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Role of Selenium in Attenuating Cardiac and Hepatic Damages Induced By the Antitumor Agent, Doxorubicin

Safinaz S. Ibrahim*, Maged A. Barakat and Hebatalla M. Helmy

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Abstract: Background and Objectives: The clinical use of doxorubicin, one of the most effective antitumor agents, soon proved to be hampered by such serious   problems   as the development of cardiomyopathy and liver damage. The current study aims at evaluating the role of trace element, selenium, in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. **Materials and Methods**: Animals were divided into normal control group and doxorubicin -treated group injecting doxorubicin i.p. as 6 equal doses of 2.5 mg/kg, twice weekly/ 3 weeks. The doxorubicin - treated animals were divided into 2 groups, one kept without further treatment (doxorubicin -group), second group, (doxorubicin + selenium) received selenium (Na Selenite) 0.5 mg/kg orally, 3 times/week/4 weeks including one week before the doxorubicin 1st dose. Serum creatine phosphokinase, lactate dehydrogenase, as cardiac damage markers, and alanine aminotransferase, as indicator of hepatic damage, were measured. Malondialdehyde and nitric oxide levels, as cardiac oxidative status indices, cardiac glutathione content, glutathione peroxidase, glutathione-S–transferase and superoxide dismutase activities, as measures for cardiac antioxidant capacity, were also investigated. Histopathological changes in cardiac and liver tissues were examined. The results were analyzed statistically by one-way analysis of variance with subsequent multiple comparisons using Tukey test. **Results:** doxorubicin induced significant increase in serum lactate dehydrogenase; creatine phosphokinase; alanine aminotransferase activities, cardiac nitric oxide, malondialdehyde levels, superoxide dismutase, glutathione peroxidase, glutathione-S–transferase activities, and reduction in glutathione content. Selenium co-administration caused significant decrease in serum lactate dehydrogenase and creatine phosphokinase levels; normalization of serum alanine aminotransferase; significant decrease in cardiac malondialdehyde, nitric oxide levels, glutathione peroxidase, glutathione-S–transferase, superoxide dismutase activities and significant elevation in cardiac glutathione content, compared to doxorubicin -treated group values. Histopathological examination of cardiac and liver tissues supported the previous biochemical results. **Conclusions:** Chronic doxorubicin administration caused cardiomyopathy and hepatic damage. Selenium co-administration produced partial, but significant, protection against cardiomyocyte damage; however, it alleviated hepatic damage-induced by the antitumor agent, doxorubicin.

Key words: Doxorubicin, cardiomyopathy, liver damage, selenium

1. Introduction

Anthracyclines rank among the most effective anticancer drugs ever developed [1]. The first anthracycline was isolated early in the 1960s from the pigment producing Streptomyces Peucetius, a species of actinobacteria [2] and   was named doxorubicin   (Dox). Doxorubicin is an essential component of treatment of breast cancer [3], soft tissue sarcomas [4] and many other cancers [5]**.** Because Dox has been shown to produce free radicals, it was suggested earlier that free radical injury might be a mechanism of Dox antitumor activity [6]. There now appears to be general agreement that oxidative stress is unlikely to be a significant contributor to the antitumor activity of Dox [7]**.** Liver is the main site of Dox metabolism, reduction of side chain carbonyl group by NADPH-

Cyto P450 yields a more polar and toxic metabolite, doxorubicinol. Such metabolite accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity of Dox [8]. The enormous value of Dox in treating a variety of solid and hematologic malignant conditions is unquestioned. However, as with any other anticancer agent, the clinical use of Dox soon proved to be hampered by such serious problems as the development of resistance in tumor cells [9] or toxicity in healthy tissues, most notably in the form of chronic cardiomyopathy and congestive heart failure[1]. These adverse effects of the drug can preclude its use in some patients and limit the duration of its use in many others [10].

Selenium (Se) plays an important biological role in living organisms, mostly through its

incorporation in a family of proteins, selenoproteins. The main biological form of Se is selenocysteine, a cysteine analog that is synthesized from a serine bound to tRNA. The biological roles ascribed to Se include the prevention of cardiovascular disease [11] and cancer [12]. In the heart, Se supplementation caused increase in the cardiomyocyte glutathione peroxidase (GPx) activity, the total antioxidant activity, glutathione (GSH) concentration and catalase activity, leading to decreased generation of reactive oxygen species (ROS)[13]. The present study aims at evaluating the attenuating effect of Se, as an adjuvant therapy, on Dox- induced cardiac and hepatic damages.

2. Materials and methods.

A- Animals:

Total numbers of 32 male albino rats of the Wister strain, weighing 170-200 g, were used in the present study. The animals were obtained from the central animal facility at the Faculty of Pharmacy, Cairo University, Cairo, Egypt. All rats were housed in a room with a controlled environment, at a constant temperature of 23 ± 1^0C, humidity of 60% ± 10%, and a 12 hrs light/dark cycle. The animals were housed in groups and kept at constant nutritional conditions throughout the experimental period. The experimental protocols were approved by the Ethical Committee of Cairo University.

B- Drugs and chemicals:

Doxorubicin HCL was obtained from Pharmacia & Upjohn, Milan, Italy. Sodium selenite was obtained from Sigma Chemical Company, USA. Other chemicals in the experiments were of analytical pure grade and supplied by British Drug House (BDH, UK), Merk (Germany) and Sigma Chemical Company (USA).

C- Experimental design:

Animals were divided into a normal control group (10 rats), receiving the appropriate volume of saline i.p, and Dox-treated group. Doxorubicin was dissolved in saline and injected i.p., as a total cumulative dose equal to 15 mg/kg, divided into 6 equal doses, 2.5 mg/kg each. They were injected twice weekly/ 3 weeks [14]. Dox-treated animals were divided into tow groups, one kept without further treatment (Dox-group), and a second group (Dox + Se) received Se, as sodium selenite, 0.5 mg/kg, orally, 3 times/week/4 weeks including one week before the 1st Dox dose[15].

D- Serum and tissue sampling:

24 hours following the last Dox injection, rates were sacrificed by decapitation. Blood sample of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at 3000 r.p.m. /15 minutes and used to determine creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). Serum CPK activity was determined using a kit provided by STANBIO, USA. CPK catalyses the transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions. NADH is provided at a rate directly proportional to CPK activity. The method determines NADH absorbance increase per minute at 340 nm [16]. Serum LDH activity was determined using a kit provided, also, by STANBIO, USA. LDH specifically catalyzes the oxidation of lactate into pyruvate with subsequent reduction of NAD to NADH. Rate of NADH formation is proportional to LDH activity. The method described determines NADH absorbance increase per minute at 340 nm [17]. Serum ALT activity was determined, using a kit provided by Quimica Clinica Aplicada, Spain[18].

Histopathological study:

The hearts and livers were removed by dissection, washed by ice-cold isotonic saline and blotted between two filter papers. Autopsy samples were taken from heart and liver in different groups of rats and fixed in 10% formol saline for 24 hrs. Washing was done, then, serial dilutions of alcohol were used for dehydration. Paraffin bees wax tissue blocks were prepared for sectioning the studied tissues. The obtained sections were stained by hematoxylin and eosin stains [19] for histopathological examinations through the light microscope.

Biochemical parameters:

10% w/v homogenate was prepared in ice-cold deionized water for the remainder of the heart tissues of the different groups.

Measurement of cardiac oxidative status indices:

A portion of the homogenate was mixed with ice-cold 2.3% KCL (in ratio of 1:1) and centrifuged at 3000 r.p.m./15 minutes. Thiobarbituric acid (TBA) – reactive substance, malodialdehyde (MDA), content was determined in the supernatant [20], depending on measuring the coloured complex formed between TBA and MDA in acidic medium. Another aliquot of homogenate is centrifuged at 17.000 r.p.m./4ºC/20 minutes. The resulted supernatant was used for the determination of nitric oxide (NO), as nitrite (NO_2^-) and nitrate (NO_3^-) concentrations [21], using Griess reagent after the enzymatic reduction of nitrate to nitrite. The Griess reaction involves the reaction of nitrite with

sulfanilamide in an acidic solution to yield a diazonium salt, followed by coupling with N-(1-naphthyl) ethylenediamine to yield a colored azo dye that can be measured colourimetrically at 540 nm. Measurement of some cardiac antioxidant systems:

A portion of homogenate was mixed with ice-cold 7.5% sulfosalicylic acid (in a ratio 1:1) and centrifuged at 3000 r.p.m/ 15 minutes. The resulted supernatant was used for determination of GSH [22]. Another part of homogenate was mixed with equal volume of ice-cold Tris- EDTA buffer (pH =7.6), centrifuged at 39.000 r.p.m./ 4º C/ 20 minutes. The supernatant was used for determination of superoxide dismutase SOD; glutathione peroxidise (GPx) and glutathione S-transferase (GST). Determination of GST activity [23, 24] depends on the ability of GST to catalyze the formation of glutathione adduct with 1-chloro,2,4 dinitrobenzene(CDNB). This adduct was measured by noting the net increase in absorbance at 340nm. Determination of GPx [25] depends on measuring the rate of oxidized GSH formation, by following up the decrease in absorbance of the reaction at 340 nm as NADPH was converted to NADP. Superoxide dismutase activity was determined [26], depending on the fact that the spontaneous autoxidation of pyrogallol, at alkaline pH less than 9.5, produces superoxide anion, which in turn enhances further oxidation of pyrogallol with a resultant increase in absorbance at 420 nm. The presence of SOD in the reaction medium retards pyrogallol autoxidation by scavenging the formed superoxide anion.

Statistical analysis:

The results were analyzed statistically by one-way analysis of variance (ANOVA test) with subsequent multiple comparisons using Tukey test. Differences were considered statistically significant at p less than 0.05. The results were presented as the mean ± standard error of the mean (SEM). Data obtained were submitted to a computerized statistical treatment using SPSS statistical package, version 17. Graphs were represented by Harvard graphics version 4 computer program.

3. Results:

Results revealed that Dox caused significant increase in serum levels of LDH and CPK, amounting to 182.4% and 183.6% respectively, as compared to the normal values (Fig. 1). Selenium co-administration caused significant decrease in the activities of LDH and CPK reaching to 139.5% and 153.6%, respectively of the control values. Figure (2) illustrated that, Dox caused a significant increase in cardiac MDA and NO contents, amounting to 183.36% and 177.7%, respectively, compared to the

control values. Concomitant administration with Se caused significant decrease in MDA and NO levels reaching to 126% and 120%, compared to the control values.

As shown in figure (3), Dox administration caused a significant decrease in cardiac GSH level reaching to 64% of the normal values. Co-administration of Se significantly elevated GSH content to about 77.9% of the control values. Figure (4) showed significant increases in cardiac activities of GPx and GST in the Dox-treated rats, amounting to 410% and 184% respectively, compared to the normal values. Meanwhile, the co-administration of Se caused significant decrease in the levels of GPx and GST to about 166% and 136% of the normal values. Figure (5) showed significant increases in cardiac activity of SOD in the Dox-treated rats amounting to 225% compared to the normal value. Co-administration of Se caused significant decrease in the level of SOD to about 172% of the normal values.

Results of figure (6) revealed that Dox administration caused significant elevation in the serum ALT level to reach 118% of the normal control level. Selenium co-administration caused normalization of the elevated ALT level.

Cardiac histopathological results showed that; in the control sections, the cardiac muscle fibers were grouped in bundles with connective tissue in between. The single muscle fiber had acidophilic cytoplasm and a central nucleus (Figure 7). In the cardiac sections obtained from rats administrated Dox, hyalinization was observed in the myocardial bundles associated with either inflammatory cells infiltration only or inflammatory cells and edema in focal manner in between the bundles. Edema was also noticed in the subendocardial layer. The subendocardial adipose tissue was infiltrated by inflammatory cells (Figures 8, 9, 10). In the cardiac sections obtained from rats administrated Dox + Se, there was mild hyalinization in the myocardial muscle bundles (Figure 11).

Examination of liver sections of the different groups illustrated that: Liver tissue of the normal group showed hepatic lobules with normal architecture (Figure 12). In case of liver sections of rats administrated Dox, congestion was observed in the central vein, in addition to kupffer cells proliferation in diffuse manner between the fatty degenerated hepatocytes (Figures 13). In case of liver sections of rats administrated Dox + Se, least liver damage was shown, just kupffer cells proliferation was observed in between hepatocytes (Figure 14).

4. Discussion:

Doxorubicin-induced cardiomyopathy has long been a serious side effect in treating human cancers, which limits the clinical dosage of Dox [27]. The mechanism of Dox-induced cardiotoxicity is attributed to the formation of ROS and subsequent changes of membrane fluidity and integrity. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to Dox cardiotoxicity [28]. Nutritional strategies designed to augment cellular defense systems have been identified as a promising approach to combat oxidative stress- associated disease conditions. In this respect, dietary supplementation with Se, potentially adjusting antioxidant enzymatic status, could offer protection in preventing free radical-induced cardiac injury. In the present study, role of trace element, selenium, in attenuating cardiac and hepatic damages induced by antitumor agent, doxorubicin was studied.

Fig (1): Effect of selenium on doxorubicin- induced alterations in serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities in rats.

Fig (2): Effect of Selenium on doxorubicin- induced alterations in cardiac malondialdehyde (MDA) and nitric oxide (NO) levels in rats.

Fig (3): Effect of Selenium on doxorubicin- induced alterations in cardiac glutathione (GSH) content in rats.

Fig (4): Effect of Selenium on doxorubicin- induced alterations in cardiac glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities in rats.

Fig (5): Effect of Selenium on doxorubicin- induced alterations in cardiac superoxide dismutase (SOD) activity in rats.

Fig (6): Effect of Selenium on doxorubicin- induced alteration in serum alanine aminotransferase (ALT) activity in rats.

Figure(7): A photomicrograph of cardiac muscle fibers of control group showing normal histological strucrure of myocardium(M) (H&E x160).
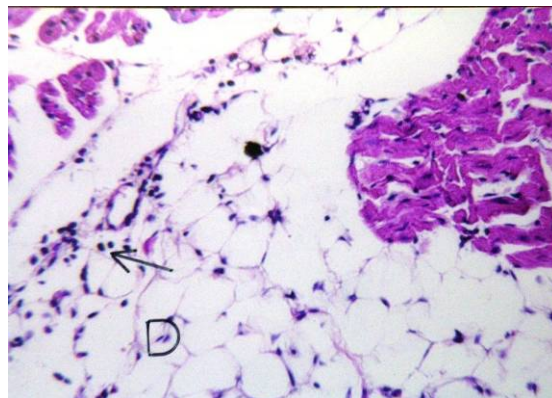
Figure(10): A photomicrograph of cardiac muscle fibers of Dox group showing inflammatory cells infiltration(arrow) in the subendocardial adipose tissue(D) (H&E x64).
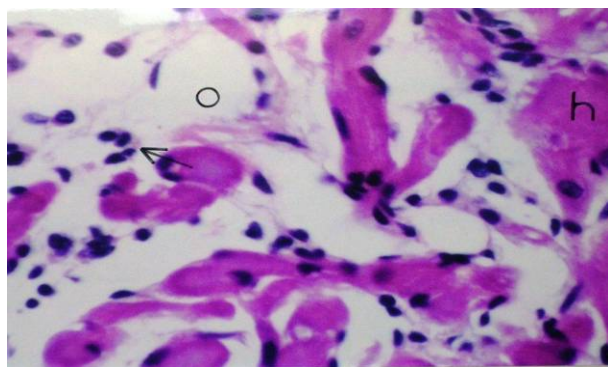
Figure(8): A photomicrograph of cardiac muscle fibers of Dxo group showing Oedema(o) with inflammatory cells infiltration(arrow) in focal manner between the myocardial bundles. (H&E x160).
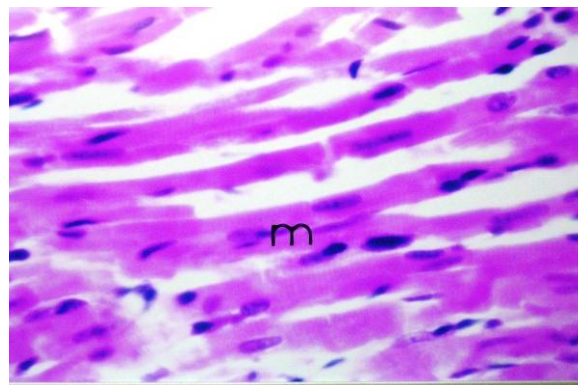
Figure(11): A photomicrograph of cardiac muscle fibers of Dox+Se group showing mild hyalinization in myocardial bundles(m) (H&E x160).
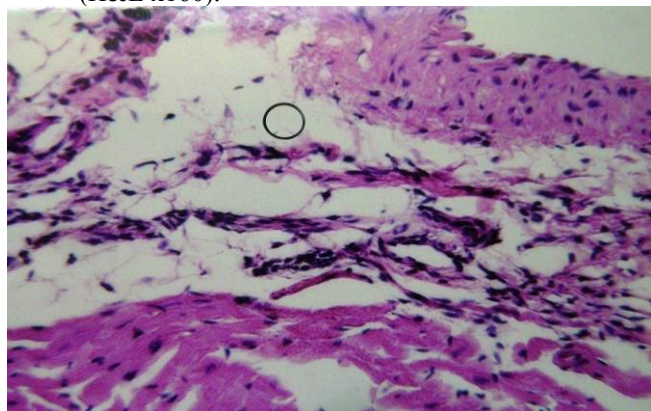
Figure(9): A photomicrograph of cardiac muscle fibers of Dox group showing Subendocardial oedema(o). (H&E x64).
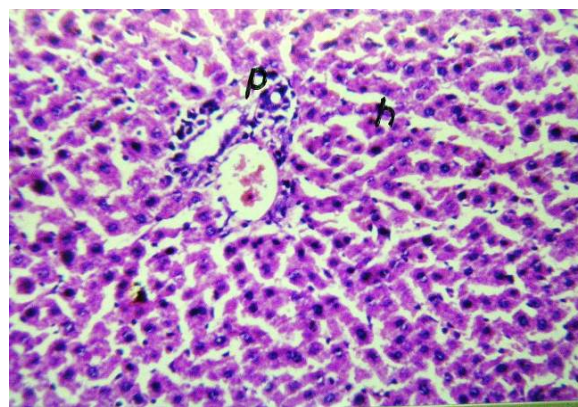
Figure (12): Photomicrograph of liver of normal group showed hepatic lobules (h) and portal vein (p) with normal architecture (H&E x64).
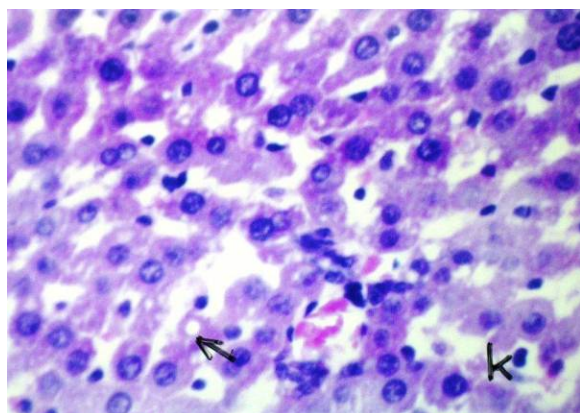
Figure(13): Photomicrograph of liver of Dox group showing diffuse kuffer cells proliferation(k) inbetween the fatty degenrated hepatocytes (arrow) (H&E x160).
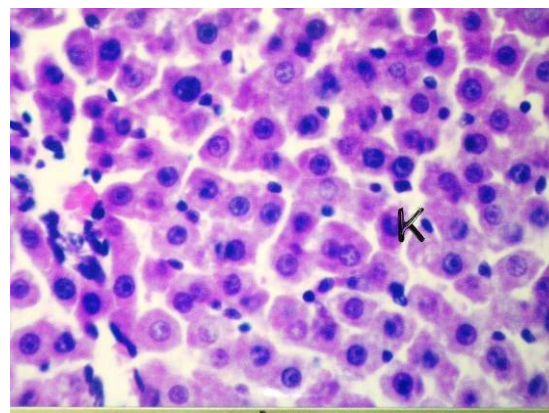
Figure (14): Photomicrograph of liver of Dox + Se group showing diffuse kuffer cells proliferation(k) inbetween the fatty hepatocytes (H&Ex160).

Results of the present study revealed that 15 mg/kg total cumulative dose of Dox induced cardiac and hepatic damages, manifested biochemically by significant increase in serum activities of LDH; CPK and ALT. Additionally, Dox caused elevation in cardiac NO, MDA levels, SOD, GPx, GST activities, and reduction in GSH content. Histopathological examination of heart and liver sections of Dox-treated animals supported these biochemical results. Selenium administration, concomitant to Dox therapy, caused significant decrease in the serum activities of LDH and CPK; cardiac MDA, NO levels, GPx, GST and SOD activities and significant elevation in cardiac GSH content, compared to Dox-treated group values, as well as normalization of serum ALT level.

The present results showed significant increase in serum levels of LDH and CPK in Dox-treated group. These enzymes are considered important markers of cardiac injury. Many previous studies have demonstrated similar results in rats following Dox administration [29, 30]. Different types of Dox cardiotoxicity can be recognized [31]: "Acute" cardiotoxicity occurs during Dox administration, however, these effects are never of major concern because these are generally reversible and/or clinically manageable. "Early chronic" cardiotoxicity develops later in the Dox treatment course and characterized by dilated cardiomyopathy, with subsequent development of congestive heart failure [32]. It is now well established that Dox cardiotoxicity may manifest even decades after the completion of anticancer treatment [33]. Co-administration of Se with Dox therapy resulted in decrease in the elevated activities of serum LDH and CPK. This finding is in harmony with this stated by Simoni et al.[34], who reported that the elevation in

serum LDH activity, as a result of hemoglobin cardiotoxicity, is significantly decreased by Se dietary supplementation. Our biochemical results are supported by the histopathological examination of the cardiac tissue, since, the marked morphological changes shown in the hearts of Dox- treated animals have been partially preserved by Se administration.

Dox therapy caused significant increase in MDA level. Previous studies reported similar results [35, 36]. This elevation might be attributed to Dox mediated oxidative stress. Heart tissue is rich in mitochondria, which occupy about forty percent of the total intracellular volume of cardiomyocytes [37]. Dox has high affinity for cardiolipin, a negatively charged phospholipid abundant in the mitochondrial inner membrane, leading to mitochondrial accumulation of Dox [38]. Under clinically relevant plasma Dox concentrations, the heart becomes a site of redox reactivity. The quinone functionality of Dox is transformed, in the presence of NADH, into a semiquinone via one-electron reduction by complex I of the electron transport chain [39]. The semiquinone form reacts with O_2 to produce a superoxide radical (O •-), whereby Dox returns to the quinone form. The cycling of Dox between quinone and semiquinone generates large amounts of O_2, which further give rise to a variety of ROS/RNS species [40]. ROS can damage membrane lipids and other cellular components and consequently lead to cardiomyocyte apoptosis or death [41]. Our results showed that lipid peroxidation induced by Dox is significantly decreased in the pretreatment of Se, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies [34, 42]. Previously, it was reported that Se supplementation can protect against free radical

damages by increasing myocardial Se content and improving the expression and activity of GPx [43].

The present study revealed a marked increase in cardiac NO level in those Dox-treated rats. This finding is in agreement with the results reported by Saad *et al* [36] who used a model of doxorubicin chronic cardiotoxicity similar to that used in our study. The increase in NO level can be explained on the basis of the ability of Dox to mediate the induction of NOS expression and NO release in heart [44]. Several reports indicate that exposure of endothelial cells to H_2O_2 promotes eNOS expression [45]. Previous studies also suggested that stimulation of endothelial cells with calcium-mobilizing agents could activate eNOS [46]. Because Dox-induced toxicity is mediated by intracellular H_2O_2 as well as the calcium influx, Dox treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis. On the same line, recent study provides evidence of upregulation of iNOS gene and protein expressions in Dox-induced cardiomyopathy[47]. The concomitant overproduction of NO and ROS is known to yield highly reactive nitrogen species, peroxinitrite, which may attack and destroy important cellular biomolecules [48]. Selenium, in the present study, caused significant decrease in the elevated cardiac NO level shown in the Dox-treated group, which is in agreement with that reported by Ayaz and Turan [49]. The exact mechanism by which Se influence cardiac NO synthases expression is unknown. Of interest in this context is the report that treatment of nuclear extracts of lipopolysaccharide-activated human T cells with relatively high concentrations of selenite inhibited nuclear factor -κB binding and thus decreased NO production [49]. This is because nuclear factor -κB is a transcription factor that regulates a number of cellular genes, such as those encoding iNOS [50].

Doxorubicin administration, as shown in our results, caused a significant decrease in cardiac GSH content, which is quiet compatible with previous studies [35]**.** The overproduction of ROS, caused by Dox administration, can account for this decrease in GSH content, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted [51]. The decrease of cardiac GSH content may also be attributed to the enhanced activities of GSH metabolizing enzymes by Dox administration, as shown in the present study. One is GPx which reduces H_2O_2 and various peroxides using GSH as reducing agent.The other is GST which consumes GSH in the conjugation of Dox toxic metabolites [52]. The present study showed that cardiac GSH concentration is higher in (Se+ Dox) -treated rats rather than those administered Dox alone,

which is in agreement with recent results [53]. Such effect might be attributed to the antioxidant properties of Se and its ability to reduce Dox- induced oxidative stress. Selenium reduces the consumption of GSH by ROS [53]. Because GSH is one of the essential compounds for maintaining cell integrity [54] and the GSH redox cycle is one of the most important intracellular antioxidant systems, the increase in GSH content could be one of the mechanisms for cardiac protection by Se supplementation [53]. In addition, we assumed that the observed increase in cardiac GSH content might be related to the decreased activities of GSH-utilizing enzymes, GPx and GST, shown in the (Dox + Se) -treated group, leading to preservation of their substrate, GSH.

Our results showed significant increase in cardiac activity of SOD in the Dox-treated rats, which is consistent with some studies [35, 55]. The increase in SOD activity can be explained on the basis that the redox cycling of Dox between quinone and semiquinone forms generates large amounts of O_2 [40], which in turn stimulate SOD as an adaptive response to counteract oxidative stress. The increased activity of SOD could lead to overproduction of hydroperoxides, in consequence, GPx might be stimulated in response to the accumulated peroxides. This assumption was supported by our results, which showed a significant enhancement in cardiac GPx activity in the Dox-treated group, and also by some authors [36]. On the same line, GPx have been reported to be over expressed in Dox-treated cells, especially those tumor resistant ones [56]. The current study revealed that Se administration caused significant decrease in Dox-induced elevation in SOD cardiac activity, which still higher than that of the normal group value. This result is in harmony with several previous studies. Selenium has been reported to decrease the elevated activity of SOD in heart as a result to cadmium toxicity [57] and hemoglobin mediated cardiotoxicity [34], which used Se dose similar to that used in our study. It is now well established that Se, through its incorporation in selenoproteins, could actively protect against free radicals generation, and hence, ROS- induced damage [58]. As a result of this protective effect, Se consumption could attenuate superoxide radical production, and consequently, decreased the activity of such antioxidant enzyme. Also, our study showed that pretreatment with Se relieved Dox induced hyperactivity of cardiac GPx, which is in harmony with Ayaz and Turan [49]. Glutathione peroxidase is one of the most active antioxidant enzymes in the myocardium [59], and selenium, present in its active site, is essential for its activity. One of the major roles of this essential trace element in the body is to act as cofactor of this key antioxidant enzyme in which it

contributes to both catalytic activity and spatial conformation [60]. Therefore, any significant modification of Se status would lead to changes in the activity of GPx and have important consequences on the susceptibly of the tissues to oxidative stress [13, 61].Selenium has prophylactic action, when it is administered before doxorubicin, it increases myocardial selenium content and improves both expression and activity of GPx. This may account for the increased cardiac GPx activity in (Se+ Dox)-treated group, compared to the normal control value, as shown in our results. Upon pretreatment with Se, myocardial tissues became already protected, and therefore, when exposed to Dox, we assume that there is no need for farther dramatic increase in cardiac GPx activity as an adaptive mechanism. This might be an explanation for the decrease in GPx cardiac activity in (Se+Dox) group value, compared to that result shown in the group treated with Dox alone. Additionally, the obtained biochemical results were supported by each other, since, as we mentioned later, Se supplementation caused decrease in the SOD activity, which consequently, leads to decreased production of H_2O_2 and hence, decreased activity of GPx enzyme.

Results of the present study revealed significant increase in cardiac GST activity in rats treated with Dox, which is in agreement with many studies [62]. GSTs are family of dimeric proteins that posses a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics [63]. It has been reported that cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes including GST [64]. Indeed, GSTs belong to phase II enzymes that in contrast to phase I, who can participate in both metabolic activation and inactivation, predominately participate in the detoxification of xenobiotics [65]. It has been known that Dox is metabolized via alkedoreductases yielding C13 hydroxyl derivative, doxorubicinol. This metabolite is actually more polar and toxic than Dox itself. Doxorubicinol accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity induced by Dox [8]. It has been reported that Dox toxic metabolites are efficiently conjugated with reduced GSH, a reaction that is catalyzed by GST [52]. Moreover, GST, due to its peroxidase activity, can serve to reduce Dox-induced peroxides [52]. In brief, GST has showed elevation after Dox injection to detoxify Dox and its metabolites and to attenuate the elevated oxidative stress [66]. The current study revealed that Se caused significant decrease in Dox-induced elevation in GST cardiac activity, which still higher than that of the normal group value. This result is in agreement with

previous studies stated that Se afforded reduction in cadmium- induced elevation in GST cardiac activity [57]. The protective effects of Se are mainly related to its physiological antioxidant properties, and hence, decreased generation of ROS and RNS. Thus, Se supplementation could decrease the activity of GST enzyme responsible for the detoxification of such free radicals.

Our results showed an elevation in serum ALT upon Dox administration which agrees with many previous studies [67, 68] and supported by the present histopathological examinations. Doxorubicin-induced hepatotoxicity might be less severe than its cardiotoxicity, which can be related to the fact that liver mitochondria, unlike cardiac mitochondria, lack the NADH-related pathway of reducing equivalents from the cytosol to the respiratory chain. As a result, liver mitochondria do not generate significant amounts of Dox semiquinones [69]. Selenium co-administration was shown to decrease the elevated serum ALT when administered with Dox, as reported previously [34]. Selenium supplementation could reduce hepatotoxicity by rendering hepatic tissues less susceptible to lipoperoxidative attack by the drug [70]. Selenium prevents hepatocyte oxidative damage and thus leakage of liver enzymes into serum as in the cases of cadmium hepatotoxicity [71]. This biochemical result is supported by the histopathological examination of liver sections of the different groups which illustrated that, in the liver sections of rats administrated Dox, congestion and kupffer cells proliferation were observed, while sections from rats administered Dox+Se showed least liver damage.

In conclusion, Se supplementation produced partial, but significant, protection against Dox-induced cardiomyocyte damage; however, such trace element could alleviate the Dox- induced hepatic damage, as evidenced by the biochemical measurements and histopathological examinations of the cardiac and hepatic tissues.

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The Impacts of Globalization on Rural Communities of *Kermanshah* Township, Iran

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Abstract: Globalization, as a process, analyzed in consideration of communicative instruments, development possibilities, and the expanded nature of capitalism. The purpose of this study was to investigate impacts of globalization on economical and cultural aspects of rural communities in Kermanshah Township. The study represented correlation research. The statistical population of this research was selected among 700 villages located in Kermanshah Township. Among these, 30 villages were selected in three different regions for gathering data by multi-stage sampling. For data analysis multivariate procedures were used. This research examined eight factors of agro-industrialization, increasing in poverty and inequality, change in employment patterns, economic restructuring, role of nation-state, role of media, cultural universalism, cultural particularize and their impacts on rural communities progress. The results showed that there was a significant correlation between independent and dependent variables (p<0.01). On the base of regression analysis independent variables can be predicted 62 percent of changes in dependent variable. Structural Equation Modeling (SEM) certificated regression analysis results. Path analysis results showed that the model fitted the data with acceptable fit indices: chi square=5.341, p=0.376, RMSEA=0.013, CFI=1.000, NFI=0.992.

1. Introduction

The province of Kermanshah is located at the west part of Iran bordering with Iraq. People are predominantly Kurdish, multi-ethnic and multi-cultural in nature. Agriculture is the main source of subsistence in this province. This area, particularly the rural regions were underdeveloped during the last regime, because the dominant development policy was the expansion of the urban areas. Industrial activities appropriated to the urban regions and assembled machineries factories imported from the industrial countries. There were not appropriate programs in utilizing and developing the local resources. This situation led to a vulgar and lop-sided economy along with misbalances between the rural and urban regions, leaving the people with anxiety, disappointment and insecurity.

Nowadays many rural populations in Iran have access to modern communicative devices such as radio, television, media, satellite technologies and improved road and transportation facilities and technologies in relation to agricultural industry. This article is a new attempt to shed a light on the life of the villagers in this trans-cultural setting, whose lives are being affected by the global economy (Dicken, 1992), and the continuing significance of state as a key agent in this process. However, the authorities are still keen about preserving the religious and cultural values in this complex, rapid-changing and unpredictable environment.

Globalization, as a process, analyzed in consideration to communicative instruments and development possibilities and the expanded nature of capitalism. Wallerstein traces the roots of this global system to the economic expansion those 500 years ago as rich nations cast their eyes on the wealth of the rest of the world. The unprecedented worldwide flow of information originating in rich nations has the predictable effect of tying far-flung societies together and fostering common pattern of global culture (Wallerstein, 1974). Globalization has increasingly observed in newspapers and reports of mass media in association to socio-economic news since the end of 1980s. Globalization rather than economy is used connected to more items. For instance, about cultural and ecological globalization is discussed too, but is underlined on economic sense of globalization (Roselius, 2004). According to definition, globalization is the process of time-space compression that it corporate people in the world-united society consciously (Golmohammadi, 2005).

A glance to globalization theories illustrate that designing of developmental discourse within developed countries has been initiated with enlightenment age in eighteen century for social uniformity of communities in western globalization project. Industrialization of San Simon (Golmohammadi, 2005), structural differentiation of Durkhiem (Abazari,1985), Rationality of Weber (Sedarati, 1993), co-modification of Marx (Ahmadi, 2003), Modernization of American Functionalists [Parsons and Levy] (Azkia, 2005), dependency theory of Neo-Marxists [Frank and Wallerstein] (So,1990), global village (Mcluhan, 1964), time-space compression (Harvey, 1993), distinction of space and time (Giddens, 1990) and globalization theory of Robertson (Robertson,1992), discuss about fundamental forces of convergence, furthermore, demonstrate lines progress and development for societies in consideration to institutive characteristics of modernity.

Globalization leads to expanded social changes in human societies. Modern society, certainly the society that is assimilated in current macro-sociology, not only is a confined and determined social system but also is a society that is made by social-spatial networks (Ershad, 2000). Thus, global content must be analyzed to understand essential aspects of social life among human societies in contemporary world (Vosoghi, 2004).

In addition to the reality of increasing global integration, also, the investigating of integration and compression in rural communities is required (Azkia, 2003). In terms of rapid socio-economical changes, it cannot be renounce the globalization impacts even in the remotest rural communities. Changing in development discourse and models of development planning (such as rural development planning in levels of micro, medium and macro) (Eftekhari, 2005), extreme dissemination of changes in employment and unemployment patterns (Taherkhani, 2005), increasing of poverty and inequality in rural regions (Sumner, 2001), expansion of media and communicative roles in rural communities (Jones and Tonts, 1995), accelerating restructuring trends (Nelson, 1999), change in nation-state roles in rural communities (Kofman, 2003), redefinition of security, human rights and democracy in different communities (Thompson, 1998), increasing consumerism and commoditization in societies, expansion cultural universalism and particularize in rural communities (Golmohammadi, 2005), are the most important issues that researchers argue about them in this paper.

The main porous of this study was to investigate the economic and cultural impacts of globalization on the rural communities of Kermanshah Township.

2. Material and Methods

This study has been performed in the field of quantitative research. Quantitative researches according to the research design (or the mode of data gathering) can be divided two following researches: descriptive and experimental researches. This article has emerged a descriptive research. Descriptive research contains a set of methods describing conditions. The implementation of this method can purely be to recognize present conditions or helping decision process (Bazargan and Sarmad, 2003). Descriptive research can be implemented by using of several methods e.g. "correlation researches". For gathering of data, multivariate methods were used, particularly administration of a questionnaire. Then, a pilot survey was conducted. The researchers tried out the questionnaires on 30 head of households who were similar to those who will be investigated in the actual research. Any problem with the draft questionnaire was corrected at this stage and the questionnaire was finalized for the real investigation.

Statistical population in this study was selected among 700 villages in Kermanshah Township. Then, 30 villages were selected among the geographical regions of *Miandarband*, *Mahidasht* and *Jalalvand* districts for data collection through multi-stage sampling. In the first sampling, these regions were selected based on the number of developmental indexes. Then, in the second stage of sampling 30 villages were chosen. Considering to the statistical population, 380 respondents were selected by Cochran formula.

In order to achieve the aims of the study, the following indicators have been selected: (1) Agro-industrialization, (2) Increasing in poverty and inequality, (3) Change in employment patterns, (4) Economic restructuring, (5) Role of nation-state, (6) Role of media, (7) Cultural universalism, (8) Cultural particularize, and (9) Progress in rural regions of Kermanshah Townships. The first eight-abovementioned indicators were independent variables and progress in rural communities was used as dependent variable. (figur1)

To determine the relationship between the variables, multivariate techniques were used. These techniques contained Multivariate Correlation, Multiple Regression and Structural Equation Modeling (SEM). In order to find out the results of the globalization impacts on the rural communities the following theoretical framework was investigated through statistical methods. The SPSS software was used for data analysis.

3. Results

To determine the correlation between criterion (dependent) and predicative (independent) variables the Pearson-coefficient of correlation was used. Table 1 shows the correlation coefficient matrix. According to the Table 1, there were significant correlation between all predicative variables and the criterion variable (p<0.01).

Table 1: Correlation matrix

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	Y
X_1	1.0	.59**	.4**	-.26**	.41**	.55**	.46**	.12*	.75**
X_2		1.0	.2*	.11*	.17**	.38**	.46*	.11*	.42**
X_3			1.0	.44**	-.17**	-.01	.14**	-.08	-.34**
X_4				1.0	-.25**	-.2**	.07	-.06	-.29**
X_5					1.0	.26**	-.04	.03	.30**
X_6						1.0	.48**	-.02	.49**
X_7							1.0	-.2**	.41**
X_8								1.0	.13**
Y									1.0

Agro- industrialization(X_1), Increasing in inequality(X_2), Change in employment(X_3), Restructuring (X_4), Change in state role (X_5), Role of media(X_6), Cultural universalism(X_7), Cultural particularize(X_8), Progress in rural community(Y)

Multiple Regression

In this research, we used stepwise method to identity the impacts of predictor (independent) variables on rural communities' progress. In the stepwise method, the strongest variables enter to equation and this program continues until error of significance reaches 5 per cent (Kalantari, 2004). In Table 2, the regression analysis has advanced until six stages. As you seen (Table 2), based on the Coefficient of Determination (R^2), six predictors (independent variables) can predict 62 per cent of dependent variable.

