors in different types of cells. It is well documented that oxygen plays an important role in development in tissues and cells and hypoxia often occurs in physiological and pathophysiological conditions, especially when rapid growth exceeds the blood supply. For embryonic stem cells, hypoxia helps to maintain cells in stably high stemness and higher levels of oxygen tensions promote cells into differentiation (Forristal et al., 2010; Ma et al., 2009). Similar result has been observed in adult cells like adipocytes, fibroblasts

and cancer cells (Kim et al., 2009a; Kim et al., 2009b; Malladi et al., 2007).

The hypoxia inducible factors (HIFs) are the key regulators found in mammalian cells cultured under reduced oxygen tension and play an essential role in cellular and systemic homeostatic responses to hypoxia. HIFs are heterodimers, composed of an alpha subunit and a beta subunit including HIF-1 α and HIF-2 α which are the major isoform of the α -subunit and share a high degree of sequence identity. Increasing evidence indicates that HIFs regulate a number of genes including glucose metabolism, cell survival, erythropoiesis, stem cell maintenance, angiogenesis related markers and resistance to chemotherapy and radiation therapy (Heddlestone et al., 2010).

Oct3/4, also called POU5F1, and SOX2 are known embryonic stem cell markers which are important transcription factors in maintaining the self-renewal of embryonic stem cells and primordial germ cells. They are not only detected in germ cell tumor, but also in different somatic tumors such as lung, gastric, colorectal, rectal, bladder, breast,

ovarian, and esophageal cancers (Ben-Porath et al., 2008; Meng et al., 2010; Nirasawa et al., 2009; Peng et al., 2010; Saigusa et al., 2009; Sotomayor et al., 2009). Comparatively, the expression of these genes is downregulated in all differentiated somatic cell types *in vitro* as well as *in vivo* (Sperger et al., 2003).

Mitochondria are powerhouses of cells and consume up to 90% of inhaled oxygen. However, mitochondria DNA (mtDNA) can be depleted *in vitro* by different methodologies and the mtDNA depleted cells (ρ^0 cells) share at least one major feature in common with tumor cells: ATP production is mainly through glycolysis, not through oxygen respiratory chain, because tumor cells, most probably tumour stem cells as well, usually locate in areas with low oxygen niche. It is well proved that the ρ^0 cells become pyruvate and uridine dependent for survival (King and Attardi, 1989; Liu et al., 2009; Olgun and Akman, 2007), indicating that these chemicals play a vital role in maintaining cells in live when cells are forced to obtain energy through glycolysis.

The common issue for cells cultivated in hypoxia condition and tumour cells *in vivo* is their niche of low oxygen tension. Under this condition cells are forced to synthesize ATP via glycolysis in cytoplasm which exhausts less oxygen. To survive better under this hypoxic condition, cells in cultivation may need different culture medium as shown for the ρ^0 cells. We, therefore, hypothesize that pyruvate and uridine addition in culture medium for tumor cells under hypoxic condition may favor their physiological growth and maintain these cells in rather "healthy" status with higher expression of stemness factors.

Therefore, in this study we firstly asked whether hypoxia could influence the expressions of the stemness factors Oct3/4 and SOX2 in the esophageal cancer cell lines KYSE70 and KYSE450. The cells were cultivated under 1%, 7% and 20% oxygen tensions, respectively, and the expressions of these factors and the HIF-1 α and HIF-2 α were examined by RT-PCR and Western blotting. We did observe that the expressions of Oct3/4 and SOX2 were significantly increased at lower O₂ tensions. We further asked whether pyruvate and uridine had similar effect on these cells and whether synergic effect of hypoxia and these chemicals could be identified in these cells. Our results clearly showed that pyruvate and uridine did have synergistic effect on the expressions of Oct3/4, SOX2, HIF-1 α and HIF-2 α , and the cells' colony formation capability under this condition was also greatly improved, indicating that the combinational application of low oxygen tension and pyruvate and uridine in esophageal cancer cells cultivation may help to propagate and maintain tumor cells with greater

stemness, which may be of importance in cancer stem cell targeting studies.

2. Material and Methods

Cell lines and conventional cell culture

Human esophageal cancer cell lines KYSE70 and KYSE450 were purchased from ATCC (American Type Culture Collection, USA) and maintained in our lab for this study. For conventional cell culture, 2×10^5 cells were seeded in 75 cm² culture flasks and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 100 units/ml penicillin and 100 μ g/ml streptomycin in a humidified 5% CO₂ incubator at 37°C.

Hypoxic cell culture

After 24 hours cultivation in conventional cell culture (allowing cells to attach onto the flasks), the cells were transferred into different incubators with different oxygen tensions. The Xvivo Closed Incubation System (Xvivo system 300C, BioSpherix, New York, USA) was used in this study to obtain accurate different oxygen tensions in different incubators. The cells were simultaneously cultivated in three incubators with 1%, 7% and 20% oxygen tensions, respectively, for variable periods of time before being harvested for additional RT-PCR and Western blotting analyses.

Pyruvate and uridine treatment

Pyruvate (sodium pyruvate) and uridine were purchased from Sigma (Sigma-Aldrich, USA). For cell culture treatment, the concentrations of pyruvate and uridine were determined solely based on our previous experience on mtDNA depleted cell experiment (Liu, Geng, and Suo, 2009). The cells were treated with 100 μ g/ml pyruvate and 50 μ g/ml uridine, respectively or in combination for 24 hours at 1% O₂ condition. Then the cells were collected for further RT-PCR and Western blotting.

MTT assay (Growth curve)

The KYSE450 and KYSE70 cells were firstly seeded at a density of 2×10^3 /ml (180 μ l/well) into 96-well microplates with complete RPMI-1640 medium and placed into incubators with either 1% O₂, 7% O₂ or 20% O₂ conditions for variable time periods for MTT analyses using the Countess Cell Counter (Electronics Countess automated Cell counter, Invitrogen, USA). After the cells in culture reached their time schedule, the 5 mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) were added and incubated at 37 °C for 4 hrs before 150 μ L of dimethyl sulfoxide (DMSO) (Sigma-Aldrich) was added to each well and mixed thoroughly. The plates were shaken for 15 min and absorbance was determined using a spectrophotometer at a

wavelength of 490 nm (μ Quant; Bio-Tek Instruments, Winooski, VT).

RT-PCR procedure

Total RNA was extracted from the cultivated cells using RNeasy Kit (Qiagen, CA, USA) according to the manufacturer's instruction. Dnase I was used in the RNA isolation procedure, in order to eliminate any DNA. The concentrations of RNA samples were quantified using a spectrophotometer (Nanodrop ND-1000, USA) at OD260/280. The RNAs were then reverse-transcribed using the Multiscribe reverse transcriptase (Applied Biosystems, Foster City, CA) according to the manufacturer's instruction. The condition for the reverse transcription was 25°C for 10min, 37°C for 120min and 85°C for 5min and ended with 4°C keeping, and the cDNAs were kept in freezer (-70°C) for later PCR running.

The cDNAs were amplified with a PCR machine (DOPPIO VWR, UK). All PCR programs started with a denaturation step at 95°C for 4 min and terminated with an elongation step at 72°C for 10 min. The primers and PCR conditions for Oct3/4, SOX2, HIF-1 α and HIF-2 α are shown in Table 1. The amplified PCR products were separated by a 7.5% polyacrylamide gel electrophoresis, stained with gelred (Molecular Probes, Invitrogen) and visualized in a syngene image system (G: BOX Syngene, USA). GAPDH and actin were used as internal controls for normalizing the expression levels in the subsequent quantitative analyses, respectively and the densitometries were analyzed by Syngene software.

Table 1: Primers and amplification conditions used for RT-PCR

Gene	Primers sequences		Amplification length (bp)	Amplification conditions
	Forward primer (5'-3')	Reverse primer (5'-3')		
Oct3/4	ACATGTGTGAAGTCGGGCC	GTGTGCATAGTCGCTGCTTG	297	60°C:32
SOX2	TTGCTGGCTCTTTAAGACTAGGA	CTGGGGCTCAAACCTCTCTC	75	62°C:35
HIF-1 α	AGTGTACCCTTAAGTACCGAGGAA	CTGAGGTGGTTACTGTTGGTATCA	113	60°C:35
HIF-2 α	GACCAGCAGATGGACAACCTGTAC	CAGAAAGATCATGTGCGCCATCTT	84	60°C:35
GAPDH	CCTCAAGATCATCAGCAATGC	TGGTCATGAGTCTTCCACG	101	62°C:28
β -actin	CTTTGATTGCACATGTTGT	GAAAGCAATGCTATCACCTC	160	62°C:28

Western blot analysis

Special attention was paid to quickly rinse the cells by ice-cold phosphate-buffered saline (PBS), and the cells were scraped into RIPA buffer (25 mM Tris HCl pH 7.6, 100 mM NaCl, 1% NP40, 1% Sodium deoxycholate, 0.1% SDS, Thermo Scientific Pierce, Germany) added with protease inhibitors (0.1 μ M Aprotinin, 1.0 mM PMSF, 1 μ M Leupeptin, 1 μ M Pepstatin) immediately before use. The samples were centrifuged at 15,000 rpm for 15min at 4°C and the supernatants were transferred to new tubes. The protein concentrations were measured with Bio-Rad

protein assay according to the manufacturer's instruction. After heated with a benchtop heater (Model 111002, Boekel Scientific, USA) at 100°C for 5min in SDS-loading buffer (500 mM Tris HCl pH 6.8; 10% Glycerol, 2% SDS, 0.6 M DTT, 0.05% Bromphenol blue), equal amount of protein (50 μ g) per sample was subjected to 4-10% SDS-PAGE and transferred to polyvinylidene difluoride transfer membrane (BIO-RAD, USA). Membranes were blocked with 5% non-fat dry milk in TBS-Tween for 60 minutes at room temperature and incubated with the primary antibodies at optimal dilution in TBST/5% milk overnight at 4°C, such as: GAPDH (0.2 μ g/ml, R&D), Oct3/4 (1 μ g/ml, R&D), SOX2 (1 μ g/ml, R&D), HIF-1 α (1 μ g/ml, R&D) and HIF-2 α (1 μ g/ml, R&D). The membranes were then incubated by secondary HRP-conjugated antibodies including anti-goat, IgG-HRP antibody (Resourced from rabbit, dilution 1:2000) or anti-mouse IgG-HRP antibody (Resource from goat, dilution 1:1000). Immuno-complexes were visualized by enhanced chemiluminescence (GE Healthcare, UK). The western blotting experiments were repeated at least three times.

Colony formation assay

The cells in 80% confluent were dispatched from cell culture flask, harvested and counted with the Countess Cell Counter (Invitrogen). 500 cells per well were plated in plates at 20% or 1% oxygen tension for 14 days, and colonies were fixed with 4% buffered formalin for 15min and then were stained with 1% crystal violet for 30min. The plates were gently washed with PBS and dried before colony evaluation under microscope. Colony number which contained more than 30 cells was counted.

Statistical analyses

Experiments data are shown as the mean \pm SD of at least 3 experiments (in duplicates) each; SPSS software (version 16.0) was used for data analysis. Quantification of band densities was performed using Genetools software (version 3.07). Statistical analysis was performed using a one-way ANOVA test and Student *t* test ($P < 0.05$ was considered statistically significant).

3. Results

Hypoxia effect

For both KYSE70 and KYSE450 cell lines, hypoxia inhibition of cell proliferation was repeatedly shown with oxygen tension-dependent manner. As shown in Fig 1, 7% oxygen tension demonstrated proliferation inhibition, 1% oxygen tension showed higher proliferation inhibition, in comparison to the cells cultured in 20% oxygen. There was a statistical significant difference ($P < 0.05$).

At mRNA level, the HIF-1 α , HIF-2 α , Oct3/4 and SOX2 were detected both at hypoxic and normoxic conditions for KYSE70 and KYSE450 cells. At 48 hours cultivation, the expressions of these four factors were significantly increased in 7% and 1% O₂ compared to the expressions in 20% O₂ cultivation, respectively (Figs. 2).

The Western blotting analyses showed weak positive Oct3/4, SOX2, HIF-1 α and HIF-2 α expressions in both KYSE70 and KYSE450 cells cultivated in 20% O₂. Comparatively, the cells in 7% oxygen tension expressed higher levels of these proteins, and the highest levels of expressions of these proteins could be repeatedly demonstrated in cells cultivated in 1% oxygen tension (Fig 3).

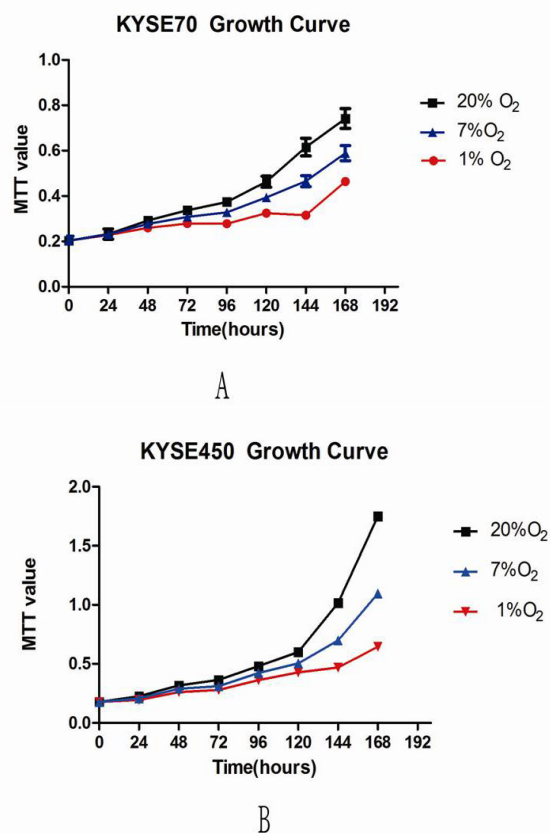


Figure 1. Growth curves of KYSE70 (A) and KYSE450 (B) cells in different oxygen tensions at variable time periods show growth inhibition under hypoxia. MTT values are shown with mean \pm SD from 3 separate experiments for both KYSE70 and KYSE450. Statistical differences were observed between different oxygen tensions for both cell lines ($P < 0.05$), cells in 7% O₂ tension show lower proliferation rate, while cells in 1% O₂ tension show lowest proliferation rate, in comparison to the cells in 20% O₂ tension.

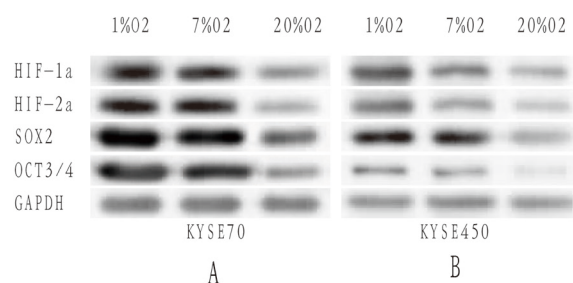


Figure 2. RT-PCR results show increasing mRNA expressions of HIF-1 α , HIF-2 α , Oct3/4 and SOX2 genes in hypoxic cultivation, in comparison to those in normoxic cultivation in variable time intervals in KYSE70 (A) and KYSE450 (B) cell lines. Representative mRNA expressions of HIF-1 α , HIF-2 α , Oct3/4 and SOX2 at 1%, 7% and 20% O₂ conditions were showed in the figure. GAPDH was used for internal loading control.

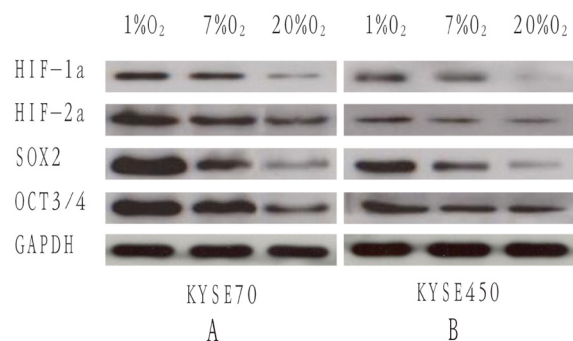


Figure 3. Hypoxia induces the protein expressions of HIF-1 α , HIF-2 α , Oct3/4 and SOX2 in KYSE70 and KYSE450 cell lines. In comparison to the cells cultivated in 20% oxygen, the cells cultivated in 7% and 1% oxygen tensions show higher levels of HIF-1 α , HIF-2 α , Oct3/4 and SOX2 as shown in Western blotting results for KYSE70 (A) and KYSE450 (B) cell lines. GAPDH was used as internal loading control. The higher levels of Oct3/4 and SOX2 are in parallel with the higher levels of HIF-1 α and HIF-2 α in hypoxic conditions.

Pyruvate and uridine increased the expressions of Oct3/4, SOX2, HIF-1 α and HIF-2 α

We next asked whether pyruvate and uridine could have any effect on the expressions of these genes. Since we discovered that the expressions of these genes were significantly upregulated 24 hours in cultivation in 1% O₂ tension, we chose this condition for further pyruvate and uridine test on these cell lines. As shown in Fig 4, on the left panels, in comparison to the expression in 20% O₂, the mRNA level expressions of these genes (Oct3/4, SOX2, HIF-1 α and HIF-2 α) were upregulated in cells

cultivated in 1% O₂ tension. However, their gene expressions were even higher in the cells treated either with pyruvate or uridine or in combination. Similar Western blotting results were repeatedly obtained as shown in Fig 4 at the right panels.

Pyruvate and uridine enhanced colony formation in hypoxic condition

Inhibition of cell growth and up-regulation of the HIFs and transcription factors under 1% O₂ were repeatedly observed in our lab for these cell lines. For one end, upregulation of these factors is ideal for enriching cells with greater stemness, but for another end the cell proliferation inhibition may be a challenge for *in vitro* studies. Since pyruvate and uridine were proved to have synergic effect on the expressions of these factors, we asked how pyruvate and uridine could influence the colony formation of these cells in different O₂ tensions. As shown in Fig 5, for both KYSE70 and KYSE450 cell lines, fewer colonies were formed in 1% O₂ compared to the cells cultivated in 20% O₂ tension. However, both pyruvate and uridine could significantly stimulate colony formation separately in 1% O₂, and the greatest stimulation could be seen for cells treated with both pyruvate and uridine in 1% O₂ tension, reaching to similar colonies as for cells cultivated in 20% O₂.

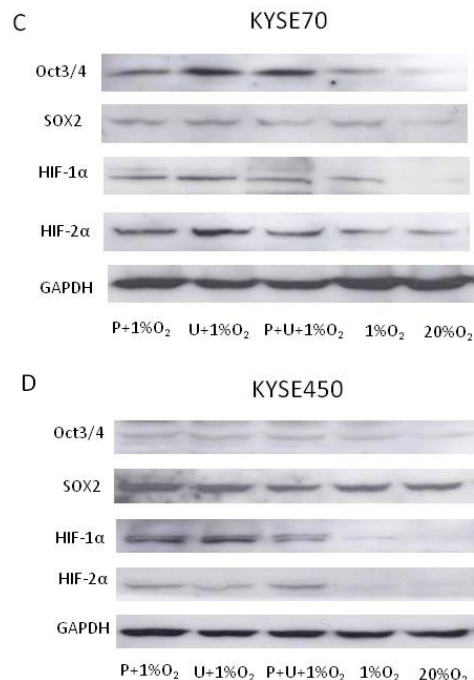
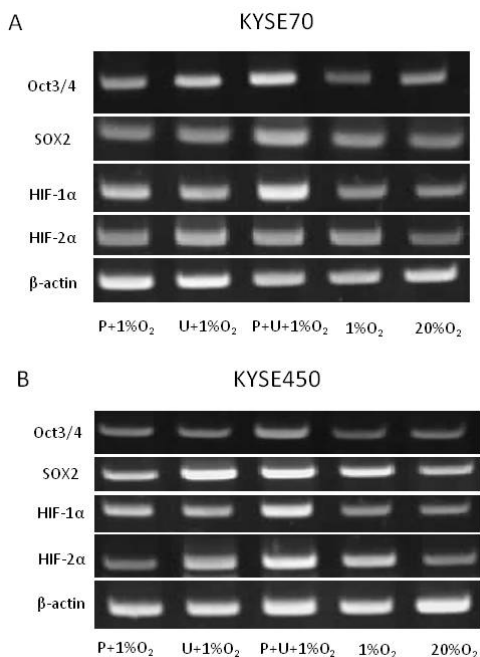
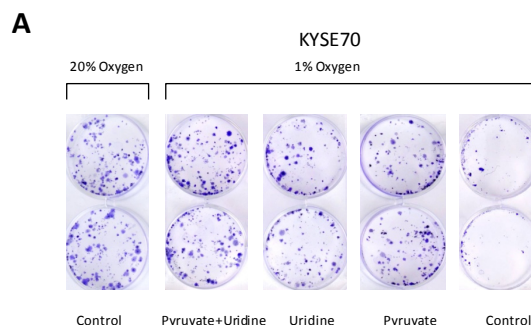


Figure 4. Pyruvate and uridine show synergistic effect on the expression induction of HIF-1 α , HIF-2 α , Oct3/4 and SOX2 in esophageal cancer cell lines. Representative RT-PCR results show increasing levels of Oct3/4, SOX2, HIF-1 α and HIF-2 α mRNA expressions in hypoxia, and even higher levels expressions in hypoxia with pyruvate and uridine, in comparison to normoxia in KYSE70 (A) and KYSE450 (B) cells. Representative Western blotting results show increasing protein level expressions of Oct3/4, SOX2, HIF-1 α and HIF-2 α in hypoxia, and even higher when pyruvate and uridine were added in the media, in compared to the expressions in normoxia in KYSE70 (C) and KYSE450 (D) cells. β -actin was used for internal loading control for all the RT-PCR examinations and GAPDH was used as internal loading control for all the Western blotting analyses. P+1%O₂ is for 1% O₂ with 100 μ g/ml pyruvate; U+1%O₂ is for 1% O₂ with 50 μ g/ml uridine, P+U+1%O₂ is for 1% O₂ with 100 μ g/ml pyruvate and 50 μ g/ml uridine; 1%O₂ is for 1% O₂ only; 20%O₂ is for 20% O₂ only.



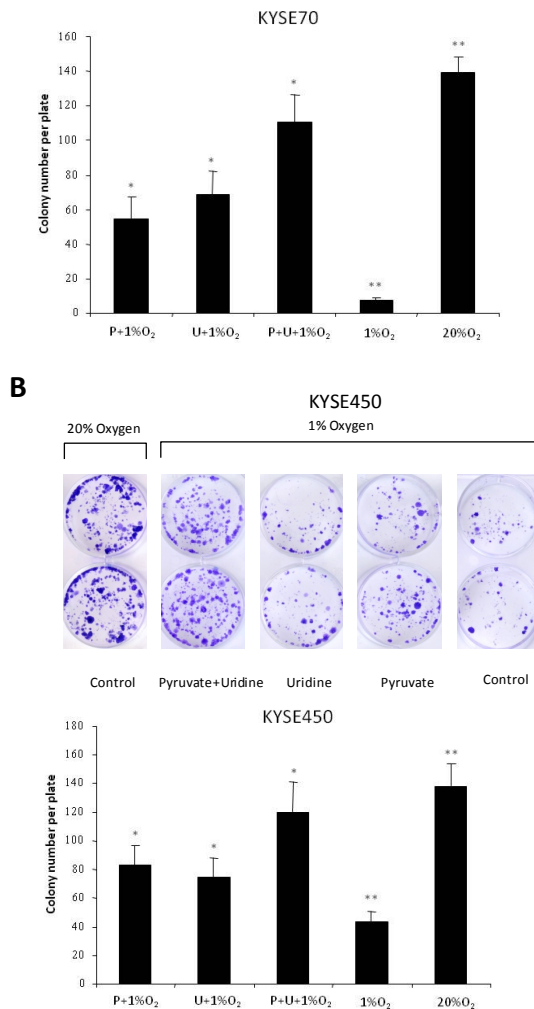


Figure 5. Pyruvate and uridine increase the ability for colony formation of esophageal cancer cells. Representative photomicrographs of colony formation assay of KYSE70 (A, up panel) and KYSE450 (B, up panel) show that cells under 1% O₂ form less colonies, in comparison to the cells under 20% O₂, while either pyruvate or uridine alone could significantly increase the number of colonies for both cell lines and cells with both pyruvate and uridine added in the medium show greater number of colonies similar to the cells under 20% O₂. The down panels in both A and B show the histograms which were created with the means \pm SD of three independent experiments. P+1%O₂ is for 1% O₂ with 100 μ g/ml pyruvate; U+1%O₂ is for 1% O₂ with 50 μ g/ml uridine, P+U+1%O₂ is for 1% O₂ with 100 μ g/ml pyruvate and 50 μ g/ml uridine; 1%O₂ is for 1% O₂ only as control; 20%O₂ is for 20% O₂ only as control. * means P<0.05, ** and means P<0.01.

4. Discussions

According to the cancer stem cells definition, these cells may generate tumors through the normal stem cell processes of self-renewal and further differentiation upon appropriate stimulation. These cells may persist in tumors as a distinct population and cause relapse and metastasis after general cancer therapies like radiotherapy and chemotherapy. It is believed that the higher stemness the tumour cells show *in vitro*, the more likely that the cells are closer to tumour stem cells. Furthermore, the stemness of tumour cells can be reflected by their expressions of the transcription factors or stemness factors (Ceselli et al., 2009;Takahashi and Yamanaka, 2006).

In order to upregulate stemness of tumour cells *in vitro*, hypoxia has been examined in different cell or tissues types during the recent years. Hypoxia often occurs inside solid tumor and exhibits more severe at the undifferentiated parts compared to surrounding tumor or normal tissues. The hypoxia inducible factors HIF-1 α and HIF-2 α are important factors activated under hypoxia and sometimes happen under normoxia as well in tumour tissues (Khandrika et al., 2009;Peng, Maihle, and Huang, 2010). It has been observed that HIFs greatly influence on the phenotypes of tumors by regulating a number of target genes such as glucose transporters, glycolic enzyme, vascular endothelial cell growth, and growth factors (Heddleston, Li, Lathia, Bao, Hjelmeland, and Rich, 2010;Keith and Simon, 2007;Pescador et al., 2010). HIF-1 α and HIF-2 α share some target genes such as VEGF, GLUT1, and ADM-1, but they have their unique targets as well: the glycolytic enzymes (PGK1, ALDA) are only targets of HIF-1 α and TGF- α , cyclin D1 appear to be HIF-2 α targets, at least in certain cell type (Heddleston, Li, Lathia, Bao, Hjelmeland, and Rich, 2010;Keith and Simon, 2007). HIF-1 α also regulates the adoptive signaling including Notch signaling, Wnt signaling, c-myc and p53 factors and HIF-2 α has an interaction with Oct3/4, c-myc and maybe KIF4 and Sox2.

The HIF-1 α and HIF-2 α were increased in several cancer cells like lung, gastric, colorectal, breast, ovarian and esophageal cancers (Bryant et al., 2010;Cao et al., 2009;Wan et al., 2009;Jung et al., 2009;Li et al., 2009;Pipinikas et al., 2008;Daponte et al., 2008;Kolev et al., 2008;Shaida et al., 2008;Shi et al., 2007). Li, Y. etc. found that hypoxia induced HIF-1 α and HIF-2 α expressions in BE1 and A549 cells and also demonstrated that hypoxia-HIF-1 α , 2 α - CCR7-ERK1/2 pathway could regulate the migration and invasion of lung cancer cells under hypoxic conditions and promote metastasis of lung cancer (Li, Qiu, Zhang, Zhang, and Wang, 2009;Wan, Ma, Mei, and Shan, 2009). It is reported that HIF-1 α increased

expression in ovarian cancer cell lines MDAH-2774 and SKOV-3 in response to hypoxia and further investigation showed that inhibition of HIF-1 α expression by specific siRNA resulted in a significant decrease in VEGF production and angiogenesis. Other groups chose the ovarian cancer cell lines ES-2 and SKOV3 under hypoxia to induce HIF-1 α overexpression (Bryant, Munkarah, Kumar, Batchu, Shah, Berman, Morris, Jiang, and Saed, 2010; Hua et al., 2009). HIF-1 α over expression was observed in primary esophageal cancers, compared to normal esophageal epithelium, esophageal cancer bone metastases and esophageal cell lines KYSE70 and KYSE450 as well (Natsuzaka et al., 2012; Zeng et al., 2011). In a clinical observation of high-grade esophageal intraepithelial neoplasia lesions, the precursor of a majority of invasive esophageal adenocarcinoma, increased HIF-1 α expression was found in relative to the respective normal epithelium, stromal cells, and benign prostatic hyperplasia (Monsef et al., 2007).

In our study, both HIF-1 α and HIF-2 α were weakly revealed in 20% O₂ tension cultivation and higher levels expressions were repeatedly demonstrated under hypoxic conditions, typically peaked at the time period 24 hours incubation at 1% and 7% O₂ in both KYSE70 and KYSE450 cells, with the highest level expressions in cells in 1% O₂ cultivation (as shown in Fig 2-4).

Previously, direct molecular links have been established between HIFs and stem cell factors such as Oct3/4, c-Myc and β -catenin. Oct3/4 occupies promoters for many developmental regulators in human embryonic cells, and with SOX2, forms a transcriptional network which has capacity in maintaining the self-renewal of embryonic stem cells and primordial germ cells (Mazumdar et al., 2009). Oct3/4 and SOX2 are not only detected in embryonic cell and germ tumor cell, but also in different somatic tumors such as lung, gastric, colorectal, rectal, bladder, breast, ovarian, and esophageal cancers (Ben-Porath, Thomson, Carey, Ge, Bell, Regev, and Weinberg, 2008; Meng, Zheng, Wang, Liu, Sui, Wu, Zhou, Ding, and Li, 2010; Nirasawa, Kobayashi, Tsuji, Kuribayashi, and Watanabe, 2009; Peng, Maihle, and Huang, 2010; Saigusa, Tanaka, Toiyama, Yokoe, Okugawa, Ioue, Miki, and Kusunoki, 2009; Sotomayor, Godoy, Smith, and Huss, 2009). Comparatively, the expression of these genes is downregulated in all differentiated somatic cell types *in vitro* as well as *in vivo* (Wang et al., 2009).

In consistence with the enhanced HIF-2 α expression under hypoxia, the KYSE70 and KYSE450 cells in our study were repeatedly shown with higher level Oct3/4 expression, both at RNA and protein levels, a link supported by the finding that

HIF-2 α binds to promoter of Oct3/4 and induces its expression and activity directly (Covello et al., 2006). In addition to induction of Oct3/4 expression under hypoxia, we observed also an induction of SOX2 expression.

Pyruvate and uridine have been used for maintaining the survival of cells with mtDNA defect or depletion. MtDNA is more sensitive to damage than nuclear DNA when DNA intercalating agents like ethidium bromide (EB) *etc.* is applied in cell culture resulting in cells without mtDNA or called ρ^0 cells. These cells are therefore forced to synthesize ATP through glycolysis. Many researches show increased tumor cell glycolysis when the cells exposure to low oxygen levels (external pressure) or even under normoxia (internal pressure). The later is due to mtDNA defect or depletion as discussed above. We hypothesize that tumor cells may need pyruvate and uridine for better survival under hypoxic conditions. We found in our study that addition of pyruvate and uridine to the medium for the esophageal cancer cell lines in 1% O₂ cultivation dramatically induced the expressions of HIF-1 α , HIF-2 α and also the stem cell marker Oct3/4 and SOX2 as shown in Fig 4.

In most cancer cells, glycolysis is responsible for about 60% of ATP production. The raised blood or serum pyruvate was frequently tested in leukemia, lymphoma, and renal cancer carcinoma. In addition, the pyruvate reduces DNA damage during hypoxia which might protect cancer stem cells. Recent studies exhibited that the pyruvate importation was enhanced in hypoxic tumor which could help to maintain pyruvate level and stabilize HIF-1 α (Roudier et al., 2007; Roudier and Perrin, 2009). Therefore, it is indicated that the cancer cells cultivated *in vitro* under hypoxia may need extra pyruvate for better growth.

We unexpectedly found that the KYSE70 and KYSE450 cells showed growth inhibition under hypoxia, although there was upregulation of the HIFs and transcription factors under hypoxic conditions. Furthermore, we also discovered that pyruvate and uridine had synergic effect on the expressions of these factors. That the better survival effect of pyruvate and uridine on mtDNA depleted cells encouraged us to investigate whether these chemicals could stimulate colony formation of these cells under hypoxia. In line with our speculation, colony formation capability of both KYSE70 and KYSE450 cells was greatly enhanced with addition of these chemicals, reaching to a similar level of the cells in 20% O₂ cultivation. The synergic effect of hypoxia and pyruvate and uridine results in, therefore, cells with greater stemness *in vitro*.

In conclusion, hypoxia induces the hypoxia inducible factors HIF-1 α and HIF-2 α and stem cell factors in esophageal cancer cell lines KYSE70 and KYSE450; Pyruvate and uridine not only have synergic effect on the expressions of these factors in these cell lines, but also enhance the colony formation capability under hypoxia, resulting in cells with greater cell stemness and good propagation possibility, indicating that combinational application of low oxygen tension and pyruvate and uridine in tumor cells *in vitro* may help to propagate and maintain tumor cells with greater stemness, which should be of importance in further cancer stem cell targeting studies.

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Effects of Organic Fertilizer on the Contribution of Nitrogen Resource to Protein, Nicotine and PEE in Tobacco Leaf

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Abstract: Background: Nitrogen is not only an important element for the growth and development of the tobacco plant but also the quality of leaf. The different types of fertilizer have different effect on the contribution of nitrogen resource to protein, nicotine and petroleum ether extract (PEE) in tobacco leaf. **Materials and Methods:** An experiment was conducted with ^{15}N -labeled KNO_3 to study nitrogen sources in tobacco plants and nicotine, protein and PEE in tobacco leaves. Two treatments were designed: chemical fertilizer was only used in treatment I, and chemical fertilizer mixed sesame seed cake was used in treatment II. The chemical fertilizer included the ^{15}N -labeled KNO_3 . From 68 days after transplant, the plants and leaves were sampled per 10 days, and then atom % ^{15}N excess was determined in both the total N and the three compounds in leaves. **Results and Discussions:** The data showed that the value of N derived from mineral soil N was much more than from fertilizer-N in biomass and the three compounds, and the amount of N from mineral soil was much more in treatment II than that in treatment I. Although the amount of $\text{NO}_3\text{-N}$ incorporation into soil was 20% more in treatment I than that in treatment II, the content of $\text{NO}_3\text{-N}$ in the biomass was almost same between the two treatments, whereas the other nitrogen forms in the biomass and the three compounds was much more in treatment II than that in treatment. Furthermore, the total $\text{NO}_3\text{-N}$ in protein and petroleum ether extract was obviously lower in treatment II than that in treatment I, indicating that sesame seed cake fertilizer not only promote N nutrition in soil to be released and utilized, but also improve the utilization rate of $\text{NO}_3\text{-N}$.

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Keywords: tobacco, isotope fertilizer, nitric compound, nitrogen resource

Abbreviation: PEE=petroleum ether extract; SSC=sesame seed cake.

1. Introduction

Nitrogen (N) is an essential macronutrient that can frequently act as a limiting factor for plant growth. Nitrogen acquisition of plants is usually dominantly by the uptake of ammonium (NH_4^+) and nitrate (NO_3^-), although soil organic nitrogen can be taken up by plants and may represent a significant proportion of total N absorption under particular ecological situations (acidic soils, low temperature environments). The mineral nitrogen content is generally greater in upper compared with lower soil layers, probably due to more favourable conditions for N mineralization in the upper part of the soil (higher content in organic matter; higher O_2 diffusion).

Because ammonium (NH_4^+) and nitrate (NO_3^-) are fundamentally different in charge, they have quite different effects on the physiological and metabolic processes of plants. Although NH_4^+ is an intermediate in many metabolic reactions, it can result in toxicity symptoms in many plants when it is supplied as the sole nitrogen source.

Nitrogen is not only an important element for the growth and development of the tobacco plant but also the quality of leaf (Zhang et al., 2010). The nitric and carbonaceous compounds should have an adequate ratio in high quality tobacco leaf (Shi et al., 1998). The nitric compounds, especially the contents and the ratio of nicotine, protein and petroleum ether extract in leaf, are crucial factors for the smoking quality (Leffingwell, 1976), and the contents of these compounds have a close relationship with the amount and type of the nitrogen fertilizer (Shi et al., 1998).

It had been reported that the tobacco plant need extra nitrogen nutrient till the flower-bud appearing stage (Zhou et al., 1996). In order to control excessive nitrogen applied to tobacco after the flower-bud appearing stage, the producer always tended to decrease the amount of nitrogen in the field, but the expected efficiency was not attained. It is still a serious problem that the high nicotine levels results in lower quality flue-cured tobacco and lower industrial availability.

In the present paper, to examine the effects of different types of fertilizer on the contribution of

nitrogen resource to protein, nicotine and PEE in tobacco leaf, the experiment with ^{15}N -labeled KNO_3 in chemical fertilizer and SSC mixed with chemical fertilizer was constructed.

2. Material and Methods

2.1 Design of the experiment and tobacco plant materials

The soil was dry fluvo-aquic soil with a pH of 7.1. Although the fertilizer including N (1.44g), P_2O_5 (2.16g) and K_2O (4.32g) was incorporated into 18 Kg of soil per pot, the nitrogen form of fertilizer was different (Table 1), and total nitrogen contained ^{15}N cone 0.73 atom % in treatment I and ^{15}N cone 0.44 atom % in treatment II. ^{15}N - KNO_3 was supplied by the Chemical Industry Institute of Shanghai.

The tobacco seedling was transplanted on 9th of May, and topping was done on 15th of July. From 68 days after transplant, Leaves, stems, and roots were separately sampled per 10 days. After initial heat treatment at 105 °C for thirty minutes, these portions were dried at 60 °C and weighed respectively. All of them together were as biomass, then ground into small pieces and passed through a 1 mm sieve. In order to obtain a representative sample for each treatment, three replications per treatment were randomly arranged. The same amount was taken out from the three replications and mixed before it was analyzed.

2.2 Method for the measurement and calculation of nitrogen source

The N % in biomass were analyzed with the method of Nelson and Sommers (1973), and the N in nicotine, protein and PEE were also analyzed with the method of Cao (1980). The atom % ^{15}N excess in biomass and the nitric compounds in leaves were determined with the Isotope Mass Spectrograph made in America.

atom % ^{15}N excess = ^{15}N % - 0.365 (nature cone),
 $^{15}\text{N}_{\text{T1}}$: cone 5.239%, $^{15}\text{N}_{\text{T2}}$: cone 3.150%, $^{15}\text{N}_0$: original cone 10.215%,
 $^{15}\text{N}_{\text{T1}}$, $^{15}\text{N}_{\text{T2}}$ cone = amount of $\text{KNO}_3 \times \text{N}\%$ in $\text{KNO}_3 \times ^{15}\text{N}$ cone / N in per pot

Table 1. The design of experiment

Treatment	Rate of SSC-N to total N (%)	SSC-N (g/pot)	NO_3 -N (g/pot)	NH_4 -N (g/pot)
T1	0	0	0.720	0.720
T2	40	0.576	0.432	0.432

3. Results

Tobacco plant growth, N uptake, amount of mineral soil N, and the contribution of different

nitrogen resource to the tobacco plants were closely related to the types of fertilizer incorporated into soil. The growth of tobacco plant in treatment I was poorer than that in treatment II during the latter period of tobacco plant growth. Mineral soil N contributing to biomass and the three nitric compounds in treatment I was evidently less than that in treatment II (Figure 1), indicating that SSC fertilizer incorporation into soil had a significant effect on promoting nutrition in soil to be released and utilized. It had been reported that SSC fertilizer can modify soil physical properties that can improve the root environment, and consequently enhance the activity of soil enzymes and increase microbiology quantities (Luo and Zhang, 1996). The N accumulation from mineral soil N in tobacco plants and the three nitric compounds was markedly more than that from fertilizer N as given Figure 1.

Protein is the mainly component of cell and catalyzer in plant metabolism, therefore, it would be reasonable that N ratio in protein was similar to biomass (Figure 1a, Figure 1b and Table 2). The changes of N source in nicotine and PEE in leaves were showed in Figure 1c and Figure 1d. There was slight different in N amount from mineral soil N in nicotine and PEE, which increased before day 88, and then declined, but the nicotine remained higher levels in treatment II than that in treatment I, and PEE N in treatment II was almost the same levels as treatment I after day 98.

The changes of N from fertilizer entering the nicotine and PEE were showed in Figure 1c and Figure 1d. Their changes course between treatments were almost same before day 98, while the amount of nicotine N and PEE N from fertilizer in treatment II sharply increased and was more than that in treatment I after 98th day, indicating that mixing SSC fertilizer could improve the fertilizer-N utilization in biosynthesis of nicotine and PEE at the latter stage period. It was suggested that SSC improved much more release of mineral soil N rapidly before day 98, and made the mineral soil N more than the fertilizer-N in soil, resulting that the tobacco plants could absorb more mineral soil N relatively (Figure 1c, Figure 1d and Table 2). Gradually the mineral soil N in the root-zone decreased at the latter stage period, and after day 98 the fertilizer-N in soil in treatment II was utilized better than treatment I (Table 3). On the other hand, nicotine and PEE were all the products of secondary metabolism (Trevor, 1974). Generally, their biosynthesis was activity at the latter stage period, so the N assimilated by tobacco plants was supplied preferentially to the synthesis of nicotine and PEE.

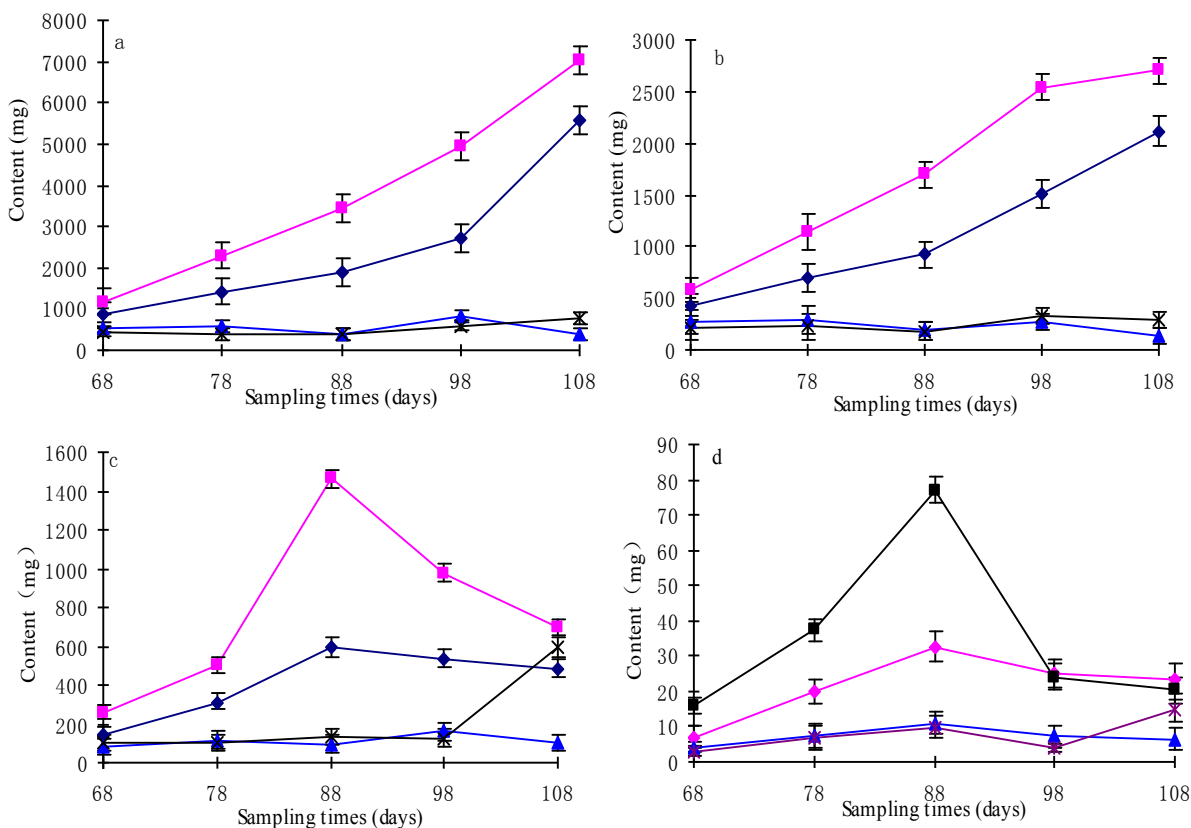


Figure 1. The N resource in biomass (a), protein (b), nicotine (c) and petroleum ether extract (d) in treatment I and treatment II, each mean being the average of three replicate pots, coming from mineral soil N (◆) and fertilizer N (▲) in treatment I; coming from mineral soil N (■) and fertilizer N (×) in treatment II.

Table 2. Rates of total nitrogen and nitrogen in three nitrogen compounds coming from soil and fertilizer (%)

Treatment	T1					T2				
Day after transplant(d)	68	78	88	98	108	68	78	88	98	108
Total nitrogen (mg)	1412.22	1997.82	2297.85	3533.94	5948.21	1639.82	2683.21	3829.71	5555.41	7818.92
From soil (%)	61.22	70.73	82.97	77.00	93.54	72.03	85.77	90.26	89.12	89.94
From fertilizer (%)	38.78	29.27	17.03	23.00	6.46	27.97	14.23	9.74	10.88	10.06
Protein nitrogen (mg)	697.36	996.69	1108.20	1774.71	2257.24	806.31	1377.29	1874.11	2873.06	2996.34
From soil (%)	60.44	69.93	83.13	84.87	93.81	72.62	83.41	90.42	88.37	90.11
From fertilizer (%)	39.56	30.07	16.87	15.13	6.19	27.38	16.59	9.58	11.63	9.89
Nicotine nitrogen (mg)	226.60	427.84	689.07	697.33	585.61	363.36	602.76	1594.88	1102.41	1292.51
From soil (%)	64.85	72.97	86.35	77.17	82.50	71.68	83.30	91.72	88.59	53.72
From fertilizer (%)	35.15	27.03	13.65	22.83	17.50	28.32	16.70	8.28	11.41	46.28
PEE nitrogen (mg)	11.26	27.24	43.66	32.61	29.94	18.96	44.57	87.02	28.01	35.02
From soil (%)	63.06	73.33	74.86	77.43	78.21	83.60	84.21	88.68	86.19	58.16
From fertilizer (%)	36.94	26.67	25.14	22.57	21.79	16.40	15.79	11.32	13.81	41.84

Table 3. Dynamic change of nitrogen nutrition in soil

Treatment	Day after transplant (d)	Total nitrogen (g)	Atom % ¹⁵ N excess (%)	¹⁵ N (mg)	Fertilizer nitrogen (mg)	Mineral nitrogen in root-zone soil (g)
T1	68	15.84	0.055	8.7120	1.6629	15.6840
	78	15.66	0.046	7.2036	1.3750	15.5225
	88	16.56	0.032	5.2992	1.0115	16.4509
	98	16.02	0.333	5.2886	1.0090	15.9191
	108	15.30	0.027	4.1310	0.7885	15.2212
T2	68	15.84	0.052	8.2368	2.6149	15.5785
	78	16.74	0.026	4.3524	1.3820	16.6018
	88	16.02	0.024	3.8836	1.2329	15.9285
	98	16.92	0.022	3.7224	1.1817	16.8018
	108	16.02	0.022	3.5244	1.1189	15.9081

Note: atom % ¹⁵N excess = $^{15}\text{N}\% - \text{Soil natural cone (0.236)}$; $^{15}\text{N} = \text{Total nitrogen} \times \text{atom \% } ^{15}\text{N excess}$; $^{15}\text{N}\%$ left in soil = $^{15}\text{N}/^{15}\text{N}$ supplied in soil; Fertilizer nitrogen = $^{15}\text{N}/\text{fertilizer cone } X^{T1:5.239\%; T2:3.150\%}$; Mineral nitrogen in soil = Total nitrogen - Fertilizer nitrogen.

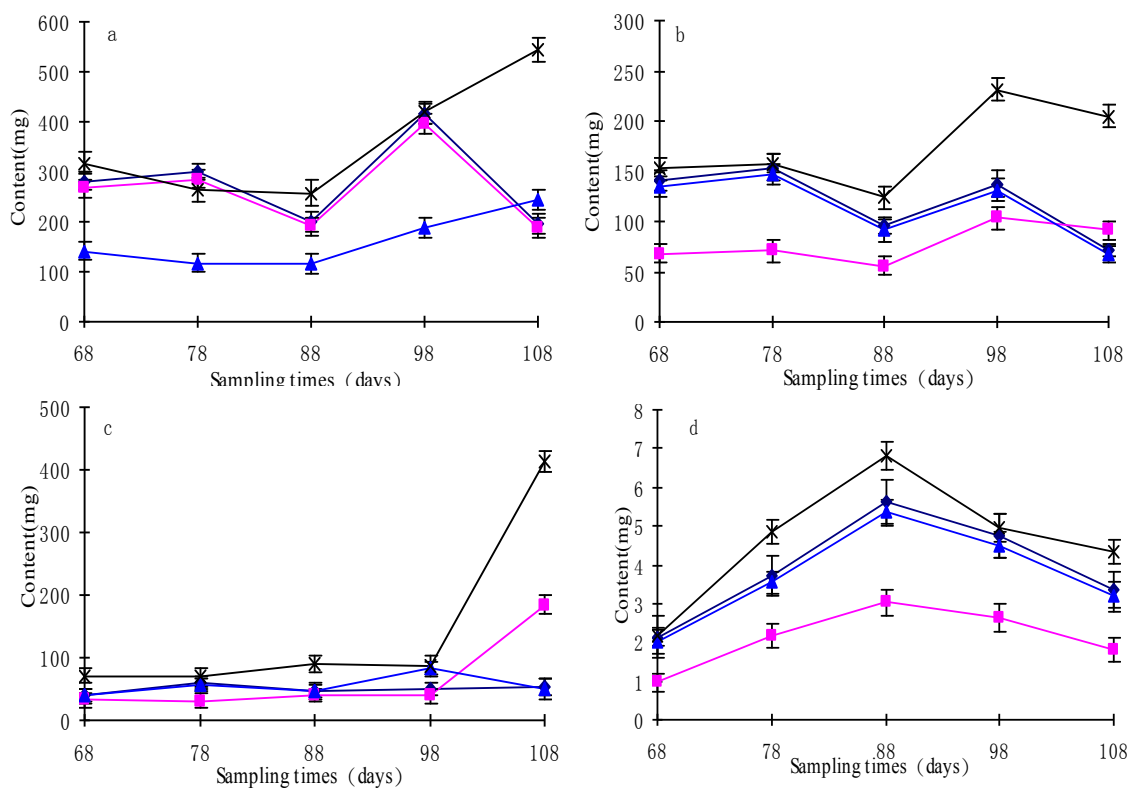


Figure 2. The nitrate-N and other nitrogen forms-N from the fertilizer incorporated into soil in biomass (a), protein (b) nicotine (c), and petroleum ether extract (d) in treatment I and treatment II, each mean being the average of three replicate pots. nitrate-N (◆) and other nitrogen forms-N (▲) in treatment I, nitrate-N (■) and other nitrogen forms-N (×) in treatment II.

The time courses of changes in the accumulation of $\text{NO}_3\text{-N}$ and other nitrogen forms from fertilizer in biomass were showed Figure 2a. Between the two

treatments, the time courses of changes in $\text{NO}_3\text{-N}$ accumulated are almost same. It was suggested that the $\text{NO}_3\text{-N}$ was assimilated and used preferentially in growth of tobacco plant, because the SSC fertilizer

whose nitrogen accounted for 40% in total N incorporation into soil was mixed in treatment II. In the other words, the NO₃-N was less 20% in treatment II than in treatment I. It was consistent with the conclusion that NO₃-N was easily assimilated and utilized by tobacco plant (Feng and Peng, 1988).

The NO₃-N entering protein or PEE in treatment I had almost the same time course changes as other nitrogen forms, and the former was only little more than the latter. Comparing with treatment I, there was higher amount of other nitrogen forms and lower amount of NO₃-N in treatment II (Figure 2b and 2d). The changes in accumulation of N from fertilizer in nicotine were shown in Figure 2c. There was not significant different between NO₃-N and other nitrogen forms in treatment I, but after day 98 the NO₃-N and other nitrogen forms increased sharply in treatment II and the other nitrogen forms was one times than NO₃-N at day 108. This suggested that in the latter stage period N assimilated from fertilizer to tobacco plant was more preferentially synthesized into nicotine, the main products of secondary metabolism in tobacco plant. From this point, mixed excessive SSC is not beneficial to the production of high quality tobacco leaves.

4. Conclusion and Discussion

The results of atom %¹⁵N excess of nicotine, protein, PEE and the biomass of the tobacco plants showed that N absorption from soil was much more than that from fertilizer. The total N, nicotine N, protein N and PEE N in tobacco plants grown in the soil mixed with SSC and chemical fertilizer was much more than that in the soil only with chemical fertilizer. Compared with the latter, greater nitrogen mineralization from the former was a result of more N available for mineralization (three times) and a higher percentage of N mineralized from SSC. The application of SSC was also an advantage for enhancing the assimilation and utilization of NO₃-N in the plants.

It was obvious that the amount of nicotine N from the soil was increased transitorily at the day 88 after transplanted, suggesting that the biosynthesis of nicotine had an excellent correlation with topping. In order to benefit the fate of tobacco leaves in the latter stage, top excision must be taken at about 70 days after transplanted, thus accelerating the secondary growth of roots, so the nutrition can be absorbed more easily from the soil by tobacco plants (Han et al., 1998). It was reflected by the kinetic curve line of utilization of soil N by nicotine in Figure 1c and Figure 2c. SSC can enhance the above effect, and offer more N for accumulation of nicotine. However, the latter was not beneficial to the quality of tobacco leaves, because it cannot accord with the N supplying

regulation required in tobacco growth. In addition, it is possible that topping can cause a decrease in auxins levels, resulting in active biosynthesis of nicotine (Wang et al., 2008). It had also been observed by Yasurmatsu (1967) that nicotine production was reduced on the addition of auxins to tobacco plants.

