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Estimate water use efficiency (WUE) and determining the period of irrigation in corn plant

Tayeb Saki Nejad

Assistant Professor Department of Agronomy Physiology, Islamic Azad University, Ahvaz branch
saki1971@iauahvaz.ac.ir; TayebSaki1350@yahoo.com

Abstract: in order to examine the effect of drought stress in different periods of plants grow on process factor along with four levels as Main plot (I_0 : Full irrigation point of FC, control, without water stress, I_1 : 75% of the amount of irrigation treatments I_0 , mild stress, I_2 : 50% of the amount of irrigation treatments I_0 , severe stress, I_3 : 25% of the amount of irrigation treatment I_0 , very severe stress and point of PWP and Sub-plots: Different growth phases: S_0 : growing phase, the establishment of the plant stem to the emergence, S_1 : natal phase: to stem the rise of coffee being resilient and end silk pollination, S_2 : grain filling phase: the end of pollen grain maturity and the emergence of black layer) in research farm of Islamic Azad University. although the frequency difference between irrigation treatments and amounts of irrigation I_0 & I_1 , 6 times water (6 = 16-22) and also in terms of grain yield in two different irrigation treatments Performance 1.5 ton per hectare there and Duncan test time difference performance meaningful shown so should be treated I_0 as the best irrigation, the number of irrigated 22 times during the growing season be introduced but if precise will be investigated is observed I_1 that 75 percent of the treated water FC state farm yield acceptable 12.8 ton in ha plots managed in a 1502 liter (26460-27962) that consume less water, saving the farm level is very valuable and Due to water shortage and drought phenomenon can be considered and it should be used now in Khuzestan IRAN irrigation takes place between 20-21 times in the same treatment that I_0 is using treatment I_1 with some loss of performance seed-saving high water is done, on the other hand WUE in these two treatments (I_0 & I_1) of Duncan test at 1% level statistics has been that this can also cause other using treatment I_1 is.

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Key words: water use efficiency (WUE), period of irrigation, corn

1. Introduction

Movement of water from the soil to the roots of plants due to osmotic movement defining a mass flow in slow and fast mode is sweating. Thus the activity is called osmotic share a little water absorption compared to transpiration. Buoyancy forces result in tension or negative pressure sap wood they are. Forces related to water absorption can be as follows(1,16):

$$A = \frac{(\psi_m = \psi_o)_{Soil} - (\psi_p + \psi_o)_{root}}{r_{soil} + r_{root}}$$

That this formula:

ψ_m Levels of metric potential,

ψ_o Osmotic potential,

ψ_p Pressure potential (MPa all the units are expressed)

The resistance r in soil water and roots is based on scm^{-1} . Water absorption by roots can be by force, osmotic (active), or be passive.

Corn plant with annual growth is very high, stem height was also relatively high and the other hand, growth and production of sufficient need and warm temperate areas planted, the purpose of important issues and significant in corn the required water supply and irrigation as well as different stages of the plant. In areas where the growth period, the rainfall to supply the full amount of water that this plant requires falling is no pupil corn field to be timely irrigation. Field Irrigation depending on atmospheric conditions such as environmental and soil moisture in the soil and with regard to temperature every 7 to 12 days once the corn must be irrigated. When the irrigation process and the amount of water carefully to avoid surgery and to provide enough water to corn root NO, thirsty plant residue and if this situation continues to plant wilting point water reached and if the time coincided with the flowering Candy and this continues for 2 days to yield 22 percent deficit if it finds and to continue for 6 days decreased the amount of product will be 50 percent. The most important and most sensitive stages of maize irrigation include (Arkel, Van H. 1978) (1):

- 1 - Bud stage production.
- 2 - Stem elongation stage.
- 3 - Male flower emergence stage.
- 4 - Female flower emergence stage.
- 5 - Grain filling stage.
- 6 - Milky stage.

Thus, in temperate zones and in the spring months, corn every 8 to 12 days in the summer and every 7 to 10 days and temperate climates and in spring every 7 to 10 days in the summer and every 5 to 7 days once a pupul can be irrigated (9,17).

2. Materials and methods

in order to examine the effect of drought stress in different periods of plants grow on process factor along with four levels as Main plot (I₀ : Full irrigation point of FC, control, without water stress, I₁ : 75% of the amount of irrigation treatments I₀, mild stress, I₂ : 50% of the amount of irrigation treatments I₀, severe stress, I₃ : 25% of the amount of irrigation treatment I₀, very severe stress and point of PWP and Sub-plots: Different growth phases: S₀: growing phase, the establishment of the plant stem to the emergence, S₁: natal phase: to stem the rise of coffee being resilient and end silk pollination, S₂: grain filling phase: the end of pollen grain maturity and the emergence of black layer) in research farm of Islamic Azad University.

Culture as wet ground do and do so before planting and irrigation land after getting proper soil moisture, and the disk began plowing, preparing the ground conducted in late June with propagation land, plow, disk, Fertilizer sprayed (before planting of ammonium phosphate fertilizer when planting of urea (nitrogen fertilizer and 200 net phosphor (P2O5) 90 kg per ha) was applied) and a disc and re-trowel handling test was performed and plots based on test design and implementation were plan for New Water the field of poly ethylene tubes were used to install water Parshal Flume was measured. Seed planted by the hand and two seeds in each hole in the first half of August (August 3, conducted in cultures and the first irrigation was on August 5 that recent history, on 5 August as planting was considered) were considered to high heat 50 ° C in August two farm surface irrigation interval of 3 to 4 days to prevent Hardpan and dry ground was to emergence and establishment of plants in farm fields as well be done.

After field planting process was performed as follows:

- 1 - Method for elimination of weeds from mechanical weeding was used three times in the weeding was done.
- 2 - Irrigation Based on the experimental treatment was conducted in the field.
- 3 - Half of nitrogen fertilizer at stem elongation stage of corn crop land as fertilizer roads was.

4 - Pests and disease was found in the field, did not receive a result of fighting convention. On all results, analysis of variance was performed

That the results tables (ANOVA) are presented and then using the Duncan test, comparison has had results with charts, Excel 2007 was conducted and analyzed data Two statistical program SPSS was performed and Mini tab.

3. Result

Examining Table1 So it seems that although the frequency difference between irrigation treatments and amounts of irrigation I₀ & I₁ , 6 times water (6 = 16-22) and also in terms of grain yield in two different irrigation treatments Performance 1.5 ton per hectare there and Duncan test time difference performance meaningful shown so should be treated I₀ as the best irrigation, the number of irrigated 22 times during the growing season be introduced but if precise will be investigated is observed I₁ that 75 percent of the treated water FC state farm yield acceptable 12.8 ton in ha plots managed in a 1502 liter (26460-27962) that consume less water, saving the farm level is very valuable and Due to water shortage and drought phenomenon can be considered and it should be used now in Khuzestan irrigation takes place between 21-20 times in the same treatment that I₀ is using Treatment I₁ with some loss of performance seed-saving high water is done, on the other hand WUE in these two treatments (I₀ and I₁) of Duncan test at a level statistics has been that this can also cause other using Treatment I₁ is (Table No. 1 & 2).

Table 1: No. irrigation (A), water consumed in one plot (B) and water in a total plot (A * B)

water in a total plot (A * B)	water consumed in one plot (B) (Liters)	No. irrigation (A)	Treatment
27962	1271	22	I ₀
26460	1653	16	I ₁
20880	2320	9	I ₂
15200	3040	5	I ₃

Table 2: Analysis of variance WUE

S.O.V	Irrigation (I)	growth phase (S)	interaction (I * S)	CV%
-	7.09**	0.87 ^{ns}	4.52**	14

Duncan test different values of treated irrigation water use efficiency in relation to three nodes, but can the average separation between treatment plant growth and there was no difference in the levels were statistically similar (Table 3).

4. Discussion

Usually leaves stage development, pollination and seed formation, which often occurs in the hot summer months, corn requires a lot of water. Frequency of irrigation depends on soil conditions, climate and ... Is between 2 to 15 times is variable. Maize cultivation in areas where annual precipitations 250 to 2500 mm are also a homebred is possible (18).

Smith (2001) as the maximum water use efficiency can be obtained when the amount of irrigation water is equal to evaporate transpiration in other words, the exact amount needed to plant the plant must be calculated (34).

Brodsky (2000) announced that the distances great impact on the efficiency of irrigation water use and should not affect these intervals so that the convention is more efficient water use reduced by 20 percent because the trend reduction in maize yield drops shows (5).

Unlike corn grain (wheat and barley) requires high heat and heat is the sun. Regions the hot summer sun and light enough to have the best performance from the point of view are produced. If the minimum temperature of 60-10 over the earth turning corns sprouts and the best growth in the normal temperature is 20-30 degrees. After corn emergence temperature of about zero tolerance and it does not hurt you see severe. Some varieties Serotinous within 90 days to produce, but primarily during the period of growth for production of 110 - 130 days. During the growing need for moisture and rainfall rate mm 600-700 needs. One kilogram of dry matter of corn requires about 315 liters of water. In different latitude, except in areas that were too cold or shorter growth period comes to action. Because corn is done in the summer to provide sufficient moisture for land preparation operations beginning wet ground field is necessary. Of course to be determined during the interval boundaries to be established. Then with heavy irrigation to wet all parts of the same field (14, 38).

The first farm irrigation immediately after planting is done that determines the planting dates is 72 hours after the first irrigation 60 to 70 percent seed grown and are outside the territory. Second irrigation for green field and achieve full moisture to seeds that are cultivated in more depth should be within 3 to 5 days after the first irrigation to be done. The third to final irrigation fertilization, irrigation 7-6 days to be considered in the late growing season and irrigation interval can be fulfilled 13-11 days.

Table 3: Comparison of average water use efficiency (WUE) using Duncan test at 1%

Treatments	WUE (g / lit)
I0	A8.51
I1	A8.1
I2	B6.7
I3	C5.2
S1	A7.45
S2	A7.88
S3	A7.91
I0 S1	A7.91
I0 S2	A7.01
I0 S3	A7.11
I1 S1	A7.11
I1 S2	A7.16
I1 S3	A 7.31
I2 S1	B6.82
I2 S2	B6.41
I2 S3	B6.88
I3 S1	C5.02
I3 S2	C5.32
I3 S3	C5.22

For advancing novel bore water should be set. Irrigation time period should be four times the water is advancing. Example: If the input water to track along the track during the 2.5 hour cover. Duration of irrigation for 10 hours is sufficient. $Time\ 10 = 4 \times 2.5$

In examining reports received from around the world by Simit (2001) two important factors limiting maize production in Asia include: stress and water deficiency Nitrogen uptake by plants during drought stress and methods to combat the increasing resistance of maize germplasm and as was following points can be investigated in this report: countries, Angola, Lesotho, Malawi, Mozambique, Tanzania, and Zimbabwe due to drought Zambia an average of 4 million tons of corn grain yield reduction has been met. Drought and the reduction factor of 10 5 percent annual yield of maize in South Africa declared. Gambols Vambola (2000) ,corn root weight between 3150 to 5070 kg per hectare in a silt loam soils are mature, the high volatility or measurement of the yen due to different amounts in different parts of soil moisture, soil compaction or mechanical pressure and different amounts of fertilizer as can be. Weight change root directly affects their performance thoracic (1999) most serious drought in 92 years in 1991 due to drought symmetry with maize flowering period decreased 4.5 million tons of grain yield respectively. (Amides et al (1999)) emergence of stress from the male flower and pistil silk increased considerably increased, causing sterility are a number of grains per panicle and seed number correlated with the flowering period showed a positive regression (Bvlans and Admydas (1996)).

Water stress reduced the number of pollen grains are well formed pollen tube less power their survival will be decreased. And drought in the calving period decreased the number of spikelet's that this reduction is due to a drop in yield has been. Spikelet differentiation rate is calculated from the following formula (Hall et al (1982)).

Period of physiological maturity (maximum dry weight) water potential of corn and wheat grains between 6/1- and 2 - Mega Pascal fluctuate and decrease water potential of this remarkable range of force matrix is increased, which has stopped growing seed search.

Eagle and single Rooney (1997) and Vrtvsky (1989)) very minor changes in water potential or osmotic potential of seeds of drought stress on dry matter accumulation stop are. (Barlow et al (1980), West Gate (1994)) effect of drought stress on enzyme activity during grain filling was studied and reported that the nitrogen and carbohydrate metabolic enzymes caused by drought that diminish

Krmvdv (1989) the lack of enzyme activity related to stopping matched DNA and mRNA copy was

compared. (Boilers (1989)) little effect of drought stress on grain filling rate affect corn, although high temperatures associated with drought stress, grain filling rate increases,

Jones, baby Stone (1987) and West Gate (1994)) Molly Lea and colleagues (2001) both ends, both as part of the effective temperature and water stress, followed by early grain maturity, and the quick, stop photosynthesis parameters such as aging of leaves, endosperm cells are involved in reduction potential. And water stress, the most important components of the plant growth in maize leaf area index, and decreases in LAI decreasing trend growth stages differentiated pistil, pollination and grain filling more than other stages of plant growth as they and reduced leaf stage, internodes elongation them know not significant.

Cimarron and colleagues (2002) research in India on the effects of drought stress on corn growth period were asked to evaluate and stated that if water stress, 7-2 days after silk emergence and start 22-16 days after emergence Silk to an end, yield components, seed number, is gaining significant decrease Tine ear and will be expanded. Also, in an article in America Magazine published in completely Agriculture announced that during the irrigation experiment in different periods; 30-20, 50-40, 70-60, 90-80, the percentage of usable soil moisture content, yield and its components in corn studied and which were reported at the time of irrigation use 30-20 percent moisture content of soil, increased ear length, number of grains, grain weight, grain yield per plant, grain yield per unit level and harvest index will be.

Gracious and colleagues (2004) had reported the effect of drought stress at flowering stage decreased or stable harvest index of corn is the harvest index increased from 0.44 in the optimal irrigation conditions, to 0.54 in irrigation regimes showed undesirable, harmful effects of drought on biomass production during the vegetative growth stages and crop increased harvest index, this work helps fill harmful, although never fully compensated if does not.

Saki nejad and colleagues (2002) state that relationship with grain yield per plant, ear number 0.94 ($r^2 =$, seed weight ($0.74 = r^2$) and number of grains ($0.89 = r^2$) has a positive correlation is, the lowest correlation with grain weight has been reported, but the correlation between grain yield and the period of emergence of flower male to female signs (ASI) 3 negative and what the gap further, the grain yield than is the effect of drought stress during Also, vegetative and flowering, the number of ear and plant water stress during drought ASI, and increased negative effect on grain yield leaves.

Corresponding Author

Dr. Tayeb Saki Nejad

Department of agriculture

Islamic Azad University, Ahvaz Branch, Iran

00989166129260

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The Expression and Regulation of GSK-3 , CDK-5 and PP2A in Differentiated Neural Stem Cells of RatsZHAO Qing-xia^{1,3}, XU Yan^{2,3}, YAN Wen-hai^{2,3}, HAN Xue-fei³, XING Ying^{2,3}

1. The Nursing College; 2. The Essential Medical School; 3. The Stem Cells Research Center
Zhengzhou University, Zhengzhou, Henan 450052, China
zzuzhaoqingxia@126.com; xingy@zzu.edu.cn

【Abstract】 Objective: To explore the expression of GSK-3 , CDK-5 and PP2A and the regulation of them by A₂₅₋₃₅ and ginsenoside Rb1 after NSCs are transformed into neurons. **Methods** Neural stem cells of the third passage were induced towards neurons; the expressions of GSK-3 (pTyr279, 216), PP2A and the regulation of them by A₂₅₋₃₅ and ginsenoside Rb1 were tested by the immunofluorescence cytochemical staining after NSCs had been induced for one week; The expressions of GSK-3 , CDK-5, PP2A and the regulation of them by A₂₅₋₃₅ and ginsenoside Rb1 were detected with RT-PCR. **Results:** Immunofluorescence cytochemistry showed that neural cells from NSCs which had been differentiated after one week can express GSK-3 (pTyr279, 216) and PP2A. A₂₅₋₃₅ can enhance the expression of GSK-3 (pTyr279, 216), meanwhile it also restrained the expression of PP2A. Moreover ginsenoside Rb1 can lessen the affect of A₂₅₋₃₅. RT-PCR found that neural stem cells which had been differentiated after one week can express GSK-3 , CDK-5, PP2A. The expression of GSK-3 and CDK-5 rose up and the expression of PP2A weakened when they were treated by A₂₅₋₃₅. However, the effect of A₂₅₋₃₅ was restrained when they were pretreated by ginsenoside Rb1. **Conclusions** NSCs which were cultured and induced in vitro can express GSK-3 , CDK-5 and PP2A; moreover A₂₅₋₃₅ and ginsenoside Rb1 can regulate the expressions of GSK-3 , CDK-5 and PP2A. It hinted that cells which differentiated from neural stem cells in vitro have protein phosphorylation regulation system of normal cells.

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Key words: neural stem cells; GSK-3 ; CDK-5; PP2A; ginsenoside Rb1; A₂₅₋₃₅

Introduction

Neural stem cells (NSCs) are cells that have self-renewal ability and multidifferentiation potential. NSCs are transformed into neurons as its immature forms changed to mature forms, which is coincident with the change of the tau protein's location and expression. Tau protein is an important microtubule-associated protein, and localizes primarily in the axon of neurons. Its major role is to promote the formation and increase the stability of microtubules, therefore it plays an important part in maintenance of the morphology and function of cells. The normal tau protein is a phosphoprotein, and it can not only influence the formation of microtubules when it is hyperphosphorylated, but also can cause various kinds of depositions to localize in the neurons, which will cause damage of the neurons and diseases of the nervous system^[1].

Tau protein's phosphorylation is regulated by the relative activity of protein kinases (which catalyze hyperphosphorylation) and protein phosphatases (which catalyze dephosphorylation). Glycogen synthase kinase-3 (GSK-3) and cyclin-dependent kinase-5 (CDK-5) belong to Ser/Thr kinase, which can promote tau protein hyperphosphorylation in vivo and vitro and play a very important role in the regulation of tau phosphorylation^[1]. The decreasing activity of

protein phosphatases, especially the activity of protein phosphatase-2A (PP2A), also plays a key part in the regulation of tau phosphorylation.

It has been proved that the mature neurons in the brain express GSK-3 、 CDK-5 and PP2A . A₂₅₋₃₅ and ginsenoside Rb1 can also regulate the expression of GSK-3^[2], CDK-5^[3] and PP2A. However, this hasn't been reported for the neurons which are from the differentiated NSCs. Thus, in this study we intend to investigate the expression of GSK-3 , CDK-5 and PP2A and the regulation of them by A₂₅₋₃₅ and ginsenoside Rb1 after NSCs are transformed into neurons.

1. Materials and methods**1.1 Main reagents**

A₂₅₋₃₅ (sigma); GSK-3 (pTyr279, 216) antibody (sigma); ginsenoside Rb1 (Chinese Medicine Identification Institute); Rabbit Anti -PP2A antibody (Beijing Zhongshan); DMEM/F12 culture medium (GIBCO); Fetal bovine serum (Hangzhou Chinese Holly Bioengineering Material Research Institute); gelose (Sigma); Marker (TaKaRa Biotechnology); AMV First Strand cDNA Synthesis Kit and PCR Kit (Shanghai Bioengineering Company).

1.2 Primer design and synthesis

RT-PCR primer was designed by corresponding software: primer of GSK-3 (Forward: 5' AACACCAACAAGGGAGCAAA 3'; Reverse: 5' GAGCGTGAGGAGGGATAA GG3'), primer of CDK-5 (Forward: 5' ATGGGGAAGGCACCTACGG 3'; Reverse: 5' TCCAGGTCACCATTGCAGCT 3'), primer of PP2A (Forward: 5' AGGTGGGAG AGTCGTCATCT3'; Reverse: 5' GTGGTAGGTATGGGCGTTGG 3') and primer of -actin for intra-contrast (Forward: 5' GAGCTGCGTGTGGCCC CT AG3'; Reverse: 5' AGTTTCATGGATGCCACAGG3'). All primers were synthesized by Shanghai Bioengineering Company.

Table 1. Sequences of primers

Gene	GeneBankNo	Primer sequence	Product size
CDK-5	NM - 080885	Forward:5'ATTGGGGAAGGCACCTACGG 3' Reverse:5'TCCAGGTCACCATTGCAGCT 3'	249bp
GSK-3	NM - 032080	Forward:5'AACACCAACAAGGGAGCAAA 3' Reverse:5'GAGCGTGAGGAGGGATAAAGG 3'	326bp
PP2A	M83297	Forward:5'AGGTGGGAGAGTCGTCATCT 3' Reverse:5'GTGGTAGGTATGGGCGTTGG 3'	455bp
-actin	AY039651	Forward:5'GAGCTGCGTGTGGCCCCTGAG3' Reverse:5'AGTTTCATGGATGCCACAGG3'	554bp

1.3 Source of animals

The newly born 24h old SD rats were afforded by Animal Center of Zhengzhou University.

1.4 Isolation, culture and differentiation of NSCs

NSCs of 24h old SD rats were isolated from the dentate gyrus of the hippocampus and cultured with DMEM/F12 culture medium containing 2 % B27, bFGF(20 ng/ml) and EGF (20 ng/ml) at 37 and 5% CO₂. Each three days the cultured cells were replaced with fresh medium. After one week NSCs began to accumulate and form neurospheres. We treated NSCs to the next passage by centrifuging the cell's suspension for 5min (1000 rpm). The third passage NSCs were induced into neurons by adding 10% fetal bovine serum and removing mitogens. Undifferentiated neural stem cells, neurons and astrocytes were identified separately with Nestin, NSE and GFAP antibodies by using immunocytochemistry.

1.5 The experimental groups

NSCs were divided into 3 experimental groups after they had been induced for one week.

- (1) The control group: cultured for another 36h without additional treatment.
 - (2) A₂₅₋₃₅ treatment group: cultured for another 24h, and then added A₂₅₋₃₅ (20 μmol/L) for 12h.
 - (3) Ginsenoside Rb1 pre-treatment group: Pre-treated with ginsenoside Rb1 (10 μmol/L) for 24h, and then added A₂₅₋₃₅ (20 μmol/L) for 12h.
- After total 36h, each group of cells was collected.

1.6 Immunofluorescence cytochemistry

Each group of cells was collected from 6-well plates, operational procedure according to the

instruction of reagent kit. The morphological changes of cells were observed under fluorescence microscope.

1.7 RT-PCR

Abstraction of RNA and synthesis of cDNA processed according to the instruction of reagent kit. Amplification by PCR carried on after synthesis of cDNA : Put the following materials into Ependoff tubes in turn: 2mmol/L dNTP 2 μl, Taq enzyme (5U/ μl) 1 μl, cDNA 5 μl, specific primer 1,2 each of 0.5 μl, -actin 0.5 μl, 10× PCR buffer 3μl, ddH₂O 17 μl. Conditions of amplification for PCR: at 94 for 3min, 94 for 45s, 55 for 45s, 72 for 1min, 35 cycles later, extending at 72 for 7min. 5 μl amplification product mixed with buffer solution was put into 1.8% gelose gel which contained EB, after electrophoresing by voltage 5-10V/cm for 50minutes, the result was observed by gel scan imaging system.

1.8 Statistics analysis

Results expression by $\bar{X} \pm S$, analysis data by ANOVA. Comparison between groups by LSD, $p < 0.05$ is significant.

2. Results

2.1 Isolation, *ex vivo* culture and differentiation of NSCs

Freshly isolated single NSCs from the dentate gyrus of newborn rats hippocampus were smaller, round and contained more opaque particles. After 3 passages, the neurospheres grew significantly bigger and the particles mostly disappeared. The specific markers of the NSCs (Nestin) were expressed on primary culture and the passed cells. After 3 days

induction, most floating neurospheres began to adhere to the bottom of the bottle and grew outwards like in the shape of a thorn. After 7 days induction, most cells of the neurospheres grew outwards and formed axons

which were interlaced with one another. Immunocytochemistry found that differentiated cells showed the specific markers of neurons (NSE) and astrocytes (GFAP).

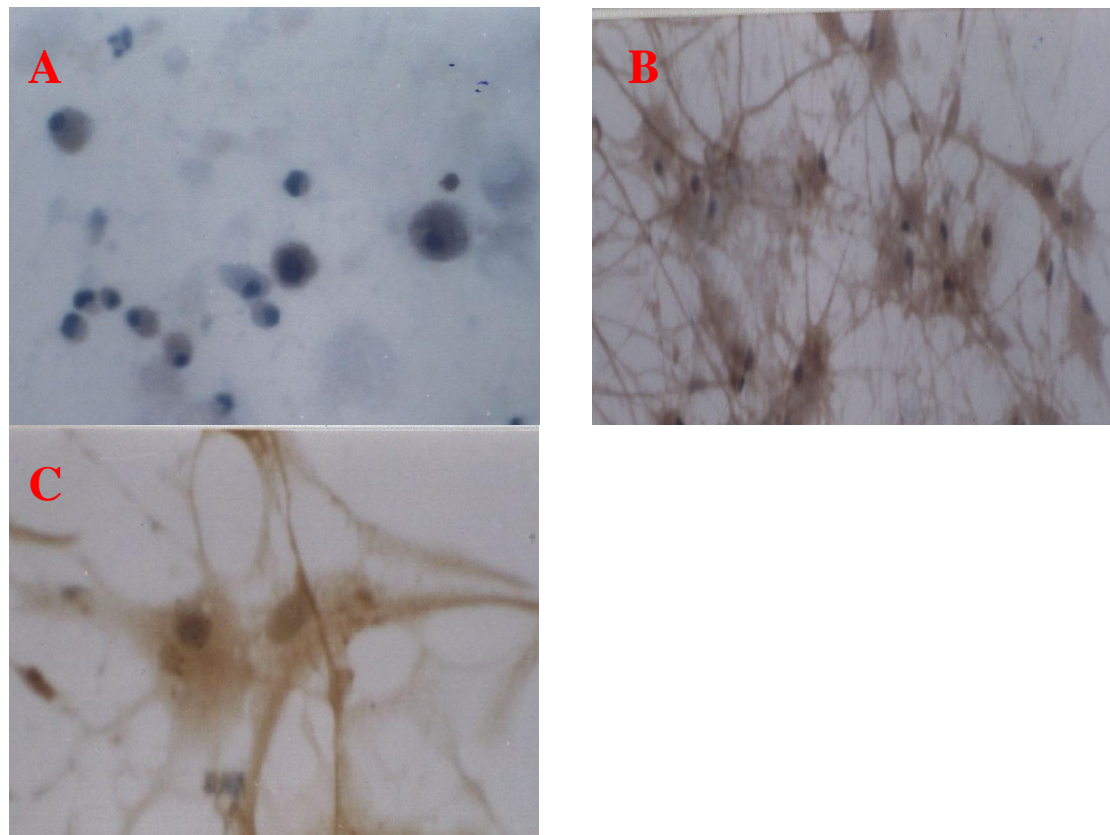


Figure 1. Immunocytochemistry results of NSCs and differentiated NSCs

A: NSCs have expression of Nestin ($\times 100$)

B: Differentiated NSCs have expression of NSE ($\times 100$)

C: Differentiated NSCs have expression of GFAP ($\times 200$)

2.2 Immunofluorescence cytochemistry

Immunofluorescence cytochemistry showed that the expressions of PP2A in the A₂₅₋₃₅ and the ginsenoside Rb1 treatment groups were all reduced more than that in the control group, especially in the A₂₅₋₃₅ group (Figure 1 A, B, C). However, the expressions of GSK-3 (pTyr279,216) in the A₂₅₋₃₅ and the ginsenoside Rb1 treatment groups were all increased more than that in the control group, and especially in the A₂₅₋₃₅ group (Figure 2 D, E, F).

2.3 Results of gene expression of GSK-3、CDK-5 and PP2A

The cells of the control group expressed GSK-3、CDK-5 and PP2A. The expressions of GSK-3 and CDK-5 in the A₂₅₋₃₅ and the ginsenoside Rb1 groups were more than those in the control group, moreover, the expressions of GSK-3 and CDK-5 were the greatest in the A₂₅₋₃₅ treatment group. The difference was considered significant ($p < 0.01$); However, the expression of PP2A was lower in the A₂₅₋₃₅ and the ginsenoside Rb1 treatment groups than the control group, and the expression of PP2A was the lowest in the A₂₅₋₃₅ group. The difference was considered significant ($p < 0.01$).

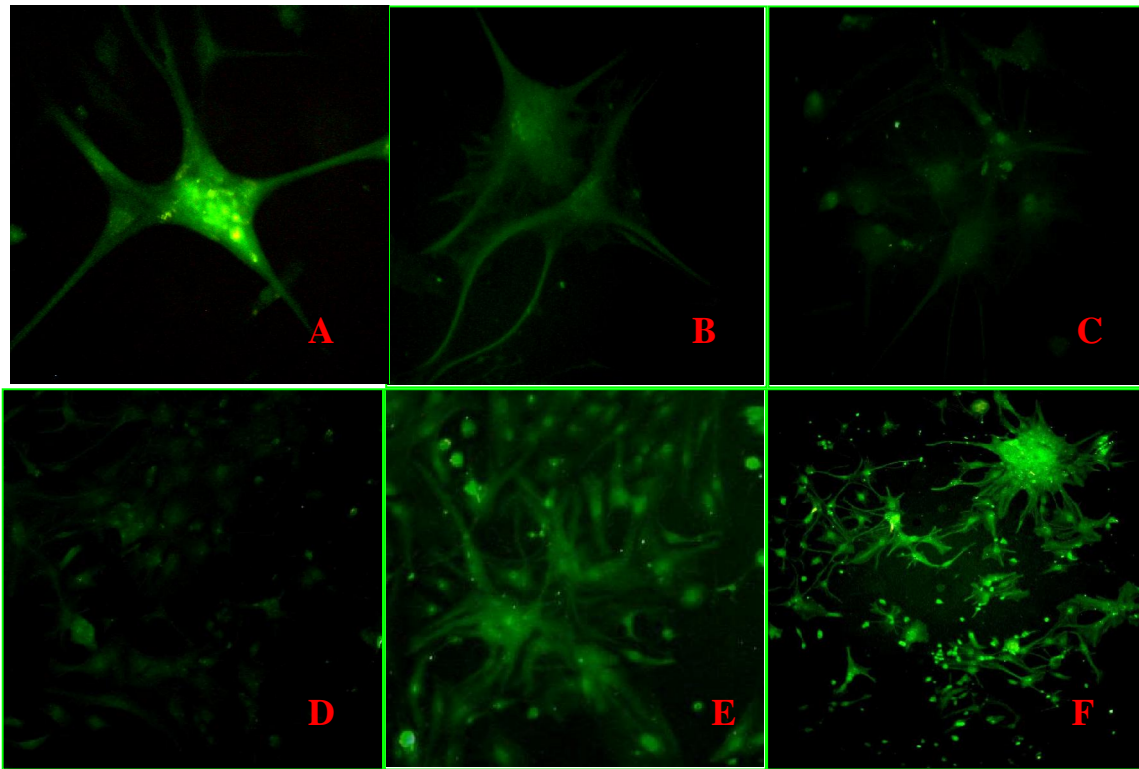


Figure 2. Immunofluorescence cytochemical staining after NSCs had been induced for one week.
 A, B, C: Staining with GSK-3 (pTry279,216) ($\times 100$)
 D, E, F: Staining with PP2A ($\times 400$)
 A and D: Control; B and E: Ginsenoside Rb1; C and F: A₂₅₋₃₅.

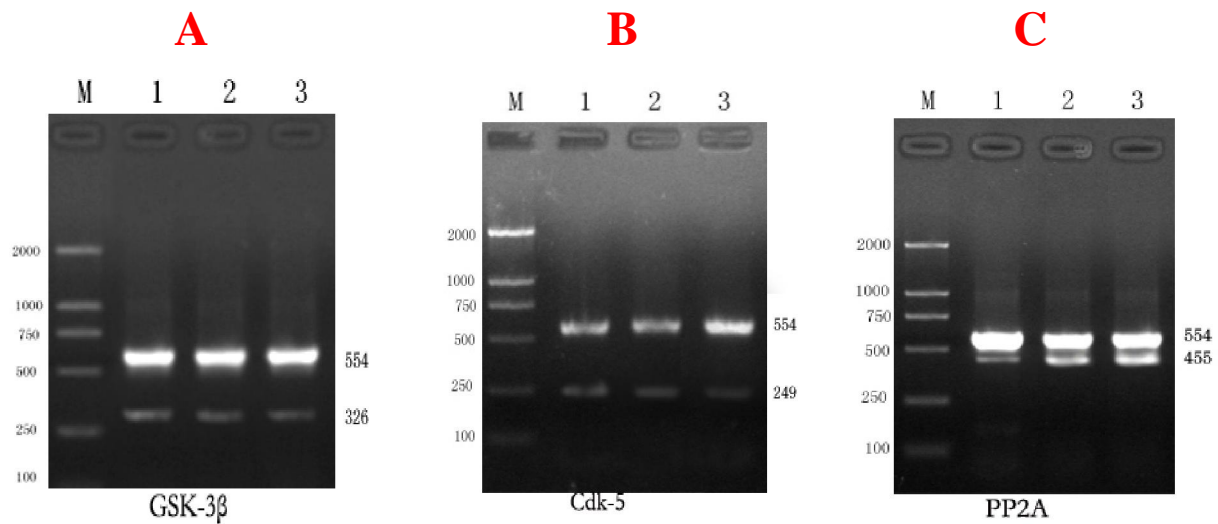


Figure 3. Expression of mRNA after NSCs had been differentiated for one week.
 A: Expression of GSK-3 ; B: Expression of CDK-5; C: Expression of PP2A .
 M: Marker; 1: A₂₅₋₃₅; 2: Ginsenoside Rb1; 3: Control

Table 2. Expression of GSK-3 /CDK-5/PP2A mRNA after NSCs had been differentiated for one week ($\bar{X} \pm S$, n=5)

group	GSK-3	CDK-5	PP2A
Control	0.074±0.011	0.084±0.011	0.714±0.087
A ₂₅₋₃₅	0.246±0.011	0.324±0.011	0.116±0.011
Ginsenoside Rb1	0.128±0.008	0.208±0.008	0.408±0.072

The difference of compare with each other is significant, $p < 0.01$.

3. Discussion

Since a long time ago it has been argued that the functional status of the neural cells differentiated from neural stem cells (NSCs) in rats. Our laboratory has already certified that the neural cells differentiated from NSCs in rats have been found K^+ current by using patch clamp technique (PCT). But it is many-sided to identify the function of neural cells. In this investigation, the neural cells differentiated from NSCs in rats expressed GSK-3, CDK-5 and PP2A by using immunofluorescence cytochemistry and RT-PCR, that is, these neural cells had regulation system of tau protein phosphorylation as same as the mature neural cells in rat's brain. So this experiment identified function of the neural cells differentiated from NSCs in another way.

Neuropathologically, Alzheimer's disease (AD) is now defined by the accumulation of two types of insoluble fibrous material - extracellular amyloid protein in the form of senile plaques and intracellular neurofibrillary lesions (NFL) made of abnormally and hyperphosphorylated tau protein. In addition to the neurofibrillary tangles (NFTs), the NFL consists of neurofil threads and dystrophic neurites that are associated with senile plaques. Although AD and its main brain histopathology, that is, senile plaques and neurofibrillary tangles (NFTs), were described a century ago, The discoveries of the major protein components of senile plaques as amyloid β -peptide and of NFTs as abnormally hyperphosphorylated tau in the 1980s initiated a new era of AD research. Recent studies demonstrate that it is the abnormal hyperphosphorylation that makes tau lose its normal function to stimulate microtubule assembly, gain toxic activity, and aggregate into NFTs^[4], upregulation of tau phosphorylation that leads to neurofibrillary degeneration^[5].

To understand the mechanism leading to abnormal hyperphosphorylation of tau in AD, protein kinases and phosphatases that regulate tau phosphorylation level must be identified first.

The kinases that most likely play a role in phosphorylation of tau in the brain include glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase 5 (CDK-5)^[6]. The activity of GSK-3 is regulated by Ser and tyrosine phosphorylation. Tyr279, 216 phosphorylation may increase the activity of GSK-3,

however, Ser 9 phosphorylation will down-regulate the activity of GSK-3^[7,8]. In our experiment showed that in A₂₅₋₃₅ treatment group the expression of GSK-3 mRNA and CDK-5 mRNA, GSK-3 (pTyr279, 216) all raised up, but in GinsenosideRb1 pre-treatment group they were restrained. This result is coincidence with that of in AD brain GSK-3 showed tendency of up-regulation..

Among protein phosphatases, PP2A has been shown to be the major tau phosphatase in the brain^[9-11]. It has been reported the okadaic-acid-induced inhibition of PP2A activity and prevents tau hyperphosphorylation in hippocampal slice cultures from adult rats^[12]. Our result also showed in A₂₅₋₃₅ treatment group the expression of PP2A mRNA cut down, but in GinsenosideRb1 pre-treatment group it was up-regulation.

These results may due to the following factors: A₂₅₋₃₅ might activate the activity of GSK-3, CDK-5 and inhibit the activity of PP2A which result in the expression of GSK-3 mRNA and CDK-5 mRNA raise up and the expression of PP2A mRNA cut down in A₂₅₋₃₅ treatment group. GinsenosideRb1 might activate the activity of PP2A and inhibit the activity of GSK-3 and CDK-5 which leads to in GinsenosideRb1 pre-treatment group the expression of PP2A mRNA is up-regulated and the expression of GSK-3 mRNA and CDK-5 mRNA is down-regulated. In conclusion, A₂₅₋₃₅ and GinsenosideRb1 may regulate the expression of GSK-3, CDK-5 and PP2A in differentiated neural stem cells of rats.

Inhibition of dysregulation of protein phosphorylation/dephosphorylation is a promising target to treat AD. Further investigation of new compounds that can inhibit abnormal hyperphosphorylation of tau will likely provide new treatments for AD.

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Correspondent to:

ZHAO Qing-xia
The Stem Cells Research Center
Zhengzhou University

Zhengzhou, Henan 450052, China
 Phone: 01186-139-3853-9808
 Fax: 01186-371-6665-8377
 E-mail: zzuzhaoqingxia@126.com

XING Ying
 The Stem Cells Research Center
 Essential Medical College
 Zhengzhou University
 Zhengzhou, Henan 450052, China
 Phone: 0371-66658377
 Fax: 0371-66658377
 E-mail: yingy@zzu.edu.cn

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11/20/2010

Self-management in Primary Dysmenorrhea: Toward Evidence-based EducationMaryam Kabirian¹, Zahra Abedian², Seyed Reza Mazlom³, Behroz Mahram⁴¹. MSc in midwifery, Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran². Faculty Member, Department of Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran³. Faculty Member, Department of Medical & Surgical Nursing, Mashhad University of Medical Sciences, Mashhad, Iran⁴. Faculty Member, Mashhad Ferdowsi University, Mashhad, IranAbedianz@mums.ac.ir

Abstract: There is not an evidence-based education that reinforces use of scientific research in the context of self-management in primary dysmenorrhea. This study was conducted to determine the effect of evidence-based education on dysmenorrheic girl's self-care behaviors and the severity of primary dysmenorrhea at dormitories of Ferdowsi University in Mashhad, Iran. The method of this study involves the following steps: A) Formulation of answerable questions to address self-care methods in primary dysmenorrhea. B) Systematic searching for the research evidence that could be used to answer the questions. C) Appraisal of the validity, relevance and applicability of the research evidence. D) Designing of the protocol of evidence-based education based on the best acquired evidence. E) Evaluation the effect of evidence-based education on dysmenorrheic girl's self-care behaviors and the severity of primary dysmenorrhea. There was a significant reduction in pain score at the first (-0.6 ± 1.7 VS 1.1 ± 2.1 $P=0.000$) and second (-1.9 ± 1.5 VS 0.1 ± 1.6 $P=0.000$) menstrual period after intervention in the evidence-based education group compared with the girls in the control group. Also, the difference in self-care behaviors between evidence-based education versus control group at the second menstrual period after intervention (105.8 ± 8.9 VS 80.4 ± 11.3 $P=0.021$) was significant. Evidence-based care leads to remarkable advances in the management of primary dysmenorrhea. Health education systems can use evidence-based education in order to promote self-management behaviours among primary dysmenorrheic girls.

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Keywords: Evidence-based Education; Self-management; Primary Dysmenorrhea

1. Introduction

Primary dysmenorrhea is one of the most common gynecological problems that require clinical and intensive self-care (Marrow, 2009). More than 70% of Iranian girls experience dysmenorrhea (Panahandaz, 2008; Afshari, 2006; Jalili, 2004; and Kamjo, 2001). Besides being a gynecological problem, primary dysmenorrhea is an important health problem concerning public health, occupational health and family practice, as it affects both the quality of life and the national economy due to short-term school absenteeism and loss of labor. Menstruation with severe pain also affects development of motherhood (Havens, 2002).

Few educational programs have been directed at improving self-care behaviors among dysmenorrheic girls. Furthermore, health professionals are generally enthusiastic about the value of the education programs on offer. However, care should be taken to ensure that the quality of education provided in the education program is based on the best evidence available.

Evidence-based education is the integration of professional wisdom with the best available empirical evidence in making decisions about how to deliver

instruction (EBE, 2010). Educational protocols are based on evidence-based care, thereby supplying the health-professionals with the latest and most effective managements (Moran, 2004).

Self-care behaviors related to dysmenorrhea refer to those actions taken to enhance comfort and to prevent or respond to the condition (Ching-Hsing, 2004). Self-care behaviors are divided into two categories: externally oriented behavior including searching for knowledge, expression of emotions, seeking assistance, control over external factors and internally oriented behavior including resource utilization and self-control being.

There is not an evidence-based education that reinforces use of scientific research in the context of self-management in primary dysmenorrhea. This study was conducted to determine the effect of evidence-based education on dysmenorrheic girl's self-care behaviors and the severity of primary dysmenorrhea at dormitories of Ferdowsi University in Mashhad, Iran.

2. Material and Methods

The method of this study involves the following steps:

- A) Formulation of answerable questions to address self-care methods in primary dysmenorrhea.
- B) Systematic searching for the research evidence that could be used to answer the questions.
- C) Appraisal of the validity, relevance and applicability of the research evidence.
- D) Designing of the protocol of evidence-based education based on the best acquired evidence.
- E) Evaluation the effect of evidence-based education on dysmenorrheic girl's self-care behaviors and the severity of primary dysmenorrhea.

2.1. Answerable questions

A "well-built" question should include four parts, referred to as PICO that identify the patient problem or population (P), intervention (I), comparison (C) and outcome(s) (O). In this study:

P=girls with primary dysmenorrhea

I=self-care education

C=no education

O=reduction in the severity of dysmenorrhea and increase the self-care behaviors.

'In girls with primary dysmenorrhea, does self-care education, compared to no education, reduce the severity of dysmenorrhea?'

'In girls with primary dysmenorrhea, does self-care education, compared to no education, increase the self-care behaviors?'

2.2. Systematic searching

We searched the Medline, EMBASE, CINAHL and Cochrane Library. Searches were limited by the publication years as follows:

- Systematic Reviews: From January 2005 to July 2010
- Randomized Controlled Trials: From January 2000 to July 2010

The Inclusion Criteria were present original data or reviews of original data, focus on girls with primary dysmenorrhea, applicable to dysmenorrhea care or prevention in Iran, conducted in humans and published in the English language. The Exclusion Criteria were: studies of inappropriate population, studies that are not clinically applicable as self-care methods of dysmenorrhea management, reviews and articles which present the author's opinion rather than evidence.

2.3. Appraisal of the research evidence

Due to the large volume of studies addressing these issues, the 6 systematic reviews used as evidence are summarized in table 1.

2.4. Designing of the protocol of evidence-based education

The protocol of evidence-based education' was designed by evaluating the available resources in the context of self- management in primary dysmenorrhea. The evidence addressing the question "Self-care in Primary Dysmenorrhea" is categorized to 6 sub-headings based on orem's self-care theory as follows: seeking assistance, expression of emotions, searching for knowledge, control over external factors, resource utilization and self-control being.

2.5. Evaluation the outcomes

In this stage of study, a randomized clinical trial was designed to evaluate the effect of evidence-based education on dysmenorrheic girl's self-care behaviors and the severity of primary dysmenorrhea. Single girls aged less than 25 years, who had experienced primary dysmenorrhea based on Verbal Multidimensional Scoring System for at least 3 months in the past half year, didn't have any prior history of gynecological disease and symptoms of secondary dysmenorrhea were eligible. The menstrual history for diagnosis of primary dysmenorrhea included the regularity of menstrual cycles (menstrual cycle length from 28 to 35 days and bleeding cycle length from 3 to 7 days) and the beginning of pain, a few hours before the onset of a menstrual period, not lasting more than 72 hours. Exclusion criteria included current or recent use of hormonal contraception, refusing to participate in educational section or fill out the follow up's questionnaires.

Girls with dysmenorrhea (n=111) from two different dormitories of Ferdowsi University fulfilled the inclusion criteria and were randomly assigned to evidence-based education (n=65) and control (n=65) groups. Subjects were asked to provide demographic information and fill out Pain Assessment Form (VAS) and Dysmenorrhic Self-Care Scale (DSCS) during their baseline cycle. After that, the evidence-based education sessions were carried out by method of small-group discussion in the intervention group. Post-test forms the same as pre-test one were completed in the end of the two follow-up cycles.

The data were analyzed using Independent t-test and Mann-Whitney by SPSS software version 11.5 (power of 80% and type I error (alpha) of 5%).

Table 1. Systematic reviews of interventions in management of primary dysmenorrhea

Author(s), year, number and type of studies included	Intervention	Population Characteristics	Outcome Measurements	Main Results
Marjoribanks et al, 2010, 2 Reviews 73 RCTs	Comparing NSAIDs vs placebo or paracetamol or each other	Girls with primary dysmenorrhea	Pain-relief	NSAIDs were significantly more effective than placebo and paracetamol, but there was insufficient evidence to determine which of them is the most effective
Proctor M et al, 2010, 5 trials involving 213 women	Comparing behavioural interventions with each other, placebo, no treatment, or conventional medical treatments	women with dysmenorrhoea	Symptom severity, daily activities and pain management	behavioural interventions (both of physical and cognitive procedures) may be effective for dysmenorrhoea
Cho SH & Hwang EW, 2010, 4 RCTs involving 458 women	Comparing acupressure with sham acupressure on non- acupoints	Girls with primary dysmenorrhea	Pain, anxiety, consumption of pain medication	Acupressure alleviates menstrual pain
Brown J & Brown S, 2010, 4 RCTs	Comparing exercise with a control or no intervention	Women with dysmenorrhea	Moo's Menstrual Distress Questionnaire	Exercise reduced the MDQ score during the menstrual phase and three observed cycles
Proctor M & Murphy PA, 2009, 7 RCTs	Comparing omega-3 fatty acids, magnesium, vitamin B6, B1, E, combination of vitamin B6 and magnesium with each other, placebo, no treatment or other conventional treatments	Girls with primary and secondary dysmenorrhea	Pain-relief	Vitamin B1 is shown to be an effective treatment, magnesium is a promising treatment, there is insufficient evidence to recommend the use of any of the other therapies in this review
Dennehy CE, 2006, (9 RCTs for dysmenorrhea part of this review)	Reviewing the use of herbs and dietary supplements	Women with menopause, premenstrual syndrome, dysmenorrhea, mastalgia, and infertility	-	Black cohosh for menopause; vitamins B1 and E for dysmenorrhea; calcium, vitamin B6, and chasteberry for premenstrual syndrome; and chasteberry for cyclic mastalgia. There were too few trials involving herbs and supplements in infertility.

2.6. Dysmenorrhic Self-Care Scale (DSCS)

It is the revised form of DSCS by Ching-Hsing et al (2004). This is a 25-item, self-administered scale that was designed based on Orem's self-care deficit theory. For each item, dysmenorrhic girls rate their self-care behavior on a 5-point scale from 1 (I never do) to 5 (I always do). The total score ranges from 25-125 and is calculated by summing the scores for each item. CVI for this scale was calculated 0.82 and Cronbach's alpha coefficient was 0.84

2.7. Ethical and confidentiality considerations

The study received ethical approval from the committee for research on human subjects of Mashhad University of Medical Sciences. All subjects gave written consent for participation. They were thoroughly explained on the purpose and procedures of the study and informed that they could withdraw from the study any time without any consequences.

3. Results

The demographic and menstrual characteristics of the subjects (n=111) in the two groups are shown in table 2. The distribution of all recorded characteristics didn't have significant difference among groups.

Table 2. The demographic and menstrual characteristics of the participants.

	Health provider-led education group	Control group	p-Value
	(n=50)	(n=61)	
	Mean (SD) or %	Mean (SD) or %	
Age (years)	21.4 (1.4)	21.7 (1.1)	0.402
BMI (kg/m ²)	22.6 (1.8)	22.7 (2.0)	0.836
Menstrual cycle length (day)	29.1 (1.7)	29.7 (2.0)	0.057
Bleeding cycle length (day)	6.1 (1.1)	6.3 (0.9)	0.302
Age of menarche (years)	13.7 (1.1)	13.3 (1.4)	0.315
Age of onset of menstrual pain (years)	15.1 (1.4)	14.8 (2.2)	0.143
Degree of dysmenorrhea			
Degree 1	10.0	6.6	
Degree 2	80.0	73.8	0.586
Degree 3	10.0	19.7	
Duration of menstrual pain (hours)	37.6 (4.8)	37.1 (6.2)	0.632
frequency of cycles combined with dysmenorrhea during the past 6 months	5.2 (1.0)	5.2 (0.8)	0.053

Table 3. comparison of self-care behavior scores between evidence-based education and control groups

	evidence-based education group	Control group	p-Value
	(n=50)	(n=61)	
Dysmenorrhic Self-Care	Mean (SD) or %	Mean (SD) or %	
Baseline Cycle	79.8 (9.1)	76.6 (12.4)	0.163
The first Cycle after intervention	100.4 (12.4)	89.1 (9.8)	0.090
The second Cycle after intervention	105.8 (8.9)	80.4 (11.0)	0.005
p-Value	0.000	0.219	

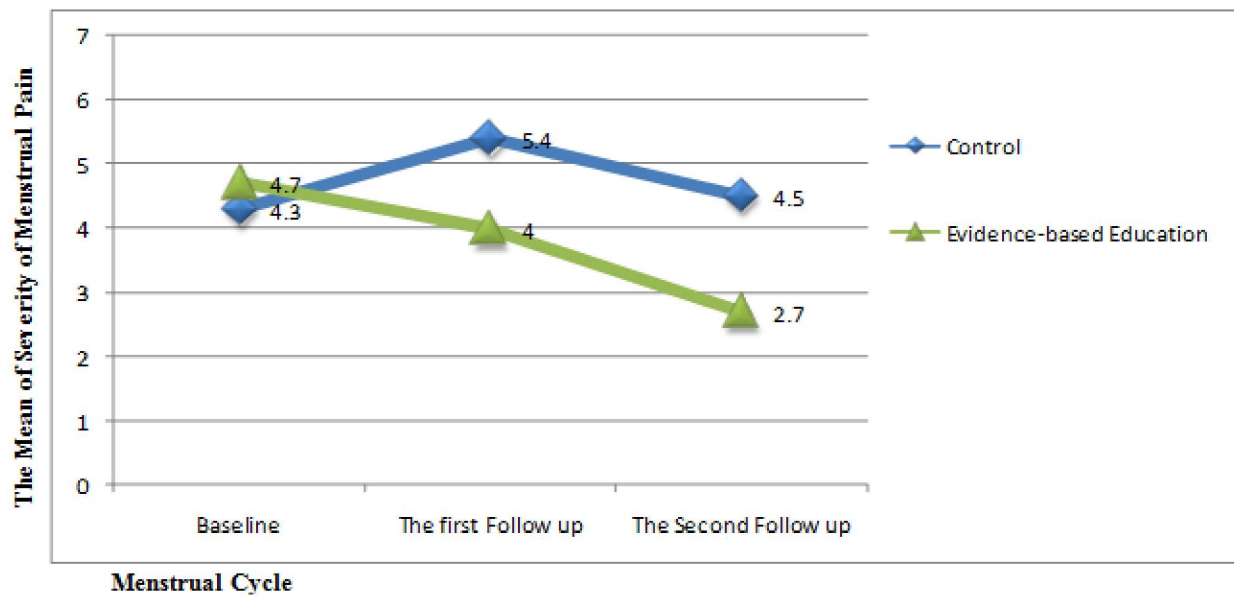


Figure 1. Comparison of severity of menstrual pain in evidence-based education and control groups

Table 3 shows the comparison of self-care behavior scores between two groups before and one and two menstrual period after intervention. There was a significant difference in self-care behavior between evidence-based education versus control group at the second menstrual period after intervention.

The mean of dysmenorrhea in the evidence-based education group decreased 0.7 score in the first menstrual after intervention and 2 score in the second menstrual after intervention. (Fig1.)

4. Discussions

There is a wide range of strategies in management of primary dysmenorrhea, but the midwives should be aware of which therapies have evidence-based support, carry a low side effect burden, and the least potential to interact with other medications. The review of trials found some evidence that NSAIDs are an effective treatment for dysmenorrhoea, though women using them need to be aware of the significant risk of adverse effects (Marjoribanks, 2010). Many dietary supplements and herbs have been proposed as being effective for primary dysmenorrhea, including black cohosh, chastetree, dong quai, black haw (*Viburnum prunifolium*), crampbark (*Viburnum opulus*), omega-3 fatty acids, vitamin E, thiamine (vitamin B1), niacin (vitamin B3), and magnesium (Proctor, 2009). In our review, Vitamin B1, E and magnesium are shown to be effective treatments and there is insufficient evidence to recommend the use of any of the other therapies (Proctor, 2009; Dennehy, 2006). Behavioural therapies focus on both physical and psychological coping

strategies reduce menstrual symptoms. Pain management training and relaxation plus biofeedback may help with period pain in general and progressive muscle relaxation with or without imagery and relaxation may help with spasmodic (acute, cramping pain) symptoms of period pain (Proctor, 2007).

The available data from RCTs suggest that acupressure reduced the pain and anxiety typical of dysmenorrhoea and there was no adverse event in acupressure treatment (Cho, 2010). In our review, the SP6 point is known as the most recommended point for treatment of primary dysmenorrhea. There are several plausible mechanisms by which exercise might be effective in the management of primary dysmenorrhea. Evidence from controlled trials suggests that exercise can reduce primary dysmenorrhea and associated symptoms, but the results should be viewed with caution due to the limited evidence (Brown, 2010; Daley, 2008).

Despite treatments being available for primary dysmenorrhea and the positive effects of self-care behaviors on treatment, the low self-care knowledge causes the high prevalence of primary dysmenorrhea. The results of this study show that evidence-based education can encourage the girls with dysmenorrhea to do self-care behaviors. The self-care behaviors increased 1.3 times; as a result, the severity of dysmenorrhea decreased 2 times in the evidence-based education group. Chiou et al (2007) evaluated the effects of systematic health education on dysmenorrhoeic girls' knowledge, attitudes and self-care behaviors and resulted a significant increase in the experimental group members' dysmenorrhea-related

knowledge and self-care behaviors. Considering the importance of self-care behaviors, it is important to use evidence-based resources when seeking information about management of primary dysmenorrhea. We hope to use of the findings of this study as a basis for further research to recognize the best evidence for self-management in primary dysmenorrhea.

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Corresponding Author:

Zahra Abedian
Department of Midwifery
Mashhad University of Medical Sciences
Mashhad, Iran
Tel: +98.9155030337
Fax: +98.5118597313
E-mail: Abedianz@mums.ac.ir

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Evaluation Of Serum Hyaluronic Acid And Matrix Metalloproteinase-2 As Non Invasive Markers Of Hepatic Fibrosis

Elham Ragab Abdel-Samea¹, Soma Sherif Abd El Gawad¹ and Mohammed Abd El Hamid Ali²

1. Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt
2. Department of Hepatology Gastroenterology and Infectious Disease, Benha Faculty of Medicine, Zagazig University, Egypt
elhamelngar@yahoo.com

Abstract: Liver biopsy is currently the gold standard for assessing liver fibrosis and non reliable non invasive approach is available, therefore a suitable serologic Biomarker is needed. Several biochemical markers have shown promise for the detection of advanced fibrosis and cirrhosis. The aim of the present work is to study the diagnostic value of serum hyaluronic acid (SHA) and matrix metalloproteinase-2 (MMP-2) as indicators for the stage of hepatic fibrosis, and to correlate the liver pathology and liver function tests with serum fibrosis markers. Eighty treatment naïve patients with chronic hepatitis C [CHC] with or without HBV and forty healthy subjects are used as a control group. The patients were divided according to Metavir classification of liver biopsy into 3 groups. Group 1 with normal biopsy (17 patients), group 2 was 35 patients with mild fibrotic changes (stage 1-2) and group 3 of 28 patients with severe fibrosis (stage 3-4). SHA level was significantly higher in patients with severe fibrosis than patients with mild or no hepatic fibrosis. (378.7±147.5, 226.2±123.7 and 85.3±52.2 pg/ml). (P<0.0001). MMP-2 was also significantly higher in severe fibrosis (group 3) than group 2 or group 1 (1196.2±119.5, 918.1±175.8 and 841.1±224.5 pg/ml) respectively (P<0.001). SHA and MMP-2 were not correlated to age, S bilirubin AST, ALT or spleen size. Group 3 was correlated significantly to the SHA and MMP, platelet count, S albumin and liver size but not correlated to AST, ALT, S. bilirubin or spleen size. The Specificity of fibrosis markers SHA and MMP-2 in prediction of severe fibrosis were 94.4% and 90.0% respectively and the sensitivity were higher to SHA 90.0% than MMP-2 80% but not a predictor of mild or normal biopsy. The cut of value of SHA, MMP-2, platelet count and prothrombin time (PT Activity), in diagnosis of severe fibrosis were 294.84 pg/ml, 1003 pg/ml., 115.084/cmm, 72.116% respectively. Measurement of SHA and MMP-2 can be used to differentiate cirrhotic from non-cirrhotic patient and can be regarded as a useful non invasive test in the diagnosis of liver cirrhosis.