Table 2: Regression Analysis

Stage	Variable	R	B	ß	p
1	Agro- industrialization(X_1)	0.750	0.518	0.864	0.563
2	Change in employment(X_3)	0.761	-0.193	-0.42	0.579
3	Cultural universalism(X_7)	0.771	0.175	0.382	0.595
4	Role of media(X_6)	0.777	0.099	0230	0.603
5	Cultural particularize(X_8)	0.780	0.086	0.216	0.609
6	Change in state role (X_5)	0.784	0.087	0.168	0.615

In consideration to Variance Inflation Factor (VIF), we can argue about co-linearity statistics. If VIF is lees than 10, co-linearity will not be significant. According to Table 3, it is considered amount of co-linearity is less than 10 for predictor variable in the last stage of regression analysis.

Considering to quantity of beta (ß) can be arbitrated ratio and proportion predictor variables in explanation of dependent variable. Quantities of beta (the sixth column of Table 2) show that per unit of variation in standard deviation of agro-

industrialization, change in employment patterns, cultural universalism, role of media, cultural particularizes and change in nation-state role can be varied standard deviation of dependent variable in order 0.518, -0.193, 0.175, 0.099, 0.086 and 0.087.

Table 3: variance inflation factor

Variable	VIF
Agro- industrialization	2.135
Change in employment	1.256
Cultural universalism	1.803
Role of media	1.671
Cultural particularize	1.114
Change in state role	1.220

Structural Equation Modeling

One of the main purposes of the multivariate techniques is expanding in explanation ability of researchers. Multiple Regression, Conformity Factor Analysis, and so on, are important instruments for researchers but have a common limitation: these methods can only investigate a separated relationship. "Structural Equation Modeling (SEM) is investigates a set of correlation relationships. This method is specially uses when a dependent variable is furthermore an independent variable in the next correlation relationships"(Sharepoor, 2005). SEM is used in different branches of science such as education, marketing, psychology, management, demography, organizational behavior and biology (Hoyle, 1995). SEM can contain covariance structure analysis, latent variable analysis, conformity factor analysis, path analysis and linear structural relations. In this study Path Analysis was used. Path Analysis is a multivariate technique to explain direct and indirect impacts between variables (Kalantari, 2004).

The logic of SEM is based on its theoretical framework. There are different indexes to goodness of fit such as Chi-Square, RMSEA (Root Mean Square Error of Approximation), CFI (Comparative Fit Index) and NFI (Norm Fit Index).

To goodness of fit, have to installation the following conditions in SEM:

1-Chi-Square should not be significant.
2-RMSEA has to be less than 0.5.
3-CFI has to be about 1.
4-NFI has to be more than 0.95.

In consideration to Path Diagram (Diagram 2), that has been demonstrated by using AMOS software, the model was fitted for the following reasons:

1-Chi-Square is not significant (P-value=.376)

2-RMSEA=.013
3-CFI=1
4-NFI=.992

Quantities of beta showed that independent variables of agro-industrialization, change in employment patterns, cultural universalism, role of media, cultural particularize and change in role of nation-state, can be explained in order 0.55, -0.16, 0.20, 0.01, 0.08 and 0.082 dependent variable. Thus, SEM can explain 0.62 progresses in rural communities according to theoretical framework of this research.
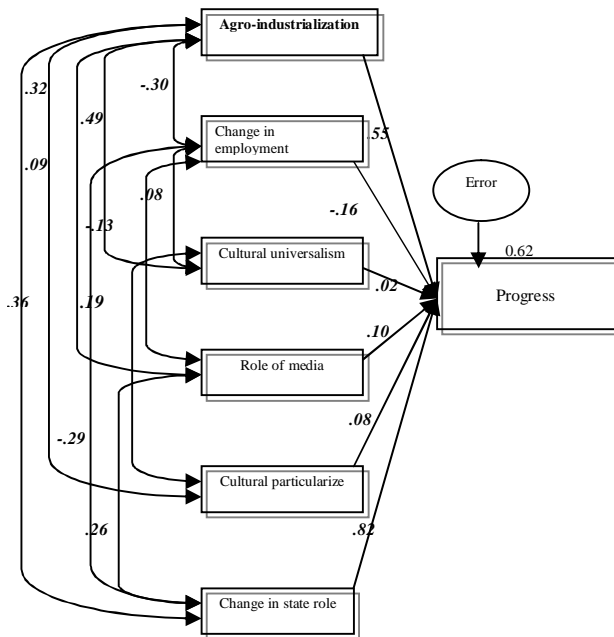
Chisq=5.341, DF=5, p=0.376, RMSEA =.013, CFI=1.000, NFI=0.992

4. DISCUSSION

Corporate globalization is a world-wide phenomenon that has rapidly come to affect the lives of many people- rich and poor, women and men, black and white, urban and rural(Sumner,2001). Globalization has rather investigated as impact-oriented approach in the recent studies. According to this approach, economical, political and cultural impacts of globalization are increasing on rural and urban communities especially in the post- cold war world (Eftekhari, 2005).

In consideration to research objectives, the impacts of globalization on rural communities of Kermanshah Township are investigated. The most expensive area in social activities is economical area. The economic impacts of corporate globalization are the most immediately obvious, and have the great repercussions. Economical globalization has

historical priority, in the same way that is the most obvious sense of globalization (Sassen, 1993). In this study, we selected agro-industrialization, increasing in poverty, change in employment patterns and restructuring as economical variables.

The industrialization of agriculture has resulted in extensive declines in the number of farms and in the number people employed in agriculture, with transitioning effects for many rural communities. The results of correlation analysis indicated that there was significant correlation between agro-industrialization and progress in rural communities (p<0.01). Agro-industrialization was entered regression analysis as the first stage and the regression analysis was confirmed by SEM. Agro-industrialization impacts happen through different ways in rural communities. Summner (2001), under pretence economical impacts of globalization, analysis agro-industrialization as a process which that replace small and medium-sized farms with large, agro-industrial one. Padavic (1993) uses fried land's description of large industrialized farm as marked by integrated production, processing and distribution of generic inputs for mass marketable food stuffs, adding that it is occurring at an increasing fast rate and replacing classic family farming. Agro-industrialization affects rural communities in other ways as well. For example, agro-industrialization input manufacturers promote diverse credit relations among farmers, which ties them to technological packages that are designed to reproduce certain commodity forms(Marsden,1989), such as products for export instead of local consumption.

One of the ways of widening the gap between rich and poor and contributing to the Age of Exclusion is debt creation. Whether it is individual or governmental indebtedness, Third-World or First-World indebtedness, all serve to keep money flowing to the wealthiest sectors of the population (Murshed, M. 2002). Increasing in poverty and inequality was analyzed as independent variable in this study. The results of correlation analysis showed that there is significant correlation between increasing in inequality and dependent variable in rural communities (p<0.01). According to economical theories of globalization, poverty and inequality in Third-World has bilaterally related to linear progress in First-World. This issue has to identify as an important variable in rural development planning in south countries. As Chambers (1998) warns, poverty is the strongest determinant factor to make deprivation trap in rural communities. Poverty and inequality often go hand in hand, especially in rural communities. Taherkhani (2005) argues that policy-making to encounter rural poverty is one of the most important priorities in rural development.

Another economic impact of corporate globalization involves changes in employment and unemployment patterns, such as indentured, child and slave labor, flexible employment and increased unemployment. As consumption intensifies in the global market, full-time, stable employment dwindles, while new forms of employment, and unemployment, increase (Sumner, 2001). The results of correlation analysis in this research showed that there was significant and negative correlation between change in employment patterns and progress in rural communities (p<0.01). This variable is entered in regression analysis as the second stage and the regression analysis is confirmed by SEM. In general, globalization erodes employment opportunities. Mc Michael (1996)argued, like many other communities around the world, rural communities have experienced the changes in employment and unemployment patterns brought on by corporate globalization such as making informal jobs, increasing in indentured and slave labor and unemployment thriving. The rate of unemployment has also increased due to corporate globalization. In this era of the "jobless recovery', the economy thrives while more and more people face destitution. In Canada, according to Leach and Winson (1995), there is strong evidence of a rather substantial and disturbing increase in the population of unemployed individuals experiencing long-term unemployment (Sumner, 2001).

Restructuring is a term often used to describe changes in economy including stagnant wage growth and increasing in part-time jobs. Foley (1994) warns that restructuring should be understood as a myth that masks the actual processes of capital reorganization. He adds that any economic restructuring which is directed by the capitalist state will be, ultimately and primarily, in the interests of capital and against the general interest. The results of correlation analysis showed that there was significant and negative correlation between restructuring and progress in rural communities of geographical region of this study (p<0.01). Restructuring has had a devastating impact on the economies of rural areas, especially in those communities that are very dependent on manufacturing activity (Nelson, 1999). For example, in the western Australian wheat belt, rural restructuring has resulted in significant changes in the employment structure, with the primary sector of the workforce declining by 30 per cent (Jones and Tonts, 1995). In the United States, restructuring is a wrenching experience for many rural communities. As kristof (2000) argues, restructuring is impoverishing small ranchers and farmers, forcing them to sell out, depopulating large chunks of rural

America changing the way Americans get their food(Sumner, 2001).

Corresponding to globalization theory of Waters (1995), economical globalization is influenced by geographical ties. But cultural globalization is less influenced by geographical ties. Thus, economical exchanges tend to localism and cultural exchanges tend to globalization. In this study, and according to theoretical framework, we selected Change in nation-state role, the role of media, Cultural universalism, Cultural particularize as cultural variables.

About twenty years ago Daniel Bell claimed that the nation-state institution, under corporate globalization, is great for small issues and is small for great issues of human communities (Bell 1987). The role of the state has changed dramatically under corporate globalization, affecting rural communities both directly and indirectly. The erosion of the power of the nation-state transforms institutions of the state from being protectors of the health and rights of people to protectors of the property and profits of corporations (Sumner, 2001). The results of correlation analysis showed that there is significant correlation between change in nation-state role and progress in rural communities (p<0.01).This variable is entered regression analysis as the sixth stage and the regression analysis is confirmed by SEM. The nation-state role is highly transited by influenced globalization in rural regions. Rosenau (1990) argues that while the nation-state has traditionally buffered civil society from the excesses of capital, there is no such protection at the global level. Ward et al (1995) contend that the role of agricultural protection is diminishing in association with a secular redefinition of the social functions of rural space to encompass distinctive consumption roles (such as residence, recreation, leisure, and environmental conservation).

The impacts of corporate globalization reach far past the economic into the socio-cultural lives of people in rural communities. Globalization, essentially, analyzed in consideration of communicative instruments and many ruralists have access to modern communicative devices such as radio, television, media, and satellite technologies and so on. The results of correlation analysis showed that there was significant correlation between role of media and progress in rural communities of Kermanshah Township (p<0.01). This variable was entered regression analysis as the fourth stage and the regression analysis was confirmed by SEM. The qualitative and quantitative expansion of media and that's impacts on human life is appraised in different ways. Mcluhan (1964) has a cultural view to globalization. He points out that the mediators in transferring of culture are more important than

content of culture, from the viewpoint of communicative sociology, and divides the social history of human based on media technologies in two periods: 1- tribal epoch, and 2- industrial epoch. In the industrial epoch, speed and volume of communications have been expanded in rural and urban communities. Jones and Tonts (1995) mention that improved technology and communicative instruments are the most important characteristics of rural societies in Third World.

Rural ways of life are incrementally giving way to "lifestyles" that based individualism, consumption, cosmopolitanism, security, cultural integration and individual freedom, which are characteristic of the experience of corporate cultural globalization. One of the most important consequences of corporate globalization is the cultural integrating of different human societies by cultural globalization. Cultural universalism attributes set of cultural changes and reactions that create based on exchange, integration, coexistence and alikeness of cultures. This concept is one of the most important impacts of globalization on rural communities. The results of correlation analysis showed that there was significant correlation between cultural universalism and dependent variable in statistical society (p<0.01). Cultural universalism was entered regression analysis as the third stage and the regression analyses was confirmed by SEM. Universalism, fundamentally, refers to principles, values and criteria that, entirely, are accurate for human and communities. As Thompson (1998) concludes, the validity principles and values directed at universalism are not related to interests such as localism, commutarianism and tribalism. Corresponding to globalization theory of Robertson (1992), the globalization process intimates the cultures together in new conditions defining through density of cultural spaces.

The local cultures percept the global culture from different ways, based on unique characteristics and situation in world system, and furthermore, the symbols of global culture represent from numerous ways in particular contexts. The process of globalization, to some extent, deconstructs necessary recourses for traditional identification, through reconstructing of time and space, opening the boundaries and expanding of social sphere. In this way crisis of identity is appeared and reconstruction of identity is required. Some people prefer traditional modes of identification to predominate crisis of identity and, thus, the social appropriate context for cultural particularizes is provided. One of the clearest impacts of corporate cultural globalization is cultural particularize, especially, in nomadic and rural communities. The results of correlation analysis

showed that there was significant correlation between cultural particularize and dependent variable in rural communities (p<0.01). Cultural particularize is entered regression analysis as the fifth stage and the regression analysis is confirmed by SEM. As Golmohammedi (2005) concludes, cultural particularize has tendency towards elements of particular culture that emphasis unique ideas a specific society, especially, in nomadic and rural communities. Giddens (1991) investigates local ethnocentrism and he argues about the possibility of reproduction of local identities by corporate globalization.

5. CONCLUSION

Finding efficient and right method to measure development and then to provide service supply in rural area is very difficult. This is result from more frequency of rural, population dispersion, rural diversity and their distribution manner in the area, their communication situation, rural special characteristics, budget and developmental credit deficiency, expert personal deficiency and rural administrative management system deficiency and ignoring rural settlements in several past decades (Rezvani and Sahneh, 2005). With regard to this issue that no program can without objective, so in planning stage, balancing developmental situation of rural districts and balancing in enjoying rate of various possibilities and services and to improve this indexes in order to provide community health are considered the key goals, goal which cause to social justice and provide sustainable development area (Khakpour, 2006).

The results indicate that out of the total Guilan rural districts in developmental situation, six rural districts were underdeveloped and more percent of villages were in less developed situation. One of the regional planning goals, is to balance develop of region and to prevent from generating under developed area. Use the results of this study to reach to above goals. As in allocation of improvement credits, allocation credits to each region can determine according to developmental degree and distance rate of each rural districts from ideal condition. So, developed rural district will receive less budget and underdeveloped rural districts will receive more budget.

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Sense of Community and Participation for Tourism Development

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Abstract: This article investigate the relationship between sense of community and level of participation in tourism activities in Shiraz, Iran. Pearson correlation has been used to determine the relationship between these variables. The findings through survey questionnaire showed that there are significant relation between sense of community and level of participation for local tourism development. According to the results, sense of community can have a catalytic effect on development of tourism industry through enhancement of local participation.
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1. Introduction

Sense of community and participation are the main factors which can effect on processes of tourism development. Without community participation and sense of community tourism development could not be achieved. Bopp et al. (2000, p. 113) defines sense of community in the following way: "sense of community refers to the quality of human relationship that makes it possible for people to live together in a healthy and sustainable way". The sense of community plays an important role in fostering community support for tourism development and may enhance its long-term sustainability as a broad basis for tourism development planning (Hall et al., 2005). Developing a sense of community contributes to participation by enabling people to feel connected and motivated to live in harmony and work together towards common goals. Sense of community can be seen as the capacity of the local people to participate in development activities (Cupples, 2005).

Tourism developers often like to encourage a sense of community among residents as a way of contributing to tourism development. This study provides a study of applying an approach of sense of community and participation for tourism development. While there is a substantial body of literature on the definition and conceptualization of sense of community (Chavis & Wandersman, 1990; Davidson & Cotter, 1989; McMillan & Chavis, 1986), only a few literatures discuss the practical application of approaches that have been successfully used to measure sense of community in different cultural contexts. Little research also has been conducted on sense of community and community participation. Local communities for tourism development requires strengthening of sense of community (Conway & Hachen, 2005).

2. Literature review

Tourism industry is often referred to as the world's largest industry and regarded as a means of achieving sustainable development and it represents significant economic, environmental, and socio cultural opportunities for many local communities (Sharpley, 2002). According to the World Travel and Tourism Council (WTTC, 2008), tourism accounts for nearly 10% of the world's GDP comprising $5.89 trillion in economic activity and is expected to nearly double by 2018 (WTTC, 2008). Harrill & Potts (2003, p. 233) believed that "tourism is an invisible industry, encompassing transportation, loading, and entertainment. Unfortunately, tourism is also invisible to many planners, so tourism development is often left to private developers and leisure service providers" (Harrill & Potts, 2003, p. 233). Tourism also is the sum of the phenomena and links arising from the interaction of tourists, governments, and local communities in the process of attracting and hosting these tourists and other visitors. Tourism research has also recently come to be a favorite research subject in community development researches (Galston & Baehler, 1995).

Tourism is undoubtedly important for local, national, and international levels. It should not form the core element of a community's economy, but is better suited to play a supplementary role to help diversify community economic activities (Godfrey & Clarke, 2000). Tourism has become a source of income generation for many communities seeking ways to improve their livelihoods. Clearly, tourism and its impacts is a multidimensional phenomenon that encompasses economic, social, cultural,

ecological, environmental, and political forces (Singh et al., 2003). A key term used in tourism development is a sense of community. Sense of community is the feeling of obligation and commitment of an individual towards other members in the community develops over time through understanding of collective values, beliefs and interests among community members. Sense of community also is a feeling of belonging to the community (Bowen et al., 2003). Sarason (1974) defined the sense of community as the interdependence between individuals and communities. Bopp et al. (2000, p. 113) defines sense of community in the following way: "sense of community refers to the quality of human relationship that makes it possible for people to live together in a healthy and sustainable way" (Bopp et al., 2000, p. 113).

The importance and need for sense of community and participation in building strong capacity communities for tourism development cannot be ignored(Aref et al., 2010). Community participation can be seen as a process whereby the residents of a community are given a voice and a choice to participate in issues affecting their lives. Whether a community participates or not is determined by a variety of factors. One such factor is reluctance to participate because the community members do not trust each other. Community participation can thus be enhanced by addressing barriers to participation while at the same time taking the necessary steps to promote the principles of sustainable participation (Theron, 2005).

Community participation in tourism development processes can support and uphold local culture, tradition, knowledge and skill, and create pride in community heritage (Lacy et al., 2002). The goal of community participation is to improve communication between stakeholders in the interest of facilitating better decision-making and sustainable development (Nampila, 2005).

Community participation also is the mechanism for active community involvement in partnership working, decision making and representation in community structures (Chapman & Kirk, 2001). It should be noted that community participation often means the involvement of people or community with the government. However, this study emphasized the participation of the community as an involvement of local people in tourism development processes. Without participation, there is obviously no partnership, no development and no program. Hence lack of community participation in decision making to implement tourism development can lead to failure in the community development (Miranda, 2007). Community participation increases people's sense of control over issues that affect their lives and also promotes self-confidence and self-awareness (Nampila, 2005). Levi & Litwin (1986, p. 26) also regard community participation as the creation of a democratic system and procedure to enable community members to become actively involved and to take responsibility for their own development, to share equally in the fruits of community development and to improve their decision-making power. Community participation provides a sense of community to take responsibility for oneself and others, and a readiness to share and interact (Aref et al., 2010). Figure 1 depicts the tourism development chain. The figure shows that progress in the outcome of community development also contributed to sense of community and tourism development. This figure also emphasized on community participation as important key for tourism development.
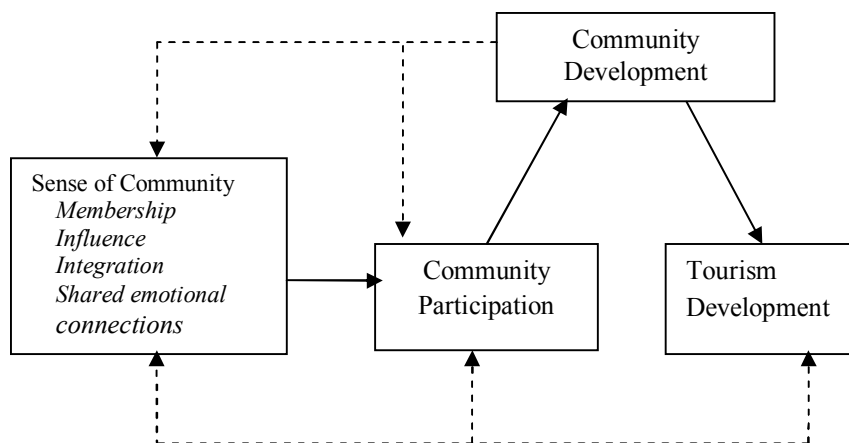
Figure 1: Tourism Development Chain : Adapted from Phillips & Pittman (2008)

Jones (1996) concludes that the development of a successful community based tourism strategy will require a sound institutional framework based on a partnership between the local community, state, the private sector and Non-governmental Organizations. In reality, however, participation is a continuum based on the degree of people's involvement in deciding or influencing the decision making process concerning the tourism development program or in its implementation. Although the types of participation are differ between the authors, the main aspects which differentiate between the stages within the typologies are the same. They share common ideas regarding the extent or the degree to which community members have the chance or are given the chance to decide for themselves. Table 1 showed the main typology of participation which had been done by Preety (1995).

Table 1: Typology of community participation in tourism development

Typology	Comments
Passive Participation	-People participation is limited to be told what is going to happened -People's responses are not taken into account -Information belongs only to external professionals
Participation in information giving	-People participation is limited to provision of information in response to questionnaires, surveys etc. designed by external agents -Findings of the research are not shared with the people
Participation by consulting	-People participation involves consultation with local people -They may take into account people's views during this process, but are not obliged to do so
Participation for material incentives	-People participate by contributing resources (e.g. labour) in return for food, cash or other material incentive -Farmers may provide fields and labour but are not involved in the experimentation or the process of learning -This is often called participation, but people have no stake -This is often called participation, but people have no stake in prolonging activities when the incentives end
Functional participation	-People participate by forming groups to meet specific objectives related to the project -Involvement may be interactive but tends to arise later in the project cycle after major decisions have been made -Institutions formed tend to depend on external facilitators, but may become self dependent
Interactive Participation	-People participate in joint analysis, development of action plans and creation or strengthening of local institutions -Participation is seen as a right and not only as a means of achieving projects goals -It tends to involve interdisciplinary methodologies that seek multiple perspectives and make use of systematic and structured learning processes -Local groups take control of local decision making and determine how resources are to be used giving them a stake in maintaining structures or practices
Self-mobilization	-People participate by taking initiatives independent of external institutions or change systems -They develop contacts with external institutions for advice and resources, but retain control of the use of resources - Self-mobilization and collective action may or may not challenge existing inequitable distributions of wealth and power

Source: Pretty (1995)

3. Methods

In order to achieve the stated objective, data had to be obtained from the leaders in the designated study area. The leaders for this study were identified in the 175 local communities with the help of the Shiraz municipality and the Islamic council of Shiraz. The data were collected through a survey questionnaire. The items in the questionnaire for this survey were measured using Likert scale. Relation between sense of community and level of participation were measured by 14 item on a 5 point Likert scale (0=never 1=seldom 2= sometimes 3=often 4= always). Pre-testing of the research instrument was carried out to examine the appropriateness and reliability of the instrument. The results were sufficiently comprehensive and verified the value of the instrument (Aref, 2010). Pearson correlation was used to interpret the data in this study.

4. Results and discussion

The questionnaire was used to obtain the primary data through a survey of 175 community leaders of Shiraz. All the respondents provided completed answers to every question. Out of the 175 leaders, 5.14% were females and 94.86% were males,

with an average age of 53.12 years. The youngest participant was 33 years and the eldest one was 72 years of age. Out of all the respondents, 26.36% state their highest level of education is diploma and 42.86% holds the bachelor's degree. This study was to determine the relationship between sense of community and their participation for tourism development. Pearson correlation used to identify this relationship. Correlation statistic is a statistical technique to measure the strength of the association that exists between two quantitative variables (Ary et al., 1996).

The Pearson correlation coefficient, r, will be used to measure the degree of linear relationship. The value of r is always between +1 and -1. A Pearson correlation addressed the relationship between sense of community (M = 18.74, SD = 3.683), with the level of community participation (M=13.95, SD= 4.184). For an alpha level of .05, the correlation between sense of community and level of community participation was found to be significant, (r = .000, N = 175, p =. 766, two-tailed). The table 1 indicates that sense of community and level of community participation are significant (r = .000, N = 175, p =. 766, two-tailed).

Table 1: Pearson correlation between sense of community and participation

Variables		Community participation	Sense of community
Community participation	Pearson Correlation	1	.766(**)
	Sig. (2-tailed)		.000
	N	175	175
Sense of community	Pearson Correlation	.766(**)	1
	Sig. (2-tailed)	.000	
	N	175	175

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

Findings through Pearson Correlation showed there is positive significant relationship between community participation and sense of community in terms of tourism development. Participation and sense of community are the main factors which can effect on processes of community development. Without these factors tourism development could not be achieved. In support with this findings of the study, David & Wandersman (1990) stated that sense of community can have a catalytic effect on local action such a community participation by affecting the perception of the environment, social relations, and one's perceived control and environment. Levi and Litwin (1986: 25) also stated that Community participation provides a sense of community to take

responsibility for oneself and others, and a readiness to share and interact.

5. Conclusion

The aim of this study was to determine the role of sense of community and participation in local tourism development in Shiraz, Iran. Sense of community is an individual's perception of being responsibility to supported by, and belonging to his/her community. Assessing sense of community is an important step in developing tourism strategies to achieve community development (Marre & Weber, 2007). Sense of community is a motivation to support local people for participation in tourism activities. Clearly, sense of community plays an influential role in the success of tourism in local communities.

According to the results, sense of community can have a catalytic effect on development of tourism industry through enhancement of local participation. Findings from this study could be used as guidelines in developing scale for measuring sense of community in other areas of tourism and community development.

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Concentration of zinc and boron in corn leaf as affected by zinc sulfate and boric acid fertilizers in a deficient soil

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Abstract: Zinc (Zn) and boron (B) deficiency is one of the most widespread micro nutritional disorders in crops, and occurs predominantly in calcareous soils of arid and semiarid regions. A field experiment with maize plant grown on Zn and B deficient soil was carried out in a calcareous soil at Fars Province, Iran. This work aimed to study the interaction effect of Zn and B on the concentration of Zn and B in the maize leaf during 2008. Treatments included five levels of Zn (0, 8, 16 and 24 kg ha^{-1} Zn added to the soil and Zn foliar spray at 0.5 weight percent of zinc sulfate) and four levels of B (0, 3, and 6 kg ha^{-1} B added to the soil and B foliar spray at 0.3 weight percent of boric acid) in a completely randomized block design. The findings showed that application of Zn, B, and Zn−B interaction on the B concentration in the leaf was insignificant. Zinc spraying increased Zn concentration in the leaf but adding Zn to the soil had no significant effect on it. Presence of B prevented from the increase in Zn concentration in the leaf; that is, an antagonism was seen between the Zn and B. Boron application decreased leaf Zn content and this increase was not influenced by the amount of Zn. Zinc use at no presence B in the soil (zero B and B spraying levels) increased leaf Zn content.

1. Introduction

Plant analysis is an important component of soil fertility and plant nutrition research. Zinc an essential element for the normal growth and metabolism of plants played very important role in enzyme activation and was also involved in the biosynthesis of some enzymes and growth hormones (Ranja and Das, 2003). Zinc deficiency is very common in many agricultural crops especially on high-pH soils. Zinc is one of the essential elements for plants, animals and humans, but it is deficient (less than 1 mg kg^{-1} DTPA-extractable Zn) in most calcareous soils and consequently in plant, animal and human diets (Cakmak 2006). The amount of extractable Zn varied markedly depending on the soils and extractants used. Zn availability is inversely related to soil pH and its deficiency in variety of plant species is frequently noted on calcareous soils with pH>8.0 (Swietlik, 1989; Ma and Lindsay, 1990; Srinivasara et al., 2008). Total Zn concentration is in sufficient level in many agricultural areas, but available Zn concentration is in deficient level because of different soil and climatic conditions. Soil pH, lime content, organic matter amount, clay type and amount and amount of applied P fertilizer affect the available Zn concentration in soil (Adiloglu, et al., 2006).

Boron is an essential micronutrient for plants, but the range between deficient and toxic B concentration is smaller than for any other nutrient element (Goldberg, 1997). Soils may contains 0.5−2 mg kg^{-1} of available B, but this represents only a small part of the total since only 0.5−2.5% of the total B in the soil is available to plants. There is also a very narrow range between B deficiency and toxicity as more than 5 mg kg^{-1} available B can be toxic to many agronomic crops (Kelling, 2010). Levels of B above the optimum range cause significant changes in the activity of numerous enzymes and consequently, the metabolism of higher plants (Shkolnik, 1974). Furthermore, B toxicity also resulted in increases in membrane permeability, and possible role of membrane integrity and structure in tolerance mechanism of B toxicity reported by Karabal et al. (2003). Boron uptake by plants is controlled by the B level in soil solution rather than the total B content in soil. Nable et al. (1997) reported that soils containing more than 5 to 8 mg L^{-1} of hot water soluble B is considered to probably cause B toxicity. The range of B concentrations in the soil solution, in which plants suffer neither toxicities nor deficiencies, is very narrow (0.3−1 mg kg^{-1}). Soil factors affecting availability of B to plants are: pH, texture, moisture, temperature, organic matter and clay mineralogy (Goldberg, 1997). Soils low in organic matter are deficient in B more often than soils with high organic matter content. When the surface sol dried out, plants are unable to fee in the zone where most of the available B in present. This can lead to B deficiency. When rain or irrigation moistens the soil, the plants can again fee from the surface soil and the B deficiency often disappears (Kelling, 2010). Soil pH is one of the most important factors that affects B uptake by plants. Many investigators (Peterson and Newman, 1976; Gupta and Macleod, 1981, Kelling, 2010) have found

that increasing soil pH by liming to above pH 6.5 reduces B concentration in many plants. B deficiency causes various changes in properties such as membrane integrity and permeability, auxin metabolism, sugar transport, lignifications in the cell wall, carbohydrate metabolism and transport, respiration (Loomis and Durst, 1992), and reduced fertility (Marschner, 1995).

Analysis of plant tissue gives a good indication of the B nutrition of plants. Excessive amounts of B can cause problems with some crops; B application in a fertilizer program should be stopped if the B concentration in the plant tissue is in the sufficient or high range. Kelling (2010) reported that, because B levels in the plant change with age, it is important to indicate the stage of development at sampling. Relative levels of B in mg kg^{-1} for corn in sample of whole plant when plant height is 15−38 cm are: < 4 deficient, 4−6.4 low, 6.5−40 sufficient, 40.1−55 high, and > 55 excessive. Also, B levels in ear leaf of corn at tassel to silking stage are: < 2 deficient, 2−5 low, 5.1−40 sufficient, 40.1−55 high, and > 55 excessive (Kelling, 2010). Deficiency or excess of B not only affects the relative values of individual elements, but it also affects the balance among certain nutrient elements within plants, causing either an increase or decrease of dry matter production (Tariq and Mott, 2007).

In nutrient culture, Graham et al. (1987) reported that low Zn treatment did not affect plant growth, but enhanced B concentration to a toxic level in barley (Hordeum vulgare). Similarly, Zn deficiency enhanced B concentration in wheat (Triticum aestivum) grown on Zn deficient soils (Singh et al. 1990). Sinha et al. (2000) noted a synergistic interaction between Zn and B in mustard (Brassica nigra) when both the nutrients were either in low or excess supply. Hosseini et al. (2007) reported that high levels of B decreased plant height and dry matter production of corn (Zea mays L.). There was a significant B and Zn interaction on plant growth and tissue nutrient concentration which were rate dependent. In general, the effect was antagonistic in nature on nutrient concentration and synergistic on plant growth. Shaaban et al. (2004) reported that increasing rates of B and Zn were applied to maize plant in calcareous soil under greenhouse conditions, Egypt. Boron concentration of plant increased with increasing B application. On the other hand, Zn concentration of plant decreased with this application. Zinc concentration of maize increased with Zn application. Whitehead (1987) found that the accumulations of zinc (Zn^{+2}) varied in different plant species. Frey et al. (2000) measured Zn concentration in shoot which was higher and reached a maximum value of 83 mM·kg^{-1} dry mass whereas total concentration of Zn in roots were lower up to 13 mM·kg^{-1}. Concentration of Zn in dry matter of corn (mg kg^{-1}) in lower leaves at tasseling stage was: 9−9.3

showing deficiency symptoms, and 31.1−36.6 intermediate (Barker and Pilbeam, 2007); in leaves at 6th node from base at silking stage was 15−24 showing deficiency symptoms, 25−100 intermediate, and 101−150 high (Deleers et al., 1985); and in ear leaf at silking stage was: < 10 showing deficiency symptoms, 20−70 Intermediate, 71−100 high, and > 100 showing toxicity symptoms.

The objective of this research was to measure concentration and uptake of Zn and B in corn leaves as affected by Zn and B application and interactions between Zn and B in plant.

2. Materials and Methods

A field experiment was conducted at the farm of F. Aref in Abadeh Tashk, Fars province of Iran, on the corn (Zea mays L.), cultivar "Single Cross 401" during 2008 cropping season. The experiment site located 200 km northeast of Shiraz on latitude 29° 43' 10" N and longitude 53° 51' 56" E and 1580 m altitude. Composite surface soil samples were collected from surface horizon (0− 30 cm) of the soil before the experiment was initiated, air-dried, passed through a 2-mm sieve and analyzed for the following properties. Selected soil chemical and physical characteristics for the soil are presented in Table 1. Particle-size distribution determined by hydrometer method (Gee and Bauder, 1986), soil pH and ECe were measured at a 1:2.5 soil/water ratio and saturated extract, respectively, organic matter (OM) content by the Walkley-Black method (Walkley, 1947). Soil available K was determined by 1 M NH_4OAc extraction and K assessment in the extract by flame photometer (Thomas, 1982). Soil P available was measured by Olsen method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption. Soil available B was extracted by hot water and measured by Azomethine-H colorimetric method (Bingham, 1982). This soil had a loam texture, pH of 8, 0.56 % organic matter, 9 mg kg^{-1} available P, 205 mg kg^{-1} available K, DTPA extractable Fe, Mn, Zn and Cu concentration were 6.2, 9.9, 0.63 and 1.4 mg kg^{-1} and available B with hot water extractable was 0.87 mg kg^{-1}.

This experiment consisted of 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels Zn (0, 8, 16 and 24 kg ha^{-1} Zn added to the soil and Zn foliar spray at 0.5 weight percent of zinc sulfate) and four levels of B (0, 3, and 6 kg ha^{-1} B added to the soil, and B foliar spray at 0.3 weight percent of boric acid). Nitrogen, P and K used at 160, 90 and 80 kg ha^{-1} according to the recommendation, from sources of urea (with 46% N), triple super phosphate (with 46% P_2O_5) and potassium sulfate (with 50% K_2O), respectively, were added to all treatments.

Half of the urea was used when planting and the remainder two times: At vegetative growth and when the corn ears were formed. Potassium and P used before planting. 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^{-1} 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. At silking stage, leaf samples were taken from the second and third leaves from the top of plant. The leaves were dried in a forced air oven at $70^{o}C$ for 48 h. Total elements were analyzed after digestion of dry and milled plant material with HCl 2 N (Wolf, 1982). The total micronutrient concentrations: Fe, Mn, Zn, and Cu were analyzed by atomic-absorption spectrophotometry (Hocking et al, 1977). All micronutrient concentrations were expressed in mg kg^{-1} DW. To measure B concentration in leaf tissues, the Azomethine-H method was followed and the mixture was read by spectrophotometry (Wolf, 1974). The concentration of B was expressed as mg kg^{-1} DW.

Standard analysis of variance techniques were used to assess the significance of treatment means. Each variable was subjected to ANOVA using the Statistical Analysis System (SAS version 8.2, SAS Institute, 2001) for each soil. Treatment (fraction) means were separated by Duncun's multiple range test (P < 0.05 level). Multiple regression analyses (stepwise procedure) were conducted to evaluate the relationships between concentration of Zn and B in leaf with other factors.

3. Results and Discussion
3.1. Soil analysis

Physicochemical characteristics of soil taken before the experiment was initiated in the May 2008 are presented in Table 1. While table 1 indicates the soil available K was high but available P was lower than the critical level suggested in scientific sources (Karimian and Yasrebi, 1995). Karimian and Ghanbari (1990) have reported the critical P level by the Olsen method in calcareous soils as 18 mg kg^{-1}. The soil Zn and B content was lower than the critical level. High soil pH and $CaCO_3$ content induce B deficiency in the surveyed area. Similar results were found by Borax (1996) and Rashid et al. (1997). In soil, the B concentration of <0.65 mg kg^{-1} and >3.5 mg kg^{-1} are deficient and toxic levels for cotton crop, respectively (Annonymous, 1981). For many crops, a DTPA-

extractable Zn level of 0.5−0.8 mg kg^{-1} has been regarded as a soil critical level below which crop production would be limited by Zn deficiency (Martins and Lindsay, 1990). The soil Mn, Cu and Fe content was above the critical level. Sims and Johnson (1991) have reported the critical levels of Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water in the soil method to be 2.5−5, 0.2−2, 1−5, 0.1−2.5 and 0.1−2 mg kg^{-1}, respectively. Chen et al., (2001) reported that Fe deficiency is likely to be observed if the Fe concentration in the soil is less than 4−5 mg kg Fe, extracted by DTPA-Calcium chloride, with a pH of 7.3. Elgbla et al., (1986) showed that critical levels of Fe, Mn and Cu in the calcareous soils were 3.8, 1.2 and 0.7 mg kg^{-1}, respectively.

Table 1. Soil physical and chemical analysis

Properties	Values
Depth of soil (cm)	0−30
Soil texture	Loam
pH	8
EC (ds m^{-1})	1.2
Organic matter (%)	0.56
Nutrients (mg kg^{-1})	
P	9
K	205
Fe	6.2
Mn	9.9
Zn	0.63
Cu	1.4
B	0.87

3.2. The leaf B concentration

Application of different levels of Zn, B and the Zn-B interaction showed no significant effect on the leaf B concentration (mg kg^{-1}) at the 5% level (Table 2). Aref (2011) studied the effect of Zn and B on the leaf Zn and B concentrations and reported that Due to a Zn and B antagonism, high amounts of Zn in the soils, prevented from increase of leaf B content by B application; also Zn application prevented from B use affecting B concentration in the leaf. The lowest and the highest leaf B content were 25 and 39.33 mg kg^{-1} DW but showed no significant difference relative to the control. The B concentration in leaf was higher than other research. So that, Mills and Jones (1996) reported that sufficiency range of B in ear leaf of maize at silking stage was 5−25 mg kg^{-1}; Of course, compared to results of other researchers was sufficient (Kelling, 2010).