A tobacco plant will be physically mature at 98 days after transplanted when the metabolism of synthesis should be lower than the metabolism of decomposition in the plant, so the amount of protein should reduce gradually, compared with before day 98. Whatever the protein nitrogen from soil or from fertilizer in treatment II was rather more than that in treatment I (Figure 1b and Figure 2b), indicating that it was adverse for the maturation of tobacco leaves in the latter stage that the excessive nitrogen of sesame seeds cake released throughout the life of the plant.

The PEE is the aromatic compound in tobacco leaves. The effect of SSC on the nitrogen of PEE can be seen clearly in Figure 1d and 2d. The N, especially from soil, supplied for PEE was more in treatment II than treatment I, suggesting that SSC was beneficial to the biosynthesis of PEE. In addition, though NO₃-N content incorporation into soil in treatment II was 20% less than that in treatment I, no difference in NO₃-N of nicotine and protein at the day 108 was observed, and the NO₃-N in PEE in treatment II was markedly less than in treatment I. This suggests indirectly that NO₃-N was used preferentially in biosynthesis of nicotine and protein in leaves. Further research needs to be done to confirm and expand these preliminary findings with regard to the rate of application of SSC.

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Inhibition of neurite outgrowth and promotion of neuronal degeneration by the atropine in Neuro2a cells

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Abstract:

Myopia, a very common human eye disorder, affects up to 80% of young adults in some East Asian countries, including in Taiwan. Higher degree of myopia is associated with a number of visual terrorized complications, including retinal detachment, macular degeneration, cataract and glaucoma. The risk of these complications rises with increasing severity of myopia. Contrasting other blinding disorders such as aged-related macular degeneration, all these myopia-related complications tend to arise mainly in young adult. Atropine, a pan muscarinic cholinergic receptor antagonist, has been used for treated myopia since long time ago. However, the influence of innervation in the eyes was still not known. To address this question, the effect of atropine on nerve innervation and cell character, the pan muscarinic cholinergic receptor antagonist treated cultured cell were examined. The neuronal neurite lengths were assay with microscopy. To study the relationship between neuronal death and atropine-treated, statistical analysis was examined. Blocked the neuronal transmission caused the cells neurite lengths decreased compared with control. It also detected that the increase in the number of nuclear condensation cells after treated atropine with microscopy. In other words, neuroblastoma cells after atropine-treated, the cultured cell death ratio was increased compared with control. These results indicated that neuronal cells treated with atropine, a muscarinic receptor antagonist, reduced the neurite outgrowth and promoted neuronal degeneration.

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Keywords: Atropine, neurite outgrowth, cell death

1. Introduction

Myopia, the most common ocular disorder in humans, affects up to 82% of young adults in Taiwan. Additionally, the decreased visual function from out of optical focus, higher degrees of myopia is associated with the risk of permanent blinding complications. These myopia-related complications, including retinal detachment, macular degeneration, glaucoma and cataract, tend to occur in young adults. Axial length of eyeball is the major determinant of refractive error [1] and has a well-known negative correlation with myopia. Restated, the longer the axial length, the more severe the myopia [2-3].

Atropine, a nonselective muscarinic cholinergic receptor antagonist, has a long history of use for treating myopia or for inhibiting axial growth of eyeball in human and animal models [4-8].

Atropine is a competitive inhibitor of the muscarinic acetylcholine receptor. It blocks the effect of acetylcholine and protects receptors from further stimulation. It has a minimal effect at nicotinic receptor sites [9-10]. The central nervous system effects observed in atropine that it is capable of crossing the blood-brain barrier.

However, the underlying mechanisms underlying the action of atropine in myopia treatment are ambiguous. At the outset, atropine was consideration to retard myopia by obstruction with accommodation through its cycloplegic effect. Another possible mechanism atropine regulates myopia is its interaction with the long-lasting increase in retinal dopamine release [11]. Otherwise, some studies also suggest that atropine-mediated growth signal may involve the retinal neural cells,

retinal pigmented epithelium, the choroids or the sclera, and effect on eyeball growth [6, 12-13].

Local reaction to atropine may derive from both toxic and allergic origins, which are not simply distinguishable by the clinical presentation alone [14]. Local toxic reaction to atropine includes conjunctival infection and periorbital dry, red skin. It evaluated the adverse reactions to atropine eye-drops among children, noted some reports of toxic reactions that resulted in eminent body temperature and dry, warm skin [15]. In some cases with red eyes and periorbital dermatitis, allergic reactions were suspected but skin allergy tests were negative. Other studies also demonstrated that atropine eye-drops toxic effects included local ocular reactions, mild systemic reactions, and severe reactions of asthmatic attack, convulsions and one of tachycardia [16].

After these years with use atropine for treating myopia or for inhibiting axial growth, studies indicated that incidence rate of myopia in Taiwan and some parts of world are not falling. Conversely, a high prevalence of myopia in young adults has been reported in Taiwan [17]. However, the influence of nerve innervation in eye balls was still not known. To address this question, the effect of atropine on nerve innervation and cell death, the pan muscarinic cholinergic receptor antagonist treated cultured cell were examined. The cell death ratio and neurite lengths were assay with microscopy. To study the relationship between neuronal death and atropine-treated, the cell morphology evaluation was examined.

2. Material and Methods

Retinoids

All-trans retinoic acid (atRA) was obtained from Spectrum Chemical Co. (New Brunswick, NJ, USA) and was deemed greater than 99% pure by reverse-phase HPLC.

Cell Culture

The murine neuroblastoma cell line (Neuro2a, American Cell Type Culture Collection), was used. Cells were cultured in Falcon dishes in DMEM supplemented with 10% fetal calf serum, 4 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml potassium penicillin G, and 100 mg/ml streptomycin sulfate in 5% CO₂, 95% air humidified incubator.

Neurite outgrowth

To induce neurite outgrowth, cells were plated at a $1.8 \times 10^4/\text{cm}^2$ cell density, and 48 h after plating the medium was replaced with 2% FCS-DMEM

containing 20 mM retinoic acid, the incubation being continued for different times up to 48 h. Control experiment showed that incubation up to 48 h with 2% FCS-DMEM, in the absence of retinoic acid, caused only a very modest outgrowth of processes. The neurite outgrowth was used to measure average neurite length (μm per cell) as well as the number of neurites per cell. For each group, cells were examined in a minimum of 5 random non-overlapping images per replicate of each treatment condition.

Atropine treatment

All drug treatments were additions to DMEM (vehicle control). Cell viability of each treatment was determined morphologically after 24, 48, and 72 h.

Cell morphology evaluation

The cells were fixed in 4% paraformaldehyde for 15 minutes, washed 3 times in PBS, and covered with cold 100% methanol for 10 minutes. The cell nucleus was stained with hematoxlin (Sigma-Aldrich). After 5 more rinses in PBS, the cells were mounted and viewed on a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

Statistical analysis

The results are expressed as the mean \pm SEM and were evaluated for significance. Statistical analysis was using a student's t-test. A *p* value of less than 0.05 was considered significant.

3. Results

3.1 Induction of neurite outgrowth in neuroblastoma neuro2a cells treated with atRA

The time course of neurite outgrowth induction by atRA and changes in morphology produced by atRA were studied in neuroblastoma cell lines. An increase in the overall length of neurites was observed in Neuro2a cells exposed to atRA at both 24 and 48 h, with the effect more pronounced at the latter time point (data not shown). Thus, neuroblastoma cell lines showed the expected increase in neurite outgrowth after treatment with atRA.

3.2 Inhibition of neurite outgrowth after blocked muscarinic receptors

To assess the effects of muscarinic receptors expressed in neuronal cells, Neuro2a cultures were treated with muscarinic anagonists atropine. Neurite lengths were slightly decreased in the different atropine treatment groups (Fig. 1B-1D) compared with the control (Fig. 1A) after 1 day. After 2 days of atropine treatment, blocked muscarinic receptors substantially reduced the neurite outgrowth of atRA

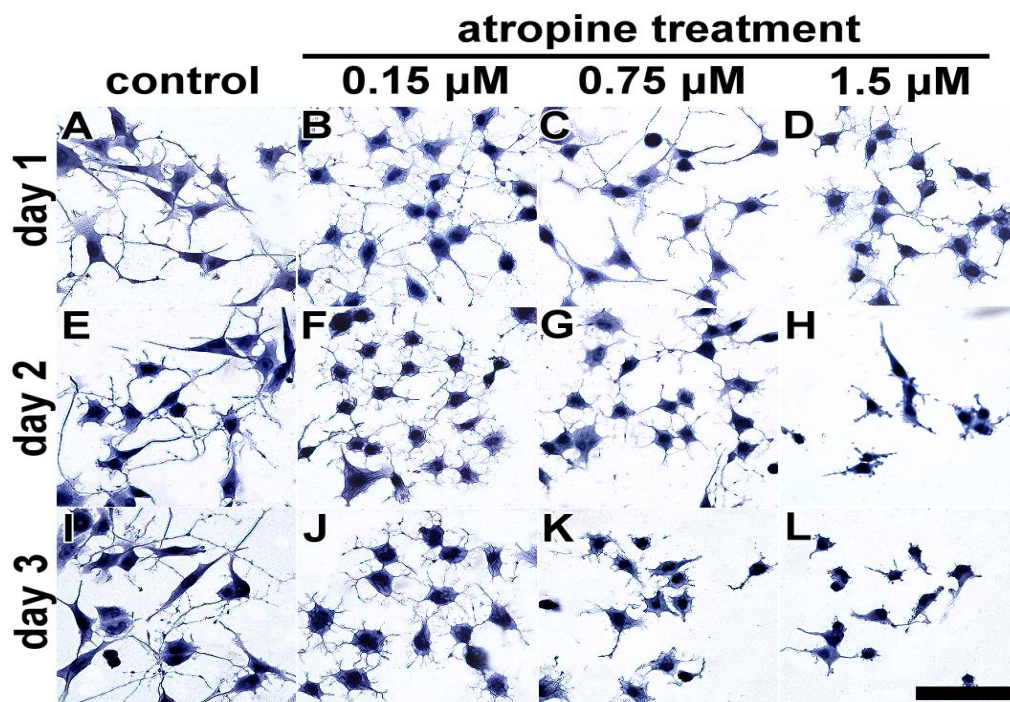


Fig. 1: Effects of atropine on the process length of Neuro2a cells

To enhance neurite outgrowth, Neuro2a cells were pretreated with 10 μM retinoic acid for 2 days. The cells were then either treated with atropine (0.15, 0.75 and 1.5 μM) or were not treated, and the neurite lengths of cells were assessed after 1-3 days. The neurite lengths were slightly decreased in the different atropine treatment groups (B, C, and D) compared with the control (A) after 1 day. However, after the cells were treated with atropine for 2 days, the neurite lengths of the cells were shorter (E-H). Moreover, the neurite lengths more significantly decreased after 3 days with atropine treatment compared with control cells (I-L). Scale bar = 50 μm .

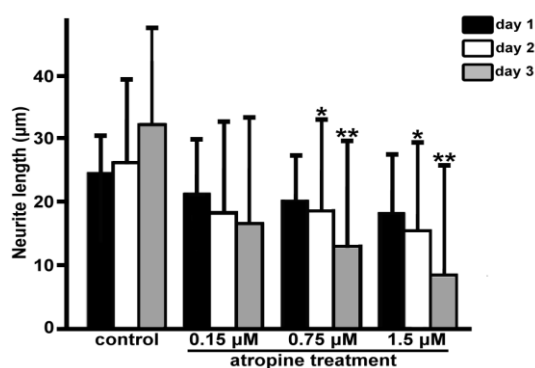


Table 1: Quantization of neurite length of Neuro2a cells after atropine treatment

The neurite length was not significantly different between any groups at 1 day after atropine treatment. Conversely, the neurite length of cells was significantly shorter on days 2 and 3 for both 0.75 and 1.5 μM atropine treatments than in the control. Quantification of neurite length showed a higher concentration of significantly shorter neurite after 2 days. ** indicates $p < 0.01$ and * indicates $p < 0.05$.

exposed Neuro2a, as indicated by cell morphology (Figs. 1E-1H). After 3 days, neurite lengths more significantly decreased in atropine treated cells (Figs. 1I-1L). It demonstrated that muscarinic receptors were involved in the regulation of axonal outgrowth as shown in the significant decrease in the axon length.

3.4 Notable differences between atropine treated and untreated cultured neuronal cells

Neuro2a cells were pretreated with 10 μM retinoic acid to enhance neurite outgrowth. The length of neurite from all the cells within an image was used to calculate the average neurite length. The neurite length did not significantly differ between any of the groups at 1 day after atropine treatment. The length decreased slightly after treatment with 0.0001% atropine for 2 and 3 days but did not significantly differ between the treatment groups. Conversely, the neurite length of cells was significantly shorter on days 2 and 3 for both 0.0005% and 0.001% atropine treatments than in the control. Quantification of neurite length showed a higher concentration of significantly shorter neurite after 2 days.

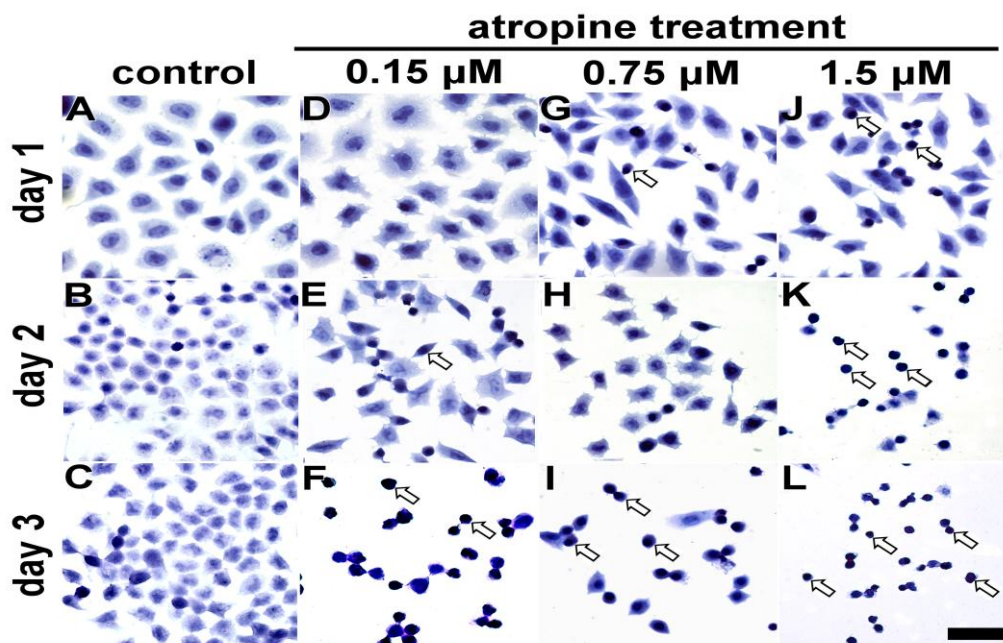


Fig. 2: Effects of atropine on the morphology of Neuro2a cells

Cellular morphology of untreated cells (A-C) and cells treated with 0.15 μM (D-F), 0.75 μM (G-I), or 1.5 μM atropine (J-L) for 1, 2, and 3 days. In the control cells (A-C), nuclei with normal chromatin structure were stained by hematoxylin. Atropine treatment of 1 day duration did not lead to significant differences in morphology among the different treatment groups (D, G, and J) compared with the control (A). However, after the cells were treated with 0.75 μM (H) or 1.5 μM atropine (K) for 2 days, cells showed nuclear chromatin condensation (arrows). At 3 days after treating the cells with different concentrations of atropine (F, I, and L), nuclear condensation (arrows) was evident in the cells. Moreover, the size of the cells decreased over time with atropine treatment. Scale bar = 50 μm .

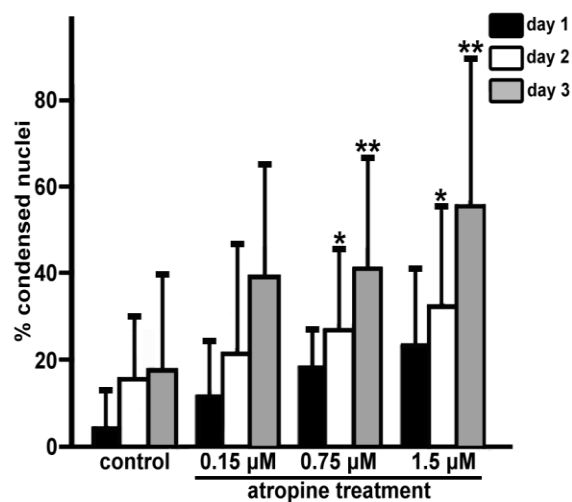


Table 2: Quantization of the percentage of nuclear condensation after atropine treatment

The percentage of nuclear condensation on days 2 and 3 of treatment in cells treated with 0.75 μM or 1.5 μM atropine was significantly higher than that in the control. ** indicates $p < 0.01$ and * indicates $p < 0.05$.

3.4 Promotion of nuclear condensation in Neuro2a cells

Cultured Neuro2a cells were either treated with atropine (0.15 - 0.75 and 1.5 μM) or were not subjected to treatment (control), and nuclear condensation was assessed after 1-3 days. The percentage of treated or untreated Neuro2a cells with condensed nuclei was assessed with microscopy. At 1 day after atropine treatment, the percentage of nuclear condensation was not significantly different between all the groups. After the cells were treated with 0.15 μM atropine for 2 or 3 days, the percentage ratio of nuclear condensation slightly increased, but no statistically significant difference was observed between the untreated groups. Conversely, percentage ratios of nuclear condensation after day 2 and 3 of Neuro2a cells treated with 0.75 μM or 1.5 μM atropine, were significantly higher than that of the control.

4. Discussion

The neural trophic factors have been shown to mediate various functions in the nervous system and in cell lines, including neuronal proliferation and

differentiation [18-19]. In vitro model systems, cells differentiate and acquire a sympathetic neuron-like phenotype in response to nerve growth factor (NGF) [20-21]. Our experiment data also indicated that blocked muscarinic receptors with the atropine, a nonselective muscarinic cholinergic receptor antagonist, substantially reduced the neurite outgrowth of atRA exposed Neuro2a, as indicated by cell morphology and statistical analysis (Figs. 1 and Table 1). The neurite length showed significantly shorter neurite after 2 days treated with 0.75 μ M and 1.5 μ M atropine.

Cells death is the dependence of on trophic factors for cellular survival. The need for trophic support is not restricted to the stage of neuronal growth but has also been demonstrated in the adult [22]. The availability of trophic factors determines which neurons die and which survive. Neurons that fail to acquire sufficient trophic factor die [23-24]. Many reports have indicated neurotransmitters, which mediate synaptic communication, can also act as trophic factors in the nervous system [25-28]. The neurotransmitter acetylcholine is abundant in the nervous system. acetylcholine effect innervated tissues via two different receptor families, the nicotinic and the muscarinic receptors [29]. In our current study, this degeneration pattern after treatment with was demonstrated with cell morphology using microscopy. Ratios of nuclear condensation after day 2 and day 3 in Neuro2a cells treated with relatively higher concentration of atropine, were significantly higher than that of the control.

Acetylcholine mediated neural functions are essential in central nervous systems and peripheral nervous systems. In particular, muscarinic type acetylcholine receptors are widely distributed in the full bodies, and play key roles in various aspects by normalizing the activity of many essential functions of the nervous system [30]. Many neurodegenerative conditions are associated with loss of acetylcholine function and increased cellular oxidative stress, for example Alzheimer's disease [31]. Stimulation of muscarinic receptors provides substantial protection from DNA damage, oxidative stress, and mitochondrial impairment. Action of muscarinic receptors prevents mitochondrial cytochrome c release, bcl-2 depletion, and bax accumulation [32]. Studies may be encountered by neurons of central nervous systems and peripheral nervous systems in growth, aging, or neurodegenerative diseases.

Atropine, a nonselective muscarinic cholinergic receptor antagonist, has a long history of use for treating myopia or for inhibiting axial growth of eyeball in human and animal models. After these years with use atropine for treating myopia or for

inhibiting axial growth, studies indicated that incidence rate of myopia in Taiwan and some parts of world are not falling. However, influences of atropine on the nerve outgrowth and neuronal cell death were demonstrated in the study. These results suggest that the treated with nonselective muscarinic cholinergic receptor antagonist, atropine, may cause the degeneration of neurons with acetylcholine receptors.

5. Conclusion

The results indicated continued neuronal survival is dependent upon the environment of the cell, signals received from neighboring cells may provide the necessary drive to encourage defenses against death programs. Blocked muscarinic receptors with the atropine, a nonselective muscarinic cholinergic receptor antagonist, substantially reduced the neurite outgrowth and promoted neuronal degeneration. It is impossible to consider these degenerative changes are related unavoidably part of their mechanism of action or an avoidable toxic effect of use for treating myopia or for inhibiting axial growth of eyeball in human.

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Effect of Nurses' Work Hours and Fatigue on Occurrence of Medication Errors in ICU and Medical Oncology Unit –Cairo University

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Abstract: Nurses are responsible for the safety of their patients. So, preventing error and maximizing quality of care for patients requires that those in health care be open to a variety of strategies for modifying work schedules and preventing fatigue that may require changes in long standing behaviors and preferences. **Aim:** To examine the effect of nurses' work hours and fatigue on occurrence of medication errors. **Subject and methods:** An exploratory, descriptive design was utilized to accomplish this study. The sample included all available nurses (n=29) were work in the two medical oncology units and (n=20) nurses work in ICU and conducted at the National Cancer Institute (NCI), Cairo- University. Tools for Data Collection were five included Socio demographic data, working hours data sheet, (CIS) chronic fatigue instrument, Need for recovery instrument and Medication errors observational sheet. **Results:** indicated that there was correlation between working hours and fatigue related to physical activity items in both units. There was correlation between medication errors and concentration fatigue subscale, motivation subscale at the ICU unit. Medication preparation errors and concentration fatigue subscale in the medical unit were also correlated. **Conclusion:** This study concluded that there was no statistical significant correlation between working hours and medication errors with both wings of errors preparation and administration. Also, the study concluded that correlation between medication preparation and administration errors and concentration fatigue subscale.

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Keywords: nurses' work hours, fatigue, medication errors, CCU.

1. Introduction

Healthcare providers are not expected to make errors, mistakes do occur, and some mistakes have resulted in serious injury or death. Each year, approximately 1.3 million patients are injured because of error during their hospitalization and more than 100000 deaths due to preventable adverse events occur. The effects of human error may be more significant for patients in critical care units (Scott *et al.* 2006). Nurses are responsible for the safety of their patients. Preventing error and maximizing quality of care for patients requires that nurses be open to a variety of strategies for modifying work schedules and preventing fatigue that may require changes in long standing behaviors and preferences (Bellebaum, 2008 and The Joint Commission, 2008).

Improving quality of medication administration process considered number-one of all nurses' priorities and the heart of health strategy to provide safe and error-free medication therapy. Medication errors are an important cause of patient morbidity and mortality, 44,000 to 98,000 patients die each year as a result of medication related problems (Mohamed and Gabr, 2010).

American Society of health – System Pharmacist (ASHP) has defined medication errors as “any preventable event that may cause or lead to inappropriate medication use or patient harm while the

medication is in the control of the health care professional, patient, or consumer”. (National coordinating council for medication error reporting and prevention, 2008). Medication administration is a complex multi-step process that encompasses prescribing, transcribing, dispensing, and administering drugs and monitoring patient response. An error can happen at any step. Administration errors account for 26% to 32% of total medication errors— and nurses administer most medications (Anderson and Townsend, 2010).

It has been reported that 78% of serious errors in the hospital are due to medications and among all serious errors, 11% are potentially life threatening (Wise, 2007). Intensive care unit (ICU) patients are at a high risk for medication errors due to the substantial quantity of medications administered, tenuous nature of the patient and complexity of the environment (Woodward, 2004). Medication administration errors in the ICU have been documented at rates ranging from 3.3 to 44.6% (Kazaoka *et al.* 2007 and Kelly, 2008). In ICU, patients experience 1.7 errors per day and nearly all suffer a potentially life threatening error at some point during their stay (Hussain and Kao, 2005).

Medication errors do occur and are a persistent problem associated with nursing practice. Reducing error rate affects overall institutional risk assessment, and this can decrease costs in institutional insurance

money (**Straight, 2008**). Clearly, medication errors are a significant and growing problem in health care. Enhanced understanding of some associated factors, such as the hospital unit and nursing shift, on which the error occurred, might assist nursing administrators to identify common patterns and improve nursing care, ensure patient safety, and contain hospital costs. Better organizational systems then could be designed and implemented to reduce potential medication errors (**Moyen et al. 2008**).

Also, **Hodgkinson et al. (2007)** and **Kazaoka et al. (2007)** identified contributing factors to medication errors as: personal, system and managerial problems. Others (**Clancy, 2004** and **Madegowda, 2007**) revealed that these events may be related to professional practice, health care products, or procedures and systems, including prescribing, ordering, communication, product labeling, packaging, and nomenclature. Thirty-two percent of nurses attributed contextual factors to medication errors: short staffing, large numbers of assigned patients, frequency of change in patient assignments, distractions during preparation of medications, high acuity, and lack of knowledge of the patient (**Bellebaum, 2008**).

A study considered the effect of nurses' work hours on medication errors done by **Scott et al. (2006)** found that 38% of the nurses making at least one error during the two month study period. When nurses worked greater than 12.5 consecutive hours, the risk of that nurse making an error almost doubled compared to nurses who worked 8.5 consecutive hours or less. Another study done by (**Rogers et al. 2004**) used log books to gather error data from nurses in order to consider the effect of work hours on patient safety, nurses worked more than 40 hours per week, and 39% of the shifts were 12 hours or more in length. Fifty-eight percent of the errors captured through the log books were related to medication administration.

Ellis (2008) stated that shift work can result in fatigue, irritability, reduced performance, and decreased mental agility. Because human beings developed for a life style of wakefulness in daylight hours and sleep during hours of darkness, changing this pattern of wakefulness and activity creates the potential for increased fatigue and sleepiness. (**Lockley et al. 2007**) added that 300% more fatigue-related medical errors that led to patient death than those working shorter shift. Fatigue has deleterious effects on all types of performance and has negative effects on alertness, vigilance, concentration, judgment, mood, and performance. Fatigue is defined as "a condition characterized by a lessened capacity for work and reduced efficiency of accomplishment, usually accompanied by a feeling of weariness and tiredness." (**Medicine, 2008**).

Finally, this study is significant as it will observe nurses who work in both an ICU and medical oncology units. These two areas of nursing have the greatest proportion of nurses working long hours. Few studies have been published investigating the associations with nurses' work hours or fatigue and incidence of medication errors. The medication administration process is an everyday part of nursing practice and is so much more than a simple psychomotor task. Because nurses play a key role in the process of medication administration, namely administering the medication to the patient, so, it is important to examine correlates of medication errors (**Maurer, 2010**).

The framework for this research is based on the work of Donabedian, in which he categorized medical care in terms of structure, process, and outcome in order to determine indicators of quality (**Aday et al. 2004**). Medication administration errors can serve as quality indicators, but more importantly, they are a proxy measure for patient safety in an institutional setting.

STRUCTURE → PROCESS → OUTCOME

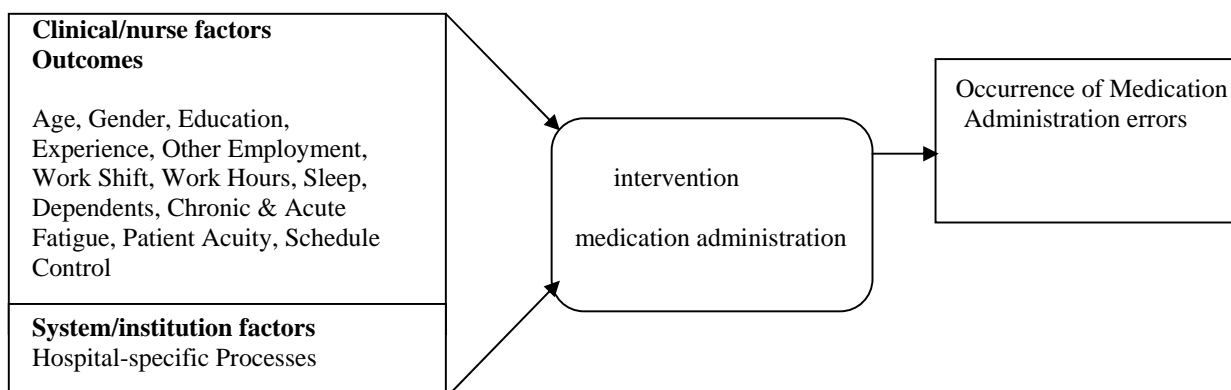


Figure 1.1: Conceptual framework for this study

2. Methodology

The aim of the study:

This study aim to examine the effect of nurses' work hours and fatigue on occurrence of medication errors.

Research design:

An exploratory, descriptive design was utilized to accomplish the study.

Sample:

The sample included all available nurses (n=29) were assigned to work in the two medical oncology units and (n=20) nurses from intensive care unit who met the criteria of inclusion which were willing to participate in the study and responsible to give medication were recruited.

Setting of the study

The study was conducted at the National Cancer Institute (NCI), Cairo- University, in the two medical oncology units and the intensive care unit.

Tools for Data Collection:

Tool I: Socio demographic data:

It was contain information related to demographic characteristics of the studied nurses and it is consisted of 7 items.

Tool II: working hours data sheet:

It was used to gather information about the working hours of the nurses during the week and the schedule of work.

Tool III: Checklist individual strength (CIS) chronic fatigue instrument:

It contains 20 items, each item is scored on a seven-point scale. There were four subscales which were subjective experience of fatigue (8 items), concentration (5 items), motivation (4 items), and physical activity level (3 items). Cronbach's alpha for reliability of the entire instrument was 0.90 and subscale Cronbach's alphas were 0.88, 0.92, 0.83, and 0.87, respectively. Validity also was proved (Beurskens *et al.*, 2000).

Tool IV: Need for recovery instrument:

It was used to assess acute fatigue, it contains 11 items and is scored dichotomously as "yes" with (1) score or "no" with (0) score. Cronbach's alpha was 0.88 (Van Veldhoven and Broersen, 2003).

Tool V: Medication administration errors observational sheet:

It contains 40 items, it was used to gather information about the medication administration process. It was divided into 2 parts (a) medication preparation errors, that contains 23 items & medication administration errors which contains 17 items. Content validity was done by four panel of experts from medical surgical and administration staff members. Each item is scored as (1) for error occurred and (0) for no error.

Methods of Data collection:

Ethical consideration

Human rights and ethical permission were obtained to conduct the study. An official permission was obtained from Cairo faculty of nursing dean and then the official permission was obtained from the National Cancer Institute (NCI), Cairo- University director. Nurses were fully informed of the study. The voluntary nature of participation was stressed as well as confidentiality. Consent was obtained from each nurse.

Pilot study

A pilot study was conducted on 3 nurses at medical oncology unit and 2 nurses in the intensive care unit, and these nurses were excluded from the study sample. The objectives of the pilot study were: (a) estimate the time necessary for nurses to fill out the entire questionnaire; (b) test the clarity of the questions (whether any question was unclear or ambiguous); (c) identify the most appropriate response categories for specific questions; and (d) Test whether there was any question that might frustrate nurses.

Data collection procedures:

Data were collected in two ways:

1. Observation checklist :

During observation, the medication administration errors observational sheet was filled out. Each nurse was observed for 3 times during 3 different days. Nurses were observed during the routine medication administration at both units. The day of observation was divided into 2 observation periods at first during medication given at 10 o'clock second, during medication given at 2 pm o'clock. These periods were selected because the majority of medications ordered were taken during these periods. So that, adequate numbers of medications for observation were feasible at the 2 proposed time periods of observation.

2. Interview questionnaires:

Each nurse was individually filling four questionnaires:

Demographic data sheet, working hours data sheet, checklist individual strength (CIS) Chronic Fatigue Instrument & need for recovery instrument to measure acute fatigue. The checklist individual strength (CIS) and the need for recovery instruments:

- It was collected by all the nurses while they are on duty, purpose of the study was explained prior to get the questionnaire sheet, and it distributed to be answered within (45- 60 minutes) then collected.
- The questionnaire was filling from 1-2 nurses per day started from November 2011 to January 2012, over a period of 3 months starting according to nurses' schedule for attendance to the hospital and availability of time for both nurses and their units.

Limitation of the study:

1. Night nurses shift were excluded from the sample because none of the observers can be available during night shifts.
2. Hawthorne effect, which is an improvement in a subject's performance when being observed may also affect the results.
3. Nurses did not had the same numbers of medication which my seem unfair among the nurses work.

Data Analysis Plan:

Descriptive statistics were used to summarize demographic characteristics of ICU and medical oncology nurses to give an overview results for the instruments. Data were revised, coded, analyzed and tabulated using the number and percentage distribution and carried out using SPSS version 16. The statistical tests used are chi square test. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

Table (1) shows that most of nurses had age less than 30 years and about (65%) of them were females in both units. (80% & 69%) of nurses were technician with less than 5 years of experience in (70% & 65.5%) in ICU & medical oncology units respectively. Also, there was no statistical significant difference between ICU and medical oncology unit in relation to socio-demographic variables.

Table (2) illustrates that total means of working hours per week were (52 & 51.4 hours) at ICU and medical oncology units respectively. Also, all nurses suffer from chronic and acute fatigue with mean scores of (79.2, 76.5 & 6.2, 6.1) at ICU and medical oncology units respectively. In addition, there was statistical significant difference between ICU and medical oncology unit in relation to fatigue related to physical activities.

Table (3) says that there was no significant correlation between working hours, both medication preparation and administration errors in both units.

Table (4) shows that there was statistical correlation between working hours and fatigue related to physical activity items in both units. In addition there was correlation between concentration chronic fatigue subscale & working hours in ICU unit.

Table (5) reflects that there was correlation between medication errors and both concentration & motivation fatigue subscale in the ICU unit. Medication preparation errors and concentration fatigue subscale in the medical oncology units were also correlated.

Table (6) says that there was correlation between years of experience and medication preparation errors in medical oncology unit.

Table (7) shows that there was statistical significant difference between level of education and medication preparation errors in medical oncology unit.

Table 1: Socio-demographic variables among nurses in both ICU and medical oncology units

Items	ICU (n= 20)		Medical Oncology units (n=29)		p-value
	N	%	N	%	
Age					
20 – <30	15	75	24	82.8	NS
30 – 40	5	25	5	17.2	
X ± SD	26.5 ± 4.1		25.3 ± 3.5		
Gender					
Male	7	35	10	34.5	NS
Female	13	65	19	65.5	
Level of education					
Diploma	2	10	4	13.8	NS
Technician	16	80	20	69	
Bachelor	2	10	5	17.2	
Years of experience					
1 –	14	70	19	65.5	NS
5 –	3	15	6	20.7	
10 –	3	15	2	6.9	
15 – 20 years	0	0	2	6.9	
X ± SD	4.7 ± 3.2		4.8 ± 5.1		

NS: no significant difference

Table (2): Compare of means between ICU and medical oncology units nurses regarding working hours per week, fatigue & medication errors

Variables	ICU		Medical Oncology units		t-test	p-value
	X	SD	X	± SD		
Working hours	52	9.9	51.4	11.2	0.167	.869
CIS subscale (chronic fatigue)						
-Subjective fatigue feeling	32.9	6.8	31.3	7.1	0.833	.415
-Concentration	20.4	3.2	20.8	3.2	0.470	.644
-Motivation	14.4	3.2	12.8	4.7	1.35	.190
-Physical activity	12	1.8	10.4	3.3	2.120	.040
Total mean scores	79.2	12.4	76.5	12.8	.900	.384
Need for recovery (acute fatigue)	6.2	1.5	6.1	2.3	0.160	.874
Medication errors						
- Medication preparation errors	3.3	2.8	2	2.4	1.406	.176
- Medication administration errors	0.5	1.2	0.2	0.7	1.07	.297

Table (3): Relationship between working hours & medication errors among nurses in both ICU and medical oncology units

Medication errors	Working hours			
	ICU		Medical Oncology units	
	r-test	p-value	r-test	p-value
Medication preparation errors	-.233	.323	.175	.363
Medication administration errors	-.256	.275	-.070	.719

Table (4): Relationship between working hours and fatigue among nurses in both ICU and medical oncology units

Fatigue	Working hours			
	ICU		Medical oncology units	
	r-test	p-value	r-test	p-value
CIS subscale (chronic fatigue)				
-Subjective fatigue feeling	-.131	.583	.117	.545
-Concentration	.442	.051*	-.010	.958
-Motivation	-.351	.129	.029	.880
-Physical activity	.534	.015*	.471	.048*
Total mean scores	-.218	.434	.297	.282
Need for recovery (acute fatigue)	-.370	.109	.243	.205

Table (5): Relationship between medication errors and fatigue among nurses in both ICU and medical oncology units

Fatigue	Medication errors			
	ICU		Medical Oncology units	
	Preparation	Administration	Preparation	Administration
CIS subscale				
-Subjective fatigue feeling	.250	.283	-.200	.334
-Concentration	.656*	.662*	.601*	-.264
-Motivation	.274	.455*	-.158	-.009
-Physical activity	-.254	-.176	.140	.058
Total mean scores	.321	.386	-.412	.141
Need for recovery (acute fatigue)	.240	.193	.142	.334

Table (6): Correlation between years of experience and medication errors among nurses in both ICU and medical oncology units

Medication errors	Years of experiences			
	ICU		Medical Oncology units	
	r-test	p-value	r-test	p-value
Medication preparation errors	.058	.807	-.395*	.034
Medication administration errors	-.043	.856	-.206	.283

Table (7): Compare of means, ANOVA test, between levels of education and medication errors among nurses in both ICU and medical oncology units

Medication errors	Level of education			
	ICU		Medical Oncology units	
	F	p-value	F	p-value
Medication preparation errors	.926	.415	3.188*	.058
Medication administration errors	.078	.925	.377	.689

4. Discussion

The aim of the current study was to examine the effect of nurses' work hours & fatigue on occurrence of medication errors. In this study, medication error means not only a deviation from the physicians' medication order as written on the patients' chart, but also, any variation from safe medication practice such as washing hands, wrong preparation of medication and wrong administration.

Regarding socio- demographic profile, the study concluded that the nurses' age in both units were in the middle adult hood, the majority of them were females and technician with years of experience less than five years. In addition there were no statistical significant difference in relation to socio-demographic characteristics between ICU and medical oncology nurses. These results were disagreed by (BHP, 2006 and Scott *et al.* 2006) created that 73.7% of nurses had an average age of 41 years, had worked in their current position for over eight years, and all of them were females.

In relation to working hours, most of the nurses had 12 hours working a day and all of them suffered from both acute and chronic fatigue. The result of the current study come into the same line with a study done by Landrigan *et al.* (2004) who reported that higher work hours per day and greater work hours per week generally lead to fatigue and more need for recovery from work.

In addition, ICU nurses perceived more fatigue regarding physical activity than medical oncology unit. The study done by Ruggiero (2003) consistent with the results of the current one whereas she found that ICU nurses are most likely suffer from fatigue, and she added that work hours, anxiety, depression and shift work are likely contributors to fatigue in critical care nurses.

As regards to medication errors the study concluded that about one third of the nurses had medication preparation errors, most of these errors

were: no hand washing and no wearing gloves. In addition, less than one fifth had administration error with most common error is not observing the patient after giving drugs. The result of the study was congruent with Bellebaum (2008) who concluded that thirteen percent of medication errors were administration without hand washing. Also, Mohamed & Gabr (2010) found that nurses work in surgical ICU not follow aseptic technique in preparing medication by making hand washing and nurses did not read medication label, also, they were depending on color and shape of medication packaging when preparing the medication and they did not follow the basic safety measures that require nurse to check the medication product for expiry date.

The study results also concluded that there was no statistical significant difference between ICU and medical oncology units in relation to medication preparation or administration errors. This result was contradicted with what was mentioned by Woodward (2004) who said that intensive care unit patients are high risk for medication errors than other patients. In addition, Bellebaum (2008) reported that a comparative study on medication error in ICUs and medical-surgical units at two hospitals was done and found that medication errors were more likely to be severe in the ICUs than the other wards.

Although the study reported that there was medication errors occurred, but it concluded that there was no statistical significant correlation between working hours and medication errors with both wings of errors preparation and administration. This result was incongruent with two studies done by Maurer (2010) who found that nurses who had worked over 12 hours had a greater mean number for medication errors compared with those who had not worked over 12 hours. The next one done by (Rogers *et al.* 2004 ; Dorrian *et al.* 2006 and Hewitt, 2010) said that the chance of making medication errors were three times higher when nurses worked 12 hours or more in a shift

than those who worked 8 hours or less. **Scott et al. (2006)** found that working more than 40 hours per week increased nurses' errors. While **Bellebaum (2008)** support the result of the current study and found that there was no statistical relationship existed between nurses' total work hours and occurrence of medication errors.

With reference to working hours & fatigue, the study revealed that when working hours increased, fatigue related to concentration and physical activity also increased. The result was contradicted with a study done by **Berger and Hobbs (2006)** which reported that there was no significant correlation between fatigue measured by CIS and the average work hours, however the study added that there was significant correlation between average working hours and need for recovery. However, **Jansen et al. (2003)** support the result of the current study, whereas she found that women who working six or fewer hours per day had significantly lower levels of need for recovery time in comparison with women who work eight hours per day.

As regards to medication errors and fatigue, the study concluded that correlation between medication preparation and administration errors and concentration fatigue subscale. A study done by **Bellebaum (2008)** come on the same vein with the current study and said that twenty – five percent of nurses responded that drug errors occur when they are tired and exhausted. **Shen et al. (2006)** and **Eills (2008)** added that fatigue has deleterious effects on all types of performance manifested in decreased alertness, vigilance, concentration, mood and judgment. However studies done by **Ayas et al. (2006)**; **Barger et al. (2006)**; **Gander et al. (2007)** and **Lockley et al. (2007)** concluded that there was no relationship found between nurses' level of fatigue and occurrence of medication errors.

Also, the study reflected a correlation between years of experience and medication preparation errors in medical oncology unit, indicating that medication preparation errors decrease when years of experience increase. **Maurer (2010)** concluded that nurses over the age of 35 years reported making fewer errors than those under age 35, though this result was not statistically significant, in addition, the researcher found fewer medication errors were reported by nurses who had been in nursing over one year or employed in the same hospital for more than one year. Nursing experience may have an important influence on patient safety. Experienced nurses are more likely to intercept errors compared with less experienced nurses. Physician inexperience and new staff are particular risk factor for medication errors (**Straight, 2008**). However, study done by (**Biron, 2009**) contradicting the current research finding and concluded that the odds of a medication administration errors were

significantly higher among nurses with more than five years of professional experience compared to nurses with less than five years of professional experience.

Finally, the study result found a statistical significant difference between level of education and medication preparation errors in medical oncology units. This result disagreed by (**Hewitt, 2010**) reported that there was no statistics regarding nurses' level of education, specific practice setting were mentioned. However, **Maurer (2010)** found that 61% (n = 208) of nurses had a masters or doctoral degree identifying themselves as advanced nurse practitioners. The majority of these advanced practice nurses would not currently be working as staff nurses in the hospital setting. The respondents' level of education was not analyzed in relation to the number of medication errors. **Joanna Briggs Institute (2006)** recommended that education and training of nurses could improve nurse competence to prevent errors beyond the skills they had already accrued.

Conclusions:

The study done on (49) nurses in the ICU and medical oncology units at National Cancer Institute, Cairo university to examine the effect of nurses' work hours and fatigue on occurrence medication errors concluded that there was statistical significant difference between ICU and medical oncology units in relation to fatigue related to physical activities, moreover, there was no significant correlation between working hours both medication preparation and administration errors in both units. There was statistical correlation between working hours and fatigue related to physical activity items in both units. Finally, there was correlation between medication errors and concentration fatigue subscale, motivation subscale at the ICU unit. Medication preparation errors and concentration fatigue subscale in the medical unit were also correlated.

Recommendation

- Preventing errors and maximizing quality of care for patients requires that those in health care be open to a variety of strategies for modifying work schedules and preventing fatigue that may require changes in long standing behaviors and preferences.
- Nurses must be encouraged to use standard protocols in drug preparation and administration.
- Continues in serves education and training specially for new nurses regarding medication administration was recommended.
- Further researches are needed to study the other correlates of medication errors
Further researches are needed to conduct in other departments in all nurses' shifts.

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Simultaneous biosurfactant production and hydrocarbon biodegradation by the resident aerobic bacterial flora of oil production skimmer pit at elevated temperature and saline conditions

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Abstract: Six aerobic thermo and halotolerant bacterial isolates from an oil production skimmer pit were evaluated for their ability to produce biosurfactants and degrade petroleum hydrocarbons simultaneously under elevated temperature and saline conditions. Phylogenetic analysis using 16S rRNA sequencing revealed that the six bacterial isolates used in the study (SKP-1, SKP-2, SKP-3, SKP-4, SKP-5 and Skp-6) were most homologous to the gammaproteobacteria *Pseudomonas* sp. VS-1, *Pseudomonas aeruginosa* strain S2QPS8, *Serratia marcescens* strain A4, *Pseudomonas stutzeri*, *Pseudomonas stutzeri* strain RA10 and *Pseudomonas stutzeri* strain BOD-3 respectively. Using previously sterilized skimmer pit sample as the sole nutrient, carbon and energy sources and at an elevated temperature of 45°C and salinity (Chloride) level of 6012mg/L, all the bacterial isolates in a mixed culture were able to grow, produce biosurfactants and degrade petroleum hydrocarbons simultaneously by removing about 92% of residual TPH in the skimmer pit within 2 weeks of exposure. This study suggests that in-situ bioremediation procedure using the resident aerobic bacterial flora of the skimmer pit that are thermotolerant and halotolerant can be developed to degrade the petroleum hydrocarbon contaminants in-situ. This bioremediation procedure can be a more attractive and cost effective option than the costly thermal treatment option that is currently in operation in the industry.

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Introduction:

Many hydrocarbon contaminated environment are characterized by extreme environmental conditions such as very low or elevated temperatures, highly acidic or alkaline pH, high saline concentrations and or high pressures. Indigenous hydrocarbon degrading microorganisms associated with such environments have been able to adapt to such extreme environmental conditions and play an important role in the biological treatment of these polluted extreme habitats (Margesin and Schinner, 2001, Muller *et al*, 1998, Niehaus *et al*, 1999 and White *et al*, 1999).

Oil production skimmer pit is one of such extreme environmental habitats because of its elevated temperature and saline conditions. Skimmer pit is a relatively cheap and simple short term storage of liquid oily wastes arising from oil production activities. The pits are dug on the ground and the base and the walls are lined with heavy oil impermeable material such as PVL, Polythene or oil resistant rubber sheeting to prevent ground water and soil contamination. The sheets are pre-lined with sands to prevent the plastic sheet from being punctured by sharp objects. As a result of frequent movement of hot liquids used for washing, flushing and cleaning of oil facility into the skimmer pit, its temperature is

usually elevated ranging between 45 and 60°C (Margesin and Schinner, 2001). Some residual chemicals and salts used in flushing the facility can also find its way into the skimmer pit. When the skimmer pit is filled up, the liquid waste are usually evacuated and treated by thermal means before disposal and this is usually an expensive process.

Most industrial operators are of the opinion that if the liquid wastes in the skimmer pit are treated biologically on the spot, it will reduce drastically the high cost of evacuation, transportation and thermal treatment and this idea motivated the conduct of the present study. Ideally if the indigenous microbial floras of the skimmer pit are capable of degrading the hydrocarbons under its elevated temperature and saline conditions, an appropriate bioremediation technology can be applied to clean up the skimmer pit thus reducing the cost of evacuation, transportation and thermal treatment of the skimmer pit liquid wastes.

Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic contaminants into less harmful, non-hazardous substances which are then integrated into natural biogeochemical cycles. The intensity of biodegradation is influenced by several factors such as nutrients, oxygen, pH value, temperature

composition, concentration and bioavailability of the contaminants as well as the history of the contaminated environment. Temperature plays a significant role in controlling the nature and extent of microbial hydrocarbon metabolism. Bioavailability and solubility of less soluble hydrophobic substances such as the aliphatics and polyaromatic hydrocarbons are temperature dependent. According to Margesin and Schinner 2001, a temperature increase affects the decrease in viscosity which in turn affects the degree of distribution and increase in diffusion rates of organic compounds, the reverse is the case with decreased temperature. Higher degradation rates are therefore expected at elevated temperatures. The increased volatilization and solubility of some hydrocarbons at elevated temperatures also affects toxicity and allows biotransformation with high substrate concentrations (Muller *et al.*, 1998, Whyte *et al.*, 1999). Microorganisms that grow optimally above 40°C are designated as thermophiles. Most thermophiles known are moderate and show an upper temperature border of growth between 50 and 70 °C but optimum growth temperature of extreme thermophiles and hyper-thermophiles occurs at 70-80 and above 80°C respectively (Margesin and Schinner, 2001). The use of thermophiles for biodegradation of hydrocarbons with low water solubility is therefore of interest as solubility and bioavailability are enhanced at elevated temperatures. It is expected that enhanced biodegradation of petroleum hydrocarbons is likely in skimmer pits because of its elevated temperature environments.

We also evaluated the potential for indigenous microorganisms found in skimmer pits to produce biosurfactants which can also enhance biodegradation. Biosurfactants are a structurally diverse group of surface active molecules synthesized by microorganisms. These molecules reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures which make them potential candidates for enhancing biodegradation and oil recovery (Desai and Banat, 1997). Biosurfactants have been used by several investigators to enhance the removal of hydrocarbons from oil contaminated environments (Huang *et al.*, 2009, Okoro, 2009, Inakolu *et al.*, 2004, Bovdoloi and Konwar, 2009).

In the present study, thermo and halo tolerant indigenous bacterial species were isolated from an oil production skimmer pit and their biodegradation and biosurfactant production potential were evaluated. The isolated bacterial species were further characterized by genetic methods using the 16S rRNA sequencing technique.

Materials and Methods:

Physico-chemical analysis of Skimmer Pit samples:

Skimmer pit samples were analyzed for total petroleum hydrocarbon (TPH), salinity, biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen, ammonia-nitrogen, phosphorus, potassium, pH and temperature. TPH was estimated by partition gravimetric method while salinity was measured as chloride by argentometric method as described in Eaton *et al.*, 1995. The BOD and COD were also determined as described in Eaton *et al.*, 1995. Phosphorus and Potassium were estimated by persulphate digestion method while Ammonia nitrogen was determined by titrimetric method as described in Eaton *et al.*, 1995. pH and temperature of the samples were measured with digital Orion pH meters and thermometers respectively.

Isolation of hydrocarbon utilizing microorganisms

Hydrocarbon utilizing microorganisms were isolated with minimal salt media as described in Mills *et al.*, 1978. The media plates contained in petri-dishes were inoculated with 0.1ml of serially diluted samples and inverted over sterile membrane filters moistened with crude oil (Escravos light) as the sole carbon source and held in the lid of the petri-dishes. The dishes were wrapped round with a masking tape so as to increase the vapor pressure within the petri-dishes and the plates were incubated at 32°C for 6 days. At the end of incubation, developed colonies were isolated, sub-cultured and further purified for the purpose of identification.

Determination of optimal growth temperatures of hydrocarbon utilizing microorganisms from the skimmer pit

Optimal growth temperatures were determined by incubating previously sterilized 250 ml samples of skimmer pit inoculated with mixed bacterial culture from previous isolates in a temperature controlled rotary shaker for 7 days. At every 2 day interval, 0.1ml of the samples was withdrawn and population density of the bacteria determined by plate counts.

Biodegradation studies using the Skimmer pit as the sole carbon and nutrient source:

Growth and degradation studies over a time course were carried out by using the skimmer pit samples as the sole carbon and nutrient sources for microorganisms. 250ml of skimmer pit samples were inoculated with 5mls of already prepared starter cultures of mixed bacterial isolates from skimmer pits grown on Mills *et al.*, 1978 media and incubated at

45°C and 50°C in a rotary shaker for 2 weeks. At every week interval, the residual hydrocarbon was extracted with methylene chloride and analyzed in a gas chromatograph.

Gas chromatographic analysis of the hydrocarbon:

1µl of the extracted oil was injected by an auto-injector (7683B series, Agilent Technologies, Santa Clara, CA) into a gas chromatograph (7890N series, Agilent) that was connected to a mass-selective detector (5975C inert XL MSD series, Agilent). The gas chromatograph was equipped with an HP-1 fused silica capillary column (length 50 m, inner diameter 0.32 mm, film thickness 0.52 µm; J&W Scientific) with helium as the carrier gas. The GC-MS system was operated as described by Agrawal *et al*, 2012.

Biochemical Characterization of Biosurfactants produced by the bacterial isolates:

Previously grown pure microbial cultures on Rosenberg *et al*, 1988 medium were centrifuged at 7000g for 30mins to separate the cells from the supernatants. The supernatants were then precipitated with hydrochloric acid as described in Umeji *et al*, 2010 and analyzed for the following;

- a. **Lipid analysis using thin layer chromatography:** Precoated silica gel (20x 20cm) plates with petroleum ether, diethylether and acetic acid (90:1:1) as developing solvents. After air drying, the plates were stained with 5% sulphuric acid in 95% ethanol followed by heating at 150°C for 30mins. The RF values of developed spots were calculated and compared with values of standard compounds in similar solvents as described in Kates (1972).
- b. **Protein analysis:** The protein content of cell extracts was determined using the method of Bradford (1976). Reagents used included Coomassie blue 9250 (0.16ml), perchloric acid (5.15ml), add distilled water to make 200ml. The reagent was stirred in a dark bottle overnight and filtered with Whatman No. 1. Filter paper. Protein extract (0.5ml) was added to 1ml cuvette + 0.5 ml of the reagent. The absorbance at 620nm was read against the reagent blank made up of 0.5ml water + Coomassie reagent. The concentration of the protein was extrapolated from the standard curve prepared with bovine serum albumin as the standard.
- c. **Carbohydrate analysis:** The carbohydrate content of the biosurfactant was estimated using

the anthrone method as described by Spiro (1966).