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Keywords: Hyaluronic Acid, hepatic Fibrosis, Matrix metalloproteinase,

Introduction

Even after a long and asymptomatic course of chronic hepatitis C (CHC), mild disease can progress to cirrhosis⁽¹⁾. Serial liver biopsies are the best way to diagnose and assess the severity of CHC and to monitor its progression. Liver biopsy can determine the degree of both the inflammatory component (grade) and that of fibrosis (histological stage)⁽²⁾. Although the liver biopsy is the key examination for the diagnosis of cirrhosis, the use of liver biopsy has several limitations⁽³⁾. There are 24% sample errors of false result. Complications with death rate of 0.015%, discomfort, and the cost of hospitalization. These limitations prevent the use of liver biopsy as a general screening procedure for cirrhosis⁽⁴⁾. Early diagnosis is essential because cirrhosis is often revealed by complication. On the other hand cirrhosis is a common disease that is frequently undiagnosed. Several indirect diagnostic tests have been evaluated⁽⁵⁾. These tests include clinical signs, biochemical parameters, echogenic signs and endoscopic signs⁽⁶⁾. In the liver,

SHA is mostly synthesized by the hepatic stellate cells and degraded by the sinusoidal endothelial cells⁽⁷⁾. The increase in SHA level occur together with the development of liver fibrosis by 2 mechanisms, first, enhancement of hyaluronan production by the activated stellate cells may contribute to the increase in serum hyaluronan levels observed in patients with chronic liver disease without cirrhosis. Later, when cirrhosis is established, reduced degradation by sinusoidal endothelial cells may cause greater hyaluronan increases⁽⁸⁾. Hepatic stellate cells are believed to be the main source of fibrillar and nonfibrillar collagens in the liver and also of certain matrix-degrading proteases (MMPs) and their specific inhibitors (TIMPs). Although some researches, studied the MMP-2 in CHC they reported that level is higher than control, they stated that measured MMP-2 values were less liver specific⁽⁹⁾. This work was to determine the diagnostic accuracy of serum markers of fibrosis (SHA and MMP-2) for the diagnosis of hepatic cirrhosis. Study of this non invasive serologic assessment of hepatic fibrosis is

to enable diagnosis of fibrosis in early stages allowing therapeutic intervention to prevent progression to cirrhosis and HCC.

Patients and Methods

The present study was performed on 120 subject (40 control and 80 patients) attending the university hospital of Benha and Mansoura from November 2008 until October 2009.

Control group: Forty healthy subjects (25 males and 15 females) selected from subjects attending for routine medical check up, the mean age (42.5 ± 8.4 years) range from 31-61 years. They were negative for HbsAg, HCVAb and HCVRNA by PCR. They had normal liver function test, no history of schistosomiasis and abdominal ultrasonography was normal.

Patient group: A total of 80 patients (47 men and 33 females), 20 to 65 years old (42.7 ± 11.81 years) were participating in the study. At entry all patients with chronic hepatitis had persistently elevated serum alanine aminotransferase (ALT) (more than twice the normal upper limit) for at least 6 months on three occasions. They had anti-HCV antibodies, and liver histological findings compatible with chronic hepatitis C. Twenty patients had evidence of coinfection with hepatitis B virus (+ve HbsAg). None had clinical, ultrasonographic or histological evidence of other causes of chronic liver disease (Wilson's disease, hemochromatosis, α -1-antitrypsin deficiency, autoimmune hepatitis or hepatocellular carcinoma). All patients had given prior informed consent. The patients were subjected to thorough history taking, clinical examination and laboratory investigation including: CBC, Liver function tests (AST, ALT, S Bilirubin, S Albumin and PT); HCV Ab by ELISA I and HCV PCR II, HbsAg, and HBcAb; Alpha-fetoprotein; Serum fibrosis markers (SHA measured by ELISA kit supplied by Corginex inc. USA. MMP-2 measured by ELISA kit supplied by Amersham UK); Abdominal ultrasonography for measurement of liver and spleen size; Rectal snip for detection of schistosomiasis; Liver biopsy for histological grading and staging. Histological features of liver biopsy specimens were analyzed according to the Metavir scoring system (1994)⁽¹⁰⁾. Fibrosis was staged on a scale of F0-F4, as follows: F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3= numerous septa without cirrhosis, and F4=cirrhosis. The histological activity, a measure of the intensity of necroinflammation was graded on a scale of A0-A3: A0=no activity, A1= mild activity, A2=moderate activity and A3=severe activity.

According to the stage of liver biopsy the patient were divided into 3 groups:

Group 1: included 17 participant with chronic HCV and normal liver biopsy.

Group 2: include 35 patients with mild fibrosis (stage 1 and 2).

Group 3: included 28 patients with severe fibrosis (stage 3 and 4).

Results

Table 1: shows some demographic data of the patients, denoting the most common causes of liver fibrosis in Egypt, as schistosomiasis, HCV and HBV.

Table 2: shows the clinical, biochemical and ultrasonographic variables of the 3 studied groups. There was a significant change in SHA, MMP-2 (figure1), platelet counts, serum albumin and PT between the 3 groups ($P < 0.05$). While age, AST, ALT, spleen and liver size, S bilirubin or PV diameter showed no changes between the different groups.

Table 3: showed the correlation between serum markers of fibrosis and clinical, biochemical and ultrasonographic variables. SHA was correlated significantly to platelet counts, PT and S. albumin, MMP-2, was correlated to liver size in addition to the above parameters. Both markers were not correlated either to age, S. bilirubin, PV diameter or spleen size.

Table 4 showed correlation between liver histology and serum markers of fibrosis. There was a significant correlation between serum SHA, MMP-2 platelet counts, PT, S. Albumin and liver size ($P < 0.05$) and histological scores. There was a very strong correlation between SHA, MMP-2, PT and the stage of fibrosis ($P < 0.001$). Whereas no correlation was found between histological stages, age, S. bilirubin, PV diameter or size of the spleen.

Table 5 and figure 2 showed the ROC curve of sensitivity curve of sensitivity and specificity of fibrosis markers and severity of fibrosis. The SHA and MMP-2 were specific in diagnosis of severe fibrosis in 94.4% and 90.0% respectively and sensitive in 90% for SHA and 80% for MMP-2. The cut off value of significant variable for prediction of severe fibrosis was 294 pg/ml, 1003 pg/ml, 115.084/cmm and 72% for SHA, MMP-2, platelet count and PT respectively (table 6).

Table (1) Demographic data of the individual in different groups

	Control (no.=40)	No fibrosis Gr1(No.=17)	Mild Fibrosis Gr2(no.=35)	Sever fibrosis Gr3(No.=28)
Bl. Transfusion	0	5	7	8
schistosomiasis	0	5	13	9
HCV	0	17	35	28
HBV	0	3	8	9

Table (2) Different variables among the studied groups

	Control(40) X \pm SD	Gr1(17) X \pm SD	Gr2(35) X \pm SD	Gr3(28) X \pm SD	F	P
MMP-2(pg/ml)	582.72 \pm 107.4	841.1 \pm 224.5	918.1 \pm 175.8	1196.2 \pm 119.5	23.231	0.000*
SHA(pg/ml)	59.80 \pm 17.4	85.3 \pm 52.2	226.2 \pm 123.7	378.7 \pm 147.5	24.292	0.000**
Age(yr)	42.5 \pm 8.4	46.0 \pm 14.8	42.1 \pm 9.6	46.6 \pm 9.1	1.156	0.322
Platelet/cmm	264.8 \pm 42.6	147.6 \pm 38.7	129.6 \pm 47.9	99.6 \pm 28.7	6.610	.003*
PT(%)	93.60 \pm 6.1	89.0 \pm 7.6	80.0 \pm 10.1	60.0 \pm 13.7	32.147	.000*
PV(mm)	10.5 \pm 0.9	13.0 \pm 1.7	13.0 \pm 1.4	13.5 \pm 0.9	1.329	0.273
Albumin(gm/dl)	3.91 \pm 0.3	3.5 \pm 0.5	3.5 \pm 0.6	3.0 \pm 0.2	6.589	.003*
Bilirubin(mg/dl)	1.01 \pm 0.11	2.3 \pm 0.8	2.27 \pm 1.0	2.58 \pm 1.1	.543	0.584
ALT(u/ml)	28.70 \pm 5.3	78.92 \pm 26.1	84.72 \pm 29.2	70.04 \pm 27.0	1.637	0.204
AST(u/ml)	25.70 \pm 5.6	84.61 \pm 24.0	87.96 \pm 25.6	74.86 \pm 27.9	1.520	0.227
Spleen size(cm)	9.94 \pm 0.6	14.45 \pm 2.7	13.88 \pm 2.9	14.71 \pm 3.3	.451	0.639
Liver size(cm)	14.94 \pm 0.5	13.74 \pm 1.7	13.74 \pm 1.7	13.47 \pm 1.5	2.542	0.088

*Significant difference between controls and patient groups.

** significant difference between the 3 patient groups

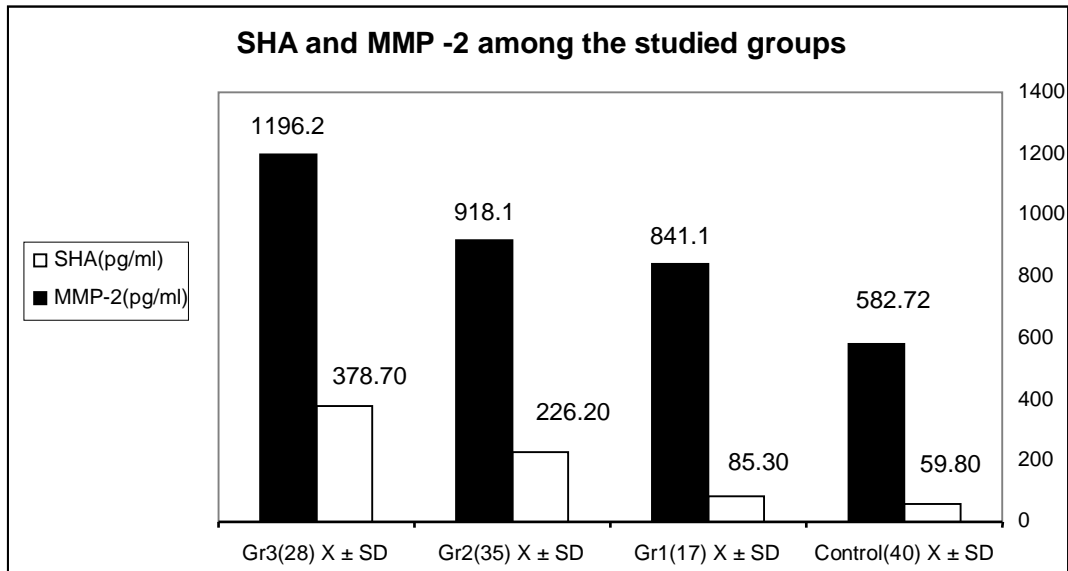
**Figure (1):** SHA and MMP-2 among the studied groups

Table (3) Correlation of MMP and SHA to other variables in 80 patients

	MMP		SHA	
	r	P value	r	P value
MMP	1		0.724	0.000
Age	0.178	0.174	0.165	0.198
Platelet	-0.613	0.000	-0.617	0.000
PT	-0.563	0.000	-0.503	0.000
S. Albumin	-0.537	0.000	-0.509	0.000
S. Bilirubin	0.033	0.803	0.144	0.27
ALT	-0.113	0.093	-0.229	0.053
AST	-0.231	0.075	-0.232	0.075
Spleen size	0.136	0.300	0.063	0.633
Liver size	-0.353	0.006	0.226	0.083

Table (4) Correlation of stage to other variables (80 patients)

P value	Chi-square	Variables
.000	22.109	MMP-2
.000	18.472	SHA
.081	10.475	Age
.001	11.077	Platelet
.002	9.710	PT
.106	2.610	PV
.005	7.772	S. albumin
.391	.736	S. bilirubin
.159	1.982	ALT
.102	2.668	AST
.411	.675	Spleen size
.024	5.118	Size liver
.076	3.140	GRADE

Table (5): Area under the curve

Test Result variable(s)	Area
SHA	.944
MMP-2	.900

Table (6) the cut off value of independent significant variable in prediction of severe fibrosis

SHA	294.8467
MMP-2	1003.4450
Platelet	115.084
PT	72.1167

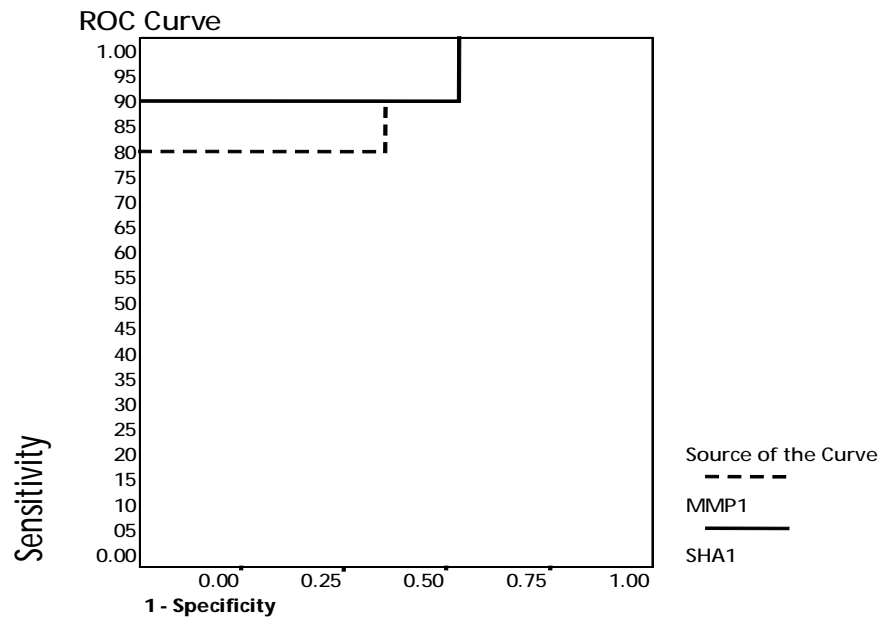


Figure (2) ROC curve of specificity of SHA and MMP in diagnosis of severe fibrosis

Discussion

Liver histology is the gold standard for establishing the severity of liver injury and fibrosis, although it is associated with complication and expense⁽³⁾. Practicing physicians are in need of simple, safe, inexpensive and non-invasively assess the severity in patients with liver disease. The serum fibrosis markers reflect the balance between fibrogenesis and fibrolysis have been proposed as a simple, non-invasive means of assessing hepatic fibrosis^(11,12). The aim of this study was to study the diagnostic accuracy of SHA and MMP-2 as indicators for the stages of hepatic fibrosis. The present study showed no significant difference of age in different studied groups. The value of age as a marker of fibrosis seems obvious, as fibrosis progression is time-dependent⁽¹³⁾. However the duration of HCV infection would be more precise indicator of fibrosis than age⁽¹⁴⁾. Platelet count were correlated significantly to severe fibrosis ($P < 0.05$). This result comes in agreement with Bonacini et al⁽¹⁵⁾. Thrombocytopenia may be related to the development of portal hypertension and the decreased production of thrombopietin. Decreased platelet count is the earliest indicator of cirrhosis⁽¹⁶⁾. Fontana and Lok⁽¹⁷⁾, found that the prothrombin index began to decrease when the Metavir fibrosis score was 2. Prothrombin index had a diagnostic accuracy that was nearly as high as that of

the best serum marker of fibrosis⁽¹⁸⁾. In present study, the serum fibrosis markers (SHA and MMP-2) were significantly different between studied groups ($P < 0.001$) and correlated significantly to the stage of fibrosis (Chi square 18.4 and 22.1). The level increased proportionally to the severity of cirrhosis. This result coincide with Kozłowska et al⁽¹⁹⁾, they reported that the measurement of SHA and MMP-2 reliably differentiated cirrhotic from non-cirrhotic and can be regarded as a useful test in the diagnosis of liver cirrhosis⁽¹²⁾⁽²⁰⁾, particularly when a liver biopsy is contraindicated. However McHutchison et al⁽²¹⁾, found no strong associations between SHA and MMP-2 and the components of the Knodell histological activity index score, they concluded that the clinical value of SHA measurement appears to be its ability to exclude cirrhosis⁽⁸⁾, low level of SHA showed good correlation with low risk of fibrosis. Also SHA and MMP-2 can be helpful in discriminating patients of chronic hepatitis from the liver cirrhosis⁽²²⁾. Gebo et al⁽²³⁾, found that SHA and MMP-2 may have value in predicting fibrosis, and were poor at predicting intermediate levels of fibrosis⁽²⁴⁾. In the present study the two fibrosis markers were not correlated to the grade of inflammatory activity ($P > 0.05$). This was previously confirmed in many article as McHutchison et al⁽²¹⁾, but disagree with Wang et al⁽²⁵⁾, who found a close

correlation between inflammation process and fibrosis, they suggest that the inflammatory process may play an important role in fibrogenesis. The serum fibrosis markers in the current study were highly correlated to PT, Platelet count, serum albumin and liver size ($P < 0.05$) but not correlated to AST, ALT, S. Bilirubin or spleen size. This result consistent with most of previous published articles⁽¹⁸⁾, found that the fibrosis markers correlated significantly with albumin, platelet count and PT but not to serum variables reflecting inflammatory activity. The receiver operating characteristic (ROC) curves in the present study shows specificity 94.4%, 90% and sensitivity 90%, 80% for SHA and MMP respectively in prediction of severe fibrosis. This result comes near the result of Xie et al⁽²⁶⁾, and Lee et al⁽⁸⁾, who found ROC curve of 93% and 72% respectively. It is not surprising that the reported rate vary widely reflecting different patient etiologies. Pares et al⁽²⁷⁾, found the ROC curve of SHA is 91.4% in patients with fibrosis. Boeker et al⁽²⁸⁾, studied the MP-2 in cirrhosis and he mentioned that the diagnostic efficiency of 92%. The cut off value of independent significant variables in detecting the stage 3 and 4 (bridging and cirrhosis) in the current study were nearly similar to the figures in a number of other studies^(26,29). However, in the present study we did not correlated the effect of gender and ethnic origin with the fibrosis markers and staging because for establishing the actual changes rate of serum markers in male and female patients requires serial liver biopsies to correlate with Metavir fibrosis score, which is difficult and also need long time observations. Also it is known that, fibrosis grading and staging scores are higher in Egyptian patients infected with HCV due to concomitant schistosomiasis infection.

Conclusion

SHA and MMP reflect the severity of fibrosis in patient with chronic viral liver disease and useful as marker of precirrhotic and cirrhotic stage. Regular determination of both CHC in patients may be used as indicators of increasing fibrosis and development of cirrhosis. The addition of some laboratory parameters as PT and platelet count in addition to serum fibrosis markers SHA and MMP-2 may add a prognostic importance. Assessment of hepatic scarring may be performed with combination of novel fibrosis biomarkers, thus eliminating the need for liver biopsy. Further evaluation needs to be performed in large patient populations. Diagnosis of fibrosis during early stages will allow early treatment, thereby preventing fibrosis progression

Correspondent:

Elham Ragab Abdul Samea

Assistant professor in Clinical Pathology
Department of Clinical Pathology

Mansoura University, Egypt

Tel. 02-0114571726

Email: elhamelngar@yahoo.com

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The Effects of Tourism on Quality of Life: A Case Study of Shiraz, Iran

Fariborz Aref

School of Management and Economics, Science and Research Branch
Islamic Azad University, Tehran, Iran
fariborz.aref@gmail.com

Abstract: Does tourism contribute to quality of life in tourism destinations? This study investigates the effect of tourism upon quality of life in Shiraz, Iran. The results revealed that tourism has the positive effect on quality of life of residents. According to the survey, the strongest tourism impacts are found to be linked with emotional well-being, community well-being, and income and employment. While health and safety well-being are found to be the least favorable in terms of the effect of tourism on quality of life. The findings of this study will aid in the planning of strategic development programs for tourist destinations.

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Keywords: quality of life, tourism, community, well-being

1. Introduction

Tourism is often viewed as an expression of human behavior (Kim, 2002). Harrill & Potts (2003, p. 233) believed that "tourism is an invisible industry, encompassing transportation, lodging, and entertainment. Tourism is the set of ideas, theories, or ideologies for being a tourist, and that it is the behavior of people in tourist roles, when these ideas are put into practice (Przeclawski, 1986). Once a community becomes a destination, the lives of residents in the community are affected by tourism, and the support of the residents is essential for the development, planning, successful operation and sustainability of tourism (Kim, 2002). Therefore, the quality of life of the residents should be a major concern for community leaders. Numerous studies have examined local residents' perceptions of the economic, social, cultural and environmental impacts of tourism (Aref, 2010). Although to date, there is a little studies about the effect of tourism on the quality of life of residents in communities (Kim, 2002). Thus, there is limited understanding of residents' perceptions of the effect of tourism on quality of life of residents in Iran. Hence, measuring the effect of tourism on quality of life of residents can help the planners and community developers for to achieving the tourism development goals in communities.

2. Literature review

Tourism is a complex industry. It provides employment opportunities and tax revenues and supports economic diversity. It has very different impacts, both positive and negative, or even mixed ones. However, from a national, regional or local planning point of view, tourism should support the

development of the quality of life of residents too (Puczko & Smith, 2001).

Tourism come in many shapes and forms such as social, cultural, economic and environmental (Godfrey & Clarke, 2000). Tourism has been a source of social-economic change in many developing countries. According to the World Tourism Organization (2009), tourism is one of the world's fastest growing industries and is one of the global engines of development. The tourism industry is an important economic activity involving different groups of community. Tourism is a multi sector, and as a means of economic, social and cultural exchange, it has many aspects and types (Mowforth & Munt, 2003). Tourism growth provides significant economic benefits such as creating employment and additional income in both host countries and tourists' home countries (Delibasic et al., 2008). It is also an industry that has many environmental and social consequences. Few have addressed the effect of tourism on enhancing the quality of life (Cohen, 1978; Gursoy, Jurowski, & Uysal, 2002; Jurowski, Uysal, & Williams, 1997; Kim, 2002; Linton, 1987; Richard R. Perdue, Long, & Kang, 1999).

The concept of quality of life is implicit in much of the academic literature on tourism impacts. Tourism academics have explored in some detail the contributions that tourism makes to various aspects of the quality of life of destination residents (Moscardo, 2009). According to Constan a (2009) tourism can increase quality of life, which is carried out in various forms: rest, relaxation, recreation, maintenance of tone, knowledge development and sense of taste for beauty, aesthetic feelings, cultivate, etc...

Quality of life is the degree of well-being felt by an individual or group of people (Delibasic et al., 2008). Quality of life has been defined by World Health Organization (WHO) as individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns (Skevington, Lotfy, & O'Connell, 2004). Quality of life is measured for many different purposes but mainly for economic and political reasons; in fact indicators of quality of life can give observers information on how well a country or a government is doing if compared to other countries or previous governments, or can be of support when taking certain decisions whose outcomes can impact the quality of life of people or when to evaluate certain strategic decisions at community levels (Delibasic et al., 2008). Historically, the early attempts to measure quality of life have come from the social indicators movement (Biderman, 1974; Parke & Sheldon, 1974). Veenhoven (2005, p. 61) claims that "quality-of-life can be measured by how long and happy people live". According to Argyle & Lu (1990), quality of life is measured by well-being, life satisfaction, made up of happiness, and absence of ill being.

According to Kim (2002) quality of life has been categorized in five domains including; material well-being, community well-being, emotional well-being, and health and safety well-being.

In the present study, material well-being, community well-being, emotional well-being, and health and safety well-being were used to measure the quality of life of the residents in the community (Kim, 2002).

Material well-being: The satisfaction of material well-being can be shared in the form of cost of living and income and employment. Three items for cost of living and four items for income and employment were used to measure residents' satisfaction of material well-being.

Community well-being: There are many aspects of community life and setting that make up people's appreciation or dissatisfaction with the greater than neighborhood area where they live. Four items were used to measure the community well-being domain.

Emotional well-being: Emotional well-being can be satisfied in the form of leisure well-being and spiritual well-being. Four items for leisure well-being and five items for spiritual well-being were proposed to measure residents' satisfaction of the emotional well-being.

Health and safety well-being: The satisfaction of health and safety well-being consists of health well-being and safety well-being. Two item for health and three item for safety well-being were used to measure the health and safety well-being domain (Kim, 2002). The Figure 1 illustrates the contribution of tourism to increase quality of life, which is carried out in various forms: material well-being, health and safety, community well-being, and emotional well-being.

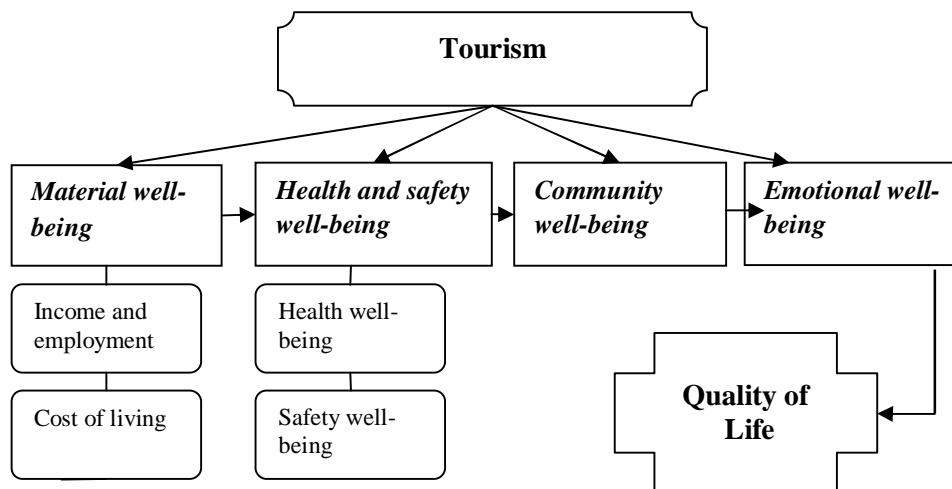


Figure 1: The effect of tourism on quality of life

3. Research Design

This study was carried out in tourism destinations in Shiraz, Iran. It uses questionnaire survey to examine the effect of tourism on quality of life through 200 residents in the communities. The questionnaire was structured around a Likert scale. Each statement was situated on a five scales with 1 representing a response of “strongly disagree” and 5 representing “strongly agree”. A face to face onsite self-administered survey was conducted using the convenience sampling method. Convenience sampling methods has been widely used by almost all the major public opinion polling groups, political groups and market research organization (Deng et al., 2010). Five variables that are presented for measurement the quality of life were; material well-being, community well-being, emotional well-being, and health and safety well-being. The items were adopted from Sirgy (2001), and Kim (2002). Pre-testing of the research instrument was carried out to examine the appropriateness and reliability of the instrument by taking 35 convenience samples. Thirty two questionnaires, a 91.42% response rate, indicated that the results were sufficiently comprehensive and verified the value of the instrument and the statements received.

4. Finding of the Study

According to the results, most respondents were highly educated (61.03), earned more than 700 USD (59.18%), male (82%) and female (18%) with an average age of 55.10 years. Majority of them (62.5%) were engaged in tourism activities. Descriptive statistics revealed that respondents rated higher on positive statements and lower on negative statements, indicating consistency in the direction of their perceptions. As shown in Table 1, the perceived impacts of tourism by residents were measured by 20 impact items embodying both the benefits and costs of tourism on quality of life. Based on the mean measures of impact items, the impact items associated with health and safety well-being have the lowest scores. Some of the health and safety well-being impacts, which are most favored by residents, are as follows: “Increase the water quality” (3.95). Following the health and safety well-being impacts of tourism, Emotional well-being impacts are evaluated relatively favorable. Some of the items comprising the factor of social and cultural are: “cultural lives” (4.49), “spare time” (4.36), “leisure life” (4.15). The findings that show that community well-being, Material well-being are the most favored impacts among the residents of the communities.

Table 1: The effects of on quality of life

Variables of QOL	Indictors	Mean	Std. D
Health and safety well-being	Health well-being		
	Increase the air quality	2.80	1.18
	Increase the water quality	3.95	0.64
	Safety well-being		
	Increase the accident rate	2.02	0.79
	Increase the crime rate	2.93	1.24
Emotional well-being	Increase the safety and security	2.70	1.02
	Spare time	4.36	0.60
	Leisure activity	4.06	0.83
	Your leisure life	4.15	0.58
Community well-being	Your cultural life	4.49	0.52
	Improve the conditions of the community environment	3.21	1.08
	Increase the community services	4.34	0.55
	Increase the community facilities	4.06	0.52
Material well-being	Improve the community residents wellbeing	4.49	0.52
	Income and employment		
	Your income at your current job	4.62	0.47
	Economic security of your job	4.50	0.60
	Your family income	4.59	0.49
	Pay and fringe benefits you get	4.53	0.58
	Cost of living		
	Increase your real estate taxes	3.38	0.94
Increase of cost of living	3.62	1.19	
Increase cost of basic necessities	2.46	1.10	

As shown in Table 1, all the indicators show that the respondents have rather positive attitudes toward tourism impacts in their quality of life in their community. Meanwhile differences among respondents were also observed. Most of the 20 attitudinal items had the max range from the minimum (1 point) to maximum (5 points), indicating a variation of individual respondents' attitudes toward tourism impacts. The size of the standard deviations of the 20 statements also indicated a moderate spread around the theoretical mean. Findings of this study supported the research question that there is positive effect of tourism on quality of life. The result reported here is consistent with previous research findings (Kim, 2002). In consist with this finding Crotts and Holland (1993) summarized that tourism has a positive influence on the quality of life of rural populations. Perdue, Long and Gustke (1991) also analyzed the relationship between tourism and quality of life of local citizen. They concluded that tourism has an influence on type of employment, cost and quality of education and health services provision. Therefore, this study concludes that tourism has the positive effect on quality of life (well-being).

5. Conclusion

The results of this study provided some explanation of tourism effect on quality of life. The results showed that respondents strongly agree that tourism has many effects on their quality of life. As Moscardo (2008) believed, lack of understanding of tourism impacts is a factor for underdevelopment of tourism in third world countries. Hence, the findings help to understanding relationship between tourism impacts and level of quality of life with support for tourism development (Aref, 2010). The findings of this investigation can assist tourism stakeholders community developers in the implementation of tourism development strategies based on residents' attitudes towards tourism impacts on quality of life in tourism destination.

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Zinc and Boron Fertilization on Concentration and Uptake of Copper and Nitrogen in Corn Grain in a Calcareous Soil

Farshid Aref

Department of Soil Science, Science and Research Branch, Islamic Azad University, Fars, Iran
farshidaref@yahoo.com

Abstract: A farm experiment was conducted to study the effect of Zn and B interaction on the concentration and total uptake of nitrogen (N) and copper (Cu) in corn grain at Fars Province of Iran. Treatments included five levels of Zn (0, 8, 16 and 24 kg Zn ha⁻¹ added to the soil and Zn foliar spray with a 0.5 percent concentration) and four levels of B (0, 3, and 6 kg B ha⁻¹ added to the soil and B foliar spray with a 0.3 percent concentration) in a completely randomized block design. A high Zn content in the soil helped increasing the concentration and uptake of N in the grain by B application; that is, at high levels of Zn, there was a synergism between B and N. Boron spraying helped with increasing the concentration and uptake of N in the grain by Zn application. There was a negative correlation between N and Cu concentration in the grain and a positive correlation between N and Cu uptake in the grain.

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Keywords: Antagonism, Deficiency, Fertilizer, Interaction, Nutrients, Synergism

1. Introduction

Nitrogen is present in a part of any protein compounds, any enzyme, metabolism intermediate compounds, compounds involved in production and transfer of matter and energy and even in the structure of ribonucleic acid that conducts the transfer of genetic traits. In addition to being involved in the structure of proteins, it makes up a part of the chlorophyll as well. Nitrogen is the first nutrient the deficiency of which being referred to when speaking about soils in arid and semi-arid regions. Sufficient supply of N is associated with high vegetative growth and dark green color (Tisdale et al., 1993). The N content in plant organs is next to carbon, oxygen and hydrogen (Tisdale et al., 1993). Agafone (1991) has reported that by increasing the use of N fertilizers, the plant's need to Cu will increase. Nitrogen causes an increase in uptake and concentration of Cu in the wheat the reason of which is attributed to a change in soil pH and, consequently, an increase in the solubility of Cu in the soil solution, increase in root volume and its extension, as well as the synthesis of compounds that may be carriers for Cu uptake. The plant's Cu is mostly involved in enzymatic activities. Its presence is necessary in enzymatic oxidase – catalase systems. Also, this element is involved in electron transfer reactions and is the activator of several enzymes (Tisdale et al., 1993). It is also involved in the metabolism of proteins and carbohydrates and N fixation (Pals and Benton, 1997). A correct balance between Zn and Cu concentrations plays a significant role in their uptake level. There are many reports on zinc-copper interaction in references. Copper is necessary for protein production. Therefore, adding it to soils with

Cu deficiency leads into an increase in their protein content. Pals and Benton (1997) state that the Cu uptake level is lower than most micronutrients.

Based on a report by Dhillon et al. (1987), in the Zn deficiency conditions, conversion of N to protein compounds is reduced and the buildup of amino acids and amids in the plant under these conditions is an evidence for the importance of Zn in protein synthesis. According to a report made by Price et al (1972), Zn deficiency in the plant is associated with RNA and ribosome reduction the result of which is a defect in protein synthesis and consequently, build up of free amino acids in the plant. Ribosomes are located on the cell RNA and are involved in protein production. Nuttal et al. (1987) report that the joint use of B and S increases the grain protein content while joint B and N use reduces the protein content and increases the oil content. Boron is effective in the metabolism of N compounds in the plant and in its deficiency, soluble N compounds, especially the nitrates, build up in the plant (Marschner, 1995). Increase in the Zn level, affects the N uptake and production in the plant (Dhillon et al. (1987). There are many reports on the effect of N on Zn uptake (Karimian, 1995; Mishra and Singh, 1996). Gupta and Patalia (1993) reported that Zn application had no effect on N uptake if N fertilizer was not used, while with using N, application of Zn increased N uptake. They attributed the increase in N uptake to an increase in the dry weight of the airborne organs. Due to the role of Zn in RNA and protein synthesis, Zn use increases the effectiveness of N in the plant (Kitagishi et al., 1987).

Hussien and Faiyad (1996) reported that by Zn application, the plant's N concentration increased.

Gupta et al. (1986) observed that with the use of 2.5 mg Zn/kg soil, the N uptake by corn increased from 53.4 mg in the control to 206.2 mg in the fertilized treatment. Many authors have reported the effect of Zn on the increase in N uptake by the plant (Salam and Subramanian, 1988; Sahu et al., 1996). Latife (1983) reported that by using zinc sulfate, the plant height, ear length and diameter, the number of grains per ear, the weight of 1000 grains, grain yield and the N uptake significantly increased. Kumar et al. (1981) stated that adding Zn to the soil reduced Fe, Mn and Cu concentration and increased Zn concentration in the plant. Many reports are made by authors on zinc-copper interaction (Cayton et al. 1983). Some authors reported that Zn use increased plant growth and reduction of Cu concentration in corn and cereals. Some authors attribute the antagonism of Cu and Zn uptake to having common uptake sites on the root surface and some have attributed the same to their antagonism in transfer of one another from the root to airborne organs (Cayton et al. 1983; Mesquita, 2000). Parker et al. (1992) showed that Zn application increased Mn concentration in the plant but had no effect on Fe and Cu concentrations. Gupta (1993) has reported an interaction between N and B.

The grain being rich in nutrients, including N and Cu plays an important role in human nutrition. The grain being rich in such elements is also an evidence for improve of the harvest, qualitatively and quantitatively. Therefore, by studying Zn and B interaction in the grain, we can find its indirect effects that arte improvement of the harvest, qualitatively and quantitatively, while enriching the grain as well. Also, it has been shown that if we use grains rich in these elements as seeds, we can improve the harvest, qualitatively and quantitatively.

The objective of the study was to evaluate the concentration and uptake of N and Cu in corn grain as affected by Zinc sulfate and Boric acid application.

2. Materials and Methods

A field experiment was conducted at the farm of Aref in Abadeh Tashk, Fars province of Iran, on the corn (*Zea mays* L.), cultivar "Single Cross 401" during 2009 cropping season. The site is located 200 km northeast of Shiraz, with latitude 29° 43' 44" N and longitude 53° 52' 07" E and 1580 m altitude. Before implementing the project sampling from the soil (0-30 cm depths) was made in order to select a zone in which the available amount of Zn and B was low (less than 1 mg kg⁻¹ extracted by methods DTPA and hot water, respectively). This soil had a loam texture, pH of 8.2, 0.59 % organic matter, 229 mg kg⁻¹ available K, 12.1 mg kg⁻¹ available P, DTPA extractable Fe, Mn, Zn and Cu concentration were

1.65, 8.14, 0.32 and 0.62 mg kg⁻¹ and available B with hot water extractable was 0.78 mg kg⁻¹.

This experiment included 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels of Zn (0, 8, 16 and 24 kg Zn ha⁻¹ added to the soil, and Zn solution spray with a 0.5 percent concentration) and four levels of B (0, 3, and 6 kg B ha⁻¹ added to the soil, and B solution spray with a 0.3 percent concentration). Due to a high pH and the high calcium content of the soil in question, a high level of Zn was used. Nitrogen, P and K used at 180, 70 and 75 kg ha⁻¹ according to the recommendation, from sources of urea (with 46% N), triple super phosphate (with 46% P₂O₅) and potassium sulfate (with 50% K₂O), respectively, were added to all treatments (plots). Half of the urea was used when planting and the remainder at two different times: at vegetative growth and when the corn ears were formed. Zinc and B, from zinc sulfate and boric acid sources, respectively, were used by two methods, adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 0.5 % zinc sulfate and 0.3 % boric acid two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L/ha using a knapsack sprayer. Each experimental plot was 8 m length and 3 m width, had 5 beds and 4 rows, equally spaced, and seeds 20 cm apart on the rows.

Analysis of the grain and soil was carried out using common lab procedures. Phosphorous in soil was measured by Olsen method, available K by 1 M NH₄OAc extraction method and potassium assessment in the extract by flame photometer, organic carbon by the Walkley and Black method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption setup. The soil available B was extracted by hot water and then was measured by spectrophotometer by azomethine-H colorimetric method. For N determination, dried grains were digested with 2 N HCl and were analyzed by micro-Kjeldahl method (Bremner and Mulvaney, 1982). Digestion method by dry burning was used to measure Cu and then they were measured by atomic absorption setup. Statistical analysis of data was made using SAS software with Duncan test.

3. Results and Discussion

3.1. Soil analysis before culture

The results of soil analysis before culture are summarized in Table 1. The P, Zn, B, Cu and Fe in the soil were low but soil available K and Mn were higher than the critical level. Karimian and Ghanbari

(1990) reported the critical P level by Olsen method in calcareous soils to be 18 mg kg⁻¹. Sims and Johnson (1991), reported the critical limits of soil Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water method to be 2.5-5. 0.2-2, 1-5, 0.1-2.5 and 0.1-2 mg kg⁻¹, respectively. Agrawala (1992) reported that the critical level of Fe, Zn, Mn and Cu in the soil by the DTPA extraction were 2.5, 0.8, 5.5 and 0.75 mg kg⁻¹ soil, respectively.

Table 1. Soil mechanical and chemical analysis

Soil properties	Values
Depth of soil(cm)	0-30
Soil texture	Loam
pH	8.2
EC (ds m ⁻¹)	2.41
Organic matter (%)	0.59
Nutrients (mg kg ⁻¹)	
P	12.1
K	229
Fe	1.65
Mn	8.14
Zn	0.32
Cu	0.62
B	0.78

3.2. Nitrogen concentration in the grain

The effects of Zn and B on the grain N concentration were insignificant at a 5% level (table 2). The study of the effect of Zn and B interaction on the grain N concentration showed that B use only at 24 kg ha⁻¹ Zn level increased the N concentration in the grain. Application of 3 kg ha⁻¹ B at 24 kg ha⁻¹ Zn increased the grain N concentration from 1.5 to 1.8 percent (20% increase as compared with no B use at this Zn level) while other B levels showed no significant effect. At other Zn levels, application of B had no effect on grain N concentration. Zinc spraying use only at the B solution spraying level increased grain N concentration from 1.55 to 1.99 percent, showing a 28.38 percent increase as compared with the no Zn use level. The lowest and the highest leaf N concentration, 1.5 and 1.99%, were observed at using 24 kg ha⁻¹ Zn and joint Zn and B spraying levels, respectively. Except for these two treatments, other treatments showed no significant difference from the control.

3.3. Nitrogen uptake by the grain

The main effect of Zn on N uptake by the grain (kg ha⁻¹) was significant at 5% level (table 3). The lowest mean N uptake by the grain at 125.99 kg ha⁻¹ was seen at no Zn level. With applying 16 and 24

kg ha⁻¹ Zn, N uptake by the grain increased from 125.99 at zero Zn level to 155.92 and 148.57 kg ha⁻¹, respectively (23.76 and 17.92 percent increase, respectively), but no significant difference was seen between these two Zn levels. Zinc spraying increased grain N uptake to 155.79 kg ha⁻¹, showing a 23.65% increase relative to zero Zn level; but there was no significant difference between the Zn spraying and applying Zn to the soil in that regard. The highest N uptake by the grain, which is 155.92 kg ha⁻¹, was seen at 16 kg ha⁻¹ Zn level.

The effect of applying different B levels on N uptake by the grain was significant at 5% level. The lowest grain N uptake, 129.07 kg ha⁻¹, was seen at zero B level. Boron use at all levels (applying to the soil and spraying) increased grain N uptake relative to zero B use. The use of 3 and 6 kg ha⁻¹ B increased grain N uptake from 129.07 at zero B level to 150.78 and 148.56 kg ha⁻¹, respectively (16.82 and 15.1 % increase, in that order); but there was no significant difference between these two B levels in that regard. Boron spraying, too, increased grain N uptake from 129.07 to 155.26 kg ha⁻¹, a 20% increase relative to zero B level; but no significant difference was seen between Zn spraying and its addition to the soil in that regard. The highest grain N concentration was due to B spraying.

Studying the effect of Zn and B interaction on grain N uptake showed that B use at highest Zn level (24 kg ha⁻¹ Zn), increased grain N uptake but at other Zn levels, it showed no significant effect on the N uptake. At 24 kg ha⁻¹ Zn level, the use of 3 kg ha⁻¹ and B spraying significantly increasing grain N uptake from 115.65 kg ha⁻¹ to 165.99 and 174.53 kg ha⁻¹, respectively (43.52 and 50.91 percent increase relative to zero B use), but the use of 6 kg ha⁻¹ B had no significant effect.

Zinc use at B spraying level, significantly increased grain N uptake but at other B levels, had no significant effect on N uptake. At B spraying level, application of 16 and 24 kg ha⁻¹ Zn, increased grain N uptake from 116.43 to 171.9 and 174.53 kg ha⁻¹, respectively (47.64 and 49.9 percent increase, respectively). Zinc spraying at B spraying level, too, increased grain N uptake to 169.38 kg ha⁻¹ (45.48% increase) but no significant difference was seen with the case in which Zn was directly applied to the soil.

The lowest N uptake by the grain, 108.83 kg ha⁻¹, was seen in the case where no Zn and no B was used (the control). The highest N uptake by the grain, 174.9 kg ha⁻¹, was seen when 6 kg ha⁻¹ B + 16 kg ha⁻¹ Zn was used, leading to 60.71% increase relative to the control.

Table 2. The effect of Zn and B on the N concentration (%) by the grain*

B (kg ha ⁻¹)	Zn (kg ha ⁻¹)					Foliar Spray	Mean
	0	8	16	24			
0	1.62 bcd	1.6 bcd	1.69 bcd	1.5 d	1.75 abcd	1.63 a	
3	1.76 abcd	1.63 bcd	1.65 bcd	1.8 abc	1.67 bcd	1.72 a	
6	1.83 ab	1.64 bcd	1.81 abc	1.69 bcd	1.71 bcd	1.74 a	
Foliar Spray	1.55 cd	1.66 bcd	1.76 abcd	1.7 bcd	1.99 a	1.73 a	
Mean	1.69 ab	1.63 b	1.73 ab	1.67 ab	1.78 a		

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 3. The effect of Zn and B on the N uptake (kg ha⁻¹) by the grain*

B (kg ha ⁻¹)	Zn (kg ha ⁻¹)					Foliar Spray	Mean
	0	8	16	24			
0	108.83 b	138.69 ab	128.51 ab	115.65 b	153.68 ab	129.07 b	
3	145.2 ab	148.07 ab	148.37 ab	165.99 a	146.28 ab	150.78 a	
6	133.5 ab	142.45 ab	174.9 a	138.09 ab	153.84 ab	148.56 a	
Foliar Spray	116.43 b	144.08 ab	171.9 a	174.53 a	169.38 a	155.26 a	
Mean	125.99 b	143.32 ab	155.92 a	148.57 a	155.79 a		

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.4. Copper concentration in the grain

The main effect of Zn and B on the grain Cu concentration was not significant at a 5% level (table 4). In the study of the effect of Zn and B interaction on the grain N concentration, it was observed that at 8 kg ha⁻¹ Zn, application of B only in spray form reduced grain Cu concentration from 4.67 to 2.67 mg kg⁻¹ (42.82% reduction) but at other Zn levels, B use had no significant effect on the grain Cu concentration. Zinc application had no effect on Cu concentration in the grain at any B levels.

No treatment showed a significant difference from the control. The highest and the lowest grain Cu concentration, 2.67 and 4.67 mg/ha, showed 19.82% reduction and 40.42% increase as compared with 3.33 mg kg⁻¹ of the control.

3.5. Copper uptake by the grain

The main effect of Zn and B on the grain Cu uptake was not significant at a 5% level but the effect of Zn and B interaction was significant at 1% level (table 5). Boron application in the form of spraying at 8 kg ha⁻¹ Zn level reduced Cu uptake by the grain

from 39.6 to 23.13 g/ha (41.59% reduction) but application of B directly to the soil had no significant effect. At a high Zn level (24 kg ha⁻¹ Zn), only B spraying increased Cu uptake by the grain from 26.43 g/ha to 44.57 g/ha (68.63% increase) but at other Zn levels, B application showed no significant effect on uptake. Probably a high soil Zn content (24 kg ha⁻¹ Zn) reduced B toxicity and, consequently, an increase in Cu uptake by the grain with B application; but a low Zn level (8 kg ha⁻¹ Zn) was not able to reduce the toxicity and, consequently, the Cu uptake by the grain with B application was reduced.

Zinc use in cases where B was not applied directly to the soil (zero and B spraying levels) increased Cu uptake by the grain but in cases where it was applied directly to the soil (3 and 6 kg ha⁻¹ B), it had no significant effect on Cu uptake by the grain. At zero B level, only application of 8 kg ha⁻¹ Zn significantly increased Cu uptake by the grain from 22.53 to 39.6 g/ha (75.76% increase). The use of 24 kg ha⁻¹ Zn at B spraying level increased Cu uptake by the grain from 25.17 to 44.57 g/ha (77.07% increase) but other Zn levels had no significant effect. Probably

due to a Zn and B antagonism, B application prevented from Zn use affecting Cu uptake by the grain.

Table 4. The effect of Zn and B on the Cu concentration (%) by the grain*

B (kg ha ⁻¹)	Zn (kg ha ⁻¹)				Foliar Spray	Mean
	0	8	16	24		
0	3.33 abcd	4.67 a	4 abc	3.33 ab	3 bc	3.67 a
3	4 abc	4.33 ab	4 abc	2.67 c	3.67 abc	3.73 a
6	3.67 abc	3.33 abc	2.67 c	3 bc	3.67 abc	3.27 a
Foliar Spray	3.33 abc	2.67 c	3.33 abc	4.33 ab	3 c	3.33 a
Mean	3.58 a	3.75 a	3.5 a	3.33 a	3.33 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 5. The effect of Zn and B on the Cu uptake (kg ha⁻¹) by the grain*

B (kg ha ⁻¹)	Zn (kg ha ⁻¹)				Foliar Spray	Mean
	0	8	16	24		
0	22.53 e	39.6 ab	30.5 bcde	26.43 cde	26.4 cde	29.09 a
3	32.93 abcde	38.57 abc	36.3 abcd	24.93 de	31.8 bcde	32.91 a
6	26.1 cde	29.13 bcde	25.9 de	24.43 de	33.17 abcde	27.75 a
Foliar Spray	25.17 de	23.13 e	32.6 abcde	44.57 a	24.23 de	29.94 a
Mean	26.68 a	32.61 a	31.33 a	30.09 a	28.9 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.6. Correlation between the concentration and total uptake of Cu and N in grain with other variables

Concentration and uptake and other variables, correlation coefficients (R) and (R²) between different variables were computed using the Pearson method and equations relating to each variable were derived using the step-by-step method. The symbols * and ** in equations denote significance at 5 percent level ($\alpha = 0.05$) and 1 percent level ($\alpha = 0.01$) respectively.

3.6.1. Nitrogen concentration in the grain

The grain N content showed a positive correlation with the leaf K content (R= 0.38), Fe (R= 0.32), Zn (R= 0.31) and B (R= 0.47*), the grain B content (R= 0.43), the uptake of N (R= 0.67**) and B (R= 0.42) by the grain, the percentage of grain in the ear (R= 0.40) and grain protein content (R= 0.99**)

and a negative correlation with the leaf N content (R= -0.38) and P (R= -0.33), the grain Cu content (R= -0.32), the ear length (R= -0.33), the ear diameter (R= -0.40) and 1000-grain weight (R= -0.38). The equations of which were:

- 1) $NG = -0.00144 + 0.176 P$ $R = 1^{**}$
- 2) $NG = -0.00186 + 0.176 P - 0.00000945 BUG$ $R = 1^{**}$
- 3) $NG = 0.00408 + 0.176 P - 0.0000143 BUG + 0.0000496 NGL$ $R = 1^{**}$

NG, P, BUG and NGL are N grain content (%), grain protein content (%), B uptake by the grain (g ha⁻¹) and number of grains in the ear length, respectively.

3.6.2. Nitrogen uptake by the grain

There was a positive correlation between N uptake by the grain and the leaf Zn content (0.41), the grain N content (0.67**), P content (R= 0.33), Mn content (0.38) and B content (0.53*), the uptake of P

(0.79^{**}), K (0.78^{**}), Fe (0.31), Mn (0.63^{**}), Zn (0.56^{**}), Cu (0.31) and B (0.76^{**}), ear weight (0.68^{**}), grain weight in the ear (0.69^{**}), total grain yield (0.88^{**}), the number of grains in the ear length (0.58^{**}), the number of grains across the ear diameter (0.31), grain protein content (0.67^{**}), and a negative correlation with leaf Mn content (-0.32) and Cu content (-0.33). The equations of which were:

$$1) \text{NUG} = -22.417 + 0.0197 \text{TGY} \quad R = 0.882^{**}$$

$$2) \text{NUG} = -145.955 + 0.017 \text{TGY} + 86.073 \text{NG} \\ R^2 = 0.998^{**}$$

$$3) \text{NUG} = -146.408 + 0.0171 \text{TGY} + 83.816 \text{NG} + 4.847 \text{CuS} \\ R^2 = 0.999^{**}$$

$$4) \text{NUG} = -139.577 + 0.0172 \text{TGY} + 81.666 \text{NG} + 6.573 \text{CuS} - 0.000374 \text{DM} \\ R^2 = 0.999^{**}$$

NUG, TGY, NG, CuS and DM denote N uptake by the grain (kg ha⁻¹), total grain yield (kg ha⁻¹), grain N content (%), soil Cu content after harvest (mg kg⁻¹) and dry matter (kg ha⁻¹), respectively.

3.6.3. Copper concentration in the grain

The grain Cu content showed a positive correlation with the leaf P content (R = 0.39), Cu uptake by the grain (R = 0.85^{**}), and a negative correlation with the percentage of grain in the ear (R = -0.30) and grain protein content (R = -0.32). The equations of which were:

$$1) \text{CuG} = 1.063 + 0.0814 \text{CuUG} \quad R = 0.854^{**}$$

$$2) \text{CuG} = 3.624 + 0.114 \text{CuUG} - 0.000412 \text{TGY} \\ R^2 = 0.988^{**}$$

$$3) \text{CuG} = 3.089 + 0.117 \text{CuUG} - 0.000436 \text{TGY} + 0.0667 \text{P} \\ R^2 = 0.991^{**}$$

$$4) \text{CuG} = 3.193 + 0.116 \text{CuUG} - 0.000403 \text{TGY} + 0.0726 \text{P} - 0.00198 \text{GW} \\ R^2 = 0.993^{**}$$

CuG, CuUG, TGY, P and GW are grain Cu content (mg kg⁻¹), Cu uptake by the grain (g ha⁻¹), total grain yield (kg ha⁻¹), grain protein content (%) and grain weight in the ear (g), respectively.

3.6.4. Copper uptake by the grain

There was a positive correlation between Cu uptake by the grain and with the leaf P content (R = 0.46^{*}), the grain Cu content (R = 0.85^{**}), the uptake of N (R = 0.31), P (R = 0.36), Mn (R = 0.41), and Zn (R = 0.39) by the grain, ear weight (R = 0.33), the total grain yield (R = 0.55^{**}), the number of grains along the ear (R = 0.44^{**}), the number of grains across the ear diameter (R = 0.43), and a negative correlation with leaf Fe content (R = -0.53) and grain K content (R = -0.40). The equations of which were:

$$1) \text{CuUG} = -1.421 + 8.955 \text{CuG} \quad R = 0.854^{**}$$

$$2) \text{CuUG} = -31.551 + 8.695 \text{CuG} + 0.00363 \text{TGY} \\ R^2 = 0.992^{**}$$

$$3) \text{CuUG} = -25.995 + 8.484 \text{CuG} + 0.00374 \text{TGY} - 0.596 \text{P} \\ R^2 = 0.995^{**}$$

$$4) \text{CuUG} = -27.144 + 8.553 \text{CuG} + 0.00348 \text{TGY} - 0.642 \text{P} + 0.167 \text{GW} \\ R^2 = 0.996^{**}$$

CuUG, CuG, TGY, P and GW are Cu uptake by the grain (g ha⁻¹), grain Cu content (mg kg⁻¹), total grain yield (kg ha⁻¹), grain protein content (%) and grain weight in the ear (g), respectively.

4. Conclusion

Application of Zn to the soil and spraying it increased N uptake in the grain. The least and the highest N uptake in the grain at 125.99 and 155.92 kg ha⁻¹, were seen at no Zn level and 8 kg ha⁻¹ Zn level, respectively. Also application of B at all levels increased N uptake in the grain. The least and the highest N uptake in the grain at 129.07 and 155.26 kg ha⁻¹, were seen at no B level and B spraying level, respectively. Boron spraying at 8 kg ha⁻¹ Zn level decreased Cu concentration and uptake in the grain; but at 24 kg ha⁻¹ Zn level, increased Cu uptake in the grain. Zinc application had no effect on Cu concentration in the grain at any B levels. Application of 8 kg ha⁻¹ Zn at zero B level, and 24 kg ha⁻¹ Zn at B spraying level, increased Cu uptake in the grain.

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Rural Cooperatives for Poverty Alleviation in Iran

Abrisham Aref

School of Humanities and Social Science, Science and Research Branch
Islamic Azad University, Tehran, Iran; abrishamaref@yahoo.com

Abstract: This paper provides evidence on the contribution of rural cooperatives to poverty alleviation in Iran. Rural cooperatives are certainly a major contributor to poverty alleviation in many countries. But, there are a significant number of barriers to effectively using rural cooperatives as a tool for poverty alleviation in developing countries. The result of this study found that rural residents have negative perception towards contribution of rural cooperatives for poverty alleviation. They referred to the dependency of rural cooperatives to government and lack of cultural capacity for group collaboration as main barriers related poverty alleviation through rural cooperatives. The finding can assist the rural developers at the local and national level for remove this problem in face of rural cooperatives for poverty alleviation.

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Key words: Poverty alleviation, rural cooperatives, rural development

Introduction

Cooperatives are the groups of people who work together voluntarily to meet their common economic, social, and cultural needs through a jointly owned and democratically controlled enterprise (Tanzanian Federation of Cooperatives, 2006). The International Cooperative Alliance (ICA) defines a cooperative as “an autonomous association of persons united voluntarily to meet their common economic, social and cultural needs and aspirations through a jointly owned and democratically-controlled enterprise”(ICA, 1995). A co-operative can therefore provide a hub for organizing particular local economic interests and/or for protecting common pool resources (Simmons & Birchall, 2008a). This suggests that rural cooperatives are, first and foremost, voluntary business associations formed by people of limited means through contribution of share capital that forms the basis of sharing out the profits that accrue from the business (Wanyama, Develtere, & Pollet, 2008). In developed countries rural cooperatives have significantly contributed to the mobilization and distribution of financial capital; created employment; constituted a forum for education and training; social welfare, and poverty alleviation and other socio-economic problems (Tanzanian Federation of Cooperatives, 2006). Prakash (1999: 47-48) also identifies the significance of rural co-operatives in rural development. From this point of view, Moradi (1999: 33-46) states that attacking poverty requires attacking many interrelated forces that keep poor people in a state of deprivation. Above all, it requires expanding people’s access to participation in decision-making and to

knowledge, training and markets and the other productivity resources for income generation such as land, technology, credit and information. The study actually implies the importance of people empowerment. It is reasonable since however well are the poverty alleviation programs without improving people’s capacity it will be worthless. In Iran, although, there are many cooperatives in rural area, but, it is alleged that cooperative does not play a significant role in poverty alleviation in Fars, Iran. Hence, this study attempts to shows the barriers of poverty alleviation through rural cooperatives in the study area.