3.3. The leaf Zn concentration

The main effect of Zn on the Zn concentration in the leaf was significant at 5% level, but the effect of different B levels on the Zn concentration in the leaf

was insignificant at 5% level (Table 3). Zinc application to the soil had no significant difference from the zero Zn level, but Zn spraying increased Zn concentration in the leaf relative to the no Zn level. Foliar spray increased leaf Zn concentration from 32.42 to 39.67 mg kg^{-1}, showing a 22.4% increase as compared with the no Zn level. In fact, most calcareous soils of Iran prevent absorption of Zn; in this soils Zn are soon rendered insoluble.

On calcareous soils where Zn and B deficiency are common, applications of Zn and B compounds to the soil have not been very successful because the Zn and B are soon rendered insoluble. Barker and Pilbeam (2007) reported that applications of nutrients by foliar spray are effective in areas of California and Arizona where soil applications of micronutrients are ineffective because elements such as Zn, Mn, and Cu are fixed in forms that are not readily available to certain crops. Foliar applications, besides resulting in higher Zn accumulation in plants, could be used to advantage if a farmer omitted the addition of Zn in the NPK bulk fertilizer or if Zn deficiency was suspected. Foliar spray applications in the early growth stages resulted in greater absorption of Zn than did those applied at later stages of growth (Gupta and Cutcliffe, 1978). This result indicates that Zn uptake, at least by corn, is more efficient through leaves than through the soil–root systems. Soils deficient in Zn frequently are low in organic matter, are sandy and/or have an alkaline pH (pH greater than 7.0) (Follett and Westfall, 2010). Foliar spray of Zn is the most common because it is the most widely deficient micronutrient. Treatment can also be quite effective if the correct material and methods of application are used (Christensen, 1982). Neutral Zn (52% Zn) and Zn oxide (75% Zn) are the most economical and effective on a recommended label basis (Christensen et al., 1978 and Christensen, 1982). There is no advantage in using chelated Zn products in sprays. They were originally intended for soil application, are more expensive, and less effective than neutral. Therefore in calcareous soils such as this region foliar application of Zn is effective than soil application.

The lowest and the highest mean Zn concentration in the leaf, 31.17 and 39.67 mg kg^{-1}, were seen at 16 kg ha^{-1} Zn and Zn spraying levels, respectively. Concentration of Zn in this research was sufficiency range according to the results of Mills and Jones (1996), they stated that sufficiency range for Zn in ear leaf of maize at silking (mg Zn per kg dry matter of plant tissue) was 25 – 100.

The effect of Zn-B interaction on leaf Zn concentration showed B application in any Zn levels had no significant effect on the Zn concentration in the leaf. The use of Zn foliar spray at B spraying level, increased leaf Zn concentration from 30.67 to 44.33

mg kg^{-1} which showed a 44.5 percent increase relative to the no Zn use at this B level, but showed no significant difference relative to the control with a leaf Zn content of 35 mg kg^{-1} (Table 3). At other B levels, Zn use had no significant effect on the leaf Zn content. Also, any treatments showed no significant effect on the leaf Zn concentration relative to the control (no Zn and no B use).

Table 2. The effect of Zn and B on B concentration in the leaf (mg kg^{-1})[*]

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Zn foliar spray	Mean of B levels
0	31.67 ab	30.67 ab	31.33 ab	30.67 ab	29.67 ab	30.80 a
3	31.67 ab	35.33 ab	34.33 ab	33 ab	33 ab	33.47 a
6	39.33 a	32 ab	30.67 ab	39 ab	32.67 ab	34.73 a
B foliar spray	32.67 ab	32.33 ab	32.67 ab	25 b	37 ab	31.93 a
Mean of Zn levels	33.83 ab	32.58 a	32.25 a	31.92 a	33.08 a	

The same letters are not significantly different in each row or in each column (p<0.05) by Duncan's test

Table 3. The effect of Zn and B on Zn concentration in the leaf (mg kg^{-1})[*]

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Zn foliar spray	Mean of B levels
0	35 abc	27.33 c	29.67 bc	32 bc	37.67 abc	32.33 a
3	31.67 bc	34 bc	32.67 bc	37.67 abc	37.33 abc	34.67 a
6	32.33 bc	35.67 abc	32.67 bc	36.33 abc	39.33 ab	35.27 a
B foliar spray	30.67 bc	33 bc	29.67 bc	36.33 abc	44.33 a	34.8 a
Mean of Zn levels	32.42 b	32.5 b	31.17 b	32.58 ab	39.67 a	

The same letters are not significantly different in each row or in each column (p<0.05) by Duncan's test

3.4. The correlation between the concentration of Zn and B in the leaf with other variables

The correlation coefficients (r) between different variables by the Pearson method and the relevant equations were obtained by the step by step method using the SPSS software. One can use each of the following equations depending on what are the variables measured and r and r^2, but the last equation derived, is the most complete equation containing dependent and independent variables and we must measure more variables to derive that equation. The

symbols * and ** in equations and correlation coefficients (r or r^2), are significance at 5% ($\alpha = 0.05$) and 1% ($\alpha = 0.01$) levels.

3.4.1. The leaf B concentration

BL = 2.63 + 14.3 NL, r = 0.62[**]

BL and NL are leaf B content (mg kg^{-1}) and leaf N content (%).There was a positive correlation between leaf B concentration and leaf N content (r = 0.62[**]), K content (r = 0.36), Fe content (r = 0.61[**]), Mn content (r = 0.42) and Cu content (r = 0.43), ear length (r = 0.38). ear diameter (r = 0.34), and number of grains along the ear (r = 0.37).

3.4.2. The leaf Zn concentration

The leaf Zn content showed a positive correlation with leaf N content (r = 0.30), K content (r = 0.36), Fe content (r = 0.36), Mn content (r = 0.45[*]), and Cu content (r = 0.84[**]), and ear weight (r = 0.33), and a negative correlation with leaf P content (r = - 0.36) and plant height (r = - 0.33). The equations of which were:

1) ZnL = 9.711 + 3.418 CuL, r = 0.84[**]

2) ZnL = 39.765 + 3.439 CuL − 0.141 H, $r^2 = 0.82$[**]

ZnL, CuL and H denote leaf Zn content (mg kg^{-1}), leaf Cu content (mg kg^{-1}) and plant height (cm).

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The Role of 4G/5G Genetic Polymorphism of Plasminogen Activator Inhibitor-1 Gene in Myocardial Infarction among Egyptians

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Abstract: Objectives: To assess weather the common polymorphic allele (4G) of plasminogen activator inhibitor-1 (PAI-1) gene is associated with myocardial infarction (MI) and with PAI-1 enzyme level in Egyptian patients. Methods: Fifty consecutive patients who presented with acute MI and 48 normal control subjects were included. Clinical features were examined, PAI-1 4G/5G gene polymorphism was detected using polymerase chain reaction and PAI-1 levels with other risk factors were determined in all subjects. Results: Patient age averaged 51 ($\pm$SD 10.4) years, 68% were men and 46% had a family history of MI. Overall frequency of 4G allele was 60.4% among patients versus 51.0% among normal controls. There was no significant difference in genotype distribution (4G/4G, 4G/5G and 5G/5G), (P=0.34) and allele frequency (P=0.191) between patients with myocardial infarction and controls. Neither carriage of 4G allele (OR=1.46; 95% CI: 0.83-2.59; p=0.191) nor 4G/4G homozygosity (OR= 1.73; 95% CI: 0.684-4.36; p=0.245) was associated with MI. There was a significant increase of PAI-1enzyme level (p<0.001) among patients than the control group and the plasma levels of the enzyme were highest in myocardial infarction patients who were homozygous for the 4G allele (4.3$\pm$3.5) with a stepwise decrease in levels as the number of 4G alleles decreased; (3.6$\pm$2.3) for 4G/5G and (2.6$\pm$1.8) for 5G/5G; however the difference was not statistically significant (F=0.82, P=0.45); and even the increased mean level of PAI-1enzyme in patients with 4G/4G genotype than in patients with 5G/5G genotype; was not statistically significant (P=0.568). The study revealed also increased frequency of smoking (P= 0.001), family history of myocardial infarction (P=0.03) and hypertension (P = 0.016) among patients than controls. In multivariate analyses, risk factors associated with MI were smoking; hypertension and high level of PAI-1 enzyme. Conclusion: The common PAI-1 polymorphism (4G) was not associated with MI in Egyptian population, however modest risk (i.e., OR, =1.46) could not be excluded. Increased level of PAI-1enzyme, smoking and hypertension are significant risk factors for myocardial infarction among Egyptians.
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1. Introduction

Plasminogen activator inhibitor-1 (PAI-1) plays a central role in modulating intravascular thrombosis and thrombolysis. It may contrast plaque growth but also promote thrombosis and plaque vulnerability provoking acute myocardial infarction (MI). It serves as the main physiological inhibitor of endogenous fibrinolytic activity by inhibiting both tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) (Kohler et al, 2000 and Agirbasli, 2005). Impaired fibrinolysis due to high PAI-1 activity has been shown to be associated with an increased risk of thrombotic events (Smith, 1998). PAI-1 over-expression may also promote development of weak plaques with thin fibrous caps by inhibiting both u-PA receptor and integrin-mediated cell adhesion and migration (Cortellaro et al, 1993). Therefore, over-expression of PAI-1 may play a critical role in the development of MI, by impairing

both thrombolysis and plaque stability (Hamsten et al, 1985). Since PAI-1 has also an inhibitory effect on the smooth muscle cell (SMC) migration and neointima formation it is possible that low PAI-1 levels may promote development of high grade coronary stenosis. On the other hand, by inhibiting cellular migration and neointima formation PAI-1 over-expression may have a protective effect against plaque growth (Collet et al, 2003).

High triglyceride levels were possibly connected with a predisposition to thrombosis through a coexisting high level of plasminogen activator inhibitor enzyme. Boncoraglio and his colleagues (2006), found that the 4G/4G genotype of PAI-1 was significantly associated with high cholesterol, but not with triglycerides. Chronic kidney disease (KCD) was also strongly associated with an increased risk of MI in the general population. The underlying mechanism behind this relationship is unclear but seems to be

independent from other common risk factors (Meisinger et al, 2006).

PAI-1 gene is located on chromosome 7 and contains eight introns and nine exons (Deng et al, 2001). A single guanosine insertion/deletion (4G/5G) polymorphism in the promoter region of PAI-1 gene at position –675 bp, may play an important role in the regulation of PAI-1 expression (Sobel, 1999). Moreover, morning increase in PAI-1 activity may be determined by 4G/4G genotype (Schneider et al., 2004). PAI-1 4G/5G polymorphism was associated with an increased (Libby et al, 2005; Onalan et al, 2008 and Isordia-Salas et al., 2009) or decreased risk of MI (Junker et al., 1998) in some studies, while no significant association was found in others (Sugano et al., 1998; Doggen et al., 1999 and Crainich et al., 2003).

The purpose of this study was to investigate the association between the deletion allele of the PAI-1 gene (4G) with myocardial infarction as well as with the plasma levels of PAI-1 enzyme among Egyptian patients.

2. Patients and Methods:

This case control study included 50 consecutive Egyptian patients (34 males and 16 females); their ages ranged from 33 to 80 years old having myocardial infarction admitted to the cardiovascular intensive care unit in Kasr El Eny Hospital. Patients had no diabetes mellitus or obesity and 48 unrelated healthy, age and sex matched volunteers (control group); their exclusion criteria were: diabetes mellitus, obesity, signs of ischemic changes on electrocardiogram and ischemic changes during maximal stress exercise test. Clinical information was obtained by history, physical examination and routine laboratory analyses. Participants were genotyped for 4G/5G polymorphism using the polymerase chain reaction analysis, and their plasma PAI-1 enzyme levels were measured. Informed consent was obtained from all participants after a clear explanation of potential risk of the study.

Blood sampling
Specimen Collection:

Venous blood samples (10ml) were withdrawn from each subject and divided into three parts:
- Two ml was collected in a tube containing ethylenediamine tetraacetate (EDTA) as an anticoagulant and submitted to genomic DNA extraction from peripheral blood leucocytes using QIA amp DNA mini kit (QIAGEN, Inc., Germany).
- Six ml was collected in a clean dry centrifuge tube. Blood was allowed to clot at 37 ° C water bath. Clot was separated and centrifuged for 10 minutes at

3000 g. Serum was divided into aliquots and stored at – 20ºC until analyzed. Samples were assayed for measurements of cholesterol, triglycerides, urea and creatinine.
- The rest of the blood sample was used for detection of serum levels of PAI-1 enzyme.

Methods

Molecular detection of 4G/5G polymorphism genotyping

To identify the plasminogen activator inhibitor-1 (PAI-1) genotypes, polymerase chain reaction amplification of promoter region containing the 4G/5G polymorphism was done. The PCR reaction used an upstream control primer (5`- AAG CTT TTA CCA TGG TAA CCC CTG GT- 3`), an allele specific primer 4G (5`-GTC TGG ACA CGT GGG GA-3`) or 5G (5`-GTC TGG ACA CGT GGG GG-3`) and a common downstream primer (5`-TGC AGC CAG CCA CGT GAT TGT CTA-3`).

Two PCR reactions were run per a sample (one for 4G allele and the other for 5G allele); i.e. each reaction contained (upstream primer, downstream primer and one primer for 4G or 5G).

The 4G allele-specific PCR reaction mixture with a total volume of 25µl contained 50-100 ng DNA, 1unit of Taq polymerase, 1x PCR buffer Taq, 0.8mmol/l $MgCl_2$, 50 µmol/L dNTPs, 200 nmol/L upstream primer and 400 nmol/L allele specific and downstream primers. The 5G allele-specific reaction was identical except $MgCl_2$ which was 1.1 mmol/l. The 4G allele thermal cycling conditions were 32 cycles of denaturation at 94∘C for 35s, annealing 65∘C for 35s and extension 72∘C for 70s. The 5G allele thermal cycling conditions were the same except that the number of cycles was 22 cycles and annealing at 58∘C. The PCR product was electrophoresed on 1.5% agarose gel stained with ethidium bromide. A 257bp control band resulted from the upstream and downstream primers. The 4G or5G allele specific primer and downstream primer generated 139bp fragment. Patients who showed amplification products with only 4G primers were 4G/4G, and with only 5G primers were 5G/5G and patients who had amplification products with both primers were 4G/5G heterozygote (Figures, 1and 2).

Biochemical analyses:

The serum levels of PAI-1 enzyme were measured by immunoenzymatic ELISA method using the Hyphen Bio-Med kit. The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PAI-1 has been pre-coated onto a micro-plate. Standards and samples were pipetted into the wells and any PAI-1 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-

linked polyclonal antibody specific for PAI-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PAI-1 bound in the initial step (Declerck et al 1998).

-Serum total cholesterol was estimated according to Röschlau et al, 1974 and triglycerides according to Fossati and Prencipe, 1982. Urea and creatinine were measured using enzymatic methods (Faweet and Scott, 1960).

Statistical analysis:

The data were analyzed using the statistical package SPSS (version 15). They were expressed as mean± standard deviation for quantitative variables and number and percentage for qualitative values. Statistical differences between categorical data like gender, genotype distribution, smoking, family history of myocardial infarction and hypertension of patients and control groups were tested using Chi Square test. For qualitative variables, independent sample t test and ANOVA (analysis of variance) with post Hoc Bonferroni test were used for normally distributed variables, while Nonparametric Mann -Whitney test and kruskal-Wallis test were applied for variables which were not normally distributed (PAI-1 level, urea and creatinine). Associations were assessed as OR and 95% confidence intervals (CI). Logistic regression analysis was used to test for significant risk factors for myocardial infarction. Values less than or equal to 0.05 were considered statistically significant.

3. Results:

Patient age averaged 51 (±SD 10.4) years, 68% were men, and 46% had a family history of MI. There were no statistically significant differences between patients and controls as regards to age and gender, however there was a significant increased

frequency of smoking (X^2=11.83, P=0.001), family history of myocardial infarction (X^2=4.7, P=0.03), hypertension (X^2=5.76, P=0.016) and increased PAI-1 level (Z=-4.42, p<0.001) among patients with myocardial infarction than controls as shown in table (1). Only 48 patients were genotyped (two of the cases had DNA amplification failure). There was no significant difference in genotype distribution (4G/4G, 4G/5G and 5G/5G) (X^2=2.16 and P=0.34) between the two groups and in spite of the 4G allele occurred more frequently than the 5G allele in the patient group (60.4% of patients vs. 51.0% of control) (OR=1.46; 95% CI: 0.83-2.59) the difference was not statistically significant (p=0.191). Regression analysis showed no significant differences between patients and controls as regard 4G4G vs. 4G5G+5G5G (OR 1.73, 95% CI: 0.684-4.361and p=0.245) and 4G4G+4G5G vs. 5G5G genotype (OR=1.99, 95% CI: 0.612-6.433 and p=0.247) (Table 2).

The plasma levels of plasminogen activator inhibitor-1 enzyme were the highest in myocardial infarction patients who were homozygous for the 4G allele (4.3±3.5) with a stepwise decrease in levels as the number of 4G alleles decreased (3.6±2.3) for 4G/5G and (2.6±1.8) for 5G/5G but the difference was not statistically significant (F=0.82, P=0.45). Cholesterol, triglycerides, urea and creatinine, showed no statistically significant difference in patients having the three different genotypes (p values were = 0.98, 0.98, 0.56 and 0.41, respectively) (Table 3) and even between patients which were homozygous to 4G/4G alleles and 5G/5G alleles, the only significant difference was detected in creatinine level (P=0.039). Logistic regression was done to test for significant predictors of MI. Male gender, age, smoking, family history, hypertension, PAI-1 level, 4G allele were involved in the regression model while only smoking, hypertension and PAI-1 level were found to be significant predictors (Table 4).

Table (1): General characteristics of patients and controls

Characteristic	Patients no= 50	Controls no= 48	Test of significance	p value
Gender Male, no (%) Female, no (%)	34 (68%) 16 (32%)	31 (64.6%) 17(35.4%)	X^2 = 0.128	0.721
Age (years) Range Mean ± SD	(33-80) 51±10.4	(35-79) 50.65±10	t= 0.162	0.871
Smoking	33(66%)	15 (31.3%)	X^2 = 11.83	0.001*
Family history of myocardial infarction	23 (46%)	12 (25%)	X^2 = 4.7	0.03*
Hypertension	23 (46%)	11(22.9%)	X^2 = 5.76	0.016*
PAI-1 level IU/mL	3.75±2.69	1.81 ± 0.45	Z= - 4.42	<0.001*

*p≤0.05 is significant.

Table (2): 4G 5G genotypic distribution and allelic frequencies; and their associations with myocardial infarction among Egyptians:

	Patients (n=48)	Control (n=48)	OR	95%CI	P value
Genotype, n (%)					
4G4G	15 (31.3%)	10 (20.8%)			0.34
4G5G	28 (58.3%)	29 (60.4%)			
5G5G	5 (10.4%)	9 (18.8%)			
4G4G vs. 4G5G+5G5G	15 (31.3%) 33(68.8%)	10 (20.8%) 38(79.2%)	1.73	0.684- 4.361	0.245
4G4G+4G5G vs.5G5G	43 (89.6%) 5 (10.4%)	39(81.3%) 9(18.8%)	1.99	0.612-6.433	0.247
Allelic frequency, n (%)					
4G	58 (60.4%)	49 (51.0%)	1.46	0.83-2.59	0.191
5G	38 (39.6%)	47 (49.0%)			

OR: odds ratio; CI: confidence interval.

Table (3): Characteristics of Egyptian patients with MI by 4G/5G polymorphism:

Parameter	4G/4G (n=15)	4G/5G (n=28)	5G/5G (n=5)	p value
Age	48.5±11.1	52±10.4	49.2±6.6	0.54
PAI-1 level	4.3±3.5	3.6±2.3	2.6±1.8	0.45
Cholesterol (mg/dl)	188±53.8	185.6±45.4	184±23	0.98
Triglycerides (mg /dl)	224.3±63.6	226.1±61.3	221±67.7	0.98
Urea (mg /dl)	44±9.1	45± 20.3	36± 14.8	0.56
Creatinine (mg/dl)	3± 2.1	2.2±3.2	1.2±0.5	0.41

Table (4): Multivariate logistic regression analysis for myocardial infarction:

	B	p-value	Exp(B)	95.0% C.I. for Exp(B)
Gender (Males)	0.479	0.360	1.615	0.578-4.509
Age	- 0.019	0.426	0.981	0.935-1.029
Smoking	-1.537	0.001*	4.650	1.878-11.516
Family history	0.705	0.105	2.025	0.863-4.751
Hypertension	1.822	<0.001*	6.182	2.436-15.866
PAI-1 level	1.292	<0.001*	3.641	2.177-6.088
4G allele	0.112	0.780	1.118	0.510-2.455
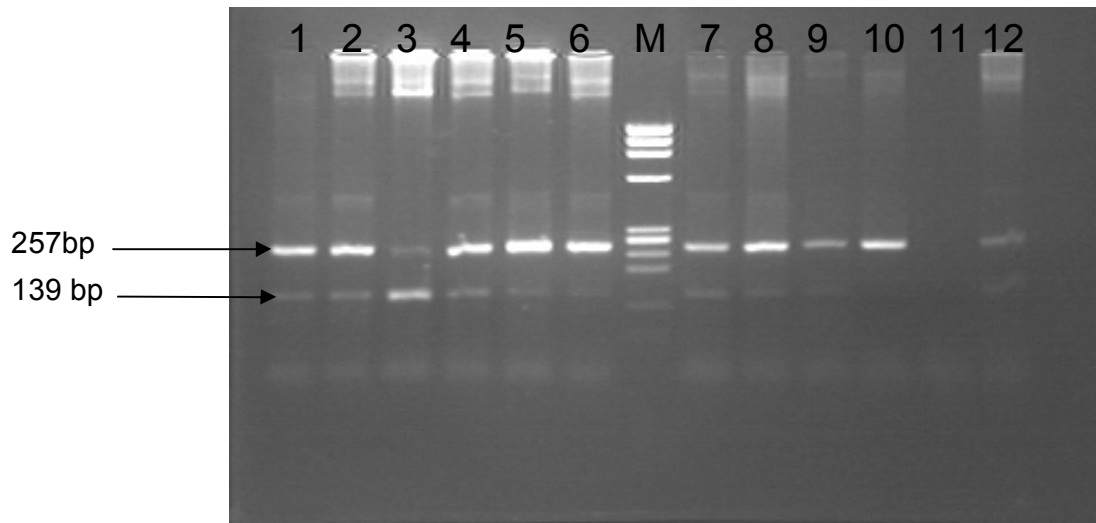
Fig (1): Example of polymorphic genotyping using 1.5% agarose gel electrophoresis of PCR amplification products. The 275 bp band corresponds to the control band and 139bp fragment to 4G band. Lane M is ØX174 HaeIII molecular weight marker. Lanes, 1, 2, 3, 4, 5, 6,7,8,9 and 12 showed 4G amplification bands. Lane 10 had no 4G band.   Lane 11 showed neither band for control nor for amplified 4G (failure of DNA amplification).
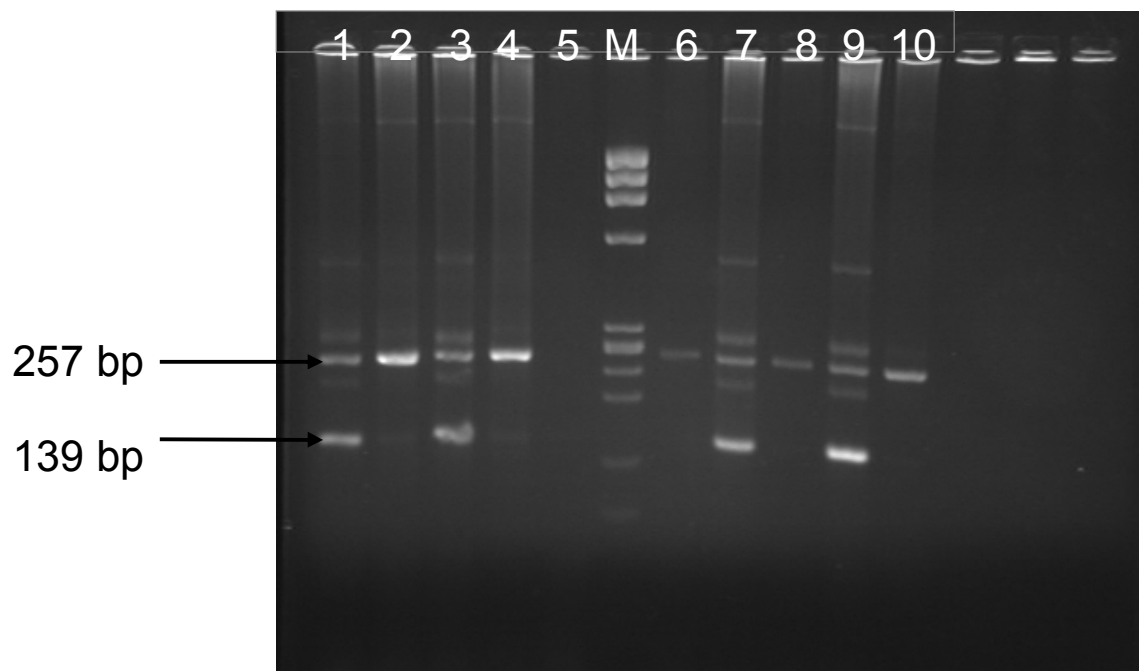
Fig (2): Example of polymorphic genotyping using 1.5% agarose gel electrophoresis of PCR amplification products. The 257 bp band corresponds to the control band and 139bp fragment to 5G band. Lane M is ØX174 HaeIII molecular weight marker. Lanes 1, 2, 3, 4, 7and 9 shows 5G amplification bands. Lane 5 shows neither band for control nor for amplified 5G (failure of DNA amplification). Lanes 6 and 8 had no 5G bands. Lane 10 shows 5G amplification band but faint in density.

4. Discussion

The etiology of cardiovascular disease is multifactorial and strongly involves genetic and environmental factors. PAI-1 is a noteworthy factor in the plasminogen activation/plasmin cascade and its level is usually related to cardiovascular disease. Evaluation of inter-individual variation of the PAI-1 level is important for the assessment of an individual's risk for thrombotic disorders (Mei et al, 2003).

Both the 4G and 5G alleles in the promoter region of PAI-1 gene; has a binding site of a common transcription activator. However the 5G allele has an additional binding site for a repressor, leading therefore to lower transcription rates and less PAI-1 activity. Thus, the transcription activity of the 4G allele is higher than that of the 5G allele (Tsantes et al, 2008).

In the present study; in spite of the frequency of PAI-1 4G/4G genotype was found to be higher in Egyptian patients with myocardial infarction than the control group, it did not show significant difference in genotype distribution (4G4G, 4G5G and5G5G) (X^2 =2.16 and P = 0.34) and also, the 4G allele occurred more frequently than the 5G allele in the patient group (60.4% of patients vs. 51.0% of control) (OR= 1.46; 95% CI: 0.83-2.59) but the difference between them was still, not statistically significant (p= 0.191). Moreover we found about two-fold increased risk of myocardial infarction associated with 4G4G +4G5G (OR=1.99) (95% CI: 0.612- 6.433) compared with 5G5G. However, the p value was still not significant (p= 0.247) and also 4G4G vs. 4G5G+5G5G (OR, 1.73; 95% CI: 0.684- 4.361; p= 0.245). However modest risk of the 4G allele (OR= 1.46) and 4G4G genotype (OR= 1.73) for MI could not be excluded.

No significant association between PAI-1, 4G and MI was also reported by Crainich and his colleague (2003), who documented the lack of association of the plasminogen activator inhibitor-1 4G/5G promoter polymorphism with cardiovascular disease in the elderly. Also; Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group(2003), found no evidence of association between PAI-1 gene polymorphisms and the development of acute myocardial infarction at a young age (under the age of 45years). Sugano et al (1998), documented that plasminogen activator inhibitor-1 promoter 4G/5G genotype is not a risk factor for myocardial infarction in a Japanese population and Ding et al (2006), found that plasminogen activator inhibitor type 1 gene polymorphisms were associated with plasma plasminogen activator inhibitor type 1 levels but not

with myocardial infarction. Despite of our insignificant result this study adjusted OR of 1.46 for the 4G allele vs to the 5G allele and of 1.73 for 4G/4G vs the other two genotypes and these observations are consistent with that PAI-1, 4G exerting at most a modest independent effect on athrothrombotic events occurring late in disease progression as the polymorphism probably require interaction with other genetic and environmental factors. Also, our study was retrospective with respect to MI events, raising the possibility of changes in prevalence of PAI-1, 4G among cases compared with controls due to differential survival rates after MI based on PAI-1, 4G carrier status. Nevertheless, prospective, matched case-control studies would be of interest.

We examined the relationship between the 4G/5G gene polymorphisms and plasma PAI-1 level among myocardial infarction patients and the control group in Egyptians. The 4G/4G homozygous subjects showed the highest plasma concentrations of PAI-1, the lowest were seen in subjects with the 5G/5G genotype, and intermediate concentrations were recorded in heterozygotes; however the difference was not statistically significant (F=0.82, P= 0.45). This may be due to wide variability of the level of PAI-1 enzyme in our sample and we recommend increased sample size in future studies to decrease this variability.

By using logistic regression analysis only smoking; hypertension and PAI-1 level were found to be associated with myocardial infarction among Egyptians.

Variability in PAI-1 plasma concentrations has been reported in different ethnic groups around the world. In some cases this appears to be governed by 4G/5G polymorphism (Dawson et al., 1991; Ye et al., 1995; Nordt et al., 2001). While in others environmental factors such as smoking are involved (Eliasson et al., 1995). Smoking by patients carrying the 4G allele may have an important impact on the frequency of MI. Anti-tobacco campaigns aimed at this group should therefore be intensified and the screening of such individuals should be contemplated. However, interaction with other, traditional risk factors is almost certainly involved in the development of MI and it is important to identify them for primary prevention early in life. The association between hypertension and MI may be related to the relevance of the Ca2+ -dependent potassium channel in the control of human blood pressure and its impact on cardiovascular disease which was evidenced by Tomás et al (2008), who found that two polymorphisms in the pore-forming alpha subunit gene (KCNMA1) were risk factors for severe essential hypertension and myocardial

infarction. We also studied the biochemical characteristics of the Egyptian patients with MI and we found only positive significance difference in creatinine level between patients with 4G/4G and those with 5G/5G polymorphisms (P=0.039), Merino and his colleagues (2000), documented that a mild to moderate elevation of serum creatinine level is an independent risk factor for stroke and MI in patients with carotid artery stenosis.

In conclusion: The pathogenesis of MI is complex and multifactorial, with multiple interacting environmental and genetic determinants. The common PAI-1 polymorphism (4G) was not associated with MI in Egyptian population however modest risk (i.e., OR=1.46) could not be excluded. PAI-1variants might affect risk only in concert with other specific environmental and genetic factors. Hence, further research on coagulation- related genetic factors is warranted, including prospective studies of 4G/5G and other PAI-1polymorphisms in large populations at risk for atherothrombotic events. Increased level of PAI-1enzyme, smoking and hypertension are significant predictors of myocardial infarction among Egyptians.

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Severe Anemia in Children Infected With Malaria in Taiz - Yemen and Its Relation to Age, Parasitaemia and Eosinophilia

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Abstract: In Yemen, about 12 million individuals live in endemic areas of malarias, out of them more than 90% were due to Plasmodium falciparum (WHO, 2001 a)

Malaria continues to be a major health problem in Yemen .Severe anemia in malaric children occurs more frequently than cerebral malaria (Laurence et al, 1994).

The aim of this work is to detect the relation of severe anemia in malaric patients < 5ys with the parasitaemia level of Plasmodium falciparum .

This research was done in Alsewedi pediatric hospital in Taiz governorate from January to September 2008,for 100 admitted cases, results, of this study indicated the strong relation of severe anemia in falciparum malaric children to age < 5ys and there was no relation between the severe anemia in falciparum malaric children to the parasitaemia level .There was strong relation between younger ages and low level of Hb (P<0.05). These finding suggest that increase level of parasitaemia not related to severe anemia in P falciparum malaria. Also younger ages <5ys has strong relation to severe anemia Hb<5g per ul in falciparum malaria.

1. Introduction

Plasmodium falciparum malaria causes 1-2 million deaths per year (WHO, 2001b), another half billion get infected but survive, most cases are found in sub-sahran Africa (Kahl, 2003).

Age and transmission intensity are known to influence the manifestations of severe falciparum malaria in African children (Idro, et al., 2006) .Malaria is one of the main causes why 3.5 millions low-birth weight infants are born each year in the region (Roger, 2000).

Reduction in severe disease and death from falciparum malaria in Africa requires new, more effective and inexpensive public health measures (Miller and Greenwood, 2002).

The burden of malaria in tropical world is estimated to involve 300-500 million episodes of acute illness and more than million deaths per year, mainly in African children. The emergence of Plasmodium falciparum resistance to widely used antimalarial drugs such as chloroquin has made malaria control and treatment much more difficult (D'AleTssandro and Butteins, 2001).

Resistance of Plasmodium falciparum to chloroquin was confirmed in most countries of Sub-Sahran Africa (Trape, 2001 & Carter and Mendis, 2002)

Highly complex and dynamic system (Craig et al., 2004). Malaria is mesoendemic and transmission is perennial, falciparum is the main species identified and A.arabiensis the main vector in Taiz (Daoud, 1988).

Malaria is one of the most common diseases in Yemen Arab Republic, Plasmodium falciparum represented > 90%, 20% of cases resistant to chloraquin (Al-Mawri, 2000).

2. Materials and Methods:

Study area:

Taiz Governorate in Yemen .It lies in the foothill and middle heights, which rang from 200-2000m elevation. The mean annual temperature between 20-3°c with little seasonal variation and relative humidity 40-60.The annual rainfall is 800-1200mm, and most of this fall in March-May and Augest-September.

In the present study used questionnaire for personal data, laboratory tests for each admitted malaric child and we get agree from each patient "consent"

The study was in Alsewedi - pediatric hospital in Taiz governorate / Yemen on 100 admitted positive Plasmodium falciparum malaria, age from 1 to 10 years regardless the sex .This study was from January to September 2008.

Peripheral blood smear (thick and thin) stained with Giemsa stain is used to identified the species, a sexual stage of parasite and the level of

parasitemia (WHO, 1985, Shute, 1988 & Garcia, 2001). Parasitaemia may be expressed as a percentage of RBCs infected, or as number of parasites present in 1 ul of blood. Since 1 ul of blood contains 5×10^6 RBCs,a 1% parasitaemia represents 50000 parasites per ul. (Moody, 2002).

Drabkins solution is used to determined hemoglobin level in g/dL(a colorimeter). Alonso et al (2002) defined severe anemia as Hb <5g per dl in patient with asexual form of P.falciparum in peripheral blood.

Counting eosinophil level:Eosinophil relative frequencies were determined by differential counting and for hospital study the absolute eosinophil count was obtained by multiplying the frequency with total leucocytes.

The eosinophil level were counted in non infected and infected children and the infected children divided as acute infection and asymptomatic infection.,the level of eosinophil also counted in infected children after cure.

The statistical analysis of data was carried out using statistical package for social science (Spss/ps).

3. Results:

The research aimed to detect the relation between severe anemia in malaric children to the age and level of parasitemia.

- According to study all the 100 positive cases were Plasmodium falciparum species.
- In present study, hemoglobin was classified into < 5g/dL and > 5-10g/dL according to (Alonso et al., 2000).

Table (1): shows the number of admitted patients according to age.

There were 45 positive cases their age < 5ys old, and 55 cases, their age from 5-10 ys , Total cases positive were 100.

Table (1): Number of malaric children according to age.

Age	< 5ys	5-10y
No	45	55
Total	100 cases	

<: less than.   >: More than. g/dL : gram per deciliter.
p/uL: parasite per micro liter.   ys: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Normal children (non infected)
Table (2): Represented the percentage of Hb in malaric children in relation to their age:

In positive cases < 5ys old a bout 15/45 cases their Hb < 5g/dL, means those cases suffered from severe anaemia, then was positive significant

correlation between the level of Hb and the age of positive case (P< 0.05) .

Table (2): Hb precent in malaric children according to age.

Age	<	5ys	5-10y	
Hb%	<5g/dL	>5g/dL	<5g/dL	>5g/dL
Cases	15	30	10	45
P.value	0.045			

<: less than.   >: More than. g/dL : gram per deciliter.
p/uL : parasite per micro liter.   ys: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Level of Hb decreased with age, but level of Hb was > 5g/dL in about 30 positive cases their age > 5 ys .In positive cases their age from 5-10 ys about 10 cases their Hb was < 5 g/dL but 45 cases their Hb > 5 g/dL .This indicated that increase the age of patients , increasing the level of haemoglobin .At end of this table, the severe anaemia was in malaric patients their age < 5 ys.

Table (3): Represented the % of Hb in relation to level of parasitaemia (PD) . The numbers of positive cases < 5 ys and their Hb < 5 g/dL, their (PD) was 100 - 250 (P/ul) . The numbers of positive cases < 5 ys and their Hb > 5 g/dL, their parasite density was 500 - 1000 p/ul.

This indicated that there was positive relation between the Hb in malaric cases and age (P < 0.05). , but no correlation between severe anaemia in malaric cases and PD (P > 0.05) . This mean high level of parasitaemia not related to severe anaemia in malaric patients . The numbers of positive cases > 5 ys ,thejr Hb level > 5g / dL and their PD was 500-1000 p/uL but the numbers of positive cases > 5 ys and their Hb > 5 g/dL and their PD was > 10,000 p/uL . Means again that no relation between severe anemia and PD .Decrease Hb not means high level of parasite in peripheral blood.

Table (3): Hb concentration level in relation to level of parasitaemia (PD) in malaric children.

Age	<	5ys	5-10y	
Hb%	<5g/dL	>5g/dL	<5g/dL	>5g/dL
No. of cases	15	30	10	45
PD (p/uL)	100-250	500-1000	500	1000
P. value	> 0.05			

<: less than.   >: More than. g/dL : gram per deciliter.
p/uL : parasite per micro liter.   ys: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Table (4): It represented the positive malaric cases with severe anemia who received blood transfusion < 5 ys. About 15 out of 45 positive cases

received blood. In positive cases > 5 ys 10 out of 55 cases received blood.

Table (4): Number of positive malaric children with severe anemia and received blood transfusion

Hb%	Blood transfusion	
	Yes	No
<5g/dL	15	30
>5g/dL	10	45
Total	25	75

<: less than.   >: More than. g/dL : gram per deciliter.
p/uL : parasite per micro liter.   ys: years .
Hb: hemoglobin.
%: percentage PD: parasite density. P:*P Plasmodium*.

Eosinophilia in Plasmodium falciparum infected children and non infected children:

About the infected child they are investigated for eosinophilia as acute infection and asymptomatic parasitemia. A significant drop in eosinophils was observed during acute illness, returning to normal value after cure, the percentage of eosinophil in non infected child was 5-5.5%.and the percentage of eosinophil in infected children in acute stage was 2.5-5%. In contrast a significant increase in eosinophil frequency was observed in children with asymptomatic parasitemia at the times of infection eosinophil frequency was scientifically higher in asymptomatic children than in clinical malaria., the percentage of eosinophilia in this group was 5-8%.