SDS Polyacrylamide gel (12%) electrophoresis:

To determine the molecular weight of proteins. After development, the gel was stained with Coomassie brilliant blue solution and allowed to stay overnight and photographed. Standard protein markers used include; Lysozyme (egg white) 14,000 DA, Beta-lactoglobulin, Bovine milk (18,400 DA) and egg albumin (45,000DA).

Petroleum products and other hydrocarbons used as emulsifier products

Crude oil was obtained from SPDC, Nigeria while Kerosine and Diesel oil were obtained from NNPC, Nigeria. Olive oil was purchased from a local supermarket in Nigeria while the rest of hydrocarbon substrates used were purchased from Merck chemicals.

Hydrocarbon substrate specificity of crude biosurfactants;

The ability of the bacterial isolates to grow on both pure and mixed hydrocarbon substrates as sole carbon source were tested on a liquid minimal salts media of Mills *et al*, 1978. All the substrates except the highly flammable ones were autoclaved before use, the flammable ones such as n-alkanes, and kerosene were sterilised by filtration before use. 100 ml of the minimal salt media was prepared in a 250ml Erlenmeyer flask and 0.1% hydrocarbon substrate was inoculated followed by the addition of 1ml of the bacterial inoculum from the already prepared nutrient broth and incubation for 48hrs at room temperature. Emulsion turbidity was measured as described in Rosenberg *et al*, 1979.

Determination of emulsification activity:

The standard emulsification assay of Rosenberg *et al*, 1979 was used in the determination of emulsification activity of the bacterial cultures used for the studies. The samples to be tested (0.5-0.1ml) were introduced into a 125ml flask containing TM buffer (20mM Tris-HCL) pH (7.0), 10mM, MgSO₄ to a final volume of 7.5ml and then 0.1ml of a 1:1 (v/v) mixture hexadecane and 2-methylnaphthalene was added. The samples were incubated at 30°C with reciprocal shaking (160 strokes/min) for 1hr. Turbidity was then determined in a Klett-Summerson photometer (fitted with green filter). One unit of emulsifying activity per millilitre is defined as the amount of biopolymer that yielded 100 Klett units in the assay mixture. Emulsion turbidity was directly proportional to the amount of biopolymer produced.

Identification of Bacterial isolates by 16S rRNA sequencing.

DNA extraction and amplification.

Genomic DNA was extracted from 42.5 ml aliquot of the samples using MP Biomedical FastDNA technique as described in the fast DNA Kit, Catalog #6540-400.

PCR amplification and purification for pure microbial cultures

Extracted genomic DNA (2µl) of the bacterial pure cultures were amplified through PCR (94 °C, 7 min; then 30 cycles of 94 °C 10 s, 60 °C 1.30 s, 72 °C 90 s; 72 °C; final hold at 4 °C) using 25µl of nuclease free water and 23 µL of PCR Master Mix comprised of (5 µL PCR buffer, 5 µL Corral load, 10µl Q solution, 1µl dNTPs, 1µl primer forward(EUB 27F), 1µL, primer reverse (EUB 1492R) and 0. 25µl, toptag). PCR product was verified on a 0.7% agarose gel and purified with a QIAquick PCR Purification Kit (Qiagen). The concentrations of the PCR products were determined on a Qubit Fluorometer (Invitrogen). The PCR products were sequenced at the University of Calgary core DNA services.

Bacterial Identification:

Sequences were compared with those in the National Center for Biotechnology information (NCBI) database by BLAST searches and the sequences for each identified bacterial isolate were deposited in Genbank under accession numbers JQ815397-JQ815404

Results:

Physico-chemical analysis;

Physico-chemical analysis of skimmer pit samples showed an in-situ temperature of 40-45°C during sampling and relatively high salinity and TPH concentrations as shown in Table 1. It is obvious that these parameters may vary during rainy season since the skimmer pit is an open pit. From the available data, it can be advanced that the skimmer pit environment can sustain microbial growth and proliferation; it can also be selective because of its elevated salinity and temperature conditions.

Table 1. Physico-Chemical analysis of Skimmer pit samples.

	Parameters measured	Concentration (mg/L)
1	Total Petroleum Hydrocarbon (TPH)	4100
2	Salinity	6012
3	BOD-5	220
4	COD	240
5	Dissolved Oxygen	4
6	Ammonia	2.60
7	Phosphorus	4.50
8	Potassium	120
9	pH	6.9
10	In-Situ Temperature	40-45°C

Isolation and Characterization of Bacterial isolates from oil production skimmer pit.

By using the minimal salt procedure of Mills *et al*, 1978, 6 different bacterial cultures were isolated from skimmer pit samples. The cultures were purified by streaking method and maintained in nutrient agar slants at low temperature (4°C) for further identification. The stages adopted in bacterial identification involved bacterial genomic DNA extraction, purification and amplification by PCR and genetic sequencing. The agarose gel electrophoresis picture of PCR amplified DNA are shown in Fig. 1. While the identified bacterial isolated with their accession numbers are shown in table 2.

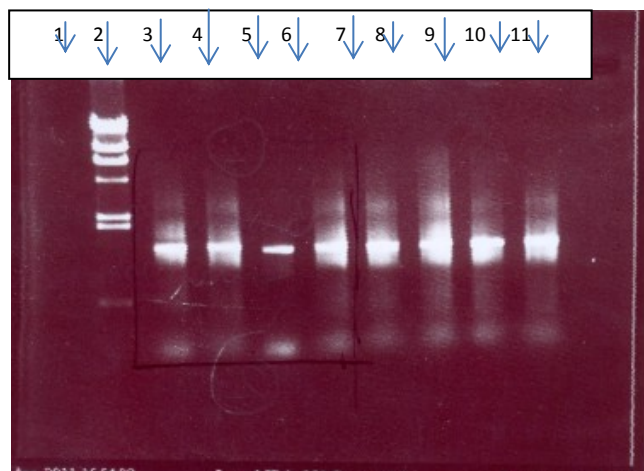


Fig. 1. Agarose gel electrophoresis picture of PCR amplified DNA showing bands that verified the presence of 16S rRNA gene in the bacterial isolates from oil production skimmer pit (Lanes 1 = Negative control, 2 = HIND 111 Ladder, 3 = SKP-1, 4 = Same as 3, 5 = SKP-2, 6 = SKP-3, 7 = Same as 6, 8= SKP-4, 9 = SKP-5, 10 = SKP-6, 11 = Negative control).

Table 2. Accession numbers of 16S rRNA genes and nearest homolog of isolates from skimmer pit samples obtained in the study

Isolate Code	GeneBank Accession Number	Closest GeneBank Homolog	% Identity	Class of nearest Homology	Name of nearest Homology
SKP-1	JQ815397	FJ497685.1	99	Gammaproteobacteria	<i>Pseudomonas</i> sp. VS-1
SKP-2	JQ815398	HQ844502.1	99	Gammaproteobacteria	<i>Pseudomonas aeruginosa</i> strain S2QPS8
SKP-3	JQ815399	JF441244.1	99	Gammaproteobacteria	<i>Serratia marcescens</i> strain A4
SKP-4	JQ815402	AM905852.2	99	Gammaproteobacteria	<i>Pseudomonas stutzeri</i>
SKP-5	JQ815403	JN585674.1	99	Gammaproteobacteria	<i>Pseudomonas stutzeri</i> strain RA10.
SKP-6	JQ815404	JN565980.1	100	Gammaproteobacteria	<i>Pseudomonas stutzeri</i> strain BOD-3

Determination of optimal growth temperatures of the bacterial isolates

The bacterial isolates used in the present study were grown and incubated at various temperatures on minimal salt broth with crude oil as the sole carbon and energy source to determine their various optimal temperatures for growth. The incubation temperatures used were 32°C (Room temperature), 45°C (Skimmer pit temperature) and 55°C (thermophilic temperature). All the bacterial isolates grew well at 32°C, and 45°C but poorly at 55°C. Isolates most homologous to *Pseudomonas* sp. VS-1, *Pseudomonas stutzeri*, *Pseudomonas stutzeri* strain RA10 and *Pseudomonas stutzeri* strain BOD-3 recorded their optimal growth temperatures at 45°C while the isolates most homologous to *Pseudomonas* sp. strain S2QPS8 and *Serratiamarcescens strain A4* had their optimal growth temperatures at 32°C. Detailed results are shown in table 2.

Table 2. Determination of optimal growth temperatures of different bacterial isolates from skimmer pit

Sample Code	Name of nearest homolog	Incubation period in days/Bacterial Population at 32°C (cfu/ml x 10 ⁴)				Incubation period in Days/Bacterial Population at 45°C (cfu/ml x 10 ⁴)				Incubation period in Days/Bacterial Population at 50°C (cfu/ml x 10 ⁴)			
		0	2	4	6	0	2	4	6	0	2	4	6
SKP-1	<i>Pseudomonas</i> sp. VS-1	0.026	33	76	54	0.015	48	84	68.60	0.018	0.008	0.001	0.006
SKP-2	<i>Pseudomonas aeruginosa</i> strain S2QPS8	0.018	46	84	68	0.028	18	28	14	0.022	0.002	0.005	0.004
SKP-3	<i>Serratia marcescens</i> strain A4	0.026	13	46	75	0.011	3.40	28	33.50	0.034	0.008	0.002	0.004
SKP-4	<i>Pseudomonas stutzeri</i>	0.011	48	88	45	0.021	38	112	63	0.016	0.008	0.002	0.006
SKP-5	<i>Pseudomonas stutzeri</i> strain RA10.	0.034	11	48	57	0.018	36	64	46	0.011	0.08	0.06	0.05
SKP-6	<i>Pseudomonas stutzeri</i> strain BOD-3	0.023	12	73	46	0.012	08	80	58	0.020	0.08	0.03	0.01

Note: Skimmer pit temperature during the period of sampling ranged from 40- 45°C.

Biodegradation studies

The mixed bacterial populations from skimmer pit comprising of SKP-1, SKP-2, SKP-3, SKP-4, SKP-5 and SKP-6 were subjected to biodegradation tests using previously sterilized skimmer pit sample as the sole carbon and nutrient source. At every weekly interval, samples were collected and the residual hydrocarbon analyzed by gas chromatograph. It was observed that after 2

weeks of exposure, the mixed bacterial populations from skimmer pit reduced the TPH from its initial value of 4000ppm at week 0 to 330ppm at week 2 (91.7% reduction). The GC chromatograms of the biodegraded oil are shown in Fig. 2.

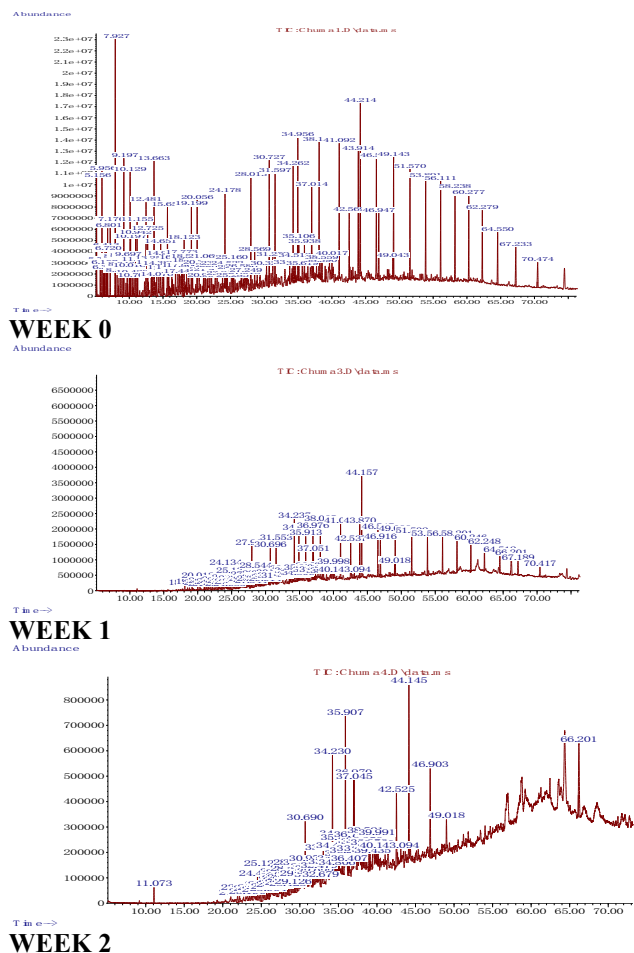


Fig. 2. Biodegradation of petroleum hydrocarbon by mixed bacterial culture isolates from Skimmer pit (Residual petroleum hydrocarbon; Week 0 = 4000ppm, Week 1 = 1100ppm, Week 2 (330ppm)

Biochemical characterization of Biosurfactants produced by the bacterial isolates;

Preliminary biochemical characterization of crude biosurfactants produced by the bacterial isolates showed that the following bacterial isolates namely; SKP-1, SKP-2, SKP-4, SKP-5 and SKP-6 had considerable concentrations of carbohydrates and lipids with no traces of protein and were therefore tentatively classified as glycolipids. *Isolate SKP-3* had both protein and lipid component without any traces of carbohydrate and was tentatively classified as lipoproteins. Detailed results are shown in table 3.

Hydrocarbon substrate specificity of crude biosurfactants.

Emulsifying properties of the biosurfactants produced by the bacterial isolates were evaluated and the various biosurfactants were found to have a very wide substrate specificity emulsifying a wide range of hydrocarbons which include aliphatic hydrocarbons ranging from pentane to octane. Aromatic hydrocarbons which were also emulsified include Benzene, Toluene, Xylene, Butyl benzene and Octyl benzene. Mixtures of hydrocarbon compounds which were emulsified include; Crude oil, Olive oil, Kerosine, Diesel oil, Hexadecane + 2-methyl naphthalene, Benzene + cyclohexane and Toluene + cyclohexane. Detailed results are shown in table 4.

Biological activity of crude biosurfactants:

The biological activity assay on the biosurfactants produced by the bacterial isolates showed relatively high biological activity of all the biosurfactants produced with optimal pH range of 7.01-7.35. The highest biological activity of 47.50 u/ml was observed in the biosurfactants produced by isolate SKP-3. Detailed results are shown in Table 5.

Table 3. Partialbiochemical characterization of bio-surfactants

	Bacterial Isolate	Biochemical composition of the Bio-surfactant			Class of Bio-surfactant Produced	Name of nearest homolog
		Protein (µg/ml)	Carbohydrate (µg/ml)	Lipid (µg/ml)		
1	SKP-1	0	260	15.60	Glycolipids	<i>Pseudomonas</i> sp. VS-1
2	SKP-2	20.30	160	33.50	Glycolipids	<i>Pseudomonas aeruginosa</i> strain S2QPS8
3	SKP-3	16.50	0	14.80	Lipoproteins	<i>Serratia marcescens</i> strain A4
4	SKP-4	0	180	12.20	Glycolipids	<i>Pseudomonas stutzeri</i>
5	SKP-5	0	110	26.50	Glycolipids	<i>Pseudomonas stutzeri</i> strain RA10.
6	SKP-6	0	240	13.50	Glycolipids	<i>Pseudomonas stutzeri</i> strain BOD-3

Table 4. Hydrocarbon substrate specificity of crude biosurfactants produced by high temperature tolerant bacterial isolates from crude oil production skimmer pit.

Hydrocarbon Substrates		Emulsion Turbidity of Bacterial Isolates (KU)					
ALKANES		SKP-1	SKP-2	SKP-3	SKP-4	SKP-5	SKP-6
1	n-Pentane	41	36	56	55	14	36
2	n-Hexane	32	48	55	68	36	53
3	Cyclohexane	28	65	44	33	52	41
4	Decane	54	38	31	14	75	88
5	Pentadecane	86	110	48	77	34	43
6	Hexadecane	55	65	96	36	48	62
7	Octadecane	23	48	42	41	33	78
Aromatics							
8	Benzene	110	67	120	86	28	130
9	Toluene	35	120	65	130	46	86
10	Xylene	56	54	80	110	130	108
11	Buthyl benzene	75	43	41	46	160	140
12	Octyl benzene	78	28	65	35	80	110
Hydrocarbon Mixtures (1:1)							
13	Hexadecane + Methylnaphthalene	130	210	160	240	110	140
14	Benzene + Cyclohexane	85	110	80	65	88	76
15	Toluene + Cyclohexane	45	160	220	41	76	48
Others							
16	Olive oil	120	80	140	53	43	130
17	Kerosine	110	220	260	130	120	210
18	Diesel oil	320	310	360	220	85	320
19	Crude oil	560	480	560	380	310	510

Table 5. Biological activity of biosurfactants produced by high temperature tolerant bacterial isolates from oil production skimmer pit.

	Code number of Isolate	Biological activity (u/ml)	Optimal pH
1	SKP-1	45.30	7.06
2	SKP-2	38.60	7.21
3	SKP-3	47.50	7.12
4	SKP-4	32.10	7.01
5	SKP-5	41.20	7.35
6	SKP-6	36.40	7.01

Discussion:

Chemical analysis of skimmer pit samples used in the present study revealed that it contained substantial micronutrients that can sustain microbial growth and proliferation even though at an elevated salinity and temperature conditions. The use of thermophiles for biodegradation of hydrocarbons with low water solubility such as crude oil is of interest as solubility and thus bioavailability are enhanced at elevated temperatures (Margesin and Schinner, 2001). Temperature plays a significant role

in controlling the nature and extent of microbial hydrocarbon metabolism because bioavailability and solubility of less soluble hydrophobic substances such as aliphatic and aromatic hydrocarbons are temperature dependent according to Margesin and Schinner (2001). Muller *et al*, 1988 and Margesin and Schinner (2001) also advanced that temperature increase affect the decrease in viscosity thereby affecting the degree of distribution and increase in diffusion rates of organic compounds. This means that higher biodegradation rates are expected at elevated temperatures as was observed in our studies.

The skimmer pit that was used in the present study had an elevated temperature that varied from 40-45⁰C and salinity concentration of 6012mg/L. These peculiar environmental conditions were able to sustain the growth and proliferation of some of the six bacterial isolates that were used in the studies. All the six bacterial isolates used in our studies grew well at both 32⁰C and 45⁰C but poorly at 50⁰C suggesting they are thermotolerant but moderate thermophiles. The 6 bacterial isolates when used as a mixed culture were able to degrade about 92% of the residual TPH of the skimmer pit within 2 weeks of exposure at 45⁰C and at a salinity (Chloride) level of 6012mg/L.

Lugowski *et al* (1997) developed a mixture of thermophilic aerobic bacteria comprising predominantly of *Pseudomonas* species that were used to detoxify hydrocarbon contaminated effluent stream at 42°C and this suggests that some *Pseudomonas* species can be thermotolerant. All the 6 bacterial isolates were able to grow well at a high salinity level of 6012mg/L suggesting that they are also halotolerant.

Another interesting aspect of the present study is the production of biosurfactant by the 6 bacterial isolates at the elevated temperature and saline conditions of the skimmer pit. All the 6 bacterial isolates used in the present study produced biosurfactants with high biological activity and wide substrate specificity. The highest biological activity of 47.50µ/ml was recorded with isolate SKP-3 which is homologous to *Serratia marcescens* strain A4 at a pH optimum of 7.2. The biosurfactants produced by the 6 bacterial isolates exhibited wide substrate specificity and emulsified a wide range of substrates from alkanes to aromatics to complex hydrocarbon mixtures. The highest emulsion turbidity in all the biosurfactants tested was recorded with crude oil.

Several authors have implicated both psychrophilic and mesophilic *Pseudomonas* species in biodegradation and biosurfactant production (Javis and Johnson, 1949, Atlas, 1981, Desai and Banat, 1997, Guero-Santos *et al*, 1984, Song *et al*, 2006) but report on the involvement of thermotolerant and halotolerant *Pseudomonas* species in biodegradation and biosurfactant production is scanty in literature. Some investigators like Kummer *et al*, 2008 have isolated halotolerant and thermotolerant *Pseudomonas* species from oil contaminated soils that are capable of producing biosurfactants and degrade hydrocarbon at a temperature of 45°C and salinities up to 6%. Margesin and Schinner (2001) have also investigated the biodegradation and bioremediation of hydrocarbons under extreme environmental conditions while Cameotra and Makkar (1998) investigated the synthesis of biosurfactants under extreme environmental conditions. Our investigation revealed that simultaneous production of biosurfactants and biodegradation of hydrocarbons can be achieved in an extreme environmental condition such as that of skimmer pit by the resident microbial flora that were able to adapt to such harsh environmental conditions. *Serratia marcescens* have also been implicated by many investigators in petroleum hydrocarbon biodegradation (Ijah, 1998, Okoro, 1999, Wongsa *et al*, 2004,) and biosurfactant production (Roldan-Carrilo *et al*, 2011, Anyanwu *et al*, 2011) but our work was the first to implicate the halotolerant and thermotolerant isolates that are homologous

to *Serratia marcescens* in both biodegradation and biosurfactant production.

Simultaneous hydrocarbon biodegradation and biosurfactant production by oil field bacteria as demonstrated by Okoro, 2009, 2010 and Mnif *et al*, 2011 can be used to gradually eliminate environmental hydrocarbon pollutants and we have further demonstrated in the present study that rapid elimination of hydrocarbon pollutants from the skimmer pit can also be achieved even at its elevated saline and temperature conditions using resident bacteria that both produce biosurfactants and degrade petroleum hydrocarbons simultaneously.

Conclusion:

The present study was able to establish that the 6 bacterial isolates from skimmer pit used in the present study were able to carry out biodegradation and biosurfactant production simultaneously at the skimmer pits' elevated saline and temperature conditions. This is an indication that in-situ biodegradation procedure can be carried out successfully by the resident microbial flora of the skimmer pit. From the economic point of view it will be cheaper to carry out an in-situ bioremediation procedure on the skimmer pit to remove the petroleum hydrocarbon contaminants than to adopt the very costly thermal treatment processes that involves costly evacuation of wastes, transportation and offsite thermal treatment.

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Role of gender (man and woman) in predicting occupational stress considering organizational commitment and personality types (Myers - Briggs) in bank staff

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Abstract : This research studies effect and role of gender (man and woman) in predicting occupational stress considering personality types (Myers - Briggs) and organizational commitment of staff of Shahr bank of Iran. Statistical Society in this research is all employed men and women in Shahr bank that 96 women and 204 men were selected by random cluster sampling. This study has been conducted using statistical analysis by correlation and stepwise regression methods and Philip L. Rice occupational stress questionnaire, Myers - Briggs personality type's questionnaire and Alan Meyer organizational commitment questionnaire have been used. The results showed that in men group organizational commitment subscales along with introversion and emotional poles are suitable predictors for occupational stress while in women group organizational commitment subscales along with emotional subscale among eight personality pole are suitable predictors of stress. Thus it is expected introverted men with emotional function that have lower organizational commitment suffer from higher occupational stress than other men and women with emotional behavior and lower organizational commitment suffer from higher occupational stress than other women.

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Key words: gender, occupational stress, organizational commitment, personality types

1. Introduction

Our behavior is the result of a natural interaction between personality and situation that most often, leads to obstacles in prediction of how individuals may behave (12). Each individual's personality is unique, i.e. aside from the similarity that exists between people; every human being has unique features that differentiate him/her from others. Various perceptions of the concept of personality present that as the time passes, the concept of personality have gone beyond its former visual and social appearance, and personality currently refers to a substantial and sustained process of each individual (Schultz, 1998). In Jung's perspective, a great deal of our conscious perception and reactions to our environment is determined by opposite introversion and extraversion mental attitudes. After recognizing various types of extraversion and introversion, he considered another integrated distinction between individuals according to which he called the psychological functions. These functions point out the different ways and contradictory understanding of the real world outside and the inner world of our mental. Jung knows the four mental functions as: Sensing, intuition, thinking and feeling (Jung, 1927).

Success of organizations and workplaces is completely dependent on effective application of human labor resources based on behavioral science and this is a challenge that has really made supervisors,

managers, practitioners and organization experts busy. Undoubtedly, circumstances under which it is tried to have something done can have an impact on the efficiency and the speed of attempts. Whenever something is done, the environment will be ready to address it in its actual dimensions spontaneously (Parsaeiyan ,2004) Researchers know organizational commitment as an important factor in understanding employees' behaviors(McMurraray, 2004). Studies have demonstrated that organizational commitment, performance of employees, external organizational data such as tendency to leave the service have an influence on the sales, profitability of the organization and staff absenteeism so that the existence of organizational commitment have resulted in an increase in profitability and a reduction in desertion; and by affecting performance of employees lead to an increase in productivity, an improvement in quality of services performed (Omalley, 2004).

Occupational stress is a phenomenon addressing which is essential in order to maintain the physical and mental well-being and health of people and increasing the efficiency of various organizations. Occupational stress has a variety of sources that can be all divided up into two categories:

Individual factors and characteristics

Working factors and characteristics(Zaharakar, 2005).

Jex & Beehr (1991) classified the reactions expressed by people dealing with stress as the following two elements:

Psychological reactions such as anxiety, anger, frustration, and job dissatisfaction

Physical reactions such as smoking, high blood pressure, and so on (Jex & etal (1991).

Ross & Altmaier believe that despite the difficulty of evaluation of the costs of occupational stress, one can achieve assured data about it. They also believe that if we look at this stress from the aspect of reactions shown by individuals regarding their employment conditions, we encounter data illustrating the impacts of common emotional and behavioral reactions with numbers and figures; for instance, alcoholism puts over two hundred million dollars on American industries annually. A mental illness such as depression that may be caused by occupational stress makes damages in forms of medical expenses and loss of working hours and production. Other costs resulting from stress, indeed, evaluate the effects of employees' performances at work, for example, absenteeism from work is estimated as a really costly problem. In fact, four percents of working hours are lost due to employees' absenteeism and the estimated financial loss is millions of dollars. Another effect of occupational stress is the destruction of close family members, particularly wives, children and other relatives that leads to loss of job opportunities and achievements and hurts the quality of life (Rass, 1996). Stress has a close relationship with lack of financial stability and working forces, especially the lack of financial security and its volatilities are among the factors affecting the occupational stress. Nowadays, the economic volatilities exert various influences on global markets and industry, among which one of the most prominent impacts is on manufacturing centers, businesses, markets, and employees at lower levels centers and is a factor for the growth of occupational stress (Nasurdin & etal 2006).

Cooper & Caret Wright (1996) also believe that each career is a potential environmental source of stress. One of the major types of stress, whose addressing seems of great importance today in order to provide welfare and physical and mental health to individuals and increase productivity in various organizations, is occupational stress. Ross & Altmaier have offered some classification in this field that includes six factors as: inner job, agents, communication in the workplace, career development, organizational factors, and the conflict between work and the house (Cooper, & etal (1996).). In a survey carried out by Princeton Survey Research Association in 1997, servants have also been under job stress comparing the previous generation at a degree of three

fourth. In 1992, one of the insurance companies of the US reported that 25% of servants know their job as the first factor resulting in stress (Sauter & etal 2005). Occupational stress, depression, and familial crisis are the three major obstacles in organizations. National Mental Health Association knows stress as the source of more than 90% of diseases that costs the organizations a fortune (Butcher & etal. 2007). According to Health and Safety Executive's reports, almost half million people in Britain experience stresses resulted by their careers and this leads to lots of illnesses for them and the trend is still upward (Jones & etal 2003).

Occupational stress has many physical symptoms for individuals. Being disposed to stress for a long period of time leads to numerous physical disorders, as a matter of fact, 70 to 80 percents of illnesses are related to stress like Cornell vascular damage, cancers, Migraines, and warts which all are the most associated with stress. Similarly, high level of stress in women causes Insomnia, wounds, and intense anxiety (Shamlou, 2003). Damages resulted from occupational stress among men and women are increasing day after day. As we know each day employed women in different occupational fields are increased and regarding men and women's different features in personality fields, occupational commitment, job interests and their special abilities predicting their occupational stress is different from other group. The researcher predicts men and women's occupational stress using Myers - Briggs personality types and organizational commitment of bank staff to explain these differences scientifically.

2. Method & Material :

This research is a fundamental research and its purpose is to explore relationships between variables and research method is correlation. To analysis data descriptive statistics method has been used like Frequency tables, graphs, calculating means, SD and also statistical indices have been used to test hypothesis by correlative method and multi variables regression. To determine type of bank staff personality, Mayers- Brigs questionnaire has been used that is inferential from Gustav Jung theory and this questionnaire is one of the most famous questionnaires in world and has been populated in Iran by Jahanian (2006). Mayers-Brigs questionnaire, sixth edition European-English volume, is an instrument with 88 questions and includes 25 questions in extroversion – introversion dimension, 19 questions in sensory- intuition, 24 questions in thinking-feeling and 19 questions in judging-perceiver dimension and it has one extra question that doesn't include test scoring (Yiannakis, C. & Taylor, N.(2009). Philip. L.Rice questionnaire (1991) has

been used to determine occupational stress level of bank staff(124). This test has 57 items with some information about occupational stress. It has been translated and standardized for the first time by Hatami (1999). Allen and mayer Organizational commitment questionnaire (1993) has been used to evaluate occupational commitment of staff. This questionnaire has been made by Allen, Mayer and Smith in 1993 to measure and evaluate three dimensions of occupational commitment includes feeling, continuous and normative commitment with 18 questions. The statistical society in present research

includes: all woman at 96 and all of the men at 204 employees of City Bank in Tehran who are working in 2011 that selected cluster randomly

3. Result

This in research, the table 1 shows correlation coefficients between the variables and their subscales are presented in two groups of men and women. Then results of entering the sex variable in the regression analysis will be discussed and regression analysis is presented step by step in two groups of men and women.

Table 1, The correlation coefficient matrix of variables according to sex

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Stress	-	0.75**	0.85**	0.73**	0.67**	0.57**	0.59**	0.50**	0.15	-0.24*	0.24*	0.29**	0.29**	0.02	0.24*	-0.20*	0.23*
2. Interpersonal relations	0.65**	-	0.40**	0.47**	0.46**	0.38**	0.40**	0.38**	0.06	-0.10	0.13	0.23*	-0.19	-0.08	0.18	-0.26*	0.21*
3. Physical health	0.82**	0.31**	-	0.42*	0.55**	0.50**	0.46**	0.37**	0.13	-0.21*	0.22*	0.21*	-0.25*	0.11	0.19	-0.02	0.11
4. Occupational Interests	0.68**	0.41**	0.42**	-	0.55**	0.41**	0.51**	0.46**	0.15	-0.19	0.15	0.26**	-0.24*	-0.21*	0.24*	0.28**	0.25*
5. Organizational commitment	0.68**	0.35**	0.57**	0.46**	-	0.89**	0.82**	0.22**	0.30**	0.31**	0.33**	0.38**	0.33**	-0.04	-0.05	0.24*	0.26**
6. Continuous Commitment	0.57**	0.25**	0.51**	0.40**	0.84**	-	0.62**	0.47**	-0.26*	0.32**	0.32**	0.44**	0.39**	-0.12	-0.02	0.18	-0.22*
7. Emotional commitment	0.47**	0.28**	0.41**	0.26**	0.74**	0.42**	-	0.43**	0.30**	0.30**	0.27**	-0.17	0.13	0.14	-0.07	0.15	-0.14
8. Normative commitment	0.47**	0.25**	0.37**	0.37**	0.63**	0.33**	0.30**	-	-0.15	0.11	-0.21*	0.27**	0.25*	-0.10	-0.04	0.27**	0.31**
9. Brigades	0.25**	0.07	0.22**	0.10	0.21**	-0.18*	0.24**	-0.08	-	0.79**	0.76**	0.02	-0.02	0.03	-0.13	-0.11	0.12
10. Extraversion	0.33**	-0.10	0.37**	0.18**	0.30**	0.27**	0.25**	0.14*	0.78**	-	0.92**	-0.12	0.11	-0.16	0.19	0.15	-0.19
11. Introversion	0.35**	0.10	0.39**	0.21**	0.31**	0.28**	0.24**	0.19**	0.78**	0.93**	-	0.12	-0.08	0.16	-0.12	-0.21*	0.26*
12. Sensing	0.07	0.01	0.14*	0.04	-0.04	-0.12*	-0.12*	-0.13*	0.11	-0.13*	0.12*	-	0.90**	0.21*	-0.05	-0.11	0.09
13. Intuition	-0.02	-0.01	-0.11	-0.10	0.04	0.03	0.07	0.10	-0.09	0.09	-0.06	0.89**	-	-0.26*	0.08	-0.01	0.06
14. Thinking	0.26**	-0.17*	-0.17*	0.27**	0.22**	0.12	0.15*	0.06	0.07	-0.09	0.08	0.12*	0.18**	-	0.59**	0.39**	0.32**
15. Feeling	0.33**	0.26**	0.24**	0.33**	0.30**	0.18**	0.18**	-0.13*	-0.09	0.07	-0.02	-0.06	0.12*	0.71**	-	0.28**	0.31**
16. Judgment	0.26**	-0.14*	0.19**	0.22**	0.29**	0.24**	0.13*	0.23**	-0.05	0.12*	0.19**	0.08	0.20**	0.22**	0.38**	-	0.93**
17. Perception	0.29**	0.13	0.23**	0.24**	0.33**	0.29**	0.17**	0.24**	0.08	0.16**	0.24**	-0.07	0.21**	0.35**	0.38**	0.92**	-

Note: Correlation coefficients in males (n = 204) are located at the bottom of the core diameter and correlation coefficients in females are located at the top of the core diameter (n =96). * P <0.05, ** P <0.01

Due to differences in the correlation coefficients in both male and female group, gender variable entered in the regression analysis as a moderating variable that results were not significant and gender variable didn't show a significant Beta coefficient (-0/02) (t = -0.38 , P > 0.05)

In the next tables, stepwise regression analysis results are presented to predict occupational stress in both men and women separately.

Regarding the above table and the last model of stepwise regression analysis for men, it is observed that Beta coefficient is significant for variables of continuous commitment (t=-5.41, p<0.01) normative (t=-4.89, p<0.01) ,emotional (t = -3.46, p <0.01), introversion (t = 3.19, p <0.01) and emotional (t = 3.15, p <0.01) and the best predictor is continuous commitment that has highest Beta (-0.32) and the lowest predicting power is belonging to emotional subscale (0.16). Occupational stress is predicted in men inversely by three subscales of organizational commitment and directly by subscales of introversion and feeling. In the next table results of regression analysis have been shown step by step in women group that is different with resulted results of men group.

Table 2, standardized coefficients, multi variable squared multiple correlation coefficient and its changes in the stepwise model of predicting occupational stress for men

Μοοοοδελ	variable	beta	R ²	ΔR ²	t	F
1	continuous commitment	-0.57	0.33	0.33	-9.09**	98.61**
2	continuous commitment	-0.47	0.42	0.09	-8.15**	71.71**
	normative	-0.31			-5.52**	
3	continuous commitment	-0.37	0.46	0.04	-5.19**	57.63**
	normative	-0.29			-6.32**	
	emotional	-0.24			-4.19**	
4	continuous commitment	-0.34	0.49	0.03	-5.81**	46.93**
	normative	-0.27			-5.06**	
	emotional	-0.22			-3.88**	
	introversion	0.15			2.90**	
5	continuous commitment	-0.32	0.51	0.02	-5.41**	41.20**
	normative	-0.26			-4.89**	
	emotional	-0.19			-3.46**	
	introversion	0.17			3.19**	
	feeling	0.16			3.15**	

** P < 0.01

Table 3, standardized coefficients, multi variable squared multiple correlation coefficient and its changes in stepwise model of predicting occupational stress for women

	Variable	beta	R ²	ΔR ²	t	F
1	Emotional commitment	-0.59	0.34	0.34	-7.01**	49.15**
2	Emotional commitment	-0.45	0.42	0.08	-5.17**	33.78**
	Normative commitment	-0.31			-3.53**	
3	Emotional commitment	-0.44	0.46	0.04	-3.61**	25.96**
	Normative commitment	-0.31			-5.14**	
	feeling	0.19			2.53*	
4	Emotional commitment	-0.30	0.50	0.04	-3.15**	22.59**
	Normative commitment	-0.24			-2.79**	
	Feeling	0.20			2.71**	
	Continues commitment	-0.27			-2.68**	

* P < 0.05, ** P < 0.01

Regarding the above table and output having four models for stepwise regression, it is observed in the last model of regression analysis that Beta coefficients for the variables of emotional commitment (t = -3.15, p < 0.01), normative (t = -2.79, p < 0.01), emotional (t = 2.71, p < 0.01), and continuous commitment (t = -2.68, p < 0.01) is significant and the best predictor is emotional commitment variable that has the highest Beta (-0.30) and the lowest predict power belongs to emotional subscale (0.20). Like men group, three subscales of organizational commitment reversely and the emotional subscale directly predict occupational stress in men. Thus in the men's group organizational commitment subscales alongside with two poles of

introversion and emotional are good predictors for occupational stress while in women group commitment subscales alongside with emotional subscale among eight personality poles are suitable stress predictors. It is expected that introvert men with emotional function that have organizational commitment, endure higher occupational stress than other men as women with emotional encountering mode and with lower commitment endure higher occupational stress than other women.

4. Conclusion

This research has been done to examine gender (man and woman) role of bank staff in predicting their occupational stress according to their personality types

and their organizational commitment. After determining personality types of men and women, the researcher has studied differences of these types in men and women related to their occupational stress and s/he determined that there are some differences between different genders in predicting occupational stress. The results of this research showed that there is meaningful difference between men and women in predicting their occupational stress. So in men group subscales of organizational commitment alongside two poles of introversion and emotional are suitable predictors for occupational stress while in women group subscales of commitment alongside emotional subscale are out of 8 personality poles that are suitable predictors for stress. So it is expected that introvert men with emotional performance who have lower organizational commitment endure higher occupational stress than other men, just as women with emotional encountering and lower commitment have higher occupational stress than other women. Consequently by using this research it can be shown that considering personality types and gender (man and woman) of normal people in society is very important in predicting occupational stress.

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Determinants of Agricultural Intensification in Southwest Nigeria

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Abstract: Declining agricultural production in many developing countries has prompted increased use of some inputs while continuous cropping prevails. This study analysed the factors promoting different forms of agricultural intensification in southwestern Nigeria. Data collected from randomly selected farmers in selected states in southwestern Nigeria were used. Results show that farmers from Osun State have the highest indices of intensification with respect to land use intensity, fertilizer use intensity and crop diversification. The censored regression showed that lost working days, use of fertilizers, crop rotation, and having more inherited land increased land use intensity while use of organic manure, minimum tillage and poverty reduced crop diversification index. Fertilizer use intensity increased with the use of minimum tillage and household size while hired and family labour use intensity increased with household size. It was recommended that in the face of increasing land degradation, farmers' access to fertilizer must be increased and efforts to reduce their poverty level must be promoted, among others.

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Keywords: cocoa, climate change, technical efficiency, stochastic frontier

Introduction

The use of land for agricultural production remains one of the strongest influences affecting environmental quality in many developing countries. Practices like unguided application of agrochemicals, bush burning and mechanized land cultivation affect the quality of soil and vegetative cover (Scherr, 1999). Policy makers are now confronted with the challenges of increasing agricultural production to stimulate economic growth and reduce poverty, while the issue of natural resource degradation requires urgent attention (Vosti, 2001).

Conventionally, intensive agricultural production can be expressed as increase in the use of inputs of labour or capital on a smallholding, in order to increase output per hectare. Alternatively, agricultural intensification can be defined as an increase in agricultural production per unit input of labour, land, time, fertilizer, seed, feed, or cash (FAO, 2004). Boserup's "induced innovation" concept asserts that increasing population stimulates increasing demand for agricultural products. As land, therefore, becomes more costly compared with labour, incentives emerge for more intensive use of land in order to reap the benefits of the enlarged market opportunities. Similarly, Cleaver and Schreiber (1994) hypothesized the "downward spiral concept". This states that poverty, overpopulation, and land degradation create a self reinforcing nexus that further promote degradation of natural resources and poverty. This is because the process of soil mining in

the form of agricultural intensification triggers soil erosion and results in decline in land productivity.

The downward-spiral and induced innovation scenarios have been found under different situations (Pender, 1998). A comparison of the downward spiral and induced innovation revealed that outcome is largely dependent on how well a society adapts to rapid population growth, globalization, market development, technological change, climatic change, and agro-ecological conditions. Forsyth et al., (1998) showed that poor people increasingly exploit natural resources as a result of population growth, limited access to land, access to marginal land of low productivity and limited resources for investment. This situation led to lack of resources for sustainable resource management and declining food production. Several authors have also noted that decline in wages and unemployment among the poor can result in increased land clearing and deforestation.

Bourn and Wint (1994) noted that in SSA, livestock biomass increases with rising levels of human population and increasing intensity of land use. It was stressed that the findings are consistent with expectations of the "Boserup hypothesis", and reflect the process of "autonomous intensification" of agricultural production (Boserup, 1981), through initial co-existence and gradual integration of animal husbandry within local farming systems.

Osemeobo (1993) evaluated the land use pattern of smallholders for cassava production in

Southern Nigeria. He found that the users' rights on land, farm yield and access to farmland all influenced land-use intensity. Okike et al., (2001) determined the factors influencing agricultural intensification in the northern part of Nigeria. The results showed that land use intensity was largely influenced by land-man ratio, herd size, rate of application of manure and fertilizers, crop labour and years of experience in mixed farming. Also, land use intensity increased as land human population ratio increased but decreased at very high land human population ratio due to the possibility of labour shortages. Also, increased fertilizer application led to increase in labour use intensity, but the reverse was the case for manure application per hectare.

Randrianarisoa and Minten (2001) found that in Madagascar, poorer households had low labour productivity, but their return to land was high. It was recommended that much economic gains would result if poor farmers have access to fertile land. Also, the analysis revealed that sound education, always influenced agricultural production. While the poor depend on upland crops, diversification into high value crops by the rich enhances economic welfare. This paper attempts to determine the factors influencing agricultural intensification in southwest Nigeria. The specific objectives are to compute some indicators of agricultural intensification and describe them, and analyse the effect of some socioeconomic and cultural factors on the indicators of intensification.

Materials and methods

The data

Data for the study were collected from Oyo, Osun and Ekiti states in the southwestern part of Nigeria. The states enjoy tropical climate with two distinct seasons – rainy season from April to October and dry season from November to March. The traditional practice of slash and burn agriculture predominates, and this is expected to be followed by a period of fallow for the soil to regain lost fertility.

The multistage random sampling procedure was used. The first stage involved random selection of three states from the southwest Nigeria. At the second stage, two Local Government Areas (LGAs) were randomly selected from each of the chosen states. The third stage involved the random selection of three villages from each of the selected LGAs, from where households were selected for interview. A total of 350 questionnaires were administered based on available cost and time. Samples were selected in proportion to the estimated population of farmers in the villages.

In Oyo State, the selected LGAs were Akinyele and Lagelu. A total of 120 questionnaires were administered out of which only 100 were good for inclusion in the final analysis. In Ekiti State, a total of 110 were administered to farmers in Ikole, and Ado Ekiti LGAs. Out of the questionnaires administered, only 100 were good for inclusion in the final analysis. In Osun State, a total of 120 questionnaires were administered in Obokun and Ife Central LGAs. Out of the administered questionnaires, 103 were good for inclusion in the final analysis. The rejected questionnaires contained insufficient information.

Analytical procedures

In order to analyse the socioeconomic/cultural factors explaining some indicators of agricultural intensification, a censored Tobit regression analysis of covariance (ANCOV) was used (Okike et al., 2001). The estimated models are stated below:

$$LUI_i = \beta_1 + \beta_2 EXP_i + \beta_3 DBB_i + \beta_4 DMC_i + \beta_5 DOM_i + \beta_6 DCC_i + \beta_7 EDC_i + \beta_8 SZE_i + \beta_9 DZTi + \beta_{10} FCI_i + \beta_{11} FCB_i + \beta_{12} FCP_i + \beta_{13} MKD_i + \beta_{14} POV_i + \beta_{15} LWD_i + \beta_{16} DFT_i + e_i \dots \dots \dots (1)$$

$$CDI_i = \varphi_1 + \varphi_2 EXP_i + \varphi_3 DBB_i + \varphi_4 DMC_i + \varphi_5 DOM_i + \varphi_6 DCC_i + \varphi_7 EDC_i + \varphi_8 SZE_i + \varphi_9 DZTi + \varphi_{10} FCI_i + \varphi_{11} FCB_i + \varphi_{12} FCP_i + \varphi_{13} MKD_i + \varphi_{14} POV_i + \varphi_{15} LWD_i + \varphi_{16} DFT_i + f_i \dots \dots \dots (2)$$

$$FUI_i = \sigma_1 + \sigma_2 EXP_i + \sigma_3 DBB_i + \sigma_4 DMC_i + \sigma_5 DOM_i + \sigma_6 DCC_i + \sigma_7 EDC_i + \sigma_8 SZE_i + \sigma_9 DZTi + \sigma_{10} FCI_i + \sigma_{11} FCB_i + \sigma_{12} FCP_i + \sigma_{13} MKD_i + \sigma_{14} POV_i + \sigma_{15} LWD_i + g_i \dots \dots \dots (3)$$

$$FLI_i = \rho_1 + \rho_2 EXP_i + \rho_3 DBB_i + \rho_4 DMC_i + \rho_5 DOM_i + \rho_6 DCC_i + \rho_7 EDC_i + \rho_8 SZE_i + \rho_9 DZTi + \rho_{10} FCI_i + \rho_{11} FCB_i + \rho_{12} FCP_i + \rho_{13} MKD_i + \rho_{14} POV_i + \rho_{15} LWD_i + \rho_{16} DFT_i + h_i \dots \dots \dots (4)$$

$$HLI_i = \rho_1 + \rho_2 EXP_i + \rho_3 DBB_i + \rho_4 DMC_i + \rho_5 DOM_i + \rho_6 DCC_i + \rho_7 EDC_i + \rho_8 SZE_i + \rho_9 DZTi + \rho_{10} FCI_i + \rho_{11} FCB_i + \rho_{12} FCP_i + \rho_{13} MKD_i + \rho_{14} POV_i + \rho_{15} LWD_i + \rho_{16} DFT_i + k_i \dots \dots \dots (5)$$

Where β_s , φ_s , σ_s , ρ_s are the estimated parameters ($s = 0 \dots 16$) and i refers to individual farmers ($i = 1 \dots 303$)
 LUI_i = land use intensity of i th farmer measured by the modified Rutherberg's index (Rutherberg, 1980).
 $LUI_i = A_i / L_i$ with A_i = number of seasons the land was cultivated by i th farmer, L_i = total number of seasons land would have been cultivated if under continuous cropping.

CDI_i = crop diversification index measured by the

$$\text{Herfindal Index which is } \sum_{i=1}^{13} \left(\frac{C_i}{\sum_{i=1}^{13} C_i} \right)^2 \text{ with } C_i \text{ being}$$

the area of land planted to *i*th crop.

FUI_i = fertilizer use intensity [fertilizer applied (kg)/land area sq meter]

FLI_i = family labour use intensity (number of family labour (man day)/land area (ha))

HLI = hired labour use intensity (number of family labour (man day)/land area (ha))

LWD_i = lost working days due to sickness

EXP_i = years of farming experience

SZE_i = household size

POV_i = poverty rate (poverty line [2/3 mean per capita expenditure of the population]/mean per capita expenditure of *i*th household)(Foster et al., 1984).

LAF_i = land area fallowing (ha)

FCI_i = food cropland areas inherited (ha)

FCB_i = food cropland areas borrowed (ha)

FCP_i = food cropland areas purchased (ha)

DCR_i = dummy variable for using crop rotation (yes = 1, otherwise = 0)

DFT_i = dummy variable for using fertilizers (yes = 1, otherwise = 0)

DCC_i = dummy variable for using cover crops (yes = 1, otherwise = 0)

DBB_i = dummy for bush burning (yes = 1, 0 otherwise)

DED_i = dummy for education (formal education = 1, otherwise = 0)

DMC_i = dummy for using mulching (Yes = 1, otherwise = 0)

DOM_i = dummy for using organic manure (Yes = 1, otherwise = 0)

DZT_i = dummy for using zero tillage (Yes = 1, otherwise = 0)

e_i, f_i, g_i, h_i, k_i = residual/error terms

Results and discussions

The results in Table 1 show that 91.75% of the house heads in all the states are male. Average age is highest in Ekiti State with 54.17 years, while all the farmers have an average of 52.83 years. In like manner, average farming experience is highest in Ekiti State with 31.17 years while farmers from all the states have an average of 28.75 years. Ekiti State records the highest average household size of 7.19 persons, while Osun State records the highest percentage of 57.28 being formally educated.

Table 1: Some Socio-economic Variables of the Farm Households in Southwestern Nigeria

Variable	Oyo State	Ekiti State	Osun State	All States
Total number of households	100	100	103	303
Male farmers (%)	96.00	92.00	87.37	91.75
Age of house head (mean)	50.22	54.17	54.06	52.83
Years of farming (mean)	26.69	31.17	28.42	28.75
Household size (mean)	6.68	7.19	6.74	6.87
Formal education (%)	52.00	57.00	57.28	55.44

The upper segment of Table 2 shows the indices of agricultural intensification. Results show that land use intensity is highest in Osun State with 95.43%, while Ekiti State has the lowest (51.20%). The three states have an average land use intensity of 71.09%. This shows that continuous cropping is most predominant among farmers from Osun State.

Table 2: Agricultural Intensification Indices and Use of Some Cultural Practices in Southwestern Nigeria.

Variable	Oyo State	Ekiti State	Osun State	All States
<i>Index of Intensification</i>				
Land use intensity (mean)	65.90	51.20	95.43	71.09
Crop Diversification (Mean)	18.48	43.19	68.19	43.53
Fertilizer use intensity (kg/sq meter)	0.0070	0.0056	0.0092	0.0073
Labour use intensity (family) (man day per hectare)	31.22	31.90	27.03	29.75
Labour use intensity (hired) (man day per hectare)	16.94	18.15	26.19	20.23
<i>Usage of land management/cultural practices (%)</i>				
Mulching	62.00	42.00	71.84	58.74
Crop rotation	64.00	48.00	88.35	66.99
Organic manure	30.00	29.00	14.56	24.42
Fertilizer application	69.00	47.00	82.52	66.34
Cover crop	25.00	29.00	24.27	26.07
Bush burning	70.00	73.00	79.61	74.26

Also, using crop diversification as index of agricultural intensification, the indices were computed. The crops planted were maize, tomatoes, leafy vegetables, okro, melon, cassava, cocoyam, plantain/banana, pepper, soybean, yam and cowpea. Farmers from Osun State have the highest output diversification index of 68.19%, while Oyo State has the lowest (18.48%). Average output diversification for the three States is 43.53%.

Intensity of fertilizer use is another form of agricultural intensification. The analysis reveals that Osun State farmers have the highest (0.0092), while Ekiti State has the lowest (0.0056). It can be deduced that allowance for fallowing as shown by land use intensity decreases where fertilizer usage is high. Intensity of fertilizer use is an average of 0.0073 kg/m² for all the three States.

Farmers from Ekiti State have the highest family labour use intensity (31.90 man-day per hectare), while those from Osun State have the lowest (27.03 man day per hectare). However, Osun State farmers have the highest hired labour use intensity (26.19 man-day per hectare), while Oyo State farmers have the lowest (16.94 man-day per hectare).

The lower segment of Table 2 shows the use of land management practices, which indirectly depict intensification. The results show that 71.84% (highest) of the farmers in Osun State were using mulching, while 42% (lowest) used it in Ekiti State. Also, 88.35% (highest) of the farmers in Osun State were using crop rotation, while 48% (lowest) used it in Ekiti State. The use of organic manure is highest in Oyo State (30%) and lowest in Osun State (14.56%). Fertilizers were applied by 82.52% of the farmers in Osun State, while only 47% use it in Ekiti State. Bush burning was most widely used in Osun State (79.61%), while cover crops were most widely planted in Ekiti State (29%).

Table 3 shows the results of Tobit maximum likelihood estimates for the determinants of agricultural intensification (estimated with Limdep 7.0 statistical package). The sigma values for all the equations are statistically significant ($p < 0.01$). This shows that the model produced good fit for the data. The Condition Index was estimated with SPSS 10.0 statistical package in order to determine the collinear variables. However, age as a variable was removed due to its high level of collinearity with farming experience. The low values for the estimated condition index reveals that multicollinearity was not a problem in the estimated models.

The estimated parameters show that the farmers that were using slash and burn method of land preparation have significantly higher labour use intensity ($p < 0.10$). This might have resulted from

employment of many family hands for such menial job of bush gathering, stumping and burning. The practice of mulching requires significantly higher use of hired labour ($p < 0.01$). This might be due to the fact that farmers that indicated mulching were those planting yam which requires ridge making. The tediousness of ridging may therefore make them employ more of hired labour. Also, the use crop rotation significantly increases land use intensity ($p < 0.01$). This can be explained from the fact that rotating crops enhances productivity of land and this may facilitate continuous cropping. Also, the use of organic manure significantly reduces land use intensity and crop diversification ($p < 0.10$). Farmers who planted cover crops have significantly higher family and hired labour use intensity ($p < 0.10$). The parameter of family labour use intensity is higher showing that farms planted with cover crops use higher family labour. This is expected because when farmlands are grown with cover crops like melon, much care is needed during weeding, and hired labour may not exercise the needed patience. As expected, those using fertilizer have significantly higher land use intensity ($p < 0.01$). Therefore, as land nutrient diminished due to continuous cropping, the farmers adopted applying of fertilizers. Hypothesis 1 is hereby rejected.

As farming experience increases, fertilizer use intensity and hired labour use intensity significantly decrease ($p < 0.05$). These findings reveal that old and experienced farmers may not be able to afford or have access to fertilizers. Also, the experienced ones, who are also the oldest could not use much of hired labour. This might have resulted from possibility of having enough children to work on the farms.

Farmers with formal education have significantly higher crop diversification index (implying more specialization) and less family labour. Education may enhance the understanding of the farmers about the expected cropping intensity and the type of crops to be grown. In like manner, the educated may be involved in some non-farm income generating activities that make them employ less of family labour.

As household size increases, crop diversification significantly increases ($p < 0.05$). Intensity of family labour use increases significantly with increase in family size ($p < 0.01$). Also intensity of hired labour use increases significantly as household size increases ($p < 0.01$). The same applies to fertilizer use intensity that has a positive sign and statistically significant ($p < 0.01$).