Literature Review

The role and potential of co-operatives have recently started to come to the fore again in discussions about poverty alleviation (Simmons & Birchall, 2008a). From this perspective, it is evident that rural cooperatives have significantly contributed to the mobilization and distribution of financial capital by creating employment and income-generating opportunities for both their members and non-members alike, given that membership is open to all persons without ethnic, class or professional biases (Wanyama et al., 2008).

The history of co-operatives provides evidence of both success and failure in poverty alleviation. However, it is difficult to sum up the strengths and weaknesses of co-operative sectors, as these vary dramatically between types, between countries, and over time (Simmons & Birchall, 2008b).

The rural cooperatives in Iran in the recent years have diversified themselves into various areas

of socio-economic activities. For certain activities, the success of which is based on the ability of the grassroots institutions to tackle them with their participatory and people-based approach, the cooperatives are considered to have an advantage over other organizations. In Iran the rural cooperatives are considered most effective organizations in rural agriculture. Similarly, because of their vast network and reach, the rural cooperatives are considered best promoters for rural development in Iran (Aref & Sarjit, 2009).

Poverty has been defined as the “denial of opportunities and choices most basic to human development to lead a long, healthy, creative life and to enjoy a decent standard of living, freedom, dignity, self-esteem and respect from others” (Hirschowitz et al., 2000, p. 54). Poverty can be reduced through rural cooperatives. Cooperatives create employment opportunities in three different ways. First, they offer direct wage employment to people who work in primary and secondary cooperatives. Secondly, cooperatives offer self-employment to members, whose participation in the economic activities that they make possible substantially guarantees a decent income. Thirdly, cooperatives also indirectly employ through the spillover effects of their activities on non-members whose income-generating activities are only viable through the transactions they have with, as well as opportunities created by, cooperative ventures (Wanyama et al., 2008). Co-operatives’ main purpose is to serve the needs of their members. Co-operatives therefore become actively involved in the process of poverty alleviation where their leaders are called upon by members to do so (Simmons & Birchall, 2008a). Rural cooperatives have been cited as a goal in rural participation for rural development processes (Aref & Sarjit, 2009). Birchall (2003; 2004) developed six arguments for the potential of rural cooperatives in poverty alleviation.

- 1) Rural cooperative values and principles provide built-in advantages for poverty alleviation
- 2) The history of co-operatives in developed countries shows great achievements in poverty alleviation.
- 3) Even though there have been failures in co-operatives in developing countries these do not indicate weaknesses in the co-operative model.
- 4) The essential nature of rural cooperative form of organization is now much clearer.
- 5) The participatory development and rural cooperative development share the same underlying principles.

6) The UN’s Millennium Development Goals and the poverty alleviation strategy of the World Bank need co-operative development if they are to succeed (Simmons & Birchall, 2008b).

In despite of these potential, there is a number barriers for poverty alleviation through rural cooperative. Jamieson and Nadkarni, (2009) identified some barriers to effectively using rural cooperatives as a tool for poverty alleviation. These barriers include:

- 1) Lack of organizational capacity of rural cooperatives to respond to the opportunities provided by rural residents.
- 2) Within the developing countries there is very little recognition of the potential of rural cooperatives by aid agencies.
- 3) Lack essential market knowledge to allow local communities to develop rural cooperatives (Jamieson & Nadkarni, 2009).

The ODI lists the different types of barriers to consider in this regard (Bolwell & Weinz, 2008).

Organizational factors can cause failures and reduce the chances of success for poverty alleviation (Bolwell & Weinz, 2008). In this sense, rural cooperatives may be more difficult to achieve than other activities in developing countries. Barriers are generally similar in these communities, but a few factors tend to be more pronounced among these communities: Lack of formal education and lack of planning (Bushell & Eagles, 2007, p. 154). As a consequence, community facilities and services may be unacceptable for rural cooperatives. Hence building capacity in rural communities is necessary for stakeholders involved in rural cooperatives development (Bushell & Eagles, 2007).

Methods

This study is based on quantitative method to investigate the barriers of rural cooperatives related to poverty alleviation. Focus group discussion (FGD) was performed to collect data from rural residents in ten villages in Fars province, Iran. FGD was used for obtaining a better understanding of participants’ attitudes (Aref, 2010b). There is no consensus among researchers on the optimal number of participants in FGD. But the ideal number in each FGD is six to ten. All respondents were male. Seventy two people were participated in FGD. They ranged in age from 29 - 78 years. The researcher explained to them the objectives of the study. The researchers examined, categorized participants responses from each focus group of villagers that were recorded in video tapes.

Table 1: Barriers of poverty alleviation through rural cooperatives

Barriers	Description
Lack of human capital	Low literacy and poor job skills.
Gender norms and constraints	Beliefs that women should not work.
Lack of social capital	Poor communities are often not represented in economic planning.
Lack of financial capital	Lack of micro credit, or revolving loan facilities.
Location	Many poor people may live remote from places
Lack of land ownership and tenure	Many poor countries have no effective rights of land ownership
lack of planning gain	Developments set up in remote rural areas
Low capacity	Poor communities may be unaware of cooperatives' role
Lack of pro-active government support for involvement by the poor	Rural cooperatives not included in development strategies, market facilities not provided, education levels low.

Source: Methodology for PPT case studies (2002)

Result and Discussion

According to the collected baseline data, farming was the most common occupation in the villages. There were overall 75 participants with an average of 58 years old. All participants; were males. They were chosen because of their engagement in agricultural activities. The questions were asked about to contribution of rural cooperative in poverty alleviation and barriers of poverty alleviation in terms of rural cooperative.

In terms of contribution of rural cooperatives on poverty alleviation most of the participants had an overall negative perception towards rural cooperatives on poverty alleviation. They believe that rural cooperatives does not have important role in their villages especially on poor people. The authors' observation also confirmed this argument. The findings showed that rural cooperatives in their villages are without any certain planning for poverty alleviation. In this way the respondent in FGD referred to variety barriers for poverty alleviation in their villages. The study refers to some common barriers which have been discussed in majority of FGD groups:

1) Lack of participation: Rural participation in rural cooperatives planning and decision making is not considered. Decisions making in rural cooperatives policy are mostly made by government. In fact the lack of really local residential involvement in the decision-making and unable rural power that exist to create a decision making for development cooperatives for poverty alleviation. The World Bank (1993) recognized lack of active participation as a reason for failure of many development attempts in developing countries (Aref, 2010a). Hence, the findings showed the lack of participation is a barrier for poverty alleviation through rural cooperatives.

2) Lack of resources: Most participants in FGD groups mentioned to lack of resource in the villages as main obstacles to rural cooperatives for poverty alleviation. These findings are supported by Atkisson et al., (2003) which referred to certain limitations such as lack of resources and planning as a common problems for development in third world countries.

3) Lack of independently: Involving the government to provide funding for rural cooperatives was other barrier.

4) Lack of cooperatives leaders' knowledge: The participants in all groups mentioned that the cooperatives leaders are without adequate knowledge for manage the cooperatives.

5) Lack of collaboration' cultures: FGD respondents believed the lack of culture of collaboration among local people was behind the failure investment for poverty alleviation. The individualism is the one feature of Iranian culture. Hence, in this situation the collaboration in cooperatives cannot be success.

5) Lack of awareness about role of cooperatives: The people through FGD though the role of cooperatives is to distribution of some goods between rural residents. Hence, they weren't aware about role of cooperatives. It also can have an effect on the apathy regards rural residents' participation in rural cooperatives.

Through the findings of this study these barriers were identified: Lack of resources, lack of collaboration culture, lack of cooperatives leaders' knowledge, dependently of cooperatives to government, were an important element contributing to limited rural cooperatives for poverty alleviation. As have been mentioned by Jamieson and Nadkarn (2009), the rural cooperatives has some barriers related to poverty alleviation in Asian countries. Hence this argument has been confirmed by this

study. According to the findings, the level of contribution of rural cooperatives in poverty alleviation in Iran is weak. In considering the application of rural cooperatives in poverty alleviation, the role of the rural leaders deserves consideration. Important role of leaders with respect to rural cooperatives would include facilitating; encourage participants, encouraging learning, and developing local skills in rural areas.

Conclusion

The purpose of this article has been to demonstrate the contribution of rural cooperatives to poverty alleviation in Iran. Overall the findings indicated that residents have negative attitude towards contribution of rural cooperatives for poverty alleviation. They referred to government policy and lack of organizational capacity as main barriers related poverty alleviation through rural cooperatives. Clearly, the described barriers may not be only specific to rural cooperatives strategy; some of them may also be considered as common general problems of rural cooperatives in other communities in Iran. Hence, it should be accepted that these barriers may be an extension of the prevailing social, political and economic structure in Iran, which have prevented communities from achieving a higher level of development. To avoid breakdowns, people's participation need to be constantly encouraged. Various methods could be applied to foster participation such as decentralization of local government. Since the government has important role in poverty reduction and rural development, it must create the basic conditions that facilitate and stimulate economic growth and development in rural areas. It also must create a favorable climate for investment and transparent economic and agricultural policies for farmers. The findings of this study can be useful for academics, researchers and all stakeholders involved in designing, assessing or promoting rural cooperatives projects which are in any way associated with general development goals.

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Awareness of Hospital Internal Disaster Management Plan among Health Team Members in A University Hospital

Hemat Abd elazeem¹, Samia Adam^{*1}, and Gehan Mohamed²

¹Faculty of Nursing, Ain Shams University, Cairo, Egypt

² Faculty of Nursing, Helwan University, Helwan, Egypt. *asamia@hotmail.com

Abstract: A disaster management plan is a formal plan of action which enables the hospital staff to respond effectively and efficiently when confronted with a disaster. The aim of this study was to assess the awareness of health team regarding hospital internal disaster management plan at a university hospital. The study was conducted in a university hospital using a cross-sectional design. It included six groups of subjects namely, medical leaders, head nurses, staff nurses, technicians, employees, housekeepers, in addition to a jury group to test validity of the study tool. A self-administered questionnaire form was used to assess staff awareness about the internal disaster management plan in the hospital. The results showed the absence of a disaster plan in the study setting and absence of a hospital evacuation plan. Also the majority of various categories of the study subjects had low awareness about all items of the disaster plan. It is concluded that there is a need for an internal disaster plan for the hospital, and the awareness of study subjects about internal disaster preparedness need to be raised. Therefore, it is recommended that the hospital administration should develop policies for disaster management and pay more attention to the problem of internal disasters and preparedness for their management. Training programs are essential for all categories of hospital staff in order to increase their awareness about disaster management.

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1. Introduction:

Disaster can be defined as a sudden extraordinary event that brings great damage, loss, destruction and injury to people and their environment (Stanhope and Lancaster, 2008). Alternatively, disaster is defined as a phenomenon that produces large-scale disruption of societal infrastructure and the normal healthcare system, presents immediate threat to public health, and requires external assistance for response (Bradt and Drummond, 2007). A disaster may be natural or human-made incident that causes destruction that cannot be relieved without assistance. According to hospital involvement, disasters may be classified into internal disasters that occur inside the health care facility or external disasters that occur outside the health care facility (Hassmiller, 2008).

At the end of the 20th century, national thinking about emergency preparedness led to two important developments: identification of the key competencies needed for effective emergency response, and increased attention to planning for and practicing emergency response. While there was a dramatic increase in both attention and funding following the World Trade Center fall of 2001 and the Anthrax events, these emergency preparedness activities were well underway prior to that time (King and Jatoo, 2005). Effective response requires a disciplined team in which each participating

individual follows clear lines of communication and performs according to clearly assigned role directions (National Incident Management System [NIMS], 2004).

Disaster management includes four phases: mitigation, preparedness, response and recovery (JCAHO, 2003). Mitigation includes any activity taken to prevent the occurrence of the disaster whenever possible (Maurer and Smith, 2005). Preparedness is defined as activities and measures taken in advance of an event to ensure effective response to the impact of hazards (WHO expert consultation, 2007). Response phase is the point at which actions are started to save lives, property, and the environment and to prevent secondary harm (National Commission on Terrorist Attacks Upon the United States, 2004). During the recovery phase efforts are started to restore the community to normal (Ciotto, 2006).

The goal of disaster management includes prevention of the occurrence, minimizing casualty number, preventing further casualties, rescuing the injured, providing first and evaluating the injured, and providing definitive care and facilitating reconstruction recovery (Abd-Elazez, 2001). Disaster management by nature is an interdisciplinary, collaborative team effort (Lundy and Janes, 2001). It requires much planning, drilling, evaluating, revising and preplanning to successfully

handle sudden events that injure humans, destroy property, and overwhelm responders. Hospitals must not only have an external disaster plan, but a plan for internal disasters as well (Afifi, 2001; Kitt *et al.*, 2005).

A disaster plan is a systematic procedures that clearly detail what needs to be don, how, when, and by whom- before and after the time an anticipated disastrous event occurs. (Carolyn, 2006 and Kein and Giannone, 2004). It describes the action to be taken in the event (Karen, 2001). Moreover, any plan must address acquisition of equipment, supplies, medicine, and even food, clean water, blankets, and shelter (Allender and spradley, 2005. The disaster plan is site specific; it is governed by factors related to nature of work performed, number of workers and contractors at site and, the hours of operation. The plan is applied to all persons on site, including workers, contractors and visitors (Salazar, 2001).

A key to disaster preparedness is that the plan must be kept both realistic and simple (Chyna, 2005). It should clearly document basic approaches to various situations, general assumptions, and critical event sequences that need to be followed (Nosalek, 2003). A disaster plan committee should include representative from medical staff, emergency room physician or trauma surgeon, administration, OR manager, nursing staff, emergency department, security, communication, public relations, medical record and admission, engineering maintenance laboratory, radiology and respiratory therapy (Clarke, 2002).

Although disaster plans should be targeted for the specific community, certain components should be included, which have to show elaboration, detail, and specifics according to the needs of the community to which it applies. So, these components have to include authority, communication, supplies, equipment, human resources, team coordination, transportation, documentation, record-keeping, evacuation rescue, acute care, supportive care, recovery, and evaluation (Higgins *et al.*, 2004).

Hospital emergency preparedness has come under scrutiny (Russ, 2005). Recent events have brought disaster medicine into the public focus; both the government and communities expect hospitals to be prepared to cope with all types of emergencies (Bartley *et al.*, 2006). Contemporary events all over the world have raised awareness of mass causality events and the need for a capable disaster response. Recent natural disasters have highlighted the poor preparedness and infrastructure in place to respond to mass causality events. In response, public health policy makers and emergency planners developed plans and prepared emergency response systems (Chockshi *et al.*, 2008). Health professionals,

including nurses will need to be personally and professionally prepared to respond to any type of emergency event. All agencies now recognize that a response to any sort of emergency requires an inter-agency, interdisciplinary response, and that nearly all emergencies have potential health consequences (Gebbie and Qureshi, 2006).

Nurses will continue to be key players in the local and national level emergency response through the 21st century. As members of the community, the basic emergency preparedness of nurses can be an example to other members of the community (Qureshi *et al.*, 2005). Nurses are also looked to as planners and policy-makers at many levels of the emergency response system. They are frequently assigned to emergency planning committees and councils, or are asked to develop the site-specific emergency plans (Gebbie and Qureshi, 2006). Therefore, they should become involved in every level of disaster management (Burger and Canton, 2007). This study aimed at assessing the awareness of health team members regarding hospital internal disaster management plan. It was carried out to answer the research question of whether health team members are aware of hospital internal disaster management plan or not?

2. Subjects and Methods

Research design

A cross-sectional descriptive design was used in this research.

Study setting

The study was conducted at A University hospital. It consists of two buildings, which contain twenty-eight departments. This hospital was selected because it is the largest hospital in the university providing various services to a large number of clients, that might increase the risks of internal disasters.

Study subjects

The subjects of this study consisted of a group of hospital staff. This group was recruited from all categories of the hospital manpower (physicians, nurse leaders, staff nurses, administrative employees, technicians, and housekeepers) in order to assess their awareness about internal disasters and disaster plan. The required sample size was calculated to determine the prevalence of any positive awareness about disasters or disaster plans of 50% or more, with a 5% absolute precision and a 95% level of confidence (Schlesselman, 1982). The estimated sample size is 384 subjects. After adjustment for a dropout rate of about 5%, it was increased to 400 subjects. The sample was recruited through stratified random

sampling technique (221 health care personnel, 99 from administrative employees, and 80 from housekeeping). The health care personnel included 56 physicians, 22 nursing leaders, 73 nurses, and 70 technicians.

Data collection tool

The data collection tool was a self-administered questionnaire form aimed at assessing health team awareness of the hospital internal disaster management plan. The tool was developed by the researchers based on review of pertinent literature, reviewed by experts, and pilot tested. The questionnaire included one part for subjects characteristics (6 questions), one part for expectations regarding internal disasters (13 questions), and a final part regarding awareness about internal disaster preparedness plan in the studied hospital (71 items categorized into internal disaster plan, hospital preparedness, special disasters precautions, preparation for outage of power/supplies, and hospital preventive measures for some disasters.

Reliability of data collection tool

Tool reliability was assessed through estimating its internal consistency. It proved to be of good reliability, where Cronbach alpha coefficient ranged between 0.52 and 0.87.

Fieldwork

To carry out the study in the predetermined hospital, letters explaining the aim of the study were directed from the Faculty of Nursing to hospital director, and nursing director in their facilities. The researchers then met with them in the hospital, and explained the purpose and the method of data collection for the study to obtain their permission to conduct the study. The fieldwork was executed from May 2007 through August 2007 for the questionnaire. The researchers visited each department in the study setting and distributed the tool to the chosen sample in their workplaces after explaining the aim of the study to them and how to fill the tool. Study subject's responses were checked against the administrator's tool to verify whether they were correct or incorrect, i.e. the administrator's responses were considered as the standard.

The principles of ethics in research were closely followed in this study. The proposal was approved by the ethics committee. The participants gave their verbal consent to participate after being briefed with the study aim and procedures. They were informed about their rights to refuse or withdraw. Anonymity and confidentiality of the obtained information was assured.

Statistical analysis

Data entry was done using Epi-Info 6.04 computer software package, while statistical analysis was done using SPSS 13.0 statistical software package. Data were presented using descriptive statistics in the form of frequencies and percentages for categorical variables. Qualitative categorical variables were compared using chi-square test. In larger than 2x2 cross-tables, no test could be applied whenever the expected value in 10% or more of the cells was less than 5. Statistical significance was considered at p-value <0.05.

3. Results:

Table 1 displays awareness of various categories of the study subjects about internal disaster plan. It points to generally low awareness about all items. According to the table, there are statistically significant differences between all categories of the study subjects in relation to telecom system ($p < 0.001$). The lowest percentages of awareness in this item were among physicians and housekeepers, while it was highest among nurse leaders.

Table 2 demonstrates the awareness of various categories of the study subjects about hospital mitigation measures for internal fire and radiation accidents. The table points to statistically significant differences among various categories of the study sample in all items of internal fire. It is evident that technicians had generally the lowest percentages of awareness about most items. However, they had the highest percentages of awareness regarding checking visitors for flammables and precautions for electric fires. Also, nurse leaders, nurses, and technicians had the lowest percentages regarding sprinklers system. As regards awareness of radiation accidents, the table shows statistically significant differences related to regular maintenance and regulations for use. It is noticed that technicians had generally the lowest percentages, particularly in relation to regular maintenance. Meanwhile, physicians had the highest percentages related to regulations for use of radioactive materials.

The awareness of various categories of the study subjects about hospital mitigation and preparedness related to infection, food poisoning outbreaks, gas explosion and earthquakes is described in Table 3. As the table shows, there are statistically significant differences among various categories of the study subjects in relation to infection control program ($p < 0.001$). Technicians had the lowest awareness about the infection control program (82.9%).

Table (1): Awareness of various categories of the study subjects about internal disaster plan (n= 400)

Plan	Group												X ² test (p-value)
	Physicians (n=56)		Nurse Leaders (n=22)		Nurses (n=73)		Technicians (n=70)		Administrative employees (n=99)		Housekeepers (n=80)		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
There is a plan	0	0.0	1	4.5	2	2.7	4	5.7	0	0.0	0	0.0	--
There is a disaster committee	1	1.8	1	4.5	0	0.0	4	5.7	1	1.0	0	0.0	--
For effective Communication: there is:													
Notification system	7	12.5	1	4.5	3	4.1	9	12.9	0	0.0	0	0.0	--
Telecom system	19	33.9	22	100.0	67	91.8	67	95.7	53	53.5	15	18.8	<0.001*
There is a disaster control centre	3	5.4	1	4.5	4	5.5	8	11.4	0	0.0	1	1.3	--

(*) Statistically significant at p<0.05

(-- Test result not valid

Table (2): Awareness of various categories of the study subjects about hospital mitigation measures for internal fire and radiation accidents (n=400)

Preventive measures	Group												X ² test (p-value)
	Physicians (n=56)		Nurse Leaders (n=22)		Nurses (n=73)		Technicians (n=70)		Administrative employees (n=99)		Housekeepers (n=80)		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Internal fire: there is:													
No smoking rules	51	91.1	18	81.8	70	95.9	56	80.0	99	100.0	78	97.5	<0.001*
No heaters in departments	51	91.1	19	86.4	64	87.7	51	72.9	99	100.0	79	98.8	<0.001*
No smoking signs	52	92.9	20	90.9	67	91.8	56	80.0	99	100.0	79	98.8	<0.001*
Visitors checked for flammables	5	8.9	5	22.7	24	32.9	28	40.0	1	1.0	1	1.3	<0.001*
Precautions for electric fires	1	1.8	6	27.3	24	32.9	26	37.1	1	1.0	0	0.0	<0.001*
Grounding	1	1.8	5	22.7	21	28.8	13	18.6	29	29.3	11	13.8	<0.001*
An alarm	6	10.7	6	27.3	18	24.7	19	27.1	28	28.3	11	13.8	0.04*
Notification phone number	54	96.4	21	95.5	72	98.6	56	80.0	98	99.0	80	100.0	<0.001*
Fire extinguishers	53	94.6	20	90.9	71	97.3	57	81.4	99	100.0	80	100.0	<0.001*
Training on the use of fire extinguishers	7	13.2	3	15.0	16	22.5	14	24.6	7	7.1	1	1.3	<0.001*
Working sprinklers system	51	91.1	2	9.1	17	23.3	12	17.1	55	55.6	66	82.5	<0.001*
Radiation accidents: there is:													
Regular maintenance of mobile X- ray units	53	94.6	21	95.5	73	100.0	56	80.0	99	100.0	80	100.0	<0.001*
Maintenance of these units	52	98.1	18	85.7	67	91.8	51	91.1	99	100	80	100.0	<0.001*
Regulations for use of radioactive materials	48	85.7	13	59.1	49	67.1	38	54.3	51	51.5	47	58.8	0.001*
Warning signs	1	1.8	2	9.1	7	9.6	14	20.0	0	0.0	0	0.0	--
Guiding signs	0	0.0	2	9.1	6	8.2	12	17.1	1	1.0	0	0.0	--
Plans to deal with radiation accidents	1	1.8	1	4.5	5	6.8	15	21.4	0	0.0	0	0.0	--

(*) Statistically significant at p<0.05

(-- Test result not valid

Table (3): Awareness of various categories of the study subjects about hospital's mitigation and preparedness related to infection control , food poisoning outbreaks, gas explosion and earthquakes. (n=400)

Special disasters precautions	Group												X ² test (p-value)
	Physicians (n=56)		Nurse Leaders (n=22)		Nurses (n=73)		Technicians (n=70)		Administrative employees (n=99)		Housekeepers (n=80)		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Infection control													
There is an infection control program	52	92.9	21	95.5	72	98.6	58	82.9	98	99.0	80	100.0	<0.001*
With a person in charge	52	100.0	16	76.2	57	79.2	53	91.4	97	99.0	79	98.8	<0.001*
Regularly revised	52	100.0	16	76.2	58	80.6	47	81.0	98	100.0	80	100.0	<0.001*
With continuing training	6	10.7	12	54.5	41	56.2	41	58.6	28	28.3	9	11.3	<0.001*
With patient isolation	0	0.0	2	9.1	4	5.5	12	17.1	3	3.0	1	1.3	--
food poisoning outbreaks:													
There is a unit for food poisoning	1	1.8	0	0.0	4	5.5	10	14.3	3	3.0	0	0.0	--
There is a nearby poisons centre	52	92.9	18	81.8	65	89.0	55	78.6	97	98.0	80	100.0	<0.001*
There is a trained team	1	1.8	0	0.0	5	6.8	11	15.7	0	0.0	0	0.0	--
There are regulations for food poisoning prevention	3	5.4	5	22.7	13	17.8	16	22.9	29	29.3	10	12.5	0.005*
Gas explosion: there is:													
Written procedures	0	0.0	0	0.0	4	5.5	12	17.1	0	0.0	0	0.0	--
Maintenance of connections	6	10.7	5	22.7	12	16.4	16	22.9	3	3.0	1	1.3	<0.001*
Maintenance of equipment	1	1.8	7	31.8	10	13.7	14	20.0	2	2.0	0	0.0	<0.001*
Medical engineering department	53	94.6	19	86.4	66	90.4	55	78.6	97	98.0	79	98.8	<0.001*
Guiding signs	1	1.8	4	18.2	7	9.6	13	18.6	2	2.0	0	0.0	<0.001*
Earthquakes:													
Regulations to deal with	0	0.0	1	4.5	4	5.5	13	18.6	1	1.0	0	0.0	--

(*) Statistically significant at p<0.05

(--) Test result not valid

The same table demonstrates a number of statistically significant differences regarding awareness about hospital precautions related to food poisoning outbreak. It is obvious that technicians had the lowest percentages regarding the presence of a nearby poisons centre (78.6%). Meanwhile, the administration employees had the highest percentage of awareness about the presence of regulations for prevention of food poisoning (29.3%).

The same table illustrates the awareness of various categories of the study subjects about hospital mitigation and preparedness for gas explosion. Statistically significant differences were revealed among various categories in almost all items of gas explosion. It is noticed that nurse leaders, and technicians had the highest percentages related to maintenance of gas connections, maintenance of gas

equipment, and guiding signs. Conversely, they had the lowest percentages in relation to medical engineering department. At the same time the awareness about regulations to deal with earthquakes was low in all categories (86.4% & 78.6%) .

Table 4 shows awareness of various categories of the study subjects about hospital preparedness for internal disasters plan related to hospital environment, emergency, evacuation, and training. As regarding the hospital environment, the table indicates very low awareness levels about all items, except for the safe exits and entries, and the phone lines. Statistically significant differences were revealed between categories regarding their awareness about equipment for calling staff, special alarms for specific disasters, phone lines for all departments, and easy access for persons in charge.

Table (4): Awareness of various categories of the study subjects about hospital preparedness for internal disaster plan related to hospital environment, emergency, evacuation, and training (n=400)

Precautions	Group												X ² test (p-value)
	Physicians (n=56)		Nurse Leaders (n=22)		Nurses (n=73)		Technicians (n=70)		Administrative employees (n=99)		Housekeepers (n=80)		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Hospital environment:													
Easy opening/ closing of exits/ entries	54	96.4	21	95.5	69	94.5	64	91.4	98	99.0	80	100.0	0.052
Enough beds	1	1.8	0	0.0	0	0.0	12	17.1	0	0.0	0	0.0	--
Stock periodically revised	0	0.0	0	0.0	1	1.4	16	22.9	0	0.0	0	0.0	--
Equipment for calling staff	0	0.0	6	27.3	11	15.1	19	27.1	5	5.1	1	1.3	<0.001*
Special alarms for specific disasters	0	0.0	3	13.6	3	4.1	14	20.0	7	7.1	1	1.3	<0.001*
Phone lines in all depts.	52	92.9	15	68.2	57	78.1	57	81.4	95	96.0	79	98.8	<0.001*
Persons in charge have easy access	1	1.8	4	18.2	7	9.6	18	25.7	10	10.1	0	0.0	<0.001*
There is a plan for traffic	0	0.0	9	13.6	6	8.2	16	22.9	6	6.1	1	1.3	<0.001*
Emergency dept with:													
Special entrance	2	3.6	17	77.3	45	61.6	42	60.0	12	12.1	0	0.0	<0.001*
Special communication system	53	94.6	18	81.8	68	93.2	58	82.9	85	85.9	79	98.8	0.0049*
Mobile X ray units	0	0.0	7	31.8	14	19.2	25	35.7	8	8.1	1	1.3	<0.001*
Enough ambulances	0	0.0	3	13.6	7	9.6	16	22.9	8	8.1	1	1.3	<0.001*
Hospital evacuation: there is:													
A plan	0	0.0	5	22.7	10	13.7	21	30.0	8	8.1	1	1.3	<0.001*
A person is in charge	2	3.6	4	18.2	7	9.6	18	25.7	8	8.1	1	1.3	<0.001*
Special exits	55	98.2	22	100.0	73	100.0	66	94.3	96	97.0	80	100.0	0.11
Special lifts	5	8.9	13	59.1	44	60.3	56	80.0	15	15.2	4	5.0	<0.001*
Arrangement with other hospitals	34	60.7	12	54.5	45	61.6	42	60.0	44	44.4	61	76.3	0.002*
Training: there is:													
Drills	0	0.0	1	4.5	0	0.0	5	7.1	0	0.0	0	0.0	--
Training plan	0	0.0	0	0.0	2	2.7	7	10.0	2	2.0	0	0.0	--
A person is in charge for media	0	0.0	1	4.5	1	1.4	7	10.0	3	3.0	19	23.8	<0.001*

(*) Statistically significant at p<0.05

(--) Test result not valid

Table (5): Awareness of various categories of the study subjects about hospital preparedness related to water/electricity/gas supply and earthquakes (n=400)

Hospital preparedness for failure of power/ supplies	Group												X ² test (p-value)
	Physicians (n=56)		Nurse Leaders (n=22)		Nurses (n=73)		Technicians (n=70)		Administrative employees (n=99)		House-keepers (n=80)		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Water supply:													
More than 1 source	27	48.2	5	22.7	16	21.9	30	42.9	32	32.3	54	67.5	<0.001*
Plans for shutdown	5	8.9	3	13.6	11	15.1	16	22.9	1	1.0	6	7.5	<0.001*
Water tanks:	51	91.1	17	77.3	63	86.3	60	85.7	99	100.0	80	100.0	<0.001*
Electricity:													
More than 1 source	52	92.9	11	50.0	47	64.4	40	57.1	85	85.9	71	88.8	<0.001*
Contingency plans	0	0.0	2	9.1	5	6.8	14	20.0	0	0.0	0	0.0	--
Generators	54	96.4	22	100.0	69	94.5	47	67.1	99	100.0	79	98.8	<0.001*
There is a special source for disasters	0	0.0	3	21.4	6	12.5	13	31.0	0	0.0	0	0.0	--
Gas supply:													
More than 1 source	47	83.9	12	54.5	55	75.3	54	77.1	83	83.8	75	93.8	0.001*
Regulations to ensure supply	1	1.8	2	9.1	7	9.6	15	21.4	0	0.0	0	0.0	--
Procedures in case of shortage	0	0.0	2	9.1	9	12.3	14	20.0	0	0.0	0	0.0	--
Contingency sources	35	62.5	8	36.4	24	32.9	36	51.4	60	60.6	42	52.5	0.003*

(*) Statistically significant at p<0.05

(--) Test result not valid

4. Discussion:

A disaster management plan is an agreed set of arrangements for preparing for, responding to, and recovering from emergencies and involves the description of responsibilities, management structures, strategies, and resource and information management. Disaster planning is about protecting life, property, and the environment (Keine and Rhyne, 2001). The present study was aimed at assessing the awareness of health team members regarding hospital's internal disaster management plan. The study was conducted in all the departments of a University Hospital and included all categories of staff.

For reasons of prudent stewardship, and because of the requirements of accreditation and licensure, hospitals must prepare for the possibility of disaster within the facility. The present study results revealed the absence of a disaster plan in the study setting. Also, the majority of various categories of the study subjects had low awareness about all items of a disaster plan. In an explanation of this lack of disaster plan, Milsten (2000) clarified that healthcare professionals have long been aware that they should make preparations for external disasters causing a surge in the number of patients. They have been less inclined to confront the possibility that disruption may occur within the facility itself and undermine their ability to provide care. Yet, these internal

disasters appear to be more common events in hospitals than are patient surges from external disasters.

Similar to the present study results regarding the low awareness of respondents of the disaster plan, O'Sullivan *et al.* (2008) indicated that studied nurses felt unprepared to respond to large scale disasters. Approximately 40% of them were unaware that their hospital has an emergency plan. Moreover, nurses reported inadequate access to resources to support disaster response capacity, and expressed a low degree of confidence in the preparedness of Canadian health care institutions for future outbreaks. Therefore, the authors recommended that more training and information were needed to enhance preparedness for frontline health care workers, and of important members of the response community. The lack of awareness about the disaster plan, and its related items is quite alarming. The shortage in the required local knowledge and capacity would lead to inability to manage disasters even if there is a written plan. In this respect, Russ (2005) emphasized that knowledge management has not been at the core of the healthcare business model despite healthcare being a knowledge-intensive business. Meanwhile, very early, Auf der Heide (1989) referred to the "paper-plan syndrome", the belief that disaster preparedness can be achieved simply through the filing of a written plan, and strongly cautioned that

such plans prove more useful in practice when they are relied upon not as documents to be suddenly consulted in an emergency, but as forms of training complemented with additional forms of preparedness.

As regards hospital prevention measures for internal fire, the present study findings showed that the presence of preventive measures for internal fire concerning no smoking rules and signs, fire extinguishers in working condition, but no training of staff in their use. This area of internal disasters has been shown to be the most expected. Therefore, preventive measures are of utmost importance. This might explain the good awareness of respondents about it. The findings are in agreement with Mostafa (2003) who pointed to a high awareness about the presence of safety precautions as no smoking regulations implemented by security. Moreover, the great majority of them were aware about the lack of tests and practical training on fire extinguishers, as well as the lack of training of newly nurses.

Concerning hospital preventive measures for radiation accidents, the present study results showed wide differences among various categories in almost all items. Nurse leaders had the lowest percentages in relation to maintenance of mobile X ray units, whereas physicians had the highest percentages related to regulations for use of radioactive materials. However, there were no plans to deal with such accidents as radiation, gas explosion, or earthquakes. In congruence with this, Mostafa (2003) reported that half of the studied nurses were aware about the daily maintenance to avoid explosions. In this regard, Michael (2003) emphasized that prearranging for fire or explosion allows the nursing to respond quickly to the victims, and triage and evacuation when needed.

According to the results of the present study, the study setting had an infection control program that is regularly revised, with a person in charge. However, continuing training was deficient, and there were no patient isolation techniques. In the same respect, Rebmann *et al.* (2008) emphasized that infection control professionals' role in disaster preparedness and response is essential, even in non-infectious disease emergencies. Meanwhile, Hui *et al.* (2007) mentioned that infectious diseases do not respect hospitals' classification system. Therefore, a minimum preparedness requirement, e.g., emergency plan, staff, beds, drugs, and equipment for infectious diseases of public health significance should be applied to all types of hospitals.

Although the university hospitals including a poisons centre the awareness of most categories of the health team about food poisoning is very low. They reported that there is no regulation for food poisoning prevention. In addition to absence of trained team to deal with. These results could be

explained by that, managers of all university hospitals are depending on the presence of a nearby poisons centre and that its team is efficient to deal with such incidents that lead to low level of hospital preparedness in all items related to food poisoning

Concerning hospital preparedness for internal disasters related to hospital environment, the present study revealed low levels of preparedness and of awareness about all items, except for the safe exits and entries, and the phone lines. Nurses and nurse leaders had generally better responses, while housekeepers were more aware about phone lines in all departments. The findings are quite unexpected given the size and status of the study setting, which is a large university hospital. In this regard, Rebmann *et al.* (2008) claimed that smaller sized hospitals were less prepared than larger facilities for internal disaster. Smaller sized hospitals were also less likely than larger facilities to have surge capacity in terms of laboratory, negative-pressure rooms, staff, or medical equipment (ventilators, surgical masks, and medication). These differences in infectious disease disaster planning most likely relate to an inequitable distribution of resources within communities, with larger facilities having more resources than smaller hospitals.

In the same vein, Masoud (2003) claimed that safety in hospital environment could only be achieved through complex coordination of efforts, integration of a wide range of scientific systems and ergonomic factors, as well as well planned process of feasibility study through medical planning design, construction, outfitting, operating, and maintaining the facility, and staffing it with trained doctors and nurses. Additionally, Mohamed and Zakaria (2003) reported that safety and welfare of patients and all employees working in the hospitals were the primary concern of all medical team, hospitals environment needed more attention to prevent accidents, reducing injuries errors and increasing organizational function. The present study findings demonstrated the absence of a notification system for disasters. Conversely, a special communication system was present in the emergency department and recognized by all categories of respondents. The findings highlight a major deficiency in communication, which is the backbone of a disaster plan. Without efficient communication, nothing could be done in disaster management. In this regard, Kaji *et al.* (2008) emphasized the role of effective communication, and mentioned that a more comprehensive approach that reflects both communication and teamwork behaviors, as well as a quantitative assessment of surge capacity, supplies, and equipment might be required for disaster management. In this same respect, Reddya *et al.* (2008) stressed that some of

the major challenges associated with team coordination during crisis management included information mismanagement, resource allocation issues, and ineffective communication.

The present study findings point to a major problem, which is the lack of a hospital evacuation plan. The only available items were those related to exits. Generally, nurse leaders, nurses, and housekeepers had the highest percentages of awareness. The findings are in congruence with Gretenkort *et al.* (2002) reported that although every hospital needs a security plan for the support of immobile patients who do not possess autonomous escape capabilities, little information exists to assist in the development of practical patient evacuation methods. The authors concluded that experiences from a hospital during an evacuation exercise should provide decision criteria for changes in the disaster preparedness plan.

Concerning arrangement with other nearby hospitals, there was some availability of such arrangement according to the present study respondents. These items of the plan are very important for efficient use of available and potential resources. The findings are in agreement with Kaji and Lewis (2006) who did a survey study for disaster preparedness among a cohort of hospitals in Los Angeles County. The results showed that about one-fourth of the hospitals had mutual aid agreements with other hospitals or long-term care facilities. The authors concluded that among hospitals in Los Angeles County, disaster preparedness and surge capacity appear to be limited by a failure to fully integrate interagency training and planning, although there is a generally high level of availability of equipment and supplies.

The results of the current study also revealed lack of hospital preparedness for internal disasters related to hospital drills, and training. These findings indicate a major shortcoming in the study setting preparedness for disaster. In this regard, Adini *et al.* (2006) mentioned that emergency preparedness can be defined by the preparedness pyramid, which identifies planning, infrastructure, knowledge and capabilities, and training as the major components of maintaining a high level of preparedness. This necessitates integrating knowledge through drills.

In congruence with these present study findings, Mostafa (2003) indicated that nurses were aware about the absence of training programs. Meanwhile, Sanders (2000) emphasized that training was obviously essential for all medical personnel, including participation in realistic drills for potential natural and manmade disasters. In any health care settings, basic medical procedures do not change

frequently, although some may be simplified or omitted in large-scale disasters.

The present study results demonstrated the presence of more than one source for power and gas supply, and to some extent for water, to be used in case of failure. Also, most respondents were aware of these sources. Nevertheless, no contingency plan was found. The consequences of power failure without contingency plans or secondary sources could be serious. In this regard, Sternberg (2003) indicated that a power outage combined with failure of the back-up generator is a particularly notorious cause of cascading failures. These present study findings are in agreement with Kai *et al.* (1994) in Japan, where 78% of the studied hospitals were found to have independent electric power generating plants. Additionally, 71% of these hospitals had reserve water supply, and 83% of them reported that it would be impossible to provide meals for patients and staff with no main gas supply. Additionally, Yamauchi *et al.* (1996) reported that only 31.7% of surveyed hospitals in Japan had manuals for power outages.

5. Conclusion and Recommendations:

On the basis of the study findings, it is concluded that there is a major deficiency in the hospital health team members' awareness regarding internal disasters and related disaster management plan. This was noticed in all categories including nursing, medical, paramedical, and auxiliaries. Therefore, hospital administration should develop policies for disaster management and pay more attention to the problem of internal disasters and preparedness for their management. Training programs are essential for all categories of the health team in order to increase their awareness about disaster management. Drills should be done regularly, with full participation of all hospital staff in order to be ready for managing any internal disasters. The designed checklist should be applied in the study setting in order to assess preparedness for disaster management, and this should be done periodically to monitor any changes, whether positive or negative, in order to take appropriate action.

Implications of the Study

This study findings help in identification of the gaps in awareness of the health team about internal disaster management and the related hospital plan. Based on the results, plans to fill these gaps and to improve staff knowledge could be developed and implemented. Additionally, the results can help decision makers and hospital administration to improve the hospital internal disaster management plan, which would lead to mitigation of losses of resources and save many lives.

Corresponding Author:

Samia Adam

Faculty of Nursing, Ain Shams University, Cairo, Egypt

*asamia@hotmail.com**6. References**

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Patient Safety: Assessing Nurses' Perception and Developing an Improvement Plan

*¹Neamatallah Goma Ahmed, ²Samia Mohamed Adam and ³Iman Ibrahim Abd Al-Moniem

¹Department of Medical Surgical Nursing, ² Nursing Administrations and ³ Paediatric Nursing
Faculty of Nursing, Ain Shams University. Cairo., Egypt.
[*nemata55@hotmail.com](mailto:nemata55@hotmail.com)

Abstract: patients' safety is a core value of healthcare service. A positive patient safety culture contributes to the environment necessary to maintain patient safety and avoid needless patient deaths. The impact of medical errors has been widely reported. The traditional blame and shame culture in healthcare organization have been criticized for being largely responsible for causing medical errors and obstructing the possibility of learning from those errors. Employees' perceptions about safety are important because organizations with strong safety culture consistently report fewer workplace injuries and fewer harmful events than do organizations with weak safety culture. The study aims to assessing nurses' perceptions of patient's safety culture at medical and paediatric hospitals and developing an improvement plan to enhance patient safety at the study settings. The study was conducted in tow university hospitals using a descriptive design. **Sample:** 148 nurses (120 staff nurses and 28 head nurses) were included in the study. **Tools of the study included** 1) Interview questionnaire sheets were used to collect the participants' characteristic data, 2) The Hospital Survey of Patient Safety Culture (HSPSC) developed by the Agency for Healthcare Research and Quality, AHRQ (2004). **Results:** nurses perceive patient safety culture more positive. There were statistically significant differences between perception of nurses working in critical care units and perception of nurses working in general wards in two dimensions. There were a statistically significant differences between perception of the staff nurses and perception of head nurses in all items related to patients' safety except organizational learning. The highest percentages of the nurses working in the general wards and critical care units scored their hospital as very good (59.5% & 33.7%, respectively). While half of the head nurses scored the hospital as acceptable (50%). The highest percentage of the staff nurses (34.2%) reported no events related to patients safety over the past 12 months. No correlation was found between head nurses age and perception of patient safety culture, while there was a positive weak correlation between staff nurses' age and their perception. **Conclusion:** nurses perceive patient's safety culture more positively. There was a statistically significant difference between nurses working in critical care units and nurses working in general wards. Head nurses perceived the patient's safety culture more positively than staff nurses did. The majority of the nurses did not report events related to patient's safety. Non-punitive environment scored high negative responses. **Recommendation:** nurses need to be encouraged to improve the reporting events related to patients' safety. Further studies are needed for testing the reliability of the suggested developed improvement plan and accordingly implementing it at the study settings.

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Keywords: Patient; Safety; Nurse

1. Introduction:

Patient safety is considered as one of the most important aspects of the healthcare. It is a global issue, affecting countries at all levels of development. It has been defined by several organizations as the freedom from accidental or preventable injuries produced by medical care (Institute of Medicine, (IOM), 1999; and Agency for Healthcare Research and Quality (AHRQ), 2005). According to a World Health Organization (WHO) report, one out of every 10 hospital patients in many developed countries experiences an adverse event which can lead to serious injury and death. The situation in developing countries is even worse (WHO, 2008).

Care is often delivered in a pressurized and fast-moving environment, involving a vast array of

technology, and daily decisions and judgments by health-care professional staff. In such circumstances, things can and do go wrong. Sometimes unintentional harm comes to a patient during a clinical procedure, or as a result of a clinical decision. Errors in the process of care can result in injury. Sometimes the harm that patients experience is serious and sometimes people die. Various studies have investigated the extent of adverse events (AbdEl-Rahman, 2004; Johnstone & Kanitsaki, 2006; WHO; 2008, and Markowitz, 2009).

Although estimates of the size of the problem are scarce, particularly in developing and transitional countries, it is likely that millions of patients worldwide suffer disabling injuries or death every year due to unsafe medical care. Patient harm

can occur as a result of a constellation of factors and circumstances. Understanding the magnitude of the problem and the main contributing factors that lead to patient harm is essential to design effective and efficient solutions for different contexts in addition to establishing a safer health system (Al-Ameri; 2000 and Milligan, 2007).

Because today's health-care context is highly complex, describing the safety culture in hospitals is an important first step in creating work environments where safety is a priority. It is a core component of healthcare quality. The safety culture of an organization is the product of individual and group values, attitudes, perceptions, competencies, and patterns of behavior that determine the commitment to, and the style and proficiency of, an organization's health and safety management. Organizations with a positive safety culture are characterized by communications founded on mutual trust, by shared perceptions of the importance of safety, and by confidence in the efficacy of preventive measures (Boyden et al., 2006).

Additionally, Safety culture refers to the "summary of perceptions that employees share about the safety of their work environment. Employees' safety-related perceptions are based on several factors, including management decision making, organizational safety norms and expectations, and safety practices, policies, and procedures. These factors all communicate an organization's commitment to safety (Milstead, 2005, Stone and Gershon 2006).

Organizations with strong safety culture have fewer employee injuries not only because the workplace has well developed an effective safety program, but also because the existence of these programs sends "cues" to employees regarding to management's commitment to safety. Evidence shows that if the organization is serious about adherence to safe work practices, then employees are more likely to comply. Safe environment support and reinforces individual safety behaviors, and this in turn further affects behavior because of the influence workers have on one another. As safety behaviors are adopted throughout an organization, increasing pressure is put on non-compliers to come "in line" (Al-Kahtani, Lund & Aaro; 2004; and Chiang & Pepper, 2006).

Hospital employees' perceptions regarding to safety are rarely formally evaluated or considered during the design or updating of safety programs. This issue is particularly important for the health care workplace because recent studies have linked global measures of a safety culture to employee compliance with safe work practices and to exposure to incidents, because exposure to incidents, regardless of the

outcome, may be extremely burdensome to employees as well as to organizations. Improving the understanding of safety climate may have far-reaching implication. (David et al., 2005; and Espin et al., 2006).

Patient safety culture is a relatively new concept in healthcare organizations. Several key features of safety cultures have been identified as applicable to health care organizations based on the studies of high reliability organizations such as nuclear power industry and naval aviation (AHRQ, 2008). These key features include: (1) A system view: Management recognizes risk is inherent in an organization's activities, and analyzes risks and errors systematically; (2) A blame-free and forgiveness environment: Individuals are willing to report errors without a fear of punishment; (3) A collaborative environment: Individuals and work groups or units collaborate effectively to accomplish organizational goals; (4) Adequate safety resources: Organizations are willing to provide resources for addressing safety concerns (Pizzi & Nash, 2001; Milstead, 2005; and David et al., 2005).

Starting point for improving safety culture is to conduct an assessment of the current culture to determine whether and how it affects patient care. A survey of the safety culture should measure aspects of the units that affect patient safety as well as attitude of clinicians and staff members. Such aspects include perceptions of leadership commitment to patient safety, the degree to which teamwork and open communication prevail, and attitudes about non-punitive response to error (Hofman and Mark, 2006; and Shostek, 2007).

Significance of the study:

patients' has become both a national and international imperative in recent years, with increased emphasis across the world on patient safety in policy reform, legislative changes and development of standards of care driven by quality improvement initiatives.

Studies of adverse events in numerous countries around the world demonstrate that, between 4% and 16% of patients admitted to hospital experience one or more adverse events, of which, up to half are preventable. Understanding why preventable errors occur is key to develop strategies by which they can be addressed and minimized. It is self-evident that safe and effective treatments and care are important in ensuring that patients get the best outcomes from their care. The international evidence also indicates that effective care is often the most efficient care (Commission of Safety and Quality Assurance report, 2008).

Aim of the study:

The study aims at assessing nurses' perceptions of patients safety culture at Medical and Paediatric University Hospitals and developing an improvement plan to enhance patient safety at the study settings.

Research questions:

Are there any differences between nurses working in critical care units and nurses working in general wards regarding their perception of patient safety culture?

Are there any differences between staff nurses and head nurses regarding their perception of patient safety culture?

2. Methodology**Design:**

A descriptive design was used in the conduction of the study.

Setting

The study was conducted in all critical care units and general wards in Medical and Paediatric University Hospitals. The medical hospital includes nine critical care units with 70 beds and 13 general wards with 400 beds. While the paediatric hospital includes three critical care units with 30 beds and five general wards with 124 beds.

Subjects:

Subjects of the study included all nurses working in critical care units and general wards in both medical and paediatric university hospitals. Total number of nurses was 250 working in three shifts. Out of the total number, 148 nurses were agreed to respond to the questionnaire representing 59.2%. Participants included 28 head nurses and 120 staff nurses. They include 83 staff nurses working in critical care units out of them 53 nurses from the medical hospital and 30 from paediatric hospital plus 37 from general wards out of them 20 working in the medical hospital and 17 nurses working in paediatric hospital. The inclusion criterion was that head nurses and staff nurses should be working at the current nursing units and position for at least 12 months.

Tools of data collection:

The following two tools were used :

Interview questionnaire sheet was used to collect the participants' characteristic data, such as; age, sex, experience, position and contact with patients. It was also used to collect organizational data such as safety training information, characteristics of the hospital, type of patients services provided.

The Hospital Survey of Patient Safety Culture (HSPSC) employed to measure nurses' perceived patient safety culture in this study, The HSPSC was adopted from Agency for Health care Research and Quality (AHRQ) 2004. It contains 12 dimensions with 42 sub items in addition to two independent questions on patient safety grade and number of events reported with single response item. Out of the 12 dimensions, two outcome dimensions including 1) Frequency of Reported Events with 3 items and 2) Overall perceptions of patient safety with 4 items. And Eight safety culture dimensions at unit level including: 1) Supervisor/manager expectation and actions promoting safety culture with 4 items , 2) organizational learning with 3 items 3) teamwork within hospital units with 4 items , 4) communication openness, with 4 items 5) feedback and communication about errors, 3 items, 6) non-punitive response to error, with 3 items, 7) Staffing with 3 items 8) hospital management support for patient safety, with 3 items , in addition to two hospital-wide safety culture dimensions including 1) teamwork across hospital units, with 4 items, and 2) hospital handoffs and transitions, with 4 items. The reliability has been examined and Cronbach's alpha ranged from 0.63 to 0.84 for each of the 12 safety culture dimensions.

Scoring system:

The instrument uses a five-point Likert scale, ranging from strongly disagree to strongly agree (or always to never). Scoring system ranged between 5 for strongly agree to 1 for strongly disagree. Positive and negative scores were calculated. The positive response percentage is the combined percentage of respondents who answered "Strongly Agree" or "Agree," or "Always" or "Most of the Time" (negative items were reversely coded before the actual calculation). Accordingly, the negative response percentage is the combined percentage of respondents who answered "Strongly Disagree" or "Disagree," or "Never" or "Rarely." The average positive percentage response score for each cultural dimension was obtained by averaging the positive response percentage on the items within that dimension.

Procedures

An official permission from each of the hospital administrative authority was obtained. Data was collected in the period from February 2009 to July 2009. A pilot study to confirm a conceptual match was carried out on five ICU head nurses and nine general ward nurses who participated and reviewed the survey instrument item by item, to find whether there were misconceptions or misunderstandings. Few items were modified based

on their comments. Nurses who were asked to respond to the instruments during the pilot study were excluded from the main study sample. Validity of the survey instruments were judged by five experts to test the feasibility and applicability of the tools. Some clarifications were added to some items. Then, the questionnaire was distributed and the purpose of the study was explained to nurses in their work settings during their shifts. Those who refused to participate in the study were replaced by other nurses. Responses to the questionnaire were collected within three weeks after several hospital visits by the investigators.

Based on the results of the survey a suggested improvement plan to enhance patient safety was developed by the investigators. The plan was distributed among seven members of a jury group including three nursing professors, three directors of general hospitals and the head of quality assurance unit at a University Hospitals for determining the validity of the suggested improvement plan. Based on the feedback of the jury modifications were made to reach to the final developed patient safety plan

Ethical considerations

The study was approved initially by the administrative authority of the University Hospitals. Verbal approval from each participant was obtained prior to the study conduction and after explanation of the purpose of the study. They were informed about their rights to withdraw at any time and that all data will be kept confidential. The permission to use the study tools and instruction sheet was obtained with written online permission from AHRQ research committee via online after explanation of the purpose of the study.

Statistical analysis:

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows. Subjects, for whom 10% or more data were missing, were excluded from the analysis. Analyzing and scoring sheets of the AHRQ were followed in tabulation and calculation. Number and percentage distribution were used to determine the highest responses and chi square-test was used to identify significant differences among nurses' perception. The confidence level chosen for the study was 95%. The differences were considered significant if the p-value was less than 0.05 at the appropriate degrees of freedom. Pearson correlation analysis was used for assessment of interrelationship among quantitative variables and scores, to assess the relationship between nurses' perception as dependent variable and nurses' age and years of experience as independent variable

3. Results:

As evident in table (1) the highest percentage of staff nurses (63.2%), their age ranged between $>20 - <30$ years with a total mean age of 30.58 ± 8.6 , while more than half of head nurses (53.6%), their age ranged between $30 - <40$ years, with a mean age of 39.2 ± 7.9 . Results also showed that 37.5% and 71.5% of the staff nurses and head nurses respectively had a Bachelor degree in nursing. The highest percentage of staff nurses held a diploma degree (55.8%). As regards the years of experience it was found that, 71.5% of the head nurses and 40% of staff nurses had experience between $10 - <20$ years in nursing. Almost all staff nurses (94.6%) had direct contact with patients.

A summary of the average of nurses positive and negative response's percentage for each dimension of the HSPSC is presented in Table (2). The highest positive response (28.9%) obtained by staff nurses working in critical care units related to supervisor/manager expectation and actions promoting safety culture dimension while the lowest (16.9%) was obtained for the non-punitive responses to error. The same table shows that the highest positive response (29.7%) for nurses working in general wards was obtained in four dimensions; overall perception of patient safety, teamwork within hospital units, non-punitive response to error and teamwork across hospital unit. While the lowest percentage (10.8%) was for the frequency of reported events. Regarding head nurses, the same table shows that, the highest positive percentage (39.3%) was obtained for teamwork across hospital units. At the same time, head nurses obtained the highest average percentage (28.2%) of positive perception among the three groups.

Table (3) represents the comparison between positive responses of nurses working in critical care units and nurses working in general wards regarding perception of patient safety culture. The table shows that there were statistically significant differences between response rate in items related to organizational learning and teamwork across the hospital units. ($X^2 = 3.75$ & 3.86 respectively at $p < 0.05$). Table (4) displays the comparison between positive responses of all staff nurses and head nurses regarding perception of patient safety culture. It is clear from the table that there were statistically significant differences between positive responses of staff nurses and head nurses in all dimensions of the survey except for the organizational learning dimension ($X^2 = 0.59$ at $p > 0.05$).

Hospital overall grade related to patient safety culture as perceived by nurses is presented in table (5). As shown in the table, almost third (33.7%) of staff nurses working in the critical care units and

slightly less than three fifths (59.5%) of staff nurses working in general wards grade their hospital as very good regarding patient safety. On the other hand, half of the head nurses (50%) categorized their hospital as acceptable.

Table (6) shows the number of events reported by the staff nurses over the last 12 months. It was clear that, the highest percentage of the total nurses (34.2%) reported no events related to patients' safety over the past 12 months. A minority (4.2%) reported 21 events or more over the last 12 months. Almost one fifth of the nurses (20.8%) reported

between 3-5 events related to patient's safety over the last 12 months.

Table (7) shows correlation between nurses' perception of patient safety culture and certain related variables. Results indicate a weak negative statistically significant correlation ($r = -0.210$) between staff nurses age and overall perception of patient safety culture, while there is no correlation between head nurses age and overall perception of patient safety culture ($r = 0.272$). At the same time, there is no correlation between staff nurses and head nurses years of experience and overall positive perception of patient safety culture.