4. Discussion

Malaria caused by *P.falciparum* remain the major life threatening parasitic infection in the world, (Durand et al., 2005).
From table 1: indicate that the age <5 yrs is more exposed to severe malaria and anaemia.

Idro et al, 2006 reported a relation between manifestation of severe malaria in children < 5yrs and transmission intensity and anaemia, which agree with our funding.

It is clear that there is a strong relation between age <5 ys and decreased Hb. (Tbale 2). Kwadwo et al., 2003, recorded that severe anemia was diagnosed in 30 individuals ranging form 3 months to 5 ys, but was primary noticed in children <24 months. These results coinced with Price et al., 2001 they stated that there was relation between decreased age and decrease Hb. They also added that children age <5ys were more likely than bolder to become anemic in Thailand.

It is clear that no relation between decreased Hb and parasitaemia level (Table 3). On the other view Kwadwo et al., 2003, showed that parasitaemia was strongly associated with lower Hb in children <2ys of age. But Al Serouri et al., 2002, not agreed with our result, they showed that parasitaemic group had a significantly lower Hb than non parasitaemic group.

And about the level of eosinophil in our study, our data indicate that *p. falciparum* infection induce eosinophilia. Our study led us hypothesis that *plasmodium falciparum* infection induces eosinophil production but that the excess production in clinical malaria is out balanced may be due to increase sequestration or destruction due to inflammatory process in the tissue, kurtzhals et al., (1998) agree with our study, they mentioned the increase in eosinophil activity in acute Plasmodium falciparum infection in association with cerebral malaria. Shanks and Wilairatanaporn (1992) said that eosinophilia in persons from malaria endemic area my represent a normal late response to malaria infection.

Jungwon et al., (2005) mentioned that hematologist should consider the possibly of pseudoeosinophilia as result hemozoin-containing WBCs and confirm the WBCs differential count by microscopy in cases of malaria.

The absolute eosinophil count was increase above the normal range in 7% of American servicemen with acute malaria eosinophilia was found in 6% before treatment and 30% after treatment of malaria (Reilly and Barrett, 1971).

In Gambian children with acute uncomplicated malaria there was reduction or absence of eosinophils in the peripheral blood at presentation and these returned to at normal numbers in all cases 3-7 days after treatment of malaria (Abdullah, 1988). No such changes were seen in patients with chronic malarial anemia. A study in Thailand found that eosinophil counts were elevated in 11% of patients with acute malaria at presentation and 93%had elevated eosinophil count by the day 7 after treatment there was then a marked reduction of eosinophil count by day -14 followed by another increase by 28 day (Camach et al., 1999).
Recommendation:

Malaria as a cause of severe anemia in our children should be given a wide attention and followed from the researchers, a cadmic staff, ancftnedical students.

Malaria is a dangerous disease we should become more a wariness to this problem.

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Molecular Biological and Biochemical Studies on Avian Influenza Virus Receptors in Different Avian Species

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Abstract: Avian influenza viruses are considered to be the key contributors to the emergence of human influenza pandemics. A major determinant of infection is the presence of virus receptors on susceptible cells to which the viral haemagglutinin is able to bind. Avian viruses preferentially bind to sialic acid α 2,3-galactose (SA α 2,3-Gal) linked receptors, whereas human strains bind to sialic acid α 2,6-galactose (SA α 2,6-Gal) linked receptors. Although ducks are the major reservoir for influenza viruses, they are typically resistant to the effects of viral infection, in contrast to the frequently severe disease observed in chickens In order to understand whether differences in receptors might contribute to this observation, we studied the expression of influenza receptors in upper and lower respiratory organs of ducks and chickens (expression of ST3Gal-III sialyltransferase and ST6Gal-I sialyltransferase genes) using semi quantitative RT-PCR. There was a marked difference in the expression of primary receptor type in the trachea of chickens and ducks. In chicken trachea, SA α 2,6-Gal was the dominant receptor type whereas in ducks SA α 2,3-Gal receptors were most abundant. This suggests that chickens could be more important as an intermediate host for the generation of influenza viruses with increased ability to bind to SA α 2,6-Gal receptors and thus greater potential for infection of humans. Chicken tracheal and intestinal epithelial cells also expressed a broader range of SA α 2,3-Gal receptors in contrast to ducks, which suggests that they may be able to support infection with a broader range of avian influenza viruses.

1. Introduction

The influenza viruses are medium-sized, comprising enveloped and negative sense RNA viruses with a segmented genome. Taxonomically, they belong to the virus family Orthomyxoviridae. There are three genetically and antigenically distinct types of influenza viruses called A, B, and C. Type A viruses are further divided into subtypes according to the combination of two main envelope glycoproteins the hemagglutinin (HA)and neuraminidase (NA). To date, 16 HA subtype (H1–H16) and 9 NA subtypes (N1–N9) have been found (Ghaleb, 2009). Influenza A virus infects several hosts, including humans, birds, swine, and horses, but individual viruses are usually adapted to sustained infection in only one species. Viruses isolated from these different species bind sialic acid through their surface glycoprotein, hemagglutinin, and require this interaction for productive infection (Gambaryan et al., 2005). The first step in the virus infection process is the recognition of cellular structures that act as specific receptors. This determines the virus tissue tropism and is performed by viral adhesion proteins (Tardieu et al., 1982) .The viral attachment to the host cell is critical for tissue and species specificity of virus

infections (Debby et al., 2007). Influenza virus initiates infection by binding of the viral hemagglutinin (HA) to sialic acid on the cell surface. (Stray and Air, 2001). The receptors for influenza viruses are sialic acids (SAs), which are usually formed 2,3 or 2,6 configuration linked to the cell-surface glycoproteins and glycolipids (Harduin-Lepers et al., 2005). Sialic acid is an essential component of cell surface receptors for a variety of microorganisms and microbial toxins (Mouricout, 1997). Sialic acid is added to the terminal sugar of glycoproteins and glycolipids by enzymes called sialyl transferases (Harduin-lepers et al., 2005).

Sialyl transferases (SiaTs) are required to synthesize all known sialyloligosaccharides (Shuichi, 1995). The ST3Gal III, preferentially transfers sialic acid in α 2,3 linkage to the Galß1-3GlcNAc disaccharidic sequence(Catherine et al.,1999). While ST6Gal-I generates an α 2-6 linkage of sialic acid to underlying N-acetyllactosamine (Weinstein et al.,1982). The differential expression of sialic acids in the mammalian respiratory tract may help to explain the low infectivity but high pathogenicity of some avian strains (Gambotto et al., 2008).

Influenza infection is initiated by virus attachment to sialic acid-containing cell-surface molecules traditionally called viral receptors. The spectrum of sialylglycoconjugates varies substantially between viral host species as well as target tissues and cell types of the same species leading to variations in the receptor-binding specificity of viruses circulating in these hosts. It is believed that a poor fit of avian viruses to receptors in humans limits the emergence of new pandemic strains (Matrosovich et al., 2008). Influenza A viruses attach to host cells by binding of the hemagglutinin (HA) protein to sialosaccharides on the host cell surface. The HAs of influenza A viruses from different host species differ in their specificity of binding. For example, HAs of human influenza A viruses preferentially recognize sialic acid (SA) α 2,6-Gal-terminated saccharides (α 2,6-SA), whereas HAs of avian influenza viruses preferentially recognize SA α 2,3-Gal-terminated saccharides (α 2,3-SA) (Connor et al.,1994). These differences generally correspond with the variation in the type of SAs expressed at important sites for influenza A virus replication in the respective host species. For example, human tracheal epithelium expresses mainly α 2,6-SA, whereas duck intestinal epithelium expresses mainly α 2,3-SA. Therefore, the type and distribution of SA is considered to be an important factor in the susceptibility of different host species to influenza A viruses (Suzuki et al., 2000). The SA recognized by influenza A virus is not only important in the host species range but also in its transmissibility (Tumpey et al., 2007). The HA protein mediates virus binding to sialic acid (SA)-containing host cell surface molecules and promotes the release of viral ribonucleoprotein complexes through membrane fusion.

Influenza virus infectivity is influenced by 2 entities:-

1- SA species (N-acetylneuraminic acid [NeuAc] and N- glycolylneuramic acid [NeuG].

2- The type of linkage to galactose (sialyloligosaccharides terminated by SA linked to galactose by an (alpha 2,6 linkage [Ac alpha 2,6Gal] or an alpha 2,3 linkage [Ac alpha 2,3Gal]) on the host cell surface (Rogers et al.,1985).

The host range selection of the receptor binding specificity of the influenza virus hemagglutinin occurs during maintenance of the virus in different host cells that express different receptor sialo-sugar chains (Yasuo, 2005). Ducks and chickens are important hosts of avian influenza virus (AIV) with distinctive responses to infection. Frequently, AIV infections in ducks are asymptomatic and long-lasting in contrast to the clinically apparent and transient infections observed in chickens. These differences may be due to the host response to AIV infection (Sean et al., 2009).

2. Materials and methods.
Bird selection and grouping:
Four groups of healthy, four weeks aged birds are classified as fellow:
1. group 1 : 5 chicken (Baladi).
2. group 2 : 5 chicken(Hubber).
3. group 3 : 5 duck(Baladi).
4. group 4 : 5 duck(Pekeni).

Tissue preparation:
- Birds were sacrified using highly sterilized scissors (180°Cfor 6 hours) to avoid RNA degradation by RNases and latex gloves weared to minimize RNase contamination.
- After excision of trachea and lung of tested birds, they were wrapped in aluminium foil and put immediately in liquid nitrogen container to make snap-freezing of tissue and minimize action of endogenous RNase.
- Samples were taken to detect the level of gene expression of ST3Gal-III(Galβ1-3(4)GlcNAcα2,3-sialyltransferase) and ST6Gal-I (Galβ1-4GlcNAcα2,6-sialyltransferase) in that organs.

Reverse transcriptase polymerase chain reaction(RT–PCR):
Using a semi-quantitative RT–PCR according to **(Mallet et al., 1995).**

A-Protocol of RNA extraction from tissue: total RNA was extracted with RNeasy Mini Kit (QIAGEN).

B-Protocol of reverse transcription polymerase chain reaction: (one step RT-PCR) by using Robus T 1 RT-PCR kit(FINNZYMES)

The protocol was as fellow:-

All components, reaction mixes and samples were kept on ice. And the following reaction component were added to a nuclease free tube placed on ice.

Table (1): Reaction set up:

RT-PCR mix component	volume
10x Robus T reaction buffer	5 μl
50 mM MgCl2	1.5 μl
dNTP mix(10mM each)	1 μl
Template RNA	5 μl
Down stream primer	10 pmol
Up stream primer	10pmol
AMV RT 5 U/ μl	1 μl
DyNAzyme EXT DNA polymerase 1U/ μl	2 μl
RNase free water	Add to 50 μl

Gently mix the components, cycling conditions have to be optimized for each amplicon. and was transferred to the thermal cycler.(2720 thermal cycler Applied Biosystems).

Cycling instructions:

1-For ST3Gal lll gene: the primer for ST3Gal lll was synthesized to amplify PCR products

that cross introns to avoid confusion between mRNA transcript and genomic DNA.
The primers used to amplify this gene are:
Forward: 5- CGGATGGCTTCTGGAAATCTGT- 3
Reverse: 3- AGTTTCTCAGGACCTGCGTGTT-5
the product size was 300 bp.

Table (2)

Cycle step	Temp	time	Number of cycle
cDNA synthesis	48 °C	30 min	1
Inactivation of AMV reverse transcriptase and denaturation of the cDNA-RNA hybrid	94 °C	2 min	1
PCR amplification Denaturation Annealing extention	94 °C 63 °C 72 °C	30 sec 1 min 1.5 min	36 cycles
Final extention	72 °C	7 min	

2-For ST6 Gal I gene::- the primer for ST6 Gal I were synthesized to amplify PCR products that cross introns to avoid confusion between mRNA transcript and genomic DNA.
The primers used to amplify this gene are:
Forward: 5-TGGGCCTTGGCAGGTGTGCTGTTG-3
Reverse: 3-AGGCGAATGGTAGTTTTTGAGCCCACATC-5
the product size was 150 bp.

Table (3)

Cycle step	Temp	time	Number of cycle
cDNA synthesis	48 °C	30 min	1
Inactivation of AMV reverse transcriptase and denaturation of the cDNA-RNA hybrid	94 °C	2 min	1
PCR amplification Denaturation Annealing extention	94 °C 50 °C 72 °C	45sec 1 min 1 min	35 cycles
Final extention	72 °C	7 min	

Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) were amplified parallely as internal control (481bp) and its sequence:
Forward: 5-ACTTGTGATCAATGGGCACGCCATC - 3
Reverse: 3-CTTCCCATTCAGCACAGGGATGAC-5

For the Glyceraldehyde 3 phosphate dehydrogenase (GAPDH) amplified by 35 cycle

using (2720 thermal cycler Applied Biosystems). Each cycle consist of :
Denaturation   94°C for 45 second
Annealing   62 °C for 30 second
Extention   72°C for 45 second
The PCR products were separated by agarose gel electrophoresis.

C-Agarose gel electrophoresis (Sambrook and Maniatis., 1989)
1- Run parameters :
- Use 1-5 volts/ cm of the tank lenth.
- Allow bromophenol blue to run 2/3 of the gel lenth before terminating the run.
2- stop the run and transfere the gel to a transilluminator, observe and photograph. Photographing using polarized camera and parameters are preferably 302 nm wave lenghth, 2500 uW / cm2, or more, and using 22 A filter.
10- Using 100 bp- DNA ladder for electrophoresis of PCR product of GAPDH,ST3Gal-III and ST6Gal-I (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000bp) from Quiagen.
11-Analysis of PCR product using GEL pro-software to detect   quantitation of bands for GAPDH, ST3Gal-III and ST6Gal-I genes.

3. Results and Discussion:

The host receptor distribution pattern in the chicken and duck upper and lower respiratory tract may be functionally significant for the evolution of viruses with a human like receptor specificity and thus for the transmission of influenza from birds and

mammals. In this work, we conducted an extensive examination on the level of expression of influenza virus Receptors in trachea and lung of two different breeds of each chickens and ducks. Their was no difference in the reported results observed due to the breed of animals, and the receptor expression was consistent between individual animals within each species. Using RT-PCR in the gene expression of ST3GAL III and ST6GALI which add sialic acids to the terminal sugar of glycoproteins and glycolipids, we found that the trachea of ducks (Baladi, Pekeni) show high expression level of ST3GAL III) while trachea of chickens(Baladi, Hubber) show law expression level. (Figure:1) but Chickens trachea (Baladi,Hubber) show very high expression level of ST6GAL I in comparson to that of ducks trachea(Baladi, Pekeni) that show lawer expression level of ST6GAL I. (Figure:3). These results were in agreement with (Suresh et al.,2009) who reported that The major species difference that they observed between chickens and ducks in the relative distribution of SA α 2-3 Gal and SA α 2-6 Gal receptors was along the tracheal epithelium. In chicken tracheal epithelium, SA α 2-6 Gal was the dominant receptor type, whereas in ducks the SA α 2-3Gal receptor was more abundant in the ciliated cells of the tracheal epithelium, it was found that the ratio of SA α 2-6 Gal to SA α 2-3 Gal in chickens trachea was approximately 10:1 whereas in duck the ratio was 1:20. The tracheal mucous glands of both chickens and ducks predominantly expressed SA α 2-6 Gal receptor type. The observed difference in dominant receptor type between chickens and ducks was confined to the upper airway (trachea). While the dominant SA α 2-6 Gal receptor expression pattern in chickens trachea was in contrast to a previous study (Wan and Perez, 2006) which, using lectin binding, found that 85% of the epithelial cells in chicken trachea were positive for SA α 2-3 Gal receptors, while only 10% were positive for SA α 2-6 Gal receptors.

Also we found that the lung of ducks (Baladi, Pekeni) showed high expression level of ST3GAL III .while lung of chickens(Baladi, Hubber) showed low expression level. but the difference between expression level of ducks trachea and lung tissues is high in case of trachea more than the lung tissue, but the expression level of chicken trachea is lower than that of lung tissue. (Figure:2). And Chickens lung (Baladi,Hubber) showed very high expression level of ST6GAL I in comparson to that of duck lung (Baladi, Pekeni) that show lower expression level of ST6GAL .

But the difference between expression level of ducks trachea and lung tissues is high in case of lung more than the trachea tissue, while the expression level of chickens lung is lower than that of trachea tissue. (Figure:4). The present results were also in agreement with the findings of Gambaryan et al (2002), who reported that human influenza viruses with SA α 2,6-Gal specificity bound to cell membranes isolated from chickens (but not ducks) tracheal cell membranes. Chicken alveolar cells expressed both receptor types. The difference in the predominant receptor across the tracheal epithelial lining in chickens and ducks could be an important contributing factor to influenza virus entry via the upper respiratory tract. In particular, such differences could impact on the susceptibility of each species to avian H5N1 influenza with its preferential tropism for infection of the respiratory tract rather than the intestines. The differences in receptor expression reported in the present study suggest that they may be responsible, at least in part, for some of the differences between ducks and chickens in the pattern of disease following influenza infection. While the presence of a virus receptor is clearly not sufficient to confirm that cells or tissue support efficient virus replication or transmission, the widespread replication of influenza virus in multiple organs has been reported in both chickens (Swayne, 1997) and ducks (Londt et al., 2008) following infection with highly pathogenic viruses.

This study suggests that some chickens and ducks tissues may facilitate entry of both human and avian viruses, with the ensuing danger of virus reassortment. However, further work is required to confirm that the tissues expressing both receptor types are able to support virus replication. The dominant presence of SA α 2-6 Gal receptor along the chicken tracheal epithelium shows some similarities to the prevalence of the receptor in mammals such as human and pig. This suggests that chickens may be important intermediate hosts for the transmission of influenza to humans, in particular for influenza viruses such as H5N1, which show a respiratory tropism in birds. Whilst much attention has been placed on the role of pigs as "mixing vessels", the potential importance of chickens for the evolution of humanised influenza viruses should not be overlooked and, as such, wants further studies. Previous studies on role of sialic acid linkage (SA α 2,3 or SA α 2,6) during influenza virus infection have shown the importance of expression of these glycans in restricting infection by viruses in different hosts.

In this work, we found the presence of both S.A α 2-3 Gal receptor and SA α 2-6 Gal receptor in chikens trachea and lung due to the expression of the two genes suggest that they may be susceptible to infection with wider range of avian influenza viruses with broader receptor specificity.
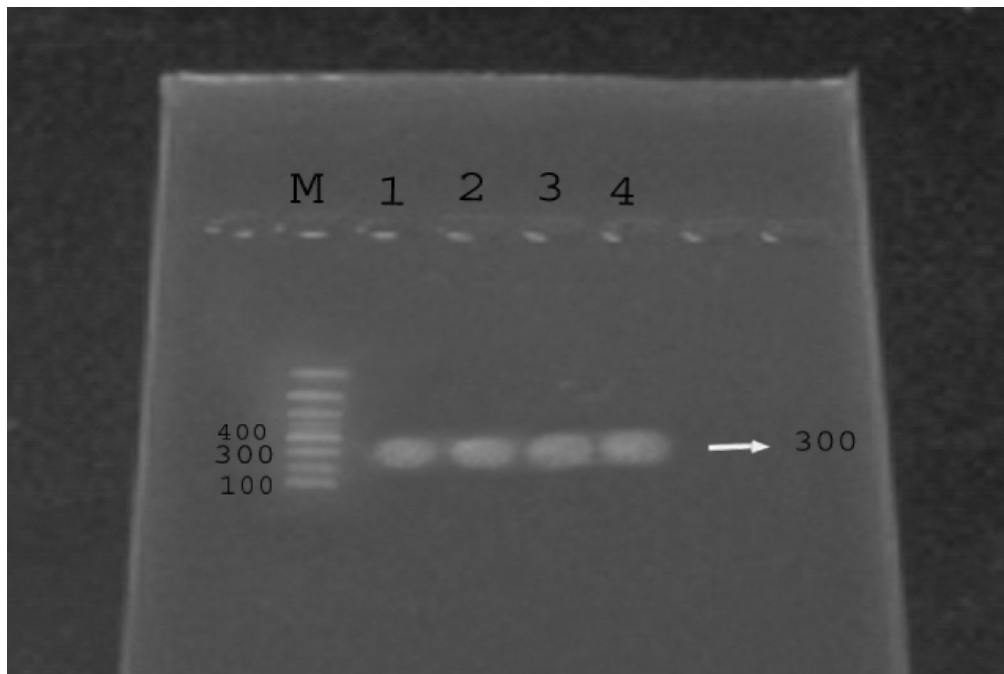
Figure (1): The elctrophoretic photograph showing the pattern of ST3GALIII mRNA expression in trachea of different birds

M: DNA ladder   Lane 1: chicken Baladi trache   Lane 2: chicken Hubber trachea
Lane 3: Duck Baladi trachea   Lane 4: Duck Pekeni trachea
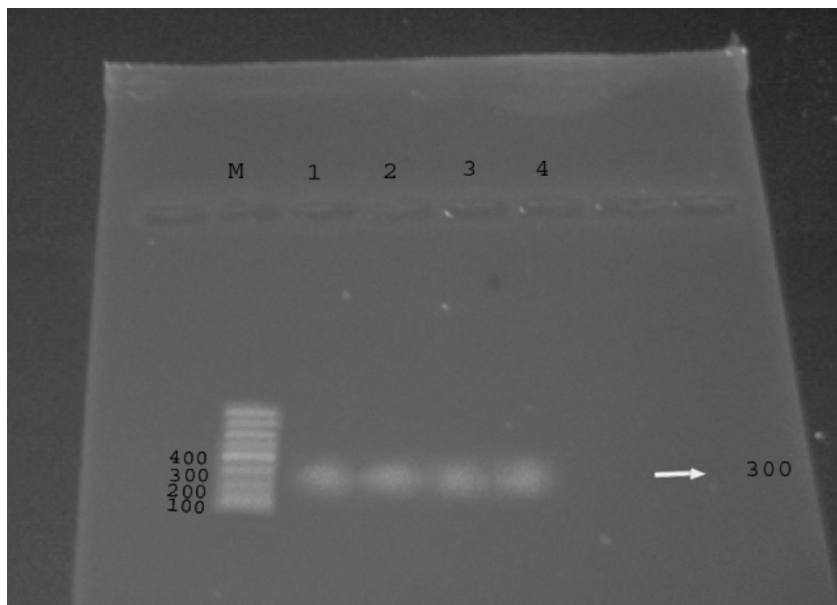
Figure (2): The elctrophoretic photograph showing the pattern of ST3GAL III mRNA expression in lung of different birds

M: DNA ladder   Lane 1: chicken Baladi trachea   Lane 2: chicken Hubber trachea
Lane 3: Duck Baladi trachea   Lane 4: Duck Pekeni trachea
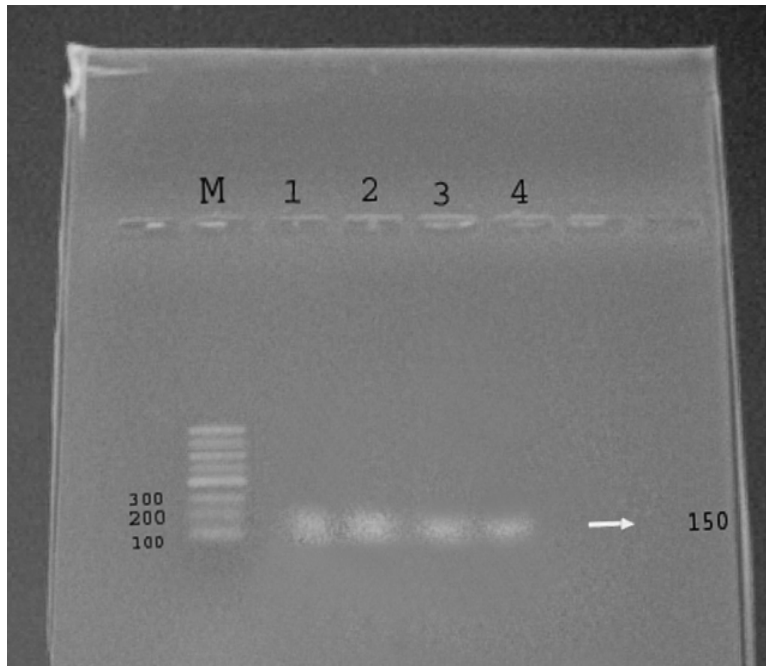
Figure (3):The elctrophoretic photograph showing the pattern of ST6GALI mRNA expression in trachea of different birds

M: DNA ladder   Lane 1: chicken Baladi trachea   Lane 2: chicken Hubber trachea
Lane 3: Duck Baladi trachea   Lane 4: Duck Pekeni trachea
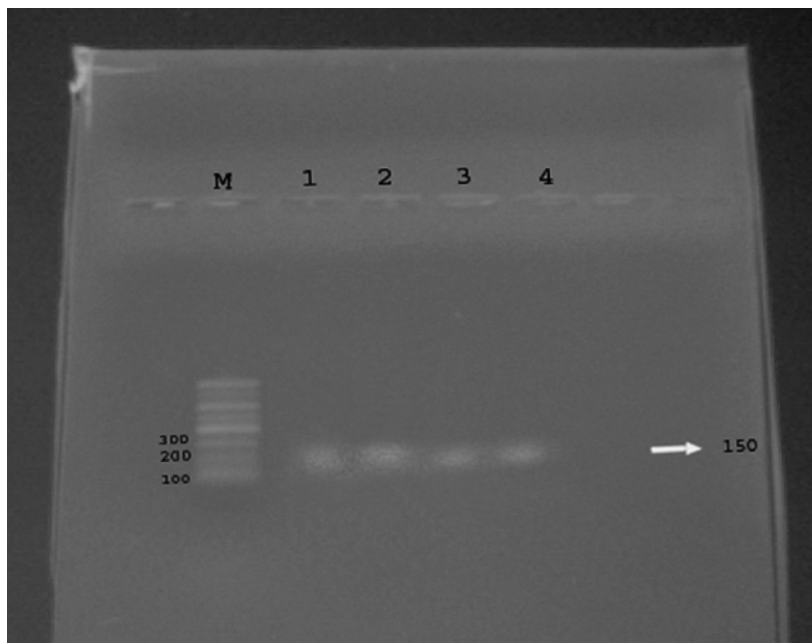
Figure(4): The elctrophoretic photograph showing the pattern of ST6GAL I mRNA expression in lung of different birds.

M: DNA ladder   Lane 1: chicken Baladi trachea   Lane 2: chicken Hubber trachea
Lane 3: Duck Baladi trachea   Lane 4: Duck Pekeni trachea
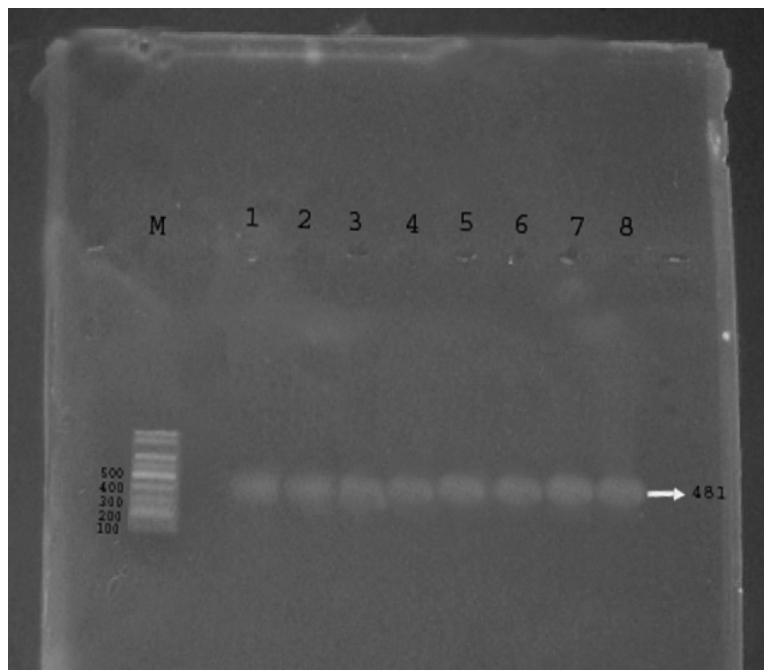
Figure(5): The elctrophoretic photograph showing the pattern of glyceraldhyde 3 phosphate dehydrogenase (GAPDH) mRNA expression in trachea and lung of different birds.

M : DNA ladder   Lane 1: chicken Baladi trachea   Lane 2: chicken Hubber trachea
Lane 3: Duck Baladi trachea Lane 4: Duck Pekeni trachea   Lane 5: chicken Baladi lung
Lane 6: chicken Hubber lung Lane 7: Duck Baladi lung   Lane 8: in Duck Pekeni lung

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Design and validation of Real Time Neuro Fuzzy Controller for stabilization of Pendulum-Cart System

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Abstract: This paper presents an application of how to design and validate a real time neuro fuzzy controller of complex a nonlinear dynamic system using the Matlab-Simulink Real-Time Workshop environment. Once the controller is obtained and validated by simulation, it's implemented to control the pendulum-cart system. Design of a neuro fuzzy controller is considered in this work because of its insensitivity to disturbances and uncertainties of model parameters. The design and optimization process of neuro fuzzy controller are based on an extended learning technique derived from adaptive neuro fuzzy inference system (ANFIS). The design and implementation of this pendulum-cart control system has been realized under MATLAB/SIMULINK environment. The experimental results demonstrate the efficiency of this design procedure and the ensured stability of the system.

I. Introduction:

Fuzzy controllers have been widely used in many control systems applications. Besides being convenient for qualitative system modeling, they are very simple conceptually [1]. They consist of an input stage, a processing stage, and an output stage. The input stage maps sensor or other inputs, such as switches, thumbwheels, and so on, to the appropriate membership functions. The processing stage invokes each appropriate rule and generates a result for each, then combines the results of the rules. Finally, the output stage converts the combined result back into a specific control output value. The most common shapes of membership functions are triangular, trapezoidal and bell curves. From three to seven curves are generally appropriate to cover the required range of an input value, or the "universe of discourse" in fuzzy jargon. The shape is generally less important than the number of curves and their placement.

Design of a fuzzy controller requires more design decisions than usual, for example regarding rule base, inference engine, defuzzification, and data pre- and post processing [2]. This paper describes the design decisions related to closed-loop neuro fuzzy controller of the pendulum-cart system. The main problems in neuro fuzzy controller design are the inference of an initial rule base and in particular the optimization of an existing rule base.

Many researchers addressed the design problem of neuro fuzzy controller. Nauck et al introduced the design of neuro fuzzy controller using backpropagation algorithm [11]. They also, presented their learning algorithm for neuro fuzzy environment NEFCON-I under matlab/simulink [22].

The pendulum-cart system is an interesting nonlinear dynamic model which has been extensively studied by control community. It represents many real world systems, such as crane at shipping port and space mission launchers. The selection of a control strategy for stabilization of such systems is a difficult design task. Optimality of the control strategy and its robustness are the main design criteria to be considered. However, due to the presence of disturbances and model parameter uncertainties, a robust behavior is more important than the optimal character of the control strategy. The efficiency of neuro fuzzy techniques to reduce disturbances makes it an excellent candidate to design a closed loop controller. With an efficient learning method, the parameters of the neuro fuzzy controller can be optimally designed. The design and validation of a neuro fuzzy controller should be assisted by a software environment that can provide the designer with functions of fuzzy logic systems and targeting a real time application.

The Fuzzy Logic Toolbox of the MATLAB technical computing environment is an efficient tool for designing systems based on fuzzy logic. The toolbox provides many functions which allow control engineers to develop and analyze fuzzy inference systems, to develop adaptive inference systems, and perform fuzzy clustering [3]. Its Graphical User Interfaces (GUIs) simplifies the steps of neuro fuzzy inference system design. Alternatively, Simulink provides fuzzy inference blocks in order to simulate the fuzzy systems within a comprehensive model of the entire dynamic system [7]. From Simulink, C code can be automatically generated for use in embedded applications that include neuro fuzzy logic [4].

This paper describes the design decisions related to closed-loop neuro fuzzy controller of the pendulum-cart model. We propose an efficient design and rule learning procedure of the neuro fuzzy controller. We also present the experimental results on the design and implementation of real time neuro fuzzy control system under matlab/simulink computation environment.

The paper is organized as follows. Sections I is this introduction. In Section II, we present an analysis of the control system development under Matlab/Simulink Environment. Section III describes the pendulum-cart set-up. Section IV presents the control algorithm, the rule base learning and its optimization. Section V evaluates the implementation and presents the experimental results of neuro fuzzy algorithm. Finally, our conclusions are drawn in Section VI.

II. Control System Development under Matlab/Simulink Environment

This work has been developed using the Mathworks tools. These tools are in varying widespread use across a number of industries for control system development [3]. Fig. 1 shows how the various elements of the MATLAB environment can be linked together to provide an integrated set of tools for control system design and experimental validation [3]. The use of these standard software tools means that, during the controller design stage, the designer only needs to model the process using the graphics tools available in Simulink without being concerned with the mechanics of communication to and from the device under test.
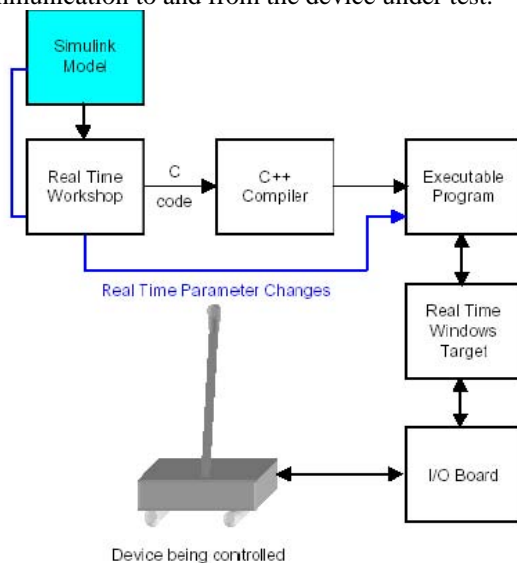
Fig. (1) Control System Development Flow Diagram.

A brief description of these tools and their use in control system development is given in next paragraphs.

Matlab acts as the application host environment in which the other mathworks products run. It provides a sophisticated set of tools for solving mathematical problems in addition there are specialized toolboxes, such as fuzzy logic toolbox which extend the Matlab functions in several different specific application areas.

Simulink is a graphics based system for modeling process, which takes the form of blocks, is fed as input into another block. Blocks perform specialized operations on the data and may be standard blocks from the Simulink library or written by the user where no suitable library blocks exists [3, 4]. Simulink model is passed to real Time Workshop.

Real-Time Workshop -RTW generates optimized, portable, and customizable code from Simulink models. Real time workshop automatically builds a C++ source program from Simulink model.

C++ Compiler compiles and links the code created by Real Time workshop to produce an executable program. The program interfaces to the outside environment via a "Target", in our case Real Time Windows Target.

Real Time Windows Target communicates with the executable program acting as the control program, and interfaces with the hardware device through an I/O board. Real Time Windows Target controls the two-way data or signal flow to and from the model, and to and from the I/O Board. When the program is running, the user may change certain of the parameters in the Simulink model, which are then passed, via Real Time Workshop, to the executable program

III-Pendulum-Cart Set-Up Description

One of the simplest problems in robotics is that of controlling the position of a single link using a steering force applied at the end. Pole-balancing systems are impressive demonstration models of missile stabilization problems [3, 4, 5, 6, 7]. The crane used at shipping ports is an example of non-linear electromechanical systems having a complex dynamic behavior and creating challenging control problems. Mathematically either is just a pendulum in a stable or unstable position. The pendulum-cart set-up consists of a pole mounted on a cart in such a way that the pole can swing free only in vertical plane. The cart is derived by DC motor. To swing and to balance the pole the cart is pushed back and forth on a rail of limited length. The vertical stationary positions of the pendulum (upright and down) are equilibrium positions when no force is being applied. In the upright position a small deviation from it

results in an unstable motion. Generally the pendulum control problem is to bring the pole to one of the equilibrium positions and preferably to do so as fast as possible, with few oscillations, and without letting the angle and velocity become too large. After the desired position is reached, we would like to keep the system in this state despite random perturbations. Manual control of the cart-pole system is possible only for simple tasks e.g. for moving the cart from one place on the rail to another. For more complicated tasks (such as stabilizing the pole in an

upright position) a feedback control system must be implemented Fig. (2). The purpose of the inverted pendulum control algorithm is to apply a sequence of forces of constrained magnitude to the cart, such that the pole starts to swing with increasing amplitude without the cart overriding the ends of the rail. Firstly the pole is swung up to the vicinity of its upright position and then, once this has been accomplished, the controller maintains the pole vertically and at the same time brings cart back to the center of the rail.
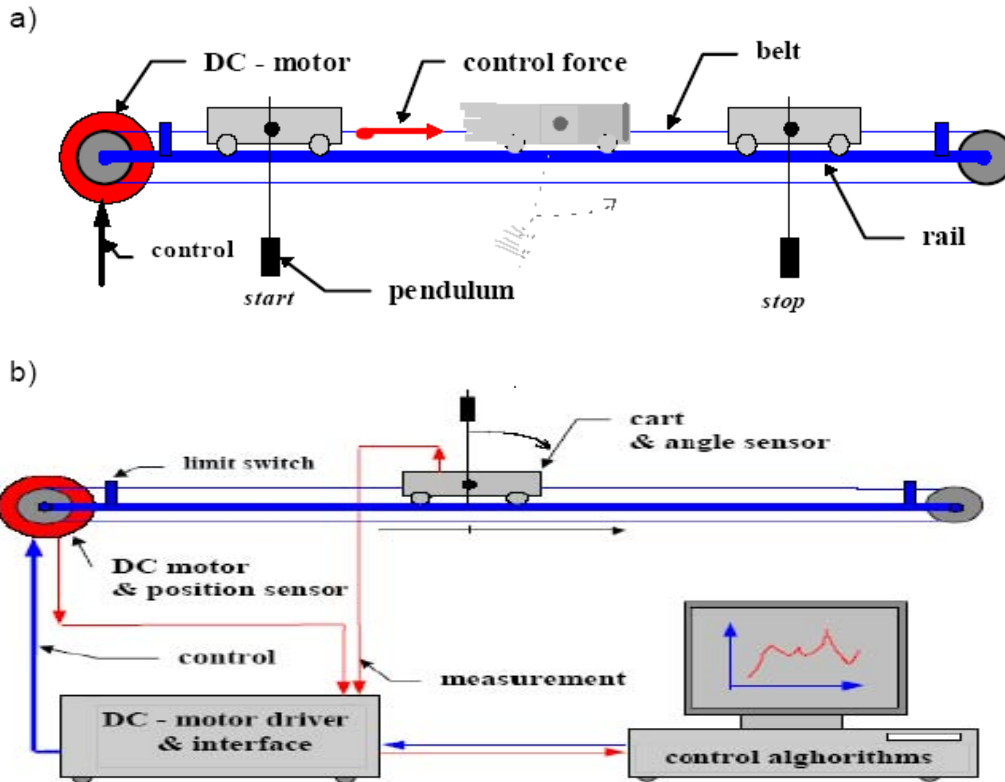
Fig. (2) Pendulum Control System

Fig. (3) Activity zones of two control algorithms

The swinging control algorithm is a heuristic one, based on energy rules. The algorithm steers the pole up thus increasing its total energy [3, 4, 19]. There is a trade-off between two tasks: to swing the pendulum to the upright position and to center the cart on the rail. Due to the presence of disturbances and parameter uncertainties, a robust behavior is more important than the optimal character of the control strategy. The switching moments are calculated according to a simple rule. The characteristic feature of control is its "bang b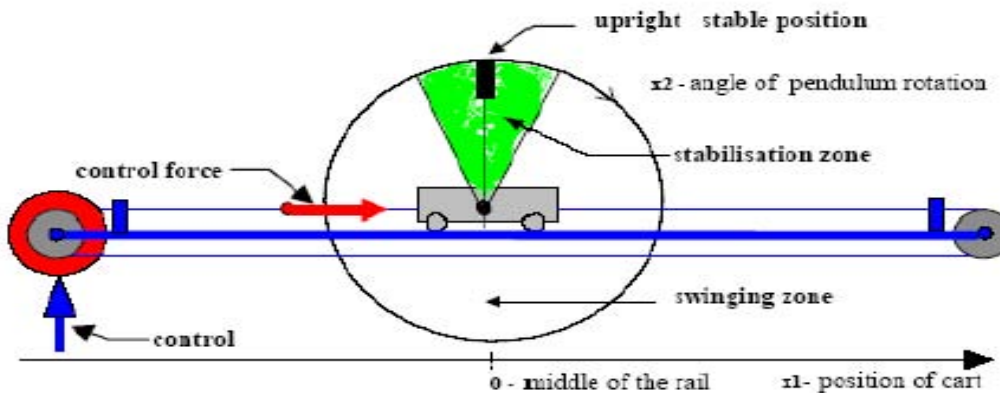ang" character. Swinging up the pole may result in over-reaching the upper unstable equilibrium point. To achieve a "soft" landing in the vicinity of the upright position ("stabilization zone" in Fig. (3), a routine called the "soft landing arbiter" checks whether the kinetic energy of the pole, minus the energy loss due to friction, is sufficient to raise the center of gravity of the pole to its upright position. If the condition is satisfied then the control is set to zero and the "bang-bang" character of the control is finished. After the pole has entered the stabilization zone the system can be treated as linear and the control is switched to the stabilizing algorithm. Due to the limited length of the rail a routine called "length control" is introduced, to reinforce centering of the cart and prevent over-running the edges of the rail. The rule is very simple. When the position given by the parameter "length" is reached, then the maximal force is applied to the cart steering it back away from this position.