Table 3: Tobit Regression Analysis of the Determinants of Agricultural Intensification in Southwestern Nigeria

Variable	Parameter for Land Use Intensity	Parameters for Crop Diversification	Parameters for Fertilizer Use Intensity	Parameters for Labour Use Intensity	Parameters for Hired Labour Use Intensity
Constant	0.2874*** (4.154)	0.4610*** (5.266)	-75.7972* (1.830)	11.6531 (1.174)	6.2025 (0.767)*
Bush burning	0.0496 (1.498)	0.0335 (0.776)	10.3575 (0.524)	8.8716** (1.848)	1.1820 (0.299)
Mulching	0.0265 (0.803)	-0.0125 (-0.291)	12.4779 (0.651)	-6.8334 (-1.417)	13.2707*** (3.337)
Crop rotation	0.1575*** (4.428)	0.0003 (0.006)	24.6973 (1.161)	3.3003 (0.634)	-3.6189 (-0.843)
Organic manure	-0.0721* (-1.826)	-0.0987* (-1.892)	-26.0865 (-1.088)	-3.3390 (-0.582)	1.9376 (0.407)
Cover crop	-0.0292 (-0.813)	0.0393 (0.846)	6.1553 (0.279)	11.7310** (2.235)	6.5776* (1.640)
Minimum tillage	-0.0016 (-0.044)	-0.1424*** (-3.019)	46.6742** (2.170)	3.7377 (0.716)	-2.9179 (-0.676)
Fertilizer application	0.1840*** (5.311)	0.0029 (0.066)	-	4.3808 (0.865)	1.4597 (0.350)
Farming experience	-0.00008 (-0.075)	-0.0001 (-0.095)	-2.0353*** (-2.830)	-0.1942 (-1.135)	-0.2738** (-1.960)
Formal education	-0.0041 (0.131)	0.0786* (1.912)	27.4867 (1.447)	-7.5224 (-1.643)	-0.8571 (-0.228)
Household size	0.0020 (0.384)	0.0160** (2.427)	16.1482*** (5.298)	2.2142*** (2.951)	2.3003*** (3.778)
Food cropland inherited	0.0396*** (4.343)	0.0112 (0.955)	2.2898 (0.423)	-5.2064*** (-3.915)	-2.4423** (-2.267)
Food cropland purchased	0.0232 (0.943)	0.0521* (1.650)	33.3411** (2.379)	-6.6866** (-1.860)	-0.9336 (-0.321)
Food cropland borrowed	0.0717 (1.428)	0.0405 (0.628)	45.2578 (1.595)	-6.9879 (-0.947)	13.4217** (2.277)
Market distance	-0.0007 (-0.138)	-0.0068 (-1.050)	1.9272 (0.631)	1.1874 (1.606)	1.6533*** (2.733)
Poverty index	0.0019 (0.095)	-0.2415*** (-8.605)	-12.6917 (-1.067)	0.7381 (0.251)	-9.8608*** (-4.019)
Lost working days	0.0069*** (3.840)	0.0030 (1.318)	-1.4155 (-1.143)	0.1569 (0.595)	-0.2168 (-1.008)
Sigma	0.2438*** (22.998)	0.3054*** (19.823)	136.2225*** (18.395)	35.3075*** (22.896)	28.3640*** (20.963)
Condition Index	16.39	16.39	15.61	16.39	16.39

Note: ***= p<0.01, **= p < 0.05, * = p < 0.10 (t-statistics values are in brackets)

As the land hectareage owned through inheritance increases, land use intensity significantly increases ($p < 0.01$). In many rural areas, inherited lands are closer to the village than any other land areas and may because of this be subjected to continuous cropping. However, as the number of land hectareage owned through inheritance increases, family and hired labour use intensity significantly decreases ($p < 0.05$). Crop diversification indices and fertilizer use intensity significantly increase with increase in cropland purchased ($p < 0.10$), while family labour use intensity decreases. Hired labour use intensity significantly increase with increase in the hectareage of land owned through borrowing.

As market distance increases, hired labour use intensity significantly increases ($p < 0.01$). Increased poverty significantly increases lower intensity of hired labour use ($p < 0.01$) and crop diversification index. Lower use of hired labour may be due to inability to afford the daily wages for hiring labour. The finding for crop diversification shows that the poor plant many crops in order to meet their basic needs. The number of days farmers lost due to sickness significantly increases land use intensity ($p < 0.01$). This is expected because sickly farmers may not be able to trek the long distance in search of fertile land. Therefore, inability to control common health problems in the rural areas may compel agricultural intensification in the form of continuous cropping.

Recommendations

Agricultural intensification in southwestern Nigeria will continue to increase due to scarcity of fertile arable land and decline in fallow periods. This study investigates the intensification processes and concludes that farmers are overexploiting the land nutrients by using continuous cropping and the agricultural production process is somehow labour intensive.

The analysis reveals that increase in the number of days farmers could not go to work due to illness will result in increased land use intensity. The need to therefore ensure that health services are provided in the rural areas can be underscored. A healthy man is able to travel far in search of good land instead of continuously cultivating the ones available near the village.

Increasing the rate of poverty will increase the number of crops that farmers cultivate on a plot of land and reduce the use of hired labour. Therefore, an attempt to reduce rural poverty is a clear way of ensuring sustainability in the agricultural production system. This is important because soil degradation can be aggravated by unguided or excessive mixed cropping. Review of the policy framework for

poverty alleviation in Nigeria with a goal of ensuring that the rural households are fully catered for is essential.

The need to promote soil conservation practices is highlighted by the findings of the study. It was found that application of fertilizer and the use of crop rotation increase land use intensity. This shows that with appropriate soil conservation technologies, intensive land use will increase. However, how sustainable this could be is beyond the scope of this paper. Moreover, planting of cover crops increases intensive use of family labour. Sustainable land use by means of planting cover crops is therefore labour intensive and any factor that hinders availability of family labour will have some adverse effect on their cultivation. Efforts set at developing a hybrid of cover crops or planting technologies that will be less labour demanding will go a long way in promoting cultivation of these soil nutrient enhancing crops.

Finally, the use of fertilizer increases land use intensity. However, this study clearly reveals that rural farmers no longer apply fertilizer in the right quantity. There is a need to ensure timely provision of fertilizers to farmers in southwestern states. Encouragement of fertilizer production by indigenous industries will also increase supply.

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Farmers' assessment of Donor support for Rain-fed Lowland Rice Production in Ashanti and Northern Regions in Ghana

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Abstract: This paper examined farmers' assessment of donor support for rain-fed Lowland Rice Production in Ashanti and Northern Regions in Ghana. A simple random sampling due to proportion was used to select 210 participating farmers from the two regions. Data for the study were collected through a structured questionnaire designed based on the objectives of the study and from literature. The sections of the questionnaire include the challenges facing the project, cooperation and adoption levels, its impact on the outputs and the funding requirement for the project. The data collected was subjected to descriptive analysis with the use of bar charts and frequency distribution tables. The results show that prominent challenges indicated by the respondents about the projects are funding, weather dependent, land tenure system, credit implementation challenge, farmer group, work system cohesion and project staff strength. Adoption rate for project interventions was high for rice cultivation activities including land development activities though farmers complained of its issue to its drudgery. Farmers yield increased to an average of 4.9ton/ha and 2.9ton/ha level for Ashanti and Northern respectively despite the challenges. The study recommends increase in project fund and also, farmer group strengthening, improving on the activities of farming support systems to bring processors and marketers and further collaboration with other relevant stakeholders to complete the rice value chain.

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Keywords: Farmer, Donor, Rain-fed, Lowland Rice, Ashanti, Northern Regions, Ghana

Introduction

According to the European Union, investments in the smallholder sector yield the best returns in terms of poverty reduction and growth (OECD, 2010). Agriculture is still the mainstay of Ghana's economy. About 35.4 % of the GDP is accounted for by agriculture and livestock, forestry, and fishing in 2010 with 65% of the population dependent on it (AfDB, 2006). This indicates the strategic importance of the sector and its overall contribution to the economy. The World Bank (2011) reported that, poverty reduction impact of growth in agriculture is three times greater than comparable growth in any other sector of economies, and Ghana as an agricultural dominated country has the propensity to reducing its poverty. About 80% of agricultural production is from smallholder family-operated farms, mainly below one hectare (Banson, 2008). Larger holdings produce mainly cash crops, such as oil palm, rubber, and pineapples. Only about one third of land suitable for agriculture is currently cultivated (AfDB, 2006). Thus, the agriculture sector (and especially the sub-sectors that produce food is critical in provision of livelihoods and incomes, and developments within this sector are most important in terms of attaining

the Millennium Development Goals such as elimination of poverty (Banson 2008).

Donor funding has been an integral part of funding sources for not only the projects in the agricultural sector but for all other sectors of the country's economy. The country's GDP has largely been supported by donors and other development partners to implement its developmental projects. This support has gradually been on the decline not only to the national budget but also to agricultural projects. This low funding poses some challenges and difficulties to the overall productivity of agriculture in the country and food security drive of governments (OECD, 2008). Donor agencies due to their own budgetary constraints and deficits now disburses low amounts of funding to the country's agricultural projects expecting the local governments to take part through their counterpart budget system as a contribution towards the implementation of the projects. But this is often difficult to fulfill by government due to their expenditure levels and low revenue levels creating serious challenges for the full implementation of the intervention projects.

The IFAD Strategic Framework (2007) defines sustainability as the "institution supported through

projects and the benefits realized are maintained and continue after the end of the project.” Ensuring that the institutions supported through projects and the benefits realized are maintained and continue after the end of the project. Projects should be sustainable from the view point of field works and knowledge adoption (technical), institutional, political, economic cash flow sustainability and above all environmentally and communally sustainable. Alternatively, government counterparts defined sustainability as sustained funding and government takeover of the services provided by donor supported projects, as well as a continued flow of capital and credit into rural areas. There are several dimensions to project sustainability, depending on the nature of a sector or a project each of these dimensions has the capacity to influence project sustainability in one or way or another. These dimensions include: continued operation and maintenance of project facilities, continued flow of net benefits, continued community participation, equitable sharing and distribution of project benefits, institutional stability and dimension, maintenance of environmental stability. According to IFAD (2009), considerations of all these dimensions are a key to sustainability of projects. Experience suggests that weakening of any one of these has the potential to jeopardize the sustainability of the entire project, in the long run especially for that of agriculture. The multi-dimensional attributes of sustainability - as stated above, imply that to enhance project sustainability, a rigorous sustainability analysis is needed at the time of formulation of a project or a programme. It is expected that such an analysis which is to be followed up by development of a sustainability strategy will assist in incorporating the elements of sustainability, right at the design stage of a project.

The Project for Sustainable Development of Rain-fed Lowland Rice Production is a joint donor and counterpart funded agricultural project by the Japanese government through Japan International Development Agency (JICA) and the Ghana Government through the Ministry of Food and Agriculture (MOFA) with total estimated funding amount of \$1.6 million for a five year period to increase the production of rice and farmers income in selected communities of the two regions of the country. With this funding and the wider scope of the project area seeking to cover over 2100 beneficiaries including farmers, rice processors and marketers in the Ashanti and Northern regions. The overall goal of the project is improvement in productivity and profitability of rice farming in rain-fed lowlands in project areas are increased. The project purpose is the

dissemination of the model for sustainable rain-fed lowland rice development is accelerated within the project areas. The expected project outputs are technical package of improved rain-fed lowland rice production is developed, methodology to improve farming support system for sustainable rain-fed lowland rice production is verified and dissemination procedure of a model for sustainable rain-fed lowland rice development, consisting of the technical package and farming support system is established. In achieving these outputs, the following thematic areas on land development, rice cultivation, extension; postharvest and marketing will be addressed. The objectives of this paper was to examine how funding has affected the operations of the project, identify and describe the challenges faced by the project, analyze the positive impact of the project on farmers/beneficiaries in the project area, examine the project funding and its contribution to the cooperation and adoption levels of the beneficiaries of the project and examine the funding requirements of the project.

Materials and Method

The study was conducted in Ashanti and Northern Regions of Ghana. As depicted in Figure 1, the site in Ashanti region falls within the semi-deciduous agro-ecological zone of Ghana and is located by latitude 6°52'N and longitude 1°51'W. The area is on an altitude of approximately 280 m a.s.l. Mean seasonal annual rainfall in this area is about 1300 mm, with a bimodal distribution pattern.

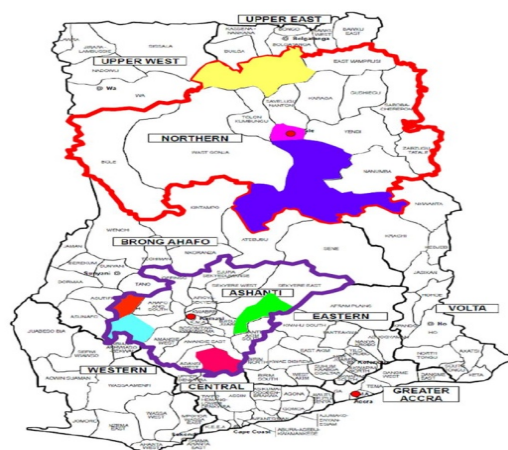


Figure 1. Project Areas marked with Colors

The population of the study is the 420 rice farmers participating in the project for the first phase. Simple random sampling technique was used to select 210 farmers for the study. The sample size was

proportional to size in Ashanti and Northern Regions as shown in Table 1. Sarantakos, (1997) argues that a bigger sample size gives better accuracy than smaller sample sizes.

Table 1: Study Population and Sample Size

PCU		Project Farmers Population (first phase)	Sample Size Selected
Region			
Ashanti		240	120
Northern		180	90
Total		420	210

Data for the study were collected through a structured questionnaire designed based on the objectives of the study and from literature. The sections of the questionnaire include the challenges facing the projects, cooperation and adoption levels, its impact on the outputs and the funding requirement for the project. The data collected was subjected to descriptive analysis with the use of bar charts and frequency distribution tables.

Results

The results on challenges facing the project was narrative while figures 2 to 5 present the results on adoption level of land development techniques, adoption level for rice cultivation techniques, adoption level of extension concepts and impact of the project on farmers. Table 1 gives the results on yield.

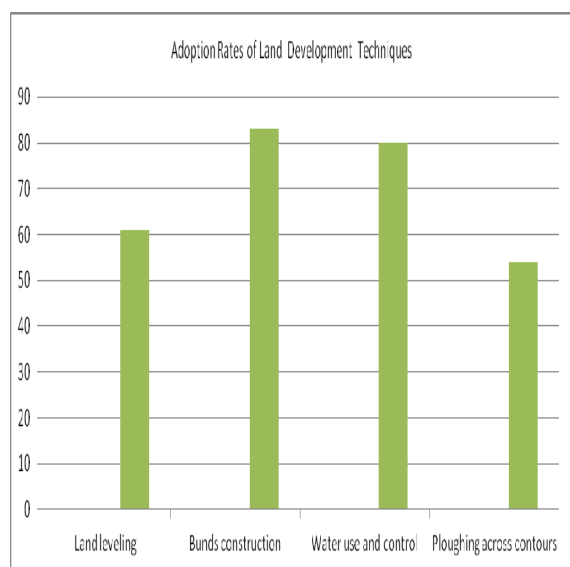


Figure 2 Adoption Level of land Development Techniques

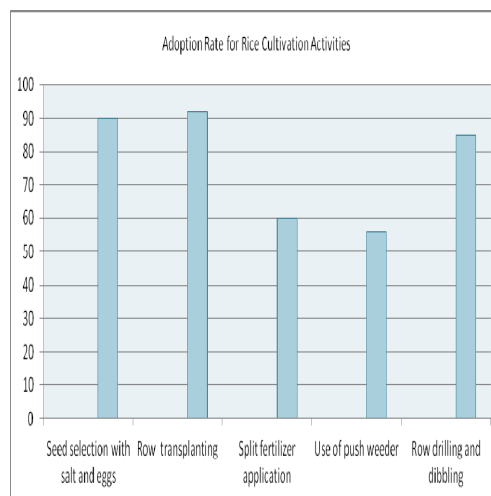


Figure 3. Adoption level for Rice Cultivation Techniques

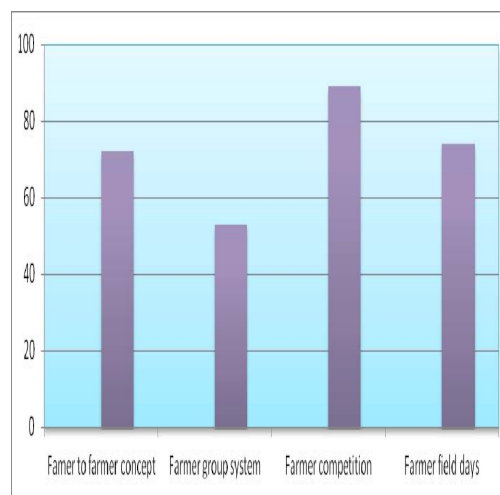


Figure 4. Adoption Level of Extension Concepts

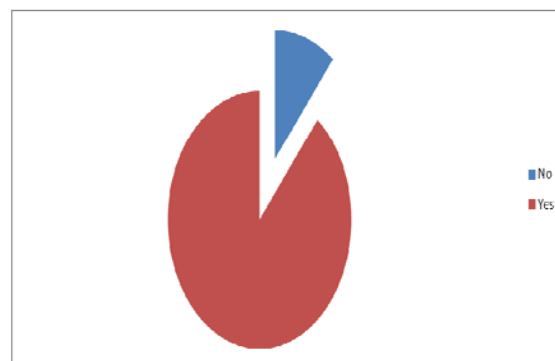


Figure 5. Impact of the Project on Farmers

Table 2. Yield Comparative Data

Region	2007	2008	2009	2010 -Project Figures
Ashanti	1.1	1.1	1.2	4.3
Northern	1.7	2.3	2.3	2.9

Discussion

Prominent challenges indicated by the respondents about the projects are funding, weather dependent, land tenure system, credit implementation challenge, farmer group, work system cohesion and project staff strength. In terms of the adoption level of technology disseminated all the techniques were above 50% level of adoption by farmers as shown in Figure 2. From Figure 2, farmers actually complained about the difficulty in developing their lands with simple tools like hoe, cutlass and manual levellers and that things could be more better and easy for them if power tillers and tractors will be made accessible to them especially for the Ashanti Region as farmers in the Northern region was used to tractors for ploughing. The low adoption for ploughing across contours could be attributed to tractor operators preferring to plough along contours as it is easier and faster but facilitates erosion as said by the land development counterpart officer. With all these, it could be deduce that with a bit more investment in the land development section, a greater part of the project output could easily be achieved.

On rice cultivation techniques, all the respondents indicated that both the land development and rice cultivation activities were easy for them when working in groups than at individual levels (figure 3). Split fertilizer and push weeders recorded lower rates due to high time as in frequency and labour demand. For the push weeders, they complained that it was slow using it for weeding as compared to their traditional hoes since they were not exposed to its use. The adoption levels for the farming support systems were not as high as others because of lots of challenges as only the farming record calendar system were well adopted. Only 52% of the respondents from the 210 sample size responded to adopting the farming record calendar promotion where all farming activities will be executed according to the calendar which was designed based long study and research. Market information relay to farmers, standardization of measurements, stakeholder linkages and rice quality forum promotions have just been started by the project and the farming support officer was hopeful that by next year adoption rates for these interventions would have been higher.

In Figure 4, concepts promoted by extension unit of the project centered on farmer to farmer extension, promotion of farmer group system, use of farmer field days for teaching specific techniques, trainings and promotion of farmer competition amongst the farmers. An interview with the farmers produced varied adoption rates for these concepts. Figure 4 shows the variations of adoption for the extension concepts. The lower adoption rate for the farmer group system was attributed to wider differences among farmers. Petty quarrels, concentration on their own individual fields other than the trial learning plot were common as said by the officer in charge of the group formation and cohesion of the project. According to the officer, the farmer competition came with incentives to the farmers that whoever adopts well the technologies disseminated by the project will be given special prizes (simple tools and inputs). This increased their flair for this concept which is bringing goods results to almost all the interventions of the project for practice and adoption by the farmers.

In Figure 5, despite the huge challenges faced by the project, it has made several positive impacts on the lives of the farmers. The farmers said their yields have started increasing from the start of the project especially for the 2010 farming season. This increase according to them was as a result of the learning and teachings of the project. Their knowledge on all the cultural practices in the field for rice cultivation from land preparation to the post-harvest activities has greatly improved. They have received several formal and informal trainings, field and off field's trainings and field exchange visits. The response to the impact of the project on the farmers as captured in figure 5 shows 74% of them agreeing that they have been positively influenced by the project with 9% believing otherwise. They believe their income increased last year due to higher yields they had and also knew more about farm record keepings on all their farm activities in addition to opening bank accounts with banks. Apart from that, the promotion of the use of tarpaulin for threshing and improved use of harvesting sickles by the project contributed to their rice paddy quality. Table 2 shows that from 2007 to 2009 rice yields from these areas of the two regions according SRID (statistics, research and information department) of MOFA indicated lower average yields from the project areas as compared to the current yield trends in the areas with the help of the project. However, farmers intimated that with supplementary irrigation infrastructure, developed lands or valleys, provision of credit facilities and farm machinery could have assisted them more greatly.

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The influence of Examinations Stress on Changes in Heart Rate regulatory Mechanism indicators in Students of the First to Third Grades Talented High School

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Abstract: Examinations are of the most important stressful factors in schools and universities and have different psycho-physiologic consequences; however, their effects in students have been less focused. To study the effects of educational pressures and examinations stress on changes in the mechanism of heart function, a test was conducted on 120 students, age range of 15-17 years of both genders. Divided in two groups, each consisting 20 students of first to third grades of high school, 60 boys and 60 girls from two high schools (one boys high school and one girls high school) in three stages (2 months before the examination(normal), 1hour before and 1hour after the examinations) in the same educational conditions in the city of Sari. Activity of heart rate regulatory mechanisms was recorded of ECG and next was processed by the method of Baevsky pulsometry variation. The statistical elaboration of the obtained data from the three stages of test has been carried out using Biostat computer programmer. Based on the results of this study, the least stress effects on changes in the heart function was during 2 months before the examination(normal) and affected activity of vagotonic and normotonic systems and the highest stress effects was observed during 1hour before the examination and affected activity of normotonic and sympathetic systems. The effects were significant at differences experimental stages ($P < 0.001$). The effects decreased during 1hour after the examination influenced normotonic system activity. The highest stress effects were observed in examination time in the first grade and third grade high school students. It showed that course volume and pressure, type of course and stress of examination time caused sympathetic system activity which influenced on changes in the mechanism of heart rate function. In comparison, in all test stages, stress effects in boys group were higher than in girl students. The results of this search shows that the factors like age, gender, personality, psychophysiological status, course volume and pressure, type of course, examination time and anxiety, individual typological characteristics and the state of autonomic nervous system affected on changes in the mechanism of heart rate function in students

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Keywords: examination stress, heart rate function, 15-17years old students

1. Introduction

Examinations are of the most important stressful factors in schools and universities and have different psycho-physiologic consequences; however, their effects in students have been less focused. As the wAxiety become one of the subjects that call for particular attention. Stress has significant effects in the educational progress and the physiological, mental and psychological conditions of student. In addition, several studies have shown that students suffer from several behavioral and psychological disorders and there are many factors in schools that cause stress in students (Dudnik, Ye. N.et.al.2007). Examinations stress is factor of disease in the students, the important ones are: the development and intensifying of previous cardiac diseases and increase of hypertension (Khatami,S and B.S.Moradmand. 1992). The process of adaptation of students to an

academic load is accompanied by the periods of recession and a pressure parameters of hemodynamic, activity regulators mechanisms of a rhythm of heart and parameters of state of health, activity and mood (Minasian,SM.et.al.2006). During an examination session, the schoolchildren were ascertained to develop emotional stress characterized by a complex of psychophysiological and autonomic nervous changes (Minasian,SM.,ES, Gevorkian., and NN. Ksadhikian. 2005.The heart rate reactions on the examination stress depended on the individual typological characteristics and the state of autonomic nervous system (Gevorkian, ES.et.al.2003) The resistance of the students to the examination stress and the type of their vegetative reactions, to a large extent, determined by both the individual-typological peculiarities of the higher nervous activity and the initial psychological state (Makarenko, MV., VS,

Lyzohub., and LI. Iukhyemenko. 2003). Since examination stressor influences blood pressure, heart beats, heart functions, the physiologic, mental and psychological situations and educational progress of students, therefore, this subject demands special attention.

2. Material and Methods

This project was carried out on 120 (60 male and 60 female) students age range of 15-17 years in three educational programs of both genders and divided in girls and boys groups. Each group consist 20 students from two high schools (one of boys and one of girls) in three stages of education program. The first test was carried out 2 months before the examination (normal), the second 1hour before the examination and the third test was taken 1hour after the examination from the same students in the same educational conditions in the city of Sari. In this test, 20 students in one classroom from each group were selected randomly in similar conditions. Also consent letter was obtained from them. In each three stages of examinations, some parameters including HR¹, AHRI², RAI³, ABI⁴, SI⁵, R-Rinterval, in both groups were measured and the data recorded (Baevsky, R.M.,O.I, Kirillov and S.Z. Klitskin). To measure and determine the electric activities and heart rate function, the monitoring electrocardiograph apparatus (Biphasic model, 200 joulesma, made in the USA, Zoll Company) was used and so were processed by the method of Baevsky pulsometry variation (1984) and that's result obtained some indicator that showed heart rate function.

To determine the AHRI, the special following formula was used:

$AHRI = 1 / MO \cdot \Delta X$; where,
MO= highest percent of number of time R-Rinterval
 ΔX ; Difference between the most and least percent of time R-R-interval

The RAI is obtained from following equation:

$RAI = AMO / MO$

AMO= highest percent of R-Rinterval

The ABI is calculated from following equation:

$ABI = AMO / \Delta X$

The SI is obtained from following equation:

$SI = AMO / 2 \cdot \Delta X \cdot MO$

Finally, the statistical elaboration of the obtained data from the three stages of test has been carried out using Biostat computer program

¹ Heart Rate

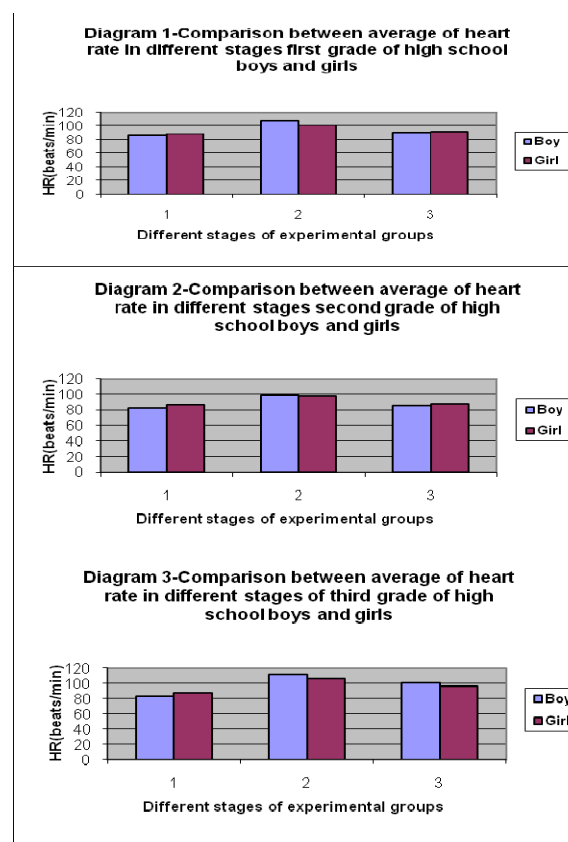
² Autonomic Heart Rhythm Index

³ Regulation Adequacy Index

⁴ Autonomic Balance Index

⁵ Stress Index

The obtained data from this study in different test stages indicated that in terms of HR, in first to third grades boys and girls groups, significant differences were observed in various test stages ($P < 0.05$). This difference was higher in the 1hour before the examination and lower in the 2 months before the examination. It was revealed that boy students have some higher levels of indicators of heart rate. The HR was higher in both groups during 1hour before the examination. The reason of increase in HR related to examination time and tired of course and so sympathetic system activity (diagram 1 to 3).



As the examination time approaches, the effects of anxiety, stress and physical and psychological status cause increase in HR. Findings of this study agree with the data obtained by Gevorkian et al. (2003), loft et al. (2007) and Zeller et al. (2004). Minasian et al. (2006) showed that periods of the highest pressure of physiological systems of an organism of students are the beginning of the first semester and examination period (Minasian, SM.et.al. 2006). Gevorkian et al.(2003) showed that heart rate reactions on the examination stress depended on the individual typological characteristics and the state of autonomic nervous system (ANS) (6). Dimitriev et al. (2008) in an

experiment during education and immediately before the examination observed change in heart rate, blood pressure and anxiety status by examining stress in students. Before the examination, more students showed increase in anxiety status, diastolic blood pressure and heart rate (Dimitriev, D.A.et.al.2008). Data of the literatures indicate that adaptive capacity of such regulatory mechanisms could be exceeded by stress. Their failure may result in the development of pathology (Andrianov, V and N.A.Vasilyuk.2001, Nidekker, I.G and B.M.Fedorov.1993). Makimbetova (2007) in a study made on 15-17 years age groups showed a decrease level (Makimbetova, C. 2007). Minasian et al. (2010) showed that boy students have some higher levels of integral indicators of heart rate and it agrees with the results of this research (Minasian, SM et.al.2010). In terms of AHRI, there was significant difference between different test stages in the first, second and third grades boys and girls groups ($P < 0.001$). In the three grades in the 1 hour before the examination it was higher than the other stages and lower in the 2 months before the examination (table 1 to 3). In during before the examination was higher AHRI indicator and was lower parasympathetic indicator (Mo), homoral (ΔX) and cardiointervals variation (Vk) than the normal stages in both groups. The reason is examinations stress that increased the sympathetic activity effects and regulatory center mechanisms of heart rate.

Makarenko et al. (2003) studied the reactions of heart rhythm in the students with their personal

Table 1-Changes of cardiac performances at different stages of first grade high school boys and girls

Parameters	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
HR(beats/min)	89.16±4.31	102.12±1.86 p<0.05	92.36±2.89	86.75±3.72	108.64±3.14 p<0.01	91.17±2.34
SI (abr.units)	98.55±9.04	146.23±12.12 p<0.001	114.8±3.06 p<0.05	121.25±10.41	226.38±16.32 p<0.001	157.61±20.6 p<0.01
AMo (%)	41.56±2.34	48.22±1.81 p<0.05	46.61±2.02 p<0.05	44.65±3.21	53.47±2.9 p<0.05	51.50±2.69 p<0.05
Mo (s)	0.81±0.021	0.71±0.023 p<0.05	0.76±0.01 p<0.05	0.82±0.032	0.67±0.021 p<0.01	0.60±0.03 p<0.01
ΔX (s)	0.26±0.02	0.23±0.01 p<0.05	0.28±0.02	0.22±0.01	0.18±0.01 p<0.01	0.27±0.02 p<0.01
AHRI (abr.units)	4.72±0.48	5.99±0.26 p<0.05	5.10±0.56 p<0.05	5.49±0.56	8.33±0.62 p<0.001	6.35±0.60 p<0.05
ABI (abr.units)	159.85±11.32	209.65±16.68 p<0.001	168.0±13.12	202.95±13.61	297.05±17.23 p<0.001	192.87±26.07
RAI (abr.units)	50.74±3.05	67.63±4.14 p<0.01	62.6±4.48 p<0.01	53.93±3.12	79.57±4.64 p<0.001	84.40±9.3 p<0.001
R-R av. (s)	0.80±0.01	0.69±0.02 p<0.05	0.72±0.01 p<0.05	0.76±0.03	0.66±0.02 p<0.05	0.68±0.03 p<0.05
R-R min. (s)	0.64±0.02	0.51±0.01 p<0.01	0.56±0.01 p<0.05	0.67±0.02	0.53±0.01 p<0.01	0.56±0.02 p<0.05
R-R max. (s)	0.88±0.01	0.76±0.02 p<0.05	0.82±0.02 p<0.05	0.90±0.02	0.72±0.02 p<0.01	0.78±0.03 p<0.05

features and difference in gender during examinations stress. The results of the test showed decrease of parasympathetic effects and increase of sympathetic effects on heart rate, it agrees with the results of this research (12). Makimbetova (2007) showed that the parasympathetic effects on cardiac rhythm is higher in higher wages in boys and 15-17 years old girls and the increase was significantly higher in boys (Makimbetova, C. 2007). In terms of RAI in the first to third grades of high school boys and girls and in each grade there was significant difference between different test stages ($P < 0.001$). In the three grades in the 1 hour before the examination it was higher than the other test stages and lower in the 2 months before the examination (table 1 to 3).

The RAI in each three grades was higher in both groups during 1 hour before the examination than the other test stages. The reason is increase of sympathetic system activity that attributed to higher percent of R-R-interval than percent of time in R-R-interval and lower Mo. The results of this research agree with the data given by Makimbetova (2007). Goliskardi et al. (2010) showed increase of AMo, SI, AHRI, RAI and ABI was more evident in boys and while tonus of the parasympathetic part of vegetative nervous system (VNS) significantly decreased (Goliskardi, R., SM, Minasian and ES. Gevorkian. 2010).

Table 2-Changes of cardiac performances at different stages of second grade high school boys and girls

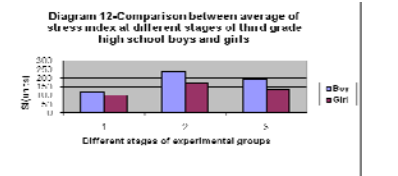
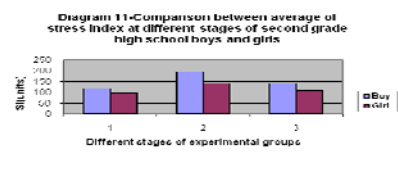
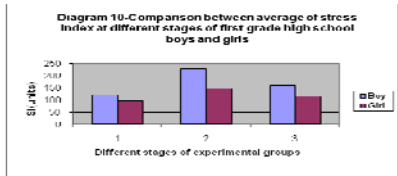
Parameters	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
HR (beats/min)	86.62±2.44	97.48±2.86 p<0.05	88.31±3.44	82.36±3.68	98.86±4.32 p<0.02	85.48±4.72
SI (abr.units)	96.24±5.32	139.54±18.6 p<0.001	110.6±6.32 p<0.05	116.8±7.4	192.96±8.6 p<0.001	140.84±4.26 p<0.01
AMo (%)	31.82±1.69	47.17±2.04 p<0.001	37.4±1.82 p<0.05	38.6±2.02	54.2±2.4 p<0.001	44.3±1.8 p<0.05
Mo (s)	0.65±0.02	0.60±0.01 p<0.05	0.66±0.02	0.70±0.01	0.65±0.01 p<0.05	0.68±0.03
ΔX (s)	0.28±0.02	0.25±0.04 p<0.05	0.29±0.03 p<0.05	0.25±0.02	0.22±0.02 p<0.05	0.24±0.03
AHRI (abr.units)	5.95±0.48	6.15±0.02	6.05±0.36	5.76±0.52	7.24±0.62 p<0.001	6.72±0.24 p<0.05
ABI (abr.units)	126.61±6.82	167.10±8.94 p<0.001	142.3±8.36 p<0.05	159.42±12.14	246.38±12.2 p<0.001	186.32±11.3 p<0.05
RAI (abr.units)	47.69±3.21	78.64±4.05 p<0.001	62.98±2.84 p<0.05	58.6±4.48	82.6±5.32 p<0.001	67.96±4.6 p<0.05
R-R av. (s)	0.69±0.2	0.67±0.01	0.71±0.02	0.73±0.04	0.65±0.03 p<0.05	0.67±0.03 p<0.05
R-R min. (s)	0.53±0.02	0.57±0.04 p<0.05	0.60±0.04 p<0.05	0.57±0.01	0.53±0.02 p<0.05	0.51±0.01 p<0.05
R-R max. (s)	0.79±0.01	0.84±0.03 p<0.05	0.88±0.06 p<0.05	0.82±0.03	0.74±0.02 p<0.05	0.74±0.04

The ABI in first to third grades high school showed significant differences between boys and girls in different test stages ($P<0.001$) and in all grades during 1 hour before the examination it was higher than the other stages and lower in the 2 months before the examination (table 1 to 3). In during before the examination was higher ABI indicator than the normal stages in both groups. The reason is effects of regulatory center systems on heart rate that attributed to the lower ΔX and effects of the highest percent of number and time of R-R intervals. It is because of examinations stress, examination volume and course learning that affect on ABI. The data of this research agree with the data of Makarenko et al. (2003).

There is a statistically significant relationship between SI regulatory systems of cardiac rhythm in first to third grades high school students ($P<0.001$) and in both groups in all grades during 1 hour before the examination it was higher than other stages and was lower in the 2 months before the examination (table 1 to 3) (diagram 10 to 12).

Table 3-Changes of cardiac performances at different stages of third grade high school boys and girls

Parameters	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
HR (beats/min)	87.98±5.64	106.12±4.76 p<0.001	96.32±4.48 p<0.05	82.80±4.22	112.24±8.04 p<0.001	101.20±6.72 p<0.01
SI (abr.units)	102.40±8.62	172.84±12.3 p<0.001	132.84±9.6 p<0.001	121.82±10.4	240.22±14.6 p<0.001	196.36±8.8 p<0.001
AMo (%)	41.61±2.3	52.30±3.1 p<0.01	48.20±2.6 p<0.05	42.82±2.4	62.41±1.6 p<0.001	56.20±2.0 p<0.001
Mo (s)	0.65±0.04	0.60±0.05 p<0.05	0.65±0.02	0.70±0.01	0.64±0.02 p<0.05	0.65±0.01 p<0.05
ΔX (s)	0.32±0.01	0.24±0.02 p<0.01	0.29±0.01 p<0.05	0.24±0.02	0.19±0.01 p<0.05	0.22±0.01 p<0.05
AHRI (abr.units)	5.13±0.68	6.94±0.41 p<0.001	5.46±0.36	5.95±0.81	8.09±1.8 p<0.001	6.68±1.2 p<0.05
ABI (abr.units)	136.67±9.1	216.42±13.8 p<0.001	165.52±8.9 p<0.01	175.66±10.2	326.8±12.8 p<0.001	243.47±11.3 p<0.001
RAI (abr.units)	63.07±0.8	86.66±4.61 p<0.001	73.85±2.2 p<0.05	60.21±1.4	98.82±3.9 p<0.001	84.92±4.1 p<0.001
R-R av. (s)	0.69±0.03	0.66±0.04	0.67±0.01	0.72±0.03	0.58±0.02 p<0.05	0.68±0.01
R-R min. (s)	0.54±0.01	0.56±0.02	0.54±0.01	0.57±0.01	0.53±0.01 p<0.05	0.56±0.01
R-R max. (s)	0.84±0.03	0.80±0.06	0.83±0.03	0.81±0.01	0.72±0.01 p<0.05	0.79±0.02



On basis heart stress indicators the reason of higher SI in boys group in second stage (1hour before the examination) is for the lower MO and ΔX and higher sympatheticotonic system activity (180≤SI≤250) (average in three grades 61.2%) and it affected on changes in heart rate mechanisms activity and in girls group is because of lower MO and ΔX and higher normotonic system activity (60≤SI≤180) (average in three grades 58.3%).

In first stage (normal) and third stage (1hour after the examination) in both groups, the SI was lower than second stage (1hour before the examination) and lower SI is because of vagotonic and normotonic systems activity (60≤SI≤180) and sympathetic and parasympathetic systems activity were in balance (table 4 to 6, diagram 4 to 9).

Table 4-Percentage of vagotonic, normotonic and sympatheticotonic at different stages of first grade high school boys and girls

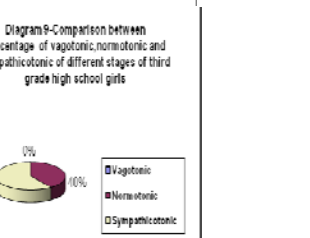
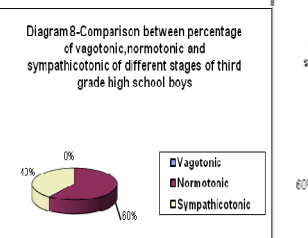
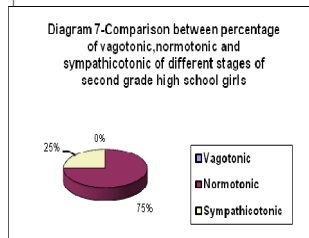
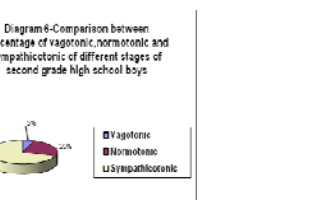
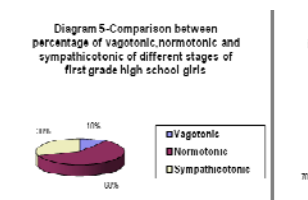
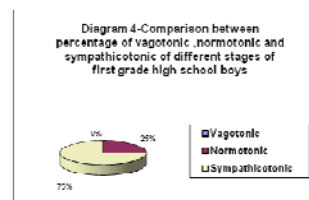
	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
Vagotonics	70%	10%	15%	10%	-	-
Normotonics	30%	60%	85%	80%	25%	65%
Sympathicotonics	-	30%	-	10%	75%	35%

Table 5- Percentage of vagotonic, normotonic and sympatheticotonic at different stages of second grade high school boys and girls

	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
Vagotonics	40%	-	5%	10%	-	-
Normotonics	50%	40%	75%	65%	60%	55%
Sympathicotonics	10%	60%	20%	25%	40%	45%

Table 6- Percentage of vagotonic, normotonic and sympatheticotonic at different stages of third grade high school boys and girls

	Girls			Boys		
	Normal	Before the examination	After the examination	Normal	Before the examination	After the examination
Vagotonics	50%	-	30%	20%	5%	-
Normotonics	35%	75%	60%	70%	25%	70%
Sympathicotonics	15%	25%	10%	10%	70%	30%



Dudnik et al. (2007) in a study reported that changes in cardiac function are under the effects of sympathetic and parasympathetic regulations, which agrees with the results of our study in both groups. The observed changes indicate that the autonomic balance shifted towards a marked predominance of the sympathetic division of the autonomic nervous system (ANS), which is known to reflect an increasing centralization of HR control and an enhanced modulating effect of the hypothalamus (Baevsky, R.M. et al. 1984, Dotsoev, L.Ya et al. 2003, Nozdrachev, A.D and Yu.V. Shcherbatykh. 2001, Shcherbatykh, Yu.V. 2000, Yumatov, E.A. et al. 2001). Goliskardi et al. (2010) in a study showed there is a statistically significant relationship between SI regulatory systems of cardiac rhythm and level of anxiety of the study participants. It was found that examination psycho-emotional stress has a significant effect on sympathetic-vagal balance of the heart rate regulation and anxiety level of students (Goliskardi, R. et al. 2010).

In before the examination stage, the increasing of sympathetic mechanisms activity indicators was resulted to higher in stress index (SI) in boy and girl groups. In comparison with normal stage, the stress index (SI) was increased in students of the first, second and third grades boys high school consecutively; 86.7 percent ($P < 0.001$), 65.2 percent ($P < 0.001$) and 97.2 percent ($P < 0.001$) and in girls consecutively; 48.4 percent ($P < 0.001$), 44.9 percent ($P < 0.001$) and 68.8 percent ($P < 0.001$).

The average of R-R interval time in the first to third grade students in each grade and different stages of test significant differences were observed ($P < 0.05$). In both groups, R-R interval time was lower in the 1 hour before the examination than 1 hour after the examination (table 1 to 3).

In this study HR was higher in the 1 hour before the examination which resulted in decreasing of R-R interval time in the 1 hour before the examination.

Makimbetova (2007) found that in 15-17 years old boys group, with increases of age the distance between RR becomes significantly longer while in lower age girls group, the distance of RR was longer. Lucini et al. (2002) studied the educational stress period of the university students and found that during stress, the RR distance lowered and the artery pressures increased significantly.

In terms of comparison, in first stage (normal), measured parameters in both groups were lower in all grades than other test stages, and most measured parameters were higher in boys group.

In second stage (1 hour before the examination) most measured parameters were higher in different grades of boys and girls than other test stages and it was a significant differences ($P < 0.001$). Most

measured parameters were higher in boys group in all grades.

In third stage (1 hour after the examination) most measured parameters were higher in different grades of boy and girl groups than first stage (normal) and was lower than second stage (1 hour before the examination) and it was a significant difference ($P < 0.05$). Most measured parameters were higher in boys group in all grades than in girl students. The most measured changes indicators of regulatory systems of cardiac rhythm in all test stages in both groups were higher in the first and third grade high school students.

3. Conclusions

The results of this study showed the highest stress effects in the 1 hour before the examination in the first grade and third grade high school students and affected sympathetic system activity. The most measured changes indicators of regulatory systems of cardiac rhythm at different stages of experiment were higher in boys group in all grades than in girl students. The factors of age, gender, and type of course, course volume and pressure, psychophysiological status, personality, individual typological characteristics, examination time and anxiety affected on educational pressure, examination stress and the state of autonomic nervous system as a results changes in the mechanism of heart rate function in the study subjects.

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Effects of Mining on Smallholder Agriculture in Asutifi District of the Brong Ahafo Region, Ghana

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Abstract: The study assessed the socio economic effects of mining on agriculture in the Asutifi district of the Brong Ahafo region. From 117 communities in the district, three communities namely Kenyasi, Ntotroso, and Ola Resettlement were randomly selected. Simple random sampling technique was used to select 120 farmers for the study. Data for the study were collected through a structured questionnaire designed based on the objectives of the study and from literature. The data collected was subjected to descriptive analysis with the use of bar charts and frequency distribution tables. The results of the socio-economic effect of mining on smallholder agriculture in the area include displacement of people and land, increased migration into mining areas, environmental damages related to water quality water quantity, tailing management, noise and dust pollution as well as ecosystem disturbances, shortage of labour availability to agriculture, and contamination of crops as well as reduction in crops' yield. [Mumuni E, Seidu Al-hassan and Oladele O.I. **Effects of Mining on Smallholder Agriculture in Asutifi District of the Brong Ahafo Region, Ghana.** *Life Sci J* 2012; 9(3):389-395]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 54

Keywords: Mining, Smallholder, Farmers, Land, Labour Ghana

Introduction

From gold to limestone and copper to oil, mining activities have been extracting commodities from the earth and providing means of livelihood to human societies for thousands of years. In recent years, the process of exploring, mining and processing various minerals, has come under tremendous pressure to improve its social, developmental and environmental performance (MMSD, 2003). In Ghana, mining started since pre-colonial times. Small-scale mining also known as “galamsey” was legalized by the Provisional National Defense Council (PNDC) law 218, of 1989. Both the small and the large scale mining contribute to economic growth in the form of taxes and royalties to the country. The nation derives the bulk of its foreign exchange earnings from gold mining which accounts for over 90% of the country's mineral export. Apart from gold, Ghana produces significant quantities of diamonds and bauxite and counted amongst the top five countries producing manganese ore in 2006. As of June 2006, sixty six (66) local and forty seven (47) foreign companies held prospecting/reconnaissance licenses in Ghana. Additionally, thirty one (31) companies had been granted mining concession leases to operate in the country. Many of the companies holding exploration licenses are focusing on gold exploration (Ghana Chamber of Mines, 2006). Mining operations compete for land use with agriculture.

Agriculture which is the back bone of Ghana's economy has suffered the most palpable neglect over the years especially in the mining areas. Many cocoa farmers have had to reluctantly give up their ancestral farmlands spanning from four to five generations to mining companies. It is usually the case that when mining companies acquire a land in a community for prospecting, the local farmers are required to abandon their farms. They are paid some compensation which never meets any economic or moral requirement for such huge losses (Agyei, 2007). Meanwhile, between 1993 and 1997 mining contributed 1.5% to Ghana's GDP as against 40% by agriculture. Again, the mining sector in 2007, 2008 and 2009 contributed 5.9%, 5.6% and 5.8% of the GDP respectively to the country's GDP, whilst the agricultural sector also contributed 34.3%, 33.9% and 35.4% to the GDP in these years respectively (GSS, 2009). In a developing country such as Ghana where about 65% of the population is engaged in agriculture as their source of livelihood, any activity like mining that claims vast arable lands will be an affront to national food security as well as sustainable economic gains and initiatives (GSS, 2009). The taxes and royalties accrued to the state from mining are insignificant compared to the gains from agriculture to the national economic development.

Several linkages have been reported by different authors between mining and socio-economic issues. Barry, (1996) noted that, employment

generated indirectly by a mining operation amounts to between 2 to 25 times the number of direct employees, in certain cases even more than that. World Bank(2001) noted that every dollar spent by a mine on operations could generate an average of 2.8 US dollars in the local economy (World Bank, 2001). The implications of mining in terms of social impacts are that those who do not earn high income or are not employed in the mining sector, (wages in the mining sector are said to be higher than the average wage in Ghana) will not be in the position to afford decent accommodation for themselves and their families or fend for themselves and their families as well. Migration of young ladies into mining communities in search of non-existent jobs may lead to prostitution with its implications for the spread of transmittable diseases including HIV and AIDS (World Bank, 2010). Environmental damage can be caused by small-scale mining and large-scale mining. Water quality water quantity, tailing management, noise and dust pollution as well as ecosystem disturbances are issues that can adversely affect the health and livelihood of the poor and vulnerable in society. In the context of mine closure, abandoned or orphaned mines normally in open pit forms causes pollution and potential public danger. There is also a problem of deforestation and land degradation from the open cast mining system by both large scale companies and artisanal small scale miners. Having cleared the lands for extraction, some mining companies make the effort of reclaiming the land by reforestation, but changes in the natural ecosystem of the land and vegetation causes the destruction of the biodiversity. This situation is aggravated by artisanal miners who clear forest and dig large trenches leaving them bare, thus exposing the soil to erosion which cannot be

used for agricultural purposes and also serving as breeding grounds for mosquitoes (Kusimi, 2007).

Agriculture is the main stay of the Ghanaian economy with 65% of the population engaged in it and Asutifi which is the study area has 77.4% of its population engaged in agriculture (GLSS, 2008). This means that any threat to any of the factors of production will have serious implication on the agricultural production sector with a consequential damage to the fragile economy. This is confirmed by the production trend of agricultural crops from 2003-2009 in the district which dropped by 1.9% together with a sharp increase in labour cost within the same period (SRID-MOFA, 2009). It is also estimated that about 9,575 individual farmers in the district with 7,500 hectares of their farmlands have been taken over by Newmont Gold Ghana Limited for gold exploration (Action Aid, 2005). This means that the practice of fallowing where farmers allow the land to regain its fertility by leaving it for a year or more without cropping on it can no longer be practiced since they are now restricted to small pieces of lands for farming if not completely lost to the miners. The main objective of this study is to assess the effects of mining on agriculture in the Asutifi District of the Brong Ahafo Region.

Materials and Methods

The Asutifi District which is in the Brong Ahafo Region occupies an area of about 1,500 square kilometers. The district is located between latitudes 6°40' and 7°15' North and Longitudes 2°15' and 2°45' West. It shares boundaries with Sunyani Municipal in the north and Asunafo in the south and to the west with Dormaa as indicated in Figure 2.

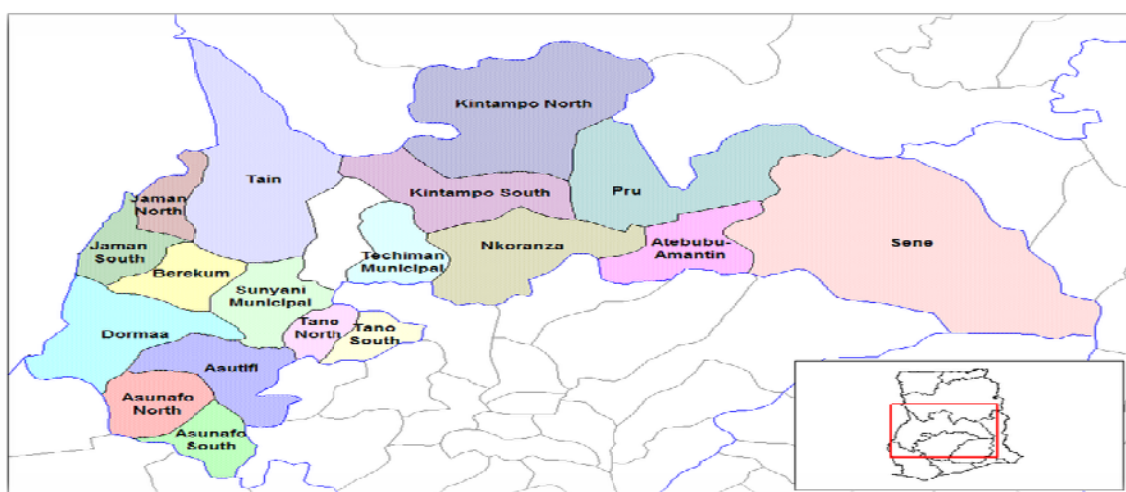


Figure 1: Map of the Study Area

The district lies within the wet semi-equatorial zone marked by double rainfall maxima with a mean annual rainfall between 125cm and 200cm. The first rainy season is from May to July and the second rainy season is from September to October when the district comes under the influence of the Wet Maritime Airmass. There is a sharp dry season between the two rainy seasons the main one coming between November and March when the tropical continental Air mass in the country sweep over the area (DDP, 2002). The district has Newmont Mining Corporation, which is a leading gold producer with operations in five continents, and developed its first project in Ghana in 2006, and it is the only mine operations of the company in Africa, which at the end of 2007 had over 17 million ounces of gold reserves, representing nearly 20% of its global gold reserves. This is expected to last for 20 years especially in the Ahafo mines (WBCSD, 2009). It operates the open pit system and currently mines from three open pits but with about 17 pits in total. It employed a total of 3,528 employees including Ghanaian contractors in 2008. Newmont have just started operations too at Akyem in the Eastern Region of the country though with low concession as compared to the Ahafo mines (WBCSD, 2009).