Table (1): Characteristics of the study subjects and the likelihood of contact with patients

Items		Staff Nurses				Total Nurses		Head Nurses	
		Nurses Working in Critical Care Units		Nurses Working in General Wards					
		No	%	No	%	No	%	No	%
Age (in Years)	>20 -	71	85.5	4	10.8	75	63.2	3	10.8
	30-	10	12	15	40.5	25	20.2	15	53.6
	40-	2	2.5	17	45.9	19	15.8	5	17.8
	50-	0	0	1	2.8	1	0.8	5	17.8
							30.58±8.6		39.2±7.9
Qualification In nursing	Bachelor	35	42.2	10	27	45	37.5	20	71.5
	Technical institute	6	7.2	2	5.4	8	6.7	0	0
	Diploma	42	50.6	25	67.6	67	55.8	8	28.5
Years of experience in nursing									
	1-<10	23	27.7	5	13.5	28	23.3	3	10.7
	10-<20	46	55.4	2	5.4	48	40	20	71.5
	20-<30	9	10.8	7	18.9	16	13.4	5	17.8
	30-<40	5	6.1	23	62.2	28	23.3	0	0
						10.89±7.7		13.9±3.6	
Contact with patients	yes	83	100	30	81.1	113	94.6	20	71.5
	no	0	0	7	18.9	7	5.8	8	28.5

Table (2): Percentage distribution of nurses' positive and negative responses to hospital survey of patient safety culture

Dimensions	Nurses Working in Critical Care Units (*n= 83)				Nurses Working in General Wards (*n= 37)				Head Nurses (*n= 28)			
	Positive		Negative		Positive		Negative		Positive		Negative	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
1. Frequency of reported events	15	18.1	10	12.0	4	10.8	10	27.0	5	17.9	5	17.9
2. Overall perception of patient safety	23	27.7	10	12.0	11	29.7	7	13.5	10	35.7	6	21.4
3. Supervisor/manager expectation and actions promoting safety	24	28.9	5	6.0	9	24.3	5	13.5	9	32.1	3	10.7
4. Organizational learning	23	27.7	4	4.8	7	18.9	5	13.5	9	32.1	1	3.6
5. Teamwork within hospital units	22	26.5	12	14.5	11	29.7	7	18.9	9	32.1	3	10.7
6. Communication openness	16	19.3	11	13.3	8	21.6	9	24.3	5	17.9	5	17.9
7. Feedback and communication about error	16	19.3	10	12.0	5	13.5	9	24.3	5	17.9	6	21.4
8. Non-punitive response to error	14	16.9	12	14.5	11	29.7	7	13.5	9	32.1	2	7.1
9. Staffing	20	24.1	10	12.0	7	18.9	10	27.0	5	17.9	3	10.7
10. Hospital management support for patient safety	20	24.1	9	10.8	9	24.3	5	13.5	9	32.1	2	7.1
11. Teamwork across hospital units	16	19.3	12	14.5	11	29.7	5	13.5	11	39.3	2	7.1
12. Hospital handoffs and transitions	17	20.5	10	12.0	7	18.9	5	13.5	9	32.1	3	10.7
Average	22.7		11.5		22.5		18		28.2		12.1	

*N.B: The scale was a 5- point likert scale. Neutral responses were not calculated

Table (3): Comparison between positive responses of nurses working in critical care units and nurses working in general wards regarding perception of patient safety culture.

Dimensions	Nurses working in critical care units (n=83)		Nurses working in general wards (n= 37)		X ²	Significance
	NO	%	No	%		
1. Frequency of reported events	15	18.1	4	10.8	1.01	>0.05
2. overall perception of safety	23	27.7	11	29.7	2.09	>0.05
3. Supervisor /manager expectation and actions promoting safety	24	28.9	9	24.3	0.27	>0.05
4. Organizational learning	23	27.7	7	18.9	3.75	<0.05*
5. Teamwork within hospital units	22	26.5	11	29.7	0.13	>0.05
6. Communication openness	16	19.3	8	21.6	0.08	>0.05
7. Feedback and communication about error	16	19.3	5	13.5	0.59	>0.05
8. Non-punitive response to error	14	16.9	11	29.7	2.57	>0.05
9. Staffing	20	24.1	9	24.3	0.08	>0.05
10. Hospital management support for patient safety	20	24.1	9	24.3	0.001	>0.05
11. Teamwork across hospital units	16	19.3	11	29.7	3.86	<0.05*
12. Hospital handoffs and transitions	17	20.5	7	18.9	0.02	>0.05

(*) Statistically significant at p<0.05

Table (4): Comparison between positive responses of total staff nurses and head nurses regarding perception of patient safety culture.

Dimensions	Total Staff Nurses (n=120)		Head Nurses (n=28)		X ²	p-value
	NO	%	No	%		
1.Frequency of reported events	19	15.8	5	17.9	4.29	<0.05*
2.Perception of patient safety	25	20.8	10	35.7	3.78	<0.05*
3.Supervisor /manager expectation and actions promoting safety	33	27.5	9	32.1	3.85	<0.05*
4.Organizational learning	30	25	9	32.1	0.59	>0.05
5.Teamwork within hospital units	33	27.5	9	32.1	3.85	<0.05*
6.Communication openness	24	20	5	17.9	5.42	<0.05*
7.Feedback and communication about error	21	17.5	5	17.9	5.71	<0.05*
8.Non-punitive response to error	25	20.8	9	32.1	4.72	<0.05*
9.Staffing	30	25	5	17.9	4.76	<0.05*
10.Hospital management support for patient safety	29	24.2	9	32.1	3.93	<0.05*
11.Teamwork across hospital units	27	22.5	11	39.3	4.02	<0.05*
12.Hospital handoffs and transitions	24	20	9	32.1	7.62	<0.05*

(*) Statistically significant at p<0.05

Table (5): Work area/unit overall grade on patients' safety as perceived by nurses.

Patients safety Grade	Nurses Working in Critical Care Units (no =83)		Nurses Working in General Wards (no= 37)		Total Staff Nurses (no= 120)		Head Nurses (no=28)	
	No	%	No	%	No	%	No	%
Excellent	19	22.9	1	2.7	21	17.5	4	14.3
Very good	28	33.7	22	59.5	50	41.7	5	17.8
Acceptable	22	26.5	9	24.3	31	25.8	14	50.0
Poor	12	14.5	5	13.5	17	14.1	4	14.3
Failing	2	2.4	0	0	1	0.9	1	3.6

Table (6): Number of events regarding patients' safety reported by staff nurses

Number of events reported over Last 12 months	Nurses Working in Critical Care Units (n= 83)		Nurses Working in General Wards (n= 37)		Total Staff Nurses (n= 120)	
	No	%	No	%	No	%
No events reported	27	32.5	14	37.8	41	34.2
2	10	12	10	27.0	20	16.7
3 - 5	16	19.3	9	24.3	25	20.8
6 – 10	17	20.5	3	8.1	20	16.7
11 – 20	9	10.8	0	0.0	9	7.5
21 or more	4	4.82	1	2.7	5	4.2

Table (7): Correlation between nurse's perception of patient safety culture and certain related variables

Items	Staff Nurses	Head Nurses
Age	r = - 0.210* P=0.021 P<0.05 S	r =0.272 P=0.161 P>0.05 NS
Years of experience	R= -0.027 P=0.891 P>0.05 NS	R=- 0.066 P=0.473 P>0.05 NS

* Significant

NS= Not significant

4. Discussion:

The aim of the present study was to assess nurses' perception of patient's safety culture at Medical and Paediatric University Hospitals and developing an improvement plan to enhance patient safety at the study settings.

The present study findings showed an overall staff and head nurses positive response to patient safety culture. This result is congruent with Singer et al. (2003) and Bscphm et al. (2008), in similar studies who found that, the overall percentage of positive response to patient safety culture was higher than negative. On the other hand this study findings were contradicting with a study conducted in Egypt by Abbas , et al. (2, et al. (2007) identified poorer perception of safety culture by nurses.

The current study results revealed that there was a significant difference between perception of nurses working in critical care units and nurse working in general wards in relation to the organizational learning environment dimension, where nurses working in critical care units perceive

this dimension more positively than nurses working in the general wards, which answers the first research question. The reason behind this could be simply explained as training and ongoing educations are usually directed to critical care unit nurses more than other unit's nurses. Similarly, a study done by Ahmed (2002) reported that, most of the training required by nurses was in the area of critical care unit.

This study finding revealed that, staff nurses perceived patient safety culture more positively than negatively. This result is comparable to previous study findings, which revealed that the overall percentage of positive responses of patient safety culture was higher than negative responses among staff nurses (Singer et al., 2003). At the same time, head nurses perceive patient safety culture more positively than staff nurses do. This result is in accordance with several previous studies (Singer et al., 2003; Kim et al., 2007; and Singer et al., 2008), which reported that, head nurses usually perceive safety culture more positively than staff nurses do. Possible reasons for this phenomenon could be explained as: first, managers have less opportunity to witness the safety

hazards, which is commonly existed in frontline situations; and second managers fail to communicate the organizational safety initiatives, policies, and expectations to the frontline staff. Another possible explanation would be the lack of a reporting culture or the mechanism to encourage reporting. Additionally, nursing managers consciously establish and reinforce the norms and attitudes related to safety practices, which could engender a positive perception of patient safety culture (Alton et al., 2006; Voqus and Sutcliffe 2007; Singer et al., 2008).

The highest percentage of staff nurses working in critical care units perceived supervisor/manager expectations and actions promoting safety culture dimension more positively than those working in general wards did. These results may be attributed to that, the critical care units' work which needs more supervision and compliance to safety standards because of the critical condition of patients. As well, this finding could be due to that, the environment of a critical area necessitates the supervision to be stronger and harder than in the general units. This finding is supported by Ahmed (2002), who concluded that, nurses working in critical areas or units always work under stress and busy tone.

The current study finding revealed a significant difference between nurses working in general wards and nurses working in critical care units as the first group respond more positively to the dimensions related to teamwork across hospital units than the last one. This finding might be explained by that, the working environment in the general wards is quiet and allows more time to communicate than in the critical ones. In this respect, Guise and Sigel (2008) emphasized that good team work is essential for the delivery of effective and efficient care in any clinical setting.

Results regarding teamwork also received the highest positive responses by head nurses suggesting that these are the areas of strength. High percentage in nurses' perception of team might reflect the evidence of effective team collaboration in the study organization. This finding could be due to that university hospital environment is providing a model of collaboration and teamwork. It could also have been the result of hospital management that is valuing nurses' teamwork. The highest positive response rate of communication and teamwork within units is congruent with what was reported by Espin et al., (2006;and AHRQ (2008).

As regards the communication openness dimension, staff nurses working in the critical care units are responding to it more positively than those working in the general wards. This result could be explained as nurses in critical care units recognize the importance of effective communication among healthcare team. This results is congruent with (Cuthbertson, et al. 2007 and Baker et al., 2009) who

concluded that effective team communication and coordination are recognized as being crucial for improving quality and safety in acute medical setting such as in ICU. Additionally, the Joint Commission of Accreditation (JCAHO) (2009), emphasised the importance of effective communication among caregivers.

The second research question was confirmed by that, there are significant differences between staff nurses' and head nurses' perception of patient safety culture. The highest percentage of head nurses had positive responses toward most of the dimensions. The reason behind that may be due to that head nurses were satisfied with their units and had a feeling that they apply more control, since they might have higher expectations for the patient safety on their units. This could be also due to that head nurses are frontline nurses in the organization and they usually recognize the importance of patient safety.

The lowest positive response to the non-punitive response to error dimension was shown by nurses working in critical care units. This result might be due to the close supervision in the critical care units, where mistakes are apparent. This finding is consistent with the AHRQ (2008) report, which showed that the cultural dimension of non-punitive response to error received the highest negative response percent (Boyle, 2004; and Kim et al., 2007). Conversely, the highest percentage of nurses working in general wards as well as head nurses responded positively toward this dimension. This result might be due to less supervision in the general wards, meanwhile head nurses may have thought that they were applying non-punitive culture in the hospital or they might not be punished by their supervisors.

The lowest percentage of positive response as identified by nurses working in general wards was related to frequency of reported events. This might be due to the low level of supervision and controlling system, and the presence of the punitive culture in the hospital. Therefore, nurses were afraid to report errors and probably afraid from being punished for making errors.

Regarding to the number of events reported by staff nurses, the current study results revealed that, little percentage of nurses reported events over the past 12 months. It is likely that this percentage represents under-reporting of safety events. In a similar study, the AHRQ (2008) stated that, an average of 48 % respondents reported the safety events in their hospitals over the past 12 months. Two possible reasons might contribute to this low reported safety events: First, it might be due to the presence of blaming or punishment culture. So, nurses choose not to report in order to avoid being punished by management and being jeered by peers (Kalisch & Aeberdold, 2006). Reporting error in a study carried out by Throckmorton and Etchegaray (2007) revealed that nurses identified the reason for not reporting error, if it is serious or if it affects the patients.

The second reason that might have contributed to low reported safety events in this study might be due to that nurses were afraid to report errors because they don't trust their management and perceived trustworthiness of managers and organizations were found to be related to patient safety culture. Congruent with the current study finding, a study conducted by Burns et al. (2006) revealed that, the major reason for nurses unwillingness to report errors were concerns about losing trust of their managers and peers. The finding is also consistent with another study, where researchers found that high manager trustworthiness facilitates open safety communication (Conchie et al., 2006).

There were differences between staff nurses and head nurses regarding to hospital overall grade on patients safety. Slightly less than three fifth of nurses working in general wards graded their hospital as very good, while only third of nurses working in critical care unit gave the same score. On the other hand, half of the head nurses graded their hospital as acceptable. these findings are in agreement with Richardson and Williams (2007), who concluded that, the head nurses usually are more experienced and they are more likely to find safety hazards in their work situations. It is also possible that the head nurses feel more comfortable in reflecting their true perceptions because they may feel more protected in their positions.

The correlation of staff nurses' age and their perception of patient safety culture showed significant weak negative correlation, this finding is extremely important because it reflects the reality of nurses concern regarding risks. This means that regardless of their reporting of positive responses their feeling of unsafe culture grows overtime.

5. Conclusion:

Almost one third of the nurses under study perceived the patient safety culture positively. There were statistically significant differences between nurses working in critical care units and nurses working in general wards regarding to patient safety culture in two dimensions. Head nurses perceived the patient safety culture more positively than staff nurses in seven dimensions. The majority of the nurses under study did not report the safety events in their work areas. There are areas of strengths and weaknesses in nurse's perception that can be improved to provide best safe culture for patient care. Non-punitive environment scored high negative response.

Recommendations:

1. Nurses are in need to be encouraged to improve reporting of events or incidents related to patients' safety.

2. Further studies are needed for testing the reliability of the improvement plan and accordingly implementing it at the study settings.
3. Developing and disseminating procedures for patient safety among all nursing staff working in critical care and general wards to assure the compliance with all appropriate standards.
4. Staff development programs should be conducted for head nurses at all levels to be aware of the significance of patient safety culture in their work areas and organizations.
5. Staff development programs are needed for all nurses working in critical care units and general wards to understand the values, beliefs, and norms about what is important in an organization and what attitudes and behaviours related to patient safety are expected and appropriate for achieving a culture of safety.
6. Head nurses are in need to be encouraged to establish non-punitive environment as well as a teamwork spirit among nursing staff working in critical care and general ward staff nurses.
7. Supporting more research efforts particularly in areas that yield the greatest benefit and that more effectively contribute to improving patients' safety and safe patients' lives.

Implication of the study

The results have implications for nursing practice; and administration, as the study considers organizational and individual factors that might influence patient safety. The outcome of this study would also be useful in supporting a culture of patient safety and quality improvement in health care service to be accredited. Additionally, it could help nurse managers to create a culture of safety and prevention of accidental harm through identification of safety dimensions , prospective analysis and follow the improvement plan.

Corresponding Author:

Neamatallah Goma Ahmed

Department of Medical Surgical Nursing, Faculty of Nursing, Ain Shams University. Cairo., Egypt.
nemata55@hotmail.com

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Detection and Quantification of *Porphyromonas gingivalis* from Saliva of Schizophrenia Patients by Culture and Taqman Real-Time PCR: A Pilot Study

^{1*}Maggie M. Fawzi, ²Mounir M. Fawzi, ²Hany M. El-Amin, ³Mahmoud H. Elafandy

¹Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

²Psychiatry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

³Prosthodontic Department, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

*mag0000eg@yahoo.com

Abstract: *P. gingivalis* is a periodontopathogen implicated in a number of systemic diseases, particularly cardiovascular disease. Little, if any, is known about the prevalence and quantity of this organism in the mouth of patients with schizophrenia who are, nevertheless, known to have poor oral health and die early from cardiovascular disease. Aim: to estimate the prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and to correlate the quantity of *P. gingivalis* with the severity of psychopathology of schizophrenia. Materials and methods: Thirty five consecutive attendees of the out-patients clinic of a psychiatric Hospital in Jeddah, with a diagnosis of schizophrenia, were assessed by the Positive and Negative Syndrome Scale (PANSS) and compared with 35 non-psychiatric controls, in terms of the prevalence and quantity of *P. gingivalis* in their saliva. For this purpose, anaerobic culture and real-time PCR with TaqMan probe were used. Results: Real-time PCR results were matching those obtained with anaerobic culture in 95.7% of cases. Using Real-time PCR, *P. gingivalis* was detected in 25 patients (78%) and 6 controls (17%) (p=0.000). The *P. gingivalis* median (range) number of copies in salivary samples of patients and controls were 5.3×10^7 (0- 2.73×10^{10}) and 1.91×10^5 (0- 6.81×10^7), respectively (p=0.009). Also, the *P. gingivalis* levels were significantly positively correlated with the scores on all the PANSS scales. Conclusion: real-time PCR, in confirmation of the results of quantitative culture, demonstrated (a) significantly higher prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and (b) positive correlation between quantity of *P. gingivalis* cells and severity of psychopathology of schizophrenia. Hopefully, the results of this pilot study will encourage further research into the relationships between oral microbiota and schizophrenia. Real-time PCR is a promising tool in this area. Hopefully too, some preventive dental programs will become an integral part of psychiatric management to meet the need of this vulnerable group of population.

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1. Introduction

Bacterial inhabitants of the oral cavity comprise one of the most complex microbial community systems associated with human body. It has been estimated that more than 700 different bacterial species inhabit the human oral cavity⁽¹⁾. These organisms, which live mainly in the saliva, have the ability to colonize the surfaces of the buccal cavity and to develop there in the form of complex biofilms⁽²⁾. They interact with each other and with their host tissues⁽³⁾. These interactions, however, are still not well understood. While the majority of oral microflora are normal/commensal bacteria, some of them are opportunistic pathogens responsible for the development of oral microbial infectious diseases such as dental caries and periodontitis⁽⁴⁾. Numerous studies have shown that these pathogens do not only cause chronic localized conditions, but may also increase the risk of various systemic diseases⁽⁵⁻⁷⁾.

One of the most pathogenic species of the entire oral flora, and perhaps the most extensively studied at the molecular level is *Porphyromonas gingivalis*. It belongs to the family *Porphyromonadaceae*, order *Bacteroidales* in the phylum *Bacteroidetes*, formerly known as the *Cytophaga-Flavobacteria-Bacteroides* group⁽⁸⁾, a black-pigmented Gram-negative, obligate anaerobic and asaccharolytic cocco-bacillus⁽⁹⁾. *P. gingivalis* is frequently found as a prominent component of the flora of subgingival lesions of adult patients with periodontitis⁽¹⁰⁾. Furthermore, *P. gingivalis* is incriminated in certain systemic conditions, such as atherosclerotic heart disease⁽¹¹⁾. Pathogenicity of this organism is attributed to a large number of putative virulence factors, such as cysteine proteinases (gingipains), hemagglutinins, lipopolysaccharide (LPS), and fimbriae by which *P. gingivalis* is enabled to invade host tissues⁽¹²⁾. *P. gingivalis*, however, is among the late colonizers of the oral cavity, a process

that is facilitated by other microbial species that provide attachment sites, as well as supply growth substrates, and reduce oxygen tension to levels optimal for growth of *P. gingivalis*⁽⁸⁾. Colonization of *P. gingivalis* is influenced by saliva, which serves as a vector for its transmission and initial entry into oral environment⁽¹³⁾. Anchoring points for *P. gingivalis* fimbriae are also provided by salivary pellicle-coated tooth surfaces. *P. gingivalis* cells, then, enmesh into networks of intercellular communication with other oral prokaryotic cells and with eukaryotic cells⁽¹⁴⁾.

In periodontitis patients, *P. gingivalis* can be detected in saliva, on the dorsum of the tongue, tonsils, buccal mucosa and gingiva, and other mucous membranes⁽¹⁵⁾, whereas in periodontally healthy individuals, this organism is usually absent, or if present, it is in low numbers⁽¹⁶⁾. Studies suggest that periodontal disease can be minimized through maintenance of oral cleanliness⁽¹⁷⁾. However, non-compliance is a major issue⁽¹⁸⁾. Though it is a universal phenomenon, non-compliance appears to affect people with severe mental illness, such as schizophrenia, considerably more than other people⁽¹⁹⁾. In these patients, non-compliance is found to be as high with non-psychiatric drugs as with psychiatric medications⁽²⁰⁾, and is probably related to the severity of psychopathology^(21, 22). These patients are further disadvantaged not only by having higher rates of physical illnesses than those without schizophrenia, but also by experiencing greater difficulty in getting adequate health care⁽²³⁾. Oral health, which is an integral part of the general health, and contributes to self esteem and quality of life, may have a low priority among these patients who are, nevertheless, liable to get dental problems⁽²⁴⁾. General self-neglect associated with mental illness, misconceptions, fear of treatment, worry about the cost of treatment, inability to access dental services and the adverse-effects of medications are also among the most commonly cited barriers to dental care^(25, 26).

To date, however, there has been relatively little research assessing oral status of patients with schizophrenia. Most of these studies have focused on institutionalized chronic patients^(27, 28) although the majority of patients are now living outside hospital. Oral health research about those attending outpatient psychiatric services is "almost non-existent"⁽²⁹⁾. Moreover, approaches have been mostly restricted to a clinical descriptive level using self-report questionnaires^(30, 24, 29) and/ or clinical dental examinations⁽³¹⁻³⁴⁾. It may be rather surprising that, despite the availability and the researchers' extensive use of various procedures to examine oral microorganisms in various populations, no previous attempts have been made, as far as we know, to detect or quantify oral opportunistic pathogens, such as *P.*

gingivalis, in patients with schizophrenia. These patients are particularly prone to cardiovascular disease⁽³⁵⁾ which is the chief cause of their excess premature mortality⁽³⁶⁾. Ironically, oral infection with *P. gingivalis* has been also strongly associated with cardiovascular disease, even after adjustment for established cardiovascular risk factors⁽¹¹⁾. However, it is not known whether there is any relation between *P. gingivalis* and schizophrenia, although researchers have tried, for more than a century, and still trying to find a role for infectious agents in triggering schizophrenia⁽³⁷⁾.

This cross-sectional pilot study aimed to estimate the prevalence and quantity of *P. gingivalis* in saliva of schizophrenia patients compared to non-psychiatric controls and to correlate the quantity of *P. gingivalis* with the severity of psychopathology of schizophrenia. We hypothesized that the severity of oral infection in patients with schizophrenia is related to the severity of psychopathology.

2. Material and Methods:

A total of 35 patients were recruited from consecutive attendees of the out-patients clinic of a large private psychiatric Hospital in Jeddah between January and July 2010, with a diagnosis of schizophrenia (F.20 of the ICD10)⁽³⁸⁾, aged 20 to 50 years, and for whom a stable regimen of antipsychotic medication was consistently prescribed for at least 3 months prior to recruitment.

The control group consisted of 35 subjects, individually matched for age and sex and randomly selected from companions of patients and from hospital employees and their acquaintances.

None of the subjects had current febrile acute infection, acute exacerbation of a chronic infection or an inflammatory disease, underlying hematologic, malignant, severe cardiac, liver or renal disease. None within the previous 12 weeks used antibiotics or had any dental or general surgery. Participants who had missing teeth and females who were pregnant or lactating were excluded from the study. Body Mass Index greater than or equal to 35 or less than or equal to 18 and blood pressure >160/100, were also exclusion criteria. Controls had no evidence of current or past history of any psychiatric disorder. A written informed consent was obtained from each participant.

Patients underwent a standardized psychiatric interview during which the ICD-10 diagnosis of schizophrenia was confirmed and the Positive and Negative Syndrome Scale (PANSS)⁽³⁹⁾ was applied by a trained psychiatrist. PANSS is a 30-item test, subdivided into three subscales: a Positive Scale (P) composed of seven items, a Negative Scale (N) composed of seven items and a General Psychopathology Scale (G) composed of 16 items.

Each item is rated on a seven point severity scale, from 1 (no evidence) to 7 (extreme).

Saliva sampling procedure

Saliva specimens were collected by expectoration into sterile calibrated medical cups. Saliva was put into Eppendorf tube, which was immediately frozen at -80 and stored until used in real-time PCR. For the detection of *P. gingivalis* by bacterial culture saliva samples were pooled in 1.5 ml of reduced transport fluid and were processed for cultivation under anaerobic conditions within 4 h of sampling. Samples were vortexed for 2 min and split. A total of 100 μ l of the sample was used for culture by tenfold serial dilution in sterile phosphate-buffered saline solution.

Culture

Serial 10-fold dilutions were prepared, and the last three dilutions were used for plating on blood agar plates (Oxoid, Basingstoke, United Kingdom) supplemented with horse blood (5%; vol/vol), hemin (5 mg/liter), and menadione (1 mg/liter) and incubated anaerobically in jars filled by the evacuation-replacement method with a mixture of gases (85% N_2 , 10% H_2 , 5% CO_2) at $37^\circ C$ for 7 to 14 days. The isolates were identified as *P. gingivalis* on the basis of Gram staining, anaerobic growth, having the typical colony color and morphology, lacking colony autofluorescence, positive hemagglutination with 3% sheep erythrocytes as well as the production of a set of metabolic enzymes (as tested with the Rapid ID kit 32A) and having a positive indole reaction. The total number of CFU of *P. gingivalis* in positive samples was determined.

Real-time PCR

Isolation of DNA

To extract DNA from the bacteria present in saliva, frozen suspensions were thawed and 100 μ l samples were used for automated DNA extraction and purification with the MagNA Pure DNA Isolation Kit III (Bacteria, Fungi; Roche Molecular Diagnostics). The protocol included 1 h of pretreatment with proteinase K (20 mg/ml) at $56^\circ C$. After isolation, the DNA was eluted in 100 μ l of elution buffer.

PCR primers and probes

The 16S rRNA sequences of the genus *Porphyromonas* were selected. The sequence of the forward primer, , was 5 -GCGCTCAACGTTTCAGCC-3 (base pairs 612 to 628); the sequence of the reverse primer, , was 5 -CACGAATTCCGCCTGC-3 (base pairs 664 to 679); and the sequence of the TaqMan probe, was 5 -CACTGAACTCAAGCCCGGCAGTTTCAA-3 (base

pairs 634 to 660) The primers and probes were purchased from Applied Biosystems (Foster City, California, USA).

Quantitative PCR assay

PCR amplification was performed in a total reaction mixture volume of 25 μ l. The reaction mixtures contained 12.5 μ l of 2 \times TaqMan universal PCR master mixture (PCR buffer, deoxynucleoside triphosphates, AmpliTaq Gold, an internal reference signal [6-carboxy-X-rhodamine], uracil *N*-glycosylase, $MgCl_2$; Applied Biosystems), 300 nM each *P. gingivalis*-specific primer, 100 nM *P. gingivalis*-specific probe. and 5 μ l of purified DNA from plaque samples. Five microliters of the DNA extracted from *P. gingivalis* W83 was used to prepare the standard curve and as a positive control; the negative control was 5 μ l of sterile H_2O .

The samples were subjected to an initial amplification cycle of $50^\circ C$ for 2 min and $95^\circ C$ for 10 min, followed by 45 cycles at $95^\circ C$ for 15 s and $60^\circ C$ for 1 min. The degradation of the probe by the DNA polymerase in each elongation step induces an increase in fluorescence that can be monitored during PCR amplification. The fluorescence signal is normalized by dividing the reporter dye emission (6-carboxyfluorescein) by the emission of the passive reference (6-carboxy-X-rhodamine). The higher the starting copy number of the nucleic acid target is, the sooner a significant increase in fluorescence is observed. Hence, this parameter can be used to compare different amplification reactions. The number of bacterial copies was calculated assuming that the genome mass is equal to 2.37 fg (femtogram=10 $^{-15}$ g)⁽⁴⁰⁾.

Statistical analysis:

Continuous data were expressed as mean (\pm standard deviation) or median (range) and were compared by use of Student's t-test, after testing for normality with a Kolmogorov-Smirnov-test and normalization by log-transformation where appropriate. Categorical data were expressed as frequencies or proportions and were analyzed with the two-tailed chi-square test. Correlations between data were analyzed using Pearson's coefficient. Data were analyzed using SPSS version 11.0.1. Software (SPSS for Windows, 2001). Two-tailed p values <0.05 were considered statistically significant.

3. Results:

Background characteristics of participants:

Background characteristics of patients and controls were not significantly different (table 1).

Severity of psychopathology in the patient group:

Mean (\pm SD) of scores of patients on the standard scales of PANSS are given in table 2.

Prevalence of salivary *P. gingivalis*:

P. gingivalis was more prevalent in salivas from patients than controls. *P. gingivalis* was detected in 78% (25 of 35) of the patient group but was found only in 17% (6 of 35) of the controls (table 3). For either group, no relationship was found between detection of *P. gingivalis* and gender, age or nationality. However, significant relationships were observed between *P. gingivalis* detection and being less educated, in a lower occupational position, not married, currently smoker and not a Miswak user (table 3).

Numbers of *P. gingivalis* cells:

Table (4) shows the results of absolute quantification of *P. gingivalis* cells determined in individual PCR runs. There is a significant difference between the number of *P. gingivalis* cells in salivary samples of patients and controls.

Relationship of the salivary *P. gingivalis* count with the severity of psychopathology in the patient group:

The salivary levels of *P. gingivalis* were significantly positively correlated with the scores on all the PANSS scales (table 5).

Comparison between PCR and culture:

Results obtained with real-time PCR were matching those obtained with anaerobic culture in 95.7% of cases (28 positive; 39 negative). A two-by-two contingency table summarizes the results (table 6). *P. gingivalis* was cultured from 28 (40%) of the 70 saliva specimens. All these culture-positive samples were also positive by the real-time PCR assay (100% sensitivity). In addition, 3 samples were positive for *P. gingivalis* by the real-time PCR but negative by culture. These samples were thawed and recultured for 14 days. Two of these samples yielded *P. gingivalis* after this prolonged culture. Of the 42 culture-negative samples, 39 were negative by PCR assay (90.3% specificity). None (0%) of the PCR-negatives was found to be culture-positive (table 6).

Table (1): Background characteristics of participants

	Patients N= 35	Controls N= 35	Significance
Gender:			
Male: N	21	21	$\chi^2=0.000$; $df=1$; $p=1.000$
Female: N	14	14	
Age (years):			
Mean (\pm SD)	29.9 (\pm 8.9)	30.2 (\pm 8.8)	$t=0.163$; $df= 68$; $p=0.871$
Age group:			
<30 years: N	22	21	$\chi^2=0.060$; $df=1$; $p=0.806$
>30 years: N	13	14	
Nationality:			
Saudi: N	16	17	$\chi^2=0.057$; $df=1$; $p=0.811$
Non-Saudi: N	19	18	
Education level:			
Intermediate or below: N	27	22	$\chi^2= 1.701$; $df=1$; $p=0.192$
Above intermediate: N	8	13	
Occupation:			
Higher: N	7	13	$\chi^2= 2.520$; $df=1$; $p=0.112$
Lower: N	28	22	
Marital status:			
Married: N	15	19	$\chi^2=0.915$; $df=1$; $p=0.339$
Unmarried*: N	20	16	
Currently smoking:			
Yes: N	22	14	$\chi^2=3.660$; $df=1$; $p=0.056$
No : N	13	21	
“Miswak”*** habitual user:			
Yes: N	15	17	$\chi^2=0.230$; $df=1$; $p=0.631$
No : N:	20	18	

* Unmarried= Never married, divorced, separated and widowed.

** “Miswak” = “Sewak”=tooth cleaning stick

Table (2): Scores of patients on the Positive and Negative Syndrome Scale (PANSS)

Scale	Score
Total	
Mean (\pm SD)	82.2 (\pm 13.0)
Positive	
Mean (\pm SD)	22.2 (\pm 3.4)
Negative	
Mean (\pm SD)	22.1 (\pm 3.1)
General psychopathology	
Mean (\pm SD)	38.1 (\pm 9.7)

Table (3): Prevalence of *P. gingivalis* by real-time PCR*

	Real-time PCR		² (df=1)	p
	Positive N	Negative N		
All subjects				
Patients	25	10	20.902	0.000
Controls	6	29		
Gender				
<i>Patients</i>				
Male	16	5	0.583	0.445
Female	9	5		
<i>Controls</i>				
Male	3	18	0.302	0.583
Female	3	11		
<i>Total</i>				
Male	19	23	0.039	0.844
Female	12	16		
Age				
<i>Patients</i>				
<30 years	16	6	0.049	0.825
>30 years	9	4		
<i>Control</i>				
<30 years	3	18	0.302	0.583
>30 years	3	11		
<i>Total</i>				
<30 years	19	24	0.000	0.983
>30 years	12	15		
Nationality:				
<i>Patients</i>				
Saudi:	9	7	3.327	0.068
Non-Saudi:	16	3		
<i>Control</i>				
Saudi:	2	15	0.673	0.412
Non-Saudi:	4	14		
<i>Total</i>				
Saudi:	11	22	3.035	0.081
Non-Saudi:	20	17		
Education level:				
<i>Patients</i>				

Intermediate or below:	23	4	10.954	0.001
Above intermediate:	2	6		
<i>Control</i>				
Intermediate or below:	6	16	4.279	0.039
Above intermediate:	0	13		
<i>Total</i>				
Intermediate or below:	29	30	14.693	0.000
Above intermediate:	2	19		
Occupation:				
<i>Patients</i>				
Higher	1	6	14.000	0.000
Lower	24	4		
<i>Control</i>				
Higher	0	13	4.279	0.039
Lower	6	16		
<i>Total</i>				
Higher	1	19	17.514	0.000
Lower	30	20		
Marital status:				
<i>Patients</i>				
Married:	7	8	7.887	0.005
Unmarried*:	18	2		
<i>Control</i>				
Married:	0	19	8.599	0.003
Unmarried*:	6	10		
<i>Total</i>				
Married:	7	27	15.047	0.000
Unmarried*:	24	12		
Current smoker:				
<i>Patients</i>				
Yes:	20	2	11.014	0.001
No:	5	8		
<i>Control</i>				
Yes:	5	9	5.666	0.017
No:	1	20		
<i>Total</i>				
Yes:	25	11	19.014	0.000
No :	6	28		
Miswak*** habitual user				
<i>Patients</i>				
Yes:	8	7	4.212	0.040
No:	17	3		
<i>Control</i>				
Yes:	0	17	6.839	0.009
No:	6	12		
<i>Total</i>				
Yes:	8	24	8.886	0.003
No:	23	15		

* Number of subjects with *P. gingivalis* /Number of subjects tested (%)

** "Miswak" = "Sewak"=tooth cleaning stick

Table (4): Number of copies (median and range values) of *P. gingivalis* in salivary samples of patients and controls assessed by real-time PCR absolute quantification.

	Patients	Controls	Significance*
Median	5.3x10 ⁷	1.91x10 ⁵	t=2.694; df= 68; p=0.009
(Range)	(0- 2.73 ¹⁰)	(0- 6.81 ⁷)	

*After normalization using logarithmic transformation

Table (5): Correlation coefficients between number of copies of *P. gingivalis* in salivary samples of patients and scores on the Positive and Negative Syndrome Scale (PANSS)

PANSS scale	r	p
Total	0.488	0.003
Positive	0.451	0.007
Negative	0.518	0.001
General psychopathology	0.431	0.010

Table (6): Detection of *P. gingivalis* by real-time PCR and anaerobic culture

Anaerobic culture result		Real-time PCR result*		Total N
		Positive N	Negative N	
Positive	N	28	0	28
Negative	N	3	39	42
Total	N	31	39	70

*Sensitivity= 100.0%; specificity= 90.3; Positive predictive value=100.0%; Negative predictive value=92.9%

4. Discussion:

We believe this study is the first to report a higher prevalence of the oral pathogen, *P. gingivalis*, in salivas from patients with schizophrenia than matched non-psychiatric controls. We used saliva because, as an oral circulating fluid, saliva is heavily laden with bacteria (108 – 109 cfu/mL)⁽⁴¹⁾. Previously, all 16S rRNA sequences of the genus *Porphyromonas* based saliva studies had utilized qualitative PCR. For the detection and quantification of *P. gingivalis* in saliva samples in the current study, however, we compared the results of a quantitative anaerobic culture method with those of a real-time TaqMan PCR assay, which is, unlike conventional PCR assays, less susceptible to PCR inhibition⁽⁴²⁾ and is suggested to provide a sensitive, efficient, and reliable approach to quantitation⁽⁴³⁾. In keeping with this suggestion, we found the sensitivity, specificity, and positive and negative predictive values of the real-time PCR to be 100, 90.3, 100 and 92.9% respectively. We conclude, therefore, that real-time PCR confirms the results of quantitative culture of *P. gingivalis* and offers promising advantages with respect to the rapidity and sensitivity of detection of *P. gingivalis* in saliva samples. Until recently, however, very little attention has been given to the quantification of *P. gingivalis* in saliva, whether of psychiatric or non-psychiatric populations.

Our results demonstrated that in both patients and controls *P. gingivalis* detection was correlated with being less educated and in a lower occupational

position. These results are consistent with previous studies which have shown that periodontitis is more common among people with low than with high socio-economic status, regardless of the indicator used⁽⁴⁴⁾. Poverty, which raises the risk of schizophrenia, especially deficit schizophrenia⁽⁴⁵⁾, reduces the chance of receiving adequate dental care, and hence, could partly explain the results. Our finding that the *P. gingivalis* detection was more frequent among unmarried than married people in both patients and controls is also consistent with other studies showing higher susceptibility to various infections among single, widowed, and separated than married individuals, independent of other demographic factors⁽⁴⁶⁾. Also, in keeping with other studies, which have indicated that smoking significantly increases the risk for the development of extensive and severe oral infections⁽⁴⁷⁾, we found significant correlation between *P. gingivalis* detection and current smoking in both patients and controls. Interestingly, habitual use of “Miswak” (the chewing stick or the traditional toothbrush in common use in Saudi Arabia and many Islamic countries) was negatively associated in this study with *P. gingivalis* detection. This should lend support to the few previous studies which have suggested that regular use of miswak is associated with good oral health⁽⁴⁸⁻⁵⁰⁾.

Correlation coefficients of the salivary levels of *P. gingivalis* with scores on PANSS were determined in this study. The results showed that *P.*

gingivalis levels were significantly associated with the severity of schizophrenia psychopathology as expressed by PANSS scores, with negative symptoms presenting the strongest correlation. The achieved results were not unexpected, considering that negative symptoms, which include symptoms such as lack of initiative (PANSS: N2), apathy, anergy, or avolition (PANSS: N4), etc., would likely lead to a reduced self-care and poor dental health, far worse than that of members of the general population⁽²⁴⁾. However, a cause and effect relationship between severities of the negative or other symptoms of schizophrenia and quantities of the oral pathogen should not be claimed by the present pilot study at least because of the limitation of its cross-sectional design.

Among participants we excluded from the study were those with evidence of cardiovascular disease. But this variable should have been important and interesting to investigate when relating to both *P. gingivalis* and schizophrenia. In addition, we did not report some rather relevant data such as details of medication history, general and dental clinical and radiographic examination findings. We did not assess the cognitive functions, although the central role of cognitive dysfunction in schizophrenia has been increasingly appreciated⁽⁵¹⁾ while there have been some suggestions that cognitive impairment may be associated with periodontal disease^(52, 53). Also, the endocrine and metabolic status of the participants, despite relevance to both schizophrenia and oral infections with a possible confounding role, was not evaluated. One more limitation is the relatively small sample size.

In summary, we conclude within the limits of this study that the real-time PCR has confirmed the results of quantitative culture and demonstrated significantly higher prevalence and quantity of *P. gingivalis* in the saliva of patients with schizophrenia compared to non-psychiatric controls. Both real-time PCR and quantitative culture have also confirmed a positive correlation between quantity of *P. gingivalis* cells and severity of psychopathology of schizophrenia. This pilot study may be the first to report such findings. It is hoped, however, that the results will encourage further research into the relationships between oral microbiota and schizophrenia. Real-Time PCR, with its capacity to produce both qualitative and quantitative results, is a promising tool in this area. We should also hope that the need of the mentally ill for more dental care will be appreciated by all concerned and that some preventive dental programs will become an integral part of psychiatric management to meet the need of this vulnerable group of population.

Conflicts of interest: None

Correspondence author

Maggie M Fawzi,
Clinical Pathology Department, Faculty of Medicine,
Zagazig University, Zagazig, Egypt.
e-mail: mag0000eg@yahoo.com

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The role of information and communication technologies (ICT) in rural development

Sharareh Khodamoradi¹ and Mohammad Abedi²

¹Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran

*Corresponding author: abedi114@yahoo.com

Abstract: Review of literature shows that intervention of information and communication technologies (ICT) in rural development initiatives are capable of development, but are not successful. Lack of community participation, absence of an integrated approach and non-inclusion of traditional knowledge systems (TKS) in the project designs are the major impediments. We therefore suggest a systems-based approach in the design of e-Governance projects, and brief some future directions. Sustained development using rural informatics is possible, only if ICT interventions are able to respond to the local needs and re-adjust as per the prevailing knowledge (Traditional Knowledge Systems- TKS) of the rural areas. To capture the needs and local knowledge prevalent at the grassroots, these interventions should preferably have an effective bi-directional link.

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Introduction:

Information and Communication technologies (ICT) have a potential for economic growth and social empowerment (Nandi, 2002). Direct or indirect application of ICT, in rural development sector has also been referred to as "Rural Informatics". Rural economies can be benefited from ICT by focusing on social production, social consumption and social services in the rural areas (Malhotra, 2001). The inculcation of a Citizen-to-Government (C2G) and Citizen-to-Citizen (C2C) interface would provide this link that would also lead to community participation in design and implementation of ICT interventions. This in return could promise better economic opportunities as well as social inclusion of rural people in the processes of governance. Such attributes in the social set up are essential prerequisites for good governance and rural development.

Globalization and technological changes, the processes in the past fifteen years have been quickly lead to a new global economy have been driven with the reinforced technology and fuel (energy) that by providing information and knowledge.

The global economy requires the kind of necessity and purpose of educational institutions. Since the current trend towards reducing incomplete information and access to accurate information is growing, other schools can not control time to transfer a set of prescribed information from teacher to student during a fixed time point are, but schools must to promote Culture of "Teaching for Learning For example, acquisition of knowledge and

continuous learning skills which make possible during the individual's life. According to Alvin Toffler, illiterate in 21st century, who was not read and write but those who do not know which fail to learn or remember are illiterate (Jauhari, 2004).

Concerns about educational quality and educational opportunities with the necessity of developing those most vulnerable are the accumulation of globalization is symbiotic. Generally, "the changes of globalization in developing countries, on low-income groups, especially women and girls and" low skill workers, as well as all groups applying for and obtaining new skills to press (Bellamy and Taylor, 1998).

In the rural context, development involves use of physical, financial and human resources for economic growth and social development of the rural economies (Burkey, 2000). The term rural development also represents improvement in quality of life of rural people in villages. As per Chambers (1983) "Rural Development is a strategy to enable a specific group of people, poor rural women and men, to gain for themselves and their children more of what they want and need." Singh (1999) defines Rural Development as "A process leading to sustainable improvement in the quality of life of rural people, especially the poor". The fact of the matter is that three quarters of the world's poor, about 900 million people are in rural areas, and the Millennium poverty target set by Millennium Development Goals (MDG), cannot be met unless the world addresses rural poverty. "Sustainable Rural Development can make a powerful contribution to four critical goals of:

Poverty Reduction, Wider shared growth, Household, national, and global food security and Sustainable natural resource management” (World Bank, 1997). Hence worldwide there is a growing emphasis on development of rural economy of the countries. Any improvement, in the social or economic status of rural areas would not just directly benefit rural poor but would also bring down the migration-pressures on cities and contribute by positive ripple effect in global stride towards development.

The process of development in a country is to be aided by its governance. The goal of governance “should be to develop capacities that are needed to realize development that gives priority to the poor, and creates needed opportunities for employment and other livelihoods” (The World Bank, 1992, UNDP, 1994). Increased number of poor, hungry or marginalized people in a country represents decrease in its quality of governance. To promote development, various studies have proposed governance in the contextual realities of each country, including veritable participation of citizens in the governmental decision-making process (Grindle, 2004; Evans and David, 2006). Several

Institutions and experts accept Governance as a reflexive process, wherein policies, institutions, outcomes and analysis interact, to maximize the process of participatory development (UNDP, 1997; Ludden, 2005; Mehta, 2006).

Information and communication technologies (ICT), including radio and television and the newer digital technologies like computers and the Internet as potentially are introduced powerful tools and activators of educational reform and changes. Different ICT, when properly applied can be developed to help access to education and the relationship between training and workshops to strengthen the increasingly digital, the quality of education also helped to create teaching and learning in an active process connected to real life high take. However, the experience of being raised by ICT in the classroom and other educational sites around the world during the last few decades proves that is not automatic fully realize the potential benefits of ICT training. (Guptaand et al, 2004)

Effective integration of ICT in the educational system is a complex process that involves not only technology but also involves educational and technical training, institutional readiness, teacher competencies and long-term investment. In fact the subject of such vital importance is that the technology to get the easiest part of it. Introduced ICT information and communication technologies are for this purpose, as a different set of tools and technology resources, used to information communicate, create, release, storage and

management have been defined. These technologies are including computers, internet, broadcasting technologies (radio and television) and telephone. In recent years started a wave of intense public interest about how computers and the Internet can become a better control to the efficiency and effect of education at all levels and in both formal and informal development (Rogers and Shukla, 2001).

But nowadays, ICT is more than a technology. Although the old technologies such as telephone, radio and television, will be less attention in the past but were used as educational tools. For example, "radio and television are used for over forty years to open and distance education. In this regard, although print remains the most expensive method and therefore available, but in developed and developing countries is provided the most prominent mechanism. Internet and computer use in developing countries still in early stages are spent and if they used are limited due to is expensive infrastructure and access to them.

Promises of ICT in education:

ICT for developing countries, are associated a potential for increased availability and quality of training and development. ICT basis and attract a lot of knowledge and its acquisition, providing unprecedented opportunities for developing countries, adding and expanding educational systems, improve policy formulation and implementation of opportunities to expand scope of work and gives poor facilitation. One of the biggest hardships that the poor are bearing the other people, who live in the poorest countries, is the sense of isolation. Communication technologies such sensory loss, are guaranteed and also has been unthinkable facilitate access to knowledge through the ways that already. However, the reality of the digital divide (the gap between those who control access technology and those who do not have access) means that the introduction and integration of ICT are challenging at different levels and in various types of training, most commitments. Failure in this struggle to become more significant gap of knowledge and the deepening economic and social inequalities (UNDP, 1997; Ludden, 2005; Mehta, 2006).

How ICT can help developing access to education?

ICT is a potentially powerful tool for developing educational opportunities, whether formal or informal is for areas already "stated (rural and dispersed populations) ethnic minorities, women, girls, disabled and old people traditionally excluded from education groups because of cultural or social reasons are also all those financial reasons or time constraints can not register in educational centers.

Any time, anywhere (defining feature ICT) capability in ICT is a passing of time and place. ICT, education or training with asynchronous features provide a time delay between education and its acceptance by students makes it possible.

1- ICT application in preparing people for the workplace:

One of the most common reasons cited for employing ICT class, better preparing students for the current generation of working environment is one in which particular ICT, Computer, Internet and related technologies, from day to day remit more prevalent. Therefore, technological literacy, or can be an effective and optimized using ICT, as a competitive edge in a labor market under globalization seems. Technological literacy skills course offered only wanted good things according to the new economy that is global. Northern Region Educational Laboratory United States, is called what the 21st century skills such identification (Nandi, 2002). Century Digital Literacy (includes functional literacy, visual literacy, scientific literacy, technological literacy, information literacy, literacy and awareness of cultural and global awareness) is thinking invention, thinking to achieve a higher rank, full argument, effective communication and high productivity.

2- Application of ICT in educational quality improvement:

Promoting education quality is a critical point. ICT can make education quality to strengthen several ways: by increasing students' motivation and entering the employment scene, by facilitating and promoting training basic skills of instructor. ICT is also a tool that transfers go when properly used, can the initiative in an environment to promote student-centered.

2-1- Motivation for learning - ICT have been merged such as video, television and multimedia software, which text, sound and moving pictures, they can create authentic content and militant students in the educational process.

Interaction and communication as well as radio waves, sound effects, songs, drama, comedy and other presentations run customs and traditions, to force students to listen and get involved in their courses are used.

Similarly, better than any ICT, network computers with Internet connection can be motivated learners with rich media integration and interaction with other ICT provide opportunities to connect the real world and participate in world events, to increase.

2-2- Facilitate learning basic skills - basic skills and concepts transfer of skills, infrastructure and higher degree of intellectual creativity as they can by ICT to facilitate oral and practical. Educational TV programs are used repeating and reinforcing tool for teaching the alphabet, numbers, colored, and other forms of basic concepts.

Most early applications of computers for education training, was reinforced and repeated skills-based mastery, curriculum content. Upgrade teacher training. ICT has been used to promote the availability and quality of teacher training (Singh, 1999).

For example, "institutions such as the Center for Teacher Education Network (CTTC) in South Korea had exploited the Internet to provide better opportunities for professional development of teachers. The center, which has financing from the government, was established in year 1997. And training courses based on the World Wide Web for primary school teachers and provides guidance.

These courses are include "computers in the information society," "educational reform" and "Future Society and Education". Specific periods of online courses with some face to face conference will be held. In China, comprehensive training of teachers based on radio and television is guided for several years by the Central Radio and Television University.

Radio and Television University, Shanghai, and many other radio and television schools in the country contributed to this work. Indra Gandhi National University in India unilateral visual conference system and audio-based two-way satellite, which was created in 1996 by print and is presented video recordings.

And will train 910 elementary school teacher and assistant coach of the 200 local Teacher Training Institute in Karnataka state, Teachers are interacting together by mutual discussions by telephone and fax (Malhotra, 2001).

3- ICT applications in education:

Organizations and educational policy planners should first of all about the desired educational outcomes (mentioned above) is straightforward. The broad objectives must choose different technologies used to go and how to apply the guidance to go. Potential of each technology varies according to how to use. Haddad and Draxler have been identified IT application in education at least five levels of:

1. present,
2. experimental proof,
3. practice and practice,
4. interaction,
5. collaboration

Each of the different ICT tools (print, audio cassette and video, broadcast radio and television, computer or Internet) may provide the most basic means and surfaces used to go to prove. Except for visual technology, practice and practice the maximum use of both technologies may be offered. Each of the different ICT (print, audio cassette and video, broadcast radio and television, computer or Internet) used to may provide the most basic means and surfaces. Except for visual technology, may be offered practice and practice the maximum use of both technologies. The other network computers and Internet, ICT interactive learning that are provided and they if only used for providing proof or go, was not realized can better their full potential (Jauhari, 2004)

4- Application of radio and television broadcasts in education:

Radio and television are used widely from year 1920 and 1950 respectively as educational tools. There are three general approaches in the application of radio and television in education (Cecchini and Scott, 2003).

1. Direct classroom teaching, in which broadcast programming substitute teachers are in a temporary basis.
2. A school broadcasting, where broadcast programming sources being provided and will provide available in supplemental education.
3. Planning the overall educational level of the community and officials of national and international public and non-formal educational opportunities offers.

The best documented example of direct class teaching approach, is education of radio interactive (IRI). The training includes exercises to learn directly prepared 20-30 minutes. Radio lessons about topics specific levels of math, science, health and language that are intended to advance the quality of education and are offered as a helpful structure to the teachers, the educational level in poor schools and no educational resources. education of radio interactive projects in Latin America and Africa have been implemented, Asia, the first project in Thailand, was conducted in 1980, and Indonesia, Pakistan, Bangladesh and Nepal to the project implementation completed in 1990(Andersen and Henriksen , 2006). What interactive radio education projects from other education programs to non-attendance is that distinguishes the primary objective is to improve the quality of learning (nor merely "to extend educational

access) and in both formal and informal form has had many successes.

Extensive worldwide research has shown that many interactive radio educations project a positive effect on learning outcomes and have educational equality. And savings in scale, a strategy that proved effective on the costs associated.

Results:

This paper is a multidisciplinary study of ICT initiatives for rural development. It emphasizes adoption of a more systematic approach for integrating Traditional Knowledge Systems (TKS) and ICT inputs to ensure sustainability of rural e-governance projects. The study of literature related to rural development and e-governance has indicated various issues impeding success of such initiatives. The main issues are lack of localization of content for rural communities and inadequate participation of rural communities in design of rural ICT initiatives. The study therefore suggests the use the systems-approach to integrate the relevant TKS along with ICT initiatives in the design of e-governance systems for rural development. This participatory approach can lead to creation of more acceptable and sustainable e-governance projects.

Regardless of the wide differences in ICT access between rich and poor countries and between different groups in the country, there are concerns that challenge the application of ICT in education with the existing differences among the lines of economic, social, cultural, geographic and gender will be broader. Everyone equal opportunities in terms of suitability for participation are necessary, but access to various factors, either as users or as producers through their sources is difficult and heavy. Therefore, the primary differences enhance and even grow. Consequently, programmers' international education is faced with a difficult challenge and how to help solve the problem and its development.

Promoting ICT in education, when done without careful study, can lead to the marginalization of those with more favorable conditions are unknown. For example, "women compared with men, because of illiteracy, lack of higher education, lack of time and mobility and poverty, controlling access to ICT and fewer opportunities for training are relevant. Also, more boys than girls' access to computers at home and school are not strange to say that if more boys than girls are willing to work with computers. The report of the University Association of American Women is that "Although some girls have an important gender gap have been limited, but today's technology, technology club, and boys in public schools while its own problems and programs are

settled girls use computers for word processing the brand". In an assessment in four African countries, the activities organized by World links remote international cooperation on projects between teachers and students in developing countries will promote, despite creating programs without regard to sex contacts, sexual inequalities remain Uganda and Ghana. In addition, while more girls than boys in relation to academic performance and advanced communication skills program will enjoy more than boys, but they were unable to perform their technological skills were. A set of economic factors, organizational and cultural differences involved in the social.

"The high ratio of students to computers and politics, whoever came first, the first is used in accordance with the girls wanted it." Girls travel restrictions in the early hours of daily work and home responsibilities are that this will limit their access. Also because local patriarchal beliefs dominate the boys are in the computer lab environment. Including proposed measures to address this discrimination, strategies to encourage schools to create "fair use" in the computer labs and the holding of meetings and sexual sensibilities conductivity decreased defense duties after school girls. ICT provides access to only a small part of the action is created equal. Equal attention should also be applied to ensure the technology really "is used by learners and ways of how well their needs will cure.

An educational program that reinforced this approach shows the overall program is bilingual. The program seeks to establish technology learning centers for bilingual teachers, students, teachers, parents and community members. Technical teams from each center three students, two teachers and the director of the Center with at least one female student and a teacher are female.

Another example of a general approach to the application of ICT in education, radio education project Gobi Women of Mongolia, which seeks to provide professional and educational structure of women's favorite courses around the nomads and their opportunities for income generation.

It contains topics such as livestock rearing, family support (family planning, health, nutrition and health) to create income in the application of local raw materials and basic skills for the job is a new market.

***Corresponding Author:**

Mohammad Abedi
 IDepartment of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran.
 E-mail: Abedi114@yahoo.com

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Evaluation of *Curvularia lunata* as an Biological Control Agent in Major Weeds of Rice Paddies

Mohammad Reza Safari Motlagh¹

¹. Department of Plant Pathology, Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Guilan Province 41335-3516, Iran. Tel:00989111384168

*Corresponding author's email: ssafarimotlagh@yahoo.com

Abstract: Common water-plantain (*Alisma plantago-aquatica* L.), arrowhead (*Sagittaria trifolia* L.) and *Echinochloa* spp. (L.) are among the most important damaging weeds of rice paddies. In this research, *Curvularia lunata* (Waker) Boedijn was isolated from the said weeds. Then, its effect in different growth stages, i.e. seed, 2-3 leaf stage (seedling) and also in greenhouse conditions was examined in *Alisma plantago-aquatica*, *Sagittaria trifolia*, *Echinochloa* spp., and five rice cultivars including 2 bred (Sepidroud and Khazar) and 3 indigenous (Ali Kazemi, Hashemi and Binam) ones in a totally random design with three replications. To do so, pure fungal colonies and a spore suspension containing 10^6 conidia/ml distilled water were used. The disease rating caused by this fungus in the 2-3 leaf stage (seedlings) of the said weeds was more than that in the rice cultivars. Also, the fungus decreased the germination of the weeds seeds. Results showed that in the evaluation of the disease rating, the studied rice cultivars showed no significant reaction to greenhouse conditions while weeds' reactions were significant. The greatest effect of *C. lunata* was on *Alisma plantago-aquatica*. The evaluation of fresh weight, dry weight and height of the said weeds and rice cultivars indicated that the above-mentioned fungus could affect these traits in weeds and rice cultivars and would reduce them. Hence, *Curvularia lunata* can be considered as a probable agent for the biological control of *Alisma plantago-aquatica*, *Echinochloa* spp., and *Sagittaria trifolia* provided that modification of rice cultivars is done with useful traits.

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Keywords: *Alisma plantago-aquatica*; biological control; *Curvularia lunata*; *Echinochloa* spp.; *Sagittaria trifolia*.

1. Introduction

Rice (*Oryza sativa*) plays a major role in the nutrition of the people around the world and after wheat is the most important agricultural product (Yamaguchi et al., 2008). There are various factors that reduce rice production, the most important of which are pests, diseases and weeds (Yamaguchi et al., 2008).