III.I System Model

The state of the system is the vector $x = [x_1, x_2, x_3, x_4]^t$, where x_1 is the cart position (distance from the centre of the rail), x_2 is the angle between the upward vertical and the ray pointing at the centre of mass, measured counter-clockwise from the cart ($x_2 = 0$ for the upright position of the pendulum), x_3 is the cart velocity, and x_4 is the pendulum angular velocity. The pendulum rotates in a vertical plane around an axis located on a cart [23]. The cart can move along a horizontal rail, lying in the plane of rotation. A control force u, parallel to the rail, is applied to the cart. The mass of the cart is denoted by m_c and the mass of the pendulum, by m_p. l is the distance from the axis of rotation to the centre of mass of the pendulum-cart system. J is the moment of inertia of the pendulum-cart system with respect to the centre of mass. T_c denotes the friction in the motion of the cart, and D_p is the moment of friction in the angular motion of the pendulum, proportional to the angular velocity: $D_p = f_p x_4$. The force of reaction of the rail V acts vertically on the cart. As the horizontal co-ordinate of the centre of mass is equal to $x_1 - l \sin x_2$ and the vertical to $l \cos x_2$, the motion equations are as follows:

$$(m_c+m_p)(x_1-l \sin x_2)''=F-T_c, \qquad (1)$$
$$(m_c+m_p)(l \cos x_2)''=V-(m_c+m_p) g, \qquad (2)$$
$$Jx_2''=(u-T_c) l \cos x_2 + VI \sin x_2 - D_p, \qquad (3)$$

$(.)''$ denotes the second derivative with respect to time t and $(.)'$ denotes the first derivative with respect to time t. The first two equations describe the translation of the centre of mass, while the third describes the rotation of the whole system around the centre of mass. After the elimination of V and simple calculations we obtain the state equations (for $t >= 0$)

$$x'_1=x_3 \qquad (4)$$

$$x'_3=\frac{a(u-Tc-\mu x_4^2 \sin x_2)+l\cos x_2(\mu g\sin x_2 - f_p x_4)}{J+\mu \sin^2 x_2} \quad (5)$$

$$x'_2=x_4, \qquad (6)$$

$$x'_4=\frac{l\cos x_2(u-Tc-\mu x_4^2 \sin x_2)+\mu g\sin x_2 - f_p x_4}{J+\mu \sin^2 x_2} \quad (7)$$

Where

$$a=l^2 + \frac{J}{m_c + m_p}, \qquad \mu = (m_c + m_p)l$$

The admissible controls are bounded such that
$$|u(t)| \le M$$

The cart friction T_c in the model is a non-linear function of the cart velocity x_3. As an approximation one can assume $T_c = f_c x_3$. The rail has a finite length and hence the cart position x_1 is bounded: The typical parameters of the cart-pole set-up are given in Table 1.

Table 1. Parameters of the pendulum-cart set-up

name of parameter	value of parameter
track limits	±0.5 m
gravity g	9.81 m/s^2
Distance between mass centre and axis of rotation l	0.017 m
mass of cart m_c	1.12 kg
mass of pole m_p	0.11 kg
magnitude of control force M	17.0 N
moment of inertia of system J	0.0136 kgm^2
friction coefficient of pole rotation f_p	negligible
friction coefficient of cart f_c	0.05 Ns/ m

The model of the pendulum-cart set-up is an example of a SIMO system: a single control input and multi outputs (states) and can be used to demonstrate the advantages of closed-loop control.

III.II Real Time Computer Control

One of the main objectives of this work is the direct implementation of designed neruro fuzzy controller in a real time process. Computer control of a real time process is presented in this section. A block diagram of a computer-controlled process is given in Fig. 4
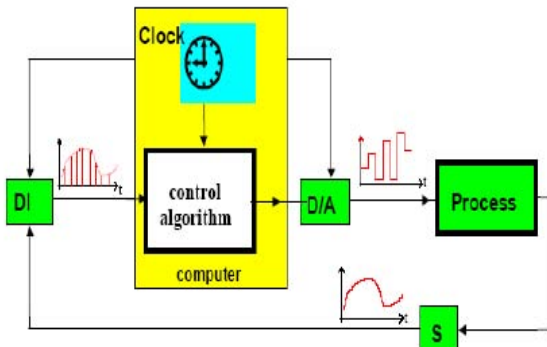
Fig. (4) Computer controlled process

The system contains six blocks: the process, sensors (S), D/A converter, control algorithm, and a clock. The software clock controls the operation of the converters and the control algorithm. The time between successive conversions of the signal to digital form is called the sampling period (T_0). The clock supplies a pulse every T_0 seconds, and the DI supplies a number to the computer every time an interrupt arrives. The control algorithm computes the value of the control variable and sends it as a number to the D/A converter. It is assumed that the D/A converter hold the signal constant over the sampling period; periodic sampling is normally used [3]. An application of the general digital control system schema for pendulum control is given in block diagram form in Fig. 5. Two process states are measured: the cart position x_1 and the pendulum angle x_2. Process states are measured as continuous signals and converted to digital by optical encoders (sensors S1 S2). The reference input (desired value of the cart position x_1) can be generated in a digital form using a desired position generator. The software timer is used to supply interrupts for the system: The basic clock activates the periodic sampling of optical decoders outputs and synchronizes the computation of controller outputs (u) and periodic digital-to-analog (D/A) conversion.
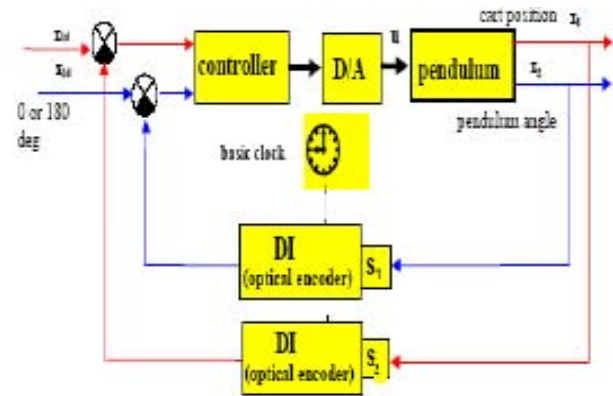
Fig. (5) Digital Control of the Pendulum-Cart System (basic block diagram).

The pendulum-cart system is controlled in real-time. The term "real-time" is often used but seldom defined. One possible definition is [4]: "Real-time is the operating mode of a computer system in which the programs for the processing of data arriving from the outside are permanently ready, so that their results will be available within predetermined periods of time; the arrival times of the data can be randomly distributed or be already determined depending on the different applications." The real-time software for pendulum control is structured around particular internal signals (events) into a set of tasks. Each task implements the processing required by a corresponding event. A task scheduler recognizes the events and activates or suspends the tasks. In the simplest case, when all tasks require processing at the same frequency, a sequential organization of the tasks can be implemented [14]. The time frame of each task is fixed. It is assumed that the longest task job takes no longer than the period of time generated by the software timer.

IV Control Algorithm

The controller in this experimental setup is based on a neuro fuzzy algorithm. The inputs of the neuro fuzzy system are pendulum angle, cart position, and the outputs are cart velocity, pendulum velocity as shown in Fig. (6). Fig. (7) Shows the initial membership function for inputs.

IV.I Rule base Learning

The learning process of the ANFIS model can be divided into two main phases. The first phase is designed to learn an initial rule base, if no prior knowledge about the system is available. Furthermore it can be used to complete a manually defined rule base. The second phase optimizes the rules by shifting or modifying the fuzzy sets of the rules. Both phases use a fuzzy error, E, which describes the quality of the current system state, to

learn or to optimize the rule base. In this work, we used the 'ANFIS Learning`-Algorithm [9, 10]. This algorithm starts with an empty rule base. An initial fuzzy partitioning of the input and output intervals must be given. The algorithm can be divided into two parts. During the first part, the rules' antecedents are determined by classifying the input values, i.e. finding that membership function for each variable that yields the highest membership value for the respective input value [13, 14]. Then the algorithm tries to 'guesses the output value by deriving it from the current fuzzy error. During the second part, the rule base is optimized by changing the consequent to an adjacent membership function, if it is necessary [15, 16, 19]. Fig. (8) Shows the viewing rules between inputs-outputs. The relations among cart velocity, cart position, and pendulum angle are introduced as shown in Fig. (9).

IV.II   Optimization   of   the   Rule   base (Implementation)

The aim of the implementation under MATLAB/SIMULINK was to develop an interactive tool for the construction and optimization of a fuzzy controller. This frees the user of programming and supports him to concentrate on controller design. It is possible to include prior knowledge into the system, to stop and to resume the learning process at any time, and to modify the rule base and the optimization parameters interactively. To optimize the rule base we choose the optimization algorithm ANFIS [10, 11, 12].

This algorithm is motivated by the back-propagation algorithm for the multilayer preceptron [8]. It optimizes the rule base by back-propagation of error. A rule is 'rewarded' by shifting its consequent to a higher value and by widening the support of the antecedents, if it's current output has the same sign as the optimal output [20, 21]. Otherwise, the rule is

'punished' by shifting its consequent to a lower value and by reducing the support of the antecedents. The inferred rule base of the system under study has 27 rules.
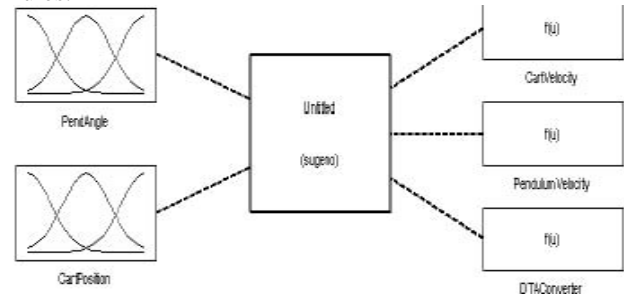
Fig. (6) Relation between Inputs-Outputs
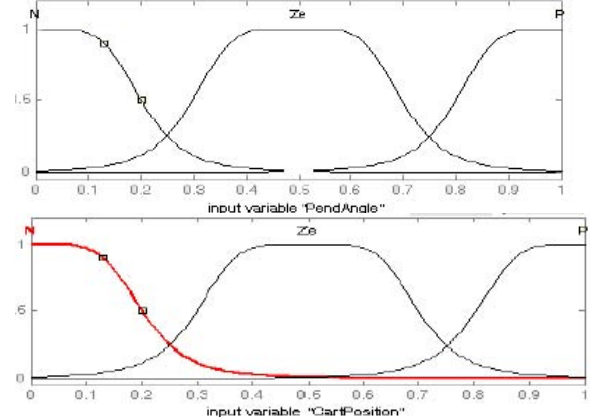
Fig. (7) Initial membership function for inputs

The reduced rule base is presented below.
1- IF (PendAngle is N) and (CartPostion is N) then (CartVelocity is N)(PendVelocity is N)(F is N)
2- IF (PendAngle is P) and (CartPostion is P) then (CartVelocity is p)(PendVelocity is p)(F is p)
3- IF (PendAngle is Ze) and (CartPostion is Ze) then (CartVelocity is Ze)(PendVelocity is Ze)(F is Ze)
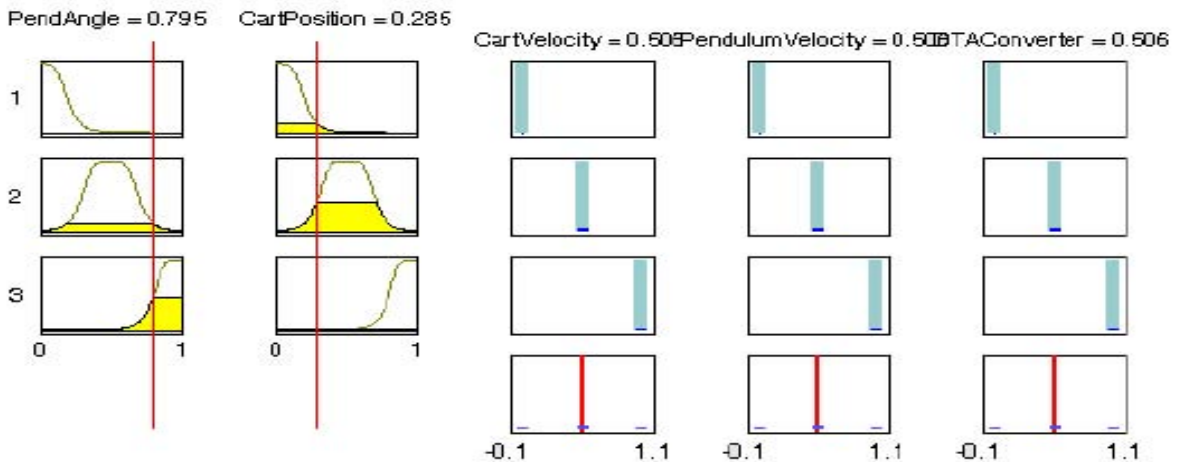
Fig.(8) Visualization of rules between inputs-outputs
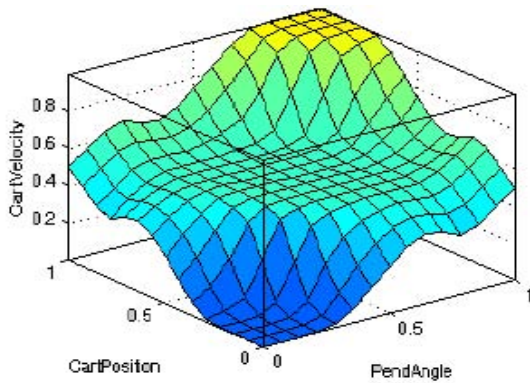
Fig. (9) The relations among cart velocity, cart position, and pendulum angle

V. Experimental Results

      To test our approach we conducted experiments on a cart-pendulum hardware interfaced with a neuro fuzzy controller implemented in Matlab/Simulink environment as previously indicated

in fig. (5). In these experiments, the cart is driven by DC motor. To swing and to balance the pole the cart is pushed back and forth on a rail of limited length. The neuro fuzzy system is used to stabilize the pendulum. Fig. (10) shows the structure of neuro fuzzy system used in the implementation. The result for this simulation of ANFIS controller system with real time inverted pendulum system is shown in following figures. Fig. (11) Represents the change of cart position with time, in another meaning this figure shows the inverse relationship between the force and stability" The higher the force the lower the stability". Fig. (12) Shows the change of pendulum angle with time, in another meaning this figure shows the direct relationship between the force and the angle of pendulum" The higher the force the higher the angle". The change of cart velocity and pendulum velocity with time is shown in Fig. (13) And Fig (14) respectively. Due to the high force generated from the initial movement it takes few seconds to reach the stability level.
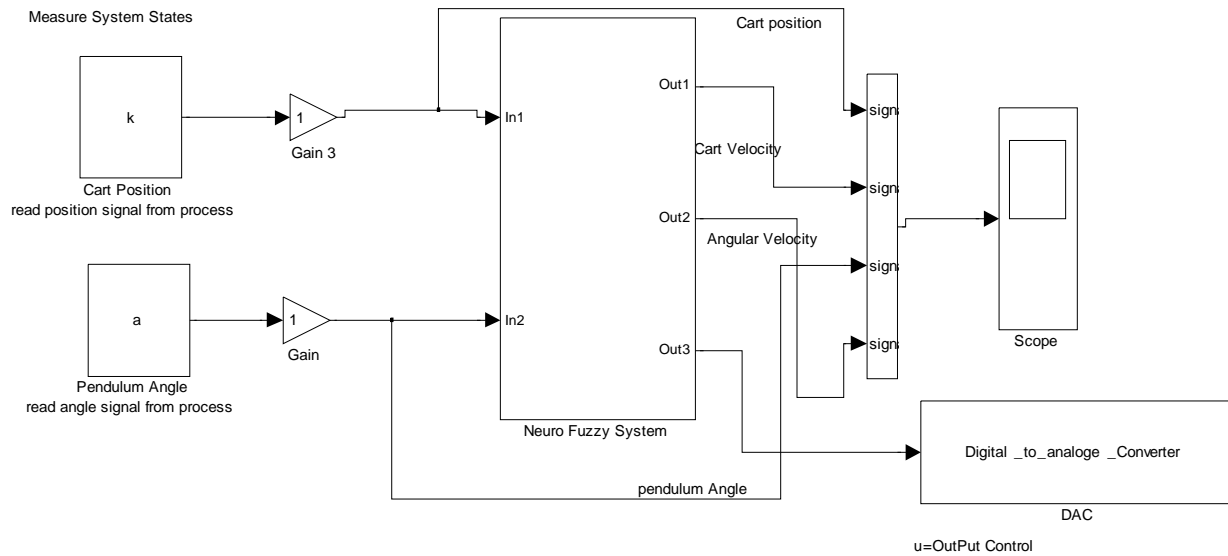
Fig.(10) The Structure of Neuro Fuzzy Simulink System used in the implementation
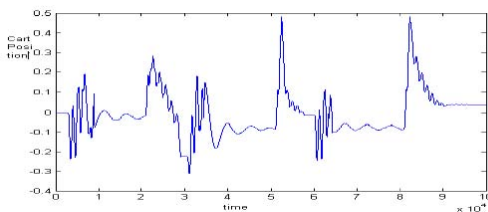
Fig. (11) The change of cart position with time.
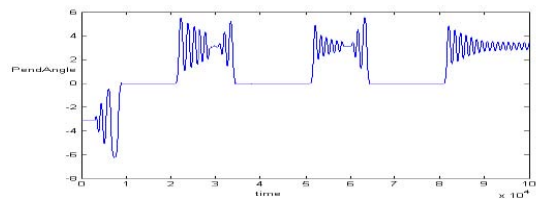
Fig. (12) The change of pendulum angle with time.
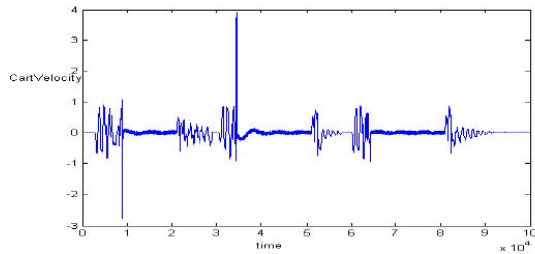
Fig. (13) The change of cart velocity with time
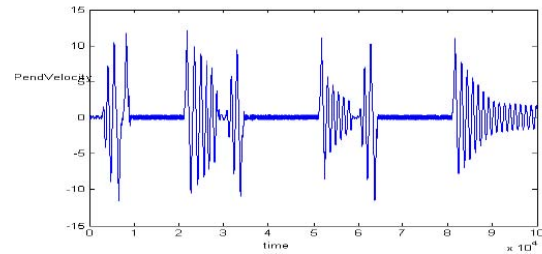
Fig. (14) The change of pendulum velocity with time
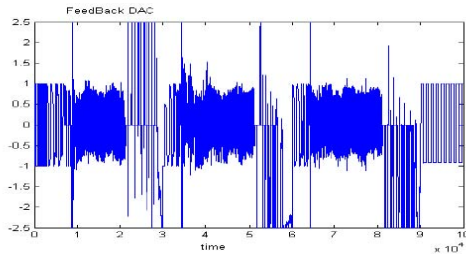
Fig. (15) The output of DTAC and inputs to process with time
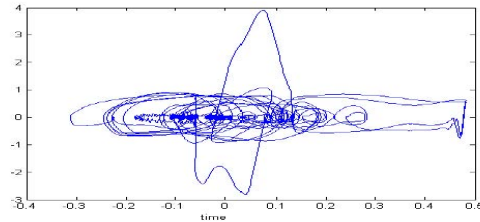
Fig. (16) The relationship between cart position and cart velocity
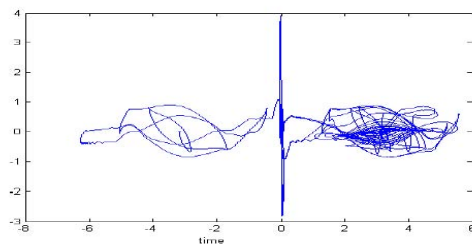
Fig. (17) The relationship between cart position and pendulum velocity
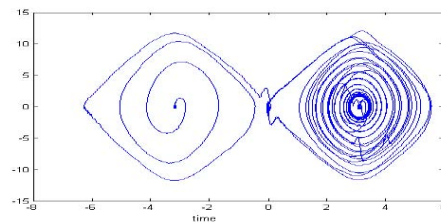
Fig. (18) The Relationship between pendulum angle and pendulum velocity

Then after few seconds the stability tends to be lost, at this moment we should give another force in order to keep the stability of pendulum at the required level. Fig. (15) displays the digital output from the computer which was converted by the digital signal to analog converter (DTAC) using data acquisition card and sending this signal as inputs to the process with time. Fig. (16) to Fig (18) show the relationship among inputs and outputs. We notice from these figures that the high velocity generated from force lead to high change in the pendulum angle and this change tend to be less when the velocity is reduced which is a positive relationship between the velocity and the pendulum angle, and force.

VI-Conclusions:

In this paper we presented the design and optimization process of neuro fuzzy controller supported by learning techniques derived from neural networks (ANFIS). The generation of rule base has been done from input output data. The implementation of this controller has been realized under the MATLAB/SIMULINK. This implementation supports the development of real time process in an easy way. One of the important conclusions in this model is that the stability of the pendulum is negatively related with the force, velocity, and angle. The design and implementation of this pendulum-cart control system has been done under MATLAB/SIMULINK environment. The experimental results demonstrated the efficiency of this design procedure and the ensured stability of the system**.**

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Analyzing Farm Management Skills in Poultry Production Enterprises in Iran

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Abstract: The main purpose of this study was to analyze managerial skills of poultry production operators in *Soumeh Sara* Township in Guilan province, Iran, which used a descriptive-analytical design. The population of this study consisted of whole industrial poultry production enterprises of *Soumeh Sara* Township (N=117) and out of them, 50 operators had been selected as statistical sample through simple random sampling. The instrument used to achieve objectives of the study was a questionnaire by reliability 0.92. Findings reveal that among the studied management skills marketing skill had been placed the least ranking means. In addition, poultry production operators had the best ability in technical skills. The average of ability rate of poultry production operators in technical area was 4.08 which indicated the high to very high ability rate of respondents in this area. According to the results, it is necessary to improve the marketing and farm management skills of farm operators through extension and participation in training activities.

1. Introduction

Studies indicate that the food of the most people of the world (especially, developing countries) is suffering from protein deficiency and since protein, especially animal protein, play important role in human nutrition, its quality and quantity must reach to ideal extent (Nikougoftar, 2003). Studies indicated that the distance of supply and demand is very high to provide the least needed animal protein. FAO had been recommended that the minimum protein which an individual must consume in a day is 65 g which from this 36g, that is 40% must provide through animal resources (Yusuf and Malomo, 2007). The consumption rate of animal protein is 22g/day in Iran and this rate is 30% less than the recommended rate by FAO.

In the recent years, hen meat had been applied to feed human and provides the needed protein, extensively. Poultry production, because of speed growth, nutrition facility, using closed space, lower density and conversion coefficient than the other portion products and also having the needed material for human body, is enjoying certain interest. But, because of no observance of correct management principles and technical standards in poultry production and breeding, remarkable damage had been done on producers in the first and then on communication and national economy (Dashti and yazdani, 1996). Generally, this branch of husbandry had been not reach to development target, because of

reasons such as low production productivity, weakness in management, inefficiency of enterprises and practicing of traditional production methods (Mirakzadeh, et al, 2010).

Today, this industry is against the severe economic practices of governments and the most rigors from governments are certain attention to management preparations in poultry enterprises in order to increase the efficiency (Oknkow & Akubou, 2001). The reason of this is that management is the hidden factor of the production and will have increasing effect. In addition to three factors of labor force, capital and land, management is introduced as the fourth factor of production which have important role in the three first factors. What is important theoretically is that in each production enterprise, due to being quality of management enterprises, it must turn to quantity for using some indexes until it can use in production function (Hamidi, 2005). Therefore, the managers of agriculture production enterprises and farmers are considered as the most efficient agent to control efficiency and productivity of enterprise. So, assessment of their performance and efficiency and also their role in realizing farm management goals play important role, So that, Amini, et al. (2007) had been introduced managers capacity and proficiency as one of the important inter organizational component in success of poultry cooperation's. In order for farm manager to act with the maximum efficiency to reach to an end, they need

one set of management skills. These managerial skills help them to perform correct selection due to financial levels, workforce, land resources and risk escaping. These skills help the farmers for access to income possible levels about what thing must be product, in which part of farm, by what method, when and how much, take informed decision (AL-Rimawi et al. 2006 and 2004).

The main purpose of this study was to analyze managerial skills of poultry production operators in Soumeh Sara Township in Guilan province, Iran.

2. Material and Methods

This study was conducted in *Soumeh Sara* Township, Guilan province, Iran. General approach of this research is quantity. With regard to the research problem which is try to study the level of managerial skills in poultry production enterprises, it performed based on survey strategy and it is enjoying of descriptive-analytical method. Statistical population of this research consisted of whole industrial poultry production enterprises (operators) of Soumeh Sara (N=117) and out of them, 50 operators selected as statistical sample. The main instrument for data collection was a questionnaire including specification and managerial questions about poultry production enterprises and individual/professional characteristic. To ensure its content and face validity, the research instrument was reviewed several times by the research group (faculty member of Animal Husbandry Department at Islamic Azad University, Rasht Branch and staffs of Guilan Agricultural Organization) and then implemented in a pilot test to measure its reliability. Questionnaire reliability was estimated by calculating Cronbach's alpha. Reliability of the overall instrument was estimated at 0.92. It meant that index had high reliability. The data were collected between April and July 2010. These questionnaires delivered to *Soumeh Sara* poultry production operators and data collected through structured interview with poultry operators. In order to measure the perspective of poultry production operators about their ability in farm management skills, 58 closed-end questions in nine skills area (planning and determine the goal, accountancy and financial management, rational marketing, seeking information, resource mobilization, risk orientation, communicative and technical) used. In order to measure respondents perspective to ability rate in each of the area in farm management, six-point scale had been used which had been ranked from 0=none, 1=very low, 2=low, 3=moderate, 4=high to 5=very high. In order to analyze data, descriptive statistic (mean and standard

deviation) and inferential methods (Friedman test) used.

3. Results and Discussion
Planning and determining the goal

The results (table 1) indicated that respondents believed that the average of their ability rate in the skills of this area in the farm management was 3.76 (SD=0.45) which indicating the moderate to high level. With regard to investigate the skills, it identified that respondents had the highest and lowest ability in predicting the required inputs rate over a production period (M=3.94, SD=0.87), and predicating and estimate the income from production over a production period (M=3.48, SD=0.76), respectively. The most important reason for low ability rate of respondents' in predicting and estimate the income from production is lacking stabilization of hen price in market and price fluctuation, and according this, producers are enjoying low ability to predict their income during one production period.

Table 1, setting priority for respondents' viewpoint on the ability of planning and goal setting skills

Rank	Planning and goal setting skills	M	SD
1	Predicting the required inputs rate over a production period	3.94	0.87
2	Ability to develop production program and to identify production targets in the short and long term	3.92	0.66
3	Having a program for hard and difficult conditions and following it	3.80	0.78
4	Predicting and estimating production costs over a production period	3.72	0.70
5	Predicting and estimating production rates over a production period	3.68	0.68
6	Predicating and estimating the income from production over a production period	3.48	0.76
	Mean	3.76	0.45

Accountancy and financial management skills

Date analyzing indicated that the mean rate of respondents ability in this area of farm management is 3.81 (SD= 0.61) which indicating the moderate to high level. The ability to record carried out production in poultry production enterprise (M=4.24) is the first priority of managers ability in this area. Also, the ability to record used inputs in poultry production enterprise (M=4.22) and the ability to record and calculate initial capital rate of poultry production enterprise (M=4.16) allocated the second and third priority of managers ability (table2).

The data of this table is confirm with table 1, because the ability to record used inputs rate in poultry production enterprise which consider as the high ability, is the first ability in the table 1, too. Respondents are not in well situation in the buy ability of general needed requirements to enjoying of

reduction price (M=3.34), relatively. One of the effective factors in this problem is liquidity deficiency and also lacking ability to effective use from various financial and credit resources.

Table 2, setting priority for respondents' viewpoint on the ability of accountancy and financial management skills

Rank	Accountancy and financial management skills	M	SD
1	Ability to record conducted production in poultry production enterprise	4.24	0.92
2	Ability to record consumed inputs in poultry production enterprise	4.22	0.93
3	Ability to record and calculate the amount of initial capital in poultry production enterprise	4.16	0.82
4	Ability to record and calculation of profit and loss in poultry production enterprise	4.08	0.95
5	Ability to create a good and effective financial accounting system	3.72	0.97
6	Fallowing-up continuing education to	3.43	0.94
7	improve financial management skills	3.35	0.95
8	Ability to effectively use of financial and credit from various sources Ability to purchase needed inputs to enjoy discount prices	3.34	1.00
	Mean	3.81	0.61

Marketing skills

The important thing after production of each product is, reaching that to consumers. Marketing misled many farmers. Sometimes, farmers leave the marketing and focuses on production stage which they better understand in, and this problem is dangerous. Successful farmers in the present time and future not only must manage the production but also marketing and financial affairs (Yaaghubi et al., 2009). Also, Maru (2003) stated that those farmers who recognize the market opportunity are enjoying more chance to success than the individual who are not recognize this opportunities. Table 3 indicate that the ability to choose the best time to sell product (M=3.50, SD=1.01) is the highest ability in this area in respondents point of view. Data analyzing indicated that the average rate of respondents ability in this area of farm management is 2.68 (SD=0.62) which impaling the low to moderate ability level.

Information skills

In order to combat to the changes of trade environment in agriculture, farmers had been found that information management is the confident way to survival (Yaaghubi. et al, 2009).

Also, Pezeshki Rad and Zamani (2005) known the using of information as the most important property of information age and they consider the obtaining of information as the most important challenges of this age. The ability to find new and

better methods to do things (M=3.61, SD=0.91) and the ability to collect the information about modern technologies of production (M=3.52, SD=1.03) had been allocated the two first priority of ability in this area, respectively.

The findings of table 4, indicated the respondents ability in this area of farm management in moderate to high level (M=3.17, SD=0.88). The ability to collect information about government policy in market, introduced as the least ability rate from operators of poultry enterprises. Maybe, one of the effective factors on this problem is permanently changing policies of government in the market and lacking stabilization of these policies.

Table 3, setting priority for respondents' viewpoint on the ability of marketing management skills

Rank	Marketing management skills	M	SD
1	Ability to choose the best time to sell the product	3.50	1.01
2	Familiarity with modern style of packaging products	3.30	0.88
3	Ability to analyze demand, supply and price of chicken	3.04	1.35
4	Familiarity with the role of cooperatives in direct sales of Products	2.53	1.24
5	Ability to analyze government policy on poultry markets	2.45	1.17
6	Ability to supply product directly to consumers (rather than selling to slaughterhouses)	1.24	1.20
	Mean	2.68	0.62

Table 4, setting priority for respondents' viewpoint on the ability of information seeking of management skills

Rank	Information Seeking skills	M	SD
1	Ability to find for new and better way to do things	3.61	0.91
2	Ability to collect information about new production technologies	3.52	1.03
3	Ability to collect information on inputs prices and market	3.06	1.42
4	Ability to collect information about government policies on the market	2.49	1.43
	Mean	3.17	0.88

Decision–making skills

Decision–making is the most important task of managers irrespective of their place and also the managers and supervisors of poultry production units are not excepted. Sometimes, taking correct and also on-time decision cause to continue living of an organization. The results (table 5) indicated that managers' ability in this area is in moderate to high extent (M=3.67, SD=0.66). In order to measure this skill, six statements had been used which among

these, operators consider their ability to take correct decision on technologies as more strengthen than the other properties (M=3.86, SD=0.91).The ability to effective use from production advisors (economical, veterinary, nutrition etc) (M=3.74, SD=0.94) was the second property which poultry production enterprises' manager found their ability in it. The ability to rapid analyzing of situations which they not faced with them up to now (M=3.50, SD=0.84), was the least reported properties from respondents, relatively.

Table 5, setting priority for respondents' viewpoint on the ability of decision–making of management skills

Rank	Decision–making skills	M	SD
1	Ability to make a good decision about technologies to use or be accepted	3.86	0.91
2	Ability to effective use from production advisors (economical, veterinary, nutrition etc)	3.74	0.94
3	Ability to use best management operations poultry production units	3.72	0.83
4	Ability to take right decisions about time or acceptance of new technologies	3.69	0.83
5	Ability to quickly identify and correct manufacturing problems and the principles to solve the problems	3.54	0.91
6	Ability to rapid analyzing of situations which they not faced with them up to now	3.50	0.84
	Mean	3.67	0.66

Resource mobilization skills

The ability to use with the least cost to obtain maximum efficiency had been stated as the index of productivity with the highest ability (M=3.84, SD=0.76) in this area of skills from poultry production units owners. Generally, the ability in this area is in moderate to high extent (M=3.71, SD=0.63).

Table 6, setting priority for respondents' viewpoint on the ability of resource of mobilization management skills

Rank	Resource mobilization skills	M	SD
1	Ability to use inputs with minimal cost to get the maximum efficiency	3.84	0.76
2	Ability to choose technologies and methods that make efficient use of resources	3.66	0.89
3	Ability to complete activities in the best possible time, shortest cycle time and maximum performance	3.63	0.81
	Mean	3.71	0.63

Risk oriented skills

Agriculture is consider as the most adventure job and in turn the farmers and owners of agribusiness units (including poultry production) have high risk in their job, so. It is necessary to enjoy from high capacity and ability in facing with crisis and high danger condition. The results of data analyzing (table 7) indicate that as many other studied area of farm management skills, respondents ability in this area is in the moderate to high talent (M=3.47, SD=0.61). The first ability of respondents in this area have attitude nature, so that the ability to understand this fact that sometimes risky ability is necessary devoted the highest average to it self (M=3.80, SD=0.73).

The ability to effective management of financial and production risk (M=3.64, SD=0.83) and the ability to predict and collecting some strategies to facing with production threading risks (M=3.41, SD=1.04) devoted the second and third ability in this area, respectively.

Table 7, setting priority for respondents' viewpoint on the ability of risk oriented of management skills

Rank	Risk oriented skills	M	SD
1	Ability to understand the fact that risk is sometimes necessary	3.80	0.73
2	Ability to effective management of financial and production risks	3.64	0.83
3	Ability to predict and develop strategies for facing the dangers condition	3.41	1.04
4	Ability to create savings, and financial support when it is necessary	3.32	0.96
5	Proper use of agricultural insurance	3.20	1.20
	Mean	3.47	0.61

Communicative skills

One of the three major skills for every manager is the ability to establish ideal and effective communication. The results of data analyzing indicated that the average of respondents' ability in this area is 3.82 (SD=0.55) (table8) which indicating the ability rate in the moderate to high extent. Among the investigated characteristics in this area, the ability to establish good, clear, exact and honest communication with others (M=4.00, SD=0.72), the ability to transfer the experiences and knowledge to new people working in poultry production enterprises (M=3.98, SD=0.74) and the ability to consider others opinion and perspective in management of unit (M=3.88, SD=0.72) is indicating the highest rate of stated ability among the respondents, respectively. In the other hand, the ability to empower others for doing affair (M=3.62, SD=0.92) is the least stated ability rate in this area which indicate the following of poultry production units operators from traditional

management style and lacing participative management moral among the respondents (table 8). Also, the ability to create positive and good relationship with buyers and sellers had been located in tenth place of abilities which also this point indicate the lacking attention to customer-oriented and its importance in human relationship management in poultry production enterprises.

Technical skills

The average of ability rate of poultry production operators in this area was 4.08 (SD=0.57) which indicated the high to very high ability rate of respondents in this area. Most of investigated indexes in this area were in good and very good situation but among these, the ability to control density (M=4.32, SD=0.62), ability to manage watering system (M=4.22, SD=0.84) and the ability to manage feeding system (M=4.22, SD=0.86) are enjoying the best situation. The respondents stated their ability in feeding formulation (M=3.83, SD=0.92) and familiarity rate with work and insurance rule (M=3.72, SD=0.78) in the lower extent than the other indexes of this area (table 9).