Newmont launched a community development fund to contribute an estimated US\$ 500,000 annually via a community foundation to support community development programs. Newmont Ghana Gold has also embarked on other community development initiatives including the provision of water, sanitation, upgrading of the local clinics and training centers, school construction, HIV/AIDS programs for the communities as well as a program on malaria prevention in the district. Again, the Ahafo Linkages Program by Newmont which operates in 12 communities has been able to generate about \$4.7 million since 2008 within these communities through local content use and involvement (WBCSD, 2009). The population of the study is all farmers in Asutifi district. From 117 communities in the district, three communities namely Kenyasi, Ntotroso, and Ola Resettlement were randomly selected. Simple random sampling technique was used to select 120 farmers for the study. Sarantakos, (1997) argues that a bigger sample size gives better accuracy than smaller sample sizes. Data for the study were collected through a structured questionnaire designed based on the objectives of the study and from literature. The data collected was subjected to descriptive analysis with the use of bar charts and frequency distribution tables.

Results

The socio demographic characteristics discussed include age, sex, education and employment status of respondents as presented in Table 1 while Table 2 shows comparison of yield levels of some selected crops with achievable yields standards. Figure 2 presents the types of labour respondents use and Figure 3 the perception on labour shortages for agricultural activities.

Table 1 Personal characteristic of farmers

Variables	Frequency	Percentages
Gender		
Female	54	45
Male	66	55
Age		
18-25	7	5.8
26-35	28	23.3
36-45	70	58.4
46-55	10	8.3
Above 55	5	4.2
Education		
Primary	70	58
Junior High school	56	47
Senior High school	14	12
Tertiary	4	3
Monthly income(GHc)		
500-1000	78	65
1001- 1500	18	15
1501 – 2000	5	4
2001 – 4999	13	11
5000 and above	30	25
Years of farming experience		
15 years ago	38	32
10 years ago	44	37
5 years ago	42	35
1 year ago	19	16
Difficulty of land acquisition		
Increased demand for land	69	57.50
Don't know	3	2.50
High cost of land	20	16.67
Land was lost to miners	28	23.33

Table 2 Comparison of Yield Levels of Some Selected Crops with MOFA Achievable Yields Standards

ESTIMATED YIELD OF SELECTED CROPS PER HECTARE(HA) IN THE ASUTIFI DISTRICT YIELD MT/HA						
CROP	2006	2007	2008	2009	Achievable Yields	Actual Yields
Maize	2.3	4.0	2.2	2.1	6.0	2.0
Rice	1.7	2.7	1.9	1.8	6.5	1.5
Plantain	13.1	13.1	13.5	14.0	20.0	13.3
Cocoyam	7.9	7.9	8.0	9.0	10.0	8.0
Cassava	18.0	16.6	16.8	17.0	48.7	18.1
Cocoa	11.2	10.3	10	10.1	12.2	10.1

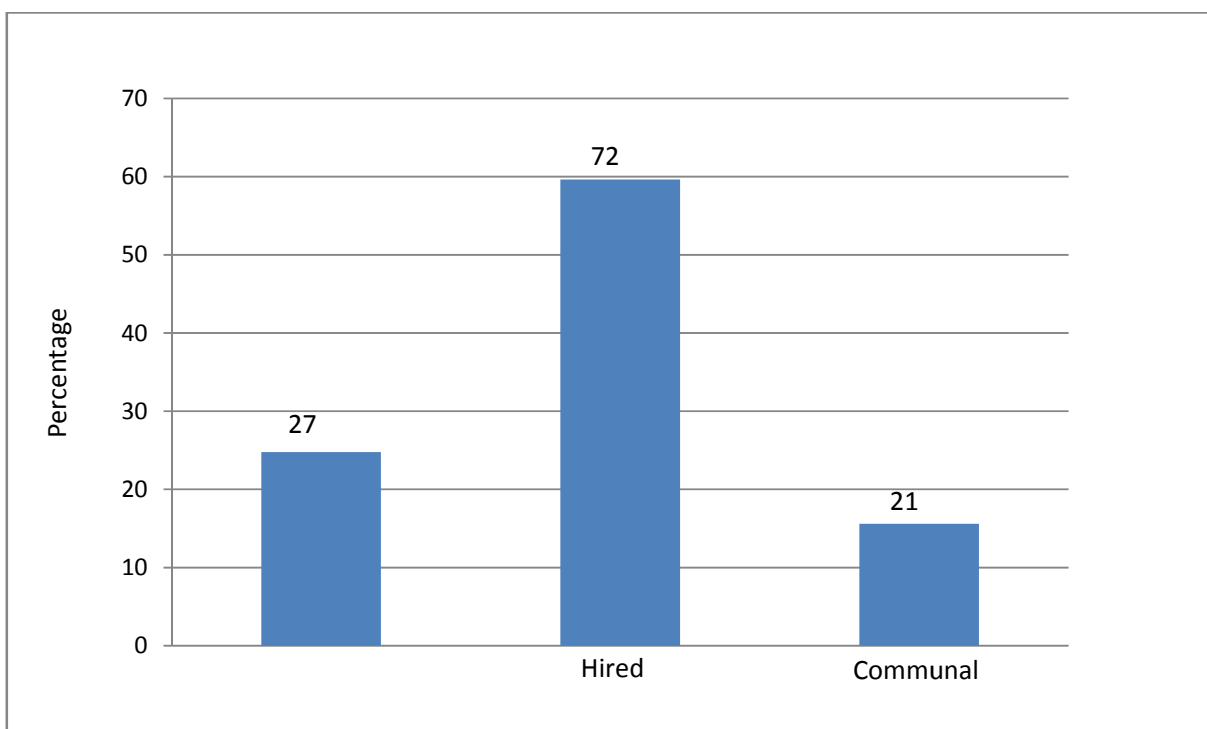


Figure 2 Types of Labour Respondents Use Family

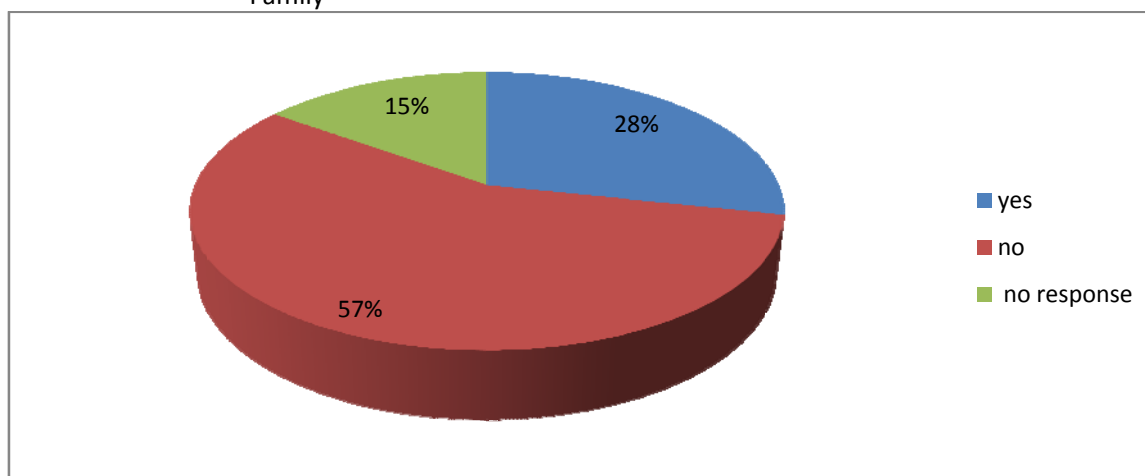


Figure 3a: Labour availability

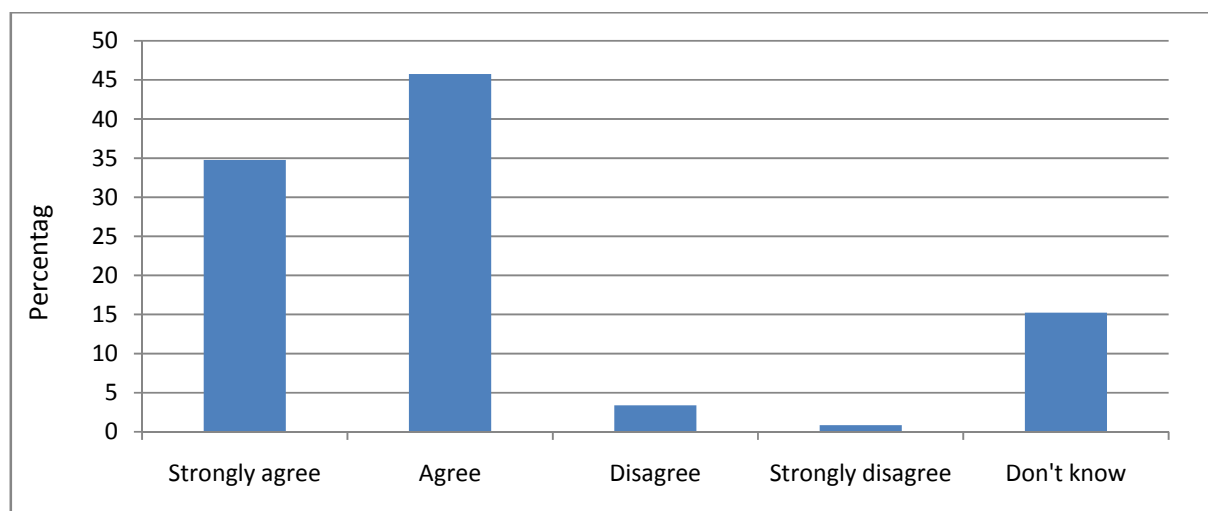


Figure 3b: Perception on Labour Shortages for Agricultural Activities

Discussion

Table 1 shows the features of farmers in the study area. Out of the total respondents 45% were women while 55% were men. This also confirms other studies that men constitute the general active agricultural working force and that of the mining sector (Amma *et al*, 2006), though with a fair representation of women. The age of the respondents was categorized into five (5) age categories as shown in table 1 below. The age category with the highest frequency was the 36 to 45 age group with about 58.4% of the total respondents. This shows that majority of the farmers in the study areas are within this category. The survey revealed that 48.9% had primary education with 39.1% attaining the Junior High School level. This indicates that most of the farmers interviewed did not have high education. Monthly income levels of respondents revealed that 54.2% earns between GH¢500 to GH¢1000 per month which constituted the majority from the study, however 9.4% of respondents earn more than GH¢2000. It was realized that farmers in this category were cultivating both cash and non-cash crops (cocoa, maize, rice cassava, plantain etc) and hence their higher monthly returns.

About 31% indicated that they started their agricultural practices about 10 years ago, whilst (32) of them making 26.7% started farming 15 years ago. Majority of the youth (35) representing 29.2% of the total respondents started farming within the last 5 years. From the survey, about 10 to 15 years ago, farmers could secure more farm lands (4 acres or more) with GH¢5 or GH¢10, or an equivalent of produce from land owners and sometimes at no cost at all. But now, the highest number of acreage a farmer could access was 3 at a cost of GH¢ 180 for a

season which is unaffordable by farmers. Some of the reasons given for the changes were, land being lost to the mining firm, increasing number of farmers in the area leading to higher demands for land and cost simultaneously in addition to general increase in land usage for other things like buildings and settlements. About 58% of the farmers attributed the difficulty in land acquisition to increase in demand of land as a result of increase in farmer numbers, whilst 23.3% attributed it to the loss of their arable lands to mining companies. It could be assumed to have contributed indirectly to the increase in the cost of lands and land fragmentation leading to more demand of land by farmers. Shortages of arable lands have been worsened by the acquisition of over 2,992 hectares of land by Newmont Ghana Gold Limited operating gold mines in the area. This has led to the increase in cost of acquiring the existing scarce arable lands for farming, implying that 77.4% of the population engaged in agriculture in the district have lost out their source of livelihood. Some of the respondents confirmed that the presence of the mining company though has created jobs for some of the people in the area have bequeathed them to small land holdings for agricultural activities limiting their ability to embark on large scale farming. Hence further expansion of existing mines or future discoveries of gold in the area could further reduce the agricultural land size in the area. According to the Ministry of Mines and Energy, approximately 30 percent of Ghana's land is currently under concession to gold mining firms, and each year more arable farming land are diverted to this use (IRIN, 2008).

The results of the socio-economic effect of mining on smallholder agriculture in the area include displacement of people and land, increased migration

into mining areas, environmental damages related to water quality water quantity, tailing management, noise and dust pollution as well as ecosystem disturbances, shortage of labour availability to agriculture, and contamination of crops as well as reduction in crops' yield. The operation at the Ahafo mines displaced about 823 households (5,185 people) of both residential buildings and cropped fields in the mine area. In addition, 878 households (4,390 people) will be economically displaced through the loss of cropped fields located in the mine area. The total number of impacted households is 1,701 consisting of 9,575 people (Planning Alliance, 2005). According to the Ghana Chamber of Mines, the mining and mineral industry injected a total of US\$2.9 billion into the economy in 2009, representing an increase of 27 percent from the 2008 figure of US\$2.3 billion. In Obuasi, Tarkwa and Kenyasi of Ghana, influx of migrants from other parts of the country to these areas are higher for works like small scale mining (galamsey) putting pressure on the social services and other amenities in these communities. Weber-Fahr, (2002) revealed in a cross-study analysis of environmental damages as a result of mining operations in 51 mining countries across the globe, that about 60% of the residents in these communities were at risk of environmental pollution. The concentration of mining operations in Tarkwa has been a major source of both surface and groundwater pollution. Four main problems of water pollution have been noticed in Tarkwa mining areas.

According to Amoah, (2003), chemical pollution of ground water and streams, siltation through increased sediment load and increased fecal matter and dewatering effects were the major water pollution problems from mining activities. Akabzaa and Darimani (2001) argued that, considerable areas of land and vegetation in Tarkwa for instance were cleared to accommodate surface mining activities which constitute over 70% of the total land area of Tarkwa. The tailing dam of one mine took a total of 6.3ha of land given an estimated per acre yield of cassava of 108,000 bags. This means the tailings dam has denied the farmers a minimum of 275,351 bags of cassava per annum. The tailings dam, plant site and feed stockpile of Ghana Australia Goldfields Limited alone affected a total of about 315 farmers currently cultivating around the area. In Akabzaa and Darimani's, view, the takeover of the dam had significant implications on the farmers' income and food security of the families due to the presence of tailing dams. Contamination of crops through poisoning as a result of waste from mining is a problem in the Obuasi area (Action Aid, 2005). TWN Africa indicates serious poisoning of local crops in

areas of historic gold mining activity, with high levels of mercury, zinc and arsenic found in local 'Obuasi oranges'. "Mercury values were up to 5 times more than EPA limits and 26 times more than World Health Organization limits. Zinc concentrations were also up to 5 and 8 times more than EPA and WHO limits in the soils and in plants. In addition, arsenic values were 24 and 1,226 times more than the EPA and WHO limits respectively as a result of the mining activities (Action Aid, 2005). With these deposits in the soil in higher concentrations, crops directly absorb the substances and assimilate it to the roots and fruits which are not safe for human consumption.

Prominent crops grown in the study areas include Cassava, maize, plantain, cocoa and rice. About 76.3% of the farmers cultivate three (3) or more acres of crops with 26% of the respondents having two acres each, with 36.4% cultivating less than two (2) acres of food crops. Almost all respondents interviewed cultivate many of the crops but 44% of the 120 respondents were growing more maize than other crops with an average yield of 2.02 metric tons per hectare. The yield trend over the past four (4) years from MOFA compared to the yield estimates from this study are captured in Table 2 below. Apart from 2007 where the yield level for maize was 4.02Mt/ha, which was quite high, the average yields for the district for maize was 2.6Mt/ha for the past five years. Other crops over the years in review showed marginal changes both in reduction and on increase as seen below.

Labour is an indispensable input in agricultural production cycle. Hired labour dominated the responses with (72) of them representing 59.6% affirmed that, it is a source of labour supply for their agricultural activities. This is a drain on the farmer's purse as an increase in the use of hired labour can lead to an increase in the cost of production. Hired labour is more effective if there is a supervision or involvement of either the farmer or the family. As a traditional practice, family labour is still being used as (27) of the farmers representing 24.7% of the respondents confirmed the use of the family labour, though it was lower than the hired labour. Communal labour use was quite low as only (21) of the farmers making 17% of the respondents resorted to using the communal system for their farming activities. It clearly showed the fading out of the "nnobua" and other communal benefits being enjoyed by the people in these communities. There is a gradual shift of family and communal labour supply to hired form of labour which is increasing the cost of crop production in the area. Cost of labour per man day of work respondents say was GH¢5 and at a peak

period could go as high as GH¢6 or more which to them is expensive because the frequencies required to undertake all the cultural practices in the farms.

Apart from being expensive for farmers to pay, 57% of the respondents said labour was not even available for farming as most of the able working youth are engaged in mining activities in the area. Twenty eight (28%) responded yes to show that there was available labour for their farming works. One respondent remarked that “ during farming season, it is extremely difficult to get labour for our farming activities as most of those we used to hire are now mine workers who are earning more than the farmers could afford”. To confirm this, the respondents were asked whether mining activities were creating labour shortages in their communities. The response showed 38.3% and 29.2% of the farmers respectively in Figure 3, indicating they agree and strongly agree with the perception that, the mining activities have created shortages of labour for their farming activities in the area.

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Abstract: One way of developing countries is Investment. Investment is use of capital in new or existing economic enterprise after obtaining a license. Attract investment capital is way to accelerate the move towards economic development and job creation, and can be used as a lever for development and economic growth. Due to our economic potential and ability and willingness of investors to attract foreign capital in our country has been unable to successfully acquire necessary. This lack of success due to a series of obstacles on the way in which investors are referred to in this paper.

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Keyword: investment, barriers, economics, development

1. Introduction

Investment leads to reform management and exchange of economical experience and implementing new technologies. In Iran to achieve a share of the large volume of capital transfers many efforts are already in the world. And Overcome many obstacles, but Iran's share of the global capital is yet scarce. Despite Iran's possession of its strategic location, which provides access to regional markets, and have the necessary resources and potential for investment has ever been a remarkable share of the funds allocated to that? Iran's economic advantages can better identify with many attractions for foreign investment there. Iran has the capability to attract foreign and Iranian investment, but has been very weak in this area.

Iran's free zones have the ability to attract large investments, But due to lack of proper infrastructure investors are not willing to invest in these areas. The infrastructure problem is that Iran isn't membership in the WTO. Membership in this organization can help resolve some problems fundraising. As long as economies are completely public, the country should not expect to have funds, because investors are working in countries with liberalized policies, deregulation and privatization. We should note that foreign investment is complementary to national investment. While national investment do not find place in your country can not expect to, Foreign capital entering to my country

2. Importance of investment

Investment has long been considered as important issue. There are Motivations that can help increase investment such as consideration of tax

incentives, investment guarantees and financial assistance to foreign investors. It seems that the policy of investee countries, especially in the field of international investment is important. Therefore, accepting the fact that financial markets of these countries spend their early stages, the importance of understanding this phenomenon in recent decades has doubled. Recognizing the potential and our ability to attract foreign capital can destroy some of the difficulties in attracting investment. So how could we mention this is that despite these obstacles, to attract foreign capital, an important issue and can be considerable.

The Impact of Investment is one of the major issues for developing countries that are often receptive foreign and domestic Investment. Investment in projects such as dams, power plants, roads, airports, and similar cases, and also directly productive projects is very important. For implementing these projects, capital requirements and for funding capital there are different ways such as borrowing from international institutions and governments, foreign investment credit (indirect) and direct foreign investment and national investment. Proliferation of technology has a pivotal role in economic development. In addition, foreign investment by multinational corporations as the main way of access to technology for developing countries. Experimental studies have shown that FDI is more stable than other types of international investors and policy-making often has a positive effect on economic growth.

Much research in the field of impact of investment in industrial growth Iran has been done that show: Percentage of exports of industrial

participate production with the percentage of foreign direct investment has a direct relationship

3. The potential advantages of investment

The potential advantages of foreign investment are:

- huge oil and gas reserves,
- abundant natural resources,
- unique geo-strategic position in the region,
- long sea borders,
- intensive consuming market and
- Young & cheap educated labor force

4. Reasons for failure to attract investment

Due to our economic potential and ability and willingness of investors to attract foreign capital in our country has been unable to successfully acquire necessary. This lack of success due to a series of obstacles on the way in which investors are referred to in this paper. The following summary of the obstacles are considered:

1. Lack of national eagerness and determination in attracting foreign investment
2. Poor performance for Iran free zones
3. Failure in performing privatization
4. Restriction of private, local or foreign banks and insurance companies
5. Extreme bureaucracy
6. Economic barriers
7. Legal barriers
8. Cultural barriers
9. Political barriers
10. Instability in policies and laws
11. High risk
12. Lack of proper cultural ground
13. Inappropriate social security which waste about 30% of Capital and increase the production costs with no economic feasibility
14. External barriers like imposed sanctions
15. Poor advertising and marketing

4.1. Economical barriers

Economic barriers such as: Excessive expansion of public sector, Currency fluctuations, Inefficient trade policies, lack of economic stability, lack of physical infrastructure, ports weakness, weakness of encourage and promote activities and services of investment, lack of adequate skilled manpower and...

4.2. Cultural and social barriers

As of the elements discouraging foreign investment in Iran, cultural and social constraints can be mentioned. Lack of a suitable technology culture,

absence of efficient information and communication networks, lack of incentives for hard work among people, lack of institutionalization of law among people, burdensome bureaucracy, traffic problems in major cities, etc., are major instances that have greatly reduced productivity in the country. Low productivity on the large and small scales indicates inability in making good use of available resources. Encouraging extravagance after oil price hike in 1974 was a major reason for wastage of rare resources. Spreading culture of optimization on large and small scales in the society would require scientific education and research from elementary school up to higher education. Another cultural barrier is the risk of spreading corruption. Preventing corruption and bribery in the society is an important issue which calls for due attention. Administrative corruption will discredit laws and regulations and lead to heavy personal and social costs.

4.3. Legal barriers

Despite the encouragement and protection of foreign investment law to attract foreign investors, implementing regulations. is faced with difficulties . Lack of coordination has caused confusion and uncertainty and complexity of various provisions of the Foreign Investor. and will intensify the ruling bureaucracy and government offices. Despite laws and regulations in many cases, due to the difficulty of implementing the above mentioned cases.

4.4. variety of Centers of decision making

Although the law has tried to encourage and support foreign investment in all relevant institutions in order to concentrate investments in one place, However foreign investors in Iran are not the only one with this organization. Government offices and institutions in Iran's decision was not only multiple, they also suffer from some kind of mismanagement and lack of coherent planning.

4.5. Administrative infraction

A major obstacle in implementing the laws and regulations, agencies and organizations at the level of corruption is Administrative infraction .This can be an obstacle in the way of investors in the country. It is likely that companies wishing to operate in Iran, despite having all the legal conditions for the start investment face to obstacles that the source is illegal behaviors.

4.6. Foreign exchange regulations

Other factor that limiting the activity of foreign investors is the issues of currency transfers. Foreign investment law requires that foreign investors use only known of currencies by central

bank. All these activities are done by banking systems that approved by the Central Bank of Iran. If the foreign investor wants no foreign exchange to the country's official currency, is required to pay their foreign orders will oversee the organization.

4.7. Political factors

The main obstacle is political sanctions against the laws of America. And then the lack of national consensus on how to attract and absorb foreign capital. This last factor has led to a lack of coherence and coordination between different devices is implemented. Other factors such as political instability, security problems, different interpretations of political independence, non-controversial and political trends in fundraising is also a political problem.

4.8. Tax Law

It was old and hindering but recently has had a remarkable decrease in tax rates. The rate of income tax decreased from 54% to 25% that covers all shareholders and company owners as well as the investing companies do not pay any income tax.

4.9 Information and communication factors

With Using information systems and telecommunications we can introduce investment opportunities in Iran in the shortest time to national and foreign investors, And marketing for projects. We will be introduced Capabilities, opportunities and foreign investment projects in different sectors of industry and mining, oil and gas, tourism, transportation and other sectors And also provides the opportunity for investors to select projects And the investment act. In this way it becomes possible for investors to register.

5. Data collection tool

Using data collection tools in the investigation is different, Because the data collection tool to the subject, purpose and research design depends.

Basis points in the method of research tools are such as: interviews, library studies and questionnaires were used for data collection. A questionnaire to identify barriers investment is designed with Likert Scale that the Likert Scale is a five point scale that by SPSS software has been analyzed.

5.1. Validity of questionnaires

Validity means that we are measuring what we want to measure. There are a number of types of validity including:

- Face Validity - whether at face value, the questions appear to be measuring the

construct. This is largely a "common-sense" assessment, but also relies on knowledge of the way people respond to survey questions and common pitfalls in questionnaire design;

- Content Validity - whether all important aspects of the construct are covered. Clear definitions of the construct and its components come in useful here;
- Criterion Validity/Predictive Validity - whether scores on the questionnaire successfully predict a specific criterion. For example, does the questionnaire used in selecting executives predict the success of those executives once they have been appointed; and
- Concurrent Validity - whether results of a new questionnaire are consistent with results of established measures.

To increase the validity of research were reviewed the research literature from the library of theses and research papers and several books. After interviews with managers and experts, research variables are identified and questionnaire was prepared. Finally questionnaire was reformed with faculty advisors consultation. We ensure that respondents understand the questions in the questionnaire does not have a problem with the final questionnaire it was distributed.

5.2. Reliability of estimates of questionnaire

Reliability means the consistency or repeatability of the measure. This is especially important if the measure is to be used on an on-going basis to detect change. There are several forms of reliability, including:

- Test-retest reliability - whether repeating the test/questionnaire under the same conditions produces the same results; and
- Reliability within a scale - that all the questions designed to measure a particular trait are indeed measuring the same trait.

Questionnaire reliability is measured using Cronbach's alpha. value 0.89 has acceptable.

6. Analysis of data

6.1. Data analysis tool

In this study, has been used statistical analysis of a specialized SPSS software And the One-Sample Test was used to check Hypothesis and Friedman test for ranked barriers.

6.2. Evaluation hypothesis analyze of questionnaire data

To check the hypothesis, the H_0 hypothesis and the H_1 hypothesis that expression is used as follows:

$$\begin{cases} H_0: \mu \geq 3 \\ H_1: \mu < 3 \end{cases}$$

H_0 : The agent is an obstacle to investment.

H_1 : The agent is not an obstacle to investment.

This research is based on tests and One-Sample Test Test Value = 3 has been done. Sig for this test Shows that mean is larger or smaller than 3. This factor is shown that a factor is a barrier to investment or not. If Sig test is less than 0.05 and lower and upper limits for both tests are positive, it means the average case is larger than 3 And this factor is an obstacle to investment. If Sig test is over than 0.05 and lower and upper limits for both tests are negative, it means the average case is smaller than 3 And this factor is not an obstacle to investment.

Evaluation The first hypothesis

H_0 : Policy is an obstacle to investment in Iran

H_1 : Policy is not an obstacle to investment in Iran

Table (1): One-Sample Test To test for Political barriers

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Policy	6.57	43	.033	.825	.57	1.08

According to the analysis that in Table (1) is shown, the test sig(P-Value) is 0.033 and is smaller than 0.05 and can be concluded that for Policy barriers is important for investment and H_0 is accepted.

Evaluation The second hypothesis

H_0 : Cultural is an obstacle to investment in Iran

H_1 : Cultural is not an obstacle to investment in Iran

Table (2): One-Sample Test for Cultural barriers

Culture	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Culture	6.26	43	.025	.861	.55	1.42

According to the analysis that in Table (2) is shown, the test sig(P-Value) is 0.025 and is smaller than 0.05 and can be concluded that Culture is important for investment and H_0 is accepted. Many people in iran have not motivation for investment and do not pursued for Investment issues.

Evaluation The third hypothesis

H_0 : legislation is an obstacle to investment in Iran

H_1 : legislation is not an obstacle to investment in Iran

Table (3): One-Sample Test for Legal barriers

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
egislation	3.42	43	.021	.895	.32	0.88

According to the analysis that in Table (3) is shown, the test sig(P-Value) is 0.021 and is smaller than 0.05 and can be concluded that legislation is important for investment and H_0 is accepted.

Evaluation The fourth hypothesis

H_0 : Information and communication are obstacles to investment in Iran.

H_1 : Information and communication are not an obstacles to investment in Iran .

Table (4): One-Sample Test for Information and communication

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Information	6.18	43	.042	.865	.97	1.28

According to the analysis that in Table (4) is shown, the test sig(P-Value) is 0.042 and is smaller than 0.05 and can be concluded that Information and

communication is important for investment and H_0 is accepted.

Evaluation The Fifth hypothesis

H_0 : Foreign exchange regulations are an obstacle to investment in Iran

H_1 : Foreign exchange regulations are not an obstacle to investment in Iran

Table (5): One-Sample Test for Foreign exchange regulations

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Foreign exchange	4.52	43	.035	.575	.32	1.33

According to the analysis that in Table (5) is shown, the test sig(P-Value) is 0.035 and is smaller than 0.05 and can be concluded that Foreign exchange regulations is important for investment and H_0 is accepted.

Evaluation The sixth hypothesis

H_0 : Economical barriers are an obstacle to investment in Iran

H_1 : Economical barriers are not an obstacle to investment in Iran

Table (6): One-Sample Test for Tax Law

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Economic	5.53	43	.013	.546	.47	0.88

According to the analysis that in Table (6) is shown, the test sig(P-Value) is 0.013 and is smaller than 0.05 and can be concluded that Economic is important for investment and H_0 is accepted.

Evaluation The Seventh hypothesis

H_0 : performance for Iran free zones is an obstacle to investment in Iran

H_1 : performance for Iran free zones is not an obstacle to investment in Iran

Table (7): One-Sample Test for Poor performance for Iran free zones

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
free zones	6.21	43	.018	.825	.78	1.98

According to the analysis that in Table (7) is shown, the test sig(P-Value) is 0.018 and is smaller than 0.05 and can be concluded that performance for Iran free zones is important for investment and H_0 is accepted.

Evaluation The Eight hypothesis

H_0 : performing privatization is an obstacle to investment in Iran

H_1 : performing privatization is not an obstacle to investment in Iran

Table (8): One-Sample Test for Failure in performing privatization

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
privatization	6.5	43	.043	.825	.57	1.08

According to the analysis that in Table (8) is shown, the test sig(P-Value) is 0.043 and is smaller than 0.05 and can be concluded that performing privatization is important for investment and H_0 is accepted.

Evaluation The Ninth hypothesis

Table (9): One-Sample Test for variety of Centers of decision making

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
Centers of decision making	6.57	43	.029	.825	.57	1.08

According to the analysis that in Table (9) is shown, the test sig(P-Value) is 0.029 and is smaller than 0.05 and can be concluded that Centers of decision

making is important for investment and H_0 is accepted.

6.3 Friedman test for ranked barriers

In this study, nine barriers were identified for investment, and Friedman test can be ranking barriers and compared barriers together.

Table (9): Friedman test for ranked barriers

barriers	Mean Rank
Political barriers	4.69
Cultural barriers	4.51
Legal barriers	4.28
Information and communication	3.66
Foreign exchange regulations	3.95
Economic barriers	4.22
Poor performance for Iran free zones	4.43
Failure in performing privatization	4.32
variety of Centers of decision making	3.70

Table (10): *Test Statistics^a*

<i>N</i>	44
<i>df</i>	8
<i>Asymp. Sig.</i>	.000

According to The coefficients obtained the Friedman test sig in the table, barriers have a different effect. Ranked in order of importance of these dimensions in the table is based on the Friedman test. As can be seen the most important aspects are

- Political barriers
- Failure in performing privatization
- Cultural barriers
- Economic barriers
- Poor performance for Iran free zones

7. Suggestions to remove barriers of foreign investment:

- Speeding the process of privatization and reducing the State dominance
- Vast advertising for foreign investments inside and outside the country
- Mobilizing all necessary resources for investment opportunities in Iran
- A careful study and reforming the protective laws

- Empirical investigation on presence of Foreign Banks in Iran
- Creating a proper and safe system to provide trustworthy statistics
- Creating special zones and facilities for foreign investors to reside
- Vast advertising to restore trust with foreign investors
- Using local and foreign facilities to attract foreign investments
- Revising in Iran investment, technical and financial assistance organization

Conclusion

The major obstacle to investment and the most fundamental problem is legal. The failure of existing laws and regulations in Iran is caused that investors do not have a clear vision of their investment. Among these problems can be pointed to some of the principles of the constitution. Investors prefer to invest in countries that are members of WTO or regional trade union. Because Membership in the WTO reduce trade barriers between member countries.

Another infrastructural barriers, lack of adequate transportation system in our country such as Short railways, locomotives and aircraft compared to other countries or for example, European countries do not allow us to enter the soil. Iran in terms of equipment and loading is low compared to neighboring countries. One successful way of attracting foreign direct investment is policy of liberalization and privatization.

- foreign direct investment can facilitate the state's economic growth which prepares the grounds to attract foreign direct investment in Iran's economy.
- The economic stability is a factor to draw foreign direct investment and instability factors such as inflation and fluctuation of foreign exchange will have a reverse effect.
- Other factors such as taxation, level of cooperation with other countries and existence of domestic capital are of other influential factors in foreign direct investment in Iran.

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The Association Between Gene Polymorphisms of Homocysteine Metabolism-Related Enzymes and Ischemic Cerebrovascular Diseases in Chinese Henan Han population

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Abstract: Background and Purpose—During the last years, several studies suggested a role for genetic factors predisposing to thrombophilia and for moderate hyperhomocysteinemia. The mutations in homocysteine (Hcy) metabolism-related enzyme genes including methylenetetrahydrofolate reductase (MTHFR) C677T, cystathionine b-synthase (CBS) 844ins68, and methionine synthase (MS) A2756G have been identified as genetic risk factors for thromboembolic events. The evidence of a role for these gene variants in the risk of ischemic stroke is controversial and it has been noticed that these gene mutations have heterogeneous distributions among different ethnic groups or geographic areas. The data on the prevalence of the gene mutations in Chinese population is not yet available. The aim of the present study was to investigate the association between these gene polymorphisms and ischemic cerebrovascular diseases in Chinese Henan Han population in a large case-control study. **Methods**—We investigated 512 cases (310 males, 202 females; mean_{SD} age, 60.58 years) as patient group with ischemic cerebrovascular disease in department of neurology in Henan province hospital were enrolled from December 2004 to July 2007 and 500 healthy subjects (274 males, 226 females; mean_{SD} age, 56.28 years) as control group in the study. All people are Henan Han Chinese and without cancer, epilepsy and hepatic or renal diseases. MTHFR C677T and MS A2756G polymorphism was genotyped by polymerase chain reaction and *Hinf*I digestion and *Hae*III respectively, while the genotypes of CBS 844ins68 was detected by polymerase chain reaction. **Results**—This investigation showed that MTHFR C677T TT-type 40%, CT-type 42.6% in patient group and TT-type 32.8%, CT-type 34.6%, in control group, and the T allele frequency was 61.3% versus 51.1% in the two groups. The frequencies of the three genotypes were significantly different between patient groups and controls ($\chi^2=30.36$, $P<0.01$). The frequency of T allele was significantly higher in patient groups than that in controls ($\chi^2=24.29$, $P<0.01$). But there were no significant differences in the frequencies of CBS 844ins 68 ($\chi^2=0.093$, $P>0.05$), MS A2756G ($\chi^2=4.101$, $P>0.05$) between the patient the and control groups. **Conclusion**—The C677T polymorphism of the MTHFR gene was associated with increased risk of ischemic cerebrovascular diseases and MTHFR C677T may be independent risk factors for ischemic cardiovascular diseases. However, the mutations of CBS844ins68 and MS A2756G was no associated with the ischemic cerebrovascular diseases in Chinese Henan Han population. The prevalences of MTHFR C677T, CBS 844ins68 and MS A2756G may vary with different ethnic groups or geographic regions. [Li AF, Zheng H, Xu YM, Zhao XJ, Zhang XM. **The Association Between Gene Polymorphisms of Homocysteine Metabolism-Related Enzymes and Ischemic Cerebrovascular Diseases in Chinese Henan Han population.** *Life Sci J* 2012; 9(3):403-408]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 56

Key Words: Ischemic cerebrovascular disease; Methylenetetrahydrofolate eductase; Cystathionine b-synthase; Methionine synthase; Gene polymorphism

1. Introduction

Ischemic cerebrovascular disease (ICVD) is a leading cause of death in the world. Over the last few years, several studies had been performed to elucidate the mechanisms of ischemic stroke, but this work was supported by the science foundation for prominent youth of Henan (No.06122001300) and Henan innovation project for university prominent research talents (N0.2005KYCX020) the etiology of ICVD is complicated and not understood completely until now. New researchs has discovered that ICVD is related

with both hereditary and environmental factors. Recently, many researchers focus on the predisposing genes of the related risk factors of cerebral infarction. HHcy is a new important and independent risk factor to atherosclerosis and cerebral infarction. The level of plasma homocysteine are influenced by hereditary and environmental factors. The three key enzymes in the homocysteine metabolism are 5,10-methylenetetrahydrofolate reductase (MTHFR), Methionine synthase (MS) and Cystathionine-βsynthase (CBS). The gene mutations of MTHFR C677T, MS

A2756G and CBS 844ins68 can result in loss or decrease of enzyme activity and consequently increase or decrease of Hcy level. Therefore, some genes that code enzymes involved in Hcy metabolism are considered to be candidate gene. But the correlation between these genes and ICVD is still controversial^[1,2,3]. In order to further investigate the correlation between the metabolism-related gene polymorphism and ICVD, the researcher detected the MTHFR, CBS and MS genes by PCR-RFLP in a large sample and observed the distribution of genotypic frequency and allele frequency among Han people in Henan province in China.

2. Material and Methods

2.1. patients and controls

This study included 512 cases (310 males, 202 females; mean \pm SD age, 60.58 years) as patient group with ischemic cerebrovascular disease in department of neurology in Henan province hospital were enrolled from December 2004 to July 2007 and 500 healthy subjects (274 males, 226 females; mean \pm SD age, 56.28 years) as control group. All people are Han Chinese and without cancer, epilepsy and hepatic or renal diseases.

2.2. Genotype detection of the MTHFR C677T, MS A2756G and CBS 844ins68

Genomic DNA were extracted from peripheral-blood lymphocytes by the standard phenol-chloroform method. Genotyping was performed according to previously described methods for MTHFR677 of the MTHFR gene, CBS ins 68 of the CBS gene and MS2756 of the MS gene polymorphism in details [4,5,6]. For the 677 C→T polymorphism, extracted DNA was amplified with the forward primer 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and the reverse primer 5'-AGG ACG GTG CGG TGA GAG TG-3'. Polymerase chain reaction (PCR) thermal cycling conditions were 2-minutes denaturation at 94°C, then 40 cycles of 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds. This was followed by 5-minutes extension at 72°C. Amplified 198-bp PCR products were digested with *HinfI* and visualized under electrophoresis on 2% agarose gel with ethidium bromide. The C allele produced a 198-bp band, and the T allele produced 175- and 23-bp fragments. For the MS 2756A→G polymorphism, DNA was amplified with the forward primer 5'-CAT GGA AGA ATA TGA AGA TAT TAG AC-3' and the reverse primer 5'-GAA CTA GAA GAC AGA AAT

TCTCTA-3'. PCR thermal cycling conditions were 2-minutes denaturation at 92°C, then 35 cycles of 92°C for 60 seconds, 56°C for 60 seconds, and 72°C for 90 seconds, with a 7-minutes extension at 72°C. PCR products were digested with *HaeIII* and visualized under electrophoresis on 2% agarose gel with ethidium bromide, resulting in a 189bp band for the A allele and 159- and 30-bp fragments for the G allele, after 2% agarose gel with ethidium bromide. For the CBS 844ins68 polymorphism, DNA was amplified with the forward primer 5'-CTG GCC TTG AGC CCT GAA-3' and the reverse primer 5'-GGC CGG GCT CTG GAC TC-3'. PCR conditions were 3-minutes denaturation at 95°C, then 35 cycles of 95°C for 60 seconds, 58°C for 60 seconds, and 72°C for 60 seconds, with a 3-minutes extension at 72°C. Mutated CBS 844ins68 fragment was 252bp

2.3. Statistical analysis

Allele frequencies in the ICVD patients and controls were determined by counting alleles and calculating proportions. The Hardy-Weinberg equilibrium analysis was calculated using the chi-square statistics for goodness of fit (1 degree of freedom). The OR with an associated 95% confidence interval (CI) was calculated to estimate the relative risk of the different genotype combinations. MTHFR, MS and CBS alleles frequencies were determined for the study and control groups, and were compared by χ^2 analysis. P values ≤ 0.05 were considered statistically significant, and all P values were based on two-tailed tests. The SPSS 11.0 statistical software program was used for all analysis.

3. Results

3.1. Mutation Identification

The genotype of heterozygous MTHFR C677T mutation (C/T) showed three bands of 198, 175 and 23bp. The mutant homozygote (T/T) showed two bands of 175 and 23 bp; the wild type (C/C) showed only one band of 198 bp (Fig. 1; the 23-bp band was out of gel). The heterozygote for MS A2756G (A/G) showed three bands of 189, 159 and 30 bp; the mutant homozygote (G/G) showed two bands of 159 and 30 bp; the wild type (A/A) showed only one band of 189 bp (Fig. 2; 30 bp band was not demonstrated here). On the gel containing PCR products of the CBS gene, the wild type (D/D) showed a band of 184 bp; the heterozygote for CBS 844ins68 (I/D) showed a 252-bp band in addition to the 184-bp band. No mutant homozygote (I/I) was found in this series (Fig. 3).

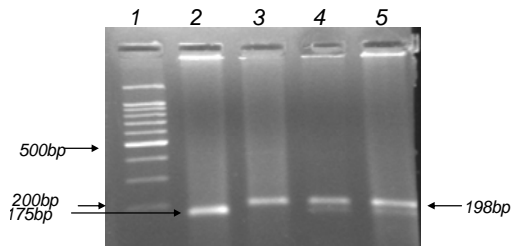


Fig.1 Genotyping of the MTHFR C677T gene

Lane 1 :100bp DNA ladder

Lane 2:TT homozygous

Lane 3:CC heterozygous

Lane 4,5:CT heterozygous

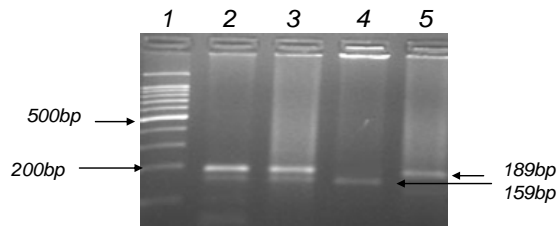


Fig.2 Genotyping of the MS A2756G gene

Lane 1:100bp DNA ladder

Lane 2,3:AG heterozygous

Lane 4:GG homozygous

Lane 5:AA homozygous

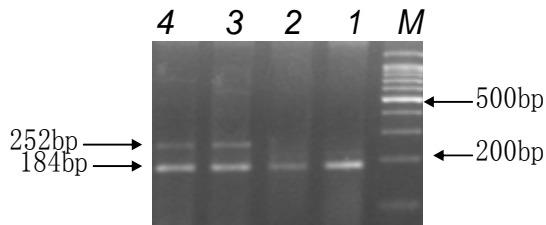


Fig.3 Genotyping of the CBS 844ins68 gene

Lane 1,2:DD homozygous

Lane 3,4:I/D heterozygous

3.2. Mutation Frequencies and Distribution in Patient and Control Groups

MTHFR, MS and CBS genotype distributions and allele frequencies for Chinese Henan Han population ICVD patients and controls were presented in Table 1,2,3. We found a genotype frequency distribution for the MTHFR, MS and CBS mutation that was as expected according to the Hardy-weiberg equilibrium. Between the patient groups and the control group, there was statistically significant difference in the distribution of MTHFR C677T genotype of the mutations ($P < 0.001$). However, The difference in frequency in the CBS allele and in the MS G allele between the patient groups and the control group there was not statistically significant. The T allele of MTHFR C677T yielded an OR of 2.583 for ICVD

(95% confidence interval (CI) 1.92-3.12). The G allele of MS A2756G yielded an OR of 0.92 for ICVD (95% CI 0.47-1.81). The heterozygous state of CBS 844ins68 yielded an OR of 0.19 for ICVD (95% CI 0.02-1.43). All of the OR values were not significant in statistical sense.

The distributive pattern for combined mutation in single individual for both MS A2756G and MTHFR C677T was summarized in Table 4. The difference in the frequency of this combined mutations between the patient groups and the control group was not significant ($P = .748$). The OR was 0.97 for ICVD (95% CI 0.46-2.48), statistical significance was not obvious. The frequency of mutation of CBS 844ins68 was relatively low (2.96%) in the overall study series.

Table1. Prevalence of the MTHFR C677T genotypes and allele frequency in patients and controls

groups	subjects(n)	Genotype frequency n (%)			allele frequency (%)	
		CC	CT	TT	C	T
Patient	512	89(17.4)	218(42.6)	205(40)	38.7	61.3
control	500	163(32.6)	173(34.6)	164(32.8)	49.9	51.1
					$\chi^2=30.36, P<0.001$	
					$\chi^2=24.29, P<0.001$	

Table2. Prevalence of the MS A2756G genotypes and allele frequency in patients and controls

groups	subjects(n)	Genotype frequency n (%)			allele frequency (%)	
		AA	AG	GG	A	G
Patient	512	455(88.9)	55(10.7)	2(0.39)	965(94.2)	59(5.8)
control	500	423(84.6)	75(15)	2(0.4)	921(92.1)	79(7.9)
					$\chi^2=4.101 P>0.05$	
					$\chi^2=3.64 P>0.05$	

Table3. Prevalence of the CBS 844ins68 genotypes and allele frequency in patients and controls

groups	subjects(n)	Genotype frequency n (%)			allele frequency (%)	
		D/D	D/I	I/I	D	I
Patient	512	496(96.7)	16(3.3)	0(0)	1008(98.4)	16(1.6)
control	500	486(97.2)	14(2.8)	0(0)	986(98.6)	14(1.4)
					$\chi^2=0.093 P>0.05$	
					$\chi^2=0.186 P>0.05$	

Table 4. Prevalence of combined mutations of MS A2756G (A/G) and MTHFR C677T (C/T or T/T) between control and patient

combined mutations	Patients	Controls	P values
AG+CT	22	27	0.748
AG+TT	20	26	
Sum	42	53	

* The P value was obtained by χ^2 test

4. Discussion

In this study we investigated whether the gene mutations of MTHFR C677T, MS A2756G and CBS 844ins68 could have an impact on ischemic cerebrovascular disease in the Henan Han population. We found the frequency of the T-containing allele was slightly higher in this study than in a previous study in Japan^[7] but was similar to that for Chinese other population^[8], demonstrating that this polymorphism is common in the Chinese Henan Han population it is likely that discrepancies in allele frequency result from ethnic or regional differences. The results of the present study indicate the T-containing allele has an increased occurrence of the ischemic cerebrovascular disease (OR=2.583), it may be expected that the MTHFR C677T mutation is a risk factor for ICVD in the Chinese Henan Han population.

Our finding of no association between the A2756G polymorphism in the MS gene and the occurrence of the ICVD, which is in agreement with the findings of other groups who did not report a direct or significant role for this polymorphism in the etiology of ICVD^[8,9,10].

MS plays an important role in the Hcy metabolism. The gene coding MS has been cloned, sequenced and

located, and, with several mutations, have also been identified. The most prevalent mutation of the MS gene is the A2756G transition, which results in the substitution of aspartic acid by glycine. Among Western Caucasian populations, the prevalence of heterozygous and homozygous MS A2756G carriers has been reported to be around 32% and 4%, respectively, and does not exhibit a significant difference between patients with coronary artery disease and healthy controls^[11,12]. Our data show that the prevalence of MS A2756G in the Chinese Henan Han population is lower than that in the Western Caucasian population, and the frequency of MS A2756G mutation in the patient groups are not different from that observed in the control group. The information indicates that the MS A2756G gene may not be an independent risk factor for ICVD in the Chinese Henan Han population.

Cystathionine beta-synthase (CBS) mediates conversion of homocysteine to cystathionine and deficiency in enzyme activity may lead to hyperhomocysteinemia/homocystinuria, which are often associated with vascular disease. A large number of polymorphisms have been reported in the CBS gene, some of which impair its activity and among these, a

T833C polymorphism in cis with a 68 bp insertion at 844 in the exon 8 is found to be associated with mild hyperhomocysteinemia in different ethnic groups^[13,14]. From our observation, the prevalence of CBS 844ins68 in the Chinese Henan Han population is evidently lower than that in Western Caucasian population. The CBS 844ins68 mutation tends to be no significant difference in the healthy individuals (2.8%) and that in the patients (ICVD, 3.3%) and yields an OR of 0.19 for ICVD, which may suggest that CBS 844ins68 is not a risk factor for ischemic cerebrovascular diseases. The carriers of all the three mutations are few in the investigated population. Thus, combined mutations of MTHFR C677T and MS A2756G can be confirmed not to be an independent risk factors for ICVD in the Chinese Henan Han population.

In conclusion, the mutation of MTHFR C677T, may be a genetic risk factor for population with ICVD, while the gene polymorphisms MS A2756G or CBS 844ins68 cannot be a genetic risk factor for ICVD in the Henan Han population of Chinese.

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Study of tris{2-(benzimidazol-2-yl) quinolinato} Aluminum

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Abstract: An emission material, tris(2-(benzimidazol-2-yl) quinolinato) Aluminum (**AIBIQ**) used for organic light emitting devices, has been synthesized. The decomposition temperature was observed at 436°C and no melting transition (T_m) of **AIBIQ** was observed up to 430°C. The emission spectrum of organic emitting device using **AIBIQ** as emitted layer exhibits a broad maximum at 596 nm. The color of the emitted light is in the orange region in the CIE coordinate of $x = 0.46$ $y = 0.46$.

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Keywords: Electroluminescence; orange light; device

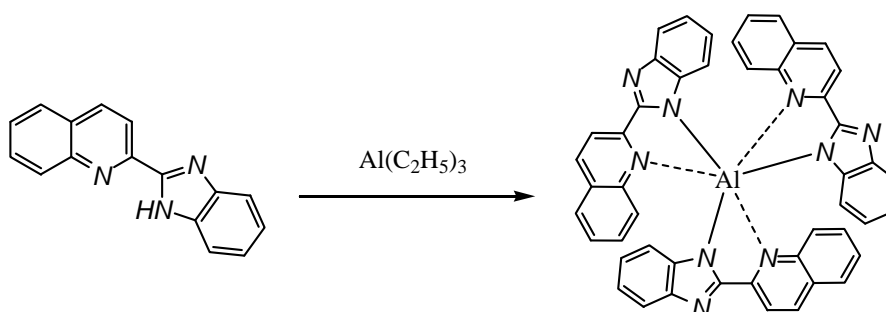
1. Introduction

Since an organic light emitting diode was reported by Tang and Vanslyke [1], LEDs based on organic or polymeric materials have generated considerable interest and enabled the development of low-cost, full-color, flat-panel displays along with other emissive products [2–5]. Luminescent chelate complexes have been shown to be particularly useful in electroluminescent (EL) displays because of their relatively high stability and volatility. The most well-known example of such chelate compounds is Alq_3 , not only a good emitter but also a highly efficient electron-transporting material, where q is the 8-hydroxyquinolinato ligand [6, 7]. Via the modification of the ligand of metal chelate compound, the emission color of a metal chelate compound may be tuned. Other properties, such as thermal stability and carrier mobility, may also be improved upon. In the present work, we report the synthesis and electroluminescent (EL) property of tris(2-(benzimidazol-2-yl) quinolinato) Aluminum (**AIBIQ**). The **AIBIQ** containing N,N-bidentate ligand instead of N,O-bidentate one such as

8-hydroxyquinoline [8]. Therefore, the thermal stability, an important character for the practical application in the electronic fields, of this metal complex is investigated by thermo-gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The organic emitting device using **AIBIQ** as emitting layer has been fabricated to study the electroluminescent property of this metal complex.

2. Experimental

The synthesis of the title compound was accomplished by following processes, as shown in Scheme 1. The triethylaluminum solution (25% w/w in hexane 1.37ml, 3×10^{-3} mole) was slowly added to 100 ml of THF solution containing benzimidazol-2-yl-quinoline [8] (2.45g, 10×10^{-3} mole) at 0°C under N_2 . After the resulting mixture was stirred at room temperature for 6 hours, 5 ml isopropyl alcohol was added to quench the reaction. The solvents were removed under vacuum condition at 5×10^{-3} Torr, and the residual solid was sublimed to purify the final product. Light green of



Scheme 1. Synthesis process for the AIBIQ complex.

AIBIQ was obtained in 75% yield. The formula of this compound has been determined by ^1H NMR and elemental analysis. The organic light emitting device, Fig. 1, using **AIBIQ** as the emitting and electron-transporting layer were fabricated on the transparent conductive indium-tin oxide (ITO) glass substrate. The organic layers and the cathode were sequentially deposited by conventional vacuum vapor deposition in the same chamber without breaking the vacuum under 3×10^{-6} Torr. In the present work, the N,N' -bis-(1-naphthyl)- N,N' -diphenyl-1,1'-biphenyl-4,4'-diamine (NPB) was used as the hole-transport material (HTM), and tris (8-quinnolinolato) aluminum (Alq_3) was employed as the electron-transporting material (ETM). The EL spectrum and the Commission Internationale de l'Eclairage (CIE) co-ordinates were measured by Pro-650 Spectroscanner (step size is 1.0 nm and bandpass is 4nm), the current-voltage (I-V) characteristic was measured by Keithley 2400 Source meter.

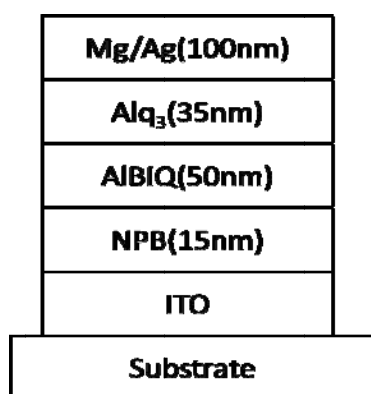


Fig. 1. The organic light emitting device (OLED) structure and molecular structures of **AIBIQ** and **NPB**.