In terms of nutrients that exist in rice paddies, these weeds are some strong competitors of rice which prevent adequate light to reach it (Anonymous, 2002). Moreover, they can be suitable hosts for many rice pests and diseases as well and if necessary controlling measures won't be taken, the resulting damages would reach up to 90% (Anonymous, 2002). The importance of weeds in rice plantation is known to be much more than in other cultivations, in a way that in the worst growth conditions of weeds, their damages have been stated to be 25% (Lindquist and Kropff, 1998). About 350 species of 150 genus and 60 families have been reported as rice weeds throughout the world (Hill et al., 1990). Using herbicides is considered as one of the best methods for controlling weeds; however, improper and unduly use of these chemicals along with the resulting pollution in the environment have limited their use and the risk of resistance to pesticides should be added to the previous issues (Holt and Lebaron, 1998). Because

of this issue, using other controlling methods such as applying natural weed-controlling microorganisms known as biological controls or bioherbicides has become necessary (Rashed Mohasel et al., 2001). Applying mycoherbicides is the most essential measure in controlling weeds with biological backgrounds, particularly in the sustainable agriculture because they target ecosystem much less than controlling factors such as herbicides (Charudattan, 1993). In addition, they perform quite selectively and cause minimum damages to crops (Charudattan, 1993). *Curvularia* species as facultative parasites are among microorganisms being used for controlling weeds (de Luna et al., 2002). In South American countries, *Curvularia lunata* and *Phyllachora* sp. have been identified as leaf spot-causing factors in *Hymenachne amplexicaulis* (Rudge) (Monterio et al., 2003).

Also, *Bipolaris*, *Curvularia*, *Drechslera* and *Exserohilum* species, as fungi which cause lesions on the leaves of *Lolium multiflorum* (L.) and *Cynodon dactylon* (L.) were evaluated (Pratt, 2006). Hence, isolates from different *Curvularia* species in *Cyperaceae* were collected and evaluated as probable biocontrol agents of weeds in rice paddies among which *Curvularia tuberculata* was quite effective in

controlling *Cyperus difformis* (L.), *C. iria* (L.) and *Fimbristylis miliacea* (L.) (de Luna et al., 2002).

The first isolation of *Curvularia lunata* from *Lolium perenne* (L.) was reported in 2007 (Goldring, 2007). Also, six pathogenic fungal species were isolated from *Echinochloa* spp. (L.) and were evaluated as controlling agents of this weed in rice that among these fungi, *Curvularia lunata* var. *aeria* and *Exserohilum oryzae* were pathogenic in rice and *Echinochloa* species (Zhang et al., 1996), while *Curvularia geniculata* was only pathogenic in *Echinochloa* spp., but not in rice (Zhang et al., 1996).

In order to modify fungal strains, which could show better efficiency in biological controlling, protoplast fusion was done between *Helminthosporium gramineum* and *Curvularia lunata* and the resulting strains effectively controlled major rice weeds (Zhang et al., 2007).

Curvularia lunata and *Curvularia aerea* have been reported as biological control agents in *Echinochloa* spp. (Tsukamoto et al., 1998). *Curvularia lunata* isolated from barnyardgrass was evaluated for controlling weeds in bean fields (Bisen, 1983). It was found that this fungus was not effective in rice cultivars, but caused disease in bean varieties (Bisen, 1983). Also, *Curvularia lunata* reduced the growth of *Echinochloa crassipes* (L.) by 15-20% (Praveena and Naseema, 2004).

In the Philippines, isolates from *Curvularia tuberculata* and *Curvularia oryzae* were evaluated as probable control agent of *Cyperus difformis* and *Fimbristylis miliacea* (de Luna et al., 2002). Inoculation with spore suspension in these fungi during the foliar stage destroyed the weeds' seedlings (de Luna et al., 2002).

Generally, the substantial condition for introducing a microorganism as a weed biological control factor is ensuring that the microorganism would not damage the main crops (Watson, 1985). Therefore, proving that these fungal isolates would not damage rice is of great significance. In this study, *Curvularia lunata* was isolated from *Echinochloa* spp. (*E. oryzicola*, *E. crus-galli* L.), *Sagittaria trifolia* (L.) and *Alisma plantago-aquatica* (L.) and as a probable biological control agent for these weeds in rice paddies of Guilan province, it was inoculated to several rice cultivars and the above-said weeds.

2. Materials and Methods

2.1. Collection and culture of fungal isolates

Leaves with symptoms of the disease weeds were collected in Guilan province of Iran, cut to appropriate sizes and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar (PDA) in Petri dishes. Then,

Petri dishes were incubated at 28°C in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and then, monoconidial isolates of the recovered fungi were maintained on half-strength PDA slants in test tubes as stock cultures (Zhang et al., 1996) or colonial of fungal placed onto sterilized filter paper, then cuts of these filters were incubated in sterilized vials at freezer on -20°C (Safari Motlagh, 2010).

2.2. Study and identification of fungi

Fungi which had grown were isolated and Koch's postulates were completed for most sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for the confirmation of identification.

2.3. Pathogenicity test

Pathogenicity tests of weeds in seedling stage were carried out in desiccators. In each of two desiccators (one desiccator as control) two Petri dishes were placed each containing 10 germinated seeds of weeds. At first, seeds of weeds were placed on moistened filter paper in Petri dishes and incubated at 28°C for 24 h in a germinator with 12h light/dark photoperiod. Then, seeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, 10 germinated seeds were planted per 10-cm Petri dishes filled with saturated soil (Zhang et al., 1996), and were incubated at temperature room. Distilled water was added to Petri dishes. Seedlings at the 2-3 leaf stage were inoculated with 10⁵ conidia per ml. To increase the surface adsorption, 1% tween-20 was applied. Evaluation of symptoms was performed 7 days after inoculation. Therefore, standard evaluation system and Horsfall-Barratt system were applied for *Echinochloa* spp. (Zhang et al., 1996; Bertrand & Gottwald 1997).

$$\text{Disease rating} = \frac{(N_1 \times 1) + (N_2 \times 2) + \dots + (N_t \times t)}{(N_1 + N_2 + \dots + N_t)}$$

Where N is number of leaves in each of rate, t is number of treatments.

Pathogenicity tests of rice seedlings were carried out in desiccator. To do so, in each of two desiccator (one desiccator as control) were placed two Petri dishes and in each Petri dish placed 10 seeds of rice, Khazar cultivar. Then, seeds were sterilized in water bath at 52-57°C and cultivated in saturated soil and incubated at 25°C. Distilled water was added to Petri dishes. After 16-18 days, seedlings containing 2-3 foliages were inoculated by suspension of spores (Safari Motlagh and Kaviani, 2008). Other conditions including concentration of conidia and evaluation systems were similar.

Pathogenicity tests of weeds in greenhouse conditions occurred as complete random design (CRD) with one treatment and 3 replications. Inoculation of weeds was performed at its 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *C. lunata* spore/ml distilled water was used. In order to increase adsorption, 1% Tween-20 was used. Weeds were planted in farm soil inside plastic pots, 2.5 cm in diameter. For each treatment, one control was assigned (Zhang et al., 1996). Pots were placed at 25-30°C, 12 D:12 L photoperiod and a relative humidity of more than 90%. This suspension was sprayed on the leaves using a sprayer. It should be mentioned that before inoculation, all pots were sprayed with distilled water. To create a relative humidity higher than 90%, treated plants were immediately covered with plastic bags for 48 hours (Ghorbani et al., 2000). Evaluation disease symptoms was done 7 days after inoculation based on lesion type and size in reaction to inoculation: 0= lesions absent, 1= small, unexpanded lesions, 2= slightly to moderately expanded lesions, 3= large lesions (Zhang et al., 1996). Then, five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated in complete random design with three replications against inoculation with *C. lunata*. In order to do so, first, rice seeds germinated and after being transferred to the greenhouse inside pots, 2.5 cm in diameter without any drain, they were planted in the farm soil. When the plants reached their 3-4 leaf stage, thinning was performed. Finally, there were 4 shrubs in each pot. Then, 2g urea fertilizer was added to the pots. At this stage, inoculation was done by a spore suspension of *C. lunata* containing 10^6 spore/ ml of distilled water with 1% Tween-20. Other environmental conditions were similar to those of the weed. Evaluation was done 7 days after inoculation for which Horsfall-Barrat system was used. Then, disease ratings were calculated (Bertrand and Gottwald, 1997). It is noteworthy that in both experiments, one control was considered for each replication.

2.4. Measuring plant fresh weight, dry weight and height

In order to measure these traits, inoculated weeds and rice cultivars along with controls were transferred from greenhouse to the laboratory. Then, shrubs were cut on the soil surface and weighed by an electric scale. This weight was recorded as their fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and for 48 hours, they were in an oven at 80-90°C. When the bags were taken out of the oven, each shrub was weighed, which was considered as its dry weight (Ghorbani et al., 2000).

2.5. Inhibition of Seeds germination test

Seeds of weeds were surface sterilized with 0.2% sodium hypochlorite solution for 2 min. After washing with distilled water, seeds were placed per 10- cm-Petri dishes containing wet filter papers. Then inoculation was done. To do so, 10 seeds were transferred to two Petri dishes containing wet filter papers (one Petri dish as control). Then cuts of fungus colonies were placed on seeds. This test was done with three replications. The Petri dishes were incubated at 28°C on a 12h light/dark photoperiod. Evaluation of symptoms was performed 7 days after inoculation and number and percent of germinated seeds was determined (Zhang and Watson, 1997).

2.6. Data Analysis

Data analysis was done using SPSS, MSTAT-C and NTSYS softwares. In order to compare average values, Duncan test was used, while for comparing the reaction of rice cultivars and weeds, the difference between the average value of each fungus-treated rice cultivars and the controls was used.

3. Results

3.1. Study of the disease rating caused by *Curvularia lunata* in weeds and rice cultivar in desiccators

Evaluation of the disease rating of *Curvularia lunata* isolates in rice, *Alisma plantago-aquatica*, *Sagittaria trifolia* and *Echinochloa* spp. in accordance with Horsfall-Barratt system using the number and sizes of the spots showed that the disease rating caused by this fungus in weeds was more than in rice and they indicated a significant difference (Figure 1). The first symptoms in weeds appeared 48 hours after inoculation. Initially, chlorotic spots appeared which later on became necrotic and connected and caused necrosis in most parts of the leaf surface. Also, cotton-like colonies of the fungus were observable on the leaves.

On the other hand, the first symptoms in rice appeared 5-6 days after inoculation. They started as small necrotic spots and in some cases, blight occurred on the tip of the leaves.

3.2. Evaluation of the inhibition of seeds germination in weeds

Results showed that *Curvularia lunata* was quite effective on the germination of the studied weeds and even inhibited it. Accordingly, there was a significant difference between controls and treatments (Figure 2).

Based on the dendrogram obtained from the cluster analysis, *Curvularia lunata* isolates were divided into two groups in accordance with the disease rating in weeds. In each group, there were two isolates. Moreover, in the second group, there were two isolates

with a similarity coefficient of more than 95% (Figure 3).

According to the said dendrogram, in terms of disease rating in rice, *C. lunata* isolates were placed in two groups. Group 1 consisted of one isolate, while there were 3 isolates in group 2. The two isolates in group 2 had a similarity coefficient of more than 95% as well (Figure 4).

Also, based on the dendrogram regarding the inhibition of seeds germination in weeds, *C. lunata* isolates were divided into two groups each having two separate isolates. There were two isolates with a similarity coefficient of more than 95% in the second group (Figure 5).

3.3. Greenhouse experiments

Results from the variance analysis of the disease rating revealed that the studied rice cultivars did not show any significant reaction to *Curvularia lunata* (Table 1). Despite the fact that in terms of the disease rating reactions of rice cultivars were not significant, based on direct observations regarding types and sizes of the spots caused by the fungus in the aforesaid rice cultivars, it was found that Sepidroud as a bred cultivar was more tolerant compared with others, while Hashemi was more affected by the fungus among the cultivars. In terms of tolerance, Sepidroud was followed by Ali Kazemi, Khazar and Binam.

In the study of dry weight, fresh weight and height of the said rice cultivars, a significant reaction was observed in all these traits (Table 2). Evaluation of the mean values of the said traits in the studied rice cultivars revealed that regarding height, there was no significant difference between Hashemi, Ali Kazemi, Sepidroud and Binam and it was only Khazar that showed such a difference (Table 2). In terms of fresh weight, there was no significant difference between Sepidroud, Khazar and Binam, but Ali Kazemi and Hashemi showed less reduction in their fresh weights (Table 2). For dry weight, the reactions of the studied cultivars were similar to those of the fresh weight (Table 2).

Evaluation of the fungus' effect on each of the studied traits in rice cultivars compared with the controls revealed that in terms of height, Ali Kazemi, Sepidroud and Binam had no significant difference between them. Moreover, compared with the controls, they showed height decreases as a result of the fungus' effect (Table 3). In terms of height, the highest and lowest height reductions were those of Khazar and Hashemi, respectively (Table 3). Regarding fresh weight, there was no significant difference between Sepidroud and Khazar, while both of them showing

reduced fresh weights compared with controls. Furthermore, Hashemi showed more fungus-induced fresh weight decrease in comparison with other cultivars. In the indigenous Ali Kazemi cultivar, fresh weight decrease was less than Hashemi, yet compared with Sepidroud and Khazar it was more. In the evaluation of this trait, fresh weight decrease was not observed in Binam. The dry weight of rice cultivars, compared with that of the controls did not show any significant decrease (Table 3).

For the evaluation of the disease rating caused by *C. lunata* in *Alisma plantago-aquatica*, *Sagittaria trifolia* and *Echinochloa* species, a significant reaction was observed (Table 4). *Alisma plantago-aquatica* was more affected by the fungus while *Sagittaria trifolia* was the most tolerant. Moreover, there was no significant difference between two *Echinochloa* species, i.e. *E. crus-galli* and *E. oryzicola* regarding the effect of this fungus, but based on direct observations relevant to the type and size of the appeared spots by the fungus, the disease in *E. oryzicola* was more severe (Figure 6).

In the study of the effect of the said fungus on fresh weight, dry weight and height of *Sagittaria trifolia*, *Alisma plantago-aquatica* and two *Echinochloa* species based on the variance analysis table, a significant reaction was observed for height and fresh weight. But for dry weight, the reaction was not significant (Table 4).

Also, based on the comparison of the traits' mean values, all the studied weeds showed a significant difference in terms of height (Table 5). In this regard, *Alisma plantago-aquatica* had the highest reduction. It was found that in terms of fresh weight, there was no significant difference between two *Echinochloa* species. However, *Alisma plantago-aquatica* and *Sagittaria trifolia* showed a significant difference. Moreover, in terms of dry weight, no significant difference was found between these weeds (Table 5). It is noteworthy that compared with controls, all studied and treated weeds showed reductions of the said traits (Table 6). Therefore, it could be concluded that the fungus caused reduction in the weeds' height, yet this reduction did not show any significant difference in the four studied weeds. Also, fresh weight conditions were similar to height changes and in terms of the dry weight the fungus caused it to decrease in all the studied weeds compared with controls (Table 6). However, in *Sagittaria trifolia*, the reduction of the dry weight was less in comparison with other weeds and compared with the controls, it was less affected by the fungus (Table 6).

Table 1. Variance analysis of disease rating and the studied traits in rice cultivars affected by *C. lunata*.

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight(g)
Treatment	4	0.388n.s	103.008**	5.397**	0.14*
Error	10	0.114	6.464	0.193	0.29
C.V.	-	14.95	3.73	11.17	32.09

** : Significance at the probability level of 1%

* : Significance at the probability level of 5%

n.s.: not significant at p=5%

SOV: sources of variations

DF: degree of freedom

Table 2. Comparison of means of the studied traits affected by *Curvularia lunata* in rice cultivars.

Cultivar	Height(cm)	Fresh weight(g)	Dry weight(g)
Hashemi	69.196 ± 1.089a	4.818 ± 0.258b	2.127 ± 0.043b
Ali Kazemi	72.416 ± 1.044a	5.817 ± 0.425a	4.182 ± 0.0296a
Sepidroud	72.333 ± 2.103a	3.309 ± 0.11c	0.678 ± 0.201c
Khazar	58.123 ± 1.200b	3.124 ± 0.186c	0.732 ± 0.072c
Binam	68.333 ± 1.622a	2.584 ± 0.163c	0.672 ± 0.059c

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 3. Comparison of the reactions of rice cultivars affected by *C. lunata* with those of the controls.

Cultivar	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
Hashemi	-2.21 ± 0.606b	-1.01 ± 0.358a	0.16 ± 0.023a
Ali Kazemi	-3.50 ± 0.5775ab	-0.6 ± 0.286ab	0.063 ± 0.021a
Sepidroud	-2.58 ± 1.21ab	-0.19 ± 0.061b	0.095 ± 0.018a
Khazar	-5.12 ± 0.919a	-0.18 ± 0.053b	0.15 ± 0.091a
Binam	-4.91 ± 0.759ab	0.36 ± 0.066ab	0.16 ± 0.043a

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 4. Variance analysis of disease rating and the studied traits in weeds affected by *C. lunata*.

SOV	DF	Squares Mean			
		Disease rating	Height(cm)	Fresh Weight (g)	Dry Weight(g)
Treatment	3	2.178*	610.230**	122.452**	1.190 n.s.
Error	8	0.328	12.080	1.451	0.329
C.V.	-	18.03	7.19	10.07	24.19

** : Significance at the probability level of 1%,

* : Significance at the probability level of 5%

n.s.: not significant at p=5%

SOV: sources of variations, DF: degree of freedom

Table 5. Comparison of means of the studied traits affected by *Curvularia lunata* in weeds.

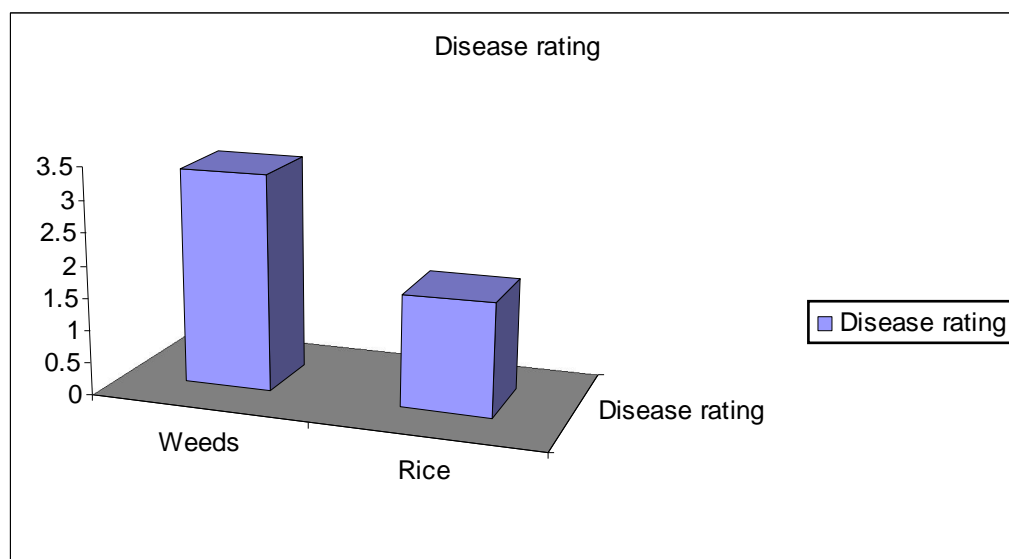
Weed	Height(cm)	Fresh weight(g)	Dry weight(g)
<i>E. oryzicola</i>	63.500 ± 2.362a	6.175 ± 0.078c	5.018 ± 0.071b
<i>E. crus-galli</i>	53.480 ± 2.992b	7.896 ± 0.406c	0.738 ± 0.056b
<i>Sagitaria trifolia</i>	46.666 ± 1.013c	20.343 ± 0.983a	2.163 ± 0.599b
<i>A. plantago-aquatica</i>	29.600 ± 0.737d	13.403 ± 0.891b	1.563 ± 0.265b

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 6. Comparison of the reactions of weeds affected by *C. lunata* with those of the controls.

Weed	Change of Height(cm)	Change of Fresh weight(g)	Change of Dry weight(g)
<i>E. oryzicola</i>	-3.29 ± 1.37a	-0.12 ± 0.054a	-0.06 ± 0.006a
<i>E. crus-galli</i>	-1.8 ± 0.32a	-0.29 ± 0.28a	-0.09 ± 0.032a
<i>Sagitaria trifolia</i>	-0.6 ± 0.3a	-0.82 ± 1.71a	-0.63 ± 0.245b
<i>A. plantago-aquatica</i>	-1.3 ± 0.83a	-0.43 ± 0.019a	-0.42 ± 0.327a

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Figure 1. Diagram of the comparison of *C. lunata* mean disease rating in rice and weeds.

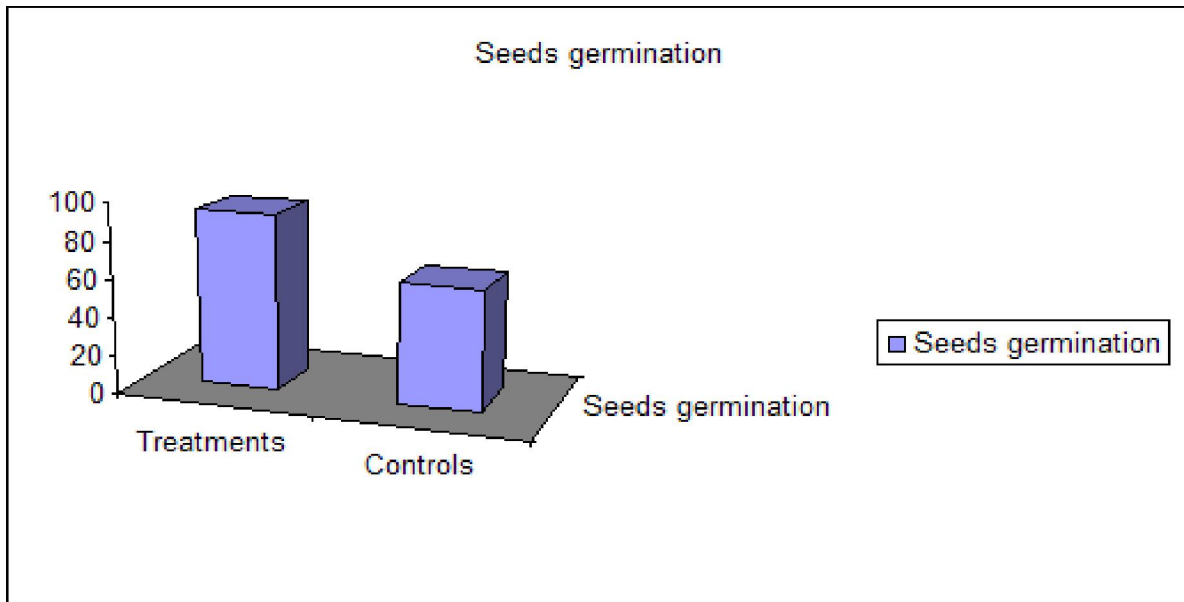


Figure 2. Diagram of the comparison of mean seeds germination percent in treatments and controls of weeds.

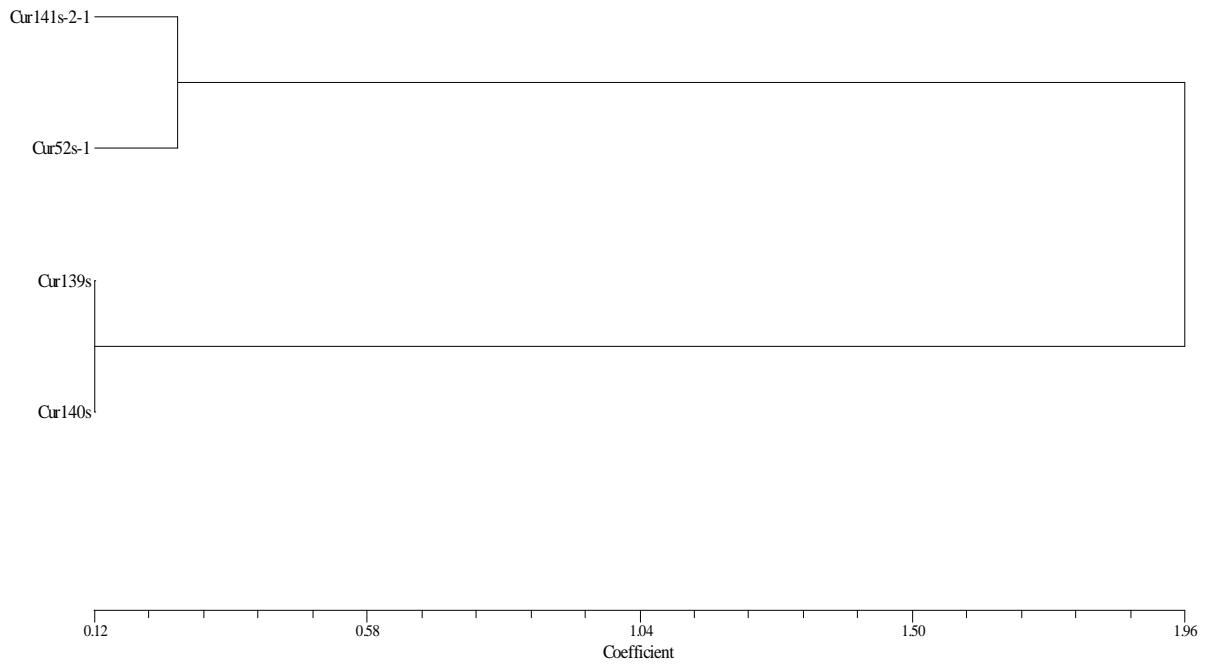


Figure 3. UPGMA-dendrogram for *C. lunata* isolates on weeds.

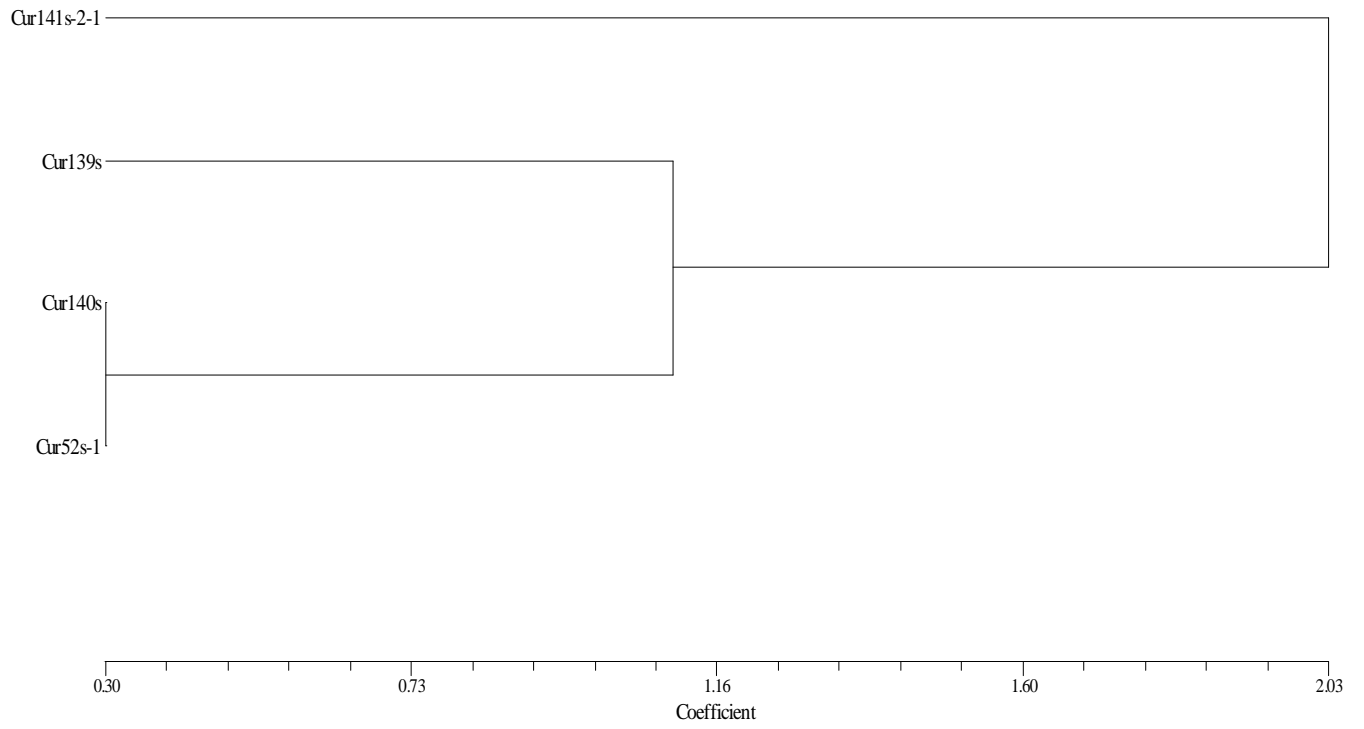


Figure 4. UPGMA-dendrogram for *C. lunata* isolates on rice.

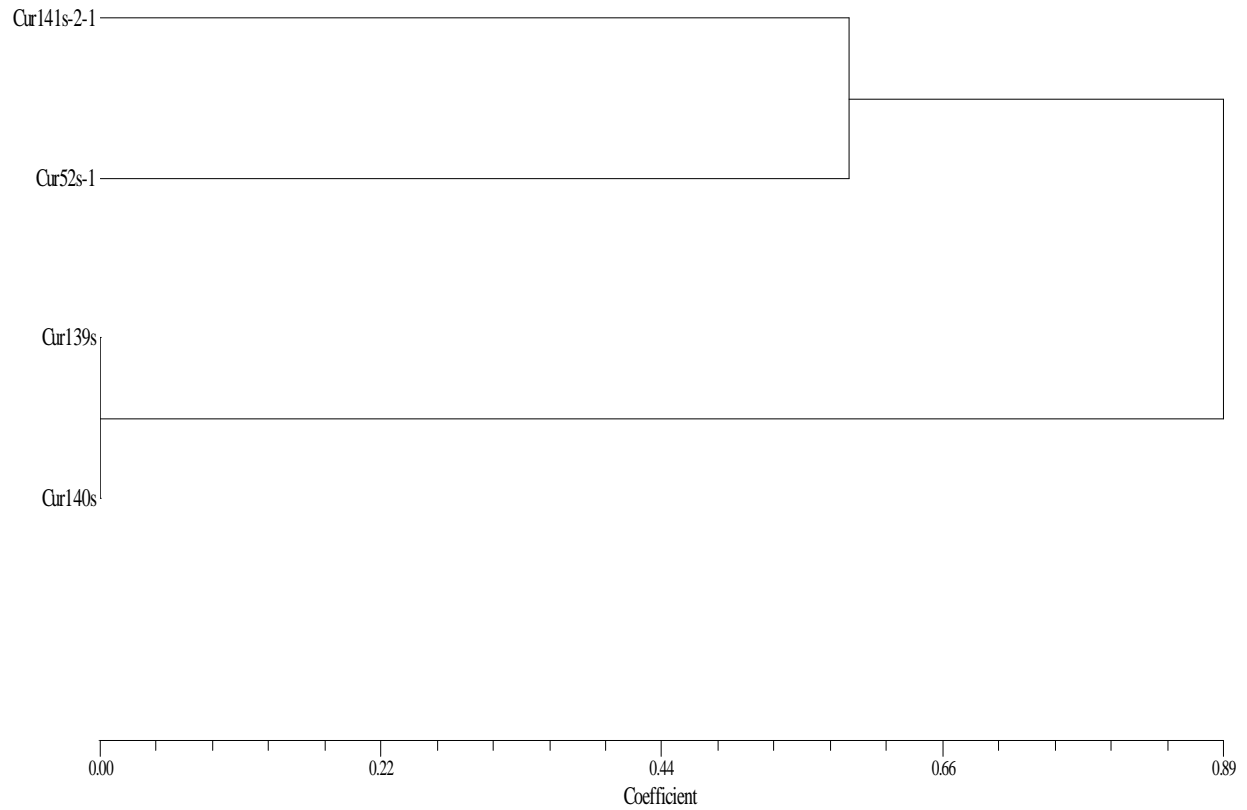


Figure 5. UPGMA-dendrogram for *C. lunata* isolates on weeds (based on inhibition of seeds germination).

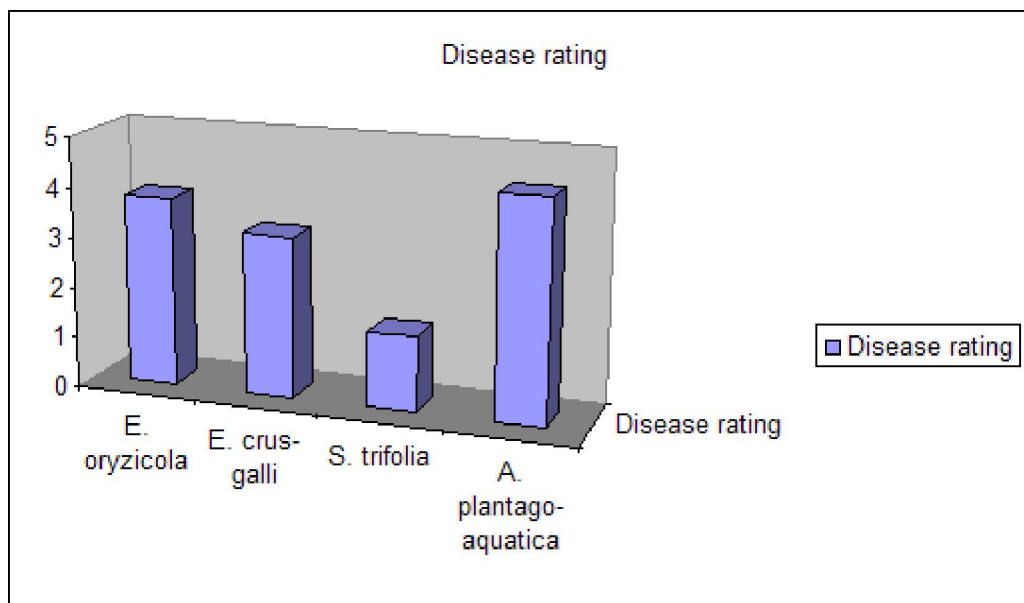


Figure 6. Diagram of the comparison of *C. lunata* mean disease rating in weeds.

4. Discussion

Based on the cluster analysis in the present study, *Curvularia lunata* isolates showed similar reactions in terms of pathogenicity and the inhibition of seeds germination of weeds and thus, were placed in similar groups. Moreover, since two bred cultivars, i.e. Sepidroud and Khazar were used along with some indigenous cultivars, the formers based on the disease rating index were more tolerant compared with Hashemi, Ali Kazemi and Binam.

Also, a study by de Luna *et al.* revealed that indigenous rice cultivars in comparison with bred ones which were exposed to *Curvularia oryzae* were more damaged (de Luna *et al.*, 2002). In the Philippines, the study of rice cultivars' reactions to *Curvularia lunata* isolated from *Echinochloa crus-galli* revealed that the fungus was not pathogenic in six bred and indigenous rice cultivars (Zhang *et al.*, 1996).

Based on the results of this research regarding the evaluation of fresh weight, dry weight and height in rice cultivars treated with *C. lunata*, it was observed that Hashemi and Alikazemi cultivars were less affected by the said fungus. This reaction could be related to less genetic diversity and more adaptability of indigenous cultivars to environmental conditions and the inoculated fungus (Kimber, 1983).

According to the results from the present study, although the disease rating was higher in Hashemi, in terms of height, fresh weight and dry weight, the cultivar was less affected by the said fungus. To elucidate this finding, one can say that the interaction between the genes in every plant's genome and the genotype of each fungal races leads to developing

different responses of each trait to a given fungus. As researches have shown, one of the main problems regarding the modification of plant resistance to a parasite while studying the biological control of weeds to develop resistant cultivars is encountering a wide range of diverse creatures with different genetic structures (Kimber, 1983). This had led to obtaining inconsistent results in some researches. For example, according to the study conducted by Zhang *et al.* *Curvularia lunata* did not cause a high disease rating in *Echinochloa* spp. (Zhang *et al.*, 1996) while in the present research, the fungus was the cause of a high disease rating in this weed, particularly in *Echinochloa oryzicola*.

Differences of the effect of a fungus as a biological control agent could depend on the environmental conditions of a geographical location, especially humidity and temperature (Huang *et al.*, 2005). For instance, *Exserhilum monoceras* that was isolated from *Echinochloa* in some farms the Philippines was effective on the weed in the region. However, when it was used for controlling this weed in rice paddies in South Korea, the response was quite different and in fact, it was not effective at all (Chung *et al.* 2005). Studies showed that different climatic conditions between the two geographical regions had led to different responses of the fungus (Chung *et al.*, 2005).

Furthermore, reactions of weeds to fungi isolated from different hosts might be different. For example, in the study of *Curvularia oryzae* isolated from *Cyperus difformis*, it was found that the host range of different weeds was effective in their responses to the fungus in

terms of traits such as fresh weight and stem length. In the said study, *Curvularia oryzae* reduced the height of *Cyperus difformis*, but it did not affect the fresh weight (de Luna et al., 2002).

Different species of one fungal genus may also have different effects on a weed. Studies conducted by Zhang et al. showed that *Curvularia lunata* and *C. geniculata* (isolated from *Echinochloa*) had different effects on *Echinochloa* and rice that is the former had no effect on *Echinochloa* yet was pathogenic in rice cultivars while the latter affected *Echinochloa* but not rice cultivars (Zhang et al., 1996). Thus for different responses of rice cultivars and weeds, different factors such as genetic diversity, geographical and climatic conditions and interactions between different weed and fungal species are effective.

Furthermore, in the study of the effect of *C. lunata* on different growth stages such as seed, 2-3 leaf stage (seedlings) and also in the greenhouse, it was observed that the said fungus was effective in weeds at all stages. This meant that the disease rating caused by the fungus in different growth stages of weeds was more than in rice. Also, it reduced germination in weeds and did not have a significant effect on the said rice cultivars.

With consideration of the effect of the fungus on some performance traits of the studied rice cultivars, using it as a biological control agent is only recommended when the modification of rice cultivars is done using desirable traits of the regional indigenous cultivars so that its probable damages to rice production are minimized.

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Corresponding Author:

Dr. Mohammad Reza Safari Motlagh

Department of Plant Pathology, Faculty of Agriculture

Islamic Azad University, Rasht Branch, Rasht, Guilan

Province 41335-3516, Iran. Tel:00989111384168

Email: ssafarimotlagh@yahoo.com

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Review the reasons for the decentralization of agricultural extension

Sharareh Khodamoradi¹ and Mohammad Abedi²

^{1,2}Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Corresponding author: abedi114@yahoo.com

Abstract: Agricultural extension is a non-formal type of education that provides advisory services by the use of educational approach in acquiring knowledge and skills to deal with the growing needs of global world. Diverse agricultural extension funding and delivery arrangements have been undertaken since the mid-1980s by governments worldwide in the name of "privatization." When agricultural extension is discussed, privatization is used in the broadest sense – of introducing or increasing private sector participation, which does not necessarily imply a transfer of designated state-owned assets to the private sector. In fact, various cost-recovery, commercialization, and other so-called privatization alternatives have been adopted to improve agricultural extension. The form and content of decentralization has dominated development discourse and public sector reform agenda in Kenya in the last two decades. The case of agricultural extension service presents decentralization in a difficult context partly due to lack of information on its possible diverse impacts especially on resource poor farmers.

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Introduction:

Throughout the world the powerful effect of agricultural development vivid on rural livelihood not only by increasing their incomes but also by releasing labor and capital that can be used in non-agricultural enterprises and goods (Johnson, 2000; Lanjouw & Lanjouw, 2001; Haq, 2003). This better utilization of skill and craft mainly dependent on the swift movement of market information, which is the main cause of agricultural extension, that serves as important tire of agriculture and rural development process. The whole process of agricultural development showed weak linkages between its different components (Sharma, 2003; Mubangizi et al., 2004) and it seems necessary to revive the shattered agricultural education, research and extension system (Khan, 2002). Agricultural extension is one of the main institutional components of agriculture as it promotes the transfer and exchange of information that can be converted into functional knowledge. It is better to say that extension is the instrument, which is helpful in developing enterprises that promote productivity and generate income in the present climate of change, which ultimately reduce poverty in developing as well as developed countries (Kaimowitz, 1990; Alston & Pardey, 1996; Carney, 1998; Wanga, 1999; Anderson & Feder, 2003).

Un-fortunately in developing as well as low income countries agricultural extension has failed in diffusing new technology to its ultimate users (Government of Malawi, 2000) and further deterioration witnessed with the passage of time (Eicher, 2001). The failure of agricultural extension services for last decades is under constant pressure to be responsive to ever-growing challenges of food production.

Over the past two decades many countries have undertaken to decentralize government functions and transfer authority and responsibilities from central to intermediate and local governments, and often to communities and the private sector. Decentralization is potentially important to agricultural knowledge and information systems, but decentralization is not an end in itself, and successful decentralization strategies must address three challenges— establishing a national framework for decentralization, developing subsector approaches, and enhancing capacities of various participants for coproduction of decentralized goods and services. Agricultural extension services are under increasing pressure to become more effective, more responsive to clients, and less costly to government. Decentralization is an increasingly common aspect of extension reforms. Field extension advisory services are well suited to decentralized approaches, but a comprehensive extension system requires a range of

extension support services and programs, some of which (strategy formulation, training, monitoring and evaluation, specialized technical support) are often best carried out at the central level.

The prime challenges in the traditional public extension systems enlisted as outdated, top-down, paternalistic, inflexible, subject to bureaucratic inefficiencies that results less ability to cope with the dynamic demands of modern day agriculture (World Bank, 2002; Obaa et al., 2005). In some countries the change is occurring with its natural pace but in many developing countries these have been accelerated by structural adjustment reforms (Chapman & Tripp, 2003).

Like other developing country Pakistan is also an agrarian country, whose economy is highly dependent on agriculture having 23% share to GDP (Government of Pakistan, 2005). But still the performance of agriculture sector at the farm level remains significantly below the potential and limited due to the weak institutional formwork in disseminating agricultural technology to the farmers (Farooq, 2005). Research scientists evolving new methods and technologies to meet the challenges of new era and the farming community also has a potential and courage to adopt but the third component i.e. agricultural extension, which serves as a technology transfer vehicle and play a significant role in increasing the productivity, farm incomes and ensure food security has been very much weak since independence (Luqman et al., 2004; Farooq, 2005). The extension services in the country have not been able to achieve their goals effectively, because of a number of bottlenecks. These include weak research-extension linkages, lack of adequate resources for on-farm demonstrations, poor mobility, inadequate research and training in extension methodology and lack of an effective system of continuing education for extension personnel at various levels (Sandhu, 1993). Among major filed crops wheat, rice, cotton and sugarcane accounts for 90.4% of the value added in major crops and 37.1% of the value added in overall agriculture (Government of Pakistan, 2005). The low production of these crops depends upon a number of factors including ineffective and isolated agricultural extension system.

All over the world agricultural extension assists the rural population of remote areas to up-lift their living standard through increase in crop production . The Government of Pakistan is well aware of this fact therefore from the Day of Independence different extension and rural development programs at national level launch by her e.g. Village Agricultural and Industrial Development Programme (Village-AID), Basic Democracies System (BDS), Integrated Rural Development Programme (IRDP) and Training and

Visit System (T & V) . Un-fortunately all these programmes were abolished one after the other because of their conventional, top down nature and inherited less effective technology transfer model (Williamson, 2002; World Bank, 2003). The last efficient extension programme was (T & V) that become ineffective due to its rigidity, top down orientation, non-responsiveness to farmers' needs, much expensive, least effective in feed back communication with farmers and un-able to meet the challenges of changing circumstances . To overcome the weaknesses and shortcomings in (T & V) system Government introduced Decentralization of Agricultural Extension reforms with the name Devolution of Power Plan to up-lift the local people's economic status through pooling all the national sources and resources at grass root level . Devolution is the complete, permanent (SPDC, 2000) and advanced form of decentralization and also helps in strengthening the functions of and empowering with more authority to the elected representatives (FAO, 2001). With the promulgation of this new system, institutional reforms have been introduced almost in all the line departments including Agricultural Extension (Luqman et al., 2004). The new system of agricultural extension, works under the supervision of district Government in which each district is managing its agricultural extension activities, where the functions of all sister organizations such as Water Management, Fisheries, Livestock, Soil conservation, Forestry, etc; are put under single manager called as Executive District Officer of Agriculture (EDO) (World Bank, 2003). The administrative changes in the setup of agricultural extension department affect the working efficiency of Extension Field Staff (EFS) in their area of jurisdiction having both positive and negative impacts (Luqman et al., 2005), while on the other hand Farooq (2005) conducted a research study in two districts of North West Frontier Province (NWFP) and observed the difficulties faced by the extension staff in post devolution framework. The major hurdles in creating difficulties for EFS in the research area were multifarious duties, double chain command and lack of administrative staff and burden of increased paper work.

Decentralizing:

Decentralization as transfer of authority and responsibility for government functions from central government to intermediate and local governments, and often to communities and the private sector has become widespread over the 1980s and 1990s. Countries with diverse systems and traditions of government have pursued decentralization initiatives for many reasons, including especially the failure of government to meet expectations under centralized

approaches to economic management and service approaches to organizing public administration. Though not yet widely applied to agricultural research and extension, decentralization strategies are potentially important to these agricultural knowledge and information systems. Decentralization is frequently viewed from one of two different perspectives (Johnson, 2000).

1. The democratic view emphasizes the aspect of empowering local people to control and direct their own public programs; and

2. The administrative view emphasizes the efficiency gains resulting from improved administration and effectiveness of public programs due to local control. Decentralization is generally expected to: encourage local financing and ownership of programs, result in more efficient and equitable allocation of government resources, provide incentives for production and service delivery, ensure lower-cost service delivery, build local capacity, and respond more effectively to local needs. (Khan, 2002).

For rural programs, decentralization offers hope for correcting the urban bias that results from the geographic dispersion of rural people, the difficulties for them to organize to promote their interests, and the discrimination against agriculture inherent in many country policy frameworks. Decentralization of agricultural extension and research seeks to increase user participation in technology programs and make programs more accountable to users. (Eicher, 2001). Enthusiasm for decentralization needs to be tempered with some caution. In small countries, decentralization may be unnecessary and in very large countries decentralization to the state or provincial level may still leave programs distant from user influence. Definitive evidence of the impact of decentralization is limited and not everyone benefits from any reform. Furthermore, decentralization does little to improve intraregional disparities, may bring oppressive elites into power, and can lead to greater inequalities in allocation of government resources. Thus, decentralization has the potential to increase access to and cost of services, but specific targeting mechanisms and strong central oversight are needed to avoid inequities in service access and quality. (Farooq, 2005).

Principles in Decentralization Reform:

Decentralization takes many forms with varied mixes of fiscal, administrative, and political decentralization. Privatization, deconcentration, and delegation initiatives can complement and reinforce an overall decentralization policy, but these do not constitute, and can in some cases work against, effective decentralization (FAO, 2001).

Four requirements for successful decentralization are:

- Providing local people with substantial real influence over the local political system and local developmental activities;
- Ensuring availability of financial resources adequate for decentralized institutions to accomplish their tasks;
- Ensuring adequate administrative capacity in local units to carry out their tasks; and
- Establishing reliable mechanisms for accountability of politicians and bureaucrats to local people.

Deconcentration is nearly always the first— and necessary—step in any process of decentralization. This puts staff from central administrations in closer contact with local people, problems, and conditions and provides a channel for local interaction with government. Unfortunately, decentralization reforms frequently stop at this point with central authorities retaining control over deconcentrated administrative structures (World Bank, 2003).

Administrative decentralization, represents a more fundamental reform that replaces existing centralized structures with a new administrative structure of local government. Transfer of power to decentralized offices increases local participation in decisionmaking and allows programs to be tailored to local needs.

Political decentralization, makes decentralized bureaucracies accountable to locally elected officials and officials accountable to the people. Elections, referenda, and local participatory decisionmaking arrangements give people direct control over government programs, but short of these formal political processes, a variety of mechanisms (reflecting “participation” more than “decentralization”) can give people influence over government programs. These include: incorporating local representatives into governance and advisory boards, client surveys, polls, and program “report cards,” and rapid rural appraisal techniques (Luqman et al., 2005).

Fiscal decentralization, is often seen as a way to reduce central government budgets by off-loading tasks a central government can no longer finance. In practice, however, decentralization is likely to result in higher costs for central budgets. Fiscal decentralization may transfer authority for expending funds, raising taxes, or borrowing, but intergovernmental fiscal transfers (IGFTs or “grants”) are usually the key means of financing decentralized programs.

Concern over local administrative capacity frequently leads central governments to impose controls that are costly to administer and that restrict local flexibility in managing funds. Experience would indicate that local governments are generally capable of assuming substantial responsibility, and

decentralized programs can provide different financing packages to communities with different levels of capacity (Williamson, 2002).

Many programs are best implemented through “coproduction” or partnerships between various actors—central government, local government, private sector, civil society, and the individual—each providing the good or service for which it has a comparative advantage. Coproduction requires clarity in division of labor and clear “contracts” between different partners. (Sandhu, 1993).

Privatization, delegation, and devolution strategies complement decentralization and, like decentralization, broaden the institutional base for administration and execution of technology programs; reduce the burden on central governments for provision of services (responsibilities in which they have been less than fully successful); and increase stakeholder participation and influence over programs. Advantages of these complementary strategies are that:

- Full privatization relieves government of responsibility for production of private goods and services with few externalities. Extension services for commercial crops grown by wealthier farmers, information on postharvest handling and processing technologies, and marketing of machinery or production inputs often fall in this category.
- Private provision of publicly financed services takes advantage of private providers’ greater efficiency and flexibility in executing programs. Government contracting of NGOs or private extension providers is a common example.
- Delegation and devolution maintains some government authority and financing, but gives implementing institutions operational flexibility and ability to specialize. Governments may delegate extension responsibilities to research institutes or devolve responsibility for commodity extension to a commodity group (Farooq, 2005).

Decentralization of Public Sector Extension:

Public extension services are being forced to change. In the 1990s agricultural extension services were attacked for being inefficient, irrelevant, ineffective, and poorly targeted. The need for reform was obvious and national systems responded with three major strategies—privatization, decentralization, and program revitalization. Although cost reduction has been the force behind many changes, the principal objective of reforms should be an attempt to improve quality of services to clients. Decentralizing extension services, when implemented effectively, can transform extension and address a range of generic problems.

Decentralized extension brings decisionmaking processes closer to clients and makes programs more responsive to user needs. Service providers become more accountable to clients and better oversight increases efficiency of operations. Decentralization itself can introduce a new dynamism in programs and can promote diversity in service providers and program approaches, thus serving as a first step toward privatization. In addition, reforms to revitalize and privatize programs can accompany decentralization reforms, which generally involve: (World Bank, 2003).

- Administrative decentralization—moving responsibilities for extension to local levels of government;
- Political decentralization—expanding user influence on program priority setting, planning, and management; and
- Fiscal decentralization—giving financial management responsibility to local governments or requiring cofinancing from local governments and producer groups.

Extension services differ from research in two important ways that affect their potential for decentralization. First, extension advisory services (field extension services) come in direct contact with clients and provide services that have a high private-goods content. These characteristics make field extension services a much better candidate for decentralization than research, which typically has a longer-term payoff. Local producers are more willing to commit resources to pay for effective extension services from which they realize immediate direct benefits. Still, there remains a need for other extension services to address “externalities”—environmental problems, food quality or safety concerns, or social equity issues (that is, special needs of small farmers)—that are in the public interest, but are not a priority for individual producers or decentralized institutions. This requires continued central support for extension. A second difference between research and extension is the scope and scale of programs. (Williamson, 2002).

Research institutions are generally smaller and more concentrated. Extension programs typically operate across the country, provide information on a wide range of technologies from various sources, and draw on traditional knowledge and farmer innovation to improve producer organization, management, production, and marketing functions. The broad demands on extension require strategies that incorporate a variety of approaches to providing services.

Despite the apparent suitability of extension service provision to be decentralized, they are often highly centralized. A World Bank study of 19

countries found that in the early 1990s 13 countries or regions showed almost no evidence of decentralization of extension services. Colombia, Jiangxi (China), the Philippines, and Nusa-Tenggara-Timor (Indonesia) were relatively highly decentralized, and Poland and Tunisia showed some decentralization. The study found that:

- When extension is decentralized there is a fairly good balance in fiscal, administrative, and political decentralization;
- Political decentralization (the role of elected officials) lags other elements of decentralization; and
- NGO involvement is moderate and farmer participation is significant in extension.

Underlying these conclusions was the fact that institutional development and civil society provide important support to decentralizing extension services. (FAO, 2001).

Recognizing Multiple Extension Functions:

National agricultural extension systems (NAESs) must incorporate a range of extension activities that vary in suitability to decentralization. Field advisory services, as the traditional extension methodology, are compatible with decentralized program strategies and in some cases are suited to private service provision or complete privatization. Other services to support field extension agents and complement field advisory services are often better suited to centralized production. (Khan, 2002).

Functions best centralized are those that:

Support national strategies and financing mechanisms; involve economies of scale and scope; serve a number of administrative regions; or require greater technical input and networking than can be managed at the local level. Services needed in a comprehensive extension system include:

- Extension policy, strategy formulation, and planning (centralized);
- Training programs for extension agents (centralized or decentralized);
- Technical specialist support to extension agents (centralized);
- Production of extension publications, audiovisual materials, guidebooks, and other materials (generally centralized);
- Monitoring and evaluation to support program quality enhancement (needed at all management levels);
- Training programs for farmers (generally decentralized);
- Market information services (centralized);
- Encouragement for (and possibly some controls on) private sector extension (privatization with mixed centralized/decentralized controls);

- Mass media campaigns, including radio, television, agricultural magazines, newspapers, and letters (generally centralized, but may be decentralized or privatized); and
- Internet and/or telephone dissemination of information and fielding questions from farmers, agribusiness, or extension agents (centralized). (Farooq, 2005).

Administrative Decentralization:

Deconcentration is intrinsic to extension services that are provided in dispersed fields and communities throughout a country. Cropping systems, markets, agroecological zones, and ethnic and cultural characteristics of farmers can vary widely within a country, and moving administration closer to field services can substantially improve program management through better understanding of local conditions. Administrative decentralization goes further by making extension programs directly responsible to local authorities. The challenge in any successful decentralization reform is that of maintaining overall program quality and coherence. Decentralized extension programs are limited if the decentralized administration lacks awareness of new technologies, sources of assistance, and extension methodologies. Although decentralized administrations can effectively integrate local institutions, organizations, and technologies into an extension system, major benefits from formal extension often come from integrating external knowledge into the local system. Lack of coordination between local administrations can be a problem. If many localities promote a single commodity, the result might be overproduction and low prices. Similarly, separate localities might finance the same feasibility studies, training programs, or extension materials. Implementing an integrated watershed or regional development plan might prove impossible if programs in each administrative region are completely independent. Other potential problems include the lack of career opportunities for extension staff in decentralized programs, and difficulties with monitoring and evaluation when local administrative units lack ability to compare targets, results, and achievements with other areas. (Khan, 2002).

Extension program quality depends fundamentally on good linkages with other programs—specialized training for extension agents and farmers, technical backstopping by subject matter specialists and information services, other extension services (mass media, fairs), and other development programs (credit programs, market development programs, input supply).

Some of these linkages can be maintained at the local level, but many require higher level coordination to ensure efficiency and quality support.

Decentralized Governance -Introducing Accountability:

Decentralizing extension by involving farmers and local government in governance of programs can improve program accountability, increase user ownership of programs, ensure relevance to local needs, improve planning and information flows, and strengthen user capabilities. Transferring program responsibilities to locally elected officials directly decentralizes program governance and accountability to local people. Perhaps equally important are alternate mechanisms that increase user participation and influence over program content and operations. Reforms that enhance farmer influence over program governance include: incorporating farmers into governance and oversight committees; adopting participatory extension approaches; involving farmers in identifying priorities, planning, and monitoring; working through farmer groups; and using participatory evaluation and feedback mechanisms for program evaluation. Decentralizing governance holds particular promise for making extension programs (and agents) accountable to users. Farmers know whether they are receiving valuable services and should have the power to demand good performance by their service providers. When farmers have authority to influence decisions on program funding, hiring and dismissing staff, and staff incentives, they are truly empowered to improve services. At a minimum, all extension programs should seek farmer feedback on the relevance, quality, and usefulness of extension services. (Chapman & Tripp, 2003).

A concern in decentralized extension systems is the degree to which governance mechanisms are representative of all farmers in an area. Women, small farmers, and ethnic or cultural minorities are often underrepresented in governance groups, but may be more in need of public services than those actually representing local interests. Disadvantages of farmer governance are the high up-front costs of participatory approaches, difficulties in ensuring true representation of participating groups, risk of aggravating conflicts or unduly raising expectations, and the possibility of program capture by elites (Farooq, 2005).

As the traditional view of extension as a function of government agencies gives way in the face of multiple service providers, an expanding agenda, and a better understanding of farmer information and innovation systems, decentralized

governance of extension services should become both easier and more important.

Fiscal Decentralization of Extension Services:

Government inability to sustain financial support for large extension systems has been a motivation for the many reforms that attempt to reduce public sector funding, introduce private financing, or eliminate government programs that compete with the private sector. Typically, these strategies tend to decentralize extension financing. Although an objective of many decentralization reforms has been to reduce government expenditures, local governments generally have limited resources and limited ability to raise funds. Central governments therefore must usually continue financing for extension services through intergovernmental financial transfers (IGFTs), and must also finance the considerable costs of reform and local capacity development. This increases total financing requirements for extension, at least over the short term. Over the longer term, decentralizing extension services might reduce government financing requirements by: (1) increasing efficiencies through better oversight and greater flexibility in funding decisions and (2) increasing cofinancing by being more responsive, and demonstrating greater benefits, to users. Cofinancing grants (IGFTs) to local governments or farmer groups are an important element of fiscal decentralization, but they present two significant problems: (Chapman & Tripp, 2003).