The investigation of respondents perspective about ability in farm management skills through Friedman test ($\chi2$=189.044, P<0.001) indicated that technical skills with the mean of 7.01 and marketing skills with mean of 1.61 have the highest and least ability in farm management, respectively (table 10).

Table 8, setting priority for respondents' viewpoint on the ability of communicative management skills

Rank	Communicative skills	M	SD
1	Ability to establish good, clear, exact and honest communication with others	4.00	0.72
2	Experience and ability to transfer knowledge to new people working in poultry production enterprises	3.98	0.74
3	Ability to consider others opinion and perspective in management of unit	3.88	0.72
4	Ability to communicate with others about the problems to achieve the desired result and the finding ways to solve them	3.86	0.67
5	Ability to assist employees to improve their skills and abilities	3.82	0.77
6	Ability to create fit between job requirements and skills of their employees	3.82	0.82
7	Ability to listen to their comments and suggestions to improve the performance	3.80	0.86
8	Ability to define specific tasks for each employee	3.80	1.00
9	Ability to avoid hegemony	3.74	0.94
10	Ability to create positive and good relationship with buyers and sellers	3.69	0.94
11	Ability to give authority to others for doing works	3.62	0.92
	Mean	3.82	0.55

Table 9, setting priority for respondents' viewpoint on the ability of technical management skills

Rank	Technical skills	M	SD
1	Ability to control density	4.32	0.62
2	Ability to manage watering system	4.22	0.84
3	Ability to manage feeding system	4.22	0.86
4	Ability to prepare hall before entering the chicken	4.16	0.79
5	Ability to manage and develop one-day chicks	4.14	0.81
6	Ability to manage the physical environment (air, adjust the heat, light and humidity)	4.12	0.85
7	Ability to control the sanitary conditions and health measures and preventive actions necessary	4.04	0.75
8	Ability of feeding formulation	3.83	0.92
9	familiarity rate with work and insurance rule	3.72	0.78
	Mean	4.08	0.57

Table 10, study of differences between mean of management skills in poultry production enterprises from the viewpoint of the operators

Rank	Management Skills	Rank Mean
1	Technical	7.01
2	Accountancy and financial management	6.06
3	Communicative	6.02
4	Planning and determining the goal	5.88
5	Resource mobilization	5.50
6	Decision–making	5.32
7	Risk oriented	4.28
8	Informative	3.32
9	Marketing	1.61

4. Conclusion

Currently weak management of production factors and economic inefficiency of production enterprises is one of the agricultural problems. Incorrect using is related to low of information and technical skills of farmers. So, paying attention to farm management and recognition its restricting factors and providing suitable executive ways, will be a good way to change in production of agricultural products and ideal use of production factors (Yaaghubi, et al., 2009). Farms operators need to management skills to take correct decisions. These skills enable them to mange their profession, effectively facing with the changes in agribusiness environment and remain in the high competition of trade environment (AL-Rimawi et al., 2006).

The results of this research indicate that among the studied management skills marketing skill had been placed the least ranking means. Until productive crops not reach to sell point and the customers have not satisfaction, production process is not complete. In the marketing skills area, the property of direct supply of product to consumers had been placed in the lowest rate of ability. In this respect, we can refer to middle-man in the poultry

market which in one hand increase the poultry price fluctuation and, at the other hand, can play role to prevent direct communication between sellers and consumers. Establishing direct delivery centers can play key role to solve this problem. Therefore, the major part of solution of this problem is to collect executive program to set out this centers and at the other hand, holding extension courses with the title of attention and given importance to customer opinion and attract the customers satisfaction can be as a training strategy for operators of poultry production enterprises. Also, low level of analyzing ability of government policy in market area is one of the properties which had been stated by respondents. Unfortunately, the most important reason of this is lacking the clear policy form government and also corrects and on-time decision in poultry market. Also, the fluctuation price of   in the world market raises this problem.

After marketing skills, information skills are in the lowest average. Generally, information role of managers is one of the three conceptual roles for them, which unfortunately, the results of this research indicating the lowering of ability level of Soumeh Sara poultry production owners in this area. Also, in this area, as marketing area, respondents found themselves less strength in collection information about government policies in market than the other properties which support the previous results.

According to the results, it is necessary to improve the marketing and farm management skills of farm operators through extension and participation in training activities (AL-Rimawi et al., 2004). Training role to improve management skills of farmers is considered as an important task to public extension service (Mattila et al., 2007).

Advisors through extension education programs can improve the management ability of farm operators, remarkably. They can help the farmers in taking better and informative decision, to acquire skills in analyzing their decision, evaluation the marketing, identify the trade opportunities and scheduling, quality and quantity of products toward the satisfaction of target market in intensive trade environment (AL-Rimawi et al., 2004 and 2006).

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Ameliorate the Drastic Effect of Ochratoxin A by using Yeast and Whey in Cultured Oreochromus niloticus in Egypt.

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Abstract: Ochratoxin A is one of the most important mycotoxins in fish feed. In the present study the effects of OTA on cultured *Oreochromus niloticus* were evaluated. Trials for ameliorate the drastic effect of OTA were done by using active life yeast and whey. The results indicted that significant ($p<0.05$) decrease in RBCS, WBCS, phagocytic activity and phagocytic index were occurred in both levels of OTA. Hypoalbuminemia, hypoproteinemia, decrease of globulin, and antibody titer as well as increase of liver enzymes, creatinine and uric acid were noticed. The histopathological examination showed that OTA caused diffuse hydropic degeneration and advanced fatty changes in liver. Tubular necrosis and hydropic degeneration of the kidneys were observed .The activation of melano macrophage centers (MMCs) were recorded. The results proved that OTA produce serious physiological, immunological and pathological effects on, *O.niloticus*. Morovere active life yeast and whey were succeed to neutralize the drastic toxic effects of OTA.

1. Introduction

Ochratoxin is a group of secondary metabolites produced by fungi of two genera: *Penicillium* and *Aspergillus*, this group include Ochratoxin A; Ochratoxin B; Ochratoxin C; Ochratoxin α, and the most toxic member is Ochratoxin A (OTA) (Ringot *et al.,* 2006).

Manning *et al.,* (2005) indicated that juvenile channel catfish fed OTA had greater mortality when challenged with *Edwardsiella ictaluri* compared with control group. Saad (2002) reported that OTA has immunosuppressive effect on *O. niloticus* and Common carp in acute (50 µg/kg fish) and chronic toxicity (10 µg/kg fish).

The role of microorganisms on detoxification of OTA has a lot of concern because they promote the hydrolysis of OTA to its nontoxic form (Ochratoxin α (OTα)) in case of ruminant (Sreemannarayana *et al.,* 1988) and non ruminant (Madhyastha *et al.*, 1992).

In many studies on OTA detoxification by yeast showed antagonistic effect on the production of OTA by fungi. Petersson *et al.*, (1998) showed that *Saccharomyces cerevisiae* inhibit production of toxin from *Penicillium verrucosum*. Péteri *et al.* (2007) found that yeast strain, *Phaffia rhodozyma,* degraded

more than 90% of OTA in 15 days at 20°C where hydrolysis it to OTα.

Moreover, yeast enhanced immune response of treated fish (Elkafoury, 2006; Reyes-Becerril *et. al.,* 2008). Useful microflora in the intestine such as Lactobacillus and Bifidobacterial can utilize the lactose for proliferation (Naghton *et al.,* 2001).

The proliferation of this species causes increase in the acidity of intestine by producing lactic acid and short-chain fatty acids formed unsuitable environment to pathogen bacteria like *Salmonida* and *Escherichia coli* (Juven *et al.,* 1991). This competition leads to excluding harmful bacteria out of the gut (Nurmi and Rantal, 1973). Consequently digestion and absorption increased and feed utilization improved (Tellez *et al.,* 1993). No available studies conducted to investigate the effect of whey on fish.

Moreover, whey protein concentrates enhanced ex-vivo lymphoid cell proliferative responses and increased in vivo antibody production (Knowles and Gill, 2002).

The aim of the present study is to investigate the effects of OTA on cultured *O. niloticus* and attempt to ameliorate the drastic effect of OTA by using yeast and whey as diet supplementations.

2. Materials and Methods:

Apparently healthy 210 *O. niloticus* with an average body weight of 40 ±5 g/fish were used. Fish was obtained from private fish farm in Alexandria governorate and kept for 21 days in circular fiberglass tanks (800L) for acclimatization and fed on a diet contained 30 % crude protein.

Water temperature was ranged 25–27C. Continuous aeration was maintained in each tank using an electric air pumping compressor.

The 210 *O. niloticus* fish were randomly allotted in fourteen fiberglass tanks (two tanks/treatment) with fifteen fish per tank. The fish treated by Ochratoxin A (OTA) in two doses according to Saad (2002), 80 µg/kg fish as low dose (LOTA) and 160 µg/kg fish as high dose (HOTA). The OTA doses performed by stomach intubations once in day zero of the experiment in all fish groups by dissolving OTA in chloroform according to Trucksess and Pohland (2001) then dissolved in corn oil (Abdel-Wahhab *et al.,* 2005) and left to evaporate the chloroform before using. The individual stomach) intubations performed by using syringe attached with butterfly cannula to get the doses through the stomach of the fish (Abdel-Wahhab *et al*., 2005). Fish in control group which fed basal diet received 0.5 ml corn oil.

Yeast (Tonilisat®): Active live yeast (China Way Corporation, Taiwan kindly supplied by EL Zahra Vetrinary), *Saccharomyces cerevisiae*, (8 X 10^9 cells/gram) was used. The yeast added in the ration by incorporating 0.5 kg/ton ration after coating it with oil according to (Elkafoury, 2006). Fish were kept under daily observation for 8 weeks.

Whey: Whey powder (Dairy Farmers Company of America New Wilmington, PA 16142 U.S.A) free fats were used in the experiment. The whey incorporated into the diet at 14%. The whey contained 11, 62, 0.5 and 11% of Protein, Lactose, Fiber and Ash, respectively.

Seven experimental treatments were designed as follows: the basal diet (BD), BD with LOTA dose (80 µg OTA/kg fish), BD with HOTA dose (160 µg OTA/ kg fish), AY diet (0.5 g/kg diet) and LOTA dose,AY diet (0.5 g/kg diet) and HOTA dose, W diet (14% of diets) and LOTA dose and W diet (14% of diets) and HOTA dose.

Every two weeks, blood samples were taken from the caudal vasculature of - fish after anesthetized with MS222 (ten fish/treatment) for hematological assay and serum separation. Total red blood cell (RBCs), white blood cell (WBCs) were performed according to the methods of Anderson and Siwicki (1995) and Hesser (1960) respectively.

Determination of phagocytic activity and phagocytic index:

Phagocytic activity was determined according to Kawahara *et al.* (1991) and Safinaz, (2001). Phagocytosis was estimated by determining the proportion of macrophages which contained intracellular yeast cells in a random count of 300 phagocytes and expressed as percentage of phagocytic activity (PA). The number of phagocytized organisms was counted in the phagocytic cells and called phagocytic index.

Clinico-biochemical determination was used to examine total protein, albumin, globulin and albumin/globulin ratio, alkaline phosphatase, glutamic-oxaloacetic transaminase, uric acid and creatinine were done according to Saad (2002) and Safinaz (2001) by using commercial kits (Biodiagnostic , Cairo, Egypt).

Evaluation of immune response of *O. niloticus* against *Aeromona. hydrophila* bacterin.

Aeromonus hydrophila isolate was used in the bacterin preparation according to the method described by (Sakai *et al.,* 1984)

The preparation of bacterin for injection was carried out according to the method of Badran (1990). The formalin inactivated bacterin cells were mixed with an equal volume of 0.85% sterile saline. Bacterial number was adjusted to Fit MacFarlan's No. 2

At the 4th week one hundred and five *O. niloticus* fish exposed to both dose of OTA and control were inoculated intraperitoneally (IP) with 0.2 ml/fish of formalin inactivated bacterin. One hundred and five *O. niloticus* fish were similarly injected IP with 0.2 ml/fish sterile saline. After 2 weeks, the injected fish received booster dose from bacterin. After 1, 2, 3 and 4 weeks post-injection with inactivated bacterin blood collection was carried out from the caudal vasculature of inoculated fish after anesthetized with MS222 for antibody determination by microagglutination test according to the method described by Badran (1990).

Histopathological studies:

At the end of experiment specimen from kidneys, spleens and livers were removed from fish of the experimental groups and rapidly placed in adequate amount of 10% neutral buffered formalin for at least 24 hrs and used for histopathological studies according to Culling (1983).

Statistical analysis:

Statistical analysis of the experimental results was conducted according to SPSS (version 16.00). Duncan's (1955) multiple range test was carried out to test the significance levels among means of treatments.

3. Results:

The effects of OTA, yeast and whey on red blood cells (RBCs), white blood cells (WBCs) count PA and PI are demonstrated in (Table 1). The red blood cells count differ significantly ($P <0.05$) all over experimental period, where OTA presented severe decrease of RBCs especially with HOTA dose and showed anemia. Meanwhile, addition of yeast and whey with both OTA doses increased RBCs count and improved the body health condition.

Significant ($P< 0.05$) differences were observed after two weeks of treatment and showed decrease of WBCs count with LOTA and HOTA doses significantly than control group and reduced insignificantly than yeast and whey treatments all over the experimental period.

The significant ($P<0.05$) differences of PA were observed at week four until the end of the experiment. The PA of HOTA dose reduced significantly than other treatments. Meanwhile, insignificant ($P>0.05$) differences were observed among LOTA dose and detoxification treatments.

The phagocytic index differ significantly from the second week of treatment, where HOTA dose recorded the lowest significant ($P<0.05$) PI and showed insignificant ($P> 0.05$) differences with LOTA dose and HOTA dose plus whey all over the experiment. The addition of yeast ameliorate the drastic effect of OTA significant ($P<0.05$) on PI especially with LOTA dose. Meanwhile, slightly improve of PI observed with HOTA dose plus yeast and LOTA dose plus whey.

The significant effects of OTA, yeast and whey on total protein (Table 2) observed at week six to eight and showed significant ($P<0.05$) decrease of total protein with both LOTA and HOTA doses treatments. Meanwhile, the addition of yeast increased total protein values with both LOTA (significant $P<0.05$) and HOTA doses. Whey addition increased total protein but not significantly ($P> 0.05$) with both LOTA and HOTA doses.

Regarding to albumin level, significant effects was observed at week six where each OTA treatments and detoxification treatments showed significant ($P> 0.05$) decrease of albumin value (hypoalbuminemia) than control group although yeast and whey improved albumin levels insignificantly ($P<0.05$) than LOTA and HOTA doses.

Insignificant ($P> 0.05$) decrease of globulin with LOTA and HOTA doses and increased in case of yeast and whey until sixth week were found. Meanwhile, at eighth week globulin decrease significant ($P<0.05$) with LOTA dose and HOTA dose.

The results of antibody titer of *O. niloticus* after vaccination with *A. hydrphila* and exposed to OTA and detoxification agents (yeast and whey) were 4, 2.67±0.33, 2±0.00, 3.33±0.33, 3±0.00, 3.67±0.33 and 3.00±0.00 in case of control, LOTA, HOTA, LOTA plus yeast, HOTA plus yeast, LOTA plus whey and HOTA plus whey respectively. The results indicated that significant ($P> 0.05$) differences were observed among other treatments and control group. However, the addition of yeast and whey to the diet increased significantly antibody titare.

Data presented in (Table 3) showed the effect of OTA, yeast and whey on the liver and kidneys function. Significant differences of GOT were observed at the sixth week of treatment where GOT values with LOTA and HOTA doses increased significantly than control and yeast supplementation treatments.

In the same time the levels of liver enzymes in case of OTA plus yeast were less than OTA only.

The results of creatinine and uric acid showed increase especially with HOTA dose than other treatments. Moreover, the addition of yeast and whey decreased creatinine and uric acid levels especially with LOTA dose.

The histopathological examination in the present study showed that LOTA dose after 8 weeks from treatment caused diffuse hydropic degeneration of hepatic cells, congestion of hepatic sinusoids and mild incidence of melanomacrophage centers (MMCs). Moreover, the posterior kidney showed mild acute cellular swelling and MMCs activationwere observed in kidney and spleen (Fig. 1, 2 and 3).

In case of HOTA dose diffuse advanced fatty changes appeared as Signet ring, atrophied of hepatic cells and activation of MMCs in pancreatic islets were recorded. In kidney, infiltration and activation of MMCs and acute tubular necrosis were recorded. Severe infiltration of MMCs to extent that total replaced of the splenic tissues were also noticed (Fig. 4, 5 and 6).

Regarding to addition of yeast to diets the drastic effects of OTA on hepatopancreas and kidneys in LOTA treatment were similar as control.

HOTA dose showed acute cloudy swelling, tubular necrosis and mild activation of MMCs. Also spleen in LOTA dose didn't affected but in HOTA dose spleen showed mild activation of MMCs (Fig. 7, 8 and 9).

Addition of whey to fish diets with OTA showed mild congestion and hydropic degeneration of liver cells in case of LOTA. Meanwhile, with HOTA dose the alteration appeared as mild fatty changes, focal lymphocytic aggregation, enlargement and hyper activation of MMCs. Kidney in LOTA dose showed slight acute cellular swelling of tubular epithelial lining with mild MMCs infiltration. The effect of HOTA dose with whey on posterior kidney

appeared as focal tubular necrosis replaced by inflammatory cells. In spleen the alteration is activation of MMCs in both OTA doses but the severity increased with HOTA dose (Fig. 10, 11 and 12).

Table (1): Effect of Ochratoxin A (OTA), yeast and whey on red blood cells (RBCs), white blood cells (WBCs) phagocytic activity (PA) and phagocytic index (PI) of blood of *O. niloticus* through out experimental period ($\overline{X}$ ±SE)

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
Total protein (g/dl)	**Control**	4.39±0.21	4.37±0.10	4.39±0.12[a]	4.46±0.06[a]	**4.40±0.06[a]**
	LOTA dose	4.14±0.31	4.05±0.22	3.73±0.08[cd]	3.55±0.09[bc]	**3.87±0.11[BC]**
	HOTA dose	3.91±0.10	3.69±0.17	3.42±0.04[d]	3.06±0.06[d]	**3.52±0.09[D]**
	LOTA dose + yeast	4.34±0.17	4.29±0.12	4.12±0.13[ab]	3.84±0.14[b]	**4.15±0.08[AB]**
	HOTA dose + yeast	4.31±0.32	4.14±0.06	3.88±0.14[bc]	3.44±0.08[bcd]	**3.94±0.12[BC]**
	LOTA dose + whey	4.22±0.18	4.20±0.18	4.02±0.20[bc]	3.65±0.25[bc]	**4.02±0.11[BC]**
	HOTA dose + whey	4.18±0.12	3.98±0.25	3.70±0.03[cd]	3.42±0.10[cd]	**3.82±0.10[C]**
	Total Mean	**4.21±0.08[A]**	**4.10±0.07[AB]**	**3.90±0.07[B]**	**3.63±0.09[C]**	**3.96**
Albumin (g/dl)	**Control**	3.10±0.11	3.16±0.21	3.06±0.10[a]	3.13±0.08[a]	**3.11±0.06[A]**
	LOTA dose	3.03±0.23	2.87±0.03	2.73±0.06[b]	2.52±0.04[c]	**2.79±0.07[B]**
	HOTA dose	2.95±0.04	2.76±0.14	2.65±0.08[b]	2.47±0.05[c]	**2.71±0.06[B]**
	LOTA dose + yeast	3.09±0.25	2.97±0.13	2.81±0.14[b]	2.73±0.05[b]	**2.90±0.08[B]**
	HOTA dose + yeast	3.04±0.33	2.87±0.09	2.75±0.06[b]	2.76±0.07[b]	**2.84±0.09[B]**
	LOTA dose + whey	3.06±0.12	2.92±0.09	2.79±0.07[b]	2.73±0.06[b]	**2.87±0.05[B]**
	HOTA dose + whey	3.00±0.04	2.81±0.06	2.67±0.04[b]	2.52±0.06[c]	**2.75±0.05[B]**
	Total Mean	**3.04±0.06[A]**	**2.91±0.05[AB]**	**2.78±0.04[BC]**	**2.69±0.04[B]**	**2.85**
Globulin (g/dl)	**Control**	1.29±0.17	1.21±0.22	1.34±0.11	1.33±0.11[a]	**1.29±0.07[A]**
	LOTA dose	1.11±0.38	1.18±0.22	0.99±0.12	1.03±0.12[ab]	**1.08±0.11[A]**
	HOTA dose	0.96±0.10	0.92±0.26	0.77±0.11	0.59±0.07[b]	**0.81±0.08[B]**
	LOTA dose + yeast	1.25±0.22	1.33±0.23	1.31±0.07	1.11±0.17[ab]	**1.25±0.09[A]**
	HOTA dose + yeast	1.27±0.07	1.27±0.12	1.12±0.12	0.73±0.13[b]	**1.10±0.08[A]**
	LOTA dose + whey	1.16±0.24	1.29±0.27	1.24±0.26	0.92±0.31[ab]	**1.15±0.13[A]**
	HOTA dose + whey	1.18±0.15	1.17±0.21	1.03±0.03	0.89±0.10[ab]	**1.07±0.07[A]**
	Total Mean	**1.17±0.07[A]**	**1.19±0.08[A]**	**1.12±0.06[AB]**	**0.94±0.07[B]**	**1.11**
A/G Ratio	**Control**	2.55±0.36	3.01±0.74	2.33±0.20	2.42±0.26	**2.58±0.21**
	LOTA dose	4.00±1.54	2.68±0.46	2.87±0.34	2.60±0.44	**3.04±0.41**
	HOTA dose	3.17±0.34	4.18±1.47	3.84±0.87	4.48±0.71	**3.92±0.44**
	LOTA dose + yeast	2.75±0.55	2.53±0.57	2.16±0.17	2.66±0.48	**2.53±0.22**
	HOTA dose + yeast	2.43±0.33	2.36±0.35	2.55±0.31	4.46±1.34	**2.95±0.40**
	LOTA dose + whey	3.00±0.58	2.63±0.62	2.67±0.68	4.50±1.54	**3.20±0.47**
	HOTA dose + whey	2.69±0.36	2.74±0.62	2.59±0.11	2.95±0.39	**2.74±0.19**

Values in the same item with different letters are significantly different.
LOTA dose (80 µg OTA/ kg fish). HOTA dose (160 µg OTA/ kg fish).

Table (2): Effect of Ochratoxin A (OTA), yeast and whey on the total protein, albumin, globulin and albumin / globulin ratio (A/G Ratio) in serum of *O. niloticus* through out experiment

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
Total protein (g/dl)	Control	4.39±0.21	4.37±0.10	4.39±0.12^a	4.46±0.06^a	**4.40±0.06^a**
	LOTA dose	4.14±0.31	4.05±0.22	3.73±0.08^{cd}	3.55±0.09^{bc}	**3.87±0.11^{BC}**
	HOTA dose	3.91±0.10	3.69±0.17	3.42±0.04^d	3.06±0.06^d	**3.52±0.09^D**
	LOTA dose + yeast	4.34±0.17	4.29±0.12	4.12±0.13^{ab}	3.84±0.14^b	**4.15±0.08^{AB}**
	HOTA dose + yeast	4.31±0.32	4.14±0.06	3.88±0.14^{bc}	3.44±0.08^{bcd}	**3.94±0.12^{BC}**
	LOTA dose + whey	4.22±0.18	4.20±0.18	4.02±0.20^{bc}	3.65±0.25^{bc}	**4.02±0.11^{BC}**
	HOTA dose + whey	4.18±0.12	3.98±0.25	3.70±0.03^{cd}	3.42±0.10^{cd}	**3.82±0.10^C**
	Total Mean	**4.21±0.08^A**	**4.10±0.07^{AB}**	**3.90±0.07^B**	**3.63±0.09^C**	**3.96**
Albumin (g/dl)	Control	3.10±0.11	3.16±0.21	3.06±0.10^a	3.13±0.08^a	**3.11±0.06^A**
	LOTA dose	3.03±0.23	2.87±0.03	2.73±0.06^b	2.52±0.04^c	**2.79±0.07^B**
	HOTA dose	2.95±0.04	2.76±0.14	2.65±0.08^b	2.47±0.05^c	**2.71±0.06^B**
	LOTA dose + yeast	3.09±0.25	2.97±0.13	2.81±0.14^b	2.73±0.05^b	**2.90±0.08^B**
	HOTA dose + yeast	3.04±0.33	2.87±0.09	2.75±0.06^b	2.76±0.07^b	**2.84±0.09^B**
	LOTA dose + whey	3.06±0.12	2.92±0.09	2.79±0.07^b	2.73±0.06^b	**2.87±0.05^B**
	HOTA dose + whey	3.00±0.04	2.81±0.06	2.67±0.04^b	2.52±0.06^c	**2.75±0.05^B**
	Total Mean	**3.04±0.06^A**	**2.91±0.05^{AB}**	**2.78±0.04^{BC}**	**2.69±0.04^B**	**2.85**
Globulin (g/dl)	Control	1.29±0.17	1.21±0.22	1.34±0.11	1.33±0.11^a	**1.29±0.07^A**
	LOTA dose	1.11±0.38	1.18±0.22	0.99±0.12	1.03±0.12^{ab}	**1.08±0.11^A**
	HOTA dose	0.96±0.10	0.92±0.26	0.77±0.11	0.59±0.07^b	**0.81±0.08^B**
	LOTA dose + yeast	1.25±0.22	1.33±0.23	1.31±0.07	1.11±0.17^{ab}	**1.25±0.09^A**
	HOTA dose + yeast	1.27±0.07	1.27±0.12	1.12±0.12	0.73±0.13^b	**1.10±0.08^A**
	LOTA dose + whey	1.16±0.24	1.29±0.27	1.24±0.26	0.92±0.31^{ab}	**1.15±0.13^A**
	HOTA dose + whey	1.18±0.15	1.17±0.21	1.03±0.03	0.89±0.10^{ab}	**1.07±0.07^A**
	Total Mean	**1.17±0.07^A**	**1.19±0.08^A**	**1.12±0.06^{AB}**	**0.94±0.07^B**	**1.11**
A/G Ratio	Control	2.55±0.36	3.01±0.74	2.33±0.20	2.42±0.26	**2.58±0.21**
	LOTA dose	4.00±1.54	2.68±0.46	2.87±0.34	2.60±0.44	**3.04±0.41**
	HOTA dose	3.17±0.34	4.18±1.47	3.84±0.87	4.48±0.71	**3.92±0.44**
	LOTA dose + yeast	2.75±0.55	2.53±0.57	2.16±0.17	2.66±0.48	**2.53±0.22**
	HOTA dose + yeast	2.43±0.33	2.36±0.35	2.55±0.31	4.46±1.34	**2.95±0.40**
	LOTA dose + whey	3.00±0.58	2.63±0.62	2.67±0.68	4.50±1.54	**3.20±0.47**
	HOTA dose + whey	2.69±0.36	2.74±0.62	2.59±0.11	2.95±0.39	**2.74±0.19**

Values in the same item with different letters are significantly different.
LOTA dose (80 μg OTA/kg fish). HOTA dose (160 μg OTA/kg fish).
Yeast (0.5 g/kg diet). Whey (14% of diets).

Table (3): Effect of Ochratoxin A (OTA), yeast and whey on the glutamic-oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), Creatinine and Uric acid of *O. niloticus* through out experimental period ($\overline{X} \pm SE$).

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
GOT (units/ml)	Control	24.67±4.06	28.33±5.07	29.67±2.51[b]	36.00±3.46[c]	**29.67± .07**
	LOTA dose	26.33±5.49	30.83±4.80	33.33±0.29[ab]	44.83±2.13[ab]	**33.33±2.39**
	HOTA dose	28.00±1.15	34.33±2.67	35.78±1.61[a]	49.00±2.08[a]	**35.78±2.06**
	LOTA dose + yeast	21.50±3.62	26.33±4.70	29.61±0.46[b]	38.67±2.40[bc]	**29.61±2.64**
	HOTA dose + yeast	24.50±1.04	27.00±2.75	31.05±1.01[b]	41.00±1.53[bc]	**31.06±2.13**
	LOTA dose + whey	23.67±0.73	30.83±4.64	32.11±0.86[ab]	41.83±1.92[abc]	**32.11±2.24**
	HOTA dose + whey	24.67±4.42	30.33±0.88	32.78±0.24[ab]	45.33±2.60[ab]	**32.78±2.44**
	Total Mean	**24.76±1.16[C]**	**29.71±1.36[B]**	**32.05±0.60[B]**	**41.67±1.11[A]**	**32.05**
ALP (IU/L)	Control	21.60±2.18	17.62±2.14	18.58±1.49[b]	18.06±1.48[c]	**18.97±0.92**
	LOTA dose	20.43±0.75	19.52±3.42	21.98±0.40[ab]	23.32±1.33[ab]	**21.31±0.92**
	HOTA dose	23.19±0.60	20.54±1.44	23.63±0.54[a]	25.10±1.58[a]	**22.32±0.89**
	LOTA dose + yeast	20.48±0.44	18.49±0.49	21.79±0.88[ab]	20.40±0.74[bc]	**20.29±0.45**
	HOTA dose + yeast	22.13±3.22	18.14±3.26	21.51±1.58[ab]	22.32±1.13[ab]	**21.02±1.18**
	LOTA dose + whey	20.00±1.86	18.70±3.24	20.38±0.79[ab]	20.45±1.14[bc]	**20.68±0.90**
	HOTA dose + whey	21.86±2.64	19.62±0.97	23.07±1.95[a]	23.47±1.29[ab]	**22.00±0.90**
	Total Mean	**21.38±0.66[A]**	**18.95±0.79[B]**	**21.56±0.52[A]**	**21.87±0.63[A]**	**20.94**
Creatinine (mg/dl)	Control	0.78±0.03	0.62±0.11	1.59±0.14[c]	1.76±0.33	**1.31±0.21**
	LOTA dose	1.10±0.25	1.61±0.62	2.10±0.04[bc]	3.06±0.31	**1.97±0.27**
	HOTA dose	1.32±0.46	1.83±0.32	3.63±0.32[a]	3.50±0.28	**2.57±0.34**
	LOTA dose + yeast	1.10±0.09	1.34±0.47	1.66±0.30[c]	2.72±0.52	**1.71±0.25**
	HOTA dose + yeast	1.07±0.21	1.36±0.17	2.43±0.37[bc]	3.02±0.14	**1.97±0.26**
	LOTA dose + whey	1.08±0.30	0.64±0.35	1.84±0.46[c]	2.57±0.97	**1.53±0.33**
	HOTA dose + whey	0.92±0.39	1.10±0.27	2.80±0.08[ab]	3.17±1.10	**2.00±0.40**
	Total Mean	**1.05±0.10[C]**	**1.22±0.15[C]**	**2.29±0.18[B]**	**2.90±0.22[A]**	**1.87**
Uric acid (mg/dl)	Control	0.83±0.26	1.26±0.26	2.25±0.45	2.19±0.31[c]	**1.63±0.23**
	LOTA dose	0.86±0.34	1.56±0.27	3.00±0.42	3.86±0.62[ab]	**2.32±0.40**
	HOTA dose	2.05±0.47	2.45±0.53	3.55±0.59	4.14±0.43[a]	**3.05±0.33**
	LOTA dose + yeast	0.93±0.23	1.29±0.27	3.04±0.41	2.98±0.17[abc]	**2.06±0.31**
	HOTA dose + yeast	0.83±0.35	1.62±0.37	2.65±0.17	3.02±0.37[abc]	**2.03±0.29**
	LOTA dose + whey	1.12±0.09	2.24±0.33	2.88±0.18	2.25±0.52[c]	**2.13±0.24**
	HOTA dose + whey	1.51±0.41	2.13±0.48	2.73±0.21	2.51±0.33[bc]	**2.22±0.21**
	Total Mean	**1.16±0.14[C]**	**1.79±0.15[B]**	**2.87±0.15[A]**	**2. 99±0.21[A]**	**2.20**

Values in the same item having different letters are significantly different. LOTA dose (80 µg OTA/kg fish). HOTA dose (160 µg OTA/kg fish). Yeast (0.5 g/kg diet). Whey (14% of diets).
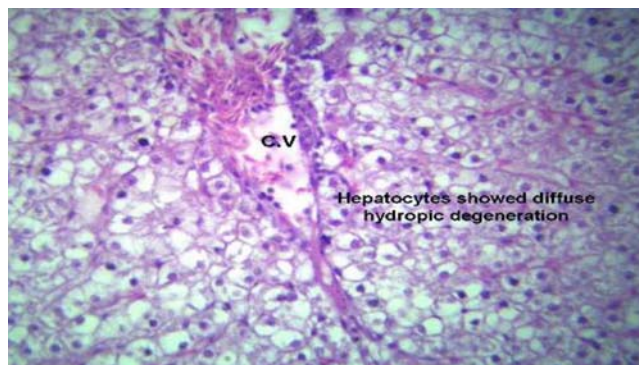
Figure 1: Liver of *O. niloticus* exposed to LOTA dose showing diffuse hydropic degeneration of hepatic cells. H&E. (X 250)
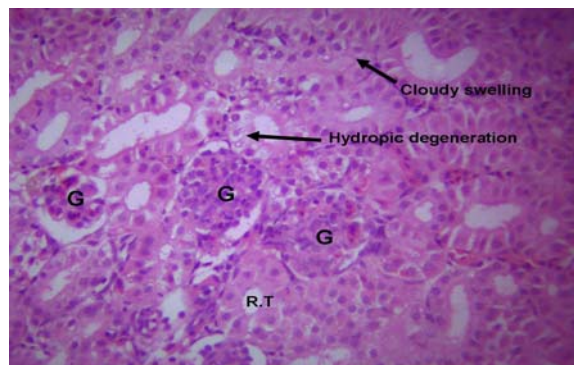
Figure 2: Kidney of *O. niloticus* exposed to LOTA dose showing mild acute cellular swelling. H&E. (X 250)
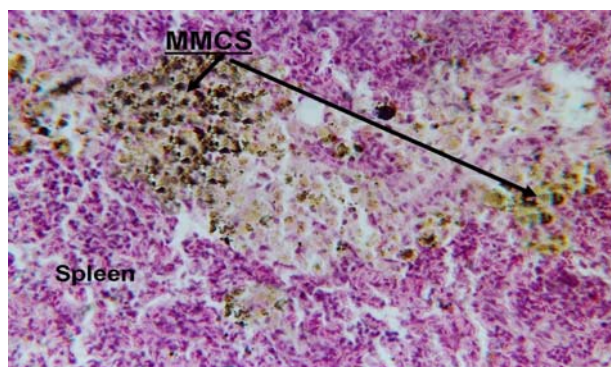
Figure 3: Spleen of *O. niloticus* exposed to LOTA dose showing activation of MMCS. H&E. (X 250)
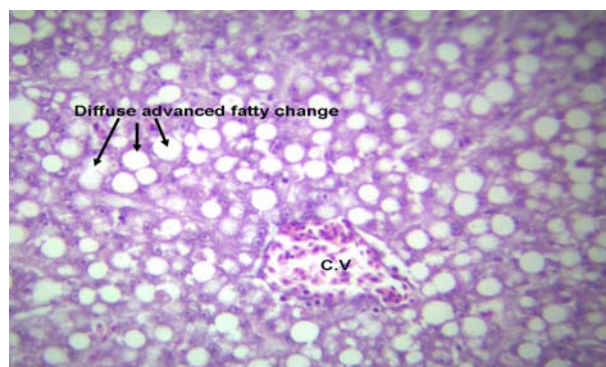
Figure 4: Liver of *O. niloticus* exposed to HOTA dose showing diffuse advanced fatty changes characterized by hepatic cells appear as signet ring. H&E. (X 250)
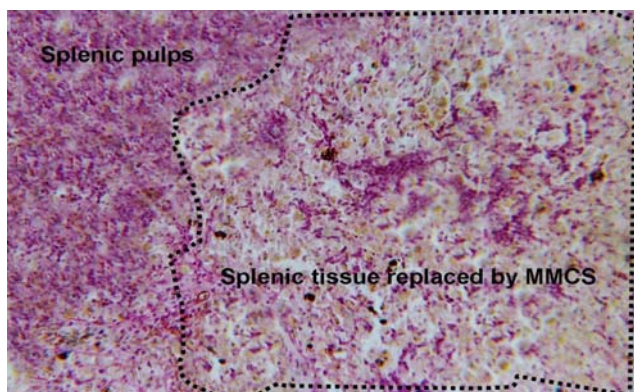
Figure 5: Spleen of *O. niloticus* exposed to HOTA dose showing severe infiltration of the splenic pulps with MMCS to extent that total replacement of the splenic tissues. H&E. (X 160)
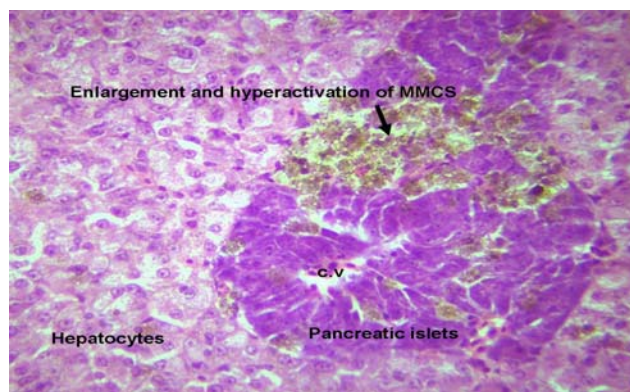
Figure 6: Liver of *O. niloticus* exposed to HOTA dose showing severe infiltration of MMCS in pancreatic islets. H&E. (X 250)
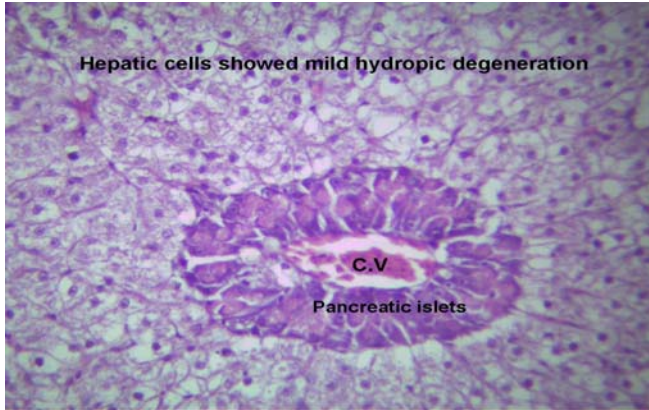
Figure 7: Liver of *O. niloticus* **exposed to HOTA dose plus yeast showing mild hydropic degeneration of the hepatic cells. H&E. (X 250)**
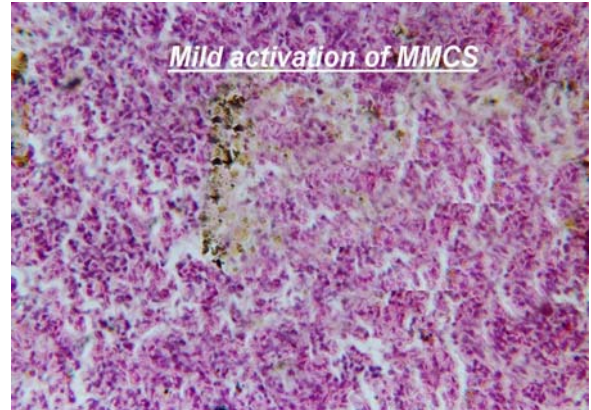
Figure 8: Spleen of *O. niloticus* **exposed to HOTA dose plus yeast showing mild activation ofMMCS. H&E. (X 160))**
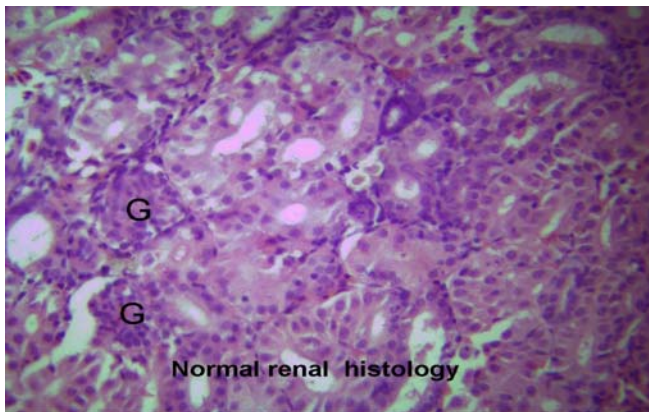
Figure 9: Kidney of *O. niloticus* **exposed to LOTA dose plus yeast showing normal renal architecture and histology. H&E. (X 250)**
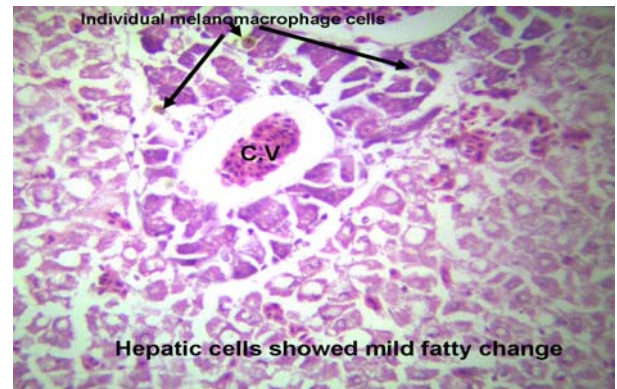
Figure 10: Liver of *O. niloticus* **exposed to HOTA dose plus whey showing mild fatty change of hepatic cells beside individual infiltration of MMC plus in pancreatic islets. H&E. (X 250)**
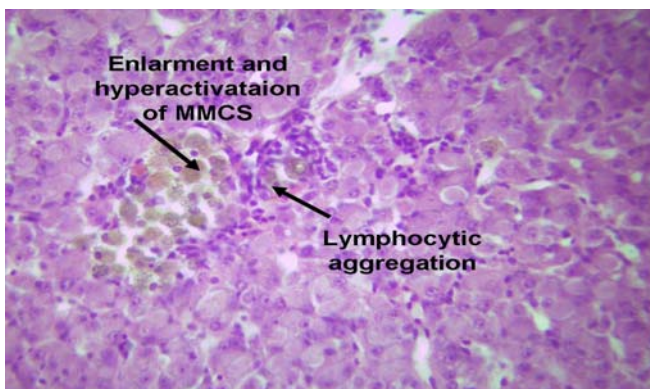
Figure11: Hepatopancreas of *O. niloticus* **exposed to HOTA dose plus whey showing focal lymphocytic aggregation and enlargement and hyperactivation of MMCS. H&E. (X 250)**
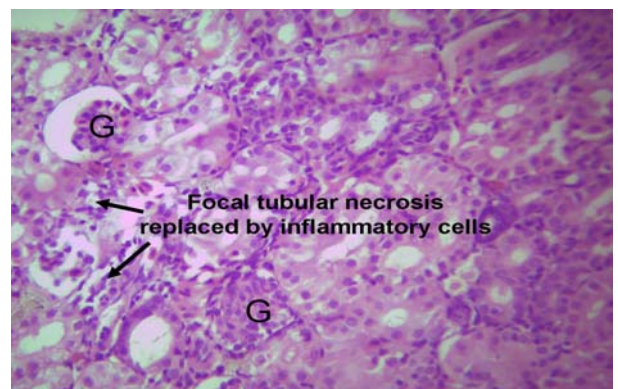
Figure 12: Posterior kidney of *O. niloticus* **exposed to HOTA dose plus whey showing focal tubular necrosis replaced by inflammatory cells. H&E. (X 160)**

4. Discussion:

The reduction of RBCs count which observed in the present study may be due to destruction of mature RBCs and inhibition of erythrocyte production due to reduction of haeme synthesis by ochratoxicosis. Also, the decrease in the RBCs may related to the elimination of RBCs from circulation as a result of ochratoxin – induced extravasations of the blood (Jordan *et al.*, 1977).