Thermogravimetric analysis (TGA) was performed on a Perkin-Elmer thermo-gravimeter (Pyris 1) under a dry nitrogen gas flow at the heating rate of $20^\circ\text{C}/\text{min}$. Glass transition temperature (T_g) and melting point (T_m) of materials were determined by differential scanning calorimetry of the Perkin-Elmer differential scanning calorimeter (DSC-7).

3. Results and discussion

Fig. 2 shows the TGA of **AIBIQ** that possesses a maximum rate of weight loss occurring at 501°C and no weight loss was observed at the temperature lower than 362°C . Above 600°C , there is about 16 wt % of residue composed of zinc ash. This aluminum complex is reasonably stable upon exposure to air and exhibited a very high thermal stability in nitrogen, which is attributed to the fact that the Al-N bond is highly

polarized [9, 10]. The melting temperature (T_m) of **AIBIQ** was not observed up to 436°C with DSC curve. The DSC and TGA results indicate that the **AIBIQ** possesses a very high thermal stability, which may serve as an advantage for the fabrication of organic light emitting device because the use of the materials with high thermal stability as the active emissive layer or carrier transporting layer may provide the device with greater longevity [11, 12].

The Photoluminescent (PL) spectra of the **AIBIQ** solutions and neat film, excited with 406 nm laser line, were illustrated in Figure 3. At low concentration, 5×10^{-5} M in DMSO, only one emission band is observed with maximum at 406 nm, corresponding to the relaxation of **AIBIQ** from the excited state of a single molecule into ground state. Besides the 460 nm band, a new emission band appeared while the concentration of **AIBIQ** increased from 5×10^{-5} to 1×10^{-3} M. This new emission band having a maximum at 470 nm is observed in the spectrum of the **AIBIQ** neat film. We have assigned this new emission band to the excimer and higher aggregates emission [13, 14] resulting from the relaxation of collision complex into the lower energy state.

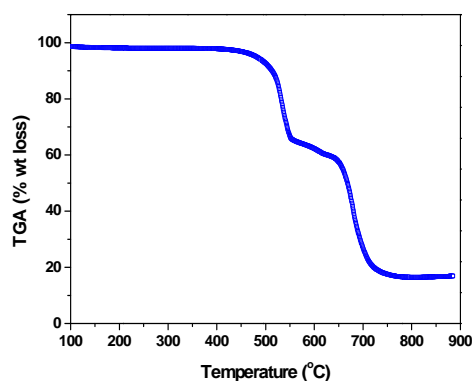


Fig. 2. TGA curve of **AIBIQ**.

The EL spectrum of organic light emitting device at the bias voltage of 12 V, Fig. 4, shows the broad emission band in the 500-700 nm region with the maximum at 570 nm. The emission is almost fixed in the orange region in the CIE coordinate of $x = 0.46$ $y = 0.46$. For the small molecular organic materials, to develop the new type of material with red emission is very important because this kind of material is very seldom prepared so far, and it is very important for the fabrication of full color display panels. The change of the spectral wavelength may be achieved also by general conception of search and design of modified materials for wide band emission consists in substitution of the backside groups by electron

acceptors like halogens etc. and different kind of donors [15, 16].

At the same time important role here may play electron-vibration interactions determining the spectral broadening of the emission lines. So the future strategy of the materials design may be in this way also.

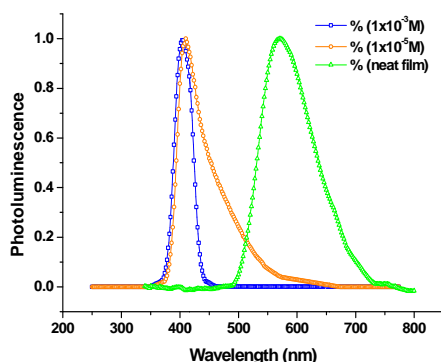


Fig. 3. Photoluminescent spectra of the AIBIQ in solutions and neat film.

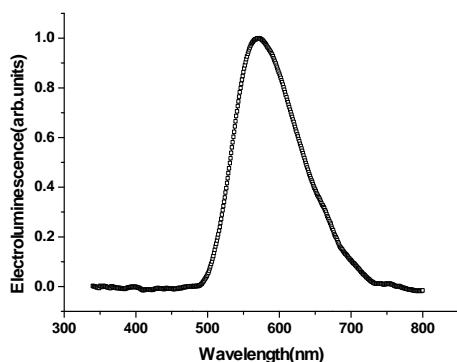


Fig. 4. EL spectrum of OLED fabricated in this work

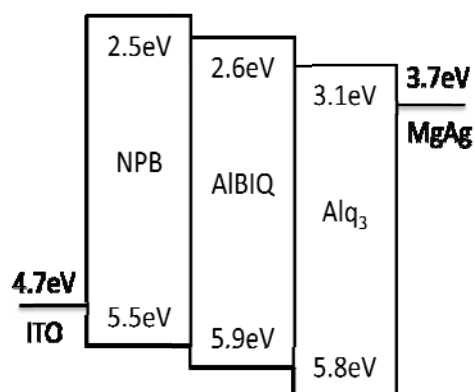


Fig. 5. Energy level diagram of OLED materials, ITO, and Mg–Ag alloy.

Figure 5 shows the energy level diagram of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of the different organic materials and the work function of cathode and anode. The LUMO energy of AIBIQ is 2.6 eV determined from the cyclic voltammetry (CV) method and the optical band gap estimated from the absorption onset. Comparing the energy level of AIBIQ with NPB, it is clear that the AIBIQ has the much higher hole injection barrier than that of NPB; in fact, it is impossible for the hole injection from ITO into AIBIQ without the assistance of NPB or some other kind of HTLs. This diagram also pointed out that the Alq₃ has the lower electron injection barrier than that of AIBIQ, so the electron injection from the MgAg into AIBIQ will be enhanced and confines the recombination zone at the interface between NPB and AIBIQ. Fig. 6 shows current-voltage and luminance-voltage characteristics of this device having a low turn on voltage of about 5.5 V for current and luminance. This device shows a brightness of 1580 cd/m² at the driving voltage of 12 V with current density of 196 mA/cm², decaying to 60 cd/m² in 120 hours.

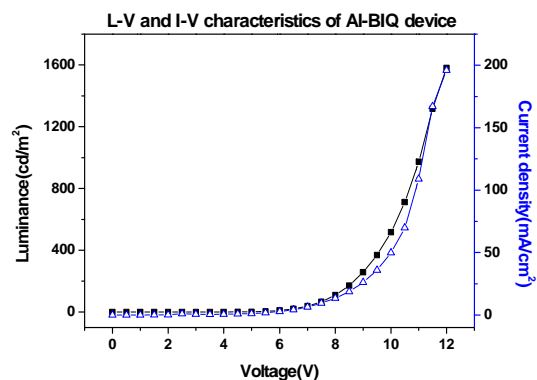


Fig. 6. Current–voltage and luminance–voltage characteristics of OLED fabricated in this work.

4. Conclusion

A novel metal complex, tris (2-(benzimidazol-2-yl) quinolinato) Aluminum (AIBIQ), was successfully prepared by the reaction of benzimidazol-2-yl-quinoline and triethylaluminum. Because of its high thermal stability and excellent electrical characteristics, AIBIQ and its related compound suggest a possible application for the use of orange-light of the organic light emitting devices.

Acknowledgements

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A novel approach for preparation of minicircle HSV amplicons by adenovirus mediated Cre-loxP recombination in mammalian cells

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Abstract: Amplicon is a plasmid backbone based helper-dependent pseudovirion vector of HSV-1 and has been used as a powerful and versatile gene delivery vehicle due to its unique features. However, like plasmid-based vectors, the major drawback of conventional amplicon for gene delivery is its transient transgene expression, which has been demonstrated that the bacteria elements in the vectors are responsible for the transgene loss event, as evidenced, minicircle DNA and minicircle amplicon devoid of bacteria sequences mediated higher and sustained gene expression both in vitro and in vivo. Nevertheless, current techniques for MC DNA preparation by inducing MC producer plasmid intra-molecular recombination in bacteria have critical limitations, including their labor-intensive, time-consuming procedure, and high contamination with input plasmids and mini plasmids. We thus herein described a novel simple approach for MC-amplicon preparation by utilizing adenovirus mediated Cre-loxP site-specific recombination and HSV helper virus supplied replication and package function in mammalian cells. This technique allows for production of MC-amplicons free of bacteria elements, making it feasible to use MC amplicon instead of conventional amplicon in gene delivery studies.

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Keywords: Cre-loxP recombination, Amplicon, Mini circle amplicon, HSV, Gene transfer, Adenovirus.

1. Introduction

Amplicons are helper-dependent pseudovirions of HSV-1 identical to wild type HSV-1 particles from the structural, immunological and host range points of view, but carry concatemeric form of plasmid DNA instead of the viral genome (1-2). Amplicon was originally developed by Spaete and Frenkel 1982 via the incorporation of a single origin of replication (oriS) and a single packaging/cleavage signal (pac) from the wild-type HSV-1 genome into a bacterial plasmid, which were termed "amplicon" (3). Therefore, conventional HSV-1 amplicon is composed of an Escherichia coli plasmid backbone carrying one origin of virus replication (OriS), one packaging signal (pac) from HSV-1 in addition to the transgenic expression cassette(s) of interest. When transfected into HSV-permissive cells and supplied with full HSV helper function, amplicons can be replicated and packaged into infectious HSV pseudovirions as a 150-kb linear vector DNA genome comprising "head-to-tail" concatenated structures of the original plasmid (4-5). Amplicon vectors possess unique features, including large transgene capacity of up to 150 kb of foreign DNA, the ability to transduce a wide variety of cell types of the most proliferating and non-dividing mammalian cells across a broad range of species, the ease of vector construction, and limited cytotoxicity and immunogenicity, thus making these vectors very appealing for preventive or therapeutic gene transfer as well as for upstream fundamental

studies (1, 6-8). Since it lacks virus coding genes and is unable to integrate into host chromosomes, the conventional amplicon does not cause insertional mutagenesis, and strongly reduces the risk of reactivation, and complementation or recombination with latent or resident HSV-1 genomes in the transduced organisms. However, before amplicon vectors can be safely and efficiently applied to human beings in gene therapy or vaccine protocols, there are still technique obstacles, such as producing large and high-titer stocks of vector particles without helper virus contamination (2), and their transient transgene expression limitation have to be overcome. Like plasmid mediated gene delivery, rapid transgene silencing, even in non-dividing cells, is the most critical shortcoming of conventional HSV amplicon vector (1, 9). Since Amplicon backbone DNA harbors multiple copies of bacteria elements (e.g. colE1 origin and antibiotic resistance gene), depending on the amplicon plasmid size, it may share the same mechanisms underlying the transgene loss with the plasmid mediated gene delivery. It has been demonstrated that the bacteria sequences in the plasmid are responsible for the rapid transgene expression suppression, as evidenced by that the gene expression mediated by a novel supercoiled minimal circular transgene cassette(s) known as the minicircle (MC) DNA devoid of bacteria sequences deriving from conventional plasmid DNA by site-specific recombination in Escherichia coli achieved a 10- to

1,000-fold enhancement compared with regular plasmids in long-term transgene expression even in quiescent tissues both in vitro and in vivo(10-11). For MC DNA preparation, several approaches have been developed by engineering different recombinases, such as the bacteriophage λ integrase(12), Flp(13-14), Cre recombinase (15-17) and phage phiC31 integrase(10, 11, 18) into the MC producer plasmid or into the bacteria genome to achieve inducible intra-molecular site specific recombination and generate recombinant MC molecules in bacteria. By adopting the phiC31 integrase mediated method, Suzuki et al 2006 generated minicircle amplicon vectors (MC amplicon) devoid of bacterial sequences and exhibited significant higher and more persistent expression at transcriptional level than conventional amplicons both in vitro and in vivo and revealed a involved molecular mechanism that the bacterial sequences in the conventional amplicon DNA triggered the whole vector chromatin inactivation (9). The findings suggested that MC amplicon might be an excellent ideal gene delivery vehicle super to the conventional amplicon and hold a promising for future gene therapy and vaccine development. However, the main shortcomings of currently used techniques for minicircle DNA preparation in bacteria have been their time-consuming, labor-intensive procedure, low-yield production and more important, high contamination with MC-producer plasmid and plasmid backbone circle (19). Therefore, the MC amplicon preparation techniques should be further improved so as to completely eliminate plasmid-amplicon contamination, simplify the preparation procedure and be capable of scale up production.

We herein describe a novel approach for the MC-amplicon preparation by adenovirus mediated Cre-loxP site-specific recombination in mammalian cells that allows for a simple and rapid production of MC-amplicons without any bacteria sequence and donor vector contamination.

2. Materials and methods

2.1 Cells, plasmids and virus

African Green monkey kidney (Vero) and 293A cells were purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The plasmid C223(Fig.1-A), a bacteria F plasmid, named bacteria artificial chromosome (BAC) plasmid carrying GFP and puromycin resistant gene cassettes and loxP sequences, and pGEM-T-Cre were gifted from Dr Yu, Washington University in St. Louis. The HSV-1 amplicon plasmid pHSV-A-DsRed (Fig.1-C) with red fluorescent reporter gene was constructed by our laboratory as described previously (20). The pAd/BLOCK-iTTM-DEST and pENTRTM/U6, a replication-incompetent adenoviral vector system for generation of adenoviral RNAi vectors were purchased

from invitrogen, (USA). The HSV-1 strain F and HSV-2 strain HG52 were obtained from Wuhan Institute of Virology, Chinese Academy of Sciences.

2.2 Construction of pAdv-loxP-OPD-loxP and generation of adenovirus

2.2.1 Construction of pENTR-MCS

Two DNA oligonucleotides as the following sequences, 5'-TCGAAGTACTACCCGCGGACGAAT TCCAGCGGCCGACCTCGAGACGGATCC-3', and 5'-TCGAAGTACTACCCGCGGACGAATTCCAGCG GCCGACCTCGAGACGGATCC-3' were synthesized and annealed to create a multiple cloning sites (MCS), with *ScaI/SacII/EcoRI/NotI/XhoI/BamHI* sites, and introduced into the *Sall* and *XbaI* sites flanked by attL1 and attL2 sites of pENTR/U6 to result in pENTR-MCS.

2.2.2 Construction of pENTR-loxP-loxP

A 1.5Kb fragment containing two loxP sites in same orientation acquired from *XmnI/ScaI* digestion of C223 plasmid was inserted into the corresponding sites of pENTR-MCS to construct an intermediate vector pENTR-loxP-loxP (Fig 1-B).

2.2.3 Construction of pENTR-loxP-OPD-loxP

A 3Kb fragment containing oriS, pac elements and DsRed expressing cassette (OPD) from *AvaII/NotI* cutting pHSV-A-DsRed was inserted into the corresponding sites of pENTR-loxP-loxP by replacing all the DNA sequences between two loxP sites except *AvaII/NotI* sequences to generate the pENTR-loxP-OPD-loxP, which was served as an entry vector for the adenovirus recombination. By the same way, the 1.5Kb DsRed expressing cassette from pDsRed2-C1 was cloned to the same site of pENTR-loxP-loxP to construct a pENTR-loxP-D-loxP as the control vector and verified its orientation consistent with that in pENTR-loxP-OPD-loxP as analyzed by *EcoRI* digestion.

2.2.4 Construction of pAdv-loxP-OPD-loxP adenovirus plasmid

To transfer the loxP-OPD-loxP structure into plasmid adenovirus vector pAd/BLOCK-iT-DEST, a LR recombination was performed following the manufacturer's instruction. Briefly, in a 0.5 Eppendorf tube, 100ng plasmid DNA of pENTR-loxP-OPD-loxP and 150ng pAd/BLOCK-iT-DEST in 8ul TE buffer were mixed with 2ul LR Clonase II enzyme (2ug/ul) and incubated at 25°C for 1 hour, then 1ul of the Proteinase K solution was added into the reaction for another 10 minutes at 37°C to inactivate the LR Clonase II enzyme. 2ul of the LR recombination reaction was used to transform DH5 α E.coli by a hot shock transformation method and the correct clone was identified by *EcoRI* enzyme digestion analysis. Also, a LR recombination between pENTR-loxP-D-loxP and pAd/BLOCK-iT-DEST was carried out to generate a negative control adenovirus plasmid

pAdv-loxP-D-loxP devoid of oriS and pac elements in the same procedure (Fig 1-D).

2.2.5 Generation of adenovirus Adv-loxP-OPD-loxP

To generate adenovirus, the plasmid DNA of pAdv-loxP-OPD-loxP and pAdv-loxP-D-loxP were digested with PacI respectively to remove the bacterial sequence and expose ITRs of adenoviral genome and then transfected into 293A cells with the Lipofectamin2000 (Invitrogen, USA) following the manufacturer's protocol. Adenovirus Adv-loxP-OPD-loxP and Adv-loxP-D-loxP were harvested after the transfected cells completely appearing CPE. The obtained adenovirus were amplified in 293A cells to get enough amount needed and the titers were determined by plaque formation assay.

2.3 Construction of pAdv-Cre vector and generation of corresponding adenovirus

The plasmid pGEM-T-Cre was digested by SacII and EcoRI to get a 2.2Kb fragment, which contained a

Cre recombinase expressing cassette driven by SV40 promoter and was introduced into the corresponding sites of pENTR-MCS to generate the pENTR-Cre. The subsequent LR recombination and Adv-Cre virus generation was carried out as the same procedure of Adv-loxP-OPD-loxP production described above (Fig 1-D).

2.4 HSV helper virus preparation

To provide recombinant MC amplicon the replication and package function, several infectious HSV virus were used as helpers, including recombinant BAC-HSV-1 strain HF, wildtype HSV-1 strain F and HSV-2 strain HG52. The recombinant BAC-HSV-1 strain HF helper virus were generated by transfecting Vero cells with BAC-HSV-1 strain HF plasmid, whereas other two wildtype HSV helper virus, HSV-1 strain F and HSV-2 strain HG52 were propagated in the Vero cells directly. All helper virus were tittered by a plaque formation assay.

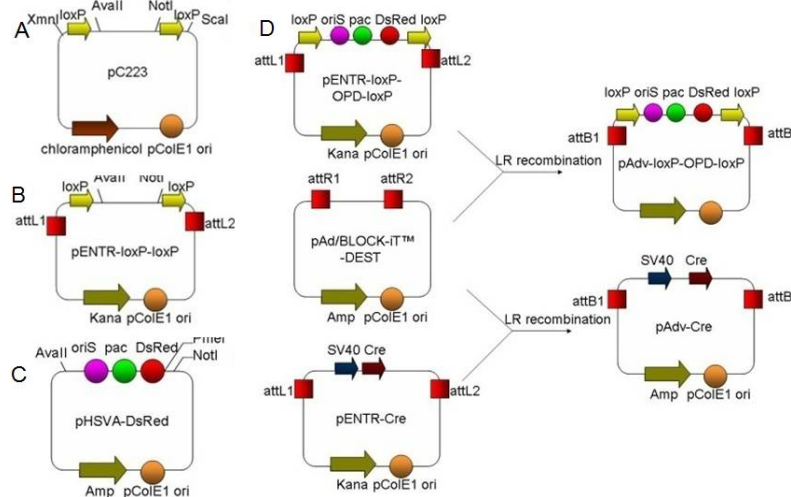


Figure 1 Flowchart of pAdv-loxP-OPD-loxP and pAdv-Cre Construction. A: The structures of plasmid pC223; B: The structures of plasmid pENTR-loxP-loxP; C: The structures of plasmid pHSVA-DsRed; D: The construction of pAdv-loxP-OPD-loxP and pAdv-Cre.

2.5 Generation and identification of mini-circle (MC) amplicons

2.5.1 Generation of MC amplicons

To generate recombinant MC amplicons and demonstrate their generation was in a recombination specific and helper-dependent manner, four different combinations were designed: Combination A., adenovirus Adv-loxP-OPD-loxP and Adv-Cre plus BAC-HSV-1-HF helper virus; Combination B., adenovirus Adv-loxP-D-loxP and Adv-Cre plus BAC-HSV-1-HF helper virus (without oriS and pac); Combination C., adenovirus Adv-loxP-OPD-loxP plus BAC-HSV-1-HF helper virus (without adenovirus Adv-Cre); Combination D., adenovirus

Adv-loxP-OPD-loxP and Adv-Cre (without HSV helper virus) respectively. The brief experimental procedure was as below: The overnight sub-cultured Vero cells grown in 6 well plate at 90% confluent were transduced with adenovirus from each combination at MOI=1. Twenty-four hours later, the cells were washed with PBS for three times to remove the residual adenovirus and infected with HSV helper virus in fresh medium at MOI=0.01 in combination A, B, and C, but in combination D, just replaced with fresh medium instead. 48 hours after HSV helper infection or fresh medium replacement, the cells together with the medium were collected and processed by three freeze/thaw cycles at $-80^{\circ}\text{C}/37^{\circ}\text{C}$ followed by a

5 minutes centrifugation at 3000g and filtration with a 0.45µm filter to remove the cell debris (Fig2).

2.5.2 DsRed fluorescence observation

The obtained supernatants from different combinations were 1000X diluted and transduced into vero cells. 24h later, the cells were examined by observing DsRed fluorescence on the inverted phase contrast fluorescence microscope (Olympus, IX71-A12FL/PH, Japan).

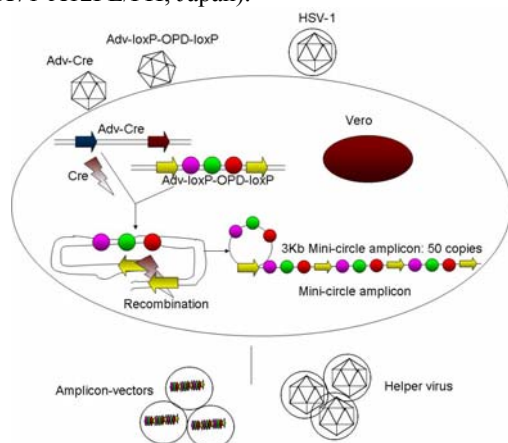


Figure 2 Schematic diagram of MC amplicon generation under the presence of HSV helper virus.

2.5.3 RT-PCR analysis of DsRed expression

In order to further identify the recombinant MC amplicons were successfully generated by adenovirus mediated Cre-loxP recombination and to verify their transducing ability and transgene expression function, we transduced vero cells with recombinant MC amplicon seed stock derived from the first round and performed RT-PCR analysis of DsRed expression at mRNA level in the transduced cells. Briefly, the obtained supernatants from different combinations were diluted 1000X and transduced into Vero cells. 12h later, the cells were washed with PBS for three times and collected for RNA extraction. The total RNA from the cells was extracted by the RNeasy mini kit (Qiagen) and treated with DNase I prior to RT reaction so as to remove any possible contaminated DNA sequence. The reverse transcription was achieved by using of SuperScript III kit (Invitrogen, USA) to synthesize cDNA with oligo-deoxythymidine primers and 1 µg RNA from each RNA preparation was used as template. After RT reaction, 200 ng of cDNA was amplified by PCR for a 336 bp band using the following primers: DsRedP1, 5'-ACGGCTGCTTCATCTACAA-3' and DsRedP2, 5'-ATCTCAGGAACAGGTGGTG-3', with the program of 95°C 4', followed 95°C 30', 53°C 30 s, 72°C 30' for 35 cycles and the final extension at 72°C for 10 minutes. In order to exclude any possible DNA contamination, a RNA PCR without DNase I digestion and without RT reaction were performed as a negative control. Beta-actin was used as an internal

control standard by amplifying a 139bp band by using of the primers: β-actinP1, 5'-GGACTTCGAGCAGGAGATGG-3' and β-actinP2, 5'-CAGGAAGGAAGGTTGGAAGAGA-3'. The PCR products were electrophoresed in a 1% agarose gel and visualized by ethidium bromide staining.

In addition, the recombinant MC amplicon derived from the first round adenovirus mediated Cre-loxP recombination process were also used to transduce Vero cells again under the presence of HSV helper virus but absence adenovirus mediated Cre-loxP recombination to demonstrate their reproducibility. We called the generated MC amplicons from this round the reproduced MC amplicons.

2.5.4 Testing the replication/package function of MC amplicons to different HSV helper virus

To test whether the recombinant MC amplicons had a universal replication and package function to different strain of HSV helpers, we performed an adenovirus Adv-loxP-OPD-loxP and Adv-Cre mediated MC amplicon DNA recombination in Vero cells, but supplied different HSV virus as helpers, including the infectious BAC-HSV-1 strain HF, HSV-1 strain F and HSV-2 strain HG52. The procedure for generation and fluorescence identification of MC amplicons was the same as that described above.

3. Result

3.1 Fluorescence identification of recombinant MC amplicon generation

By co-transduction of Vero cells with Adv-loxP-OPD-loxP and Adv-Cre and supply BAC-HSV-1-HF helper virus, we successfully generated recombinant MC amplicons in the first round recombination process, we called the recombinant MC amplicon seed stock, which were demonstrated to possess transducing ability, mediating transgene expression function and reproducing ability when supply with full HSV helper function. As shown in Fig 3, the DsRed fluorescence was observed in Vero cells transduced with obtained supernatants from combination A, but not in the cells treated with that from any other combinations, including B, C, and D, indicating that successful generation of MC amplicons was dependent on the Cre-loxP mediated site specific recombination and HSV helpers provided replication and package function, whereas without oriS/pac in combination B, without Cre in combination C and without HSV helpers in combination D could not generate any MC amplicons. By transducing Vero cells with the recombinant MC amplicons derived from the first round recombination process and providing BAC-HSV-1-HF helper virus, but no giving adenovirus, as no more adenovirus mediated Cre-loxP recombination needed, we demonstrated that the recombinant MC amplicons were reproducible under the presence of HSV helper function and called the

generated MC amplicons in this way the reproduced MC amplicons.

3.2 RT-PCR analysis of recombinant MC amplicon generation

To verify the observed DsRed fluorescence under fluorescent microscope as a results of MC amplicon mediated DsRed expression, we performed a RT-PCR analysis to examine DsRed expression at mRNA level in Vero cells with supernatant from different combinations. In Fig 4, the RT-PCR results showed that the DsRed gene expression at mRNA level was only found in Combination A, not in any other combinations, completely consistent with the fluorescence observation results and thus further demonstrated that generation of MC amplicons was Cre-loxP site-specific recombination required and HSV helper-dependent. Since RNA direct PCR failed to amplify the DsRed sequences, we thus concluded MC amplicons mediated a functional DsRed transgene expression.

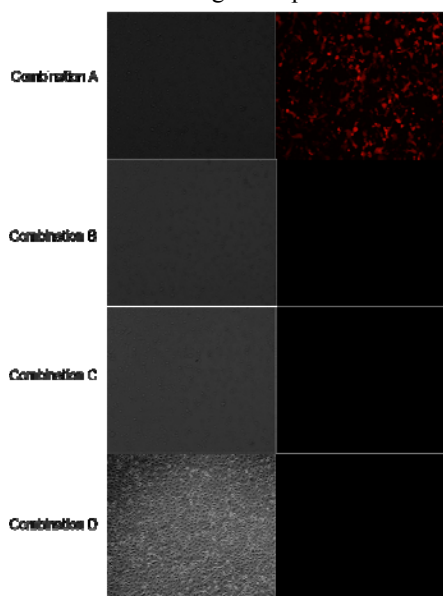


Figure 3. DsRed fluorescence observation in Vero cells treated with 1000X diluted supernatants derived from different combinations under inverted phase contrast fluorescent microscopy, the left panel was Vero cell images taken in the Bright-field and the right panel was Vero cell images taken in the Dark-field (X100). The DsRed fluorescence was observed in combination A.

3.3 The replication and package capacity of MC amplicon to different HSV helpers

By using the MC amplicon derived from BAC-HSV-1 strain HF and recombinant BAC-HSV-1-HF virus as helpers, we successfully generated MC amplicons by adenovirus mediated Cre-loxP recombination. To test the replication and package capacity of recombinant MC amplicon to

different HSV helpers, we performed the experiments for MC amplicon generation with the same recombination procedure by co-transduction of Vero cells with Adv-loxP-OPD-loxP and Adv-Cre adenovirus, but supply different HSV-1, and HSV-2 virus as helpers. As shown in Fig 5, we achieved the similar success in MC amplicon generation and demonstrated their transducing ability and transgene expression ability by fluorescence observation of functional DsRed gene expression under fluorescence microscope. These results indicated that our recombinant MC amplicon possessed universal replication and package function to different genotype and immuno-type HSV helper strains.



Figure 4. RT-PCR analysis of DsRed gene expression in Vero cells treated with 1000X diluted supernatants derived from different combinations in the first round. A-D represent Combination A, B, C, and D respectively. The upper gel image showed RT-PCR results of DsRed expression except in lane A1, a RNA PCR from Combination A as a negative control. The bottom gel image was the RT-PCR results showing of β -actin expression.

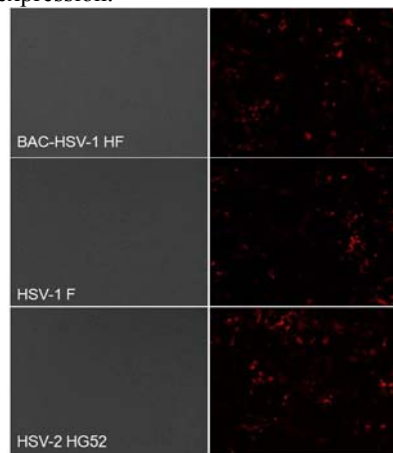


Figure 5. DsRed fluorescence observation under inverted contrast fluorescent microscopy in vero cells transduced with 1000X diluted recombinant MC amplicons generated in the first round with different HSV helper virus. the left panel showing bright-field and the right panel showing Dark-field (X100). The DsRed fluorescence was observed in Vero cells transduced with MC amplicons generated by adenovirus mediated Cre-loxP recombination but with different HSV virus as helpers.

4. Discussions

HSV amplicon has been regarded as a versatile gene delivery vehicle and holds great promise in clinical gene therapy and vaccine development (1-2, 9). Due to carrying concatemeric bacteria elements in the vector, which can result in transgene silencing and trigger chromatin inactivation of the whole vector, the conventional amplicon mediates only transient transgene expression, even in non-dividing tissue culture cells (1, 9). Therefore, MC DNA-based HSV amplicon (MC amplicon) lacking bacteria sequences represents an ideal and excellent pseudovirion gene delivery platform superior to conventional amplicon as it mediated high and persistent gene expression both in vitro and in vivo. Actually, the bacterial sequences in the gene delivery vectors are dispensable for gene transfer application, but just necessary for plasmid vector production in bacteria (21). Furthermore, the antibiotic resistance genes in the vector may also cause serious biological safety problems and the regulatory agencies thus recommend totally avoiding the use of antibiotic resistance markers (22-23). In order to achieve a higher and long-term transgene expression and reduce the possible safety risk, the best way is to completely remove the bacteria elements from the gene delivery vectors, including an antibiotic resistance gene and an origin of replication.

To realize the purpose addressed above, several methods have been employed in the MC DNA preparation by using different integrases to induce intra-molecular recombination in MC producer plasmid in bacteria and followed by a isolation procedure to eliminate the contamination of minicircle producer plasmid and circular plasmid backbone from bacteria lysates, however, all of these methods have disadvantages, including labor-intensive, time-consuming preparation procedure, low yield, and high contamination of plasmid and mini plasmid. Although Chen et al very recently improved the MC DNA preparation technology by engineering a bacteria strain with inducible integrase phi-C31 and restriction I-sacI, that allowed for more simple and fast to generate MC DNA like a routine plasmid preparation, however, it still remained a low level contamination of minicircle producer plasmid and circular plasmid backbone (19). As a fact, it is very difficult and even attainable to completely remove the contamination by digestion and purification method, as all of them are circular DNA molecules except different sizes. Since HSV amplicon is reproducible during propagation process under the presence of HSV genome or helper virus, any contamination, even very small amount, will be amplified round by round during the scale up steps. In this study, we have successfully developed a novel approach for MC amplicon preparation by adenovirus mediated Cre-loxP recombination in mammalian cells.

Cre recombinase is a bacteriophage P1-derived integrase catalyzing site-specific recombination between direct repeats of 34 bp loxP sites (24). In our MC amplicon preparation system, the oriS, pac and reporter gene or/and transgene cassette(s) were flanked with two loxP sites in the same orientation in the adenovirus genome, and when adenovirus delivered Cre was expressed, a recombination event was achieved between two loxP sites and resulted in two independent DNA molecules, one was recombinant mini circles DNA carrying a oriS sequence, a pac signal, a transgene expression cassette(s) and a single 34 bp lox footprint, and another was a linear adenovirus genomic DNA. The recombinant MC DNA was then replicated in a mono-directional, rolling circle-like fashion to generate a head-to-tail concatemer of DNA composed of tandem repeats of the MC DNA and cleaved/package around 150 kb linear fragment into MC amplicons under the presence of HSV helper virus. We developed a one-step MC-amplicon preparation techniques, that is MC DNA recombination and subsequent MC amplicon replication/package were achieved in the same mammalian cells, instead of two-step procedure, MC amplicon DNA recombination in bacteria first, and then MC amplicon replication/package in mammalian cells separately. In our system, an automatic selection mechanism is involved for the MC amplicon package, that is only MC amplicon DNA can be replicated and packaged into MC amplicons, whereas linear adenovirus vector is unable to be packaged, excluding the possibility of bacteria DNA and adenovirus DNA contamination.

While MC DNA was replicated and packaged into MC amplicons, in theory, sustained expressing Cre post MC DNA recombination might also mediate intra-molecular recombination between loxP sites within the replicated concatemeric MC DNA sequences randomly and result in more MC DNA molecules or large circular DNA harboring multiple MC DNA repeats entering mono-direction rolling circle like replication again. It is not clear if persistent Cre expression has any affection on MC amplicon packaging, however, as a fact, we achieved a 10^6 /ml TUs of recombinant MC amplicons in the first round recombination process indicating that Cre seemed to have no ruin effect on the MC amplicon packaging, but perhaps have an amplification effects as more MC DNA entering replication cycles, consequently leading to more and more MC amplicons generation. To further investigate the effects of Cre sustained expression on MC amplicon production, we next will engineer a tightly controlled Cre expression system and MC amplicon loxP system in the same adenovirus vector to study the Cre expression dynamic and related yield of MC amplicons. Once the recombinant MC amplicons were produced in the first round, no more adenovirus

mediated recombination process was needed as recombinant MC amplicons like seeds, were able to be reproduced during the roundly propagation process under the presence of HSV helper function.

Adenovirus has been widely used as a gene delivery vector for gene therapy and vaccine developments with advantages such as high transduction efficiency, extremely high viral titers (10^{10} - 10^{13}) and capable of generation on large scale (25). Taking the advantages of adenovirus vectors, we have demonstrated the feasibility by adenovirus mediated Cre-loxP in generation of MC amplicons. Due to the limited capacity of replication-defective adenovirus, the maximum insertion size of MC amplicon DNA is less than 8 kilobases. For large size MC amplicon DNA recombination, an alternative selection is to use helper-dependent adenoviral vectors (or named gutless adenoviral vectors) as donor vectors instead of replication-defective adenovirus, which is capable of delivering up to ~37 kb size of insertion (26). Actually, it is rare to deliver transgene large than 37 kb size for regular gene transfer studies.

In this study, we used replication competent HSV-1 virus as a helper for MC amplicon generation to demonstrate this technique the feasibility and to verify the universal replication/package function of recombinant MC amplicon to different genotype and immune-type of HSV helper virus, HSV-1 HF, HSV-1 strain F, and HSV-2 strain HG52. Although helper free amplicons could be generated by transfection of HSV permissive cells with pac sequence deleted HSV genomic DNA or oversized BAC-HSV genome (27), however, the critical obstacle is the difficulty in producing high titer helper free amplicons in large scale. Due to replication defective adenovirus vectors and the gutless adenovirus vectors with replication defective adenovirus as helpers are able to be produced in large scale, therefore, a simple and large production of the adenoviral vectors in our system will replace the complicated minicircle DNA preparation procedure for MC amplicon production.

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Explanation of the rate of biological environment health based on social capital components of students in Tabriz Medical and Dentistry University in 2010

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Abstract: This study examines the relationship between social capital and environmental health of student in Tabriz University of Medical Sciences. This study is a survey of the correlation. Statistical population of this research includes 1,700 people (all of the medical and dental students in Tabriz University of Medical and Dentistry Sciences) as reported by the university authorities in 1389, which 313 of them are studied by method of randomly proportion according to Cochran's formula. Students of other majors of the university are questioned by stratified sampling method. The following results were obtained after data collection using achieved questionnaires of social capital and environmental health and content validity measurement (the referees' opinion) and reliability: there was a relationship between the components of social capital (cohesion - trust - partnership – relationship) and social capital in general, with the environmental health. Cohesion and social trust explained 38% in environmental health from the perspective of the students.

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Keywords: Social capital ,biological environment health ,cohesion, trust

1. Introduction

With more and more increasing earth's population and human impact on environment and urban development, the earth is facing a crisis. Negative urban environmental effects are increasing to the extent that it is considered the biggest threat to these ecosystems. Unsustainable consumption of resources (ecological, economical and social) is worrisome. And one of the consequences is being threatened and lack of consistency in the quality of urban life. It means that if we continue to this waste structure with the same situation, in the coming years we are facing a crisis. This instability in the fields of natural resources, technology, urban development, human rights, society, culture and... . Human in all fields, plans to reduce speed to reach crisis. Part of it depends on culture and society (quoted Asgarzadeh, 1382: 1). Social capital is a new concept which today has an effective role in health and, urban management development. This social phenomenon is called to a set of norms existing in society which provides a kind of social order and trust and confidence for people through interaction and leads to a social and cultural development of the health of the community. Twenty-year outlook of the country,

considers a feature of Iranian society having health, welfare, food security, social security, equal opportunity, the appropriate distribution of earnings, strong institutions of family, away from poverty and corruption and discrimination and the benefiting of the appropriate Biological environment.

Since nowadays more than 50 percent of the factors affecting the health are made up of social factors such as education, employment, poverty and, looking at health scope is a social-cultural approach as well. Iran's major cities are encountered with numerous challenges, including population density, housing, establishment of large industries, establishment of the main units of goods production and distribution, pollution and poor environmental conditions, lack of amenities and facilities for waste collection and treatment, unemployment, addiction, and traffic. This problem reminds the need to serious attention to the environmental health and urban health. "Social capitals are intangible assets considered mostly important in people's daily life: good faith, friendship, empathy and social interaction between individuals and families who make up a social unit." (Australia Productivity Commission research report, 1387: 10) . Based on this, the health

of urban society nowadays is considered as an intangible and rare asset for residents. Through interaction with citizens and everyday life together, we can prove good faith of managers and urban executives toward sustainable urban development including urban health. So the main question of this study is that to what extent is the relationship between social capital components and environmental health.

Human concerns of environmental aspect in national, regional and global dimensions were evident when industrial development and use of few renewable and non renewable resources increased in the biosphere. Development is organically associated On the one hand with industry and technology and on the other hand with environmental degradation. If we don't pay attention to an important task such as environment beside industrialization, economic development will not only result, but also many involvements will occur, which sometimes will totally spend the benefits of an industrial activity for the community in the long-term, to offset damage of it. While in many cases with the use of compatible technology, the environment will be protected and using the residual wastes from other industrial activities, not only to avoid wasting natural and human resources mostly, but also by reserving non-renewable resources of a country and environmental protection will help to fund the Community Economic. Undoubtedly, industrial development and evolution has been provided many facilities in human societies. But it may also provide different environmental problems for the community equivalently. These problems are not always due to technology itself, but they are made because of lack of attention to the adverse results and incorrect application of technology and finally the lack of attention to prevailing rules and regulations. (Derakhshan, 1388: 2). In the traditional view of development management, economic, physical and human capitals played a significant role. But at the present time for further development we need the social capital more than economic, physical and human capital, because without these assets, using other assets of the improvements will not be made optimally (Mobasheri, 1387: 2). Actually the purpose of the continuous and constant improvement of health in a growing community interested in the welfare of urban life in many parts of social capital requires some thought and research.

1-Through continuing interacts with citizens; municipal managers and chief executives must try not to allocate charges with lower priority to urban development funds.

2- Social capital and strengthening it through trust and Reinforcing norms of the health

providing, and also consistent activities of authorities in charge of health urban management with the other institutions, can provide a kind of social order and collective and strong spirit for socialization and institutionalization of sustainable health behaviors in citizens to build a healthy and joyful city.

3- Indeed, the institutional authorities and the health-related fields should in the form of long- and short-term strategies provide executive and effective programs in urban centers to institutionalize hygiene observation and human health in different dimension with direct and indirect supervision continuously, stable and effective.

4- Encourage associations and non-governmental organizations (NGO) to socializing good health, and freezing non-normative behaviors in observing urban health is another goal of urban management activists related to strengthening social capital and making civic environment healthy.

5- Developing and sustaining accurate healthy behaviors in a good urbanization life through the means of educational, social, cultural and other tools is executable with the association of media, press, and other tools of urban organizations to establish sustainable urban development which is effective to explain the relationship between social capital and health aspect (Sharbatian, 1388: 5).

According to Putnam, relationship between social capital and urban health is important for four reasons: "First, citizens' various social networks make financial assistance available for citizens to reduce their urban lives' anxiety. Second, citizens social networks together and the relation of these social networks with executive management of urban health is improving standards of health and social and individual hygiene in citizens' life. Third, urban social networking in the form of NGO can better provide health services to citizens who are applying to. Fourth, interaction and ongoing social activities of urban management with residents in the area of social, personal, psychological and physical health causes revolve more active immune system against any urban hazard (Shojaei Baghini '1387:86). Marcuse suggests reconciliation with nature. It does not mean return to the era "before the new" or "organic" between man and nature. In this matter Marcuse's position is clear according to Horkheimer and Adorno. In his opinion "natural freedom" does not mean return to the stage before technique cognitive, but it means progress toward using achievements of technique cognitive civilization to rescue human and nature from devastating abuse of science and technology which is in the service of exploitation.

Instead, he talks about the rule of "liberation" on the nature which is against "oppressive" specific to the new industrial societies

and He says, "No free society could be assumed not to have coordinated efforts to alleviate persistent pain of humans on the natural world. The "rule of liberation" means "civilize" humanizing nature by permutation of material (and cultural) liberations from the "beast" or "raw nature" in the shape of parks, gardens, agricultural lands, perspectives and resources. Social capital is a set of social norms which is made through citizens interact with urban management series. Accordingly, urban management should provide social, psychological and physical health for citizens. Such activity increases the sense of responsibility and citizens' conscience to protect the urban health. Urban health and social capital leads to the development of health social norms in the minds of citizens' through mutual Interaction and action which executives have with the citizens. Social capital associated with the health of urban citizens, is formed through parameters such as trust, interactions and increase the collective spirit toward future. In Iran this association is somewhat wasted because of false politicization of managers. The goal of relation of these two phenomena is to continue Interaction of citizens and officials to reduce the wasteful costs of urban health and strengthen norms affecting health.

Kavachy and colleagues examined the relationship between social capital and social health, using crime as an indicator of social well-being. They have argued in their study that 2 series of social characteristics affect the level of crime: degree of relative deprivation in society and degree of coherence in social relations among citizens. They have tested their own conceptual framework using ecosystem data related to violent crimes and financial crimes in the United States of America and they have concluded that violent crimes (murder, assault, theft), have consistently mild relations with relative deprivation (income inequality) and indicators of social capital. In their research areas with high crime rates have been shown to have higher mortality rates.

Harfam and colleagues (2003, quoted Alizadeh ghavi fekr, 1388) in a research titled psychological health and social capital examine relationship between psychological health social capital. The population sample is 1,168 people between 25-15 years old whose psychological health is measured with a scale of 20 species. Also, social capital variable is measured in both structural and cognitive dimensions. The result of this study is that social capital in both the structural (civic participation, membership and activities in formal and informal organizations) and cognitive (trust, reciprocity, norms) dimensions affect people's health.

In a research by Dansby (2001) in California, entitled "Environmental health risk assessment negotiating for the" right to know "for public,

Quantitative assessment of health risk is a way to estimate the probability that being exposed to environmental pollutants will cause certain effects of against health, effects such as cancer. One of the usages of this method is the rule of "right to know "for public about toxic air in California. This article studies methods in which a valid evaluation about health risk is done and challenged during the implementation of public policy in California. Field work and analysis of available documentation shows that despite official restrictions in risk assessment procedures, Investors, hold some negotiations, but still express their competitive approach in performing risky telecommunications policy. An abstract of the results of these methods have been combined based on different organizational structures considering the uncertainty. In addition, this article shows that this political process leads to sustainability assessments and raises political negotiations as a fundamental technical level. Struggle and changes existing in interpretations are better described by organizing programs about Inconsistent values in the democratic accountability and technical efficiency.

In their research Shelz and Northridge (2003) in New York entitled "Social factors in health: industrial implications in promotion of environmental health, present methods for sociology and environmental and social contagious studies to find a mechanism contributing in social factors with different environmental exposures or health Equality. They speak of a conceptual framework for environmental health promotion which considers Social process of dynamics and rebuilds potentially transfers Environmental inequality and produced health-related disparity. The purpose of this study is developing community health by addressing various aspects of social processes and physical environments. Finally they recommend us to move from environmental treatment strategies to improve the health of our environment, because this improvement is stable and clearly designed to reduce disparities in health, environment and community.

Saduq Vaniny (1387) Ecological Geography of Shahi Beheshti University, Environmental Science Research Institute, conducted a study after the RIO Summit, entitled: the attitude of staff to 21th Agenda of World Summit on sustainable development and environmental Topics. In this study, the population sample of 120 was chosen randomly among statistical population. Results show that the 8 factors as underlying components of sustainable development estimate 3 / 82% of the variance. The results also show that from the view of experts the most significant dimension is conservation of natural resources and environmental management. Therefore,

we conclude that the attitude to environment is more than social and economic dimension.

Research questions:

1 – How much has components of social capital, social capital and environmental health among examined students?

2 - How much has been the contribution of each component of social capital in explaining student's environmental health?

2. Material and Methods

This research considering control of its conditions, was a descriptive - correlated survey study [1].

The statistical population and method of determining sample size:

The statistical population of this study includes all students in Tabriz Medicine and Dentistry University in 1389 whose number was 1,700 according to statistics reported by the university authorities. A statistical sample of 313 people was determined using the Cochran formula and different majors of university were questioned by stratified sampling method.

Tools of measuring variables:

In this study, a made questionnaire is used to measure social capital and its components and also the environment health, So that questions were designed in Likert spectral-scale after studying theoretical topics and indexing variables.

Validity and reliability of variables:

For validation of questionnaires, formal credit and several experienced teacher' comments were utilized and for reliability of variables, Bach's Crohn coefficient alpha was used separately, So that the pilot study was conducted among 30 students and finally variables were respectively gained to the high resolution 7 / 0.

Findings:

Research Question 1: How much has been the rate of components of social capital, social capital and environmental health among examined students?

Table 1 shows the descriptive indicators including frequency, mean and standard deviation and the rate of studied sample in social capital variables and its components and also environmental health. As can be seen in the chart, the highest rate is for community involvement and the lowest is for social trust. The rate of social capital is totally 71% which is more than average. Also the rate of environment is about average and 61%.

Research hypothesis: there is a relation between social cohesion, social trust, social participation, social communication and overall between social capital and environmental health.

Table 1: descriptive indicators of tests in studied variables

Rate (percent)	Standard deviation	average	Lower score	Highest score	numbers	variables
%69	3/11	11.08	0	16	309	Social cohesion
%86	4/34	26.4	12	40	309	Social trust
%80	4/84	34/18	9	45	309	Community Involvement
%71	8/71	63/84	26	85	309	Social communication
%71	16/12	13/5	47	186	309	Social capital
%61	14/35	90/05	32	131	309	Environmental Health

Table 2 shows that the calculated correlation coefficient on the level (000 / 0) is significant in all the theories. Thus the all hypothesis of the research is confirmed and there is a relation between social cohesion, social trust, social participation, social communication and overall between social capital and environmental health.

Table 2: Correlation coefficients of variables of social capital and environmental health components in tests

environmental health	variables
0/6 0/000	Social cohesion
0/33 0/000	Social trust
0/25 0/000	Community Involvement
0/32 0/000	Social communication
0/45 0/000	Social capital

Research Question 2: How much has been the contribution of each component of social capital in explaining student's environmental health?

Table 3 indicates the remaining components of social capital variables in the form of regression. Multiple correlations with the students' environmental health is computed to 62/0 and the coefficient of determination to 384 /0 and the net coefficient of determination to 38 /0 and total components of social capital explains 38% of environmental health for students. Table 4 indicates that social cohesion with the beta (557 / 0) and social trust with the beta (139 / 0) has significant share in explaining environmental health.

Table 3: Multiple correlation coefficient for students' environmental health

Standard error of estimate	Adjusted R Square	R Square	R	form
11/30	0/380	0/384	0/620	1

Table 4: Remaining coefficients of variables in the form of regression by step by step method

Significant level	ratio T	Standardized coefficients	Raw coefficients		order of variable Independent entry in the form
		Beta	Std.Error	B	
0/000	12/11		4/08	49/504	Fixed amount
0/000	11/61	0/557	0/22	2/56	Social cohesion
0/004	2/904	0/139	0/15	0/46	Social trust

(Social trust) 139/0 + (social cohesion) 557/0 = environmental health

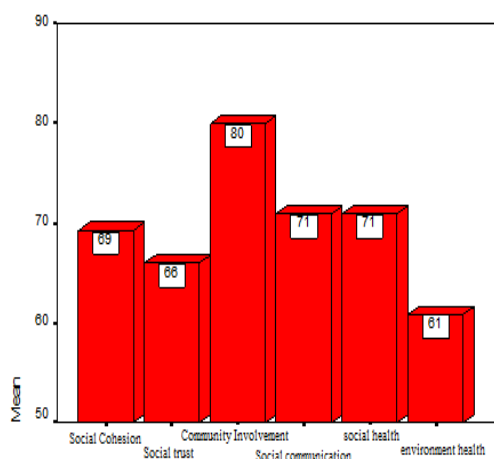


Figure 1: Diagram of the rate of research variables

Results:

The study results were in line with Putnam, Volkak, and Kavachy's ideas. According to Putnam's theories, social capital associated with urban health was important for several reasons: various social networks of Citizens provided financial assistance for citizens in such a way so that it can reduce anxiety of urban life. Social networks of Citizens with each other and their social communication with executive management of community strengthened health standards and urban living of citizens. Urban social networks (NGO) were better able to provide health and hygiene services.

Putnam believed that social capital and various aspects of social organization such as trust, cohesion and networks can improves efficiency of the society. He also argued that faith and interact communication of members in a network are resources which exists in actions of members of the community. Improvement of environmental health on which the posterity life depends, could be provided through social relationships and social trust. Actually the more the Partnership between government and people in community, the better environmental health could be seen. Also according to theories of Habermas, Giddens and Marcuse on environmental health and human reconciliation with nature, we could implicate correlation and direct relation between social capital and environmental health. Based on the social parameters, trust was an indicator and perhaps the most important indicator among other parameters of social capital which led to sustainable social development in measurement of social capital and citizens urban health and caused strengthening other components too. Kavachy also believed that impact of some elements such as social-environmental mechanism related to benefits of social integration and high levels of social trust in collective level would improve the urban health status of residents in individual and public health dimensions. According to Volkak's theories, social capital in 3 dimensions of his opinion (internal-group Relationships, connected or external social capital and communicative) effected citizens welfare by emphasizing on public relations between citizens in different levels of power. Results of experimental background indicated that features of social capital have a direct relationship with health and the higher the social capital of society, the more health gained by community and environmental health. According to results or this research some issues were recommended to improve environmental health:

- 1- To effort maintaining cleanliness of the city as much as possible by proper training and notifying people considering technology and urban pollution.
- 2- To help improving environmental health by establishing local NGOs in every town and village and long-term planning.
- 3- Society managers to take steps in order to restore and maintain environmental health, considering specified budget and adopting practical strategies to provide the necessary infrastructures in society and collaborating with responsible organizations such as municipalities and environmental protection organization.
- 4- If people's participation increases in Administrating urban community affairs,

sense of responsibility for environmental health protection will be higher.

- 5- Social cohesion is a preparation to observe environmental health. sense of commitment increases With increasing consistency and environmental health will improve To the same extent.
- 6- Planning executive practical actions of community directors to Strengthen social capital underlies improvement and promotion of environmental health by public education, Incentive and punitive programs in observing environmental health principles privately and publicly.

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Devising sustainability criteria in new towns of Iran

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Abstract: New towns in Iran tend not to be sustainable and have appropriate structures because of a great deal of problems such as lack of exact determination of legal status for new towns, uncertain construction expenses' supply resources, lack of cooperation and harmony between different organizations and lack of substructure facilities. To compare the present situation in these towns in Iran with the goals considered in this research we can claim that: most new towns have not achieved their goals fully and the confusion dominant in Iranian new towns approves this claim. The aim of this research is to identify the sustainability criteria as the means of measuring the sustainability of Iranian new. To do so, first we carried out library studies based on documents and analyzed them and then the different sustainability approaches were identified according to guidelines 21 of sustainability criteria of Iranian new towns. The results of this research are as follows: introducing sustainability criteria in Iranian new towns and describing the tools of measuring sustainability by identifying sustainability criteria based on pressure framework, present state, and response.

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Keywords: Iranian new towns, sustainability criteria, town sustainability, sustainable envelopment

Introduction:

The idea of establishing new towns which started in developed countries after the industrial revolution and is used in developing countries like Iran was aimed to meet the physical development requirements resulted from developing urbanization and organizing the metropolitans. Regarding the pass of a quarter after Islamic Revolution in Iran and the changes it brought about, the urban population in Iran is about 40 millions and it has been estimated to reach 100 millions in the year 1400 (2021). The number of towns was about 300 in the year 1961 and it is more than 950 now and it will become 1900 in 2021 (Nourinejad, 2006, P: 349). New towns were approved to achieve goals such as the direction of overpopulation and decentralization of big cities and metropolitans and responding the need to get houses and apartments for the people with low income after Islamic revolution happened (Ghamami, 2007, P: 87). It seems that while there have been much efforts to implement residential areas and place the population in new towns, they have not been successful in achieving the goals (Shieh, 2006, P: 151). Although some of them have been seemingly successful, the efficiency and independence of life has encountered with several problems such as the severe dependence on main cities. Generally, work place, education, amusement, common needs' supply and even the treatment of a great deal of inhabitants in new towns

are responded by the main cities which are located near them; thus, we are afraid that if the importance of this issue is underestimated the dependence level of these new towns will result in the lack of their success and finally they will be changed into a dormitory and even the permanence of this problem will result in the evacuation of inhabitants and in-hospitality of these new towns.