- Many local organizations lack capacity to plan, manage, and evaluate extension programs and lack the contacts and financial management capacity to procure needed services; and
- Resource-rich farmers are better able to cofinance services and capture program benefits, even if program objectives are to assist weaker elements of rural society. Still, many new initiatives are using subgrants of various types for local subprojects, and future program design can draw on this experience

Decentralization programs must address these two problems. Training and orientation, program promotion, and support services are critical to enable target clients and local organizations to take over extension responsibilities under new decentralized systems. Later, as programs are implemented, a strong monitoring and evaluation system is needed to provide management with information necessary to understand who is benefiting from the program and what real impact it is having (Farooq, 2005).

Conclusion:

Decentralize extension services where possible, with emphasis on giving users control over program planning, implementation, and evaluation.

- Provide for adequate centralized support systems for decentralized extension services, especially support for training, subject matter specialists, and production of extension materials.
- Adapt strategies to local institutional environments to accommodate country legal frameworks, political traditions, administrative structures, and social and agroecological conditions. Extension strategies can emphasize decentralization when there is already a strong political decentralization in the country, but should proceed cautiously when decentralization is not yet well established.
- Determine on a case-by-case basis whether decentralized services should be managed by local governments, community/producer organizations, or local governments in conjunction with producer/community organizations.
- Provide clear division of responsibilities between the different levels of government and other program participants.
- Develop procedures for policy formulation and priority setting in mixed systems to reconcile central government financing and policy objectives (poverty alleviation, food security, and environmental conservation) with local peoples' priorities that emerge from the decentralized program governance.
- Provide for needed fiscal transfers from central government to decentralized implementing agencies to finance decentralized extension services, recognizing that over the short term decentralization rarely reduces requirements for central government financing.
- Structure fiscal transfers to give users maximum influence over programs and to promote institutional pluralism in service provision. This empowers users and develops capacities in a range of public and private providers, such that the most competent institutions are able to provide the services.
- Provide for extensive planning, promotion of the rationale and principles behind reforms, and training in new operational procedures before launching decentralization reforms.
- Provide for needed investments in development of local capacity (local governments, executing agencies, community or producer groups), as such implementation capacity is critical to success of decentralization reforms.
- Establish effective systems to monitor and evaluate decentralized programs, and ensure that the data are available at all appropriate levels. Central monitoring should be sensitive to equity issues and the possibility of local elites capture of programs, thus excluding services to the poor or women.

***Corresponding Author:**

Mohammad Abedi

Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran.
E-mail: Abedi114@yahoo.com

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Ceftriaxone versus Chloramphenicol for Treatment of Acute Typhoid Fever

Osama Mohamed Hammad¹, Tamer Hifnawy^{2*}, Dalia Omran³, Magda Anwar El Tantawi⁴ and Nabil Isaknder Girgis⁵

¹Tropical Medicine Department, Faculty of Medicine, Beni Suef University-Egypt.

²Public Health & Community Medicine Department Faculty of Medicine, Beni Suef University-Egypt.

³Tropical Medicine Department, Faculty of Medicine, Cairo University, Egypt.

⁴Bacteriology Department, Abbassia Fever Hospital.

⁵Former NAMRU3, Cairo, Egypt.

*daliaomran2007@yahoo.com

Abstract: Typhoid fever is a global health problem, with an estimated 20 million cases and 700,000 deaths annually. In Egypt, since the beginning of the 1980s, there had been an increase in the prevalence of multidrug resistance to the first line antimicrobials used in the treatment of the disease such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (TMP-SMX) and thus other drugs, the fluoroquinolones and third generation cephalosporins, had to be evaluated for their efficacy in the treatment and their side effects. The aim of this study was to compare the efficacy of chloramphenicol, which was the classical drug for treatment of acute typhoid fever in Abbassia fever hospital (AFH), with ceftriaxone which became a first line drug for treatment of it after the appearance of multidrug resistant (MDR) isolates of *Salmonella typhi* (*S. typhi*) in the last fifteen years. As a part of the study we investigated whether or not the organisms were still sensitive to the quinolones and third generation cephalosporins. We also investigated if multidrug resistant (MDR) typhoid fever was still a problem in Egypt. A phase IV open label, prospective, randomized clinical trial study was implemented in the period between March 2007 and June 2009. Fifty two patients with positive blood culture for *S. typhi* were included in this study. They were 32 (62%) males and 20 (38%) females ranging in age from 3 to 47 years (mean±SD: 22±8.5years). Drug sensitivity tests showed that 4 (8%) of *Salmonella typhi* isolates were resistant to chloramphenicol and 18 (35%) and 21 (40%) isolates were resistant to ampicillin and TMP-SMX respectively. Two (4%) isolates were resistant to chloramphenicol, ampicillin and TMP-SMX. No isolates were resistant to ciprofloxacin or ceftriaxone. Twenty seven (52%) patients were treated with chloramphenicol and twenty five (48%) patients were treated with ceftriaxone. All patients were cured. The mean time (mean±SD) for patients to become afebrile was 3.3±1.2 days for ceftriaxone and 5.8±1.2 days for chloramphenicol. In patients treated with ceftriaxone the time taken to become afebrile was shorter with chronic infection as compared to those treated with chloramphenicol (P value= 0.0001 95% CI= 1.831-3.169). From this study, it can be concluded that: ceftriaxone was associated with a significantly shorter period of defervescence making it the drug of choice for treatment of typhoid fever. There is a marked reduction of the prevalence of MDR *Salmonella typhi* isolates and marked increase in the susceptibility of these isolates to chloramphenicol, returning it to be one of the drugs that could be used in the treatment of acute typhoid fever. No drug resistance to ceftriaxone and ciprofloxacin was reported after many years of using them for treatment of acute typhoid fever. Due to high degree of resistance to ampicillin and TMP-SMX, they should not be used as first line drugs for treatment of acute typhoid fever.

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Key words: Typhoid fever, Multidrug resistance, Chloramphenicol, Ceftriaxone.

1. Introduction

Typhoid fever occurs in over 20 million cases annually, with at least 700,000 deaths. The main burden of disease is in developing countries, particularly the Indian subcontinent and South East Asia (1). Historically, the infection was treated with chloramphenicol, ampicillin or trimethoprim-sulfamethoxazole (TMP-SMX). However, the widespread emergence of antibiotic resistant *Salmonella typhi* (*S. typhi*) has presented an important

public health problem during the past decades (2). In Egypt, chloramphenicol resistant *Salmonella typhi* was first reported in 1981 (3). Mourad et al. (4) found that 43% of *Salmonella typhi* isolates at Alexandria fever hospital were multidrug resistant (MDR) isolates. In another study done in Egypt, Wasfy et al. (5) found that 71% of patients with typhoid fever had MDR *Salmonella typhi* isolates. Recently, *Salmonella typhi* strains resistant to quinolones and third

generation cephalosporins have been documented by many authors (6-7).

Typhoid fever caused by MDR organisms is a significant public health and therapeutic problem as a large number of cases of MDR typhoid fever occur in childhood and are accompanied with significantly high morbidity and mortality rates (8).

The aim of this study was to evaluate the efficacy of chloramphenicol, which remained for many years as the drug of choice for treatment of acute typhoid fever in Abbassia Fever Hospital (AFH) and compare it to ceftriaxone which became the main drug for treatment of typhoid fever after the appearance of MDR isolates in the last fifteen years. As a part of the study we investigated whether or not the organisms were still sensitive to the quinolones and third generation cephalosporins. We also investigated if multidrug resistant (MDR) typhoid fever was still a problem in Egypt.

2. Patients and Methods

A phase IV open label, prospective, randomized clinical study was implemented in the period between March 2007 and June 2009. After having their informed consent to participate in our study, fifty two patients with acute typhoid fever in Abbassia Fever Hospital (AFH) "The main fever hospital in Cairo Governorate, Egypt" were included in this study.

Our Inclusion criteria were to have a diagnosis for typhoid fever with a positive blood culture for *Salmonella typhi* and a consent to participate in this study. Criteria for exclusion were patients with deteriorated general condition, hyperpyrexia (40.5 C or above), hypotension, meleana, bleeding per rectum and or disturbed level of consciousness

All recruited patients were subjected to: Careful history and thorough clinical examination, complete blood picture. On the day of admission to the hospital before initiation of antibiotic therapy, an aliquot of each patient blood was collected and inoculated onto bi-phasic blood culture bottles and incubated at 37°C. Bottles were checked daily for 7 days and when growth was noted, an aliquot of blood was streaked onto MacConkey and blood agar plates to allow for final identification of the organism by using standard serological and biochemical methods (9).Widal agglutination test was done to all patients (10).

Susceptibility of *Salmonella typhi* to ampicillin (10 ug), chloramphenicol (30 ug), TMP-SMX (25 ug), ciprofloxacin (5 ug) and ceftriaxone (30 ug) was performed using the disc diffusion Kirby-Bauer method (11).

Twenty seven (52%) patients were randomly allocated to be treated with chloramphenicol (50 mg/kg/day orally or intravenously) given 6 hourly till defervescence (primary outcome measure) and for a further 5 days (secondary outcome measure). The time of defervescence was defined as the time interval from starting an appropriate antimicrobial chemotherapy until the documentation of normal body temperature (8).

Twenty five (48%) patients were randomly allocated to be treated with ceftriaxone parenterally (80 mg/kg/day for children and 2 gm/day for adults) given once daily for 7 days.

Any patient infected with a strain resistant to the drug with which he was being treated, was shifted to another drug to which the isolates were sensitive and was not included in final analysis of results.

Patients presenting with complications (gastrointestinal hemorrhage or perforation, toxic myocarditis, hepatitis) were excluded from the study.

Subjects were randomized with equal distribution to the 2 treatment regimens using block of 6 and randomization envelopes were prepared by the biostatistician

This study was open label, therefore no blinding procedures were required.

Patient was considered cured if there was no fever, abdominal tenderness, toxic look or tympanic abdomen at the end of treatment course.

Statistical analysis:

Regarding our sample size, a time frame was applied to recruit all cases of typhoid fever diagnosed clinically with confirmed laboratory diagnosis from the period between the 1st of March 2007 till the end of June 2009 after signing an informed consent to participate.

Descriptive summaries were presented using summary statistics for continuous (quantitative) variables and frequency for discrete (qualitative) variables.

Data were collected coded and analyzed using SPSS software version 15 under windows XP. Unpaired student t-test was used to compare time of defervescence between those who were treated with chloramphenicol versus ceftriaxone. The threshold of significance was fixed at the 5% level.

No interment analysis was done and the final analysis was conducted at the end of the study after all patients had completed the study protocol.

Ethical Consideration: All patients participating in this study were asked to sign an informed consent form describing all study procedures, risk and benefits. For children and minors "less than 21 years" parent guardian informed consent was taken

3. Results:

Fifty two patients of acute typhoid fever with positive blood culture for *Salmonella typhi* were enrolled in this study. They were 32(62%) males and

20(38%) females ranging in age from 3 to 47 years (mean±SD 22±8.5 years). The clinical picture of these patients upon admission is shown in table (1).

Table (1) Clinical picture of (52) acute typhoid fever patients

Symptoms	Number (%)		
	Chloramphenicol treated patients No. = 27	Ceftriaxone treated patients No. = 25	Total No. = 52
Fever	27 (100)	25 (100)	52 (100)
Abdominal discomfort	22 (81)	18 (72)	40 (77)
Headache	21 (72)	19 (76)	40 (77)
Epistaxis	13 (48)	14 (56)	27 (52)
Cough	16 (59)	16 (64)	32 (62)
Vomiting	12 (44)	11 (44)	23 (44)
Diarrhea	9 (33)	8 (32)	17 (33)
Signs			
Fever	27 (100)	25 (100)	52 (100)
Toxic look	21 (78)	22 (88)	43 (83)
Abdominal tenderness	23 (85)	21 (81)	44 (85)
Splenomegaly	22 (81)	18 (72)	40 (77)
Abdominal distension	20 (74)	19 (76)	39 (75)
Hepatomegaly	10 (37)	10 (40)	20 (38)
Jaundice	0	1 (4)	1 (2)

The hematological profile and Widal agglutination test results are shown in table (2). Normal hematological profile was seen in most of the

patients. Thirty eight (73%) and forty patients (77%) had anti-O antibody and anti-H titers of $\geq 1/160$ respectively

Table (2) Haematological profile and Widal agglutination titer of (52) acute typhoid fever patients

Complete blood picture	Range	Mean	
Haemoglobin	5.5-14.8 gm%	11 ± 1.8	
Total white blood cell count	2.3 - 11.4X 10 ³ / cmm	5 ± 2.3	
Platelet count	46-458 x 10 ³ / cmm	185 ± 87.4	
Widal agglutination titer	Chloramphenicol treated patients (27) No. (%)	Ceftriaxone treated patients (25) No. (%)	Total patients (52) No. (%)
Anti-O = 1/80 - Anti-H = 1/80	1 (4) - 2 (7)	1 (4) - 2 (8)	2 (4%) - 4 (8%)
Anti-O = 1/160 - Anti-H = 1/160	7 (26) - 5 (19)	5 (20) - 5 (20)	12 (23%) - 10 (19%)
Anti-O = 1/320 - Anti-H = 1/320	6 (22) - 9 (33)	5 (20) - 6 (24)	11 (21%) - 15 (29%)
Anti-O = 1/640 - Anti-H = 1/640	8 (30) - 8 (30)	7 (28) - 7 (28)	15 (29%) - 15 (29%)
Anti-O $\geq 1/160$ - Anti-H $\geq 1/160$	21 (78) - 22 (81)	17 (68) - 18 (72)	38 (73%) - 40 (77%)

Drug sensitivity tests revealed that 4 (8%) of isolates were resistant to chloramphenicol and 18 (35%) and 21 (40%) isolates were resistant to ampicillin and TMP-SMX respectively. Two (4%) isolates were MDR resistant to chloramphenicol,

ampicillin and TMP-SMX. No isolates were resistant to ciprofloxacin or ceftriaxone (table 3). Seven isolates had no resistance to any of the tested five drugs.

Table (3): Antimicrobial susceptibility patterns of 52 Salmonella typhi isolates

Susceptibility pattern	Number of isolates	%
Any resistance		
Chloramphenicol	4	8
TMP-SMX	21	40
Ampicillin	18	35
Ciprofloxacin	0	0
Ceftriaxone	0	0
Multidrug resistance (MDR) to chloramphenicol, TMP- SMX and ampicillin	2	4
Isolates with no resistance to any of the five tested drugs	7	13

There were no reported complications throughout the study.

All patients were cured. The mean time (mean±SD) of defervescence for ceftriaxone and chloramphenicol was 3.3±1.2 and 5.8±1.2 days respectively. P value= 0.0001 95% CI= 1.8-3.2. Ceftriaxone was significantly associated with a short time of defervescence compared with chloramphenicol

4. Discussion

Enteric fever continues to be a major public health problem, especially in the developing countries of the tropics. The sensitivity pattern of *S. typhi* is changing and there is re-emergence of sensitivity to chloramphenicol but rising resistance to ciprofloxacin (12). In this study, 4%, of the isolated strains of *Salmonella typhi* were resistant to chloramphenicol, ampicillin and TMP-SMX. In a study done by Mourad et al.(4) MDR *Salmonella typhi* isolates were detected in 15 (43%) of 35 patients with culture positive *S. typhi*. Wasfy et al.(5) studied 537 *S. typhi* isolates collected between 1990-1994 in Egypt; 71% of isolates were MDR. This period represented the peak of MDR reisolates in Egypt. In another study done in Abbassia Fever Hospital, Wasfy et al. (2) reported that MDR *Salmonella typhi* increased from 19% in 1987 to 100% in 1993, but it subsequently decreased again to only 5% by the year 2000. In Fayoum Governorate "One of Upper Egypt governorates", MDR *Salmonella typhi* isolates were detected in 26 (29%) of 90 patients with culture positive *S. typhi* (13). Decline of MDR *Salmonella typhi* isolates were reported in many studies world wide and was reported to be 5.6% by Chitnis et al. (14), 5% by Pokharel et al. (15), 18.6% by Ray et al. (16) and 22% by Cooke et al. (17). In Imbaba fever hospital, Giza province, Egypt El-Din et al. (18)

reported that 25% of *Salmonella typhi* isolates were resistant to chloramphenicol.

In our study, 8% of the isolates were chloramphenicol resistant. Due to the development of MDR isolates, there was a decrease in the use of chloramphenicol for treatment of typhoid fever in Egypt and this, in addition to the use of more-effective antibiotics could have caused a decrease in the prevalence of persons with chronic infection in the community and hence the circulation of resistant strains. The improvement in susceptibility of *Salmonella typhi* to chloramphenicol (although its lower performance compared to ceftriaxone), will cause it to be re-considered as one of the drugs of choice for treatment of typhoid fever in Egypt. Similar studies should be considered in some parts of the world where medical resources are limited. Chloramphenicol has a cheaper price and well established efficiency. (2,14,19). In this study, 35% and 40% of isolates were resistant to ampicillin and TMP-SMX respectively and this is in agreement with that reported by Srikantiah et al. (13). Until improvement in the susceptibility of *Salmonella typhi* to these two drugs, they should not be used as a first line drugs for treatment of typhoid fever. None of our *Salmonella typhi* isolates were resistant to ciprofloxacin or ceftriaxone. This was in agreement with Wasfy et al. (2) and Ray et al.(16). Resistance to ciprofloxacin (3%) and ceftriaxone (2%) were documented by Srikantiah et al. (13) in the Fayoum governorate, Egypt. Resistance to ciprofloxacin was reported by Butt et al. (20); Capoor et al. (6) and Dimitrov et al. (7).

The main symptoms in our 52 patient with acute typhoid fever were fever (100%), headache (77%), vomiting (44%) abdominal discomfort (77%) cough (62%) and epistaxis (52%). The main signs were fever (100%), toxic look (83%), abdominal tenderness (85%), abdominal distention (75%), splenomegaly (77%), and hepatomegaly (38%). These symptoms and signs agreed with Abdel Wahab et al. (21)

As regards the blood picture, our patients showed anemia (mean hemoglobin ±SD 11±1.8 gms %), within normal white blood cell count (mean 5±2.3) and within normal blood platelets (mean 185±87.4). Anemia may be due *Salmonella* endotoxaemia. Within normal white blood cell count is similar to that reported by Abdool Gaffar et al.(22). In accordance with our results, The peripheral blood changes did not influence the outcome of the disease, since all patients recovered completely after treatment (23).

In our patients anti-O \geq 1/160 and anti-H \geq 1/160 titers were detected in 73% and 77% of the patients respectively. This was considered as a

significant titer suggestive of acute typhoid fever in Egypt and this is in agreement with Hassanein et al. (24) and Frimpiong et al. (25). The results of Widal test should be interpreted in concerns with a patients clinical presentation in making a diagnosis of typhoid fever. Both the somatic and flagellar agglutinins are important for this purpose (26).

Both chloramphenicol and ceftriaxone were effective for treatment of our 52 patients with acute typhoid. Ceftriaxone was significantly associated with a shorter time of defervescence compared to chloramphenicol. This agrees with other studies (8, 21).

From this study, we concluded that ceftriaxone was significantly associated with short time of defervescence making it the drug of choice for treatment of acute typhoid fever. There is marked reduction in the prevalence of MDR Salmonella typhi isolates and marked increase in susceptibility of these isolates to chloramphenicol, returning it to be one of the drugs of choice for treatment of acute typhoid fever. No drug resistance to ceftriaxone and ciprofloxacin were reported after many years of using them in the treatment of acute typhoid fever. Due to the high degree of resistance to ampicillin and TMP-SMX, they should not be used as first line drugs for treatment of acute typhoid fever.

Correspondence author

Dalia Omran, Department of Tropical Medicine,
Faculty of Medicine, Cairo University, Cairo, Egypt
Tel: + 010 0087802

daliaomran2007@yahoo.com

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Exposure of Adult Male Rats to Cadmium: Assessment of Sexual Behaviour, Fertility, Aggression as well as Anxiety like Behaviour with Special Reference to Biochemical and Pathological Alterations.Mervat M .Kamel^{1*}, Abeer H. Abd El Razek¹, Kawkab A . Ahmed² and Gehan M. Kamel³¹Department of Veterinary Hygiene and Management, ²Department of Pathology, ³ Department of Pharmacology .
Faculty of Veterinary Medicine, Cairo University, Cairo , Egypt .* mevy58@yahoo.com

Abstract: Because Cadmium is widely used in industry and in our daily life, it's likely that many people are exposed to it . The main aim of this study is to further investigate the effects of cadmium on sexual and aggressive behaviour as well as anxiety – like behaviour in adult male rats. Forty five male Wistar rats weighing (140-160 g) were administered CdCl₂ in drinking water at one of three concentrations: 0, 5 and 50 mg / L for a period of 12 weeks .Sex organs tissues (testes, prostate glands & seminal vesicles), representing all treatments were taken for biochemical , histopathological examination and male fertility assessment (semen quality analysis). Results revealed marked impairment in sexual activity with noticed influence on both territorial aggressive behaviour and anxiety – like behaviour in males exposed to CdCl₂. These behavioural alterations were paralleled by biochemical changes, showing that CdCl₂ at concentrations (5mg) and (50 mg) induced a serious decrease in the level of testosterone and a significant elevation in serotonin. Additionally, increased oxidative stress in testicular tissue. Poor semen quality (sperm count, sperm motility, sperm viability) was observed in the treated male rats. furthermore, histopathological alterations were observed in the testes, prostate gland and seminal vesicles of the cadmium treated rats . Our results strongly suggest that Cd intoxication produces adverse effects on sexual behaviour , aggression , fertility and anxiety – like behaviour , with biochemical and pathological alterations in adult male rats.

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Keywords: Cadmium chloride (CdCl₂), sexual behaviour, aggression, anxiety–like behaviour, fertility, testosterone, serotonin, SOD, TBARs, histopathological changes.

1. Introduction

Cadmium is one of environmental pollutants arising from electroplating, fertilizers, pigment and plastic manufactures. Therefore it is easily contaminate the soil, plants, air and water (Ognjanovic *et al.*, 2008). Humans and animals can easily expose to cadmium toxicity by consuming plants, water and air. Cadmium absorbed and accumulates in various tissues (Casalino *et al.*, 2002, Waisberg *et al.*, 2003) even red blood cells (Kostic *et al.*, 1993), the heart (Zikic *et al.*, 1998) and the skeletal muscle of rats (Pavlovic *et al.*, 2001). Cadmium is considered as ubiquitous toxic metal that induce oxidative damage by disturbing the peroxidant – antioxidant balance in the tissue (Ognjanovic *et al.*, 2008). Moreover, Cadmium is a recognized reproductive toxicant and has been reported to reduce male fertility and altered sexual behaviour in both humans and rodents (Thomas and Brogan, 1983). Most animals with scrotal testes are susceptible to cadmium – induced testicular toxicity (King *et al.*, 1999). Testes are included among the most target organs for cadmium intoxication (Stajn *et al.*, 1997).

Rodent testes are more susceptible to cadmium toxicity than liver, as manifested in testicular damage without pathological changes to other organs (Adaikpoh and Obi 2009). Exposure to cadmium can negatively affect the male reproductive system via degenerative changes in testes, epididymis, and seminal vesicles (Ibrahim and Sameh 2002). Recently, Azoospermic persons were found to have higher serum and seminal plasma cadmium level compared with oligospermic ones (Oluyemi Akinloye *et al.*, 2006). Also positive relationship was found between cadmium exposure and asthenozoospermia in a rat model (Benoff *et al.*, 2008).

Since aggressive behaviour are controlled by androgenic hormones (Clark and Henderson, 2003). It was hence concluded that the different parameters of aggression and violent are likely to be involved in this study. Exposure to toxic metals has been reported to affect aggressive, antisocial violent behaviour (Melvyn, 1995). Moreover, oral exposure to cadmium can cause anxiety and fear as well as alterations in the biochemical activity of the brain (Bull, 2010).

To the best of our knowledge, only a few studies have addressed the effect of orally administered cadmium chloride on both sexual and aggressive behaviour in male rats. Furthermore, the involvement of various arrays of measurement to evaluate anxiety and emotionality in rats treated with cadmium is not well implemented.

Thus, the objectives of the current study were to evaluate the impact of elevated levels of cadmium chloride in drinking water on sexual and aggressive behaviour in adult male rats. Moreover, biochemical and histopathological evaluation were carried out to detect the degenerative changes and oxidative damage in male reproductive system. Additionally, long – term changes in anxiety – like behaviour were monitored.

2. Material and Methods:

2.1. Animals and housing:

Forty five Wistar male albino rats weighing 140- 160 g.were used in this study. Animals were raised in the Animal House Unit in Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages with stainless steel wire lids (bedded with wood shavings). Food and water were supplied ad libitum. Rats were housed at a controlled temperature of $21 \pm 1^\circ\text{C}$, 60 % humidity and under a 12 -hr - light: 12- hr- dark schedule. Animal care was in compliance with the applicable guidelines from Cairo University Policy on Animal Care and Use.

2.2. Administration of Cadmium:

Cadmium Chloride (CdCl_2) in crystalline form was obtained from Sigma Chemical Company (Sigma, Aldrich). CdCl_2 was dissolved in tap drinking water at different concentrations, namely ; 5 mg / L (low dose) and 50 mg / L (high dose) (Waalkes *et al.*, 1999). Male rats were randomly assigned to three groups of 15 animals each (control or experimental groups). Experimental male rats were provided access to drinking water containing CdCl_2 for 12 weeks .The control group received tap water only.

2.3. Behavioural assessment:

2.3.1. Sexual behaviour testing:-

Each male rat was tested for sexual behaviour with a stimulus female (induced estrus).Female rats of the same strain were used in this experiment receiving subcutaneous treatment of estradiol benzoate 5.00mg/rat and progesterone 0.5mg/rat dissolved in 0.2ml sesame oil 52 and 4 h before the test sessions, respectively. Male rat was observed alone for 5min, an estrus female was then introduced in the centre of the arena and the behaviour of the male was then recorded. The time to the first mount and the number

of mounts. Also ejaculatory latency, number of ejaculation and post-ejaculatory interval (latency period) were observed (Cagiano *et al.*, 1998; Nabil and Zeyad 2005).

2.3.2. Aggressive behaviour testing:

A rectangular observation cage (45×27× 40 cm) was used for aggression assessment. A stud male rat was placed in the testing arena for 10 days. A second male (control or CdCl_2 treated) was then placed in the test arena with the stud male for 5 min. and the following parameters were recorded:lateralization, boxing bouts ,fights with stud male ,tooth chattering and leaping (Batainch *et al.*, 1998 and Nabil and Zeyad 2005).Animals were observed between 09:00 and 15:00 hr. and all behavioural measures were monitored by a single observer unfamiliar with the cadmium treated males.

2.3.3 .Fear and anxiety measurements:

The elevated plus-maze was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze considered a measure of the strength of a fear drive (Trullas and Skolnick,1993).The apparatus consists of 4 crossed arms, two open arms (50×10× 0 cm)and two closed arms (50×10×30 cm).The maze was elevated 0.65m above the floor. The rat was placed in the centre of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 minutes was recorded. All testing took place during the first 6 h of the dark phase of the L: D cycle. After each trial the maze was wiped with a cloth dipped in 70% ethyl alcohol and allowed to dry (Kierstin, 2003).

2.4. Male fertility assessment:

2.4.1. Sex organs weight:

Five rats from each group were sacrificed at the end of experiment. The testes, prostate gland and seminal vesicles were dissected and weighed in relative to body weight.

2.4.2. Semen quality analysis:

Seminal content of epididymis was obtained by cutting of the cuda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted 10 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the progressive motility and sperm concentration (Bearden and Fluquary, 1980). One drop of the suspension was smeared on a glass slide and stained by Eosin – nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities (Miller and Pass 1952). Abnormal head

and tails were evaluated by using the criteria of Okamura *et al.* (2005).

2.5. Biochemical assessment:

2.5.1. Testosterone and Serotonin assay:

On completion of all behavioural assessments, five male rats per treatment were randomly selected to obtain blood samples (orbital plexus of rats). Blood was then centrifuged at 2000 rpm for 15 min and the serum was stored at -20 °C. Testosterone was estimated in the serum as described by Ismail (1986). Also, serotonin was assessed by an improved Miller's fluorophotometry method (Zhang *et al.*, 1994).

Briefly, 1.8 ml acidified *n*-butanol (adding 0.85 ml 12 M HCl per liter *n*-butanol saturated with NaCl) was added to 0.2 ml serum, vortexed for 5 min, centrifuged at 3000 rpm for 10 min. 1.5 ml supernatant was collected, added with 1.5 ml *n*-heptane and 0.5 ml 0.1 M HCl, vortexed for 5 min, centrifuged at 3000 rpm for 5 min. After the supernatant phase (mainly *n*-heptane) was discarded, 0.25 ml aqueous phase, drawn from the bottom, was mixed with 0.05 ml 82.4 mM (10 g/l) L-cystine and 0.75 ml 60 mg/l *o*-phthalaldehyde (OPA, prepared with 10 M HCl), and kept in boiling water for 10 min, then put into icy water to stop reactions. Fluorescence was measured in a spectrofluorometer (1420 Mutilabel HTS, PerkinElmer, USA) using 96-well plate. Excitation and emission wavelengths were 355 and 460 nm, respectively. The standard line was made using serotonin creatinine sulfate complex dissolved in 0.01 M HCl following the same procedure above.

2.5.2. Oxidative stress assessment:

Testicular tissue specimens were homogenized in 9 fold volumes phosphate buffered solution (PH 7.4). The homogenate was then centrifuged at 4000 rpm for 15 min at 4 °C and the supernatant was kept at -80 °C until used in the studied enzymatic assay.

2.5.2.1. Determination of super oxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity was measured according to Giannopolitis and Ries (1977) by means of SOD assay kit (Cayman, MI, USA) according to manufacturer's instructions. The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. The SOD assay measures all the three types of SOD (Cu/Zn, Mn, and FeSOD). Enzyme activity was determined as the amount of the enzyme required to induce 50%

inhibition of nitro – blue tetrazolium (NBT) reduction rate.

2.5.2.2. Testicular lipid peroxidation :

The level of lipid peroxidation in terms of TBARS formation (nmoles / min / mg protein) was determined (Esterbauer and Cheeseman , 1990). One volume of testicular homogenate was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm.

The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. Since 99% TBARS was malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

2.6. Histopathological studies:

Specimens from testis, prostate gland and seminal vesicle were collected from all experimental groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70-100%) and then prepared using standard procedures for Hematoxylin and Eosin staining as described by Bancroft *et al* (1996).

2.7. Statistical analysis:

Statistical analyses were performed by using SPSS statistical software package (SPSS, 2006). Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test.

3. Results:

3.1. Effect of CdCl₂ on sexual behaviour in male rats:

The results presented in table (1) shows the effect of CdCl₂ on the parameters related to male rats sex-behaviour. Group of rats administered high CdCl₂ concentrations had a significantly prolonged time to the first mount and a significant decrease in the number of mount (p<0.05). In addition, Cd cl - treated rats have shown a significant increase (p<0.05) in ejaculatory latency compared to their counterparts in the control group. Decrease number of ejaculations and increase post-ejaculatory interval was significantly (p<0.05) seen in male rats exposed to high concentrations of CdCl₂ compared to those exposed to low doses of CdCl₂ and rats in control group.

3.2. Effect of CdCl₂ on aggressive behaviour

The parameters of territorial aggression in adult male rats are demonstrated in table (2). High CdCl₂ group displayed lateralization and boxing bouts ($p < 0.05$) fewer compared to control group. A significant reduction ($p < 0.05$) in the number of tooth chattering and leaping was observed in treated rats (high and low doses) when compared with the control group. Although high CdCl₂ seemed to reduce fighting significantly by male rats with the stud male rat, this effect was not significant in low CdCl₂ group.

3.3. Effect of CdCl₂ on fear and anxiety:

The effect of CdCl₂ treatment on the number of entries in closed and open arm in the elevated plus-maze has been summarized in table (3). There was a significant ($p < 0.05$) increase occupancy in closed arms by exposed rats to different CdCl₂ concentrations as compared to the control rats. Furthermore, the treated rats spent more time in closed arms while no significant differences in the number of entries of both closed and open arms among the control rats.

3.4. Effect of CdCl₂ on male fertility:

3.4.1. Sex organs weight:

A significant decrease in the relative body weights of testes, seminal vesicles and prostate glands of CdCl₂ treated male rats as compared with those in the control group (Table 4).

3.4.2. Semen quality analysis:

Semen characteristics are shown in table (5). There was a significant decrease in sperm cell concentration, percentage of sperm motility and viability in CdCl₂ treated rats compared to the control group. Sperm cell abnormalities in the form of detached head and coiled tail were increased significantly ($P < 0.05$) in male rats administered CdCl₂.

3.4.3. Testosterone and serotonin assay:

Testosterone serum level was significantly decreased in CdCl₂ treated male rats as compared with those in the control group ($p < 0.05$). While serotonin was significantly increased when CdCl₂ was administered to male rats (Table, 6).

3.5. Oxidative stress parameters:

SOD activity and TBARs formation in testicular tissue of rats are presented in table, 7. The level of TBARs formation was significantly higher in case of CdCl₂ treated rats. SOD activity was significantly lowered in CdCl₂ treated rats compared to CdCl₂ free rats.

3.6. Pathological examination:

3.6.1. Gross Pathological examination:

There were no obvious gross pathological alterations were observed in testes, prostate glands and seminal vesicles of rats in all groups.

3.6.2. Histopathological examination :

3.6. 2.1. Testes:

Microscopically, examined testes of rat treated with low dose of Cd revealed degeneration of spermatogonial cells lining seminiferous tubules and the tubules lumen were filled with degenerated germ cells (Fig. 1). Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema were also noticed (Figs. 2 & 3). Also multinucleated spermatid giant cells (symploids) were observed in the lumen of seminiferous tubules (Fig. 4). Testes of rats treated with high dose of CdCl₂ revealed more or less similar histopathological changes to the previous group. Those alterations described as marked degeneration and vacuolization of seminiferous epithelium (Fig. 5), necrosis of germ cells with complete absence of spermatozoa as well as interstitial oedema (Fig. 6), interstitial haemorrhage and necrosis of leydig cells (Fig. 7). Meanwhile, testes of CdCl₂ free rats revealed no histopathological changes (Fig. 8).

3.6. 2.2. Prostate glands:

Examined sections of rat treated with low dose of CdCl₂ showed slight hyperplasia of epithelial lining prostatic acini (Fig. 9) associated with interstitial edema. Meanwhile, prostate gland of rats treated with high dose revealed hyperplasia of prostatic epithelium, interstitial edema and hemorrhage (Fig. 10) associated with intestinal fibrous connective tissue proliferation (Fig. 11). However, prostate gland of control, untreated rat revealed no histopathological alterations. (Fig. 12).

3.6. 2.3. Seminal vesicles:

Microscopically, examined sections of rats treated with low dose of CdCl₂ revealed congestion of all blood vessels (Fig. 13), hyperplasia of epithelium lining (Fig. 14) as well as interstitial edema and hemorrhage (Fig. 15). Moreover, sections of rats treated with high dose of CdCl₂ showed marked hyperplasia of epithelial lining, interstitial edema and hemorrhage (Fig. 16) accompanied with leucocytic cells infiltration (Fig. 17). No histopathological changes were noticed in examined sections of CdCl₂ free rats (Fig. 18).

Table (1) : Effect of CdCl₂ on sexual behaviour in adult male rats .

Parameters	Control	Low dose	High dose
-Latency to 1 st mount(s).	113.60±19.125 ^{ab}	104.71±16.867 ^{abc}	241.25±41.486 ^{bc}
- No. of mount.	14.83±3.400 ^{ab}	13.50±2.217 ^{ab}	10.00±2.047 ^c
-Ejaculatory latency(s).	242.00±30.550 ^a	314.67±55.247 ^{bc}	375.29±30.552 ^{bc}
- No. of ejaculation.	10.50±3.085 ^{ab}	7.50±1.190 ^{ab}	2.71±0.474 ^c
- post ejaculatory Intervals (s).	69.83±9.005 ^{ab}	67.46±10.240 ^{ab}	153.24±35.986 ^c

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (2) : Effect of CdCl₂ on aggressive behaviour in adult male rats .

Parameters	Control	Low dose	High dose
-No. of lateralization.	3.55±0.78 ^{ab}	1.7±0.68 ^{ab}	0.78±0.32 ^c
-No. of Boxing bouts.	3.36±1.00 ^{ab}	1.88±0.61 ^{ab}	0.80±0.35 ^c
-No. of fights with Stud male.	1.80±0.66 ^{ab}	1.50±0.59 ^{ab}	0.02±0.02 ^c
-No. of tooth chattering.	0.73±0.23 ^a	0.10±0.01 ^{bc}	0.20±0.01 ^{bc}
-No. of leaping.	0.36±0.15 ^a	0.02±0.01 ^{bc}	0.10±0.10 ^{bc}

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (3) : Effect of CdCl₂ on fear and anxiety measurements in adult male rats .

Parameters	Control	Low dose	High dose
-No . of entries in open arms.	3.56±0.37	2.64±0.47	3.53±0.40
-Time spent in open arms(s).	96.67±9.67 ^a	49.46±12.37 ^{bc}	55.80±10.60 ^{bc}
- No . of entries in closed arms.	3.67±0.52 ^a	5.82±0.66 ^{bc}	5.53±0.57 ^{bc}
- Time spent in Closed arms .	103.78±14.35 ^a	125.53±15.72 ^{bc}	150.46±18.35 ^{bc}

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (4): Effect of CdCl₂ on sex organs weight in adult male rats. (gm/100g. b.wt.)

Organs	Control	Low dose	High dose
Testes.	1.566±0.068 ^{ab}	1.268±0.745 ^{abc}	1.164±0.171 ^{bc}
Seminal vesicles.	0.789±0.046 ^a	0.580±0.336 ^{bc}	0.584±0.036 ^{bc}
Prostate gland.	0.372±0.027 ^a	0.226±0.0132 ^{bc}	0.189±0.0123 ^{bc}

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (5) : Effect of CdCl₂ on semen quality in adult male rats .

Organs	Control	Low dose	High dose
- Sperm count (10 ⁶ / ml)	69.60±2.49 ^{ab}	63.60±2.80 ^{abc}	56.40±2.29 ^{bc}
-Motility (%) .	85.20±1.77 ^a	64.20±3.54 ^b	48.60±2.73 ^c
-Viability (%) .	91.60±2.34 ^a	66.40±2.73 ^{bc}	55.20±2.06 ^{bc}
-Total sperm	5.20±0.37 ^a	11.40±0.68 ^{bc}	19.60±0.93 ^{bc}
Abnormalities (%)			

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (6): Effect of CdCl₂ on serum testosterone and serotonin in adult male rats.

Parameter	Control	Low dose	High dose
-Testosterone (ng/ml)	2.49±0.892 ^a	1.89±0.095 ^b	1.48±0.017 ^c
-Serotonin(ng/ ml)	58.92±2.77 ^a	70.30±2.17 ^b	93.78±2.71 ^c

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

Table (7) : Effect of CdCl₂ on testicular lipid peroxidation and oxidative states in adult male rats.

Parameter	Control	Low dose	High dose
-SOD (µmol/min/mg protein)	0.09±0.002 ^a	0.08±0.002 ^b	0.07±0.002 ^c
-TBARS (nmol/ mg protein)	0.11±0.004 ^a	0.13±0.003 ^b	0.16±0.005 ^c

a-c values within row with unlike superscripts differ significantly (p < 0.05) ; according to ANOVA .
Values represent mean ± SEM.

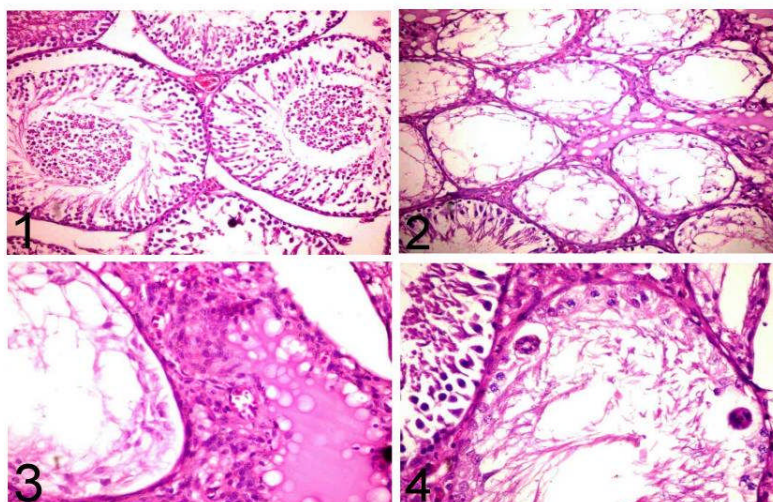


Fig. (1): Testis of rat treated with low dose of Cd showing degeneration of spermatogonial cells lining seminiferous tubules and the tubules lumen filled with degenerated germ cells. (H & E X 200).

Fig. (2): Testis of rat treated with low dose of Cd showing Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema. (H & E X 100).

Fig. (3): Testis of rat treated with low dose of Cd showing Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema. (H & E X 200).

Fig. (4): Testis of rat treated with low dose of Cd showing multinucleated spermatid giant cells (symplasts) in the lumen of seminiferous tubules. (H & E X 200).

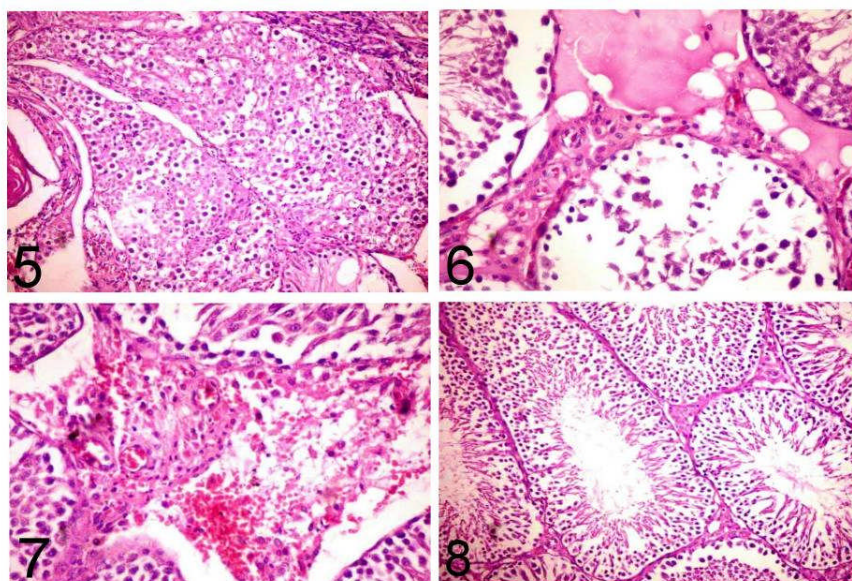


Fig. (5): Testis of rat treated with high dose of Cd showing marked degeneration and vacuolization of seminiferous epithelium with pyknosis of their nuclei. (H & E X 200).

Fig. (6): Testis of rat treated with high dose of Cd showing necrosis of germ cells with complete absence of spermatozoa as well as interstitial oedema. (H & E X 200).

Fig. (7): Testis of rat treated with high dose of Cd showing interstitial haemorrhage and necrosis of leydig cells. (H & E X 200).

Fig. (8): Testis of control, untreated rat showing no histopathological changes. (H & E X 200).

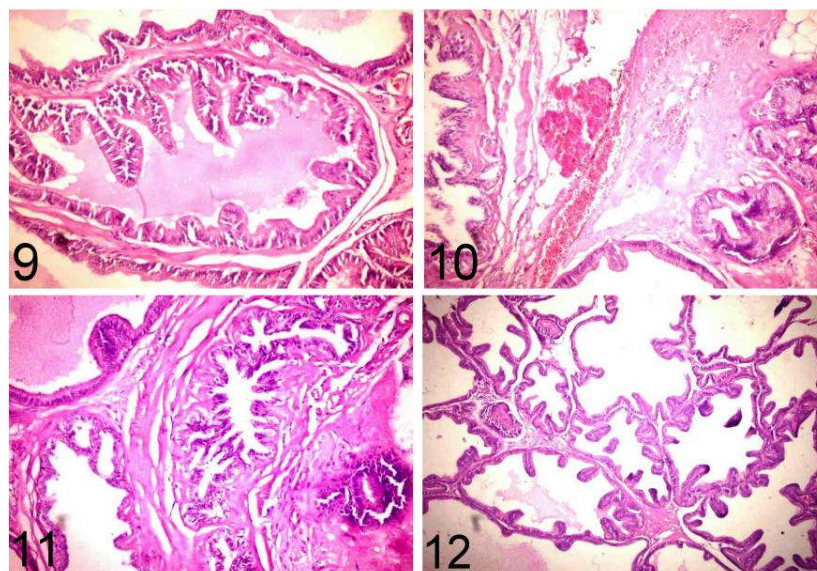


Fig. (9): Prostate gland of rat treated with low dose of Cd showing slight hyperplasia of epithelial lining prostatic acini. (H & E X 200).

Fig. (10): Prostate gland of rat treated with high dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).

Fig. (11): Prostate gland of rat treated with high dose of Cd showing interstitial fibrous connective tissue proliferation. (H & E X 100).

Fig. (12): Prostate gland of control, untreated rat showing no histopathological changes. (H & E X 100).

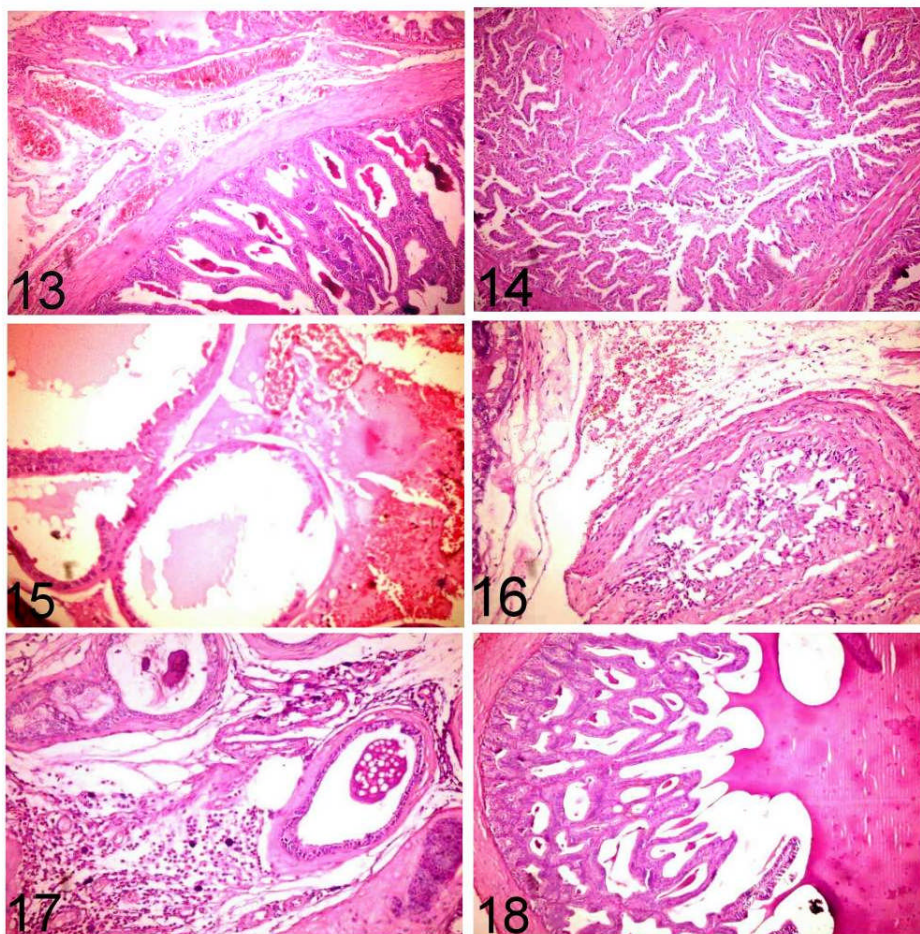


Fig. (13): Seminal vesicle of rat treated with low dose of Cd showing congestion of all blood vessels. (H & E X 100).

Fig. (14): Seminal vesicle of rat treated with low dose of Cd showing hyperplasia of epithelium lining. (H & E X 100).

Fig. (15): Seminal vesicle of rat treated with low dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).

Fig. (16): Seminal vesicle of rat treated with high dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).

Fig. (17): Seminal vesicle of rat treated with high dose of Cd showing interstitial leucocytic cells infiltration. (H & E X 100).

Fig. (18): Seminal vesicle of control, untreated rat showing no histopathological changes. (H & E X 100).

4- Discussion:

In this study, effects of exposure of adult male rat to CdCl₂ administered in drinking water on parameters of sexual behaviour, aggressive behaviour as well as anxiety and fear were investigated. Our results revealed that, male rat sexual behaviour was suppressed after the ingestion of CdCl₂ solution as evidenced by prolongation of the latency to first mount and reduce the number of mounts and ejaculations. Moreover marked increase in the latency to first ejaculation and post – ejaculatory interval in

the Cd treated groups. These data were in agreement with John *et al* (1994) who suggested that exposure of male rats to cadmium is associated with alterations in sexual functions (copulatory and erectile dysfunction) .

The other main finding, is that oral administration of CdCl₂ markedly abolished territorial aggressive behaviour in adult male rats namely, a suppression in boxing bouts, fight with stud and lateralization.

The results suggest that sexual and aggressive behaviour are very susceptible to the toxicity produced by CdCl₂ and could be explained by the direct or indirect effect of cadmium on the testes and the influence on androgen biosynthesis. As testosterone plays a key role in sexual arousability as well as cause of aggressive behaviour in males and this in turn is equated with violence (Kathrin, 2001). Our data presented in this work strongly indicates a serious decrease in the level of testosterone in the exposed groups of male rats. This might be regarded to the degenerative testicular changes exerts in the testicular tissue as showed in histopathological examination in the present study, suppression of testicular enzymes and/or decrease in leutenizing hormone (LH) secretion by the pituitary gland. In this respect, 17- α -hydroxylase and 17-20 lyase enzymes are responsible for normal testicular steroidogenesis. Their function is controlled by cytochrome P-450 which is represented in high concentration in the leydig cells and is highly affected by Cd toxicity (Maines 1984).

Concerning, LH and steroidogenesis Ellis and Desjardins (1982) and Fatma *et al.* (2009) reported that LH acts upon the leydig cells of the testis and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis. Cd administration significantly increased nitric oxide (NO) production (Waisberg *et al.*, 2003) leading to decrease in testosterone synthesis in the leydig cells through acting centrally on the pituitary gland and inhibiting LH secretion (Dobashi *et al.*, 2001) . Our findings are in accordance with previous reports from Piasek and Laskey, (1994) that have demonstrated lowering in steroidogenesis in Cd treated female rats. Further evidence derived from a study for Murugesan *et al.*, (2007) where poor pituitary LH secretion with reduced leydig cell steroidogenesis was reported in highly contaminated environment. Here, the marked inhibition of sexual behaviour in Cd treated male rats, might be attributable to the reduction of testosterone level .Corresponding to the results of Gunn *et al.* (1970) , where testosterone treatment can prevent the inhibitory effect of cadmium on mating behaviour six to nine days after cadmium injection .

As testosterone is important in sexual arousability, also serotonin (5-HT) is involved in copulatory behaviour and ejaculation. In the study of Rastogi *et al.*, (1977) and Antonio *et al.*, (1988) , significant increase in serotonin(5-HT) levels in pup brains after cadmium exposure was reported. Fernandez *et al.* (1992) noted impairment of sexual behaviour by 5- HT microinjection into the Medial Preoptic Area (MPOA) by a large dose. Additionally,

Mas *et al.* (1995) reported that 5- HT is released more laterally in the preoptic area (POA) , after ejaculation , that these high levels of 5- HT may lead to the sexual quiescence that follows ejaculation .Similar results reported by Rosen *et al.*, (1999) where serotonin(5-HT) was regarded as inhibitory to male sexual behaviour.

Contrary to our findings, the reduced level of serotonin (5-HT) was detected by high cadmium intake in pups exposed to cadmium via the milk from exposed dams (Kierstin, 2003). This contradiction in CdCl₂ effect on serotonin might be due to the level of exposure of cadmium, timing in age at exposure and individual sensitivity to the chemicals.

Testosterone and serotonin have been the two most researched chemical messengers with regards to aggression (Marco *et al.*, 2005). Here, marked impairment in aggression was a significant remark for CdCl₂ group and this could be attributed to the decline in testosterone level. There are numerous reports about testosterone and aggressive behaviour .Bermond *et al.* (1982) showed that the intraspecific aggressiveness in male rats in the same cage is influenced by testosterone and is reduced by castration. Additionally, Marco *et al.* (2005) reported that inter- male aggression in rats, mice, monkey and man is controlled by testosterone. Testosterone induced suppression of DA (dopamine) turnover in the medial preoptic nuclei and anterior hypothalamic nuclei may well be involved in androgen- dependent aggression and copulatory behaviour (Simpkins *et al.*, 1983).

The brain chemical serotonin has long been known to play an important role in regulating anger and aggression. In this study, rats exposed to CdCl₂ showed a significant elevation in serotonin and a concomitant impairment in territorial aggression. According to the serotonin (5-HT) deficiency hypothesis of aggression, highly aggressive individuals are characterized by low serotonin (Caramaschi *et al.*, 2007). Further studies revealed that deficit in serotonin activity correlates with impulsive and aggressive behaviour (Moeller *et al.*, 1996 and Pier *et al.*, 2005).

The results of this study, show a significant decrease in the relative sex organs weight (testes, seminal vesicles, and prostate gland) in CdCl₂ treated rats. Barbara *et al.* ,(2008) recoded that rats treated with higher doses of cadmium for 12- 15 months showed a marked reduction in absolute weight of testes and impairment of seminiferous tubules . Moreover , the wide array of abnormalities observed when histopathological sections of the testes were examined , provides further evidence for the reduced

sexual activity and reduced fertility of treated male rats. Our histopathological examination revealed that the examined testes of the treated rats showed marked degeneration & vacuolization of seminiferous epithelium, necrosis of germ cells, interstitial hemorrhage and necrosis of leydig cells. Moreover, prostate gland of rats treated with high dose declared hyperplasia of prostatic epithelium, interstitial edema and hemorrhage. In addition to congestion of all blood vessels & hyperplasia of epithelial lining, interstitial edema & edema of seminal vesicles. Among the proposed mechanisms of Cd toxicity on the testes are circulatory failure due to vascular damage and decreased utilization of Zn by spermatogenic cells due to competitive action of cadmium (Amara *et al.*, 2008). There is paucity of information on the effect of cadmium on the lipids of the testes. Consequently, there is a lack of information also on the role of cadmium-induced lipid changes in testicular function. Adaikpoh and Obi (2009) concluded that the mechanism of cadmium toxicity of the testes and prostate may involve elevation of cholesterol levels in these organs.

The present study shows decrease in sperm count, sperm motility, sperm viability and increase in sperm abnormality (detached head and / or coiled tail) in male rats treated with high dose of CdCl₂. These detected reduction and abnormalities in sperm could be the result of reduced spermatogenesis and the histopathological alterations observed in the testes of the treated rats. Similar results reported by Neveen *et al.* (2007), where exposure of adult male mice to CdCl₂ significantly decreased sperm counts, total number of sperms per mg of testis, daily sperm production efficiency. In addition, Bench *et al.* (1999), reported that cadmium has a detrimental effect on testicular function (stages of spermatogenesis) that could result in reduced sperm production leading to reduced male fertility. Cadmium is known as competitor of calcium, which is essential for sperm motility regulation (Beyersmann and Hechtenberg, 1997, Martelli *et al.*, 2006). Further evidence derived from a study for Benoff *et al.* (2008) where aberrant sperm motility was correlated with altered expression of L-type voltage – dependent calcium channel isoforms found on the sperm tail, which regulate calcium and cadmium influx.

Also, the increased oxidative stress resulted from Cd intoxication in testicular tissue might be responsible, at least in part, for poor semen quality, testicular damage and impairment of fertility. Moreover, it has been reported that the oxidative stress affect the sperm cell via interfering with the membrane fluidity which is the main factor for sperm motility and fusion with the oocyte (Kim and Parthasarathy 1998, Aitken, 1995). Toxic effect of Cd

on testes is known to deplete glutathione and protein – bound sulphhydryl groups, which results in enhanced production of reactive oxygen species (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide (Waisberg *et al.*, 2003). In the current study, rats exposed to CdCl₂ showed a significant reduction in the activity of antioxidant enzyme (SOD) and a concomitant enhancement in lipid peroxidation (TBARS), in accordance with earlier reports in cadmium intoxication in rats (El- Missiry and Shalaby, 2000; Turner and Lysiak 2008, Fatma *et al.*, 2009; Kanbura *et al.*, 2009).