Moreover, Shalaby (2004) found a significant reduction in RBCs of *O. niloticus* feed contaminated diet with OTA.

Decrease of WBCs count with LOTA and HOTA doses significantly than control group and reduced insignificantly than yeast and whey treatments all over the experimental period were noticed. The decrease of WBCs may be due to the immunosuppressive effects of OTA. Saad (2002) reported lymphopenia in case of acute and chronic exposure of *O. niloticus* to OTA.

This change usually associated with acute stage of haemolytic anemia (Chang *et al.,* 1979) and destructive effects of OTA on spleen, kidney and liver (Smith and Hamilton, 1970). Moreover, Easa (1997) confirmed these results by recording depletion of hematopiotic elements due to the effects of OTA.

The PA of HOTA dose reduced significantly than other treatments. Meanwhile, insignificant (P >0.05) differences were observed among LOTA dose and detoxification treatments. Control showed the highest significant (P<0.05) PA value.

The phagocytic index differ significantly from the second week of treatment, where HOTA dose recorded the lowest significant (P<0.05) PI and showed insignificant (P> 0.05) differences with LOTA dose and HOTA dose plus whey all over the experiment. The addition of yeast ameliorate the drastic effect of OTA significant (P<0.05) on PI especially with LOTA dose. Meanwhile, slightly improve of PI observed with HOTA dose plus yeast and LOTA dose plus whey.

Saad (2002) who found that OTA (10,000 ng/kg fish) decreased phagocytic activity and phagocytic index in *O. niloticus* after eight weeks of treatments.

The decrease of PA and PI by OTA may be due to the stress effect of OTA on *O. niloticus* (Pickering, 1981). This lead to increase level of serum cortisol which leads to suppression of phagocytosis process (Khalil, 1998).

The increase of Phagocytic activity by addition of yeast may be attributed to enhancing the phagocytic and oxidative activities of kidney phagocytic cells Sakai et,al (2001).

The significant effects of OTA, yeast and whey on total protein observed at week six to eight and showed significant (P<0.05) decrease of total protein with both LOTA and HOTA doses treatments. Meanwhile, the addition of yeast increased total protein values with both LOTA (significant P<0.05) and HOTA doses. Whey addition increased total protein but not significantly (P> 0.05) with both LOTA and HOTA doses.

In general, OTA disruptive total protein level in *O. niloticus* and addition of yeast and whey improved total protein level especially with HOTA dose. Moreover, total protein levels decreased significant (P<0.05) with term of exposure.

OTA and detoxification treatments showed significant (P> 0.05) decrease of albumin value (hypoalbuminemia) than control group. Although LOTA and HOTA doses decreased albumin level significant (P<0.05) than control, yeast treatments and whey with LOTA dose.

The results showed insignificant (P> 0.05) decrease of globulin with LOTA and HOTA doses and significant (P> 0.05) increase with yeast and whey until sixth week. Meanwhile, at eighth week globulin decrease significant (P <0.05) with LOTA and HOTA dose.

The reduction of plasma total protein may be due to liver damage caused by OTA where all plasma protein synthesis usually occurs in liver except gamma globulins which are produced by lymphocytes (Coles, 1986 and Khalil, 1998). This reduction may be interpreted to the inhibitory effect of OTA to protein synthesis (Ringot *et al*., 2006).

The hypoproteinemia and hypoalbuminemia my be attributed to three main causes: hepatic insufficiency, renal loss (protein-losing nephropathy), and gastrointestinal loss (protein-losing enteropathy) Carlye-Rose, (2002). Moreover, OTA found to be hepatotoxic (Gagliano *et al.* 2006), nephrotoxic (Saad, 2002), and increase the permeability of gastrointestinal tract (McLaughlin *et al.,* 2004) which interpreted the decrease of total protein and albumin with OTA treatments in the present study.

Globulin is the building source of antibody where called immunoglobulin (White, 1986). So globulin used as immune indicator and the decrease of its level in the present study with OTA treatments revealed the immunosuppressive effects of OTA. Elkafory (2006) reported increase in fish serum proteins (total protein, albumin, globulin and A/G ratio) received yeast with diet.

Antibody titer reduced significantly with HOTA dose than control group. Insignificant (P >0.05) differences were observed among other treatments and control group. In eighth week (the

forth week after vaccination) a significantly ($P< 0.05$) decreased of antibody titration was observed with LOTA and HOTA doses compared to other treatments. However, the addition of yeast and whey to the diet increase significantly antibody titration especially with HOTA dose.

Regarding to the overcome of detoxification agents to OTA on antibody titer where significantly increase of antibody titer was observed with yeast supplementation. Yoshida *et al.* (1995) showed that *Saccharomyces cerevisiae* was a source of nucleic acids and β-1,3-glucans which have been recognized to effectively enhance immune functions of African catfish. Also, Anderson *et al.* (1995) reported that Baker's yeast, *S. cerevisiae*, contains various immunostimulating compounds such as β-glucans, nucleic acids and mannan oligosaccharides. Moreover, Glucan treatment in fish enhanced the expression of interleukin 1 and complement activity Engstad *et al.* (1992).

In case of whey, which increase antibody titer may be due to whey act as source of biologically active molecules. Several of which are known to impact on the immune system (Knowles and Gill 2002). The biological components of whey protein, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, demonstrate a range of immune-enhancing properties (Horton, 1997).

Also, whey protein concentrates found to be enhanced humoral immunity, with significantly elevated serum and intestinal tract antibody responses to orally administered antigens (Rutherfurd-Markwick, *et al.,* 2005).

The significant differences of GOT observed at the sixth week of treatment where GOT values with LOTA and HOTA doses increased significantly than control and yeast supplementation treatments. Alkaline phosphatase showed significantly ($P<0.05$) different at sixth week where control group decreased significantly than other treatments.

The increase of serum transaminases may reflect myocardial and hepatic toxicity leading to extensive liberation of the enzymes in to blood circulation (Fuchs *et al.,* 1986). These results agreed with Saad (2002) who found significant increase of serum aspartat aminotransferase and alkaline phosphatase with OTA treatment on *O. niloticus*

The increase of liver function enzymes in case of LOTA and HOTA doses may be due to the toxic effect of toxin in liver cells. Moreover the liver used to be the site of detoxification of the OTA to 4(R)-and 4(S) - hydroxyochratoxin A (Stormer and Pederson, 1980). In the same time the level of liver enzymes in case of OTA plus yeast were less than OTA only. This may be indicated that yeast

decreased the toxic effect of OTA on liver and in the same time increase liver function.

The increase of creatinine and uric acid in serum of ochratoxicosis fish my be attributed to renal disturbance associated with damage of proximal tubules and thickening of the glomerular basement membrane caused by OTA which lead to reduce the ability of kidney to produce concentrated urine (Marquadret, 1996). Moreover, kidney is the main target organ of OTA genotoxicity, where induced DNA single-strand breaks and DNA adducts in kidney (Pfohl-Leszkowicz,et.al, 1993).

Saad (2002) found that in case of OTA on acute and chronic toxicity in *O. niloticus* causes severe destruction of the proximal tubules of the posterior kidney and hydropic degeneration of the tubular epithelium.

Creatinine is a protein produced by muscle and released into the blood hence removed by the kidney and the increase of creatinine levels indicated to decrease of kidney function (Zotti *et al.,* 2008). The histopathological alteration which confirmed in case of LOTA and HOTA in the form of activation of melanomacrophage centers in liver and spleen atrophied of hepatic cells, severe fatty changes, and cellular degeneration of kidneys could be attributed to the toxic effects of OTA (Saad 2006).

Similar results obtained by Manning *et al.,* (2003) in case of catfish fed dietary concentrations of 2.00 to 8.00 mg OTA/kg which revealed increase incidence and activation of MMCs centers in hepatopancreatic tissue and posterior kidney.

Orrenius and Bellomo (1986) demonstrated that lipid perox idation which caused by OTA may be an early event in hepatotoxicity, which results in structural changes in the cell membrane and allow an influx of cellular calcium to cause changes in metabolic activity within the cell and ultimately cause cell necrosis.

Also the activation of the MMCs considered as indicative on the degree of the tissue damage (Roberts, 2001).

Regarding to addition of yeast to diets of ochratoxicosis fish elemenate the drastic effects of OTA on hepatopancreas. Also spleen in LOTA dose didn't affected but in HOTA dose spleen showed mild activation of MMCs .

Addition of whey to toxicated fish diets affects the histological findings as follow; Hepatopancreas in LOTA dose are congestion and hydropic degeneration. Meanwhile, with HOTA dose the alteration appeared as mild fatty changes, focal lymphocytic aggregation, enlargement and hyper activation MMCs. Posterior kidney in LOTA dose showed acute cellular swelling of tubular epithelial lining with mild MMCs infeltiration. The effect of

HOTA dose on posterior kidney appeared as focal tubular necrosis replaced by inflammatory cells. In spleen the alteration is activation of MMCs in both OTA doses but severity increased with HOTA dose.

The histopathological examination results concluded that yeast more effective than whey in minimize the destructive effect of ochratoxin in the most affected organs (hepatopancreas, kidney and spleen) especially at the LOTA dose.

Moreover, yeast reduce the presence of potentially pathogenic bacteria by competitive exclusion and causes intestinal microbial balance of the host organism and confer various beneficial effects include immunostimulation and enhance disease resistance (Gatlin *et al.,* 2006).

The detoxification effect of yeast on OTA may be revealed to the ability of yeasts to secrete an enzyme related to carboxypeptidases which convert OTA to OTα (non toxic form) (Péteri *et al.*, 2007) by the cleavage of the peptide bond between isocoumarin and phenylalanine in OTA moiety (Marquardt, 1996). Furthermore, yeast cell wall was an effective adsorbent for OTA (Ringot *et al.*, 2007) which may reduce OTA absorption from the fish gastro intestinal tract and excluded with feces.

Molnar *et al.* (2004) found that yeast strain of the genus *Trichosporon* from the hindgut of the termite, refers to important characteristics to detoxify mycotoxins such as OTA. Since, fish gastric microorganisms able to transform mycotoxin to non toxic form in various environmental conditions (Guan *et al.*, 2009). Moreover, yeast showed antagonistic effects to OTA production and growth of OTA producing fungi (Petersson *et al.,* 1998 and Masoud & Kaltoft, 2006).

In conclusion, OTA proved to produce drastic effects on physiological and pathological levels of *O. niloticus.* Meanwhile, active yeast and Sweet whey were successed to neutralize the drastic toxic effects of OTA.

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Agricultural Cooperatives for Agricultural Development in Iran

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Abstract: Agricultural cooperatives are a significant form of business enterprise. The role of agricultural cooperatives as a critical dimension of market structure in agriculture must periodically be assessed to determine the future viability of the cooperative form of business. This paper aims to study the opportunities, challenges of agricultural cooperatives for agricultural development in Iran. The implication of this study arises from the fact that there has been little research carried out on the agricultural cooperatives. The findings of this study enrich the knowledge concerning agricultural development through rural cooperatives in third world countries; especially in Iran. Life
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Keywords: agricultural cooperative, agriculture development, rural development

Introduction

Development is one of the main goals that all communities try to achieve in order to improve the living standards for individuals in those communities. (Mohamed, 2004). Agricultural cooperatives have played an important role in rural development through development of agriculture. The agricultural cooperatives are considered to be the most important organizations that pay attention and try to support the rural development in general and the agricultural development in special through the activities and services achieved for the sake of farmers (Mohamed, 2004).

The International Cooperative Alliance (ICA, 2010) 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, 2010). Agricultural cooperatives have also taken steps to implement new strategies to enter the 21st century (Prakash, 2000). Cooperatives are user-driven businesses that have contributed greatly to the development of one of the world's most productive and scientific-based agricultural systems. Cooperatives as distinct forms of business rely upon members to work together towards collective goals (Lasley et al., 1997). They have played an important role in strengthening market access and competitive returns for independent farm operators during the 20th century. They adapted their operations to agricultural technological innovations, such as the use of fertilizers, plant and livestock breeding, agricultural mechanization, electricity and other new sources of energy, and to new information systems.

Agricultural cooperatives in Iran

The term "cooperative" first was coined in the early 19th century and has evolved as an economic and organizational form since then. Some of the most noted scholars studying cooperatives include great economists John Stuart Mill, Leon Walras, Alfred Marshall and Charles Gidei. Additionally, like the position of cooperatives in capitalist societies, "the study of cooperatives in modern economics has been relatively marginal but persistent, with occasional upsurges of interest"(Kalmi, 2003; Noruzi & Westover, 2010). In developing countries attempts to organize farmers into cooperatives have often failed, although cooperatives have the potential to supply farm inputs and market farm products that are both important for agricultural development (Hoyt, 1989; Ortmann & King, 2007). Agricultural cooperatives in Iran have a forty year history. Like many other rural communities all over the world, Iranian farmers helped one another and participated in group activities including cultivation, weeding, irrigation and harvest. Such cooperation was a collective cultivation while preserving rights of ownership and individual profit from agricultural land. Villagers still practice this type of cooperation that seems to be more natural than working in the form of agricultural cooperatives. These traditional cooperatives are considered a high-profile partnership in human community as well as in Iranian society, though it is called differently among different cultures. Some old-fashioned cooperatives were named *Boneh* or *Haraseh, Wareh* and so on. Within the past couple of years, social and economic developments in the fabric of rural community,

particularly by introducing agricultural machineries, extension of deep and semi-deep water wells, and using engine pump has diminished traditional group work or changed its framework, which resulted in creation of new form of cooperation and partnership in purchase, maintenance and sharing agricultural machineries (Ministry of Cooperatives, 2010). The launch of formal rural cooperatives in Iran dates back to inclusion of some articles in the Trade Law of 1924. In 1935, cooperatives initiated their formal activity in terms of corporation and registration and a rural cooperative society was established in Garmsar. Since birth of the first cooperatives in Iran up to 1941, three rural cooperatives with a membership of 1050 farmers came into existence. Based on reference studies on cooperatives of this period, those who had visited western countries for acquiring knowledge and technology, had first been introduced to economic and social organizations. By 1941, Reza Khan Pahlavi, the then Iranian monarch, assigned his Minister of the Interior to chair a delegation and visit near and far cities and villages. The mission was to develop cooperatives through raising people's awareness about cooperative rules and concepts and to conduct related trainings to civilian staffs. Also, they wished to stimulate the spirit of collective responsibility among all nationals, particularly the producing classes of society. The government took some measures including distribution of state-run factories' products to consumer cooperatives. However, the outbreak of the Second World War overshadowed all national and private plans. But promotion of cooperatives with the same mentioned particulars continued in two angles during post-war era.

In 1962, by virtue of Note 2, Article 165 of the Act on Land Reforms, the farmers who received agricultural land had to already register as a member of rural cooperative society. As a result, more than eight thousand rural cooperatives were established within a short period of time, which later merged together and formed about three thousand cooperatives. Since 1967 that was announce as the year of cooperation, consumer, distribution and… cooperatives recorded a significant development quantity-wise. Thereafter, Central Organization for Rural Cooperatives of Iran (CORC), National Central Cooperative Organization, Ministry of Cooperatives & Land Reforms were established. In 1971, Cooperative Societies Act was approved, parts of which are still binding as the basis of cooperative operation (Ministry of Cooperatives, 2010).

Cooperatives for Agricultural development

Cooperatives have played an important role in the development of agriculture in industrialized countries. Cooperatives have also played an important role in rural communities, where they are an integral part of the social fabric. They encourage democratic decision making processes, leadership development and education (Prakash, 2000). Agricultural cooperatives have played an important role in the Asian rural landscape for decades, and have become an integral part of its social structure. Cooperatives have contributed greatly to the development of modern national and systematized agricultural production-base, helped enhance self-sufficiency of major staple foods, and strengthened farmers' household economy by facilitating market access and competitiveness, adapting their operations to agricultural technological innovations and encouraging democratic decision-making processes, leadership development and education (Hermida, 2008). An agricultural cooperative is considered as one of the important economical and social organizations in rural societies. It plays an important role in the agricultural development through providing the farmers with production inputs, such as fertilizers, seeds and chemical substances, etc. In addition, it holds guide symposiums for the farmers to acquire them with the necessary knowledge and skills about the agricultural new methods that aim at increasing the agricultural production and, therefore, promoting the rural society. Agricultural cooperatives also have a significant role of rural development and poverty reduction as well. All over the world, cooperatives play a major role in the agricultural and food industries in individual nation-states. For example, in the Netherlands, in 2001, cooperatives processed 84% of all milk and 63% of all sugar beets, supplied 54% of all compound feed, provided 87% of all credit to farmers, and sold 95% of all flowers and potted plants (NCR, 2002). Additionally, about 60% of all fruits and vegetables produced in the Netherlands were sold through co-operative auctions or marketing cooperatives (Bijman & Veerman, 2000; Noruzi & Westover, 2010). The role of agricultural cooperatives in agriculture must periodically be assessed to determine the future viability of the cooperative form of business. Nasr (1995) conducted a study about the role of the agricultural cooperatives in new lands. His study has adopted the concept of agricultural cooperatives effectiveness as a multi–dimensional concept, which includes four dimensions, which are goal attainment, organizational productivity, economic efficiency, and organizational adaptation. The Branch of Agricultural and Irrigation in the National Council for Production and Economical Affairs (1998) also conducted a study that dealt with the importance and functions of the cooperatives in the agricultural development. The study stressed that the agricultural cooperation is an

economic and social system that aims at raising the agriculture workers living standard and improving the levels of their production and performing important social roles. The study resulted in some recommendations for promoting and developing the agricultural cooperative structure .The most important ones are:

1. The cooperative structure independence from the government and the role played by the administrative body is only confined to supervising and directing without intervening in the activity or administration of the cooperatives.

2. The agricultural cooperatives play an effective role in the field of providing the productive inputs and the requirements of the agricultural production for peasants at suitable prices and time.

3. Allowing the cooperatives to import the agricultural production from abroad.

4. Allowing the cooperatives to contract and buy directly the requirements of production from factories or traders.

5. Providing financing necessary for the agricultural cooperatives,

6. Paying attention for holding the general assembly's on time as it is determined by law,

7. Distributing the rest of surplus to the cooperative members as a return for them according to their dealing with the cooperative.

8. Training leaders, board members, employees and managers of the cooperatives (Mohamed, 2004).
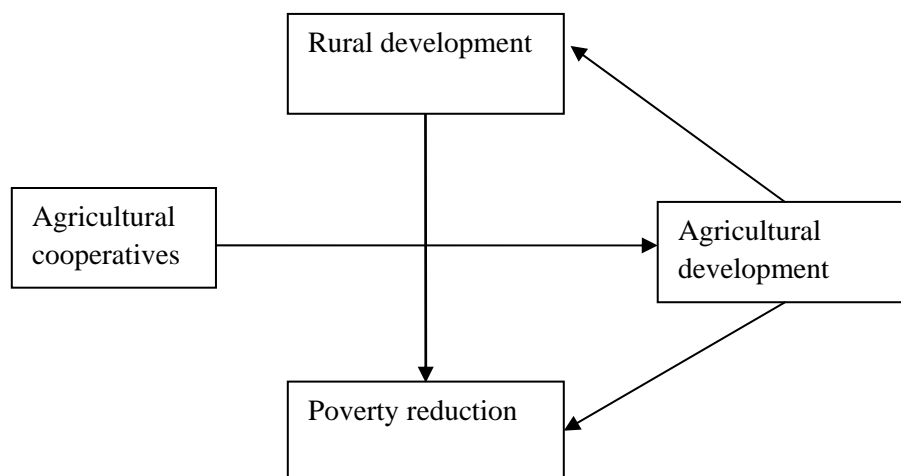
Figure 1: Contribution of cooperatives in agricultural development

Challenges for Agricultural cooperatives

In the past, agricultural cooperatives could play a substantial role in collecting and selling its members' production through a simple valorization on protected markets. But, the changed environment with new economic, social and political rules urges them to develop strategies similar to those of the private sector facing open markets. The great challenges of today and tomorrow are those of the internationalization of markets, of innovation, of environmental conservation and of food security. In the context of trade liberalization and globalization, the cooperative approach is regarded as one of the best instrument of self-protection for small farmers mainly due to its self-help concept and member's participation. Mohammad (2004) also stated some importance barriers of agricultural cooperative for agricultural development Lack of budget, Lack of agricultural production requirements (seeds –

pesticides – fertilizers) on time , quantity and prices suitable for farmers, Disallowing cooperatives to contract directly to buy the requirements of production from agricultural companies and factories, Lack of farmers trust in the agricultural cooperatives as a result of the low quality of services provided in addition to increasing the prices of the agricultural production requirements in the agricultural cooperatives. Hamad (1994) also classified problems and obstacles into the following:

Problems related to financing
-The shortage in the agricultural cooperatives capital,
-High rate of interest on loans that the cooperatives get from banks.

Problems related to the cooperative marketing of agricultural products
-The lack of necessary financing for marketing the agricultural crops.

-The agricultural cooperatives role is only confined to collect crops from farmers and selling them again for commission.

-The lack of marketing information either on the local or international market

-The lack of stations for sorting the agricultural products, packing, drying and preserving

-The lack of the suitable warehouses for storing the agricultural crops.

Legislative and legal obstacles

-Disallowing the agricultural cooperatives from establishing great agricultural productive projects but after getting the approval of the authority Ministry of Agriculture supervises.

-Low rate of interests to dealings expected to be distributed.

-Enforcing peasants to join the agricultural cooperatives as members.

-The multiplicity of the administrative authorities that supervise the agricultural cooperatives (Mohamed, 2004).

Conclusion

Cooperatives have played an important role in the development of agriculture in industrialized countries. It appears that many of these agricultural cooperatives are adapting their operations to the rapidly changing economic environment characterized by technological change, industrialization of agriculture and growing individualism. In Iran, the success of agricultural cooperatives in the past was promoted because they served as agents of agricultural marketing boards and the Land Bank, which provided subsidized loans to commercial farmers. Small-scale farmers in the former (less-developed) homelands did not have access to these cooperatives and their services for political reasons (Ortmann & King, 2007). Although cooperatives were established in the former homelands, many did not survive due mainly to poor management, lack of training, conflict among members and lack of funds. It is essential for agricultural cooperatives to remember that they should be attentive in inspiring, motivating and supporting its members as well in ensuring the sustainability of the production of their members.

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Assessment and Prediction on the Eutrophic State of a Drinking Water Source*

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Abstract: To evaluate the eutrophic state of Xiliu Lake (a water source for Zhengzhou city) and establish prediction system to monitor and predict the eutrophic condition of this water resource. Environmental factors including water temperature (WT), secchi depth (SD), water depth (WD), chemical oxygen demand (COD_{Mn}), total nitrogen (TN), total phosphorus (TP), chlorophyll-a (Chla), algae cell density (ACD) and cyanophyta cell density (CCD) were measured continuously in two sampling sites that were selected in Xiliu Lake. Trophic level index ($\sum$) (TLI($\sum$)), linear correlation and stepwise multiple regression with a significant-value cut-off of $\alpha=0.05$ were used to analyze the data. The result showed that the TLI($\sum$) of Xiliu Lake increased with seasonal changes. Three regression equations of standardized Chla, standardized ACD and standardized CCD were obtained respectively. Tests on these regression equations showed that there was a good correlation between practical value and predictive value of standardized Chla. The correlations for standardized ACD and CCD were even better. In a conclusion, the nutritive condition of Xiliu Lake was eutrophic. A prediction system was established for monitoring the eutrophic condition of surface water through using the three regression equations of standardized Chla, standardized ACD and standardized CCD, which can be used to predict the trend of Chla in Xiliu Lake within a certain range.
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Keywords: eutrophic state; environmental factors; stepwise multiple regression; water resource

1. Introduction

With the advance in industry and agriculture as well as the increase of population, the eutrophic condition of polluted water body becomes more and more serious. It has been reported that different eutrophication levels exist in 53%, 28%, 48%, 41% and 54% of lakes in Europe, Africa, North America, South America and Asian Pacific area respectively[1]. The eutrophic condition of freshwater in China has been very seriously since 1990 including Yangtze River, Yellow River, Taihu Lake, Chaohu Lake and so on[2-4].

Polluted by waste water containing nitrogen and phosphate, the water quality becomes worse, and the ecological balance is destroyed, which further leads to reproduction of algae that excessively consumes oxygen in water and makes fishes and planktons die due to lack of oxygen. In turn, their bodies are decomposed and pollute the water again. Given such a serious environmental challenge, it is necessary to establish novel scientific evaluation methods for managing and protecting water environment.

So far, most studies have focused on algae and toxins, but there has been no study about the relationships between proliferation of algae and environmental factors in Henan. Therefore, we

decided to carry out a study on Xiliu Lake that is the water resources of Zhengzhou, which will provide crucial information for safe water supply in Zhengzhou. Currently, the main methods for evaluating the eutrophic state of lake include trophic state index (Carlson index), amended trophic state index and comprehensive nutrition state index (TLI($\sum$)). TLI($\sum$) is simple and convenient, scientifically sound and easily mastered, and therefore we adopted this index to analyze the trophic state of Xiliu Lake. This study will provide crucial information for safe water supply in Zhengzhou.

2. Material and Methods
2.1 Study Sites

Xiliu Lake in Zhengzhou was formed by Yellow River flowing through sedimentation basin many decades ago. The lake has a surface area of 150 ha, and it is the major water source for residents in Zhengzhou city.

As for sanitation state surrounding Xiliu Lake, within the range of 2,000 m, there is a shelter belt in the west of sampling site NO.1, while there are shelter belts in both west and east sides of sampling site NO.2, but these shelter belts do not work well. As a result, there are many pollution sources

including agricultural pollution, soil losses, rubbish, and sewage.

2.2 Sampling procedure

Two sampling sites were selected in Xiliu Lake. The first one was at the entrance of a storage pool of Shiyuan water factory, and the second was 1,000 m away from the storage pool. The monitoring time was from March to October, and the sampling time was at 8:00～9:00Am. During the sampling, 7.5L water was collected at 5 m below water surface with a collecting device of 2.5L, and then put into polythene bottles and glass bottles quickly. Meanwhile, air temperature was measured.

2.3 Detected items and methods

The following items were measured in the study: water temperature (WT), secchi-depth (SD), water depth (WD), chemical oxygen demand (COD_{Mn}), total nitrogen (TN), total phosphorus (TP), chlorophyll-a (Chla), algae cell density (ACD), and cyanophyta cell density (CCD). WT was measured with a deep water thermometric (Shanghai Medical Instrument Factory, China). SD and WD were measured with Saishi disc[5]. COD_{Mn} was measured with the method of acidic potassium permanganate [5]. TN was measured with the method of alkaline potassium persulphate digestion UV-spectrophotometric[6]. TP was measured with the method of ammonium molybdate spectrophotometric[7]. Chla was measured with the method of spectrophotometric. ACD and CCD were measured by counting the number of blood corpuscle discs.

2.4 Statistical analyses

Trophic level index（∑）(TLI[∑]), linear correlation and stepwise multiple regression analyses were used, and a significant difference was defined when α = 0.05. Comprehension eutrophic state index was defined as in previous studies [8,9]. The data were analyzed with Excel and SPSS (version 11.0). The dataset of the five water-quality indexes at the two sampling sites in Xiliu Lake were expressed as mean ± standard deviation, and the evaluation criterion was the III standard according to GHZB1-1999 and GB3838-2002 (Environmental Quality Standard for Surface Water), (III standard as the abbreviation in the following text). SD showed a decreasing tendency with the increase of water pollution, and the amount of exceeding the standard was calculated with the formula: (certified value – mean value)/ certified value; the constituent ratio of dominate algae was expressed as pie chart; Chla, ACD, CCD were transformed by ln(X+1), then the multiple-linear regression analysis was performed between the environmental chemical-physical factors and ln(X+1). The parameters for the regression equations were as follows: statistic value of Durbin-Watson was about 0～4, residual error value interdependency was about 2, condition index < 30, mean square expansion factors of independent variable ≯ 10, tolerance of independent variable ≮ 0.1, multidimensional characteristic root ≠ 0.

3. Results
3.1 The Eutrophic State of Xiliu Lake

Table 1 shows the changes of TLI(∑) in three seasons. In spring, TLI(∑) at the two sites were 48.43 and 49.74 respectively, which were close to the maximum value of mesotrophic state (50). In summer, TLI(∑) were 58.04 and 58.91 respectively, which were close to the maximum value of slight eutrophic state (60). In autumn, TLI(∑) were 62.43 and 60.33 respectively, which exceeded to the limit value of medium eutrophic state. Thus, the nutritive state of Xiliu Lake varied from the maximum of mesotropher, the maximum of light eutropher to the minimum of middle eutropher. These results suggested that TLI(∑) of Xiliu Lake increased with seasonal changes.

Table 1.Trophic State of Two Sampling Sites and Xiliu Lake in Different Seasons

Sampling sites		TLI					TLI(∑)	trophic state
		Chla	TP	TN	SD	COD_{Mn}		
Spring	NO.1	12.32	8.65	11.21	9.05	7.20	48.43	Mesotrophic state
	NO.2	13.40	8.27	11.69	8.95	7.43	49.74	Mesotrophic state
Summer	NO.1	17.30	10.06	11.41	10.01	9.26	58.04	Oligotrophic state
	NO.2	17.80	9.61	11.46	10.56	9.48	58.91	Oligotrophic state
Autumn	NO.1	19.49	11.79	11.56	10.32	9.26	62.43	Mesotrophic state
	NO.2	18.80	9.94	11.77	10.87	8.95	60.33	Mesotrophic state
Spring		12.91	8.46	11.46	8.98	7.32	49.14	Mesotrophic state
Summer	Xiliu	17.56	9.86	11.44	10.27	9.41	58.55	Oligotrophic state
Autumn	lake	19.17	11.02	11.68	10.61	9.11	61.58	Mesotrophic state

3.2 Stepwise multiple linear regression between environmental factors and standardized Chla, ACD and CCD

Standardized Chlα has a positive correlation with standardized WT, COD_{Mn} and TP, but it has a negative correlation with SD (Table 2). The equations are as follows:

$\ln(\text{Chla} + 1) = 0.390 + 0.134 \times \text{WT}$ ($R = 0.912$, $F = 112.967$, $P < 0.01$)
$\ln(\text{Chla} + 1) = -0.442 + 0.660 \times COD_{Mn}$ ($R = 0.853$, $F = 61.398$, $P < 0.01$)
$\ln(\text{Chla} + 1) = 1.918 + 18.165 \times \text{TP}$ ($R = 0.669$, $F = 18.601$, $P < 0.01$)
$\ln(\text{Chla} + 1) = 6.209 - 3.298 \times \text{SD}$ ($R = 0.836$, $F = 53.556$, $P < 0.01$)

Table 2. Linear Correlation Matrix between Standardized Chla and the Environmental Chemical-Physical Factors in Xiliu Lake

Item	WT	SD	COD_{Mn}	TN	TP	$\ln(\text{Chla}+1)$
WT	1.000					
SD	-.812**	1.000				
COD_{Mn}	.849**	-.841**	1.000			
TN	-.013	.135	.091	1.000		
TP	.521**	-.566**	.479*	.339	1.000	
$\ln(\text{Chla}+1)$	.912**	-.836**	.853**	.112	.669**	1.000

Note. *. $P < 0.05$; **. $P < 0.01$.

Table 3. Stepwise Regression Results of Standardized Chla and the Environmental Chemical-Physical Factors in Xiliu Lake

Site	Selected variance	Stepwise regression equation	coefficient of determination	F	P
NO.1	1 WT 2 TP 3 SD	$\ln(\text{Chla}+1)=1.905+9.450\times10^{-2}\text{WT}+4.533\text{TP}-1.240\text{SD}$	0.833	34.883	.000
NO.2	1 WT 2 TP	$\ln(\text{Chla}+1)=0.364+0.111\text{WT}+9.092\text{TP}$	0.896	95.120	.000
Xiliu lake	1 WT 2 TP 3 COD_{Mn}	$\ln(\text{Chla}+1)=-0.114+8.348\times10^{-2}\text{WT}+6.874\text{TP}+0.193\ COD_{Mn}$	0.900	62.674	.000

Table 3 shows stepwise regression equations of standardized Chla and the environmental chemical-physical factors in Xiliu Lake. It can be seen that at different sampling sites, the screening factors which have significantly positive correlation with standardized Chlα were different.

At sampling site NO.1, three factors were selected: WT and TP were the positive correlation factors, while SD was the negative correlation factor. At sampling site NO.2, two factors were selected: WT and TP were the positively correlated factors ($P < 0.01$ in regression equations). In Xiliu Lake, WT, TP and COD_{Mn} affected standardized Chla and they were selected ($P < 0.01$ in regression equation).

Fig. 1 shows the testing results based on the regression equations of standardized Chlα in Xiliu Lake. We calculated the predictive value by substituting the environmental factors of WT, TP and COD_{Mn} into the regression equation in Table 3. Then, the value was fit with the practical value. As shown in Fig. 1, the correlation between practical value and predictive value was better, so the regression equation can be used to predict the trend of Chla in Xiliu Lake within a certain range.
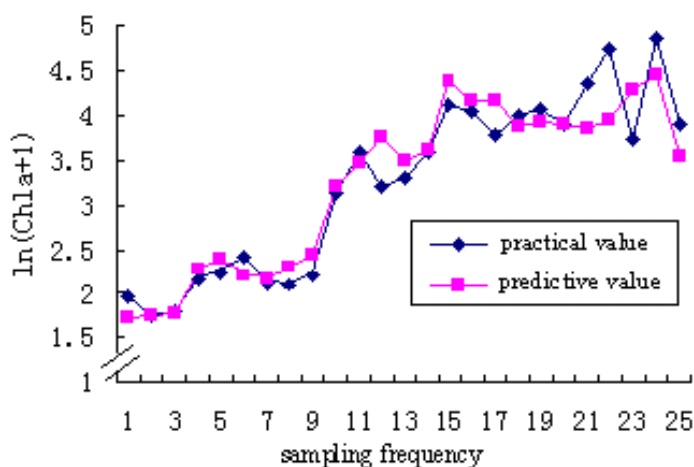
Figure 1. The Fitting Curve between the Practical Value and Predictive Value of Standardized Chla in Xiliu Lake.

3.3 Stepwise regression analysis of standardized ACD, CCD and the environmental chemical-physical factors

Table 4 shows the linear correlation matrix between standardized ACD, CCD and the environmental chemical-physical factors that we studied.