Utilizing sustainable development criteria is one of the effective methods to establish sustainable new towns. The aim of this system is to use sustainable development criteria globally and locally regarding the different valuation dimensions and environmental, social and economical dimensions. Different countries have changed this system regarding their own environmental conditions and in other words, they have localized it. But the basis for all of them is Agenda 21 and criteria which have been posed by United Nations as the principles and basics.

The main goal in this research is to achieve urban sustainability in Iranian new towns according to the sustainability criteria application. Other aims of this research include the identification of sustainability criteria in three dimensions of economy, social-environmental, and supplying the tools to measure sustainability. Thus, first we studied the application of sustainability criteria according to Agenda 21 and then study the problems of new towns and choose the sustainability criteria for new towns and select some

criteria based on different sustainability approaches and Hart's checklist.

By choosing the framework used by development and economic co-operations' organization as a pattern (pressure system, present state, response), sustainability criteria of Iranian new towns are adjusted in three fields of economy, society, and environment. They also were used as sustainability measurement tools to identify the status of new towns.

Research method:

The research method in this paper is content analyzing and reasoning and deduction method has been utilized to adjust philosophical discussions regarding the urban performance limits. Thus, we have tried to use Agenda 21 as a fundamental issue and identify sustainability criteria in Iranian new towns in three dimensions of economy, society, and environment based on the framework used by organization of economical co-operations and development (pressure, state, response).

Assessing the amount of sustainability in new towns

Sustainability criteria:

In the description posed by organization of economical co-operations and development, a criterion is: a parameter or a set of parameters gained which can represent information about an environment or a phenomenon and beyond the related parameters (Organization of economical co-operations and development, 1993).

Criteria are the hypotheses related to what has been studied and they are not directly observable (Chevalier, et al. 1992). Spangenberg stated that:

criteria are used to implement tools for guidance in sustainable development policies (including criteria controlling and the communications and their results generally) (Spangenberg, et al. 2002, 61-77).

The national project of working on the criteria was commenced in 1989 by focusing on criteria and three different aspects of sustainable development such as environmental, maintenance of systems of ecological life support, humans' health and their welfare in sustainable development of natural resources.

The first set of criteria were published by organization of economical co-operations and development (OECD) in late 1980s and early 1990s, even before Rio-de-Janerio summit and was continued in some other countries. Canada was one of the countries which published environmental criteria set in 1995. Then Dutchmen worked on environmental criteria for several years and the criteria became the main core of programming their environmental-national development and still they are considered as the main policy makers in the country. United Nations formed a workshop towards the movement for establishing sustainable development in Rio in 1992 and introduced chapter 40 of Agenda 21. According to the convention, implementing the required activities was enforced by sustainable development commission. Diamanti & Zanon believe that: in every country the criteria are affected by quality of life and environmental, local, economical, cultural, and natural factors and each operation should be adjusted with the local conditions regarding sustainability criteria (Diamanti & Zanon, 2000; 299-310). The main incidents in relation with sustainability criteria are presented in table 1.

Table 1: Historical approach towards sustainability criteria

Source: writers

Field subject	Sustainable Development Criteria
Roots	1925: the emergence of the term "urban ecology" in school of Chicago 1941: Linderman introduced the theory of ecosystem.
Preliminary concepts	1971: Club Rem published "halt to the growth". 1972: United Nation's summit was held in Stockholm, Sweden about ecosystem entitled: "Eco Development".
International concepts	1980: Sustainable Development was first posed by UICN. 1987: The famous "Brantland" report borrowed the concept of "sustainable development". 1989: Focus on environmental criteria started.
Future steps	1990: First sustainability criteria were published. 1992: Agenda 21 was held in United Nation's summit in Rio. 1996: The biggest set of criteria including 120 criteria was published in Britain. 1997: Rio+5, determined valuation of how to implement calendar 21 in New York.
New searches	1999: Emergence of sustainable criteria, overall structure and methodology by United Nations. 2001: Ecosystem criteria were presented by OECD (Organization of Economic co-operations and Development).

Different approaches of sustainability in new towns:

Sustainable development is a new field which considers both politics and culture concurrently and

emphasizes on both economic flourishing and business and industry flourishing. It supports ecosystem and coexistence with environment and supports the equal rights of human beings and deals with both local and international issues of the countries. In fact sustainability has changed to be a criterion in the new era. If once the development was confined to productivity and economic growth, today it has changed its nature in such a way that it is believed that development is a comprehensive and multi-dimensional issue which is applied not only in economics, but also in qualitative developing the life of human beings, ecosystem and changing the cultural beliefs of people (Keshtkar, 2010).

Different approaches of sustainability in new towns affect sustainable development criteria selection greatly. One of the most important factors in sustainable development approach is the concept of sustainability. In dynamic systems such as human societies, sustainability is considered to be an issue of equilibrium during the pass of time (Dahl, 1995; 17-19). Sustainability is a concept which can not be measured easily. Wheeler states that: "a sustainable development is the one which improves the long-term ecological and social health of cities and townships" (Wheeler, 1998; 436). According to the above definition, we can suggest the main approaches in

three economical, social, and environmental fields as follows:

New towns as living complexes are preplanned and their approach to develop and rebuild the cities can be effective and the considerable effect of it is called sustainability. In dictionary definition, a new town is a planned living complex, a town, township or a complex which starts from zero point and develops with a predicted program and almost has a plan (Pascaline, 2004; 45). The existence of a lot of problems in towns, and new towns forces us to change our view about urban life due to the inconsistency of environmental, social, and economical conditions. The problems of new towns and suburbs are almost the same. Some of the most important problems are lack of access to appropriate transportation system, lack of having urban centers, lack of urban growth, and absorbing poor citizens (Ghalehnooue, 2006; 285). The selection of a concise approach which can contain all the present methods for development and measurement of sustainable development is highly important. Regarding the three main approaches of sustainability and the table of sustainable development commission, sustainable development criteria based on Agenda 21 suggest the following different applied approaches for the new towns:

Table 2: different sustainability approaches

Source: Writers

No.	Sustainability dimensions	Different sustainability approaches
1	Environmental	<ul style="list-style-type: none"> - Preserving the quality and supporting sweet water resources - Preserving the beaches - Complementary approach and ground resources' management - Sensitive ecosystems' management, fight against draught and desertification - Sensitive systems' management; mountains' sustainable development - Sustainable agriculture development and urban development - Fight against jungle destroying - Supporting living species' variety; appropriate environmental biotechnology management - Preserving the atmosphere of earth - Appropriate management of environmental wastes and leftovers - Poisonous materials' management - Appropriate management of dangerous environmental leftovers - Appropriate management of radioactive leftovers
2	Social	<ul style="list-style-type: none"> - Fighting against poverty - Population dynamics and sustainability - Education, general awareness and understanding development - Preserving and developing human health - Sustainable human settlement development
3	Economical	<ul style="list-style-type: none"> - International cooperation for increasing sustainable development rate in countries an their domestic policies - Changes in consumption patterns - Financial resources and mechanisms - Transfer of appropriate technology, cooperation and capacity implementation
4	Organizational and official	<ul style="list-style-type: none"> - Complementary environment and development in decision making - Sustainable development sciences

		<ul style="list-style-type: none"> - National mechanism and international cooperation to create capacity in developing countries - Cooperation between international institutions - Presenting information for decision makers - Reinforcing the role of main groups
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It should be noted that besides the three main factors of sustainability, there exists a fourth dimension called organizational and official dimension which is presented in Agenda 21. Thus, the 4th approach is included in the discussion. The aim of this research is to utilize sustainability approaches as a study system, implementing sustainable development criteria in a global measure to the local measure in different valuation continuums and different social, environmental, economical, and cultural dimensions.

The value and importance of sustainable urban development criteria:

The criteria are important components in measuring the total sustainable development. The overall principles of measurement were suggested in 1996 in a conference in Blagio, Italy. Utilizing the criteria is the implementation of tools to direct the policies in sustainable development which includes controlling the criteria and the relationships and their results in general measurement (Spangenberg, et al., 2002; 61-77).

Criteria should present fundamental information for environmental and economical policies in the future and act as preserving against satisfaction from the environment (Smith, 2002; 305-310). Additionally, sustainable development planning should be based on environmental and biophysical criteria and efficiently describe the comparative potentials of development and environmental barriers (Schultink, 2000; 47-58).

If we suppose that the public institutions can not plan the needed strategy for sustainable development without having the required knowledge about the system (Ronchi, et al., 2002; 197-210), we can utilize the criteria of environment and sustainable development for improving the decision making about environmental issues under the conditions of indefinite variables (Levy, et al., 2000; 79-86).

Urban sustainability criteria are the main factors to create communication of performance status, a factor of sustainability which reflects the basic status and a foundation for economical, social, and ecological health of a society during the pass of different generations (Whorton & Morgan, 1975; Clark & Wilson, 1994). Every operation should adjust with the present local conditions regarding the fact that whenever the conditions change in a country, the quality of life is affected by factors such as local, environmental, economical, cultural and natural

(Diamanti & Zanon, 2000; 299-310). The point which should be considered here is that: if highly valued and important criteria are used in determining sustainable development criteria, a chance can be emerged to inform the people about how to deal with their desired expectations and responsibilities in the future. On the contrary to theoretical and independent criteria which are not favored by the public for their value and credibility or one dimensional criteria which are expressed by technical scholars and small and valueless social groups, those criteria which are gained by the totality of the society deserve more participation on the part of individuals to preserve their benefits (Farjaam, 2006; 31). Thus, sustainable development criteria should develop to prepare a strong basis for decision making in all of the levels and play a main role in environmental sustainability and development systems.

The goals of using sustainable development criteria in new towns:

The goal of using sustainable development criteria is to direct the new development decisions. It is done through the implementation of criteria over a set of social tendencies, values and future desires. Some societies express the present issues and problems in dealing with redevelopment decisions and react against the creativity of urban engineering and development investors and skill-oriented development plans through working on opportunities and social capabilities. Meanwhile, it is believed that the new development methods cause more controlling over the society. On the whole, although most of new towns in Iran are plan-oriented, often the towns didn't progress according to the preplanned programs and thus many problems emerged. We can exactly identify the problems by using sustainability criteria in planning systems of new towns and thus administer the needed strategies based on the predicted problems. Regarding the selection of criteria based on sustainability approach we can solve the problems of new towns and direct them towards sustainability.

The fundamentals of sustainable development criteria selection:

As sustainability means to find a way to create equilibrium between economy, society and environment, the development of a set of criteria for sustainable development should be in a state of

equilibrium. Thinking about this issue can create hundreds of criteria and making decisions about their number is a difficult task. One of the methods and principles used in criteria selection is Hart's checklist. We have used Hart's checklist in this research. To organize the criteria, there are 4 common methods which are defined as a framework. One of the framework's methods is "system pressure, present state, response" (figure 1). This method was used by United Nations in sustainable development field. Using this framework presents the second phase's analysis which can be used by policy makers. The framework, "system pressure, present state, response", can show the relationship between human activities, environmental status and also a working framework for long-term sustainability aspects. The most important point in the above framework is that: it can not respond to the predetermined goals well.

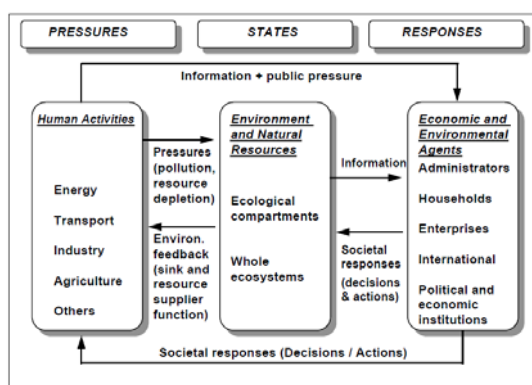


Figure 1: the total framework of the model of "system pressure, present situation, response"

Source: (Peter Hardi, Models and Methods of measuring Sustainable Development Performance, 1995)

Study process:

To assess the sustainability in new towns, first we should study the problems in three aspects of economical, social and environmental. Although new

towns have gained economical and social success in some parts of the world, most of them have encountered great problems in starting point, during the development process and after development regarding the legal regulations which can be generalized to other new towns too. Some of the problems in new towns are national-regional and some are related to specific new towns. Besides the overall social problems and problems resulted from the first era of new towns, some of the problems are resulted from the lack adjustments of planning principles based on structural characteristic of human societies in the last 50 years and the characteristics of these societies which are dominant today. Daneshpour believes that: "the important and major problems of new towns are as follows: the problem of achieving self-sufficiency, the problem of achieving a state of equilibrium, the problem of resolving settlement needs and job self-sufficiency, the problem of imbalance in age structure of the population, the problem of lack of settlement of households with low incomes in new towns, the problem of land ownership and plants constructed, the problem of depreciation of structures, the problem of financial helps' stop in the legal framework, the problem of condensation and accessibility, the problem of transportation, the problem of planning and designing fundamentals, the problem of insecurity of settlement in new towns, the problem of varied management structures of neighboring buildings, the problem of lack of people's participation in new towns, the problem of ecosystem costs in new towns" (Daneshpour, 2006; 79).

Today a variety of approaches are used to achieve the criteria, but the overall structure for all of them is nearly the same and they differ only in the procedures and details of the characteristics of every region such as goals and regional policies. In this paper, the selection of sustainability criteria has been done regarding Agenda 21, the problems of new towns and using Hart's checklist:

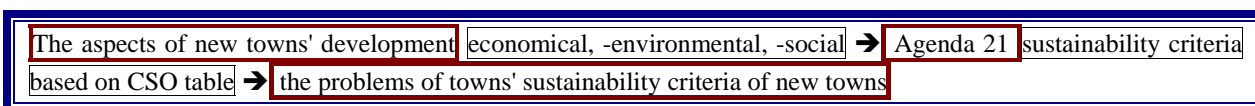


Figure 2: sustainable development concept towards sustainability criteria
Source: writers

The exact identification of criteria helps us to understand the problems better and implement the policies and investments according to the type economical, social, and environmental conditions in the future. As it has been shown in figure 2, based on discussions in Agenda 21, and considering the problems on new towns we can choose the criteria. It should be noted that the problems posed are overall and criteria selection has been done generally. We can choose the criteria regarding the special conditions of each region and accessibility of the data.

Table 3: sustainable development criteria based on the problems of new towns Source: writers
Pressure (1), State (2), and Response (3) criteria

Problems	Sustainability criteria			
	Social	Economical	Environmental	organizational
1- the problem of achieving self-sufficiency	1- rate of unemployment 2- poverty rate 3- the national capital consumptions for substructures	1- annual rate of energy consumption 2- renewable energy consumption rate 3- capital added for sustainable development	1- fossil energy consumption rate 2- environmental assessment 3- sustainability training	1- management system depended on the central government 2- access to information 3- people's participation
2- the problem of achieving a state of equilibrium	1- labor working groups 2- job ranking equilibrium 3- management considering social equilibrium	1- goods import 2- import goods share 3- capital amount added for internal production	1- annual rate of fossil energy consumption 2- renewal amount 3- natural resources' management	1- lack of complementary planning 2- multi-directional planning amount 3- sustainable development strategies
3- the problem of resolving settlement needs and job self-sufficiency	1- urban population growth rate 2- amount of land per person 3- cost of substructures per person	1- cost for house 2- cost of land per person 3- programming for cheap house	1- amount of construction 2- changing conditions of land 3- natural resources' management	1- planning for appropriate land for the population 2- planning for the present land 3- planning scenes for the future
4- the problem of imbalance in age structure of the population	1- population growth- births and deaths 2- the balance between population and jobs 3- population control plans	1- rate of unemployment 2- cost consumed for education 3- cost for population control education		1- lack of planning for real population capacity of new towns 2- presenting population control data 3- country population planning
5- the problem of lack of settlement of households with low incomes in new towns	1- labor working groups 2- social classes' equilibrium 3- cheap house planning	1- house price compared with incomes 2- the amount of cheap houses 3- the interest rate of cheap houses in annual programs of country's financial planning	1- the amount of using non-local materials and work force 2- houses adjustable with the materials accessible 3- using local materials and local workers in house construction	1- planning for cheap house 2- obligatory program in building cheap houses 3- planning for future cheap houses
6- the problem of land ownership and plants constructed	1- land ownership by public sector 2- the amount of private ownership 3- privatization of land	1- land price increase 2- investing on urban land 3- strategies to control land price	1- land conditions' change 2- land condition based on the concise plan 3- governmental control on the type of application adjusted with environmental conditions	1- the policy to change land application 2- the amount of interference and management of natural resource 3- strategies needed in land ownership
7- the problem of depreciation of structures	1- increasing old and abandoned house structures and population absorbing centers 2- population absorption and public places 3- revitalization of abandoned texture	1- cost of restoring exhausted texture 2- annual consumption for improvements 3- reinforcing improvement and reconstruction	1- structures' exhaust 2- the amount of exhausted buildings compared with out space 3- planning to restore exhausted textures regarding the environment	1- appropriate management of constructions 2- control of governmental organizations over constructions regarding the technical issues 3- planning to restore exhausted buildings
8- the problem of financial helps' stop in the legal framework	1- privatization of urban structures 2- acceptance amount of privatization by people 3- gradual privatization	1- reduction of government's financial resources 2- the amount of governmental financial resources in investments 3- financial helps for house cooperatives	1- texture destroying 2- inappropriate status regarding urban plans 3- texture restoring regarding the environment	1- reduction of governmental financial resource 2- governmental investment 3- planning for gradual reduction of financial helps
9- the problem of condensation	1- dissatisfaction of people of accessing urban services	1- the cost of access to urban services 2- the high rate of	1- high environmental damage for broadness	1- increasing official centers and managerial agents 2- the number of

and accessibility	2- the time of accessing urban services 3- increasing urban services in lanes	costs to deliver goods because of transportation 3- reduction of goods transportation cost	2- rate of land change 3- increasing urban condensation	official centers 3- centralization of management system
10- the problem of transportation	1- dissatisfaction about distances and lack of public transportation 2- the amount of public transportation 3- increasing public transportation	1- increasing private automobiles 2- cost of transportation 3- investing in urban transportation	1- increasing pollutant gases resulted from transportation 2- condensation of pollutants in urban areas 3- increasing public transportation and using green fuel and transportation without automobiles	1- automobile production increase 2- planning for automobile production and automobile imports 3- planning to increase public transportation
11- the problem of planning and designing fundamentals	1- lack of attachment of inhabitants 2- lack of identity for urban spaces 3- planning urban spaces according to what people want	1- low efficiency of space regarding profitability 2- high economical activity of the space 3- economical encouraging policies	1- environmental pressures resulted from lack of adjustment 2- cooperation with environmental conditions 3- designing regarding the environmental resources	1- potential scientists and engineers in the field 2- sustainable development strategy 3- complementary program for environment and economy
12- the problem of insecurity of settlement in new towns	1- rate of unemployment 2- immigration rate 3- increasing social security	1- households' economic status 2- economic development status of the region 3- policies to improve welfare	1- natural damages' rate 2- house conditions adjusted with the environment 3- harmony of houses with weather conditions of the region	1- regions contaminated by dangerous leftovers 2- condensation of pollutants in urban areas 3- exhaust of greenhouse gases 4- exhaust of sulfur oxides
13- the problem of varied management structures of neighboring buildings	1- social divergence 2- social context convergence regarding social classes 3- unitary management regarding social context	1- high class-differences 2- convergence of context regarding economy 3- efficient taxation system	1- divergence of urban structure regarding environment 2- adjustment of structure with the environment and natural bed 3- environmental planning before administration	1- lack of harmony in planning for areas and lanes 2- environmental complementary program 3- sustainable development strategy
14- the problem of lack of people's participation in new towns	1- attachment to government's policies 2- elections by the government 3- reinforcing people's participation in policy making and planning	1- governmental and private investments' amount 2- investments by private companies 3- encouraging policies for private investments	1- lack of observing the environment and weather as a national capital 2- amount of people's participation in environmental sustainability 3- policies for using people's cooperation in preserving environment	1- lack of awareness of government's programs among people 2- the amount of encouraging people to participate in government's activities 3- educational programs and informing the people by the government about those programs
15- the problem of ecosystem costs in new towns		1- waste of agricultural lands 2- costs consumed for substructures 3- economical yields compared with ecological cost	1- manipulations in environment to create substructures 2- harmonizing the design of new town with area conditions 3- harmonizing planning and designing of new town with area	1- environmental programming and locating new towns 2- the adjustment amount of new town administration and appropriate environmental planning 3- development scheme of new town regarding the appropriate environmental planning

As it can be seen in the table above, the proposed criteria for new towns equal 174. A certain criterion was suggested for each problem regarding sustainability, and for each field three different criteria

as: pressure, state, and response were proposed. One of the prevalent problems in sustainability criteria usage in policy makings is the interactive interference of each criterion in the system which indicates the

lack of transparency of the system. Another disadvantage of working with criteria is lack of in time decision making (figure 4). The framework "pressure, state, response", is used to present an efficient system regarding sustainability measurement to make the criteria applicable in the system. If we use this framework in new towns, we can study the conditions in these new towns based on state criteria. And finally we can propose the required policy makings and decision makings by using these criteria. The framework for "pressure, state, response", in the system is defined as follows:

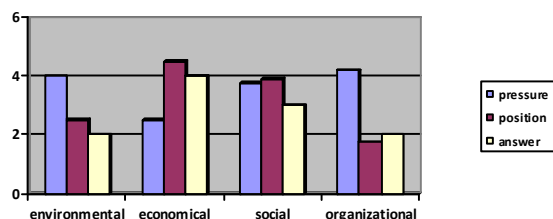


Figure 3: sustainability measurement tools

Source: writers

Regarding the above figure and the total framework for "pressure, state, response", we can say that: in most cases, pressure criteria have a reversed relationship with conditions in society and they have a direct relationship with society's reactions. In other words, if the pressure is high in a society, the conditions are inappropriate and most likely policy makings in that society is weak. In order to study a society exactly we should collect the data by using the proposed measurement tools and assess the status of the society. The figure above is simply introducing the tools and the numbers are not real.

Conclusions:

Regarding the studies carried out, we can conclude that the existence of a lot of problems in new towns is an attempt towards environmental, social, and economical in-sustainability. Choosing a concise approach which can lead our new towns towards sustainability is absolutely necessary. Utilizing sustainable development criteria in social, economical, and environmental dimensions, is an appropriate approach in this trend which is suggested by the term "an efficient method" regarding the in-sustainability of new towns in this research. To present a sustainable urban pattern by using sustainability criteria identification in new towns, the following methods were experienced and these results were achieved:

Introducing sustainability criteria for Iranian new towns regarding the problems of Iranian new towns based on Agenda 21.

Selecting appropriate sustainability criteria for environmental, social, and economical conditions of new towns in Iran by using Hart's checklist.

Identifying sustainability measurement tools by determining sustainability criteria based on "pressure, state, response" framework.

It should be noted that the application of the tools prepared was applicable in each of Iranian new towns and accordingly we can determine 1. Pressures imposed on the system, 2. The present status of the system, and 3. Administrable policy makings, in order to modify the system towards sustainability. To achieve these goals it is suggested that we should collect the data by using field studies and studies related to organizations and studying the needed statistics and then deal with measuring the place status of each of three sustainability fields by the proposed tools.

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6/5/2012

Iron and folic acid supplements for pregnant women

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Abstract: Anemia specially Iron deficiency anemia, and Neural tube defects which are preventable with preconceptional folic acid supplementation and iron supplementation during the second and three trimester, are the most common problems in pregnancy. In a cross-sectional study, 300 delivered mothers who were hospitalized in one of the obstetric hospitals in Tabriz, Iran, 1387, were reviewed. The researcher completed the questionnaires and then the data were analyzed in SPSS software. In this study the mean age of the mothers was 27.5 years old, with a range of 15-45 years. The mean of the pregnancy interval was 2.5 years, 23% of mothers had no insurance. In this study, each mother took 170 of different kinds of iron supplements, during a mean of six months, and took 72 of folic acid supplements (tablets) during 2.6 months. 4.3 percent of mothers have received no iron supplementations. 8.9 percent of mothers did not take any folic acid. In this study 8 percent of them started using folic acid supplements before conception. Most of mothers provided the supplement from drugstore and the often offered to complication. The supplementation was not associated with anemia in this study. There was an association between education level, numbers of children and pregnancy grade with supplementation.

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Keywords: Iron supplement; Folic acid supplement; Pregnant women.

1. Introduction

The principal goal of a health system is to improve society members' health and try to maintain this improved health. Health system should use planning and exact studies in order to recognize effective and purchasable interferences and make them available for the users' population. The major function of a health system is rendering services. The presence of sufficient skills in incumbent individuals and health workers in each level of service rendering and also having enough drugs in the country will enhance health throughout the country. On the contrary, if services are rendered undesirably due to incorrect organizations there would be major defects and in some cases although the resources are available and financial supplies and just distributions are carried out, the wrong arrangements will result in defects in rendering those services [1]. In our country, rendering health services to pregnant mothers has started in more than three decades ago and the primary health observations includes a continuum of services, through which family health services (mother and child) are considered to be among the most important ones [2].

In cities the presenters of these services are health houses. Also in governmental sector, they are centers and health bases and in private sector, they are offices, women scholar's clinics and obstetricians rendering the services [3].

Studies carried out in the year 2006, shows that a high percent of pregnant women were covered

[1]. Meanwhile, iron and folic acid compliments have been considered to be very important in services for mothers. Because neural tube defect is one of troubles which can be avoided to a great extent, by using iron and folic acid compliments almost three months before pregnancy or immediately after being informed of pregnancy [4]. In a study the preconceptional prescription of iron and folic acid compliments was advised to avoid neural tube defect for some groups of women, but it has been observed that a low percentage of pregnant women consumed acid folic in at least 4 weeks prior to conception in first 3 months of pregnancy [5]. Also a group of Danish pregnant women were advised to receive 400 microgram folic acid besides iron in their preconceptional period to avoid neural tube defect and in this case also, a low percentage of them did so and this led researchers to think about finding new ways to inform women about the importance of this issue [6]. It has been claimed in some studies that some women suffer from shortage of iron and folic acid before pregnancy and need excessive amounts of these compliments when they are pregnant [7]. Although shortage of iron and folic acid compliments results in neural tube defect and anemia, the seriousness of the issue is different in different countries and it is observed more in poor countries. For example, although some reports about neural tube defect have been published as a result of shortage of iron and folic acid compliments in

Philippines, Vietnam and Cambodia, the seriousness of it has been different for each [8].

Also the studies carried out in our country shows different statistics for different areas. This can depend on different variables and the only way to be informed about it is to do similar studies in different regions of the country to find the extent and real cause of this problem [3, 9]. A study carried out in the year 2004 among pregnant women referred to health centers in south of Tehran showed that %36 of pregnant women were suffering from anemia in their latest pregnancy, %3 didn't consume iron pills at all and using period among %37.6 has been less than 6 months and the using period of folic acid has been complete only for %13.4. The commonest reasons for not using are: digestive symptoms (%42.9), forgetfulness (%25.7) and not presenting by health and treatment centers (%25.7) [10]. Another study about iron shortage anemia among Kashmar high school girl students in the year 2003 showed that anemia amount based on hemoglobin and hematocrite criteria equals 24.32 and 21.62, respectively [11]. A study carried out by Jabbari & et al. in Tabriz and Zanjan indicated that the average time period of using iron and folic acid among pregnant women were 3.9 month and 1.2 month, respectively [1]. Additionally the spread of unwanted pregnancies in our country has been estimated to be %32 in our province and %18 in Fars province, respectively [12, 13]. Low level of using folic acid among pregnant women and the receipt of %85 iron needed in food patterns of households in our province [14], and the novelty of iron helping programs in our country [15], and increasing the congenital abnormalities such as neural tube defect which includes %40 of abnormalities in Iran [16], shows the importance of more studies about this issue.

Regarding thousands of papers throughout the world about the improvement of the quality of rendering health services especially for pregnant mothers presented to avoid death and realization of congenital abnormalities and the emphasis of global and local organizations about the importance of using complements during pregnancy periods and although complements are accessible and inexpensive, unfortunately we observe that there are a percent of pregnant mothers who do not pay attention to this issue and do not consume any type of complements during the critical periods of pregnancy or they do not consume them completely and in time. Meanwhile, World Health Organization (WHO) has obliged all countries in the world since many years ago to present primary health cares about all members of societies especially pregnant women and children in order to enhance and maintain consistent health. Now it is observed that these cares in all

countries of the world are implemented in any possible way. But negligence of pregnant mothers towards the importance of this issue has resulted in lots of babies and mothers' death, light weighted and anemic children are born, babies are born with different abnormalities especially neural abnormalities, early child born and ..., all of which could be avoided and controlled.

Thus, a study was designed in Tabriz in order to recognize the present issues and problems about how health services should be rendered in governmental and private sectors in Iran to study a number of women who have born babies in city hospitals and analyze the different dimensions of this fundamental issue and present probable practical strategies to improve and maintain and develop consistent health especially among pregnant mothers by knowing service qualities.

2. Procedure

In this paper, a discrete point descriptive-analytical study of pregnant women in Tabriz has been carried out for the year 2011. Our statistical society included pregnant mothers who were accepted in women's hospitals in Tabriz. Our study sample involved 300 mothers born babies and accepted in university and non-university hospital in Tabriz who have been selected randomly and investigated.

2-1- Research environment:

Our research environment and data collection included Alzahra, Taleghani, 29th of Bahman, Zakaria, Shafa and Shams hospitals.

2-2- Data collection method:

Data collection in this research has been carried out in the form of live interviews and filling out the questionnaires by the researchers in 8 to 10 days and 2 hours in each day in each one of the hospitals mentioned above. It should be noted that the questionnaire developed included two parts of demographic and general data related to the previous pregnancies, main information about the present childbirth, service receipt location, type of prescriptions and using complements and also primary data related to the babies that have been documented with the profiles of the pregnant mother giving childbirth.

2-3- Data Analysis method:

The variables investigated in this research included: mother's age, education, occupation, previous pregnancy and previous childbirths, abortion cases, insurance type, childbirth location, desired or unwanted present pregnancy, health care receipt location, health care reception time span, the amount of hemoglobin before and after childbirth, the time period of using iron and folic acid complements

and the amount of using complements, term or pseudo-term of the baby, congenital abnormalities and first minute weight of the baby.

It should be noted that recording the amount of hemoglobin before and after childbirth was carried out by studying the profiles of mothers in hospitals. The desired or unwanted pregnancy, which was not very clear for most women, was extracted easily by referring to information related to family schemes filled by families in the questionnaires. Also the data about abortion which was not mentioned by most women could be easily accessed regarding the pregnancy grade and the number of living children. Also the data related to the babies were recorded in their baby profiles. The data collected were entered into SPSS software and analyzed. In the following parts we will investigate the results found in this research.

3- Results:

This part includes findings resulted from statistical analysis of data gained from 300 women after childbirth in women's hospitals of Tabriz in the year 2011 by using the advanced SPSS software. These findings were classified in two parts of demographic and specific findings.

3-1- Demographic Findings:

3-1-1- studying age distribution of mother under investigation showed that the average age is $\mu=26.60 \pm 5.76$. In this sample, 21 mothers, i.e. %7.3 have had pregnancy under 18 years old and 21 mothers, i.e. %7.3 have had pregnancy over 35 years old. The other %85 fell in the age range of 18 to 35 years old.

3-1-2- studying the pregnancy order of mothers showed that 149 mothers (%49.67) were first time pregnant, 83 mothers (%27.67) were in second pregnancy and the others were ranked in third or higher orders (Figure 1).

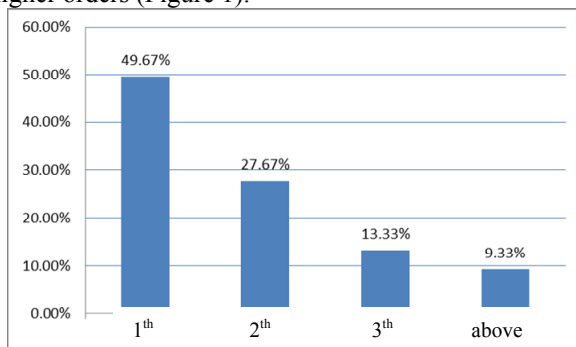


Figure 1: Pregnancy Rate

3-1-3- in this study the time span between childbirths showed that the time period between the previous pregnancy had been 1 to 3 years among 33 mothers, among 37 ones it had been 3 to 5 years, and

among 63 ones it had been 5 to 10 years and among other 19 ones it had been more than 10 years. (Figure 2).

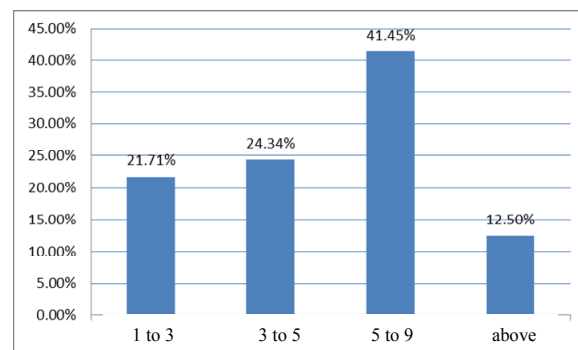


Figure 2: Distance to next pregnancy

3-1-4- Babies' maturity:

- 68 cases (%89.3) of babies, term
- 26 cases (%8.7) of babies, pseudo-term
- 1 case (%0.3) of babies, post-term
- 4 cases (%1.3) of babies, dead

3-1-5- Babies' weight:

- %7.5 of babies (22 cases) was born with less than 2500 grams of weight.
- %1.9 of babies (6 cases) was born with more than 4000 grams of weight.
- Others have had the ideal weight between 2500 and 4000 grams.
- The average weight of babies was $\mu = 3074.9 \pm 508.7$

3-1-6- Upgrade in first minutes:

- In this study 261 cases (%87) of babies were born with upgrade 0.9.
- 16 cases (%5.4) with upgrade of 0.8.

3-1-7- Congenital Abnormalities:

- %97.7 of babies investigated (293 cases) didn't have any abnormalities.
- %2 of babies (7 cases) was born with congenital abnormalities which were chromosome abnormalities, microclina and harelip.

3-2- Specific Findings:

Findings about the amount of using iron and folic acid complements by pregnant women in Tabriz show that pregnant women should have started using iron pills in the 4th month of pregnancy as quoted in the Act 2526 dated 18th. April, 2005, and in this study it was observed that the average time of using iron pills by mothers has been $\mu = 3.47 \pm 1.59$, and that each mother had consumed $\mu = 169.68 \pm 56.55$ iron pills (table 1). Among mothers being

studied, 3 persons (%1) consumed 2 iron pills daily and others had consumed 1 pill every day.

Table 1: Iron consumption amount

The number of iron tablets	Frequency	Percent	Valid Percent	Cumulative Percent
0	14	4.7	4.7	4.7
30	4	1.3	1.3	6.0
45	2	.7	.7	6.7
60	3	1.0	1.0	7.7
75	2	.7	.7	8.4
80	1	.3	.3	8.7
90	9	3.0	3.0	11.7
100	1	.3	.3	12.0
105	2	.7	.7	12.7
120	5	1.7	1.7	14.4
135	1	.3	.3	14.7
150	18	6.0	6.0	20.7
180	176	58.7	58.9	79.6
200	1	.3	.3	79.9
210	38	12.7	12.7	92.6
225	1	.3	.3	93.0
240	6	2.0	2.0	95.0
255	1	.3	.3	95.3
270	14	4.7	4.7	100.0
Total	299	99.7	100.0	
Missing System	1	.3		
Total	300	100.0		

(16 cases) consumed for 6 months and the rest (%23.9) had consumed folic acid for less than 3 months (table 4). Each pregnant mother had consumed folic acid pills for an average amount of $\mu = 2.60 \pm 1.34$ months. Among 300 mothers under investigation, only 24 mothers (%8) had started folic acid consumption before pregnancy.

Table 2: Folic acid consumption amount

The number of Folic acid tablets	Frequency	Percent	Valid Percent	Cumulative Percent
0	34	11.3	11.4	11.4
15	3	1.0	1.0	12.4
20	1	.3	.3	12.7
30	36	12.0	12.0	24.7
45	12	4.0	4.0	28.8
55	1	.3	.3	29.1
60	37	12.3	12.4	41.5
Valid 70	1	.3	.3	41.8
75	3	1.0	1.0	42.8
90	147	49.0	49.2	92.0
120	3	1.0	1.0	93.0
135	2	.7	.7	93.6
150	3	1.0	1.0	94.6
180	16	5.3	5.4	100.0
Total	299	99.7	100.0	
Missing System	1	.3		
Total	300	100.0		

Regarding folic acid, pregnant women should have started using folic acid 3 months prior than pregnancy as quoted in the Act 2526 dated 18th April, 2005, and in this study it was observed that the average time of using folic acid by mothers has been $\mu = 1.15 \pm 1.44$, and that each mother had consumed $\mu = 71.72 \pm 42.32$ folic acid pills (table 2). Among mothers being studied, only 2 persons (%1) consumed 2 folic acid pills daily.

The statistical analysis of time period of iron and folic acid complements' consumption by pregnant women in Tabriz showed that %4.3 of pregnant mothers didn't consume iron pills at all, %63.5 consumed for 6 months (according to Act 2526), %23 consumed for more than 6 months and the rest (%9) consumed iron pills less than what was needed (table 3). In our study, each pregnant mother had consumed iron pills for an average amount of $\mu = 5.98 \pm 1.63$.

Regarding folic acid, %8.9 (26 cases) of pregnant women didn't consume any pills at all. %59.2 (173 cases) consumed it for 3 months, %5.5

Table 3: Iron consumption time period

Iron intake duration (months)	Frequency	Percent	Valid Percent	Cumulative Percent
Valid .00	13	4.3	4.3	4.3
1.00	1	.3	.3	4.7
2.00	1	.3	.3	5.0
3.00	2	.7	.7	5.7
4.00	2	.7	.7	6.4
5.00	21	7.0	7.0	13.4
6.00	190	63.3	63.5	76.9
7.00	43	14.3	14.4	91.3
8.00	8	2.7	2.7	94.0
9.00	18	6.0	6.0	100.0
Total	299	99.7	100.0	
Missing System	1	.3		
Total	300	100.0		

Table 4: Folic acid consumption time period

Folic acid intake duration (months)	Frequency	Percent	Valid Percent	Cumulative Percent
.00	26	8.7	8.9	8.9
1.00	29	9.7	9.9	18.8
2.00	41	13.7	14.0	32.9
3.00	173	57.7	59.2	92.1
Valid 4.00	2	.7	.7	92.8
5.00	4	1.3	1.4	94.2
6.00	16	5.3	5.5	99.7
7.00	1	.3	.3	100.0
Total	292	97.3	100.0	
Missing System	8	2.7		
Total	300	100.0		

4. Discussions

However the results of this paper shows better condition in supplementary serving, it has main difference in level of mothers Hb, supplementary usage, percentry of seeing public hospitals and seeing public health centers according to others studies researches done in this area.

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Awareness level of use of Information Communication Technologies tools among Extension officers in the North- West Province, South Africa.

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Abstract: A simple random sampling technique was used to select 169 extension officers to examine their level of awareness of information communication technologies in North West Province, South Africa. Data were collected with structured questionnaire and analysed using frequency counts, percentages and multiple regression analysis. The results show that majority of the extension officers were male (76%) with the mean age of 44.6 years, married (79%) and 82.5% were Christians. Forty one percent of the extension officers had Diploma as their educational qualification and a mean of 16.7 years as working experience. The results revealed that out of the 37 ICT tools listed, extension officers indicated high level of awareness of nine tools, which include mobile phones (1.79), computer (1.68), internet (1.77), overhead projector (1.62), fax machines (1.60), organization e mail (1.58), fixed telephone (1.52), personal email (1.52) and organization website (1.50). Significant determinants of awareness level were religion ($t = 1.91$, $p = 0.58$); constraints to ICT use ($t = 1.78$, $p = 0.78$); importance of ICT tool ($t = 1.93$; $p = 0.63$) and 2 were significant at 0.05% which were competence on ICT use ($t = 3.50$; $p = .001$); ($t = 2.0$, $p = .003$). The study recommends that more information communication technologies should be made available to extension officers, so that they will become more aware of the use of ICT in extension work as tools that can gather and disseminate agricultural information.

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Key words: South Africa, extension officers, information communication technologies, tools, awareness.

Introduction

Information and Communication Technologies (ICTs) are all technologies used for the widespread transfer and sharing of information. ICTs are rapidly consolidating global communication networks and international trade with implications for people in developing countries. ICTs can be used to enable, strengthen or replace existing information systems and networks. ICTs in agriculture promote and distribute new and existing farming information and knowledge which is communicated within the agricultural sector since information is essential for facilitating agricultural and rural development as well as bringing about social and economic changes (Swanson and Rajalahti, 2010). Agricultural extension, which depends to a large extent on information exchange between and among farmers on the one hand, and a broad range of other actors on the other, has been identified as one area in which ICTs can have a particularly significant impact. There is growing recognition that farmers and members of rural communities have needs for information and appropriate learning methods that are not being met (Greenridge, 2003; Lightfoot, 2003), and these have been lacking in South Africa. In the midst of this change, extensionists are grappling with the question of how best to harness ICTs to improve rural livelihoods. Meera et al (2004) had noted that as a

result of the emerging new paradigm of agricultural development, old ways of delivering important services to citizens are being challenged; traditional societies are also being transformed into knowledge societies all over the world.

Agricultural improvement in South Africa, especially among small scale and resource-poor farmers, requires a major effort to improve the quality of extension services available to farmers. DAFF (2008) reported that currently, the Extension Recovery Plan (ERP) is being implemented in all the nine provinces in the country. This is predicated on the fact that agricultural extension bridges the gap between available technology and farmers' practices through the provision of technical advice, information and training. Without these, farmers' ability to adopt new technologies and plant varieties, which would benefit their production and incomes, would be limited. South African farmers receive much advice and information from other farmers and/or private input suppliers, and many also benefit from radio and television programmes, agricultural trade magazines, shows and demonstrations. Despite the different roles and functions that agricultural extension and advisory service should play, much leaves to be desired for the use and integration of ICTs in the agricultural extension and advisory services in South Africa (DAFF, 2009).

The development and improvement of agriculture worldwide, with specific reference to the African continent and South Africa in particular, requires a paradigm shift on communication and information dissemination. Swanson and Rajalahti (2010) and Rivera and Sulaiman, (2009) posted that extension services, either general or more specialized exist in many countries to provide information, advice and educate communities relating to many facets of rural life and its improvements. In South Africa, the Department of Agriculture, Forestry and Fisheries (DAFF) introduced the Extension Recovery Plan (ERP) in 2007. The introduction of the ERP was necessitated by the 2006 research findings and consolidated recommendations on the report by DAFF on the state of extension and advisory services in South Africa which highlighted the challenges and constraints facing extension and advisory services. The important role played by extension services in providing linkages and support to agricultural research in information and technology for farmers and farming communities has been crucial to agricultural successes in most developed countries. Public extension services have been ineffective in reaching farmers and farm communities with information and technologies needed to ensure food security and sustainable development (FAO, 2004). The situation is exacerbated by lack of skills by extension officers in using ICTs to promote new farming technologies, to enhance the flow of farming information relating to inputs, finance and marketing activities amongst others and bridging the rural digital divide. IFAD (2002) reached the conclusion that extension services in Africa have failed to address the needs of small-scale farmers. In another study, Richardson (2006) argues that agricultural extension services that provide agricultural information do not work effectively in Africa. These shortfalls may be due to changes in the extension

process that have resulted in the shift to the facilitation and brokerage of information, communication and advocacy services. This range of services, meant to improve rural livelihoods, can benefit from the applications of ICTs.

Materials and Method

The study was carried out in North West province, South Africa. The study population included all extension officers (200) in the province. A simple random sampling technique was used to select 169 extension officers from which data were collected. A structured questionnaire was designed based related literature and objectives of the study and comprised 37 items categorized as awareness level of information communication technologies. Validity of the instrument was ensured through a panel of experts in the Departments of Agricultural Economics and Extension and extension professionals from the Department of Agriculture and Rural Development, South Africa. The questionnaire had a reliability coefficient of 0.92 using the split half technique. Data were analyzed with Statistical Package for Social Sciences (SPSS) using frequencies, percentages, mean and multiple regressions.

Results

Table 1 shows the personal characteristics of extension officers in North West Province, South Africa. Table 2 shows the mean and standard deviation of 37 ICT tools on awareness levels of ICT tools among extension officers which were rated on a 2-point scale of Yes (2), and No (1). The result of multiple regression analysis of relationships between extension officers' socio-economic characteristics and awareness level of ICT tools were presented in Table 3.

Table 1. Personal characteristic of extension officers.

Personal characteristics	Description
Gender Predominantly	male 76%
Age Mean	44.6 years SD = 5.40
Marital status	79% married
Religion Predominantly Christianity	82.5%
Educational level Predominantly diploma	41% , BSc =15%
Household size Mean	4.8 persons SD = 1.20
Working experience Mean	16.7 years SD = 4.50
Living in job location Predominantly	Yes 79%, , No 21%
Job designation Predominantly	Extension officer 53%,Senior/Chief agricultural technicians 36%

Table 2: Awareness of specific ICT tools among extension officers in the Northwest Province

ICT Tools	Yes	No	Mean	SD
Mobile phones	150 (88.8)	19 (11.3)	1.79	.59
Computer	139 (82.2)	30 (17.8)	1.68	.71
Internet	136 (80.5)	33 (19.5)	1.77	1.65
Overhead Projector	131 (77.5)	38 (22.5)	1.62	.73
Fax Machines	129 (76.3)	39 (23.1)	1.60	.75
Organization e mail	128 (75.7)	41 (24.3)	1.58	.76
Fixed Telephone	124 (73.4)	45 (26.6)	1.52	.81
Personal email	119 (70.4)	50 (29.5)	1.52	.78
Organization website	118 (69.8)	51 (30.2)	1.50	.80

Table 3 Determinants of awareness level of ICT tools by extension officers

	B	Std. Error	Beta	t	Sig.
(Constant)	7.333	9.187			
Gender	-1.620	2.346	-.034	-.691	.491
Age	.016	.158	.007	.103	.918
Marital Status	-.558	.771	-.035	-.723	.471
Number of children	-.070	.847	-.005	-.082	.934
Religion	2.534	1.325	.083	1.912	.058
Educational qualification	.000	.599	.000	.000	1.000
Studying for a higher degree	-.480	1.789	-.011	-.268	.789
Household size	.134	.466	.014	.288	.774
Working experience	-.137	.124	-.070	-1.106	.271
Living in job location	2.603	2.225	.050	1.170	.244
Place of residence	-2.390	1.614	-.063	-1.481	.141
Number of farmers covered	5.057E-5	.001	.002	.035	.972
Distance to farmers	.002	.002	.045	1.054	.294
Use of ICT	-.242	.150	-.100	-1.616	.108
Constraints to ICT use	.415	.233	.104	1.776	.078
Effect of e- readiness on officers	.144	.237	.030	.609	.543
Effect on information access	.033	.063	.026	.520	.604
Importance of ICT	.164	.088	.173	1.872	.063
Competence on ICT use	.423	.121	.430	3.502	.001
Accessibility to ICT	-.056	.157	-.052	-.357	.722
Availability of ICT	.374	.126	.337	2.970	.003
F	22.55				
p	0.00				
R	0.87				
R squared	0.76				
Adjusted R squared	0.73				

Discussion

From Table 1, majority of the extension officers were male (76%) with the mean age of 44.6 years, married (79%) and 82.5% were Christians. Forty one percent of the extension officers had a diploma as their educational qualification and a mean of 16.7 years as working experience. There was a mean of 4.8 persons per household and 79% live in their job location, rural or peri urban notwithstanding. In terms of job designation 53% were extension officers. Zwane (2009) reported similar findings that that

extension officers in Limpopo province of South Africa were mainly males, between 40 to 49 years, and had Diploma as their educational qualification. Bembridge, (1991) also reported similar findings in terms of the personal characteristics of extension officers in South Africa. Table 2 shows the 37 ICT tools that extension officers are aware of. The results in Table 3 revealed that extension officers were more aware of the nine ICT tools out of the listed 37 ICT tools. Prominent information communication technologies among extension officers were mobile

phones (1.79), computer (1.68), internet (1.77), overhead projector (1.62), fax machines (1.60), organization e mail (1.58), fixed telephone (1.52), personal email (1.52) and organization website (1.50). This finding is similar to Adesope et al. (2007) who noted that in the Niger Delta area of Nigeria, about 98 percent of the extension agents indicated they were aware of information technologies, while 2.3 percent were not.

Seepersad (2003) reported that cell phones are fairly common among extension employees in Trinidad and Tobago, but added that cell phones have not been used in an organized way by agricultural organizations. This finding is also supported by Agwu et al. (2008) that 63% of extension officers in Enugu state, Nigeria had high level of awareness of the major ICT tools. This means that most of the respondents were aware of information technologies especially as they concern Agricultural Extension work. In Table 3, the independent variables were significantly related to awareness level with the F-value of 22.55, $p < 0.05$ showed that there was a strong correlation between independent variables and awareness level. The result further predicted a 76% of the variation in the awareness level of ICT among extension officers. Significant determinants were religion ($t = 1.91$, $p = 0.58$); constraints to ICT use ($t = 1.78$, $p = 0.78$); importance of ICT tool ($t = 1.93$; $p = 0.63$) and 2 were significant at 0.05% which were competence on ICT use ($t = 3.50$; $p = .001$); availability of ICT tools ($t = 2.0$, $p = .003$). It implies that more male extension officers will be more aware of the information communication tools than female extension officers. Agwu and Uche-Mba (2010) in Enugu State, Nigeria, revealed that the researchers, extension workers and farmers had favourable perceptions of the role of ICTs in agricultural development. The lower level of the constraints experienced by the respondents, the higher the awareness level of ICT tools will be experienced. Similarly, the higher the realization of the importance of ICT, the competence and the availability of ICT tools to extension officers the more they become aware of the information communication tools. The study showed that the most common information communication tools among officers in North West Province, South Africa were mobile phones, computer, internet, overhead projectors, fax machines, organization e-mail, fixed telephones 73.4%, personal email and organization website. Significant determinants of extension officers' level of awareness of ICTs were religion, constraints to ICT use, importance of ICT tool, competence on ICT use and availability of ICT tools. This information communication technologies highlighted will help extension officers to be more aware of their use in the

promoting, gathering and disseminating agricultural information.

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The effect of the Extended Parallel Process Model of childbirth education for decreasing the rate of Caesarean section among Iranian women

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Abstract: Despite the fact that childbirth by caesarean section (CS) does not provide significant health benefits for either the mother or the newborn, there has been a noticeable upward trend in CS births in Iran over the last two decades. The aim of the current study was to determine the effect of using the Extended Parallel Process Model for childbirth education on decreasing the number of births by CS among Iranian women. This field study consisted of three steps. In step one; a formative evaluation was conducted using a pre-test questionnaire based on the Extended Parallel Process Model (EPPM). In step two, a structured childbirth education program was developed based mainly EPPM. In step three, evaluation of the outcome was conducted by comparing the scores from the post-test questionnaire and CS rates between two groups. Two hundred and eighty-four low-risk pregnant women from antenatal clinics participated in and completed the study, and they were assigned to experimental and control groups that consisted of 145 and 139 women, respectively. Follow-up pairwise comparisons using paired t-test between the comparative groups indicated significant statistical changes for the outcome variable, i.e., the intention to have vaginal birth, from the pretest to posttest in the experimental group's score ($p < 0.001$, 95% CI = -3/7 - -2/8), but such a finding was not observed in the control group. Furthermore, the rate of caesarean delivery was significantly decreased in the experimental group compared with control group (66.2% and 48.2%, respectively) and the odds of giving birth by CS was 2.1 times greater in the control group ($p < 0.001$). Using EPPM-based childbirth education was found to effectively lower the rate of intended and actual caesarean births. The findings also indicated that childbirth preparation programs that place emphasis on promoting mothers' self-awareness and self-confidence increased the probability of their having normal childbirth.

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Keywords: Extended Parallel Process Model (EPPM); caesarean delivery; Iran

1. Introduction

Caesarean section (CS) can be a life-saving operation in some special circumstances; however, pregnancy and childbirth are both normal and physiologic processes that often occur without intervention (Campero, 2004). In recent years, CS rates have increased dramatically some developed countries and in several developing countries (Leone, 2008). According to a statement issued by the World Health Organization (WHO), there is no justification for the 10-15% increase in the CS rate (A. Scarella, 2011); even so, the goal of reducing CS birth rates has not been achievable in some developing areas, such as Iran. Notably, the caesarean birthrate in Iran

has increased significantly, from 35% in 2000 to 46% in 2009 (Maternal Health office, 2010). Although more than 95% of all deliveries take place in hospitals where they are assisted by educated midwives, medical interventions, such as episiotomy, perineal shaving, and intravenous lines, are included as routine and common practices during childbirth. Furthermore, the services of a doula are not common, and the spouse is forbidden to be present for the birth in all teaching hospitals and most private hospitals. Obviously, midwives are the only source of support for a woman in labor, but they often must engage in many duties and accompany other women in labor. In spite of the availability of certified childbirth

educators, maternal education is provided only briefly during short prenatal visits, and most of the educators do not take it upon themselves to educate women about feasible methods for relieving the pain of labor and childbirth. Thus, it is not surprising that the lack of information and some misconceptions

about childbirth that the mother-to-be might have could lead to excessive fear and anxiety, thereby leading her to choose an alternative plan of “painless” and “easy” childbirth, such as CS (Tork Zahrani, 2008).