Since cadmium classified as neurotoxic substance and generally impairs enzymes involved in the synthesis of neurotransmitters (Murphy, 1997). The serotonergic system is involved in anxiety response (File *et al.*, 2000). In the present work, increasing anxiety was evidenced by increased number of entries in closed arms and the time spent in Cd intoxicated rats. Our results are in line with data of (Bull, 2010) who stated that sub chronic oral exposure to cadmium can cause anxiety and alterations in the biochemical activity of the brain in laboratory animals. Also, Leret *et al.* (2003) recorded that, the intoxicated rats with cadmium and lead acetate, showed an increase on indices of anxiety on the elevated plus – maze. These long – term changes in anxiety – like behaviour can be related to dopaminergic and serotonergic alterations detected in hippocampus. Most of us are probably aware that serotonin plays an important role in depression and anxiety and people with low levels of serotonin are more likely to become depressed. Recent research indicates that the reverse may actually be the true and that people with too much serotonin in certain parts of the brain may develop depression. Evidence derived from a study for Lowry and Hale (2010) where there are subgroups of serotonin neurons that are overactive were observed in depressed patients, rather than underactive. Our results, showed high serotonin level in Cd treated rats in comparison with the control male rats. Similar results reported by Rastogi *et al.* (1977) and Antonio *et al.* (1998), where a significant increase in serotonin (5 – HT) levels in pup brains after pre- and post natal cadmium exposure. The chemical hypothesis of anxiety and depression comes from rat models. As rats have up to 8 times more serotonin in brain regions associated with anxiety disorders and clinical depression – the nucleus accumbens, prefrontal cortex, hippocampus and hypothalamus – than controls (Zangen *et al.*, 1999). Edwards (2005) stated that scientists believe that dysregulation of neurotransmitters such as serotonin, dopamine and gamma aminobutyric acid or GABA, have been implicated in anxiety disorders and phobias. Also, he added that the data on fear and anxiety show dense

serotonin input to the amygdala. In contrast to our results, Kierstin (2003) stated that a marked decrease in serotonin and its metabolite 5-hydroxyindoleacetic acid was observed in cerebral cortex and hippocampus in pups exposed to cadmium via the milk from exposed dams. The contradicting results may be due to differences in experimental design e.g. cadmium dose, timing in age at exposure or measurement of outcome and brain regions investigated.

There is definitive medical and scientific evidence, that testosterone elevations were associated with reduced male anxiety (Jeremy *et al.*, 2002). Cassandra and Cheryl (2007), confirmed that sexual experience is associated with lower levels of anxiety-like behaviour and higher levels of androgen secretion. Rats exposed to CdCl₂ showed higher levels of anxiety-like behaviour associated with lower levels of testosterone. Gonadectomy (GDX) in male rats would increase anxiety-like behaviour, an effect which would be reversed by systemic administration of dihydrotestosterone (DHT). Testosterone reduced anxiety-like behaviour through actions of its 5 α -reduced metabolite, & DHT (dihydrotestosterone) (Cassandra and Cheryl, 2006).

In conclusion, where testes is greatly targeted to damage by cadmium intoxication. Along with evidence derived from our study, where exposure to cadmium constitutes a great threat being associated with reproductive injurious effects. Hence, concern should be directed to limit the inadvertent incorporation of cadmium in human-consumed products.

Correspondence author

Mervat M. Kamel

Department of Veterinary Hygiene and Management
Faculty of Veterinary Medicine, Cairo University,
Cairo, Egypt.

mevy58@yahoo.com

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Vitamin D Receptor Gene Polymorphism and Growth Pattern in Egyptian Rachitic ChildrenSomaia Ismail¹; Moushira Erfan^{2*}; Manal Abd EL-Salam³; Sanaa Kamal²; Soheir Ibrahim³; and Hala Nasr¹¹Medical Molecular Genetics Department, Human Genetics Research Division, National Research Centre (NRC)²Biological anthropology Department, Medical Research Division, National Research Centre (NRC)³Pediatric Department, Faculty of medicine (for girls) AL-Azhar university*moushiraz@yahoo.com

Abstract: Nutritional rickets may be caused by either calcium or vitamin D deficiency. Vitamin D affects skeletal metabolism indirectly. The association between Vitamin D receptor (VDR) gene polymorphisms and genetic and environmental factors plays a role in the majority of cases. Several studies reported association between rickets and VDR gene polymorphism and growth parameters. Vitamin D affects skeletal metabolism by regulating calcium and phosphate homeostasis. The aim of this study was to examine the association between VDR gene polymorphism and vitamin D deficiency in Egyptian children with rickets and assess the relationship between the VDR gene polymorphisms and growth parameters. The study included 42 (16 girls and 26 boys) patients recruited from AL-Zharaa hospital, AL-Azhar University. Their age ranged from 4-36 months. Forty eight healthy individuals matched in age and sex with patients was recruited for comparison. VDR gene *Apal*, *FokI*, and *TaqI* polymorphisms, biochemical and growth parameters were studied. Results showed that the most common VDR genotype was *Ff* among patients and *Aa* among controls, with no significant differences. The allele frequency showed significant increase in the “F” (*FokI*) allele in patients compared to controls (33.3% vs 20.8%, P=0.04). While, there were no significant differences between patients and controls in frequency of *TaqI* and *Apal* alleles. The frequencies of combinations of VDR genotypes for the *FokI*, *Apal*, and *TaqI* polymorphic sites, were significantly different between rachitic and control subjects (p <0.01). The *AaFfTT* genotype was the most frequent one among the rachitic group, while the *AaFfTT* is the most predominant in the control group. The *FF* and *tt* genotypes were associated with reduced SDS of weight and height. This denotes that the VDR polymorphism has functional significance on growth parameters. In conclusions, the study shows that there is a relation between VDR gene polymorphisms and susceptibility to rickets. These results might help in risk assessment of rickets and in predicting response to treatment.

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Keyword: Vitamin D; Receptors; Gene polymorphism; Rickets, Egyptians

1. Introduction:

Nutritional rickets is gaining the attention of public health professionals and clinicians world wide as the disease remains an endemic problem in many developing countries and has re-emerged in a number of developed countries, where it was thought that the disease has been almost eradicated (Thacher *et al.*, 2006). In Egypt, vitamin D deficiency rickets continues to be a public health problem despite abundant sun shine all the year (Pettifor, 2008). Limited sun shine exposure due to more time indoors to watch TV and work on computer or avoiding sun shine internationally for fear of air pollution and skin cancer development have been reported as causes (Chesney, 2001; Hatun *et al.*, 2007). Vitamin D regulates calcium and phosphate homeostasis in the body and has a positive impact of bone mineralization (Holick, 2006). The active form of vitamin D (1, 25-dihydroxyvitamin D3) exerts its effect on the target

tissues through the VDR. Many tissues contain the VDR and thus the active form of vitamin D is expected to affect these tissues and cells like epidermis, macrophages, prostate, pancreas, parathyroid gland ((Fischer *et al.*, 2000; Bora *et al.*, 2008; Valdivielso and Fernadez, 2006; Bikle, 2007).

The emerging field in nutrition science, so called nutritional genomics (nutrigenomics) draws attentions to the fact that certain conditions of diseases may be linked to polymorphisms that individual carry. Presence of certain polymorphisms renders the most susceptible for certain disease even in the presence of recommended intake of offending nutrients whether is so for vitamin D and calcium is not clear, there have been some studies conducted in Africa indicating a possible risk between VDR polymorphisms and rickets (Fischer *et al.*, 2000; Bora *et al.*, 2008).

Vitamin D has direct effects on the skeleton, and the active metabolites regulate differentiation,

proliferation, and migration of osteoblasts and of chondrocytes of the epiphyseal growth plate, cells determining skeletal growth. VDR polymorphism could rather be related to growth and parameters of body constitution (Suarez *et al.*, 1997; Minamitani *et al.*, 1998; Lorentzon *et al.*, 2000).

The vitamin D3 receptor (VDR) is an intracellular hormone receptor, which specifically binds to the active form of vitamin D (1, 25-dihydroxyvitamin D3 or calcitriol). It interacts with target-cell nuclei and produces a variety of biologic effects. The VDR protein is encoded by the VDR gene, which is linked to 12q13.1. VDR gene is about 100 kb, consists of 9 exons and has highly polymorphic sites. Several polymorphisms in the VDR gene have been reported so far, including FokI, TaqI, and ApaI. FokI, which is a translation start codon polymorphism, is located in exon 2, and due to the T to C transition. The other polymorphism, which is localized in exon 9, is TaqI and ATT codon is converted to ATC, but either of them encodes isoleucine amino acid. ApaI is an intronic polymorphism, which is G/T transition, localized in intron 8 (Audi *et al.*, 1999; Uitterlinden *et al.*, 2004). The aim of this study was to examine the association between VDR gene polymorphism and vitamin D deficiency in Egyptian children with rickets and assess the relationship between the VDR gene polymorphism and growth parameters.

2. Patients and Methods:

This study included 42 rachitic children, 26 boys and 16 girls and 48 non rachitic control group, 23 boys and 25 girls matched in sex and age. They were selected from those attending the out patients clinic of AL-Zahraa hospital, AL-Azhar University. Their age was ranged from 4-36 months. A formal consent letter from the parents of each child was obtained after explaining to them the whole procedure. The study was approved by the Ethics Committee of the Hospital. The studied groups; cases and control were subjected to full history taking with special emphasis on the type and quantity of milk fed during infancy, calcium and vitamin D supplements, sun exposure (2 hours of sunlight per week). Clinical examination was performed with special stress on clinical signs of rickets; cranioitabes, rachitic rosary, Harrison groove, delayed closure of fontanels, muscular hypotonia, spinal deformity, pigeon chest or bowed legs. Children with chronic renal, hepatic, malabsorption disorders, congenital bone deformities, hypophosphatemia were excluded. Anthropometric measurements were taken including: weight, height or length, head circumference, waist and hip circumference, and mid arm circumference. The anthropometric measurements and instruments used followed the International Biological Programmer

(IBP) (Tanner *et al.*, 1969). Measurements were taken on the left side of the body. Body mass index (BMI in kg/m²); and relative head circumference (head circumference / height) were calculated. Physical growth was assessed for each child by determining the standard deviation scores of weight, height, BMI, head and mid-upper arm circumference, using the Egyptian growth reference data (Ghalli *et al.*, 2002). We calculated standard deviation score (SDS) independent of sex and age (child measurement minus population mean/population SD). Radiological assessment of rickets in all cases for wrist and ankle was done. Serum calcium, phosphate and alkaline phosphates levels were measured by standard methods. Peripheral venous blood samples were collected on EDTA. Genomic DNA was extracted from peripheral white blood cells using salting out procedure (Miller *et al.*, 1988). DNA was amplified by polymerase chain reaction (PCR) and examined (by specific restriction enzymes) using the restriction fragment length polymorphism (RFLP) technique. The VDR genotype of each subject was identified according to the digestion pattern and alleles according to the presence (f, t, and a) or the absence (F, T, and A) of the FokI, TaqI, and ApaI, restriction enzyme cleavage sites, respectively. Each VDR markers were amplified as following:

FokI polymorphism

Patients and control subjects DNA was amplified by PCR reaction in 25 µl total volume for FokI containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F: 5'-AGC TGG CCC TGG CAC TGACTC GCT CT-3' and R: 5'- ATG GAA ACA CCT TGC TTC TCC CTC-3', 1.5 mM MgCl₂, 0.5u taq polymerase (fenzyme), and using 50-100 ng of DNA as template. The temperature sittings were as follows; five min at 94°C, followed by 35 cycles of 95°C for 60 sec, 68°C for 60 sec and 72 °C for 2 min followed by 72 °C for 7 min as a final extension step.

The PCR product was electrophoresed on 2 % agarose gel stained with ethidium bromide. PCR product 265 bp were visualized on UV transilluminator with using molecular weight marker to determine the quality of PCR products. Then conduct 10 µl of PCR product to 1 unite restriction enzyme (FastDigest Fok-I, Fermentas) at 37 °C for 15 min, followed by 65 °C for 3 min for digestion. After digestion were loaded the products on 2% agarose gel stained with ethidium bromide to identify the digestion pattern. The FF genotype, homozygote of common allele its meaning absence of restriction site and showed one band at 265 bp. The ff genotype (homozygote of infrequent allele) generated two fragments at 196 bp and 69 bp. Presence of three

fragments at 265 bp, 196 bp and 69 bp was appearance as Ff.

TaqI polymorphism

The PCR cycle conditions were initially denaturalized at 94 °C for 4 min, followed by 35 cycles at 94 °C for 60 sec, 68 °C for 60 sec and 72 °C for 2 min followed by 72 °C for 7 min as a final extension step. In total volume 25 µl were containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F;5'- CAG AGC ATG GAC AGG GAG CAA-3' and R: 5'-CAC TTC GAG CAC AAG GGG CGT TAG C-3', 1.5 mM MgCl₂, 0.5u taq polymerase (fenzyme), and using 50-100 ng of DNA as template. The PCR product was electrophoresed on 2 % agarose gel stained with ethidium bromide. PCR product 600 bp were visualized on UV transilluminator with using molecular weight marker to determine the quality of PCR product.

The PCR product was digested with the restriction enzyme Taq-I (fastDigest-Taq-I, fermentas) 10 unit for 15 min at 37 °C and followed by 65 °C for 3 min according to manufacturer's instructions. The digested samples were added to loading dye and size fractionated by electrophoresis in a 1.5 % agarose gel. Visualization after ethidium bromide staining was accomplished by UV transilluminator. Taq-I digestion revealed one obligatory restriction site, the homozygous TT (absence of the specific Taq-I restriction site) yielded bands of 500 bp and 210 bp. The homozygous tt exhibited 210 bp and the heterozygous Tt 290 bp fragments.

ApaI polymorphism

The PCR cycle conditions were initially denaturalized at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 sec, 65 °C for 30 sec, and 72 °C for 2 min and final extension at 72 °C for 4 min. In total volume 25 µl were containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F:5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3' and R: 5'-CAC TTC GAG CAC AAG GGG CGT TAG C-3', 1.5 mM MgCl₂, 0.5u taq polymerase (Fenzyme), and using 50-100 ng of DNA as template. The PCR product was electrophoresed on 1.5% agarose gel stained with ethidium bromide to check the quality of reaction. The amplified 2000 bp PCR product was subjected to Apa-I restriction enzyme (FastDigest, Fermentas) for digestion. 10 µl of PCR product was digested with 10 units of Apa-I restriction enzyme in a 20 µl total volume using green buffer at 37 °C for 15 min and followed by 65 °C for 3 min. The Apa-I enzyme digested product was loaded on a 1.5% agarose gel stained with ethidium bromide. The 2000 bp was digested as a common allele A (wild type) and presence of restriction site resulting in 1700 bp and 300 bp was assigned as infrequent allele

(mutant allele). Genotypes were exhibited as homozygote's for common allele AA and homozygotes for mutant allele aa. Presence of 2000 bp, 1700 bp and 300 bp fragments was exhibited as heterozygotes Aa.

Statistical Analysis

Statistical presentation and analysis of the results were carried out using SPSS software version 11. Statistical tests used included chi-square test, student's t test, analysis of variance, and tukey tests. Correlations were tested between VDR gene polymorphisms, growth pattern and biochemical markers of vitamin D deficiency rickets.

3. Results:

The present study was performed on 42 patients with rickets and 48 healthy individuals, with the mean age 11.9±3.3 months and 15.3±3.3, respectively. DNA was obtained from both groups for identification of their VDR genotypes and allelic frequency. The Hardy- Weinberg equilibrium was satisfied to verify the allelic frequency. Table 1 and Fig.1 show the distribution of each allele in patients and controls. It was *F* (66.7 %), *f* (33.3%), *T* (59.1%), *t* (40.9%), *A* (61.3%) and *a* (38.8%) in patients and it was *F* (79.2 %), *f* (20.8%), *T* (59.3%), *t* (40.7%), *A* (59.7%) and *a* (40.3%) in controls. The most common VDR genotypes were *Ff* (47.6%), *Tt* (45.5%), and *Aa* (60%) among patients and *FF* (52.08%), *Tt* (41.8%), and *Aa* (67.7%) among controls, with no statistical significant differences. The allele frequency showed significant increase in the “*f*” (*FokI*) allele in patients compared to controls (33.3% vs 20.8%, *P* < 0.04). While, there were no significant differences in frequencies of *TaqI* and *ApaI* alleles between patients and controls.

Table 2 shows the frequency distribution of combinations of VDR genotypes for the *Fok I*, *Apa I*, and *Taq I* polymorphic sites in patients and controls. Statistical analysis shows significant difference between the rachitic and control subjects (*p* < 0.015). In the rachitic patients, the *AaFfTT* genotype is the most frequent (23.3%), followed by the *AAFFTt* genotype (13.3%). Regarding the control group *AaFfTT* is the most predominant (40%). Tables 3 and 4 show the associations of VDR *Apa I*, and *Taq I* and *FokI* genotypes with biochemical parameters, in patients and controls, respectively. Table 3 shows that patients with the *FF* genotype had significant decrease in serum phosphate compared to patients with the *ff* genotype (*P* < 0.05). Also, patients with *tt* genotype had significant decrease in serum phosphate compared to patients with *TT* genotype (*P* < 0.05). There were no significant differences between VDR genotypes in control group (Table 4).

Table 5 shows the mean SDS of growth parameters in rachitic children by vitamin D receptor (VDR) genotypes. The values of SDS for the weight, height, BMI, as well as mid-upper arm circumference (MUAC) lied at the lower limits of reference Egyptian growth data for all VDR genotypes with no statistical significant differences. However, the delay of weight SDS and height SDS is more pronounced in FF genotype compared to other genotypes in the rachitic children. Also, the SDS of weight and SDS of height in patients with tt genotype were delayed, with statistical significant difference when compared to other genotypes in the rachitic children (Fig.2&3). Head circumference measurements show normal SDS values.

The amplified products of the VDR gene are shown in Fig. 4. Panel A shows Fok-I digestion. Absence of Fok-I restriction site 265 bp was assigned as the common allele F and the genotype was considered as homozygous FF as in lane (2&4). The presence of Fok-I restriction site 196 bp and 69 bp was assigned as mutant allele f and the genotype was homozygous

ff as in lane 1. Presence of 265, 196 and 69 bp indicated that genotype is heterozygous Ff as in lane 3.

Panel B shows Apa-I digestion of the amplified products of the VDR gene. Absence of Apa-I restriction site 2000 bp was assigned as the common allele A and the genotype was considered as homozygous AA as in lane (2). The presence of Apa-I restriction site 1700 bp and 300 bp was assigned as mutant allele a and the genotype was homozygous aa as in lane 1. Presence of 2000, 1700 and 300 bp indicated that genotype is heterozygous Aa as I lane 3(data not shown).

Panel C shows Taq-I digestion of the amplified products of the VDR gene. Absence of Taq-I restriction site 520 bp was assigned as the common allele T and the genotype was considered as homozygous TT as in lane 3. The presence of Taq-I restriction site 320 bp and 200 bp was assigned as mutant allele t and the genotype was homozygous tt as in lane 2. Presence of 520, 320 and 200 bp indicated that genotype is heterozygous Tt as in lane1.

Table 1. VDR genotype Distribution & Allelic frequency in patients and control

<i>Group</i>	<i>VDR Genotypes (%)</i>			<i>P value</i>	<i>Allele frequency (%)</i>		<i>P value</i>
	<i>FF</i>	<i>Ff</i>	<i>ff</i>		<i>F</i>	<i>f</i>	
Rachitic children	42.8%	47.6%	9.5%	0.16	66.7%	33.3%	0.04
Normal children	52.1%	22.9%	6.2%		79.2%	20.8%	
Rachitic children	36.4%	45.5%	18.2%	0.93	59.1%	40.9%	0.55
Normal children	37.2%	41.8%	20.9%		9.3%	40.7%	
Rachitic children	30%	60%	10%	0.76	61.3%	38.8%	0.49
Normal children	25.8%	67.7%	6.5%		59.7%	40.3%	

Table 2. Distribution of VDR genotypes among patients and control

VDR genotype	Cases (%)	Control (%)	Total (%)	P value
Homozygous			2%	.015
AAFFtt	3.3%			
aaffTT	3.3%		2%	
aaFFTT		5%	2%	
AAffTT	3.3%		2%	
Heterozygous			6%	
aaFFTt	6.6%	5%		
AaffTT		5%	2%	
AaFftt		15%	6%	
AaffTT		5%	2%	
AaFFtT	13.3%		8%	
AaFFTT	23.3%		14%	
AaFFtt	10%		6%	
AaFFTt	10%	20%	14%	
AaFFTT	6.6%	40%	20%	
AAffTt	3.3%		2%	
AAFFtT	3.3%		2%	
AAFFTt	13.3%	10%	12%	

Table 3. Biochemical parameters of children with rickets by VDR genotypes

VDR genotype	Calcium (mg/dl) Mean±SD	Phosphate (mg/dl) Mean±SD	Alk.Ph (IU/liter) Mean±SD
ApaI aa	8.6 ± 0.49	3.57 ± 1.24	730.25 ± 63.69
	7.72 ± 1.12	3.66 ± 0.96	627.0 ± 33.85
	8.29 ± 1.17	4.03 ± 1.11	499.0 ± 243.18
FokI ff	8.17 ± 1.05	4.17 ± 1.19	574.5 ± 219.72
	7.95 ± 1.12	4.16 ± 1.26	518.85 ± 355.53
	8.10 ± 1.29	3.34 ± 0.67*	653.77 ± 346.95
TaqI tt	7.65 ± 2.03	2.83 ± 0.18*	284.35 ± 116.08
	8.07 ± 1.01	3.86 ± 1.20	587.06 ± 382.57
	8.08 ± 0.95	4.19 ± 1.13	468.08 ± 296.84

* P < .05

Table 4. Biochemical parameters of normal children by VDR genotypes

VDR genotype	Calcium (mg/dl) Mean±SD	Phosphate (mg/dl) Mean±SD	Alk.Ph (IU/liter) Mean±SD
ApaI aa	8.6 ± 0.56	5.0 ± 0.56	131.0 ± 12.72
	8.92 ± 0.83	4.8 ± 0.94	210.75 ± 45.45
	9.13 ± 0.53	4.56 ± 0.90	195.37 ± 123.35
FokI ff	9.0 ± 0.29	4.00 ± 0.46	272.0 ± 0.54
	8.60 ± 0.38	4.87 ± 0.69	163.71 ± 59.03
	9.26 ± 0.49	4.65 ± 0.82	194.00 ± 107.27
TaqI tt	9.17 ± 1.09	3.80 ± 0.54	268.75 ± 154.57
	8.87 ± 0.29	5.01 ± 0.46	148.44 ± 35.29
	9.12 ± 0.25	4.57 ± 0.67	180.75 ± 65.09

Table 5. The mean SDS of growth parameters in rachitic children by VDR genotypes

<i>VDR genotype</i>		<i>Weight SDS Mean ± SD</i>	<i>Height SDS Mean ± SD</i>	<i>BMI SDS Mean ± SD</i>	<i>HC SDS Mean ± SD</i>	<i>M UA SDS Mean ± SD</i>	<i>HC /Ht SDS Mean ± SD</i>
ApaI	aa	-1.54 ±1.29	-1.12±1.20	-1.31±0.76	0.62±1.8	-0.85±1.8	1.81±1.20
	Aa	-1.56±0.43	-1.11±0.97	-1.32±0.98	0.64±1.29	-0.96±0.58	1.83±1.37
	AA	-1.15±1.29	-1.12±1.20	-0.62±1.76	-0.52±1.8	-0.25±1.8	1.05±1.24
FokI	ff	-0.93±0.78	-0.51±0.75	-0.53±1.80	0.8±0.97	-0.52±0.99	1.31±0.87
	Ff	-0.94±0.73	-0.52±0.79	-0.67±1.02	0.7±1.27	-0.18±0.99	1.32±1.65
	FF	-1.92±0.78	-1.86±0.75*	-1.19±1.80	-0.6±0.97	-1.23±0.88	1.70±0.87
TaqI	tt	-1.96±0.01	-2.38±0.70*	-1.66±0.01	-0.22±0.01	-0.79±0.01	2.22±0.01
	Tt	-1.17±1.28	-1.24±1.17	-0.59±1.77	-0.05±1.93	-0.67±0.99	1.55±1.89
	TT	-1.02±0.28	-0.32±0.95	-0.37±0.34	0.68±0.15	-1.19±0.79	1.03±1.17

* P< .05

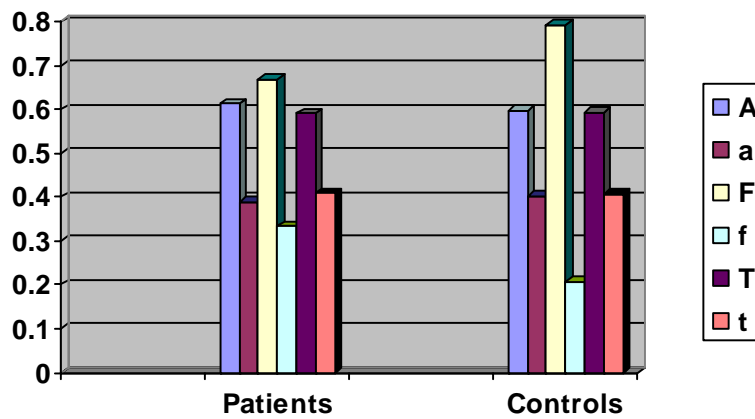


Fig.1. VDR Allelic Frequencies of Patients and Controls

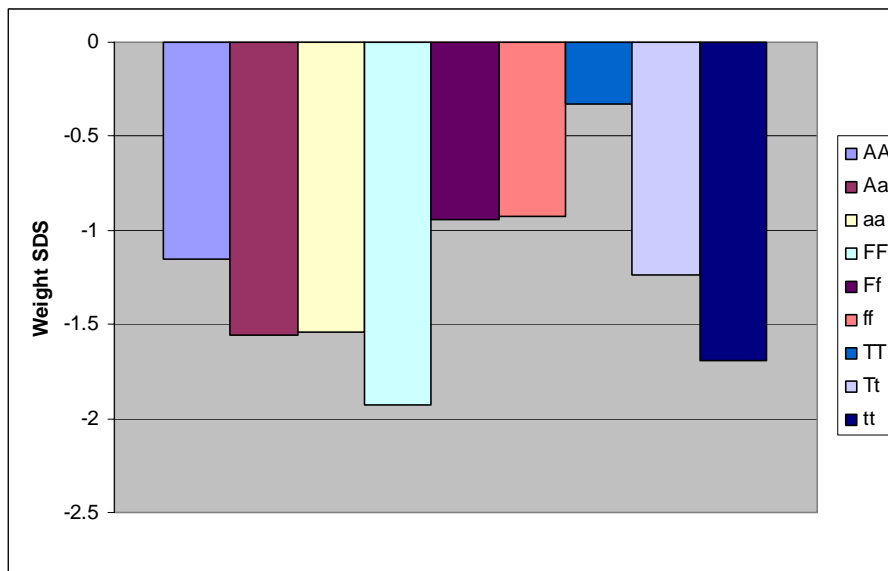


Fig.2. Mean SDS of weight in rachitic children by vitamin D receptor (VDR) genotypes

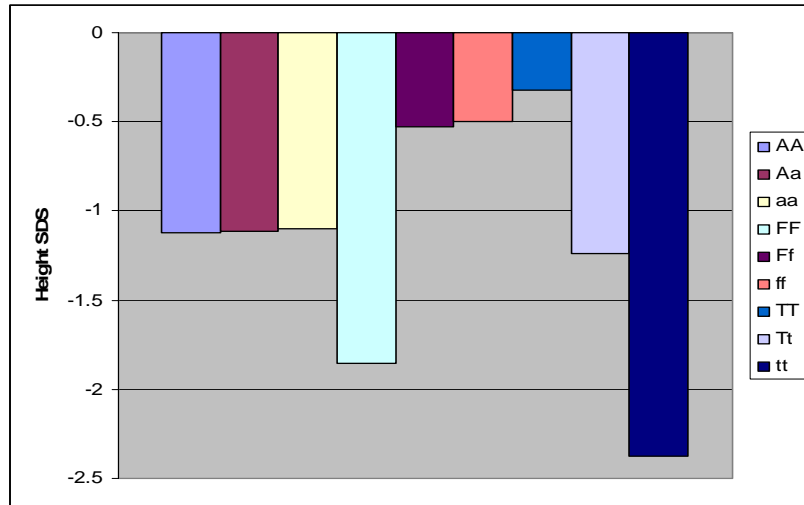


Fig.3. Mean SDS of height in rachitic children by VDR genotypes

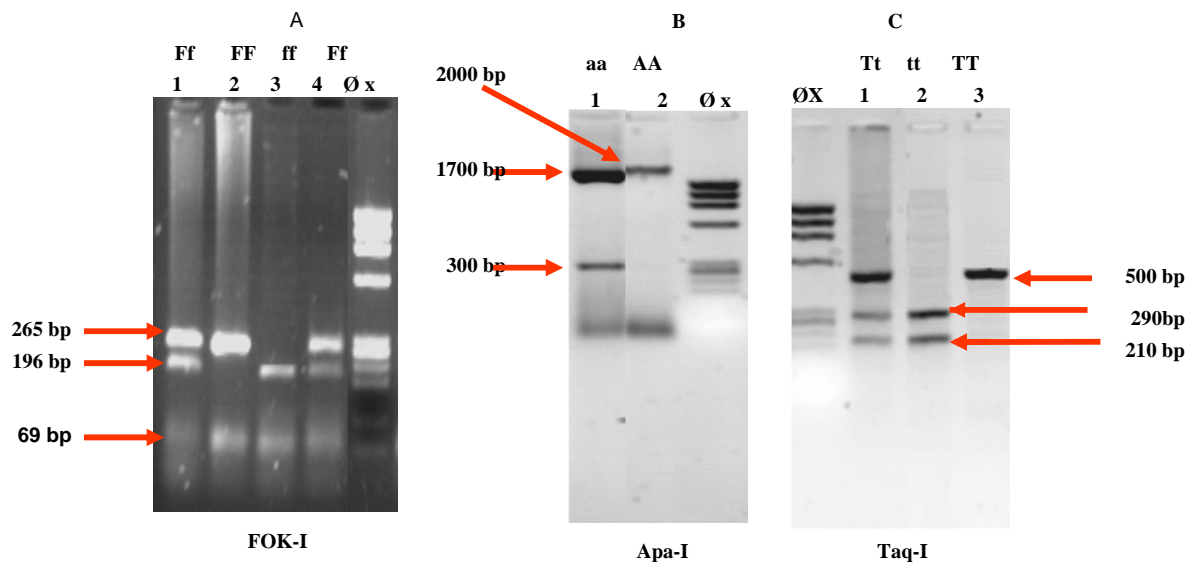


Fig. 4. PCR-RFLP analysis of the 3 VDR polymorphisms

Panel A Shows Fok-I digestion of the amplified products of the VDR gene. Absence of Fok-I restriction site 265 bp was assigned as the common allele F and the genotype was considered as homozygous FF as in lane (2&4). The presence of Fok-I restriction site 196 bp and 69 bp was assigned as mutant allele f and the genotype was homozygous ff as in lane 1. Presence of 265, 196 and 69 bp indicated that genotype is heterozygous Ff as in lane 3.

Panel B Shows Apa-I digestion of the amplified products of the VDR gene. Absence of Apa-I restriction site 2000 bp was assigned as the common allele A and the genotype was considered as homozygous AA as in lane (2). The presence of Apa-I restriction site 1700 bp and 300 bp was assigned as mutant allele a and the genotype was homozygous aa as in lane 1. Presence of 2000, 1700 and 300 bp indicated that genotype is heterozygous Aa as I lane 3(data not shown).

Panel C Shows Taq-I digestion of the amplified products of the VDR gene. Absence of Taq-I restriction site 520 bp was assigned as the common allele T and the genotype was considered as homozygous TT as in lane 3. The presence of Taq-I restriction site 320 bp and 200 bp was assigned as mutant allele t and the genotype was homozygous tt as in lane 2. Presence of 520, 320 and 200 bp indicated that genotype is heterozygous Tt as in lane1.

4. Discussion:

Nutritional rickets may be caused by either vitamin D or calcium deficiency. Genetic and environmental factors play a role in the majority of cases (Baroncelli *et al.*, 2008; Pettifor, 2008). The association between vitamin D receptor (VDR) polymorphisms and several diseases in different populations has been investigated (Park *et al.*, 1999; Ozaki *et al.*, 2000). The VDR gene polymorphism has been widely used as a genetic marker for diseases related to calcium metabolism. Several polymorphisms in the VDR gene which are able to alter the activity of VDR proteins have been described (Filus *et al.*, 2008). Expression and nuclear activation of the VDR are necessary for the effects of vitamin D. Several genetic variations have been identified in the VDR (Valdivielso and Fernandez; 2006). The VDR gene affects the activity of the receptor and subsequent downstream vitamin D mediated effects (Gao *et al.*, 2010).

The most common genotype frequency in the present study in the Egyptian control group is *Aa* (67.7%) but this percentage is higher or nearly similar to other populations such as France 50 % (Garnero *et al.* (1995), Mexican, California 55%(Sainz *et al.*, 1997), Indian 44% (Bid *et al.*, 2005), black Pennsylvanian 46%(Zmuda *et al.*, 1997) and Chinas 36%(Kung *et al.*; 1998). This may be due to the difference in the ethnic background of the population being studied. *Apal* polymorphism is localized in 3' regulatory region and is in linkage disequilibrium with 3'UTR. It is an intronic polymorphism, affecting neither splicing site nor transcription factor binding site. In this study we demonstrated that the frequencies of "A" allele and *AA* genotype were increased and *Aa* genotype was decreased in rachitic children compared to controls. This is in agreement with the study done by Bora *et al.* (2008) in the East of Turkey on vitamin D deficient rickets. However, the study of Wei-Ping *et al.* (2005) on Chinese children reported that the distribution of *Apal* polymorphism was balanced between rickets and controls. Likewise Kaneko *et al.* (2007) indicated that VDR polymorphisms among cases did not differ significantly from those of controls in Mongolia. Although these polymorphisms seem to be non-functional; they can be used as a marker to detect a functional allele due to the linkage disequilibrium. The 3'UTR region of the VDR gene is involved in the regulation of gene expression, so these polymorphisms may play an important role in mRNA stability. It may be normal to find the different allelic frequencies among patients, due to the different ethnic background of the patients in different countries (Bora *et al.*, 2008).

FokI is an exonic polymorphism, which leads to T/C transition, and variant alleles generate two VDR gene products that differ in length by three amino acids (Ferrari *et al.*, 1998; Gross *et al.*, 1998). Many Studies have been performed to determine whether there is a difference in functionality between these receptor variants. Arai *et al.*, (1997) concluded that the *F* allele (short form) functioned better than the *f* allele (long form) in transactivation assays using a transfected vitamin D responsive element (VDRE)-reporter gene construct. Also, Remus *et al.*,(1998)and Jurutka *et al.*, (1998) reported that the *F* allele (producing a shorter VDR) has higher transactivation activity, possibly because of better ability to dimerize with retinoid X receptors and bind to transcription factor II B (TFIIB), a coactivator of vitamin D transactivation. In contrast, Gross *et al.*, (1998) were unable to detect a difference in VDR affinity or abundance, messenger RNA (mRNA) stability, or transactivation activity between *ff* and *FF* cells.

In the present study, no significant difference was found in VDR genotypes (FokI) in patients against controls. This is in agreement with the study done by Baroncelli *et al.*, (2008) on Turkish and Egyptian rachitic children. Lu *et al.*, (2003) studied the VDR gene (FokI) in Chinese rachitic and control subjects and they found a significant difference in the frequency distribution of VDR genotypes (FokI) between the two groups and in *F* allele frequency. Our study showed significant increase in *f* allele frequency of FokI polymorphism in patients, however there was no significant difference in the frequency distribution of VDR genotypes (FokI). The *F* allele confers a transcriptionally somewhat more efficient VDR (Arai *et al.*; 1997), and its decreased prevalence in patients suggests that it may increase predisposition to rickets in children. Fischer *et al.*, (2000) studied the VDR genotypes in Nigerian Children and found that "*F*" allele was more abundant in rickets subjects. In Turkey, the frequency of the *F* allele was increased and that of the *f* allele was decreased in patients against controls (Baroncelli *et al.*; 2008). The frequency of the *Ff* genotype in the present control group (22.9%) is lower than other populations such as France 47 % (Correa *et al.*, 1999), Mexican California 48 % (Gross *et al.*, 1996), Indian 49% (Bid *et al.*, 2005) and Japanese 51% (Minamitani *et al.*, 1998).

This result may be explained by the small number of subjects in this study or it might be because of the differences in ethnic backgrounds.

The current study demonstrated no significant difference in allele and genotype frequencies of TaqI among rickets and controls. Likewise, Fischer *et al.*, (2000) and Kaneko *et al.*, (2007) reported that neither allele nor genotype frequencies of TaqI were

significantly different between rickets and controls in Nigeria and Mongolia populations, respectively. In contrast, Bora et al., (2008) found a significant increase in *TT* and *tt* genotypes and decrease in *Tt* genotypes in Turkish rachitic children. *TaqI* polymorphism is localized in 3' regulatory region and is in linkage disequilibrium with 3'UTR. *TaqI* is exonic polymorphism that does not affect the amino acid sequence of encoded protein. Although these polymorphisms seem to be non functional, they can be used as a marker to detect a functional allele due to the linkage disequilibrium. The 3'UTR region of the *VDR* gene is involved in the regulation of gene expression, so these polymorphisms may play an important role in mRNA stability. Frequencies of combinations of genotypes at different sites were not significantly different between rachitic and community subjects in Nigerian (Fischer et al; 2000). In contrast our findings showed that the frequencies of combinations of *VDR* genotypes for the *FokI*, *ApaI*, and *TaqI* polymorphic sites in both groups, were significantly different between rachitic and control subjects ($p < 0.015$). Uitterlinden et al., (2004) reported that it is possible that different allelic frequencies and *VDR* genotypes among populations can occur due to the gene-gene and gene-environment interactions.

In the present study, the most common *VDR* genotypes were *Ff* among patients and *FF* among controls. These findings support the evidence that *FF* genotype is advantageous for good bone mineralization and the prevention of rickets (Arai et al.; 1997 and Remus et al.; 1998). Children with the *FF* genotype had increased intestinal calcium absorption and increased bone mineral density compared with *Ff* heterozygotes and *ff* homozygotes. In healthy adolescents greater calcium absorption was found in *FF* homozygotes, compared with those of *ff* homozygotes and *Ff* heterozygotes (Ames et al., 1999); however, the positive effect of the *FF* genotype is limited whether dietary calcium is severely restricted (Abrams et al., 2005). Baroncelli et al., (2008) suggested that it is the interaction of *VDR* polymorphism with reduced calcium intake and vitamin D status that could determine the individual susceptibility to developing rickets in Egyptian patients.

The polymorphisms in the *VDR* gene might cause mild defects in *VDR* function and cause rickets (Malloy et al., 1999). The data of Suarez et al., (1997) and Minamitani et al., (1998) indicated that *VDR* polymorphism could be related to parameters of body growth. In the present study, the values of SDS for the weight, height, BMI, and MUAC among the studied rachitic group showed values that lies at the extreme lower ends of the reference Egyptian growth data

(Ghalli et al., 2002). This is in agreement with the study of Robinson et al., (2004) on rachitic Australian children, who reported that rickets has a negative impact on growth and the cases presenting with nutritional rickets had a lower weight SDS. Bora et al., (2008) recorded that, the Turkish vitamin D deficient rickets patients are at risk of growth retardation. Vitamin D is likely to regulate growth via effects on bone size (Lorentzon et al., 2000). It is critically important for the development, growth, and maintenance of a healthy skeleton throughout life (Holick, 2003). It affects skeletal metabolism indirectly via regulating calcium and phosphate homeostasis through stimulation of intestinal absorption of these ions (Bouillon et al., 1995).

The weight SDS and height SDS among *VDR* genotypes for polymorphisms in *ApaI* were nearly of the same values in our patients. As regards the *FokI* genotypes the delay is more pronounced in the rachitic children with *FF* genotype. These results explain that growth delay in rickets is more influenced by *FokI*. Studies on polymorphism in the *VDR* gene suggested a role in skeletal mineralization, with the restriction fragment length polymorphism *Ff*, as defined by the endonuclease *FokI*, conferring a greater transcriptional *VDR* activity for the *F* than the *f* allele (Arai et al., 1997; Thakkinstian et al.; 2004).

In the present study patients with the *FF* genotype had significant decrease in serum phosphorus, compared to patients with the *ff* genotype. Also, patients with the *FF* genotype showed pronounced delay in height as compared to the other genotypes with statistical significant difference ($P < 0.05$). Likewise, Lu et al., (2003) reported that *FF* genotypes were more common in patients suffering from vitamin D deficient rickets. This denotes that the polymorphic variation at the *FokI* *VDR* locus has functional significance. It has also been observed that there is a relationship between growth and *FokI* *VDR* polymorphisms in a population (Minamitani et al, 1998; Tao et al., 1998).

Tao et al., 1998 reported that Girls with genotype *TT* were heavier and taller than those with *tt*. This is in agreement with our study on the weight SDS and height SDS among *VDR* genotypes for polymorphisms in *TaqI*. We found that the patients with rickets with *tt* genotype were shorter than patients with *TT* with a statistical significant difference. In England, Keen et al., (1997) reported a significant association between female infant weight and a *TaqI* polymorphism within the *VDR* gene. They concluded that *FokI* and *TaqI* have a determinant effect on bone mineral metabolism and growth in rickets and the frequency of *VDR* polymorphisms in the rachitic children may determine growth delay in rickets.

Mutations affecting genes implicated in vitamin D metabolism or vitamin D receptor (VDR) functions are responsible for severe alterations in skeletal growth. These polymorphisms appear to be associated unequivocally with biochemical variables of calcium and phosphate metabolism polymorphisms and might cause mild defects in VDR function (Lu et al.; 2003). We therefore postulated that VDR polymorphisms might predict susceptibility to develop rickets in Egyptian children

The findings of the present study indicate that the growth in rachitic children may be regulated by mechanisms that are mediated through vitamin D and its receptor. The results reinforce the suggestion that VDR polymorphisms may play an important role in parameters of phospho-calcium metabolism and growth in rickets. This might help in risk assessment of rickets and in predicting response to treatment. Moreover, if a relationship could be established between certain polymorphisms and vitamin D deficient rickets personalized dietetic approach of nutrigenomics, will be applied to carriers of these particular polymorphisms and might be supplemented with more than the recommended daily dose to prevent the development of rickets.

Correspondence author

Moushira Erfan Zaki

Biological Anthropology Department, Medical Research Division, National Research Centre (NRC)
moushiraz@yahoo.com

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Consideration of the effects of extraction of lead (Pb) by EDTA on the mechanical properties of contaminated bentonite soil

Vahidreza Ouhadi ¹, Amirhoushang Omid ²

¹ Professor in Civil Engineering, Department of Civil Engineering, Faculty of Engineering, Bu-Ali Sina University, Hamedan, Iran

² MSc. Student of Civil Engineering, Department of Civil Engineering, Faculty of Engineering, Bu-Ali Sina University, Hamedan, Iran
omidi.buali.university@gmail.com

Abstract: Contamination of soils by lead (Pb) metal is one of the most common environmental problems globally. Soils washing and extracting lead (Pb) by chelating agents, EDTA for instance, is one of the most effective methods for remediation of lead (Pb) from contaminated soils. Based on previous studies, entering contaminants into the soil in the soil-water system can affect the physical-mechanical properties of soil. Soils that contain higher portions of bentonite are affected more than others. Remediation of the soil and eliminating the contaminants can affect mechanical properties of the soil. This study is based on a series of experiments, which have been conducted in a laboratory. The main focus of this study is to consider the effects of extracting lead (Pb) by EDTA on mechanical properties of contaminated bentonite in different concentrations, 5-100 cmol/kg-soil. By using bentonite soil, lead (Pb) as a contaminant and EDTA as a chelating agent, a series of environmental experiments (adsorbing and extracting the contaminants) have been conducted. Also mechanical experiments were performed, including sedimentation, consolidation, and liquid limit. According to the extracted results from this study, in contaminated bentonite with low concentrations of lead (Pb) (10 cmol/kg-soil approximately) after remediation of contaminants, behavior of the soil has changed and its mechanical properties become similar to natural bentonite soil before the contamination. But in higher concentrations of lead (Pb) (50-100 cmol/kg-soil), there is not a significant difference between mechanical properties of soil before and after remediation. In the other words, reversibility and changes in the mechanical properties after remediation by EDTA can be seen only in contaminated bentonite with low concentrations of lead (Pb) (10 cmol/kg-soil approximately).

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1. Introduction

As a result of increasing world population and development of the agricultural and industrial fields in most of the countries, significant parts of the soils have been contaminated by organic and inorganic contaminants (Jones, 1991). Heavy metals (HMs) are the most common inorganic contaminants in urban areas and mines industrial wastewater (Kim, 2003; McIntyre, 2003). Among the heavy metals, lead (Pb) is the most abundant and most toxic contaminant (Yong, 2001). During the last decades significant progress and developments have made in the remediation of soils. One of the most effective methods for remediation of contaminated soils that contains lead (Pb) is soil washing and extracting the lead (Pb) from the soil by chelating agents. Chelating agents, by creating stable complexes with heavy metals, change the phases of these contaminants into liquid and extract them from the soil. Chelating agents have the least effect on the physical-chemical and biological properties of the soil (Lim et al., 2004). Based on previous research, Ethylene Diamine

Triacetic Acid (EDTA) is one of the most common and strongest chelating agents and it is used for extracting the lead (Pb) from contaminated soils (Lestan et al., 2008; Zhang et al., 2008). Because of high Cation Exchange Capacity (CEC) existing clay minerals in the soil are able to interact with heavy metal contaminants in the industrial wastewater and adsorb them by different mechanisms (Harter, 1983). Cation Exchange Capacity (CEC) is a criterion for determining the soil's ability for cation exchange and saving it. Because bentonite contains a high amount of montmorillonite and CEC and negative charge it is applied in environmental geotechnical projects (such as soil barriers in the landfill centers), liner systems, Geosynthetic Clay Liner (GCL) and clay liner for burying radioactive wastes (Pusch, 1995). As a result of the alkaline property of most of clay soils and low solubility rate of lead (Pb) in alkaline ambient, chelating solutions such as EDTA are used instead of pure water for washing contaminated soils that contain lead (Pb) (Lestan et al. 2008; Peters, 1999). As observed in previous studies, the mechanical

properties of clay soils such as shear strength, consolidation, and permeability are strongly influenced by changes in pore water characteristics (concentration and capacity of cations, pH of system) and changes in their intermolecular forces (Meri & Olson, 1981; Ouhadi et al., 2006; Yong 1992). Also experiments that are performed on the bentonite soil in this study have shown adsorbing heavy metal contaminants such as lead (Pb) and zinc (zn) change the pore water characteristics and intermolecular forces and as a result, mechanical and engineering properties of soil will be changed (Ouhadi et al., 2006).

As mentioned above, it seems inevitable to consider the extraction of heavy metal contaminants from soil and its effects on the pore water characteristics and finally changes in mechanical and engineering properties of clay soil. Various studies have been conducted during the last few years about remediation of heavy metals by using EDTA. These studies contain issues such as impacts of various factors on efficiency of remediation (pH, EDTA, concentration of contaminants) and reuse of washing solution. A lack of data about changes in mechanical properties of soil after remediation of contaminants by using chelating agents can be seen. In this present study, impacts of remediation of lead (Pb) contaminants by EDTA chelating agents on the mechanical properties of clay bentonite soil have been considered.

2. Material and Methods

2.1. Bentonite

Some physical properties and environmental geotechnical of the bentonite used in this study are shown in Table 1. The commercial name of applied bentonite for this research is Plateau of Iran Bentonite, and it is provided by Iran Barit Company.

Table 1. Physical properties and environmental geotechnical of Bentonite (Ouhadi et al., 2006)

Parameter	Data
Soil Classification	CH
LL (%)	314.5
PI (%)	283.3
CEC(cmol/kg-soil)	68.2
Gs	2.79
pH	9.5
(Specific surface area)	413
Clay	76%
Silt	23%
Sand	1%
Water content (air-dried)	5.9
Water content (oven-dried)	7.1

2.2 Ethylene Diamine Triacetic Acid (EDTA)

EDTA sodium salt (Na₂EDTA), chemical formula of C₁₀H₁₄N₂Na₂O₈.2H₂O, has been used for this study.

2.3 Lead (Pb)

For simulating the contamination of the soil, Pb(NO₃)₂ solution was applied by various concentrations.

2.4 Providing contaminated samples

For consideration of interaction between lead (Pb) and bentonite and determining the rate of adsorbing and saving lead (Pb) in the sample soil, and also preparing contaminated samples, at the beginning, the proper amount of dry soil (2 g for environmental tests and 100 g for mechanical tests) was weighted with accuracy of 0.001 g and was accumulated in suitable containers (50 ml centrifugal tubes or 1.5 liter plastic bottles) and then lead nitrate contamination solution (1-250 cmol/kg-soil) was added to soil. The ratio of suspension solution and soil suspension must always be constant, 1: 10. It helps to compare all the tests with each other (environmental and mechanical). Samples were kept at room temperature for 96 hours. These samples were put in the horizontal shaker for two hours every day during this time to satisfy the equilibration and interaction conditions. After 96 hours, samples that were in the 150 ml tubes were centrifuged by 4000 rpm for 15 minutes and liquid and solid phases were separated and then analyzed by using atomic adsorption. By subtracting the amount of remaining lead (Pb) in the liquid phase from the amount of lead (Pb) in the contamination solution, the amounts of the remaining lead (Pb) in the sample contaminated soil were calculated.

2.5 Method of remediation of contaminants and preparing the samples

After preparing the contaminated bentonite samples, those with suitable concentration (0.01-0.1 molar) were selected and were exposed to various concentrations of EDTA for remediation. All the contaminated samples (both inside the tubes and inside the bottles) were dried in the oven at 35 degree Celsius and then washed with a washing solution with determined concentration and also a constant ratio of 1:10 for washing solution – contaminated soil. To find the optimal amount of EDTA for full remediation of lead (Pb) from the soil, each sample was washed with EDTA with various concentrations (1, 5, 10, 50 and 70 cmol/kg-soil). The equilibration step and washing the contaminated soil is similar to what was explained in the previous section (preparing contaminated samples). The only difference was the use of EDTA solution as lead (Pb) nitrate solution and the use of contaminated dried soil as natural soil.

Finally, extracted solution from the samples inside the centrifuge tubes was analyzed by atomic adsorption. The samples inside the 1.5 liter bottles were poured inside the Teflon tubes with internal diameter of 7 cm and height of about 15 cm for mechanical tests. The Teflon tubes are fabricated with longitudinal slots for 10-15 cm draining and an external diameter of less than 7 cm to do the easier loading for extracting the additional water and preparing the initial samples for mechanical, liquid limit, and consolidation tests. After loading the halgheye tahkim, an intact sample was prepared from samples inside the Teflon tubes and the rest of sample was used for liquid limit test. For sediment testing, 500 ml of suspension from inside the 1.5 liter bottles was poured in the graduated cylinder with a capacity of 1000 ml and then the volume of suspension inside it was increased by adding distilled water up to 1000 ml. Then, samples inside the graduate cylinder were located on a smooth surface for 24 hours to observe the leak rate.

3. Results and Discussions

3.1 Environmental tests

3.1.1 Considering interaction between bentonite and lead (Pb) contaminant

According to the results that are shown in Fig.1, for concentrations around 0.07 molar (70 cmol/kg-soil) that are equivalent to the concentration of CEC of soil, almost all of the heavy metal ions inside the electrolyte remained in the soil. Up to this concentration, the graph has a linear behavior and the slope of the graph is equal to 45 degree and it means the amount of lead (Pb) inside the initial solution and amount of adsorbed lead (Pb) by the soil are equal. By increasing the concentration of contamination to more than CEC, the bentonite sample is not able to save all the ions of the heavy metal, lead (Pb), and adsorption trend (slope of the graph) decreases drastically. According to constant level of adsorption of lead (Pb) in the soil for concentrations of more than 0.07 molar (70 cmol/kg-soil), an interval of concentrations from 0.001 molar up to 0.01 molar (1-100 cmol/kg-soil) was chosen to prepare the contaminated samples in this study.

3.1.2 Extracting lead (Pb) from contaminated bentonite by EDTA and determining the optimum amount of lead (Pb)

The amount of extracted lead (Pb) that is obtained from washing contaminated soil by distilled water is presented in Figure 2. For the entire tests, the constant ration of 1:10 (solution: soil) is applied. As can be seen, the amount of extraction of lead (Pb) goes up by increasing the concentration of EDTA. Also, it is obvious that the EDTA solution in each

specific concentration has extracted a specific amount of lead (Pb) from soil that is equal to its own concentration. Actually, the minimum required concentration for EDTA (optimum concentration) for perfect extraction of the lead (Pb) contaminant from the contaminated soil is exactly equivalent to the existing contaminant in the soil. The equivalent concentration is defined as the condition in which the EDTA concentration is equal to the contaminant concentration in soil. For example, the equivalent concentration for 30 cmol/kg-soil means that the bentonite soil contains 30 cmol/kg-soil lead (Pb) contaminant and this contaminated soil is washed by EDTA solution with a concentration equal to 30 cmol/kg-soil and the whole amount of lead (Pb) inside the soil is extracted and the soil contains no more contaminant.

3.2 Behavioral and mechanical tests

3.2.1 Sedimentation test

For observing effects of lead (Pb) contaminant on structures of the bentonite, sediment tests for bentonite samples were performed for various concentrations of lead (Pb) heavy metal. Twenty-hours hours after pouring the suspension samples inside the similar graduated cylinders, some photos were taken and the photos are shown in Fig 3. According to Fig 3, by increasing the concentration of lead (Pb) and replacing it as alkali metals in the soil, repulsive forces between the particles and the negative charges of the particles are decreased. As a result of this phenomenon, a more concentrated and flocculated double layer is created. By mounting the concentration of the contaminant, flocculating of the system increases sharply and the solid phase of soil get separated from the liquid phase rapidly. Levels of leakage suspension after 24 hours in bentonite after complete remediation of contaminants are shown in Fig 4 by an equivalent concentration of EDTA. Despite the complete remediation of the lead (Pb) from the bentonite for all concentrations, significant differences can be seen. This is because of the effects of interchangeable cation on the soil. By increasing the equivalent concentration, the amount of existing lead (Pb) in the soil was mounted. Based on previous studies, sodium montmorillonite satisfies the double-layer theory in comparison with calcium montmorillonite and it also acts dependent to double-layer and intermolecular forces (Mitchell, 1993). Here, by increasing the concentration of the lead (Pb) in the soil, the amount of sodium is decreased and its impressibility of double-layer thickness and intermolecular forces decreases. Extracted results from this study have reasonable similarities with previous research (Mesri & Olson, 1971).

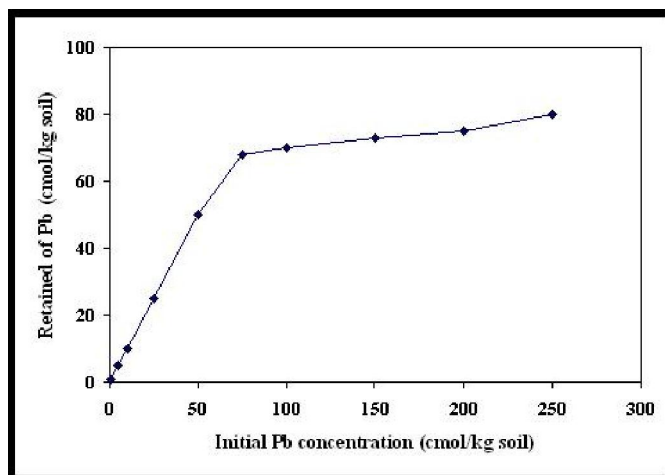


Fig 1. Specifications of adsorption and saving the heavy metal of lead (Pb) in the bentonite clay soil

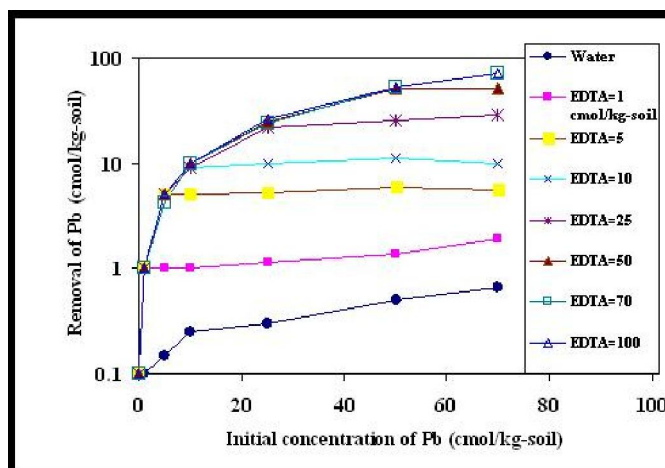


Fig 2. Amounts of extracted lead (Pb) by various concentrations of EDTA

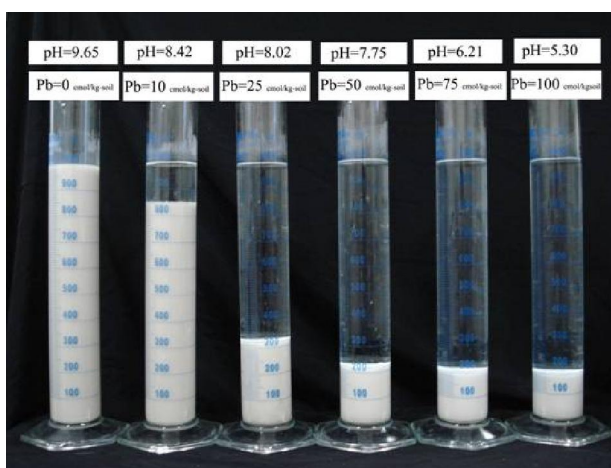


Fig 3. Comparison between effects of lead (Pb) adsorption on sinking levels of Bentonite for various concentrations after 24 hours

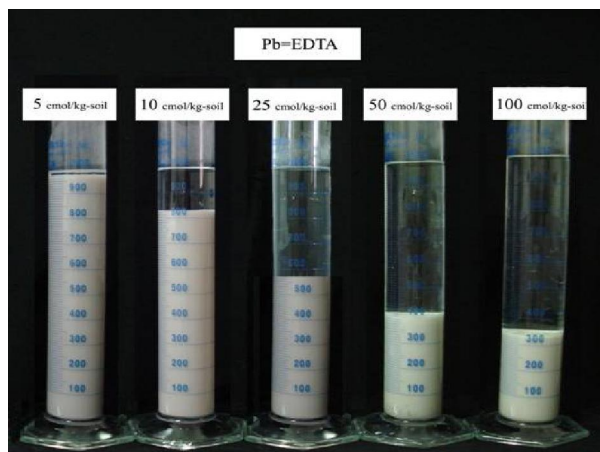


Fig 4. Comparison between levels of sinking of bentonite for various equivalent concentrations after 24 hours

3.2.2 Liquid limit test

Effects of the various concentrations of the lead (Pb) contaminant on the liquid limit of bentonite are shown in Fig 5. As indicated, by mounting the concentration of lead (Pb) contaminant, a significant downward trend appears in the liquid limit of contaminated soil. Levels of extracted liquid limit from the test performed on the bentonite with complete remediation of lead (Pb) are illustrated in Fig 6. By comparing Fig 5 and 6 it is clear that that after remediation of lead (Pb), only in low concentrations (5 and 10 cmol/kg-soil) significant changes near to liquid limit appear and after complete remediation of the lead (Pb) liquid limit of contaminated soil approaches the liquid limit of

natural bentonite. In high concentrations (50 and 100 cmol/kg-soil) even after complete remediation of lead (Pb) from the soil there are no significant changes in liquid limit of the soil. Increasing initial liquid limit in concentration of 5 cmol/kg-soil is related to fully saturating the montmorillonite with sodium and different rheological behavior in low concentrations of sodium (Van Olphen, 1977; Sivapullaiah, 2000). By decreasing the amount of existing sodium in the soil and dissimilar behavior of the soil with the double-layer theory and intermolecular forces, it is clear that irreversibility of the bentonite characteristics to the natural conditions reduces drastically.