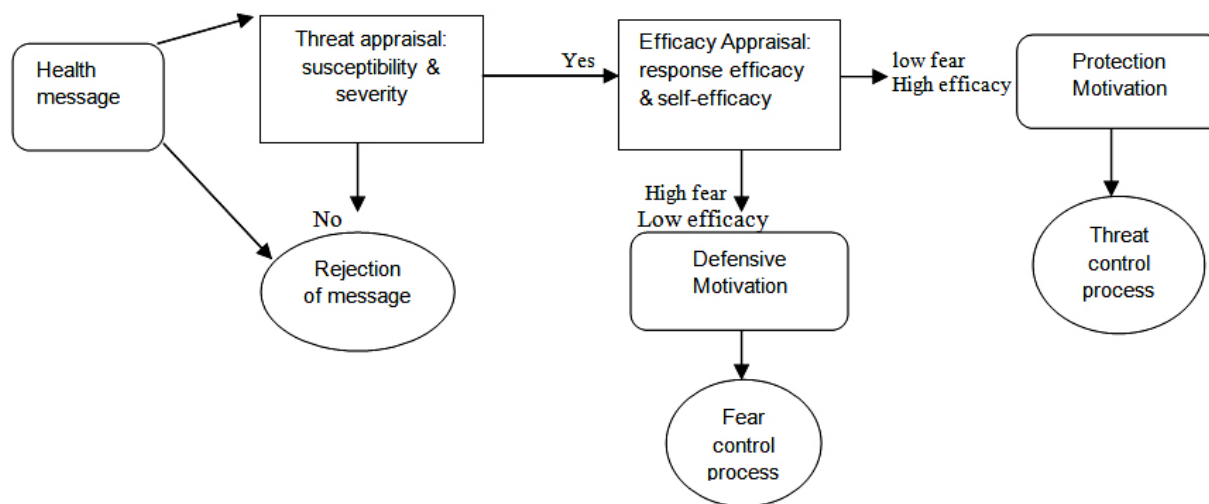


Figure1. Simple Description of Witte’s Extended Parallel Process Model for fear appeals (according to Witte, 2001)

It has been proposed that the negative effects of excessive fear could be reversed through medical intervention by trained healthcare providers who could educate and support women in all stages of pregnancy and labor (Campero, 2004). Appropriate training during pregnancy would provide opportunities to guide the mother to better understand her abilities, to control herself with less fear and apprehension, and also to benefit from valuable methods that can help her complete a healthy pregnancy and have a safe delivery. In addition, training courses could provide appropriate conditions for mothers and their families to speak to their counterparts and the trainers about their fears and concerns and share their thoughts and experiences, which could go a long way toward decreasing their concerns most of the time (Bascom, 2002). However, the effects of general, prenatal education have been debated extensively due to the methods and materials used in the educational process (Fraser, 1997; Gagnon, 2009).

It is proposed that educational models based on fear appeals theory can be effective in motivating people to change some health-related behaviors. In this field, the Extended Parallel Process Model (EPPM) has been used and found to be a useful method for appraising the knowledge and perceptions of a target population regarding an issue in order to

develop effective messages and interventions. This model, first proposed by Witte in the early 1990s, offers a condition in which fear appeals succeed or fail in motivating people to behave in certain ways relative to accepting or refusing a health-risk message (Witte, 1992). To date, however, it has not been used to reduce unnecessary caesarean births, but research suggests that this model can and does work under certain conditions (Witte, 1992; Witte, 1996; Witte, 1997). Fear can be a barrier to behavior change, such as when audiences are so frightened that they cannot act, so they deny the treat and engage in fear control. However, they can be motivated to change if they are taught to appraise their abilities and the efficacy of the message so that, when they perceive a risk, they are motivated to reduce it. From a public health standpoint, it was of great importance to determine, prior to the research, whether the participants were already engaged in danger or fear control processes. This determination was essential so that we could be able to assess during planned interventions whether or not if the messages had the intended effect. At some critical point, perceptions of threat may exceed perceptions of efficacy, and people will shift into fear control processes and begin focusing on how to deter their fear instead of thinking about the threat (Witte, 2001). Briefly, the recommendations contained in a health-risk message are accepted when danger

control dominates, and they are rejected when fear control dominates (Figure 1).

The aim of this study was to determine the effect of childbirth education based on the Extended Parallel Process Model on decreasing the rate of caesarean births among Iranian women. The present study was performed in Shahroud, a populous city in northeastern Iran where there has been an increasing trend of caesarean births over the past decade. In Shahroud, the CS rate was 57.5% in 2010, and elective caesarean births contributed to a significant proportion of this percentage (Statistical report of registered births of hospitals in Shahroud, 2011).

2. Material and Methods

2.1. Study design

The present research is a field study that included three sequential collections of data from antenatal clinics in Shahroud. The first step was a formative evaluation to determine participants' knowledge and perceptions of threat and efficacy using a structured inventory based on EPPM. The second step comprised the random allocation of clinics as clusters to the experimental and control groups. Then, childbirth education was conducted for the experimental group by trained birth educators. In the third step, we evaluated our educational interventions regarding participants' intention to give birth vaginally and the actual mode of birth as outcome variables using post-test questionnaires between the two study groups.

2.2. Study population

The participants in our study were pregnant women who sought prenatal services at one of 10 outpatient clinics affiliated with the Shahroud University of Medical Sciences. Based on Witte's recommendation (Witte, 2001), we needed at least 150 participants in order to assess the relationship between the variables of the questionnaire. Hence, by means of the maternal health information provided by the clinics, women who met the inclusion criteria were recruited. At the time of the visit, the aims of the study were described to eligible women, and willing participants entered into the study and completed the questionnaires after they provided signed, informed-consent forms. The inclusion criteria are outlined in Table 1. Gestation times exceeding the twenty-first week were considered because the probability of an abortion or a miscarriage is reduced significantly after this time. Also, since vaginal birth rarely, if ever, is performed in Shahroud and many other cities in Iran after there has been a previous caesarean surgery, we had to exclude women who had an earlier caesarean birth. Participants had the right to withdraw from the study

at any time for any reason, and they were reassured concerning the confidentiality of the data that were collected.

Table 1- Inclusion criteria of the study

Low risk pregnancy with any diagnosed prohibition for vaginal delivery
Maternal age : 20-40 years
Gestational age: 21th–28th weeks' gestation
having singleton pregnancy
negative history of previous CS
ability to read and write in Persian

2.3. Measurement instrument

We used the Risk Behavior Diagnosis (RBD) Scale in this study (Witte, 2001) to identify whether the participants were engaged in a fear control process or a danger control process. The RBD Scale is a 12-item survey that is theoretically grounded in the EPPM. The original scale asks three basic questions/phrases about the participants' perceptions of susceptibility, severity, response efficacy and self-efficacy on a 7-point scale ranging from "1-strongly disagree" to "7-strongly agree." The questionnaire used in the study consisted of two separate parts for measuring the data, i.e., Demographic and EPPM items. The EPPM items included perception of threat (severity, susceptibility) with five statements in each domain and perception of efficacy (response efficacy, self-efficacy) with six and seven phrases. In addition, we assessed the participants' knowledge of modes of birth and the mode they intended to use with nine and five phrases, respectively. In this study, "threat" was defined as such common caesarean complications as higher probability of post-partum hemorrhage, infection, readmission of mother/baby to the hospital, impaired maternal attachment, and delay in breast feeding. Likewise, "Efficacy" was described as feasible and effective coping behavior skills that promote women's self-efficacy for childbirth and coping ability in reducing anxiety and pain during labor. Since validation of the scale in judgment was based on quantitative evidence (Yaghmaie, 2003), inclusion of at least five to 10 experts was useful for reviewing and judging the appropriateness of items to the domain of content using a content validity index (CVI) (Polit, 2007). The group members consisted of nine experienced faculty members who had specialized in the subjects related to our study. They evaluated and rated items based on relevance, clarity, and simplicity on a four-point scale. In the next step, in order to improve the clarity of the responses, the remaining items were presented to 25 pregnant women from two different health centers to obtain a convenient sampling. However, few revisions were

conducted. Finally, we tested the internal consistency of the EPPM inventory using Cronbach's alpha and correlation. For this purpose, the inventory was given to 50 pregnant women with different socioeconomic characteristics and gestational ages from another health center. Cronbach's α coefficients were 0.71, 0.76, 0.78, 0.70, 0.69, and 0.81 for Susceptibility, Severity, Response efficacy, Self-efficacy, Knowledge, and Intention sub-scales, respectively.

2.4. Data collection and interventions

At the beginning of the first step, in order to decrease the likelihood of bias in the selection and to minimize information exchange between the participants in order to have a clean intervention, five out of 10 clinics were assigned randomly to the experiment, and the other five clinics were assigned to the control group. We conducted continuous sampling that lasted from November 2010 to March 2011, and 300 eligible, pregnant women completed the pre-test questionnaires to provide a formative evaluation (150 persons in each group). The women completed the pre-test questionnaires in a private room at the health centers. Then, the women in the experimental group participated in antenatal childbirth education and were trained by certified and experienced birth educators. They attended six sessions in one of the five experimental clinics, and one session was allocated to providing education for the fathers. In this single session, the educators dealt with addressed the mothers' physiological and mental health during pregnancy, the childbirth process, and parenthood in order to achieve more support by their spouses. The courses related to preparation for childbirth were held with 8-10 pregnant women for a period of 60-90 minutes. The central content of the training classes was allocated to presenting the evidence-based information about potential risks of unnecessary CS as a major surgery and presenting the benefits of normal birth for both mother and the newborn. This approach was utilized to provide realistic information that indicated that unnecessary CS could be regarded as a potential threat that should induce rational fear. Following that, in order to avoid rejection of the messages and motivate audiences to avert the threat, health messages were presented mainly to introduce coping behavior skills, such as non-pharmacological pain-reduction skills during labor and birth. The participants practiced the feasible birth skills (breathing exercises, self-massage, guided imagery, and relaxation) at the end of each session under supervision of the trainers. Finally, similar to the pre-test questionnaires, the posttests were completed by participants at the sixth session of the class, using five statements to assess the women's intentions to have a normal birth. All

sessions were conducted by means of a lecture, asking and responding teaching methods, and watching training films.

The participants of the control group received routine pregnancy care. They were given the pre-test questionnaire at the twenty-first through the twenty-eighth weeks of gestation, and they completed the post-test questionnaire at least eight weeks after the pre-test questionnaire was completed and before the thirty-seventh week of pregnancy. All participants received necessary maternal care at the time of their appointments, and they were controlled for maternal-neonatal general health during pregnancy and were referred to an obstetrician in case of any threatening health symptoms. The researcher followed up the study by proceeding to explore any problems and to support trainees with their method of delivery by making phone calls to each participant and also to the birth trainer in each health center.

2.5. Ethics review committee approval

Ethical approval was obtained from the ethics committee of the institution (i.e., Shahroud University of Medical Sciences' Ethics Committee) concerned with code 890/07 on 2/15/2011. Meanwhile, while awaiting receipt of Ethics Committee's approval for the study, 10 midwives in the targeted settings were trained for the recruitment of the eligible women and for the collection of data. We considered the following important points with respect to the collection of data:

- 1- All of the participants were assured that their responses would be kept confidential.
- 2- Participants were informed that their participation was voluntary and that they could withdraw at any time.
- 3- Any participant who needed emergency care would be referred to emergency obstetrics services immediately.

2.6. Statistical analysis

Descriptive statistics including means, standard deviations, and X² techniques were used to assess the components of the questionnaire and compare the variables between the groups. Evaluations of outcomes (intention to have NVD or CS delivery) were compared using the paired t-test and the chi-squared technique for group comparisons.

3. Results

Table 2 outlines the participants' characteristics and the significance values associated with the chi-squared assessment that evaluated differences between the experimental and control groups. The groups did not differ significantly in any of the demographic measures, indicating that the

sample allocation procedure that was used was effective.

Table 2. Sample characteristics and baseline measures between two study groups

characteristics	Experiment N(%)	Control N(%)	X2, P value
Age groups			
20-24	66 (45.5)	62 (44.6)	3.6,p=0.721
25-30	66 (45.5)	65 (46.7)	
>30	13 (9.0)	12 (8.9)	
Education level			
Primary school	30 (20.7)	27(21.1)	2.98, p=0.81
High school	76 (52.5)	69(49.6)	
College	39 (26.8)	43 (30.9)	
Occupation			
Housewife	105 (72.4)	98(70.5)	9.13, p= 0.13
employee	40 (27.5)	41(29.5)	
spouse's education level			
Primary school	51(35.2)	48 (34.5)	8.12, p= 0.1
High school	69 (47.6)	68 (49)	
College	25 (17.2)	23(16.5)	
spouse's occupation			
Official employee	39 (26.9)	44(31.6)	11.27, p= 0.257
Self employee	106 (73.1)	95(68.4)	
Number of pregnancies			
primigravidae	119 (82.1)	112 (80.6)	0.63, p= 0.88
multigravidae	26 (17.9)	27 (19.4)	
Gestational age groups(weeks)			
21-24	84 (57.9)	79 (56.8)	10.21, p= 0.266
25-28	61 (42.1)	60(43.2)	

Table 3. Detail of the EPPM scale between experimental and control groups before intervention

Variable (number of items)	Possible range	E group	C group	t, P.value
	<i>M ± SD</i>	<i>M ± SD</i>		
Susceptibility(5)	5-35	21.51 ± 5.7	21.6 ± 4.3	1.34 (p=0.12)
Severity(5)	5-35	26.7 ± 5.21	26.34 ± 5.11	1.59 (p= 0.09)
Response efficacy(6)	6-42	36.51± 3.89	36.01 ± 3.21	1.68(p=0.092)
Self efficacy(7)	7- 49	39.84 ± 5.99	39.04 ± 5.62	1.64 (p= 0.1)
Knowledge(9)	0-9	5.69 ± 1.67	5.43 ± 1.87	1.74(p=0.082)
Intention(5)	0-5	28.36 ± 3.48	28.78 ± 3.75	-1/38(p=0. 16)

The participants were 25 ± 3.9 years old on average and most of them were housewives who had a high school diploma and were pregnant for the first time. Table 3 demonstrates the mean scores obtained by the participants of the two groups of study from the pre-test variables and before the training interventions were initiated. Two independent sample t- tests showed that there were no statistically significant differences between the groups according to the variables' mean scores. Moreover, we calculated the critical points (discriminating value) in the two groups by subtracting the "threat perception"

from "efficacy perception." Comparing the critical value revealed no significant difference between the study groups. The fact that this value was positive means that the perceived efficacy exceeded the perceived threat (resulted in danger control processes). However, perceptions of susceptibility in both groups were lower than the perceptions of severity.

Within a period of interventions, 145 women in the experimental group completed the study, while three dropped out for medical reason and two withdrew, and 139 in the control group

completed the study, while 11 were excluded. Therefore, 284 women completed the post-test questionnaires. Reasons for excluding participants included constant breech-birth presentation (3), placenta previa (2), pre-term labor (4), diabetes mellitus (2), and missing data (3).

Table 4. Comparison of the scores concerning intention to have vaginal delivery between the experimental and control groups.

Group/ Scale	Mean \pm SD P.value	paired t-test ,
Experiment		
Pre-test	28.35 \pm 3.48	t= -14.25 , p<0.001
Post-test	31.61 \pm 3.02	
Control		
Pre-test	28.78 \pm 3.75	t= 0.84 , p= 0.39
Post-test	28.12 \pm 3.58	

Follow-up pairwise comparisons using the paired t-test between the comparative groups indicated significant statistical changes for the outcome variable, i.e., intention to have a vaginal birth, from the pretest to posttest in the experimental group's score ($p < 0.001$, 95% CI = -3/7- -2/8), but such a finding was not observed in the control group (Table 4). In addition, Table 5 suggests that the rate of normal delivery in the experimental group was significantly more than in the control group, and the odds of caesarean birth in the control group were up to 2.1 times greater than in the experimental group (X², $P = 0.001$, 95% CI = 1.41- 2.76). The caesarian birth rate among the first-time pregnant mothers was significantly greater than among multigravid women (X², $P = 0.003$). This finding remained significant when adjusted for social characteristics using the Mantel-Hansel procedure. In addition, the elective CS rate was greater in the control group than in the experimental group; however, this difference was not statistically significant.

Table 5. Comparison of mode of childbirth between the two study groups

Group	Mode of birth		X ² value
	NVD N (%)	CS N (%)	
Experiment (n=145)	96 (66.2)	49 (33.8)	X ² , P=0.001 OR= 2.1
Control (n=139)	67 (48.2)	72 (51.8)	
Total (n=284)	163(57.4)	121(42.6)	

4. Discussions

The results of this study showed that using methods based on the logic model of fear appeal is more effective than existing birth-training programs for decreasing the women's intention to have caesarean births. Although fear appeals tend to be viewed with doubt in health education, the results of this and some other experimental studies reflect the fact that using such models will be efficient under special conditions (Witte, 1997; Barnett, 2009; Basil, 2008). In the other words, fear appeals work when accompanied by high efficacy messages. We found that there was no difference between comparable groups before the interventions and that women tended to see themselves as invulnerable to undesired outcomes (post-caesarean complications), but they were aware of the severity of the negative consequences of such major surgery. It has been stated that "when perceptions of threat are high, a minor stimulus may be needed to initiate action" (Redding, 2000). Also, at the beginning of the study, our participants demonstrated higher confidence (efficacy), indicating great acceptance of proposed messages. It implied that women believed that they had the ability to perform the coping responses and that these responses were effective in minimizing the risk. They exhibited a confident manner concerning their intention to plan a safer delivery.

As childbirth has been described as a painful event that induces fear and anxiety (Cheung, 2007), various methods have been introduced to reduce the level of anxiety and pain associated with labor and childbirth, including childbirth education. Using the fear appeal theory, we attempted to shift the existing fear of normal birth into the fear of complications following unnecessary surgical birth, while dominantly emphasizing positive messages on the promotion of self-efficacy and the feeling of being in control during childbirth. Therefore, the perception of efficacy was reinforced by effective coping behaviors. Feeling that one is an active participant in labor and birth instead of a passive object, increases her self-confidence and helps her promote the feeling of being in control over a painful childbirth (Ip, 2009). Since both self-accomplishment and vicarious experience enhance the perception of self-efficacy (Bandura, 1977), our intervention provided an opportunity for pregnant women to develop and experience their coping skills and to be appreciated and admired by their trainers and peer group.

In some situations, inter-individual interactions and learning together with other counterparts cause a sharing of experiences and mental and emotional perspectives between the learners that can effectively change the attitudes and

beliefs of the participants concerning healthy behavior (Wang, 2011).

The finding that the experimental group demonstrated greater intention to have vaginal births than the control group after learning coping skills was supported by former studies that also ascertained a more positive behavior toward normal childbirth (Saisto, 2001; Wang, 2011; Fathian, 2007). In addition, the lower incidence of caesarean delivery in the experimental group than in the control group confirmed similar results from previous studies, which indicated that prenatal instructions, especially group education, provided support to women during childbirth and contributed significantly to reducing initial birth by CS (Campero, 2004; Wang, 2011). The effectiveness of using these coping strategies for pain relief during labor was not measured in the experimental group; nevertheless, improved intention to have a vaginal birth and actual increases in the rate of vaginal births compared with the same intent and decisions in the control group implied that programs that concentrate on the fear appeal theory, accompanied mainly by raising the individual's self-confidence, were appropriate and effective.

Although we found no significant relationship between women's social characteristics and their preferred method of delivery, the results of a few Iranian studies and studies conducted abroad indicate that the prevalence of caesarean births increases when socio-economic status improves (Campero, 2004; Ahmad-Nia, 2009; Angega, 2006). This study was limited by the fact that we did not perform individual randomization; however, we made that decision in order to minimize the probability of information leakage among participants who attended the same clinic.

In sum, the results reported in this paper indicate that structured childbirth education based on EPPM can work effectively either to improve maternal intention to have a normal birth and to decrease the rate of caesarean births. The findings also suggested that the probability of inclination to have a normal birth increased when programs specifically designed to prepare women for childbirth promoted the mothers' self-awareness and self-confidence. The results of this study raise questions about the existing components of prenatal education and how midwives can be involved to plan innovations in antenatal programs and act as childbirth educators more effectively than before. It is important for maternity caregivers to be aware of the benefits of empowering women by providing them with coping strategies they can use during labor. Further experimental investigations must be conducted to establish whether community education based on fear appeal models can lead to reductions in

unnecessary caesarean births and the negative health complications that such births may induce.

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Concordance of Serum Creatinine to Estimated Glomerular Filtration Rate in Determining Early Chronic Kidney Disease in Malaysia

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Abstract: Little is known about the accuracy of serum creatinine (SCr) in identifying early chronic kidney disease (CKD) in the primary care setting. Thus, this study aims to examine the concordance of SCr to estimated glomerular filtration rate (eGFR) in detecting early CKD. This is part of a randomly selected 10-year retrospective, observation cohort study of patients registered with the Department of Primary Care Medicine Clinic at the University of Malaya Medical Centre. A SCr $\geq 132\mu\text{mol/L}$ and eGFR $< 60\text{ ml/min}$ are used as the cut-off points for impaired renal function. Kappa statistic is used to test the inter-rater agreement of SCr with eGFR. A total of 1100 subjects were recruited. The mean age, SCr and eGFR were 66 ± 9 years, $86\pm 42\mu\text{mol/L}$ and $70\pm 30\text{ ml/min}$ respectively. The concordance between SCr and eGFR was poor as 363 (35.5%) patients had normal SCr but abnormal eGFR. Kappa value was 0.022 ($p < 0.001$). Screening for CKD using SCr fails to detect an additional third of patients with impaired renal functions. Hence using eGFR is a better way to identify early CKD.

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Keywords: Creatinine; Estimated Glomerular Filtration Rate; Chronic Kidney Disease; Concordance; Malaysia

1. Introduction

Chronic kidney disease (CKD) is an independent risk factor of cardiovascular disease (1, 2, and 3) where a majority dies from cardiovascular disease before succumbing to end stage renal failure (4). Detection of early asymptomatic stage of CKD is important because intervention can retard the progression of CKD to end stage renal disease (5, 6). Furthermore, drug adjustment is important for those with the later stages of or more advanced but yet asymptomatic CKD. This is important in order to prevent adverse drug events and minimize additional renal injury (7). This is particularly important in view of the rapidly increasing prevalence of CKD in Asia and throughout the world (8-11).

In Malaysia, there has been an exponential increase in the incidence of CKD and the number of ESRD patients needing renal dialysis. The reported dialysis prevalence rate increased from 4 per million population in 1980 to 365 per million in 2002 and 391 per million in 2003 (10). This increase in the rate of dialysis is partly due to rapid economic growth in Malaysia, leading to a more sedentary lifestyle and obesity in both adults and children. As a result of this there has been an escalating increase in the incidence of diabetes and hypertension, which are major causes of CKD. (10, 12, and 13).

Currently, most clinicians use serum creatinine (SCr) as a measure to determine the presence or absence of CKD as it is convenient and

does not require any mathematical manipulation. However, it is well known that SCr can still remain within the normal range in spite of the glomerular filtration rate (GFR) being less than $60\text{ mL/min per } 1.73\text{m}^2$ and it can also be within normal even when half or more of the normal adult kidney function has been lost (14,15).

A complete review of the literature did not show any studies that have been done to examine the difference in diagnosis of CKD using serum creatinine compared to using estimated GFR in a multi-ethnic population in Malaysia. Thus, the aim of the study was to examine the prevalence and predictors of patients with CKD, who are missed by using SCr instead of eGFR at a primary care multi-ethnic population setting.

2. Material and Methods

The current research is part of a 10-year retrospective cohort study of patients registered with the Department of Primary Care Medicine Clinic at the University of Malaya Medical Centre. The data presented here is that for the year 2007. The original cohort consists of a total of 1547 adult patients who were randomly selected based on the systematic randomisation sampling number generated by a computer programme. Baseline data was collected in 1998, and follow-up data collected in 2002 and 2007 at five-year intervals. All the patients with complete data on the serum creatinine and eGFR were selected for this study. Patients with incomplete

data for serum creatinine or eGFR were excluded.

Age, weight, serum creatinine and socio-demographic variables were obtained from patient records. SCr was measured as part of the routine medical care for patients with diabetes and hypertension in this hospital-based primary care clinic. A SCr $\geq 132\mu\text{mol/L}$ and eGFR based on the Cockcroft-Gault (C-G) formula of $< 60\text{ ml/min}$ are used as the cut-off points for impaired renal function (14,16, and 17). The Modification of Diet in Renal Disease (MDRD) and C-G formula are the two most common formulae used to classify the severity of CKD. There is consensus on the accuracy in assessing CKD by using the MDRD and C-G formula amongst multiethnic Asian population. However the MDRD formula has been shown to be less accurate than the C-G formula in assessing early CKD (18). Thus, the C-G formula was used in this study as we believe that outpatients are usually in the early stages of CKD rather than at the more advanced stages

In order to compare the difference in the prevalence of CKD using serum creatinine or the eGFR, patients were divided into four categories in the following manner; overt normal renal function (normal SCr and normal eGFR), covert normal renal function (abnormal SCr but normal eGFR), covert renal dysfunction (normal SCr but abnormal eGFR) and overt renal dysfunction (abnormal SCr and abnormal eGFR).

All analysis and calculations were performed using the SPSS version 19 (SPSS IBM New York,

United States). Continuous data are described as mean and SD or median and interquartile range (25-75th percentiles). Chi square test and t-test were used to analyse the categorical and continuous data. The kappa test statistics was used to test the inter-rater agreement of serum creatinine to eGFR where the kappa value is interpreted as in Box 1 (19).

Box 1. Interpretation of kappa value in our study

Kappa value	Interpretation *
< 0.20	Poor agreement
0.21 – 0.40	Slight agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

* The level of significance was set at $p < 0.05$

3. Results

Out of the 1547 original cohort entered into the study, 1100 remained for follow-up at the end of 10 years. Only those with complete records for this aspect of the study were included. 65% were women and the ethnic distribution was 43% Chinese, 31% Indian and 25% Malay. The mean age and weight were 66 ± 9 years and $64.9 \pm 0.4\text{kg}$ respectively. The median SCr and eGFR was 77.0 (range 30 to 586) $\mu\text{mol/L}$ and 65.9 (range 10 to 285) ml/min respectively

Table 1. Concordance of serum creatinine to estimated glomerular filtration rate in year 2007

		eGFR 2007*		Total, N
		Abnormal renal function (<60ml/min)	Normal renal function ($\geq 60\text{ml/min}$)	
Creatinine 2007	Abnormal renal function ($\geq 132\mu\text{mol/L}$), n (%)	96(99)	1(1)	97
	Normal (<132 $\mu\text{mol/L}$), n (%)	356(35.5)	647(64.5)	1003
Total, N, (%)		452(41.1)	648(58.9)	1100

* eGFR: Estimated Glomerular Filtration Rate

Table 2. Concordance of serum creatinine to estimated glomerular filtration rate stratified by age in year 2007

	Age (years) of patients				
	40-49 (n=49)	50-59 (n=214)	60-69 (n=423)	70-79 (n=318)	80 and above (n=96)
Overt normal renal function, n (%)	46 (97.9)	188 (91.7)	278 (71.6)	126 (45.2)	9 (10.7)
Covert renal dysfunction, n (%)	1 (2.1)	17 (8.3)	110 (28.4)	153 (54.8)	75 (89.3)
Kappa value, n	0.79	0.482	0.295	0.159	0.029

Table 3. Concordance of serum creatinine to estimated glomerular filtration rate stratified by body mass index in year 2007

	BMI (kg/m ²) of patients		
	Underweight (BMI<18.5) *	Normal (18.5≤BMI<23)	Overweight (BMI>23)
	n=168, mean age 66.9 years	n=153 mean age 68.1 years	n=574 mean age 64.9 years
Overt normal renal function, n (%)	93 (62.0)	68(48.2)	380 (72.8)
Covert renal dysfunction, n (%)	57(38.0)	73 (51.8)	142 (27.2)
Kappa value, n	0.238	0.127	0.327

* BMI: Body Mass Index

The prevalence of CKD based on SCr was 8.8% and 41.1% based on eGFR. Table 1 shows the concordance of SCr to eGFR. More than one third (35.5%) of those with normal SCr actually had abnormal eGFR. All except for one patient who had an abnormal SCr had an abnormal eGFR. The kappa coefficient was 0.022, which shows poor inter-rater agreement between the SCr and eGFR and this poor inter-rater agreement is significant ($p<0.001$). There was a big difference in renal function between the overt normal renal function group where the eGFR was normal (88 ± 27) ml/min compared to the covert renal dysfunction group which was "true" impaired function (eGFR was 48 ± 9) ml/min. This difference is statistically significant ($p<0.001$). This discordance is further confirmed by the sensitivity of only 21.2% of SCr in detecting renal impairment despite its high specificity (99.8%). In other words, when the SCr is abnormal, it is almost certain that the patient truly has renal impairment (20). The positive predictive value of the SCr in this study population is 98.9% while the negative predictive value is 64.5%.

The prevalence of covert renal dysfunction increases with age. Furthermore, the kappa value was worse in older people (Table 2).

Table 4. Predictors of covert renal dysfunction in multivariate analysis in year 2007

Variables	Adjusted OR*	95% CI**
Age, year	1.07	1.050,1.099
Weight, Kg	0.95	0.939,0.969
eGFR C-G, ml/min	1.06	0.934,0.955

* OR: Odds Ratio,

** CI: Confidence Interval

* Adjusted for age, weight, estimated glomerular filtration rate, diastolic blood pressure, systolic blood pressure, ethnicity, sex and serum creatinine.

The prevalence of covert renal dysfunction increased with body weight amongst patients who are

The prevalence of covert renal dysfunction dropped from 51.8% in the normal weight to 27.2% in the overweight group. Overall, it can be seen that the prevalence of covert renal dysfunction is lower in the overweight group than in the normal or underweight group (Table 3). A multivariate logistic regression analysis (Table 4) shows a significant association between age, weight, eGFR and covert renal dysfunction. For every increase of one year in age, the odds of developing covert renal disease was 1.07 (Odds ratio (OR) 1.07, 95% confidence interval (CI) 1.050-1.099). An increase of one kilogram in weight will reduce 0.95 odds (OR 0.95, 95% CI 0.939-0.969) and a reduction in eGFR of 1ml/min will increase 1.06 times odds (OR 1.06, 95% CI 0.934-0.955) of developing covert renal dysfunction. However, there was no significant association between covert renal dysfunction and ethnicity, nor with sex, systolic blood pressure, and SCr level

4. Discussion

The prevalence of CKD based on SCr in this study population was low where else the prevalence of CKD based on eGFR was nearly five times higher. Furthermore, this study shows that the concordance of SCr with eGFR is rather poor. Using SCr, instead of the eGFR will fail to detect as many as a third (35.5%) of patients with seemingly normal SCr but who, in fact, have impaired renal function. This is much higher than that reported in another study, which was also done on outpatients, where the it was 13.9% (21). This difference could be due to the lower cut-off points for abnormal eGFR that was used in the other study (50ml/min in other study vs. 60ml/min in our study). The other reasons could be our study population were older (66 ± 9 versus 57 ± 9 years) and thinner (64.9 ± 0.4 kg versus $74 \text{ kg} \pm 0.4$ kg). A further reason could be due to presence of other co-morbidities like hypertension and diabetes seen in our study population as these conditions further compromise renal function.

Despite that eGFR is not the gold standard to measure actual renal function, it serves as a reliable surrogate, particularly in a primary care setting, as it is less expensive and easier to do than the gold standard inulin test. Furthermore, several studies have supported the use of the eGFR as a reliable measure of renal function (16, 17, and 22). Primary care physicians can play an important role in identifying CKD early in order to slow down the progress of CKD by early nephrology referral or implementing interventions that can delay the progression of renal impairment (23-27). As can be seen from this study, the eGFR is better than the SCr in identifying CKD particularly in thin and older patients, age 60 years or older. This can be explained by the fact that muscle bulk is the main contributor of SCr levels and older individuals have less muscles giving a seemingly low SCr (16). Hence, the eGFR should be used to determine renal functions especially if patients are older and also thin.

The limitation of this study is that there was no comparison of eGFR against the gold standard of inulin testing. However, there are enough studies that have consistently supported the use of eGFR in its place as it closely mirrors the "true" renal function that uses the gold standard inulin test (22, 28). The alternative is to use the MDRD formula which is more accurate than C-G equation and does not require knowing the patient's weight (29, 30, AND 31). However, it was not used in this study as the applicability of the MDRD equation in adjusting medication doses has not been validated in many countries (30, 32). Furthermore, the MDRD cannot be calculated using a conventional calculator.

In summary, the finding of this study shows that there is a wide discordance between SCr and eGFR. Screening for CKD using SCr fails to detect an additional third of patients with impaired renal function. Therefore, eGFR rather than the traditional SCr should be used as a measure of renal function, particularly in the elderly and thin patients. There is no difference amongst the ethnic group in terms of usage of this formula. The higher rate of detection of early CKD using eGFR indicate that routine evaluation of kidney function should include reporting the eGFR rather than the SCr alone.

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Effects of lead pollution in SY River on children's intelligence

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Abstract To investigate the influence of lead pollution in SY River on children's intelligence and provide a scientific data for governance of the SY River and protection of the residents, especially children. The polluted area and control area were selected randomly from less than 5 km and more than 20 km away from the SY river basin, respectively. Concentrations of lead in the river water, drinking water, soil, grain and vegetables were measured by atomic absorption spectrophotometer (flame technique). There were 154 children, aged from 8 to 13, were recruited from two areas. Combined Raven's Test (CRT) was used to measure the intelligence of the children. The concentration of lead in SY River was exceeding the standards of surface water quality. Compared to the control area, the concentrations of lead were significantly higher in the polluted area among drinking water, soil and vegetables ($P<0.05$). Blood lead levels were significantly higher in children who were living in polluted area than those living in control area ($P<0.05$). Similarly, children who were living in polluted area had significantly lower IQ than those living in control area ($P<0.05$). After controlling for confounders, an inverse association was observed between blood lead concentration and IQ scores ($\beta=-0.293$, $P<0.05$). These data suggested that the lead pollution in SY River were still serious and had entered the body by soil, drinking water and vegetables. Finally, our study suggested that environmental lead exposure had affected the children's intelligence to a certain extent.

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Key words: Water pollution, Lead, Children, Intelligence

1. Introduction

Lead is a common industrial toxin and environmental pollutant and can enter the human body by mouth, skin and respiratory tract, et al. What's more it can affect the nervous system significantly, especially on the central nervous system. With the rapid development of industrial and urbanization process, more and more developing countries have paid attention to the adverse effects of lead pollution on people's health, especially on children's nervous system (Matte TD, 2003). Children aged from 8 to 13 were in the second peak of growth and development, and more vulnerable to lead poisoning because of incomplete brain development and poor detoxification function. Most research showed that 40% to 50% of lead that children intake were from food, while adults only 5% to 10% (Markowitz M, 2000). A Clinical study have shown that there was a significantly negatively relationship between blood lead and IQ. When the concentration of blood lead was 100 μ g/L to 200 μ g/L, the average of IQ would decrease 1 to 3 points, while the average of IQ would drop 5 to 10 points when the blood lead was greater than 300 μ g/L (Lidsky T I and Schneider J S. 2003). However, more and more studies began to focus on the relationship

between low levels of lead exposure and children's IQ. Bellinger DC (2008) found that no level of lead exposure appears to be "safe" and even the blood lead levels which were lower than 10 μ g/dL in children are associated with neurodevelopment deficits. A series of literatures also showed that even if children's blood lead levels below 10 μ g/dL, they can appear significant neurological dysfunction (Pamela J. Surkan, et al. 2007, and Marek Jakubowski, 2011). Tellez-Rojo(2006) and Jusko(2008) reported that children's IQ which were inversely associated with blood lead levels, decrease or deteriorate greater with increasing blood lead levels of <100 μ g/dL than that of \geq 100 μ g/dL.

Huai River is the third largest river in China which runs through Henan, Anhui, Shandong and Jiangsu provinces. It has a total length of 1,000 km which covers an area of 270,000 km², and about 150 million people live here. Since the 1980s, the water pollution in Huai river was getting worse and worse, and the accidents which caused by water pollution occurred frequently(Li X, 2009). After 90 years, the problems of water pollution in Huai River were concerned by the different levels of environmental protection departments and the total pollutants in Huai River were decreased. However, there was a serious

rebound of pollution in recent years(Wang G.Y, 2008). SY River is located in the hinterland of Henan province which is the largest tributary of the Huai River. Various types of wastewaters from 31 cities were poured into the river without any sewage disposal. Some researches showed that SY River had lost its self-purification capacity because of receiving so many municipal and agro-industrial wastewaters(Wang W.J, 2009, and Gao H.L, et al. 2010).

The effect of Huai River pollution on health of local people is a major issue of social concern. There were many reports about the effects of Huai River pollution on the health of local people(Zhang H.C. 1989, and Zuo Z.J., et al. 2009). However, research about the impact of lead exposure in SY River on children's intelligence has not been reported. Therefore, children's intelligence and the concentration of blood lead were measured to explore their relationship on basis of the environmental level of lead in SY river water, drinking water, soil, grain and vegetables.

2. Material and methods

2.1 Location and objects selection

This study was conducted in S county which is the last county that SY River runs through in Henan Province. The polluted area and control area were selected randomly from less than 5 km and more than 20 km away from the SY river basin, respectively. There were no differences between the two villages about the social and natural factors like economic situation, educational standard and geological environments et al. None of these two sites was exposed to other potential neurotoxins that are recognized as contaminants influencing IQ value. School children aged 8 to 13 years old who were grade 3 to 5 were selected by cluster sampling. 73 of them were recruited from the polluted area and 90 were from the control area. For the current study, we finally excluded 9 children who didn't complete the questionnaire because of their parents were not at home, resulting in 154 subjects eligible for our study. 69 of them were from the polluted area (30 males and 39 females) and 85 were from the control area (43 males and 42 females). In addition, an informed written consent of participation in the study was signed by the parents of the children. The local Ethics Committee of the human subjects' research, Zhengzhou University, approved this study.

2.2 Questionnaire and quality control

A questionnaire was designed for children's intelligence which was very susceptible to many factors. Father's occupation, mother's education, children's daily intake of protein (especially milk and eggs), family income and other potential confounding factors were included in the questionnaire.

Investigators were trained in advance and parents of children were visited by face to face interview survey.

2.3 Blood samples

The subjects were requested to fast for at least 10 hours before they came for the study. The fasting blood samples, totaling 5 ml were drawn into vacuum tubes. Immediately upon collection, samples were stored on ice. At the end of each daily collection, samples were centrifuged to separate serum and stored at -80°C until analysis. Blood lead was analyzed with Polarograph (Metrohm Ltd., Switzerland). Each sample was measured three times and added standard two times. The mean recovery rate was 98.6%.

2.4 Environment samples

River water samples were collected from three sampling sections which were set up at S county. The upstream section (US) was set up at the place where the SY River just enters the city, the midstream section (MS) was set up at the Huaidian sluice gate and the downstream section (DS) was set up at the place where the SY River runs out of the town. Samples were collected from each section by quartering in the dry season (December to February) and wet season (July to September). Drinking water, soil, grain, and vegetables samples were collected from five positions (east, south, west, north and central) of two villages, respectively. Three samples were collected from each position. The collection and preservation of samples were accordance with the standards of China (GB/T 5750.2-2006). Leads in all samples were assayed by atomic absorption spectrophotometer (flame technique). Each sample was measured three times.

2.5 Assessment of intelligence

Children were administered to take the Combined Raven's Test-The Rural in China (CRT-RC3) to evaluate their intellectual ability. CRT is a recognized intelligence test which is more effective because there is no limit to cultural, ethnic, language and other factors, and the results are simple and intuitive as well. In 1985, the CRT was revised in China and achieved a high degree of reliability and validity(Yin J, 2007). The seven categories of this test scores are as follows: ≤ 69 retarded (low); 70-79 borderline (below average); 80-89 dull normal (low average); 90-109 normal (average); 110-119 high normal (high average); 120-129 superior (good); ≥ 130 very superior (excellent).

2.6 Statistical analysis

The database were established using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark) and the data were doubled enter into the database by different people. All the data were

analyzed using SPSS 12.0 software (SPSS Inc., Chicago, United States). Kolmogorov-Smirnov test and Levene test were used to inspect the normality and homogeneity of variance of all data. The blood lead was a log-normal distribution and the concentrations of lead in drinking water, soil, grain, vegetables and blood and the IQ of children in two areas were compared using Independent-Sample *t*-test. Mean concentrations of lead in river water, mean blood lead levels and mean IQ in different groups were compared using ANOVA. The chi-squared test was used for comparison between qualitative variables of the groups studied. Spearman's rank correlation coefficient *r* was used to determine the relationship between blood lead and IQ scores. In addition, we performed a multiple linear regression model to analyze the association of blood lead with IQ

scores which controlled for potential confounding factors. Significance level was $\alpha=0.05$.

3. Results

3.1 The concentrations of lead in SY River

The river water samples were gathered and detected both in dry season and wet season. The mean concentrations of lead were presented in Table 1. When comparing the mean concentrations of lead in river water, no statistically significant differences were observed in the three sections ($P>0.05$). Compared with the standards of surface water quality (GB 3838-2005), the contents of lead in river water were exceeding class IV both in wet and dry seasons.

Table 1. The results of lead in river water in wet and dry seasons ($\bar{x} \pm s$, $\mu\text{g/L}$)

	n	Wet season	Dry season
US	24	52.1 \pm 8.9	75.3 \pm 11.4
MS	24	70.4 \pm 12.8	54.3 \pm 5.4
DS	24	64.8 \pm 7.8	74.0 \pm 13.3
<i>F</i> value		2.587	3.662
<i>P</i> value		0.155	0.091

3.2 The concentrations of lead in environmental samples

The concentrations of lead in soil, grain, vegetables and drinking water were shown in Table 2. Compared with control area, the mean concentrations of lead in soil, vegetables, grain and drinking water of polluted area were significantly higher ($P<0.05$). The concentrations of lead in the samples of control area were below the standards of relevant quality in China. But in the polluted area, the concentrations of lead in soil, grain, drinking water and vegetables were exceeding the standards. The standard of soil, grain and drinking water quality were GB 15618-2008, GB 2175-2005 and GB 5749-2006, respectively.

Table 2. Concentrations of lead in soil, grain, vegetables, and drinking water in two areas ($\bar{x} \pm s$)

	Soil(mg/kg) (n=60)	Grain($\mu\text{g/kg}$) (n=30)	Vegetables($\mu\text{g/kg}$) (n=30)	Drinking water($\mu\text{g/L}$) (n=30)
Control area	68.01 \pm 52.28	180.75 \pm 114.18	582.11 \pm 197.58	7.200 \pm 1.202
Polluted area	90.00 \pm 55.89	325.31 \pm 155.19	815.64 \pm 380.81	17.200 \pm 8.311
<i>t</i> value	-2.300	-3.001	-2.177	2.663
<i>P</i> value	0.023	0.005	0.037	0.029

3.3 The basic situation and blood lead levels of children in two areas

Children's age and sex in two areas were demonstrated in Table 3, and the mean concentrations of blood lead of children in two areas were shown in Table 4. Mean age of the children in two areas had no significant difference ($P>0.05$) and similar results were found in children's sex ($P>0.05$). Children who were living in polluted area had significantly lower IQ than those living in control area ($P<0.05$). Similarly, blood lead levels were significantly higher in children who were living in polluted area than those living in control area ($P<0.05$).

Table 3. Children's age and sex in two areas

	Age (yr, $\bar{x} \pm s$)	Male	Female
Control area (n=85)	10.51 \pm 1.24	48	37
Polluted area (n=69)	10.89 \pm 1.18	30	39
$t(\chi^2)$ value	1.967		2.572
<i>P</i> value	0.051		0.109

Table 4. Mean IQ and blood lead levels of children in two areas

	IQ (scores)	Log(Blood lead)($\mu\text{g/L}$)
Control area (n=85)	111.00 \pm 12.65	1.67 \pm 0.188
Polluted area (n=69)	106.75 \pm 11.81	1.77 \pm 0.17
<i>t</i> value	-2.126	3.227
<i>P</i> value	0.035	0.002

3.4 Age and IQ scores of children with different blood lead levels

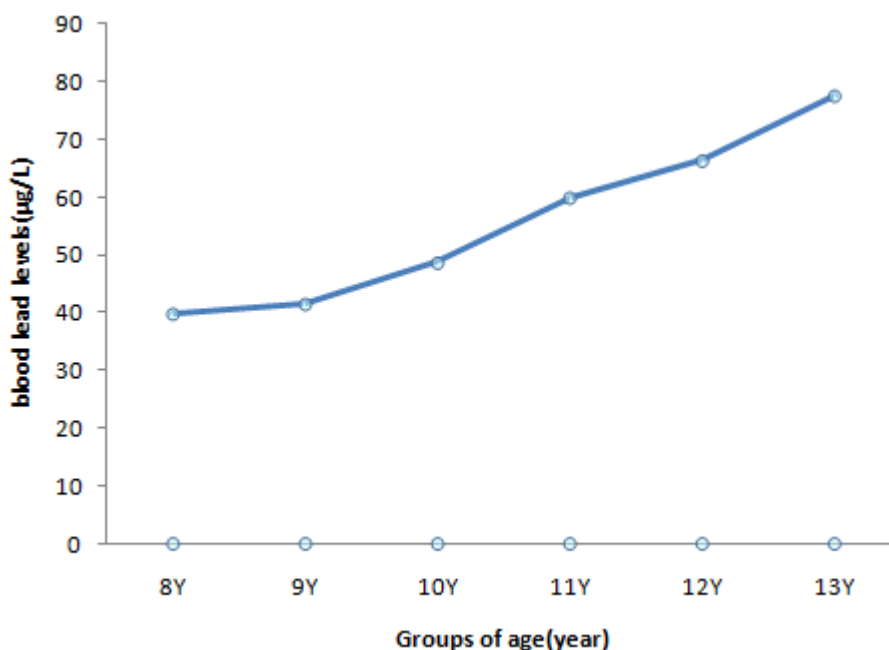
According to the blood lead levels of children, four groups were divided as follows: <30, 30-50 $\mu\text{g/L}$, 50-75 $\mu\text{g/L}$ and >75 $\mu\text{g/L}$. The ages and IQ scores of children with different blood lead levels were shown in Table 5. Statistically significant differences of age were observed between the four groups. Moreover, children with high blood lead level had significantly older than those with low blood lead level ($P<0.05$). Compared the mean IQ scores of the four groups, the differences between each of them were statistically significant ($P<0.001$). Meanwhile, IQ scores were significantly reduced in children with blood lead levels increased ($P<0.05$).

Table 5. Age and IQ of children with different blood lead levels ($\bar{x} \pm s$)

	N	Age (yr)	IQ scores
Blood lead levels			
<30($\mu\text{g/L}$)	18	10.12 \pm 1.39	120.38 \pm 11.25
30-50($\mu\text{g/L}$)	48	10.22 \pm 1.14	112.75 \pm 10.04
50-75($\mu\text{g/L}$)	56	11.07 \pm 0.95	107.67 \pm 10.84
>75($\mu\text{g/L}$)	32	11.09 \pm 1.35	99.51 \pm 11.841
<i>F</i>		8.126	16.654
<i>P</i>		<0.001	<0.001

3.6 Blood lead levels of children with different ages

According to the ages of children, six groups were divided. The tendency chart of blood lead levels changing with age was shown in figure 1. Blood lead levels were increasing with age. Compared the mean blood lead levels of the six groups, the differences between each of them were statistically significant ($F=6.358$, $P<0.001$).

**Figure 1. Blood lead levels of children with different ages**

3.7 The association between blood lead levels and IQ scores

To test the association between lead in blood and IQ scores, Pearson's correlation analysis and multiple

regression models were calculated. Results are shown in Table 6. The coefficient (r value) was -0.55, but adjusted for feeding patterns, father's occupation, mother's educational, family income, children's daily intake of protein (especially milk and eggs) and other potential confounding factors, the coefficient (β value) was -0.293 ($P < 0.001$).

Table 6 Multivariable model results for IQ by blood lead, adjusted for confounding variables (n=154)

	Coefficients	P
	Pearson	
Log(Blood lead)	-0.55	<0.001
	Adjusted model	
Log(Blood lead)	-0.293	<0.001
Feeding patterns	0.013	0.852
Mother's educational	0.019	0.786
Fother's educational	0.014	0.846
Family income	0.127	0.062
Father's occupation	0.072	0.295
Mother's occupation	-0.097	0.192
Breakfast	0.071	0.297
Picky eaters	-0.094	0.169
Milk intake	0.055	0.421
Eggs intake	0.080	0.240
Sleeping time	-0.044	0.523

4. Discussions

4.1 The lead pollution in SY River and its diffusion paths

The results of this study showed that the concentrations of lead in SY River were exceeding the standards of surface water quality. The groundwater which was the source of drinking water for the nearby residents had been contaminated by lead in SY river via the spread horizontally, infiltrate vertically, as well as dissolved by rainwater. And the soil were contaminated because of irrigation. The grain and vegetables could gain the lead from the contaminated soil. Eventually, the contents of the lead in drinking water, soil, grain and vegetables in the polluted area were significantly higher than in the control area.

Lead contamination in environmental will give rise to increase of lead content in groundwater and soil, and all the crops in the severely polluted areas will contain lead. Study had shown that various heavy metal pollutants were detected in the groundwater near the SY river at depth of 20-50 m even over 200 m, and their concentrations showed a downward trend with the increasing distance from the river (Xu X.L, 2007). Other research also indicated that the pollutants in groundwater shifted primarily through the role of convection-diffusion (Huang X.X, 2008). In addition to that lead could be filtrated, adsorbed, degraded and so on by the soil. Finally, the pollutants were stayed in the soil. And the farther away from sources of pollution the lower concentration of pollutants in soil. Contaminants in soil entered into crops through bacterial decay and the absorption of plants etc.

4.2 Children's blood lead levels and exposure pathways

This study showed that blood lead levels were significantly higher in children who were living in polluted area than those living in control area. Meanwhile, the mean blood lead levels of older children were significantly higher than younger children. These results indicated that blood lead levels were not only related to the dose of environmental exposure, but also had a certain relationship with the exposure time. The greater dose of environmental exposure and the longer exposure, the higher blood lead levels of children had. Therefore, lead in environment had entered into children's body via drinking water, vegetables, food and soil et al. and the blood lead levels were related to the time of environmental exposure, the longer the children exposure, the higher blood lead levels they had.

Lead in the environment can enter the human body through the respiratory tract, digestive tract, skin and other ways. Song Huaqin (1993) found that 90% to 98.5% of lead that daily intake of children in Beijing were from gastrointestinal tract, while respiratory tract only 1.5% to 10.0%. Children are more susceptible than adults by environmental lead pollution. When the lead reached the digestive tract, children could absorb 30% to 75%, nevertheless adults only 11% (Farley D, 1998). Another way that children intussuscepted lead is hand to mouth. According to related reports, the blood lead levels will increase 2.3 μ g/L when the natural logarithm of lead in dust of children's living environment augments one unit (Schillins R.J, 1988). Lead can pass through the placental barrier and enter into children's body. Some literatures showed that the levels of lead exposure to mothers during pregnancy could affect children's intelligence and physical

development seriously (Takashi Yorifuji, et al, 2011, Susan Claire Edwards, et al, 2010, and Wieslaw Jedrychowski, et al, 2008). They all declared that children can be affected by environmental pollutants before birth. Reinhard Kaiser et al. (2001) had shown that children's blood lead levels increased with age, which was consistent with the results of this study, may be related to exposure time and accumulation in body.

4.3 Influence of lead exposure on children's intelligence

The results showed that children who were living in polluted area had significantly lower IQ than those living in control area. Meanwhile, IQ scores were significantly reduced in children with blood lead levels increased. We found that exposure to lead in blood was associated with reduced IQ scores before and after adjusting for confounders. These results indicated that the intelligence of children who were living in contaminated area were affected by lead to some extent. And there was a significant negative correlation between the level of lead exposure and children's intelligence.

There were many reports about the relationship between lead exposure and children's intelligence in worldwide. Hu Qiansheng had researched the relevance of children's intelligence and contents of lead in deciduous teeth in the context of different environments of lead contamination. The results showed that children's teeth lead levels were significantly different in different contamination background, and there was a significant negative correlation between teeth lead levels and children's IQ ($\beta = -0.382$, $P = 0.006$) (Hu and Dong, 1997). Another study also displayed that the relationship between children's blood lead levels and intelligence was a significant negative correlation ($r = -0.789$, $P < 0.001$) (Guan Y.Q, et al. 2005). Yuan Liu (2010) had reported that children's mental retardation were significantly associated with lead content in the environment where their mother living. Another research also showed that children with mental retardation were related to the soil lead levels (Suzanne McDermott, et al. 2011). A series of studies in abroad had shown that even the blood lead level $< 100 \mu\text{g/L}$ which is a criterion in public health advisories of the U.S. Environmental Protection Agency and the Center for Disease Control could give rise to neurological disorders (Lisa M. Chiodo, et al. 2004, and Meeyoung O. Min, et al. 2009). Therefore, there was no safe limit value for lead exposure on children's intelligence and our findings were consistent with it.

Considering Children's intelligence is affected by many factors, our study conducted a questionnaire to comprehend the potential confounding factors which could affect children's IQ. Some researches displayed

that mother's education, father's occupation, family income, children's daily intake (especially eggs and milk) and sleep time were the potential confounding factors of intelligence. After correction of these factors, the correlation coefficients between blood lead levels and IQ would reduce (Jose A. Menezes-Filho, et al. 2011, and Shen X.M., et al. 1998). This study found that correlation coefficients were -0.55 and -0.293 before and after adjusting for confounders, respectively. It also suggested that it's important to consider of the potential confounding factors when we analysed the impact of lead exposure on children's intelligence. Consequently, it's a gordian technique to choose the potential confounding factors and it can determine the authenticity and reliability of the final results.

5. Conclusions

In summary, the results of this study showed that the lead pollution in SY River was still very serious, and had entered into children's body via drinking water, vegetables and soil et al. Meanwhile, the lead can accumulate in children's body and elevated blood lead levels. Ultimately, children's intelligence was affected seriously. This study also verified the significant negative correlation between the level of lead exposure and children's intelligence. Considering the limitation of this study, it is essential to replicate these findings in different village near the SY River with larger sample sizes as well as more detail information of children's intelligence.

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