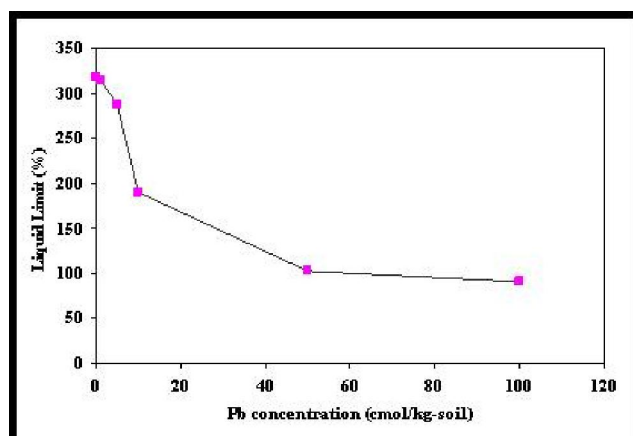


Fig 5. Effects of lead (Pb) contaminant on liquid limit of contaminated bentonite

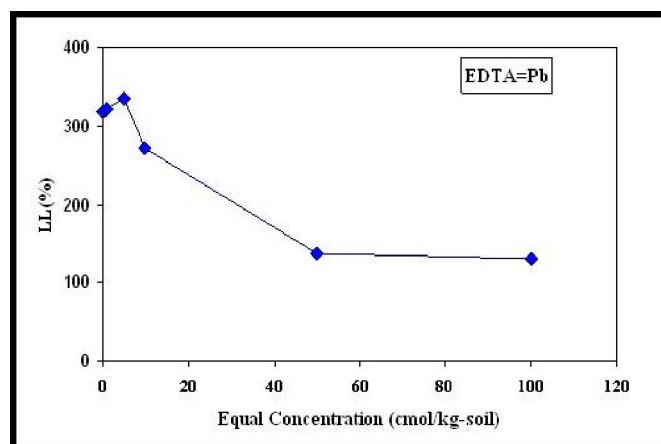


Fig 6. Liquid limit of bentonite by EDTA in various equivalent concentrations

The reason for little differences between equivalent concentrations of 50 and 100 cmol/kg-soil could be the clay minerals that are saturated by lead (Pb) and bivalent alkaline cations in equivalent concentration of 50 cmol/kg-soil. In the soil that contains 50 cmol/kg-soil of lead (Pb) in addition to 50 cmol/kg-soil lead (Pb) inside the soil, there are also 20 cmol/kg-soil bivalent alkaline cations and it fills the rest of soil adsorption capacity. The statistics revealed no significant differences between the two soil samples.

3.2.3 One-dimensional consolidation test

The graphs related to the void ratio against the consolidation pressure for various concentrations of lead (Pb) contaminant in the contaminated soil are illustrated in Fig 7. Similar to what earlier results, by

increasing the concentration of lead (Pb) contaminant and replacing it with existing sodium and calcium in the soil there was a significant decrease in the ratio of initial pores of the soil and consolidation properties of the contaminated soil.

Graphs related to ratio of pores in respect to the consolidation pressure in the bentonite after remediation of the contaminants by various concentrations of EDTA are illustrated in Fig 8. By comparing Fig 7 and 8 and regarding the complete remediation of lead (Pb) contaminant from all the samples, by increasing the equivalent concentration, a significant descending trend for the ratio of pores of the soil can be seen. With respect to the previous theory, because of the existence of lead (Pb) contaminant in the bentonite, intermolecular forces do not affect the bentonite significantly.

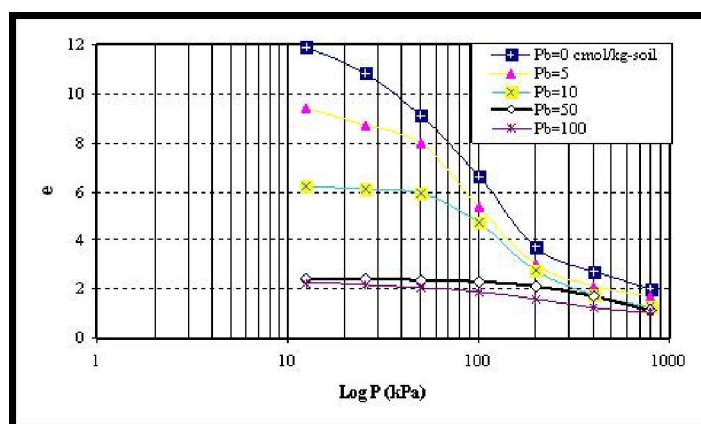


Fig 7. Curves related to ratio of void on consolidation pressure of the contaminated bentonite for various concentrations of the lead (Pb)

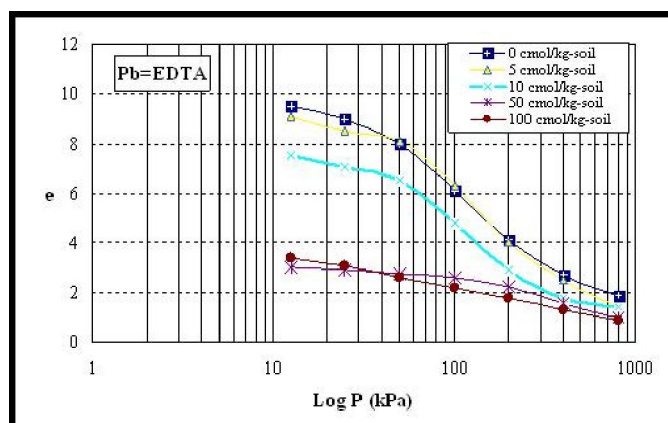


Fig 8. Curves related to ratio of void on consolidation pressure of the bentonite for various equivalent concentrations

Also, it can be seen that only in the low concentrations (5 and 10 cmol/kg-soil) EDTA was able to remediate the soil by extracting the lead (Pb) from the contaminated soil and made the properties of the contaminated soil similar to the natural bentonite by replacing lead (Pb) with sodium. Mounting the concentration of contaminant, properties of soil before and after remediation of soil do not change significantly and it has a perfect corresponding to the results of previous tests.

4. Conclusion

Effects of remediation of lead (Pb) contaminant by EDTA chelating agent on the behavioral and mechanical properties of bentonite soil were considered in this study. Based on the experiments of this study these results were extracted:

1. EDTA chelating agent is able to extract lead (Pb) contaminant from the contaminated bentonite exactly equal to its own concentration.
2. The optimum amount of EDTA for remediation of lead (Pb) from the bentonite soil is exactly equal to concentration of existing contaminant in the soil.
3. Mechanical properties and behavior of the contaminated bentonite (with low concentrations of lead (Pb), around 10 cmol/kg-soil) have changed after remediation by EDTA and have approached to the natural bentonite.
4. Mechanical properties and behavior of the contaminated bentonite (with high concentrations of lead (Pb), between 50- 100 cmol/kg-soil) have not changed significantly after remediation by EDTA and irreversibility in the properties of the soil have not been seen.

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Corresponding Author:

Amirhoushang Omidi
Civil Engineering Department
Faculty of Engineering
Bu-Ali Sina University, Hamedan, Iran
Tel: +98.8118257410
E-mail: yahidouhadi@yahoo.ca

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Nurse – physician collaboration: A comparative study of the attitudes of nurses and physicians at Mansoura University Hospital.

Karima A. EL Sayed¹ and Wafaa F. Sleem²

¹Department of Nursing Administration, Tanta University - Egypt

²Department of Nursing Administration, Mansoura University - Egypt

dr_wafaasleem@yahoo.com

dr.Karimaahmed@yahoo.com

Abstract: Collaboration and team work between physicians and nurses is crucial for patient care and morale. Each team member has his own perspective regarding assessment and plan of care for a patient and only through collaboration and exchange of information can appropriate treatment plans be made. The study aims to study attitudes of nurses and physicians regarding nurse-physician collaboration in general medical and surgical units at Mansoura University Hospital and to measure differences in attitudes of nurses and physicians regarding nurse – physician collaboration. All nurses and physicians (n=135) available at time of data collection, who were working in medical and surgical units. Data were collected by using Jefferson scale of attitudes toward nurse-physician collaboration. The Jefferson scale has four subscales that measure:(a) shared education and teamwork (7 items with responses from 1-4, with a subscale score range of 7-28) ;(b) caring versus curing (3 items with a subscale score range of 3-12) ;(c) autonomy (3 items with a subscale score range of 3-12) and physician dominance (2 items, with a subscale score range of 2-8). Results revealed that the total scores indicated that nurses have more positive attitudes toward nurse- physician collaboration than physicians.

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Keywords: Collaboration, Jefferson scale, surgical units, nurse-physician collaboration.

1. Introduction

Today's health care delivery system challenges all of us to provide care that is patient-centered, efficient, effective, safe, timely, and easily accessible ⁽¹⁾. To meet this challenge, quality and safety become everyone's business. Lindeke and Sieckert ⁽²⁾ demonstrate that maximizing nurse-physician collaboration holds promise for improving patient care and creating satisfying work roles. Indeed, we now know that we need to maximize all that occur within a multi-disciplinary health care team.

The physician-nurse relationship is stressful. Both perceived and real differences in power and status between physicians and nurses can lead to problems when these health care providers do not agree on a patient's plan of care. Traditionally, the profession of medicine has emphasized expertise, autonomy, and responsibility more than interdependence, deliberation, or dialogue. Nursing, on the other hand, has emphasized hierarchy and bureaucracy, though emphasis on these has diminished along with deference to physicians⁽³⁾. Although in the past, nurses were used to following orders and not giving them, they have learned to adapt their approaches with physicians to accomplish their patient care goals. The different emphases that Physicians and nurses have towards patient care may lead to strained physician-nurse relationships, which may in turn

compromise patient, unless the physicians and nurses develop collaborative relationships ⁽⁴⁾.

Collaborative teams developed with the basic premises that each member of the team possessed different skills but had the same philosophical goals on patient care. Combining skills and defining roles allowed for a comprehensive approach to care of a specific group of patients. Collaborative teams work in every locale, practice setting, and specialty. The way the team functions is defined by the participants on the basis of the needs of the population that are being cared for. Lack of communication and collaboration has been cited as a reason for poor patient outcomes ⁽⁵⁾.

Collaboration is defined as the process of joint decision-making among independent parties, involving joint ownership of decisions and collective responsibility for outcomes ⁽⁶⁾. It is a process whereby two or more people come together to discuss a common problem. Each participant has the self-confidence to share knowledge and information on an equal basis, and mutual respect is given to each opinion. The focus remains on the needs of the patient, and negotiations result in a plan of care ^(7,8). So that collaboration and teamwork between physicians and nurses is crucial for patient care and morale ⁽⁹⁾. Each team member has his or her own perspective regarding assessment and plan of care for a patient, and only

through collaboration and an exchange of information can appropriate treatment plans be made. In addition, physician-nurse collaboration and positive relationships have been identified as major factors contributing to positive patient outcomes and quality care⁽¹⁰⁻¹²⁾.

Concerning collaboration is a complex process that requires intentional knowledge sharing and joint responsibility for patient care. Sometimes it occurs within long-term relationships between health professionals. Collaboration has a developmental trajectory that evolves over time as team members leave or join the group and/or organization structures change^(12,13). Each health care profession has information the other needs to process in order to practice successfully. In the interest of safe patient care, neither profession can stand alone, making good collaboration skills essential⁽¹⁾.

Kramer and Schmalenberg⁽¹⁴⁾ stated that collaborative partnerships are worth the effort because they result in better outcomes for patients as well as personal growth for collaborators. Certain characteristics of nurse-physician relationships correlate directly with patient care quality. Research carried out at fourteen hospitals which had achieved "Magnet" designation from the American Nurses Credentialing Center (ANCC) also indicated that healthy collaborative relationships between nurses and physicians were not only possible, but were directly linked to optimal patient outcomes. The researchers proved a positive correlation between the quality of physician-nurse relationships, (as evidenced by measures of collegiality and collaboration, and the quality of patient care outcomes.

According to Fagin,⁽¹⁵⁾ collaboration is vital not only for the benefit of patients, but also for the satisfaction of health care providers. Collaboration between physicians and nurses is rewarding when responsibility for patient well-being is shared. Professionalism is strengthened when all members take credit for group successes. Unfortunately the contribution of nursing towards the bottom line is often not easy to identify. Physicians have often been viewed as the primary generators of income for hospitals. However, nurses are also substantial revenue producers. The invisibility of nursing may occur because differences in income and gender have historically impacted the balance of power between nurses and physicians.

Appreciation of the unique knowledge of contributing disciplines and a clear understanding of the unique contributions of nursing to care can demonstrate that nurses play an important role in achieving the positive patient's outcomes that occur categories of collaborative strategies. Thus this study was conducted to study attitudes of nurses and

physicians regarding nurse-physician collaboration in general medical and surgical units at Mansoura university hospital.

Aim of the study

The study aims to study attitudes of nurses and physicians regarding nurse-physician collaboration in general medical- surgical patient care setting at Mansoura Main University Hospital

2. Material and Methods

Materials:

Design: Descriptive design

Setting:

The study was conducted at Mansoura Main University Hospital in all general medical units (n=3) and all surgical units (n=5). Mansoura Main University Hospital affiliated to teaching university hospital and occupied with 1860 beds.

Subjects:

Two groups of subjects included in this study to achieve its aim.

They were all staff nurses (n=97) and all physicians (n=38) available at the time of the study who were working in the pervious general medical and general surgical units.

Tools:

The data for the study were collected by using Jefferson scale of attitudes toward nurse-physician collaboration. Jefferson scale was developed by researchers at Jefferson medical college, Philadelphia, Pennsylvania⁽¹⁶⁾. Which it consists of two parts:

First part: Personal data of nurses and physicians. It includes gender, specialty, age and years of experience.

Second part: It included 15 statements, which were grouped under four subscales, i.e., shared education and teamwork (7 statements), caring versus curing (3 statements), nurses' autonomy (3 statements) and physicians' dominance (2 statements).

Scoring:

The response was on a four-point, Likert-type scale from strongly agree (4) to strongly disagree (1):

The two items identified as "physician's dominance" questions are reserved scored, with a higher factor score given to a lower numerical answer and vice versa. The higher the total scores on this scale, the more positive the respondent's attitude toward physician-nurse collaboration. A higher factor

score on "physician's dominance" indicates a rejection of a totally dominant role by physicians in aspects of patient care. A higher factor on the "nurses' autonomy" dimension indicates more agreement with nurses' involvement in decisions about patient care and policy. A higher factor score on "shared education and teamwork" indicates a greater orientation toward interdisciplinary education and interprofessional collaboration. Finally, a higher factor score on the "caring versus curing" dimension indicates a more positive view of nurses' contributions to psychosocial and educational aspects of patient care⁽¹⁷⁾.

Methods:

- A permission to conduct the study was obtained from the responsible authority of Mansoura Main University Hospital after explanation of the study' aim.
- Tool was translated into Arabic for (nurses) and was tested for its content validity by five faculty members in nursing administration.
 - Pilot study was conducted on five nurses and five physicians (whom are not included in the study) to test the reliability of the tool. Accordingly minor changes were made for few statements.
- The questionnaire was distributed to the study nurses and physicians. Time needed to complete the scale was 10-15 minutes.
- Total time taken for data collection to complete the study was two months starting July 2009.
- Ethical consideration: all participants interviewed for explaining the purposes and procedures of the study, and they have the right to withdrawal from the study any time during the study. Oral consent to participate was assumed by attendance of filling questionnaire sheet.

Statistical analysis:

Computerized data entry and statistical analysis were fulfilled using the statistical package for social sciences(SPSS).Data were presented using descriptive statistics in the form of frequencies percentages, means and standard deviations for quantitative variables. Quantitative data were compared using the student t-test in case of comparisons between two groups. Statistical significance was considered at p-value <0.05. Person correlation analysis was used for assessment of the inter-relationships among quantitative variables.

3. Results

Table (1) describes demographic characteristics of the study subjects. A total of 135 participants, of which 97 were nurses and 38 were physicians. All nurses were female and all physicians were male.

Regarding specialty 40 nurses with 15 physicians were working in medical units while 57 of them were working in surgical units with 23 physicians. The mean age of nurses was 32.81 years, with an average of 15.62 years of nursing experience. The mean age of physicians was 27.21 years with an average of 2.50 years of experience.

Table (II) shows the surgical nurse's and physician's mean total score was higher at 50.68 (SD= 4.23) than the medical nurse's and physician's mean total score of 49.84 (SD = 4.64) when the two units group's scores were compared, they were not found to be significantly different ($t=1.0888$, $p<0.279$).

Table(111) shows the nurse's mean total score was 51.21(SD=4.32) compared to the physician's mean total score of 48.11 (SD= 3.83). The nurse's mean total score was shown to be significantly higher than the physician's mean total score ($t = -3.87$, $p<0.00$), indicating that the nurse's attitudes toward nurse-physician collaboration were more positive than the physician's.

Also mean scores were compared between the nurse and physician groups in relation to four collaboration factors ("shared education and team work", caring versus curing", "nurses autonomy", "physician's dominance"). In the "shared education and team work" factor (i.e., a higher score indicates a greater orientation towards interdisciplinary education and interprofessional collaboration, the nurse's mean score of 24.80 (SD =2.11) was significantly higher than the mean score of physicians at 2.55(SD=2.06). In the "nurses autonomy" factor (i.e., a higher factor score indicates more agreement with nurse's involvement in decisions pertaining to patient care and policy), the nurse's mean score of 10.44(SD = 1.40) was significantly higher than the physician 's mean score of 9.32 (SD = 1.53).

In the "caring versus curing" factor (i.e., higher score indicates a more positive view of nurses contributions to the psychosocial and educational aspects of patient care), the physician's mean score of 9.13 (SD = 2.07) higher than the nurse's mean score of 8.86(SD = 1.93). In the final factor score in the ' physician's dominance " dimension ((i.e., a higher factor score indicates a rejection of a totally dominant role by the physician in aspects of patient care), the physician's mean score of 7.11 (SD= 1.01) was relatively equal to nurse's mean score of 7.103 (SD=0.85).

Table (IV) shows there was positive correlation between collaboration factors for nurse-physician collaboration and their experience ($r = 0.202$) while negative correlation was found with their age. Two collaboration factors were positively correlated with

experience namely; shared education ($r = 0.252$) and team work and nurses autonomy ($r = 0.251$).

Table (1) Demographic characteristics of the study subjects.

Demographic characteristics	Study subject (n =135)	
	Nurses (n =97)	Physicians (n = 38)
Gender		
- Males	----	38
- Females	97	----
Specialty		
- Medical	40	15
- Surgical	57	23
Mean Age in Years	32.81	27.21
Mean Years of Experience	15.62	2.50

Table(II) Attitude of Nurses and Physician toward Nurse-Physician Collaboration Factors according to specialty

Collaboration Factors	Specialty		p-value
	Medical (n=55) $\bar{x} \pm SD$	Surgical (n=80) $\bar{x} \pm SD$	t-test
Shared education and team work	24.00±2.17	24.29±2.44	0.703
Caring versus curing	8.98±2.03	8.90±1.93	-0.237
Nurses autonomy	9.84±1.68	10.33±1.38	1.854
Physician's dominance	7.02±0.89	7.16±0.89	0.923
Total	49.84±4.64	50.68±4.23	1.088

Table (III): Comparison of Nurses and Physician toward Nurse-Physician Collaboration Factors

Collaboration Factors	Nurses (n = 97) $\bar{x} \pm SD$	Physician (n =38) $\bar{x} \pm SD$	p-value t-test
Shared education and team work	24.8041±2.11	22.5526±2.0625	-5.60*
Caring versus curing	8.86±1.93	9.13±2.07	0.73
Nurses autonomy	10.44±1.40	9.32±1.53	-4.104*
Physician's dominance	7.103±0.85	7.11±1.01	0.013
Total	51.21±4.32	48.11±3.83	-3.87*

- Statistically significant at $p < 0.05$

Table (IV): Correlation coefficient between Nurse-Physician Collaboration Factors and Demographic Characteristics as reported by Studied Subjects (n =135)

Collaboration Factors	Demographic Characteristics	
	Age (r)	Experience (r)
Shared education and team work	0.143	0.251**
Caring versus curing	-0.207	-0.056
Nurses autonomy	0.148	0.251**
Physician's dominance	0.044	0.033
Total	0.123	0.202*

4. Discussions

Collaboration is rare when there is a wide difference in power between the groups or individuals involved. Many think of collaboration as a form of cooperation, but this is not an accurate definition. In collaboration, problem solving is a joint effort with no superior-subordinate, order-giving order-taking relationship. True collaboration requires mutual respect, open and honest communication, and equitable, shared decision making powers⁽¹⁸⁾.

The findings of this study indicated that there is significant differences existed between nurses and physicians in the medical surgical patient care setting with regard to attitude toward nurse-physician collaboration. This mean total score on the selected survey tool indicated that nurses in this study demonstrated significantly ($P < 0.05$) more positive attitudes toward collaboration than did the physicians. This is due to a significant ($P < 0.05$) barrier to nursing autonomy is related to the attitudes of health care practitioners. Factors such as tradition, the subordination of nurses to physicians, socialization within health care facilities, sexism and stereotyping, and the apprenticeship model of nursing education are found to affect the attitudes of physicians and nurses alike⁽¹⁹⁾. Traditionally, the predominately male physician group gives the orders for patient care, and the predominately female nursing group carried out the orders. Physicians were "in charge", and nurses learned to defer to them and follow their lead. These traditional views on the nurse-physician relationship can affect caregiver's attitudes toward nurse-physician collaboration.

This result supported by Barrere and Ellis⁽¹⁰⁾ who mentioned that, as knowledge concerning the nurse's role increased, important positive changes took place in the nurses' attitudes toward collaboration. Also physician's knowledge about a nurse's role can affect their attitudes toward collaboration. In addition, Macdonald and Katz⁽²⁰⁾

explained that limited knowledge about the nurse practitioner's role in patient care adversely affected physician's ability to envision collaborative practice.

The afore mentioned findings, are consistent with those of previous studies that demonstrated nurses had a more positive attitude toward collaboration than did physicians⁽²¹⁻²⁵⁾. While the findings differed, however from, the findings of four studies in which physicians rated collaboration higher than did nurses^(26&27).

Compared to physicians nurses in the present study indicated a more positive attitude toward nurses' involvement in making decisions about patient care and policy and a greater orientation toward interdisciplinary education and interprofessional collaboration. The researcher attributes this finding to the influence of culture on attitudes toward nurse-physician collaboration. Nurses in the present study practiced in a more hierarchical model of professional practice. Supporting this explanation, Hojat et al.⁽²¹⁾ and Hojat et al.⁽¹⁷⁾ stated that both American and Israeli nurses, who worked in a complementary model of professional roles, expressed more positive attitudes toward collaboration than did their Italian and Mexican counterparts, who practiced in a more hierarchical model of professional practice. These findings were consistent with previous studies by, Thomson,⁽²⁴⁾ and Sterchi,⁽²⁵⁾

However, related to physicians' dominance, both nurses and physicians demonstrated more neutral attitudes. These findings indicated the trend toward more positive attitude related to collaboration versus the more traditional attitudes of the physician as the primary authority in patient care decisions. Also of note, the average age of nurse and physician participants in this study indicated that they were of the same generation.

These findings replicated the findings of Hojat and colleagues⁽¹⁷⁾ and demonstrated similar

trends to other study findings (King & Lee, ⁽²⁸⁾ Rosenstein, ⁽²²⁾ and Thomas et al., ⁽²³⁾ This study was particularly important because it was specific to the medical-surgical setting, where minimal research had been completed previously on this topic.

Results revealed that physicians scored slightly above agree and nurses scored slightly below agree on nurses possessing the ability to assess and respond to patient psychosocial and educational needs. According to the researcher point of view, this could be attributed to lack of organizational support for nurses contributions to the overall quality of patient care lead to bad collaboration between nurses and physicians. In this respect, Erickson and Clifford⁽²⁹⁾ mentioned that increased education of nurses and organizational support for their contributions to the overall quality of patient care lead to better collaboration between nurses and physicians. These findings are differed from Sterchi, 2007, who mentioned that nurses with exhibiting more positive attitudes toward caring versus curing roles.

The present study revealed no differences in attitude toward collaboration based on nursing specialty. Although surgical unit participant's total scores in this study indicated a more positive attitude toward nurse-physician collaboration than medical unit the result were not significant. This could be attributed to the very nature of surgical care, in which nurses must coordinate care with various multidisciplinary caregivers, may faster greater teamwork and collaboration. A similar result was found with Chaboyer and Patterson ⁽³⁰⁾ who founded that nurses who specialized in working in the intensive care unit perceived greater levels of physician-nurse collaboration than did hospital generalist nurses.

Regarding correlations between nurse-physician factors and length of experience, the present study revealed that positive correlations were found between scores of total collaboration factors and length of experience. Results indicated that the physicians' and nurses' attitude toward collaboration became more positive with increased years of experience .one possible explanation for the physicians' improvement in attitude toward collaboration with increasing experience might be the result of a greater knowledge concerning the nurses' role.

Macdonald and Katz ⁽²⁰⁾ founded that limited knowledge of nurses' role adversely affected the physicians' ability to visualize collaborative practice, as did Barrere and Ellis, ⁽¹⁰⁾.

Recommendations:

Based on the results of the present study, the following recommendations are suggested:

- Initiating and developing mutually respectful inter-professional relationships between nurses and physicians. This can be done through inter-professional education in their curriculum to increase understanding of complementary roles of nurses and physician, and encourage establishment of an interdependent relationship between them.
- Encourage programs that promote interaction between medical and nursing students help these future professionals understand each other's roles and responsibility.
- Involving both nurses and physicians in the recruiting efforts of an organization could help improve the understanding of the needs and values of each group.
- Providing cross-disciplinary shadowing opportunities for nurses and physicians to provide mutual understanding of roles, and enable both groups to better envision collaborative practice .
- Shared continuing educational, in service programs and workshop especially these with a focus on teamwork and communication.
- Forums to disseminate the result of research on collaboration can provide opportunities for open discussion and problem solving, thus creating an ongoing awareness of the need for improved collaboration, especially in the physician group.
- Joint participation in the orientation process for both new nurses and physician.

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Analysis of different techniques in Participatory Rural Appraisal (PRA)

Mohammad Abedi¹ and Sharareh Khodamoradi²

¹Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran

²Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Corresponding author: skhodamoradi2007@yahoo.com

Abstract: PRA is intended to enable local communities to conduct their own analysis and to plan and take action. PRA involves project staff learning together with villagers about the village. The aim of PRA is to help strengthen the capacity of villagers to plan, make decisions, and to take action towards improving their own situation. Participatory Rural Appraisal (PRA) is considered one of the popular and effective approaches to gather information in rural areas. This approach was developed in early 1990s with considerable shift in paradigm from top-down to bottom-up approach, and from blueprint to the learning process. In fact, it is a shift from extractive survey questionnaires to experience sharing by local people. PRA is based on village experiences where communities effectively manage their natural resources.

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Keywords: Participatory Rural Appraisal (PRA)

Introduction:

PRA is a methodology of learning rural life and their environment from the rural people. It requires researchers / field workers to act as facilitators to help local people conduct their own analysis, plan and take action accordingly. It is based on the principle that local people are creative and capable and can do their own investigations, analysis, and planning. The basic concept of PRA is to learn from rural people. Chambers (1992) has defined PRA as an approach and methods for learning about rural life and conditions from, with and by rural people. He further stated that PRA extends into analysis, planning and action. PRA closely involve villagers and local officials in the process.

Similarly, Rapid Rural Appraisal (RRA) reflects the new thinking about development, needs, and people oriented responsibilities. It is a process that is highly systematic and structured, relying on interdisciplinary teamwork and special strategies for data collection and analysis such as triangulation, probing, and iteration. Some critics consider RRA to be a quick and dirty technique (Guijt, 1998).

There are a wide range of participatory tools and techniques available. People can use these tools and techniques according to their situation or needs. Generally, the application of different tools may vary from one situation to another. However, the process for conducting RRA/PRA remains the same.

PRA techniques (Gibson, 1992):

The most common methods are the following:

1- Diagramming, Mapping and Modeling:

- transects
- maps (resource, social, farm)
- venn diagrams
- seasonally analysis
- historical analysis (time lines, trend lines, activity profiles)
- 2- Ranking and scoring
 - pair wise ranking
 - matrix ranking
 - matrix scoring
 - well-being analysis and wealth ranking
 - proportional piling
 - pie charts (injera charts)
- 3- Problem analysis
 - identification and specification
 - causal chaining
 - prioritization

-Direct observation -- Observations are related to questions: What? When? Where? Who? Why? How?

- Observe a particular topic or theme for a particular piece and time
- Record observations as soon as possible
- Guidelines for making observation
- Decide on the major theme or topic to be observed
- Decide before hand where observation will take place. When and how long you will observe (Pottier, 1992).

_ Do it yourself -- Villagers are encouraged to teach the researcher how to do various activities. The researcher will learn how much skill and strength are required to do day-to-day rural activities, gaining an

insider's perspective on a situation. Roles are reversed: villagers are the "experts" and attitudes are challenged.

Participatory mapping and modeling -- Using local materials, villagers draw or model current or historical conditions. The researcher then interviews the villager by "interviewing the map." This technique can be used to show watersheds, forests, farms, home gardens, residential areas, soils, water sources, wealth rankings, household assets, land-use patterns, changes in farming practices, constraints, trends, health and welfare conditions, and the distribution of various resources (Ekins, 1992).

Maps and Models – Diagrams:

Spatial data is analyzed through diagrams, maps and models. The techniques are pictorial or symbolic representation of information.

The purposes of diagrams are:

- a tract and focus attention of discussion group
- represent objects on processes
- stimulate an open discussion
- provoke an effective group work
- facilitates education and information exchange
- assist in decision making
- help remember important points
- help in monitoring and evaluation

Community Sketch Maps

The purposes of community sketch map or a model: is a visual representation of what the community perceives as their community space. This include showing the shape (appearance) of the community, boundary and all the major features as understood and known by the community (Scrimshaw and Gleason, 1992).

The map shows where resources, activities, problems and opportunities are located, as well as the dimension and scope of issues to be investigated. It is critical to understanding the boundaries and characteristics of the community involved.

Topographical data (elevation, slope, drainage etc.)

Topographical data are basic when drawing a map of community, so is information on soils, vegetation, water availability, road, schools, health facilities etc. There are different sketch maps known for different purposes. Some of them include (Dunn, 1992):

A. Social maps: Specific type of topical map representing households according to certain indicators.

- Indicates where people live and how many people live in an area
- Social and residential differences in status and wealth
- Buildings where people live or work, uses of space in a house

B. Physical and resource maps: drawn by the people to show natural resource of an area, location and use of natural resources.

- fields and land uses
- physical land features
- water location, quality and use
- soil types, uses, location

C. Topical maps: specific topic maps are drawn to draw attention to a particular type of information of the area, example:-

- location of forest resources
- soil types
- different crops grown
- houses and the number of people live in
- social & economic infrastructures etc.

D. Farm sketch: Making a farm or compound sketch highlight details that would otherwise be lost in a smaller scale maps.

Procedures for collecting spatial data

Who draws the maps?

The community members or their representatives together with the PRA team and the local extension field staff undertake this exercise. The various parties having different but complementary ideas to the process.

HOW?

The community members are the best experts of their area. While it is tempting for a team member to take charge and save time by drawing the map, it is advisable to let ordinary villagers draw the map on the ground. Literacy is not necessary in order to draw a map of one's place. The PRA team should explain the process clearly. The sketch map is drawn using their fingers, sticks and other locally available materials such as pebbles, leaves, and flowers. The community should be guided through questions to draw the map of their community territory of application (IUCN, 2001).

Community sketch map helps in defining micro-zones, knowing about disparities in wealth, differences in land use. This exercise provides to locate areas where particular problems are thought to be prevalent. The map is also used to lay the transect route. While the map is still on the ground the community members mark the most representative cross section of the community.

How should one proceed to sketch maps or models?

Before:

- Decide what type of map you want
- Bring people together who will have some knowledge about the area and can contribute
- Choose suitable time and place
- Bring materials with you on which you can copy a map drawn on the ground (Scoones, 1993).

During:

Try to minimize your own participation be an observer?

- Encourage by asking open questions
- Encourage the use of different materials, i.e. flowers, twigs, sticks etc
- Be patient! (Swift and Umar, 1991)

After:

- Make a copy of the map or model, including mapper's names
- Try drawing the same type of map with different groups of people. i.e. one group of women, a group of old men and the young
- Keep it simple
- Orient it appropriately
- Cross-check the map, compare with what you see
- Draw it in the area of study with the local people.

Transect walks and guided field walks -- The researcher and key informants conduct a walking tour through areas of interest to observe, to listen, to identify different zones or conditions, and to ask questions to identify problems and possible solutions. With this method, the outsider can quickly learn about topography, soils, land use, forests, watersheds, and community assets (Drummond, and Nontokozo, 1992).

Transect walk

A transect is a walk or a series of walks through an area with local informants to learn of the range of different condition, problems and opportunities in each of the area. It shows a cross section of the area as observed by the walk (Pretty, 1993).

Purpose?

Transect provides mapping information beyond that collected during the initial reconnaissance and verifies the information on the sketch map. It adds detail on specific characteristics (slope drainage, vegetation, water, soils other sources) that further verifies the PRA team's understanding of the area.

What?

The transect should include more detailed and specific information than the sketch map, such as data on cropping pattern, trees, vegetation, farm size, problems and opportunities.

Who?

The PRA team, community representatives, and local extension staff take charge of this exercise. People encountered along the route should casually be interviewed to provide information on other observed conditions.

How ?

PRA team uses a simple technique of reviewing the community sketch map to estimated the line of greatest diversity. When the community members. Complete drawing of the sketch map, while the map

is still on the ground, they debate, with the guidance of the PRA team about which route to take. The transect walk following the agreed route could be done on the same day the map is drawn: while walking:

-Assign responsibilities for observations and note taking to team members, for instance the agriculture should note soils, cropping patterns etc. the water officer should be responsible for water points, scope, and drainage, the social scientists should observe socio-economic indicators etc.

-The transect route can be subdivided and assigned to two or three smaller team so that a single team need not to walk the entire length

-Proceed along the designated route taking time for brief and informal interviews of residents in each of the ecological zones, time to discuss the critical issues already identified in the transect.

- At the end of the exercise, complete detailed notes and construct a chart similar to the example shown below (Gueye, 1991).

Application

Information gathered will help to verify the information on the sketch map. It will help the community and the PRA team to identify and related opportunities. It will also show the most pressing problem, which may require a thought feasibility study and additional technical data. The information displayed in the transect will be used during the development of the community action plan and to organize stratified sampling for further studies (Cornwall, 2008).

Mobility diagram

The community can get goods and services from different places. Some resources will be available within the PA, others on the boundaries of the community. People daily, weekly and occasionally fetch for those resources in and out of their area. Therefore they travel long distances under difficult situations.

Purpose

The diagram is used to understand the places traveled, resources collected and to identify the persons traveling. The resource centers could have problems and the road and means of transportation may not be appropriate, PRA team needs to know the critical goods and services that people travel to fetch for.

What?

The community center and the places of goods and services are listed. The pull factors and the reasons for not having the resource at the center are recorded. The team tries to understand the condition of the resources, and opportunities to establish within the community. The community members may face difficulties on the road and may also be inconvenient

to get goods and services at the destination(Uphoff, 1992).

Who?

The PRA team identifies individuals or groups who travel to other places on a purpose. Discussion with men and women travelers can give a clue about resource scarcity(Mukherjee, 1992).

How?

- Select informants who travel for resources
- Write down as many places as possible visited and resources the most wanted.
- The difficulties faced on the way and at the destination discussed
- The informants mark on the ground the starting point and destination for different resources or services.

Application

The PRA team and informant record resources in short supply and the reasons for not having in the community. The community action plans include ways and means to get critical resources in the future. The difficulties faced on the road and at the resource center (destination) taken due consideration for improvement(Clayton, 1997).

Seasonal calendars -- Variables such as rainfall, labor, income, expenditures, debt, animal fodder or pests, and harvesting periods can be drawn (or created with stones, seeds, and sticks) to show month-to-month variations and seasonal constraints and to highlight opportunities for action. An 18-month calendar can better illustrate variations than a 12-month calendar(Hahn, 1991).

Seasonal calendar

The seasonal calendar attempts to establish regular cycles or patterns of activities and occurrences within a community over 12 months.

Purpose

A seasonal calendar helps present large quantities of diverse information in a common time frame. It compares community activities. Month by month, across sectoral boundaries. It identifies cycles of activity that occur within the life of community on a regular basis, and helps determine whether there are common periods of excessive environmental problems or opportunities over the course of normal year. These yearly cycles are important in determining for example; labour availability, timing for project activity, potential absorptive capacity for new activities, times of diseases and food shortage and variation of cash flow.

What

The actual themes to be recorded will vary from community to community some of the more

commonly used topics include annual rainfall, water availability, cash and food crops, livestock, labour demand, food shortages, and human, crop and animal diseases. The calendar should show times when problems may be acute, variation in labour demand by gender and age etc.

Who

Data for seasonal calendars should be collected from community groups. If a community has two or three distinct ecological zones, groups should be selected from each so that differences in cycles are reflected in the calendar. Efforts should be made to diversify informants from community groups i.e. men and women, informal leaders, young and old residents.

Before:

- Identify what type of seasonal pattern you wish to learn.
- Find one or more people able and willing to share their knowledge and their views
- Find a suitable space, enough space and shade
- Explain the purpose of exercise carefully

During

- Ask when their year starts
- Ask how they divide the year
- Use local calendars
- Religious festivals
- Agricultural operation
- Have the informant mark the unit on the ground, floor or a paper
- Probe
- Compare quantities
- Ask the participant to mark which month is or are the most extreme
- Continue comparing each month with extreme until the whole year is completed

Materials

- Any kind of material can be used to indicate the duration or amounts i.e. seeds, stones, beans, soil, sand, leaves, pods, ash etc.

After

- Make a permanent record
- Analyze the information from different calendars
- Compare the months to identify periods of stress and comfort

Application

Information collected during the drawing of the seasonal calendar is very rich, not only in terms of what is put down by the community, but also in what comes out from the discussions during the process. For example seasonal calendar data provides information on opinions and attitudes of the community towards certain activities. These include attitudes towards gender allocation of labour, gender ownership and control of resources, etc. such information helps the PRA Team and the community

to prepare the community action plan (Chambers, 2004).

Gender daily calendar:

Purpose

Most daily activities in traditional rural societies are managed along gender lines. There are activities that are specifically performed by women, men or children. In some communities gender role divisions are still pronounced. In such cases it is necessary for the PRA team to be aware in order not to be seen as interfering with the community cultural norms specific gender roles so that new programmers are not introduced to overburden an already overworked group. Introducing gender awareness in PRA helps a community to begin examining itself (NCAER, 1993).

Who

Community members both men and women, young and old should be in attendance. PRA team members, men and women and local extension staff in the analysis of gender roles and responsibilities.

How

It is better if the community is allowed to lead gender related discussions. The PRA team facilitates discussions through a neutral process of mapping out a gender daily calendar. Men and women discuss on each daily activities on agreed season (raining or dry season). The groups on their timetable, from the time they wake up in the morning to the time they got to sleep in the evening.

Application

Gender daily calendar provides a clear picture of who does what in the community. It will help in the formulation of the community Action Plan. The community will become aware that unless some changes in gender relations are effected rural development will not proceed as quickly as they would like it to be (Holland, 1998).

_ **Daily-activity profiles** -- Researchers can explore and compare the daily-activity patterns of men, women, youth, and elders by charting the amount of time taken to complete tasks.

_ **Semi structured interviewing** -- A semi structured interviewing and listening technique uses some predetermined questions and topics but allows new topics to be pursued as the interview develops. The interviews are informal and conversational but carefully controlled (Chambers, 1994).

Semi structured interviews (SSI)

SSI is a guided interview here the major topics and a few key questions are formulated before the interview. But many new additional are asked during the interview based on answers to the key question.

Types of SSI:

1. The individual interview

- Get representative information about the society form individual informants
- Ask individuals at a time

2. The key informant interview

- Get specialized information from one or group of persons about the community
- Informants with specialized knowledge

3. Group interviews:

- Useful for obtaining general information about the community
- Better for cross checking information
- Group interviews require very careful preparation
- The ideal group is 8 – 15 people

_ Types, sequencing, and chain interviews --

Individual, pair, and group interviews are combined in a sequence to take advantage of key informants and specialist groups.

Using secondary sources

- Secondary sources of information include previously written documents maps, diagrams, tables etc
- Review secondary sources before beginning field survey is census data, aerial photos, marketing reports, etc.
- In reviewing secondary sources, you should keep summary notes, in the form of short paragraphs, diagrams, charts, etc
- In reviewing secondary sources, you should keep summary notes, in the form of short paragraph, diagrams, charts, etc.
- Be as critical as possible in reviewing secondary sources
- To develop understanding of local livelihoods
- Short period of time

Interview guides and checklist

- Formulate open – ended question and themes for free discussion
- Explore what farmers think about the theme
- Allow two way communication
- Learn as you go along rather than to answer specific questions of limited range
- Use the six helpers What? Why? How? Who? When? Where?
- Use simple questions with single idea
- Probe to explore more in depth, to stimulation
- Avoid ambiguous, leading, dead and direct questions scheduling and arrival
- Select open person to lead or control the interview
- Be sensitive to farming work or other work schedule
- Try not to disrupt working activities
- Agree on a team contract: what time to begin work cash morning? Who will take notes? (Write this in your notebook)

Beginning the Interview

- Will the team stand or sit on chairs with the informant sitting on the ground?
- Sit down in a suitable place & shade
- Begin with the traditional greeting in the local manner
- Explain who you are. Describe the purpose of your visit do not imply any promise
- If the informant is busy ask when it would be appropriate to return
- The team should say we are here to learn and mean it
- Spend some time in casual conversal
- Begin your questioning by referring to something or someone visible.
- Deal with more sensitive issues when confidence is built(Pottier, 1992).

Directing the flow of Interview

- Do not interrupt each other
- Write down new questions to ask latter on
- Ask one question at a time
- Take your time, allow your response to answer completely before moving on.
- Probe explore

Recording the interview

- Record during an interview
- Ask permission from your informants before you start writing things down or tape recording the discussion
- What to record

The interview setting

- Where was the interview held?
- Who was interviewed?
- Was it a group or individual?

Record what you see

- The condition of the farmers field
- Type of a house, possessions, access to water; indicators of wealth, health

Record what is said

- The details of an interview
- Do not attempt to analyze responses in your head and record an interpretation
- Record the conduct of the interview
- Assign an interview observer

Was it:

A Fact: Something definitely known to have occurred or be true

An opinion: judgment or belief base on grounds short of proof

A rumor: general talk, report of doubtful accuracy.

- Was the interview relaxed and open?
- Was it dominated by any individuals?
- Did the interviewer bias the response
- Judge the responses quickly
- Cross – check by comparing responses against other sources of information

- “Look wonder, questions”

Closing the interview

- Summarise the discussion briefly
- Look around the homestead or farm
- Ask respondents, if they have other issues to be looked in to
- Thank respondents graciously
- Take a few minutes with your colleagues to reflect on the interview and compare thoughts and impressions
- Make any additional notes you feel are necessary
- Fill in the blanks in your notebook while the interview is fresh in your mind
 - Once done move on to the next household; or groups

_ **Permanent-group interviews** -- Established groups, farmers' groups, or people using the same water source can be interviewed together. This technique can help identify collective problems or solutions.

_ **Time lines** -- Major historical community events and changes are dated and listed. Understanding the cycles of change can help communities focus on future actions and information requirements.

Time related data analysis**Time Lines**

A list of key events in the history of the community that helps identify past trends, events, problems, and achievements in its life.

Purpose

The time line helps the team to understand what local or national events the community considers to be important in its history. The time line is prepared through discussion wit a small groups or elders. The significant events in the history of the community hitherto kept in oral form are now documented.

What

The time lines go back as many generations as villagers can recall. Time line records could include, forest history, diseases, diets etc. These discussions provide a good opportunity to ask elders about previous trends and traditional responses, as well as about possible opportunities to resolve current problems.

Time lines are recorded by the community elders and any other long term resident. The team can only assist by asking proving questions. The composition of the community institutions, such as church groups, selfgroups, political units or the local administrations. Both women and men should be included(Ingilis, 1990).

How

Group discussions are preferred to interviews of key individuals because they encourage dialogue among the community members and Addis, helping them

remember events from the distant past. The elders should be asked to identify that shaped and influenced individual and the community activity large sheets of paper and felt pens should be used to write in large letter in the local language. If there is difficulty in establishing dates for particular events, try to relate them to a renown event.

Application

This data re-in forces the community's perception of the evolving problems and the possible opportunities to be considered in the preparation of the CAP.

Trend Lines

Trend lines are helpful to understand the resident's perception of significant changes in the communities over time.

Purpose

Trend analysis will help the community to and PRA team to:

- Learn from the community how it views change over time in various sectors
- Integrate key changes into a village profile, which well simplify problem identification and
- Begin to organize the range of opportunities for the community to consider.

What

Information could be collected on trends over the past ten to forty years in the areas that support the community. A core set of trends should include changes in water availability, soil loss and fertility, deforestation and tree planting, grazing, employment rates food production and population.

Trend lines can be discussed with community representatives including elders, long term residents, leaders of church groups, women groups, and self help groups. If possible, all ecological zones in the study site should be represented.

How

The following steps are important for proper generation of trend lines.

1. PRA team should carefully explain the measuring of trend lines to the community groups.
2. Explain the concept of trend lines using simple graphs. Demonstrate the meaning of the two lines. Show them how time moves from left to right along the horizontal axis, and how the rate of increase/decrease in resource use is indicated on the upright vertical axis.
3. When the community members have understood the concept, ask one of them to draw the two lines on the ground. Once this is done, years should be indicated at equal intervals along the horizontal line.
4. Use the discussion of trends to probe for explanation of the changes. This will help identify underlying problems and traditional activities to

correct the situation. Find out what solutions have been tried in the past and how well they have worked.

Application

Trend lines provide useful baseline information for researchers operating on micro level follow ups of such aspects as population, food availability, school enrollment etc. It forms the basis upon which problem identification and options assessment is made during the preparation of the community action plan

_ **Local histories** -- Local histories are similar to time lines but give a more detailed account of how things have changed or are changing. For example, histories can be developed for crops, population changes, community health trends and epidemics, education changes, road developments, and trees and forests.

_ **Local researchers and village analysts** -- With some training, local people can conduct the research process (for example, collect, analyze, use, and present data; conduct transects; interview other villagers; draw maps; make observations).

_ **Venn diagrams** -- To show the relationship between things, overlapping circles are used to represent people, villages, or institutions; lines are added to reflect inputs and outputs.

Venn Diagram (Institutional analysis)

Venn diagramming is a method to find out who, what person or organizations are important in and for a community.

Purpose

To identify groups and institutions operating in the community and to show how they interact with each other To show the degree of their cooperation and involvement in development programs. To discover their important or influence on decision making in the community.

What?

Venn diagrams have been used with in PRA in institutional context to discuss:

- The role and significance of various institutions
- Levels of communication between organizations
- The role of project bodies and their intervention Improving missing links between existing organizations,
- Potential for working through existing organizations, which ones and with which links.
- Potential roles for new organization
- Formal and non-formal groups and their levels of cooperation
- Communities perceptions of the institutions, always

Who?

The PRA team, key informants as elders, religious leaders, extension staff and other knowledgeable person take the responsibility of listing and

evaluation of individuals and institutions influencing decision making of the community.

How?

List institutions in the community and discuss importance of each institution and what they do. Make different sized circles and not which circle represents each institution i.e. big circle very important and decision maker, small circle with little importance. During overlapping the circles, the size of the circle indicates the importance of the institution, the distance between the circles indicate the degree of contact between institutions. For instance a large overlap high interaction. No overlap distant relationship.

Application

Identify individuals, groups or institutions. Important in the lives of people and establish close relationship with them. Provide the necessary support and effectively utilize their skills and experiences.

– **Participatory diagramming** -- People are encouraged to display their knowledge on pie and bar charts and flow diagrams.

– **Wealth and well-being rankings** -- People are asked to sort cards (or slips of paper) representing individuals or households from rich to poor or from sick to healthy. This technique can be used for crosschecking information and for initiating discussions on a specific topic (for example, poverty). The technique can also be used to produce a benchmark against which future development interventions can be measured or evaluated (Blackburn, 1999).

Ranking and scoring

Presentation:

A way in which various kinds of things can be compared according to different qualities people value. It places in an order of what is more or what is less important.

Purpose

Ranking methods allow us to see individual and group priorities among a number of alternative problems or solutions. It helps to generate reasons why people choose one item from the other.

What

People could use three different ways to generate a criteria for comparison and make up their choices.

- (1) preference ranking
- (2) pairwise ranking
- (3) direct matrix ranking
- (4) and direct matrix scoring

Preference ranking method helps to quickly get a good idea of what people think are the priority problem or preferences. The criteria attached to make up a choice is used to consider in the action plan. Individuals or groups vote on the items from most

important to least important item. The choices could be between crop varieties, water points, food diets, livestock species, problems, solutions and many different issues, which require preferences. Paired wise ranking is used to compare between two items and make up a choice. It is more useful for exploring the reasons why people prefer one possibility over another. The moment a preference is made lots of criteria are explored to compare items using a group of criteria before a choice. Direct matrix ranking is used to list items to be compared along horizontal line and criteria on the vertical line to rank choices from most important to least important (i.e. 1st, 2nd, 3rd, 4th etc) In this case frequency of the items valued as the 1st choice helps to make up a final decision. Direct matrix scoring helps to attach a score to a comparable items against each criteria listed before a choice. A comparison could be made out of a score of 10 (for instance) a comparison could be made between many items against one criteria set, and attach a score out of a maximum of 10 to items to be chosen. The frequencies of the highest scores (closer to 10) attached against many criteria helps to make up a decision for preference.

Who

Ranking and scoring could be done with individuals, households, community members deliberately selected and with mixed group of men, women, traditional leaders, local officials, extension workers etc. The group combination depends upon the issues to be ranked. Who should decision on the issues to be compared? Leads to the choice of informants.

How

The groups for discussion lists items to be compared. Let them generate either directly or thorough pairwise comparison criteria for ranking. Putting in an order of importance or ranking could be done through ranking order, scoring or key voting, from the most to least important. Thorough courting frequencies list in ranked order the items to be compared and make up a decision. The final choice could be made through group of criteria or a single but most important criteria. Some times, the period for ranking (emergency) or vested need to the item may influence decision-making procedures. While listing criteria, do not mix up. PRA teams criteria with those of the informants. Use positive criteria for comparison

Application

Community action plans are developed on the basis of peoples preferences. The problems, solutions technical inputs etc are arranged on the interests of the users (Appleyard, 1998).

– **Direct-matrix pair-wise ranking and scoring** -- Direct-matrix pair-wise ranking and scoring is a tool used to discover local attitudes on various topics.

People rank and compare individual items, using their own categories and criteria, by raising hands or placing representative objects on a board. For example, six different shrubs can be ranked from best to worst for their fuel, fodder, and erosion-control attributes. Other resources can be ranked in terms of taste or marketability. Wealth ranking can be used to identify wealth criteria and establish the relative position of households (Carmen, 1996).

_ **Matrices** -- Matrices can be used to gather information and to facilitate or focus analyses and discussions. For example, a problem opportunity matrix could have columns with the following labels: soil type, land use, cropping patterns, and available resources; and rows with the following labels: problems, constraints, local solutions, and initiatives already tried.

_ **Traditional management systems and local-resource collections** -- Local people collect samples (for example, of soils, plants). This can be an efficient way to learn about the local biodiversity, management systems, and taxonomies.

_ **Portraits, profiles, case studies, and stories** -- Household histories or stories of how a certain conflict was resolved are recorded. This can provide short but insightful descriptions of characteristic problems and how they are dealt with.

_ **Key probes** -- A question addressing a key issue is asked of different informants, and the answers are compared. The question might be something like "If my goat enters your field and eats your crops, what do you and I do?"

_ **Folklore, songs, poetry, and dance** -- Local folklore, songs, dance, and poetry are analyzed to provide insight into values, history, practices, and beliefs.

_ **Futures possible** -- People are asked how they would like things to be in 1 year and to predict what will happen if nothing is done or if something is done. People's desires, wishes, and expectations are revealed.

_ **Diagrams exhibition** -- Diagrams, maps, charts, and photos of the research activity are displayed in a public place to share information, facilitate discussions, and provide an additional crosschecking device. The exhibition can inspire other villagers to take part in research activities.

_ **Shared presentations and analysis** -- Participants are encouraged to present their findings to other villagers and to outsiders, providing another opportunity for crosschecking, feedback, comment, and criticism.

_ **Night halts** -- The researchers live in the village during the research process. This facilitates all interactions between the outsiders and the villagers, invites change in the outsiders' attitudes, and allows

for early-morning and evening discussions, when villagers tend to have more leisure time.

_ **Short questionnaires** -- Short and issue-specific questionnaires can be useful if conducted late in the research process.

_ **Field report writing** -- Key findings are recorded before "leaving" the village. (This assumes that the community has consented to having the research data leave the village.) Brief summaries are made of each diagram, model, and map, as well as of the process involved in creating them.

_ **Survey of villagers' attitudes toward PRA** -- To improve the PRA process and techniques and maintain realistic expectations, the researcher asks the villagers what they expected and what they learned from the PRA research process.

_ **Intriguing practices and beliefs** -- Indigenous practices and beliefs are noted, even if they are based on myth or superstition. Even practices that are unusual or don't fit in with conventional scientific thinking are worth exploring because they are meaningful to local people.

CONCLUSION:

As a result of the PRAs, the communities are expected to attain many benefits including:

- Expressing their own ideas and concerns;
- Organizing their knowledge about the past and present;
- Identifying as a community their problems, the causes of these problems and possible solutions;
- Developing a common plan to address these problems;
- Developing the ability to use their own resources more effectively and attract more resources from the outside.

The academicians/researchers involved in the PRAs are expected to get the following benefits:

- Developing better understanding of rural environments and social as well as economic dynamism taking place there;
- Appreciating the fact that communities are capable of analyzing their problems and outlining possible solutions to their problems;
- Participating in designing possible solutions to community problems;
- Utilizing the results of the PRA work as a research output for publications and presentations;
- Building their research and problem investigation capabilities;
- Supporting their classroom discussions to students with practical examples from the PRA findings.

The main objectives of the current PRA are:

1. empowerment of rural communities by assisting them to systematically utilize their local knowledge

to identify problems and strengths, develop skills of analysis, and design appropriate mechanisms for intervention by themselves and/or by development agents;

2. advancement of understanding by academicians/researchers of local knowledge and acknowledgement of the capacity of communities to gather data, conduct analysis, and identify as well as prioritize problems and solutions;

3. utilization of the research questions/problems identified during the PRAs for further investigation;

4. documenting and presenting the outcomes of the PRAs to development agents (governmental and non-governmental) and other stakeholders so that they could undertake interventions in line with the findings.

PRA consists of a series of participatory exercises which help community members better assess their history, resources, and overall situation as concerns agriculture, health, marketing, credit, coping mechanisms, education, and other important areas.

During the conduct of the PRAs, rural communities in the selected villages will gather information on the resources they already possess; organize their knowledge; share experience among themselves; learn from each other; identify and prioritize local development needs; and develop action plans which respond to these needs.

***Corresponding Author:**

Sharareh Khodamoradi

Department of Agricultural Extension Education,

Science and Research Branch, Islamic Azad

University, Tehran, Iran.

E-mail: skhodamoradi2007@yahoo.com

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