The relationships of standardized ACD and standardized COD_{Mn} with TP were positive; the relationships with SD and WD were negative. The regression equations were following:

$\ln(ACD + 1) = 4.719 + 0.182 \times WT$ ($R = 0.824$, $F = 48.612$, $P < 0.01$)
$\ln(ACD + 1) = 6.514 + 28.512 \times TP$ ($R = 0.698$, $F = 21.892$, $P < 0.01$)
$\ln(ACD + 1) = 12.945 - 4.838 \times SD$ ($R = 0.816$, $F = 45.966$, $P < 0.01$)
$\ln(ACD + 1) = 19.247 - 5.298 \times WD$ ($R = 0.711$, $F = 23.466$, $P < 0.01$)
$\ln(CCD + 1) = 1.293 + 0.295 \times WT$ ($R = 0.866$, $F = 69.071$, $P < 0.01$)
$\ln(CCD + 1) = 4.307 + 44.674 \times TP$ ($R = 0.711$, $F = 23.460$, $P < 0.01$)
$\ln(CCD + 1) = 13.973 - 7.123 \times SD$ ($R = 0.781$, $F = 35.869$, $P < 0.01$)
$\ln(CCD + 1) = 23.438 - 7.894 \times WD$ ($R = 0.688$, $F = 20.627$, $P < 0.01$)

Table 4. Linear Correlation Matrix between Standardized Algae Cell Density, *Cyanophyta* Cell Density and the Environmental Chemical-Physical Factors in Xiliu Lake

Item	WT	SD	WD	TN	TP	ln(ACD+1)	ln(CCD+1)
WT	1.000						
SD	-.812* *	1.000					
WD	-.633* *	.766* *	1.000				
TN	-.013	.135	-.004	1.000			
TP	.521* *	-.566* *	-.405*	.339	1.000		
ln(ACD+1)	.824* *	-.816* *	-.711* *	.045	.698 * *	1.000	
ln(CCD+1)	.866* *	-.781* *	-.688 * *	.068	.711* *	.978* *	1.000

Note.. $P < 0.05$; **. $P < 0.01$.

Tables 5 and 6 show stepwise regression equations of standardized ACD, CCD based on the environmental chemical-physical factors in Xiliu Lake.

As shown in Table 5, the screening factors that had significantly positively correlations with standardized ACD at the two sampling sites are different. At sampling site NO.1, three factors were selected: WT and TP were the positively correlated factors; while SD was the negatively correlated factor. At sampling site NO.2, two factors were selected: TP was the positively correlated factor, while WD was the negatively correlated factor ($P < 0.01$ in regression equation). In Xiliu Lake, both WT and TP were selected for standardized ACD ($P < 0.01$ in regression equation).

Table 6 shows that the same screening factors were significantly positively related with standardized CCD at both sampling sites, and these two factors (TP and WT) were selected ($P < 0.01$ in regression equation). For Xiliu Lake, WT and TP

affected standardized CCD, and they were selected ($P < 0.01$ in regression equation).

Three regression equations of standardized Chla, standardized ACD and standardized CCD and the environment physical and chemical factors are as follows:

(1) $\ln(\text{Chla} + 1) = -0.114 + 8.348 \times 10 - 2 \times \text{WT} + 6.874 \times \text{TP} + 0.193 \times \text{COD}_{\text{Mn}}$, $P < 0.01$.
(2) $\ln(\text{ACD} + 1) = 4.524 + 0.140 \times \text{WT} + 15.071 \times \text{TP}$, $P < 0.01$.
(3) $\ln(\text{CCD}+1) = 1.004 + 0.232 \times \text{WT} + 22.364 \times \text{TP}$, $P < 0.01$.

After putting the environmental factors of WT and TP into the regression equation for Xiliu lake in Tables 5 and 6, the predictive value was gained, then the value was fit with the practical value. The result showed in Figure 2.

Table 5. Stepwise Regression Results of Standardized Algae Cell Density and the Environmental Chemical-Physical Factors in Xiliu Lake

Site	Selected variance	Stepwise regression equation	coefficient of determination	*F*	*P*
NO.1	1 WT 2 TP 3 SD	ln(ACD+1)=6.653+0.119WT+8.782TP-1.523SD	0.793	26.779	.000
NO.2	1 TP 2 WD	ln(ACD+1)=14.667+34.936TP-3.899WD	0.735	30.563	.000
Xiliu Lake	1 WT 2 TP	ln(ACD+1)=4.524+0.140WT+15.071TP	0.778	38.559	.000

Table 6. Stepwise Regression Results of Standardized *Cyanophyta* cell density and the environmental chemical-physical factors in Xiliu Lake

Site	Selected variance	Stepwise regression equation	coefficient of determination	*F*	*P*
No.1	1 WT 2 TP	ln(CCD+1)=1.404+0.231WT+14.603TP	0.811	47.349	.000
No.2	1 WT 2 TP	ln(CCD+1)=0.478+0.258WT+24.588TP	0.787	40.539	.000
Xiliu Lake	1 WT 2 TP	ln(CCD+1)=1.004+0.232WT+22.364TP	0.842	58.773	.000

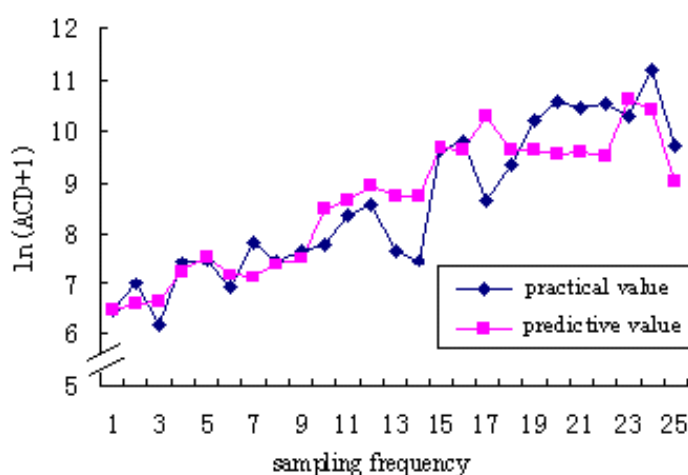**Figure 2.** The Fitting Curve between the Practical Value and Predictive Value of Standardized Algae Cell Density in Xiliu Lake.

4. Discussions
4.1 Relationship between environmental factors and standardized Chla, ACD and CCD

Chla is an important pigment in plant photosynthesis, which represents the primary productive force and has been considered as a key parameter for biomass and capacity of plankton[10]. It has become a crucial biological index for monitoring eutrophic water quality. Some studies show that there is a good correlation between ACD and the concentration of Chla[11,12]. Suitable WT can promote the growth of algae, and the dominant algae varies with the change of WT[13]. SD is also a key environmental factor affecting Chla, ACD and CCD, because it directly influences illumination intensity that leads to the change of productive layers [14]. COD_{Mn} represents the content of organic materials oxidized by potassium permanganate. In general, the change of nutriment concentration has the same phase as that of organic contamination content, and there is a lot of organic contamination when the biomass is higher in water. Thus, the relationship between Chla and COD_{Mn} indicates the trend of cooperation between each other, but it can not show that the organic is the limiting factor for the algae growth [15].

As seen from the molecular formula of algae bioplasm ($C_{106}H_{263}O_{110}N_{16}P_1$), there are some N and P content given C, H and O, so the nutrition containing N and P is essential for the plankton growth. Some reports show that N and P are the determining factors that affect the growth of planktons: planktons tend to be P-restricted but not N-restricted [16-18]. Our results showed that TN did not affect Chla, ACD and CCD; and TP had significantly positive correlations with Chla, ACD and CCD.

We obtained the predictive equation for standardized Chla, ACD and CCD in Xiliu Lake. These equations indicated that the key factors affecting standardized Chla, standardized ACD and standardized CCD were WT, TP and COD_{Mn}, WT and TP, WT and TP, respectively. In order to validate these equations, we calculated the predictive values and compared it with the measured ones. We found that the curve fitting of predictive value and measured value of standardized Chla was good, and the curve fitting of predictive value and measured value of standardized ACD and CCD was even better.

Among the environmental factors in the predictive equations, TP and COD_{Mn} can be controlled by people, so it is critical in decreasing and controlling these two factors in order to prevent the eutrophication of Xiliu Lake.

4.2 Equation established between standardized ACD, CCD and the environmental chemical-physical factors

We observed significantly positive correlations of standardized Chla with WT, COD_{Mn} and TP, and significantly negative correlations with SD in Xiliu Lake. Through the stepwise multiple regression analysis, three significant correlated factors were selected at sampling site No. 1: WT and TP were the positively correlated factors; while SD was the negatively correlated factor. Two factors were selected at sampling site No. 2: WT and TP were the positively correlated factors. In Xiliu Lake, WT, TP and COD_{Mn} were the most significantly correlated factors with standardized Chla.

Significantly positive correlations were found between standardized ACD, CCD and WT, TP，and significantly negative correlations were found between standardized ACD, CCD and SD in Xiliu Lake. The stepwise multiple regression analysis on ACD showed that three significantly correlated factors were selected at sampling site No.1: WT and TP were the positively correlated factors, while SD was the negatively correlated factor. Two significantly correlated factors were selected at sampling site No.2: TP was the positively correlated factor, while SD was the negatively correlated factor. For Xiliu Lake, WT and TP were the most significantly correlated factors for standardized ACD. The stepwise multiple regression analysis on CCD showed that two significantly correlated factors were selected for both sampling sites: WT and TP were the positive correlated factors. For Xiliu Lake, WT and TP were the most significantly correlated factors for standardized CCD. The inspective results of three regressive equations showed that the coincidence of practical value and predictive value of the standardized Chla was good. And the coincidence of practical value and predictive value of the standardized ACD and CCD was better. The contents of Chla, ACD and CCD could be predicted by the multiple equations. The fitting of practical value and predictive value are relatively coincidence.

In a conclusion, this study established a prediction system for monitoring the eutrophic condition of surface water using three regression equations of standardized Chla, standardized ACD and standardized CCD based the environment physical and chemical factors. These regression equations can be used to predict the trend of Chla in Xiliu Lake within a certain range, which will provide crucial information for safe water supply in Zhengzhou.

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Interaction of TIM4-TIM1 decreases the function of CD4^{+}CD25^{+}Treg in intestine in food allergic mice

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Abstract: Research in the area of food allergy has advanced rapidly in recent years, however, the mechanism of food allergy remains unclear. It has been shown that the T regulatory cells play an important role in preventing the allergic responses in the intestine. TIM1 (T cell immunoglobulin and mucin domain protein TIM 1 interacted with its endogenous ligand of TIM4 may attenuate the oral immune tolerance and lead to hypersensitivity reactions in the intestine. Forty male BALB/c mice fed on the OVA-free diet were randomly divided into five groups, and eight mice were used for each group: A group of mice were sensitized by intraperitoneal injection (ip) with Staphylococcal enterotoxin B (SEB) + Ovalbumin (OVA) and the other four groups of mice were separately treated with normal saline (NS), anti-TIM1 (2 μg/mouse) +SEB + OVA, anti-TIM1 (10 μg/mouse) +SEB + OVA, anti-TIM4 (10 μg/mouse) + SEB + OVA, on the 0, 3rd and 9th day; and all of the mice were challenged by means of lavage with SEB+ OVA (except NS) on the 7th and 14th day. Mice were treated intraperitoneally with the same doses anti-TIM4 or anti-TIM1 30 minutes before gavage. Twenty-four hours after the last gavage, the mice were killed and subjected to immunologic analyses.   The expressions of Foxp3 mRNA in the jejunum and spleen decreased significantly in SEB+OVA group compared with those treated with NS, but the expressions of TIM4 mRNA increased significantly (P<0.05). The levels of TGF- 1 in serum and jejunum decreased significantly compared with those treated with NS. The expressions of Foxp3 mRNA in the jejunum and spleen and the levels of TGF- 1 in the serum and jejunum were significantly higher in anti-TIM1+SEB+OVA and anti-TIM4+SEB+OVA groups compared with SEB+OVA group (all P<0.05). The function of Treg cells can be suppressed in the intestine by sensitization that can be prevented by pretreatment with antibodies against TIM1 or TIM4.
[Xin-Ting Wang, Zhi-Qiang Liu, Peng-Yuan Zheng, Ping-Chang Yang, Yu Luo, Gao-Feng Lu, Li-Li Zhang .Interaction of TIM4-TIM1 decreases the function of CD4+CD25+Treg in intestine in food allergic mice. Life Science Journal. 2011;8(1):93-99] (ISSN: 1097 – 8135). http://www.lifesciencesite.com.

Key Words: Food allergy; CD4^{+}CD25^{+}Treg cells; Oral tolerance; TIM protein

1. Introduction

As many as 2% to 6% of the populations in the world have food allergy (FA) and food antigen-related disorders; its prevalence keeps increasing especially in last 20 years (Vierk KA, 2007; Sicherer SH,2009) Symptoms of food allergy vary from slight abdominal discomfort to life-threatening anaphylactic shock (Bahna- -SL, 2003) . However, the etiology and immune mechanisms of intestinal allergic diseases remain poorly understood. FA is generally regarded as a collapse of the balance between intestinal immune system and food antigens, resulting in a skewed Th2-dominant intestinal immune response. It is believed that under normal circumstances, the gut immune system is relatively hyporesponsive to food antigens and commensal bacteria, this state of hyporesponsiveness (tolerance) is maintained by a number of important mechanisms including the integrity of gut epithelium and the presence of tolerogenic dendritic cells (DCs) and regulatory T cells (Strobel- -S, 2006) . Thus, it is conceivable that any perturbation to the homeostasis between luminal antigens and the gut immune system might lead to gut inflammation and food allergy.

CD4^{+}CD25^{+}Treg cells were subset of regulatory T cells and they play an important role in maintaining the stabilization of internal homeostasis, anti-infection, oral tolerance, introduction of transplantation tolerance and autoimmune disorder. Reduction of Treg number and impairment of Treg function can breach the balance of oral tolerance (Wiest R, 2003). Recently the forkhead-winged helix family transcription factor, Foxp3, has been shown being specifically expressed in murine CD4^{+}CD25^{+}Treg cells, and appeared being a 'master gene' controlling the development and suppressive function of CD4^{+}CD25^{+}Treg cells, and previous studies reported that Foxp3 gene mutation or decreased expression could lead to Treg cell dysfunction (Powrie- -F, 2003; Coombes JL, 2005; Fantini MC,2006) .

The TIM (T-cell immunoglobulin domain and mucin domain) family was discovered (McIntire JJ, 2001) in 2001 and received much attention due to its location on mouse chromosome 11B1.1, a genetic region associated with multiple diseases including asthma, allergy and autoimmunity (Kuchroo, V. K,

2003). The TIM family consists of eight genes in mouse (TIM1–8) and three genes in human (TIM1, 3, and 4). Among these members, TIM1 is constitutively expressed on CD4$^+$ T cells; its ligand TIM4 is expressed in DCs. The interaction of TIM1 and TIM4 could promote Th2 cell polarization (Meyers JH, 2005; Umetsu SE, 2005). In addition, studies showed that TIM1-specific agonist antibody decreased the suppressive capacity of natural regulatory T cells, in line with a reduction in their expression of Foxp3, GITR (glucocorticoid-induced tumour-necrosis factor (TNF)-receptor-related protein), CTLA4 (cytotoxic T-lymphocyte antigen 4) and IL-10 (Degauque N, 2008) . However, the functional status of Treg cells and the impact of TIM1 on Treg cells have not been reported in FA, we speculated that Treg cells were dysfunctional and TIM4 interaction with TIM1 might attenuate the function of Treg cells and oral tolerance, which might be one of the important mechanisms leading to FA.

With the goal of elucidating the immunogenesis FA, we established a murine model of SEB+OVA allergy model and pretreatment with anti-TIM1 and anti-TIM4 to further study the functional status of Treg cells and the impact of TIM1 in food allergy mice.

2. Materials and Methods
2.1 Materials

Staphylococcal enterotoxin B (SEB; synthesized by Department of Biotechnology, Zhengzhou University, Zhengzhou, China). Ovalbumin (Sigma Aldrich, MO, USA). Monoclonal Anti-mouse TIM-1 Antibody(R&D, USA). Monoclonal Anti-mouse TIM-4 Antibody (BioLegend, USA). BALB/c mice were purchased from Experimental Animal Center of Henan Province. The experimental procedures were approved by the Animal Care Committee at Zhengzhou University.

2.2 Murine Model of Intestinal Allergy and anti-TIM1 anti-TIM4 intervention

Forty 6~8 wk male BALB/c mice fed on the OVA-free diet were randomly divided into five groups, and eight mice were used for each group: A group of mice were sensitized by ip injection with SEB (10 μg/mouse) + OVA(20 μg/mouse) and the other three groups of mice were separately treated with normal saline (NS), anti-TIM1(2 μg/mouse) +SEB + OVA, anti-TIM1 (10 μg/mouse) +SEB + OVA, anti-TIM4 (10 μg/mouse) + SEB + OVA, on the 0, 3rd and 9th day; and all of the mice were challenged by means of ig with SEB+ OVA (except NS) on the 7th and 14th day. Mice were treated intraperitoneally with the same doses anti-TIM4 or anti-TIM1 30 minutes before gavage. Twenty-four hours after the last gavage, the mice were killed and subjected to immunologic analyses.

2.3 RT-PCR

The total of RNA was isolated from mouse jejunum and spleen using TRIZOL Reagent (Invitrogen, USA). The primers and PCR conditions included TIM4, forward: 5'-AGGGTCCGCCTCACTAC-3'; reverse: 5'-TCCCGTCTTCATCATCCC-3' (55   404 bp); -actin: forward, 5'-GAGACCTTCAACACCCCGC-3'; reverse,   5'-CCACAGGATTCCATCCCAA-3'(59   , 446   bp);   Foxp3:   forward: 5'-AGGAGAAAGCGGATACCA-3';   reverse: 5'-GAAGGACATACCCAGAAGC-3' (55   349 bp). The normalized value for Foxp3 and TIM4 mRNA expression was calculated as the relative quantity of Foxp3 and TIM4 divided by the relative quantity of -actin.

2.4 ELISA

Determination of OVA sIgE (ADL, USA), IL-4, TGF- 1, and IL-10(SHANGHAI WESTANG BIO-TECH INC, LTD) in the sera, the peripheral blood were kept standing at the temperature of 37   for 1 h, then centrifuged at a low temperature of 4 . Levels of OVA sIgE, IL-4, TGF- 1 and IL-10 in the sera were quantified using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions.

2.5 Immunohistochemistry

Cryosections were prepared from jejunal segments and stained with anti-TGF- 1 monoclonal antibodies by SP (streptavidin-peroxidase). Some paraffin wax block were used for HE (hematoxylin-eosin) staining. The results were observed in the mucosa of all samples from different groups of mice.

2.6 Statistical analysis

Data were expressed as the means ± SD. All statistical analysis was performed using SPSS statistical version 13.0. Differences between groups were determined with one-way ANOVA; non-parametric data were compared by the Kruskal-Wallis. a P value of less than 0.05 was considered to be statistically significant.

3. Results
3.1 SEB+OVA sensitization increased the levels of IgE and promotes Th2 responsiveness

Antigen-specific antibodies and Th2 cytokines play a critical role in intestinal allergic reactions (Owen CE, 2007) and are important diagnostic parameters in diagnosing and evaluating

therapeutic efficiency in patients with allergic diseases (Venarske D, 2003). Therefore, we assessed OVA-specific antibodies of IgE and IL-4 in the serum. The results showed that OVA-specific IgE and IL-4 increased significantly in mice sensitized to SEB+OVA (Table 1). Eosinophils are the major effector cells in hypersensitivity reactions, to further strengthen our observation, we examined the numbers of these cell types in the intestine. The results showed that the numbers of eosinophil increased significantly in mice sensitized to SEB+OVA (Figure 1). These results might indicate the successful establishment of murine food allergy model.

Table 1. Levels of IgE and IL-4 in serum in different groups (n=8, mean±SD)

group	IgE (ng/L)	IL-4 (ng/L)
Normal saline control group	0	41±7[a]
SEB + OVA group	197±58[c]	66±6[c]
anti-TIM1 group (10 μg/mouse)	87±8[a]	50±9[a]
anti-TIM1 group (50 μg/mouse)	48±16[a]	40±7[a]
anti-TIM4 group (50 μg/mouse)	50±18[a]	44±6[a]

[a]P<0.05 vs SEB + OVA group , [c]P<0.05 vs normal saline control group
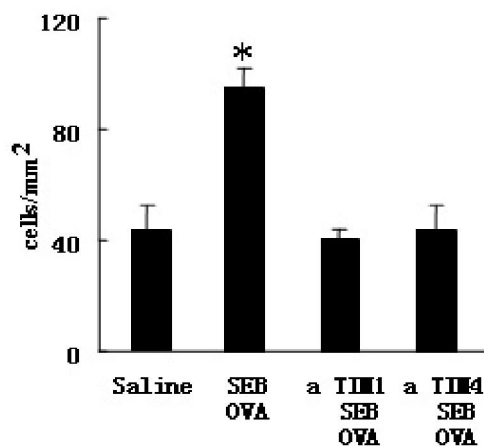
Figure 1. Pretreated with anti-TIM1 (or TIM4) antibody reduces the number of eosinophils. The jejunal segments were processed for the hematoxylin-eosin staining. Positively stained cells were counted under a light microscope. Bars indicate the numbers of eosinophils per mm2 jejunal tissue in different groups. Data were expressed as mean ± SD. *P<0.05, compared with saline group. aTIM1 (or aTIM4): Pretreated with anti-TIM1 (or TIM4) antibody.

3.2 SEB+OVA sensitization decreased the expressions of Foxp3 mRNA in the jejunum and spleen

Since Foxp3 plays an important role in the lineage commitment of Treg cells and deficiency of Foxp3 results in severe intestinal inflammation (Barnes MJ, 2009), we postulated that the expression of Foxp3 in the intestine might be compromised in food allergy mice. By the approach of RT-PCR, we found that expressions of Foxp3 mRNA in the jejunum and spleen decreased significantly in SEB+OVA group compared with those treated with NS (0.401±0.145 vs 0.732±0.162, P<0.05; 0.407±0.082 vs 0.691±0.145, P<0.05). Of interest, the results indicate that pretreatment with anti-TIM1 and anti-TIM4 effectively could restore the expressions of Foxp3 mRNA in jejunum and spleen (P = 0.000, P = 0.000) (Figure 2), that was prevented by pretreatment with different doses of anti-TIM1 in a dose-dependent manner (Figure 3). These results indicate that allergic responses interfere with the expression of Foxp3 in the intestine; the interaction of TIM1/TIM4 plays a role in the regulation of expression of the Foxp3 gene.
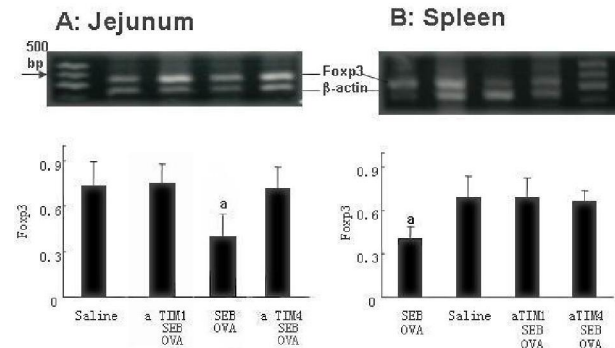
Figure 2. Expressions of Foxp3 mRNA in jejunum and spleen in different groups The average optic density of the electrophoresis bands were obtained by Bandscan. Bars indicate the ratio of the average optic density of Foxp3 mRNA divided by the average optic density of -actin mRNA in different groups. Data were expressed as mean ± SD. [a]P <0.05 vs normal saline control group.

3.3 SEB+OVA sensitization increased the expressions of TIM4 mRNA in jejunum

TIM4 is the natural ligand of TIM1. Interaction of TIM1/TIM4 promotes Th2 polarization (Meyers JH, 2005; Yang PC, 2007). Based on these previous studies, we postulated that the expression of TIM4 in the intestine might be increased. Indeed, expressions of TIM4 mRNA in jejunum increased significantly in SEB+OVA, anti-TIM1+SEB+OVA and anti-TIM4+SEB+OVA group compared with those treated with NS (P=0.004, P=0.007, P=0.033) (Figure 4). There was no significant difference between anti-TIM1+SEB+OVA and

anti-TIM4+SEB+OVA group. The results indicate that sensitization can markedly increase the expression of TIM4 gene in the intestine and disruption of TIM4-TIM1 pathway may not impact it.
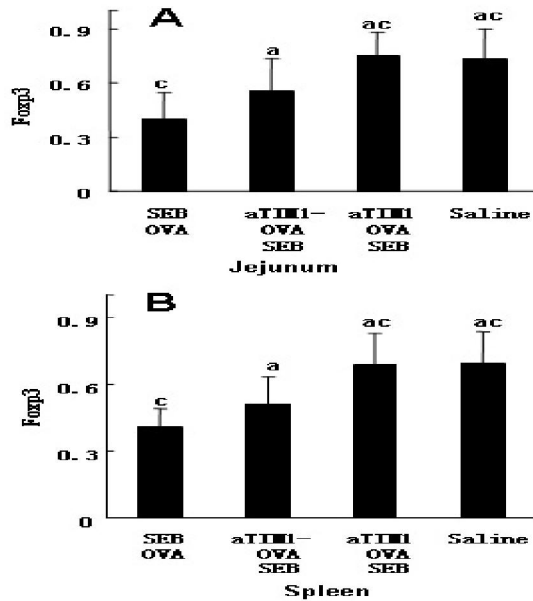
Figure 3. Expressions of Foxp3 mRNA in jejunum and spleen in different doses of anti-TIM1 groups. aTIM1-: Pretreated with anti-TIM1 antibody (10μg/mouse). aTIM1: Pretreated with anti-TIM1 antibody(50μg/mouse). The average optic density of the electrophoresis bands were obtained by Bandscan. Bars indicate the ratio of the average optic density of Foxp3 mRNA divided by the average optic density of -actin mRNA in different groups. Data were expressed as mean ± SD. [a]P <0.05 vs SEB+OVA group, [c]P <0.05 vs aTIM1- group.
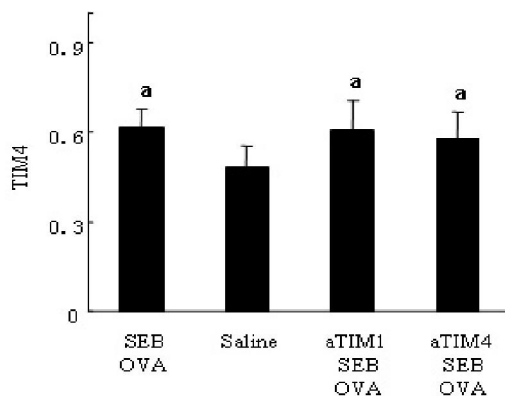
Figure 4 Expressions of TIM4 mRNA in jejunum in different groups The average optic density of the electrophoresis bands were obtained by Bandscan. Bars indicate the ratio of the average optic density of TIM4 mRNA divided by the average optic density of -actin mRNA in different groups. Data were expressed as mean ± SD. [a]P <0.05 vs normal saline control group.

3.4 SEB+OVA sensitization decreased the levels of TGF- 1 in serum

TGF- and IL-10 are the major effector molecules with which Tregs suppress other effector T cell's activities. Since the gene expression of Foxp3 was decreased as shown above, we postulated that the serum levels of TGF- and IL-10 might be suppressed under an allergic environment. As shown by ELISA, the levels of TGF- 1 in the serum decreased significantly in SEB+OVA group compared with those treated with NS, anti-TIM1 and anti-TIM4 (P=0.012, 0.010, 0.000). And the levels of TGF- 1 in the serum increased in response to different doses of anti-TIM1 stimulation in a dose-dependent manner. The levels of IL-10 was significantly higher in SEB+OVA group compared with those treated with NS (P = 0.001), but there's no statistical significance in other groups (Table 2).

Table 2. Levels of TGF- 1 and IL-10 in serum in different groups (n=8, mean±SD)

group	TGF- 1(ng/L)	IL-10(ng/L)
Normal saline control group	8342± 488[ae]	40± 6
SEB + OVA group	7859± 126[e]	46± 5[c]
anti-TIM1 group (10 μg/mouse)	8058± 97[a]	42± 2
anti-TIM1 group (50 μg/mouse)	8356± 222[ae]	43± 5
anti-TIM4 group (50 μg/mouse)	8628± 455[ae]	43± 3

[a]$P<0.05$ vs SEB + OVA group, [e]$P<0.05$ vs anti-TIM1 group (10 μg/mouse), [c]$P<0.05$ vs normal saline control group

3.5 Number of TGF-β1-expressing cells is reduced in the intestine

TGF-β1 plays an important role in the immune regulation in the intestine. Since the serum level of TGF-β1 was decreased in sensitized mice, we speculated that the number of TGF-β1 expressing cells might be decreased as well. To this end, jejunal segments were processed and examined by immunohistochemistry. A number of TGF-β1^{+} cells were localized in the jejunum of mice treated with saline that were scarcely seen in mice sensitized to SEB+OVA. Pretreatment with antibodies against TIM1 or TIM4 could prevent the suppression of TGF-β1^{+} cells in the jejunum by sensitization (Fig.5).

3.6 Pretreated with anti-TIM1 (or TIM4) antibody reduced allergic responses

Above of these results demonstrate that pretreated with anti-TIM1 (or TIM4) antibody might

restore the function of Tregs. Indeed, the sensitized mice have profuse liquid stool, and the symptom was recovered by pretreating with anti-TIM1 (or TIM4) antibody. Importantly, we found that pretreatment with antibodies against TIM1 or TIM4 diminished the levels of IgE and OVA-specific Th2 cytokine responses in the serum (Table 1). As expected, the numbers of eosinophils were reduced in anti-TIM1 and anti-TIM4 group compare with that of sensitized mice (Figure 1). These results showed that blocking the interaction of TIM1/TIM4 is effective to abolish allergic responses.
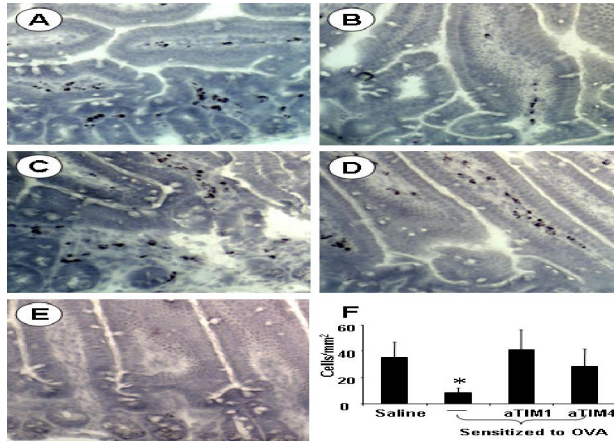
Figure 5 Sensitization reduces the number of TGF-β1-expressing cells in the intestine. Representative immunohistochemistry images from mice treated with (A) saline, (B) both SEB and OVA, (C) both SEB and OVA and pretreated with anti-TIM1 antibody, (D) both SEB and OVA and pretreated with anti-TIM4 antibody. The dark stained cells are TGF-β 1-expressing cells in panel A-D. Positively stained cells (dark staining) were counted under a light microscope. Panel E is isotype IgG staining. F, bars indicate the numbers of TGF- 1 cells per mm2 jejunal tissue in different groups. Data were expressed as mean ± SD. *P<0.05, compared with saline group. aTIM1 (or aTIM4): Pretreated with anti-TIM1 (or TIM4) antibody.

4. Discussion

In recent years, rapid progress has been made in the understanding of pathogenesis of food allergy. Researches have focused on the imbalance of Th1/Th2 cell, hygiene hypothesis, combined effect of bacterial toxins and food protein and oral tolerance (Yazdanbakhsh M, 2002; Liu T, 2006; Faria AM, 2005). However, the pathogenesis and mechanisms leading to food allergy still remain to be further understood. The discovery of TIMs and their bioactivities on immune regulation in food allergy has brought about new ideas. Each of TIM genes is predicted to encode a type of membrane protein with a similar structure, consisting

of a signal sequence followed by an immunoglobulin variable region (IgV)-like domain, a mucin-like domain, a transmembraner region and an intracellular tail (McIntire JJ, 2001). TIM proteins have played a significant role in autoimmune disease, hypersensitivity diseases and transplantation tolerance. Mouse CD4^{+} T cells expressed TIM1, TIM4 is its endogenous ligand, expressed in dendritic cells, and the pathway of TIM1-TIM4 play a crucial role in the differentiation of CD4^{+} T cells (Liu T, 2007), but its influence on Treg cells was little understood. Treg cells have played an important role in the maintenance of oral tolerance. Dysfunctional Treg cells could impair oral tolerance and promote FA (Ganeshan K, 2009). Therefore, it's necessary to elucidate the function of Treg cells and the impact of TIM1on Treg cells in FA.

Degauque et al. have confirmed that the activation of TIM1 can reduce the function and quantity of CD4^{+}CD25^{+}Treg cells *in vitro* experiments, resulting in destruction of transplantation tolerance (Degauque N, 2008). We speculated that the similar mechanism existed in FA. Co-stimulation with SEB and OVA could promote the expression of TIM4 on DC (Venarske D, 2003), we have also confirmed it in the present experiment, and TIM4 interaction with TIM1 might abolish the function of Treg cells in FA. After pretreatment with anti-TIM1 or anti-TIM4 in SEB + OVA allergic mice, we found the expressions of Foxp3 and TGF- 1 was significantly restored in a dose-dependent manner, these results suggesting that the activation of TIM1 could down-regulate the function of Treg cells in FA. Antigen-specific antibodies and Th2 cytokines are important diagnostic parameters in diagnosing and evaluating therapeutic efficiency in patients with allergic diseases (Yang PC, 2007), the results showed that pretreatment with antibodies against TIM1 or TIM4 diminish the levels of IgE and OVA-specific Th2 cytokine responses in FA mice. Pathologic diagnosis is a valid indicator of FA, mast cells and eosinophils are the major effector cells in hypersensitivity reactions, in the present studies showed that there were a large amount of eosinophils in the allergic mice jejunum segments, while these cells were reduced significantly in anti-TIM1 and anti-TIM4 groups. These results demonstrated that pretreatment with anti-TIM1 or anti-TIM4 is effective and the disruption of TIM4-TIM1 pathway might be an optimal target for the treatment of FA.

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In the previous study we found that SEB play an important role in the pathogenesis of allergy (Liu T, 2007), and SEB could enhance the TIM4 expression in DC and had a dose-dependent manner, and SEB-conditioned DCs could induce Th2 cell polarization, application with anti-TIM1 or anti-TIM4 could block the induced Th2 cell polarization (Khattri R, 2003). We previously found co-exposure to both SEB and OVA significantly promote the expression of TIM4 in DCs. In addition, we detected other bacterial products such as Staphylococcus peptidoglycan and pertussis toxin could also promote the expression of TIM4 in DCs (Liu T, 2006) . Another study showed that concurrent *in vivo* exposure to both cholera toxin and peanut extract can induce the development of FA (Feng BS, 2008), the previous studies and our findings together suggested that concurrent *in vivo* exposure to both pathogenic microbial toxin and food antigen are crucial in the development of intestinal allergy. However, the complex pathogenesis of FA keeps unknown and further research is needed.

Our experiments confirmed that the function of Treg cells was decreased in food allergic mice. Pretreatment with anti-TIM1 or anti-TIM4 can restore Treg cells' function and abolish the intestinal allergy, suggesting that the pathway of TIM4 - TIM1 may cause dysfunction of Treg cells. In summary, the activation of TIM1 plays a key role in CD4$^+$ T cell polarization in FA, not only can it promote Th2 cells polarization, but also reduce the function of Treg cells, while block the pathway of TIM1 and TIM4 can effectively restore the balance of oral tolerance and dampen allergic responses.

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Comparative Study between Inflorescences Characteristics, Pollen Viability, Germination and Dimensions of Tommy Atkins, Kent and Keitt Mango Cultivars

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Abstract: The present study was conducted at orchard located at the side of Alexandria desert road (Cairo - Alexandria, Km. 140), at Nubariya region during 2007 and 2008 seasons. The three experimented mango cultivars were Tommy Atkins, Kent and Keitt. The trees of the three studied cultivars are grafted on seeded rootstocks, attained nine years old and grown in sandy Soil .Trees of all mango cvs. were planted at 3×5 meter apart and subjected to the same horticultural practices. The objective of the present study is comparing inflorescences characteristics, pollen viability, germination and dimensions of Tommy Atkins, Kent and Keitt cultivars grown under Nubaria conditions. There were significant differences among studied cultivars in the length of panicles. Keitt cv. was found to have longest panicle compared to the other cvs. Meanwhile the lowest value was found in Tommy Atkins cv. The highest value of panicle width was found in Keitt cv. meanwhile, the lowest value was found in Tommy Atkins cv. Keitt cv. had the highest Number of branches per panicle followed by Tommy Atkins cv. meanwhile Kent cv. had the lowest values. The highest number of flowers per panicle was found in Tommy Atkins cv. in the two seasons, meanwhile, the lowest value was found in Kent cv. Tommy Atkins cv. had the largest percentage of perfect flower per panicle followed by Kent cv. meanwhile Keitt cv. had lowerst percentage in the two seasons. The highest percentage of male flowers per panicle was detected in Kent cv. in the first season and Tommy Atkins in the second season. The lowest sex ratio was shown in Tommy Atkins cv. inflorescence in both seasons. Followed in a decreasing order by Keitt and Kent inflorescence. Keitt cv. was found to have the highest percentage of malformed panicles per tree, followed by Kent cv. while, Tommy Atkins cv. had the lowest percentage. Keitt cv. showed the highest percentage of pollen grains viability followed by Tommy Atkins and Kent cvs. Kent cv. recorded the highest percentage of pollen germination meanwhile, Keitt and Tommy Atkins cvs. recorded the lowest percentage of pollen germination in first season and Keitt cv. in second season. Pollen diameter was lower in Tommy Atkins cv. compered to Keitt and Kent cvs.

1. Introduction

Mango (*Mangifera indica* L.) is one of the important fruits of the tropical and subtropical countries zone of the world. Mango grows on a wide range of climatic and soil conditions in the world where grows in Africa (Egypt, Nigeria, South Africa, *etc*.), Asia (India, Pakistan, Indonesia, *etc*.), Australia, North America and South America (Litz, 1997).

There are many factors that influence yield, maturity and quality of fruits the, same cultivar can attains different characteristics in different growing conditions. Even in the same region, different environmental conditions at different years can affect maturity and quality of the fruit (Devilliers, 1998).

Mango inflorescence is a pyramidal big flowering shoot called panicle, it's length varies from few inches to two - three feet. The inflorescence bears two types of nearly sessile flower, perfect (hermaphrodite) flowers and male (staminate) flowers. Number and percentage of distribution of both types of flowers per inflorescence vary according to many factors such as cultivar, year, and time of blooming, part of inflorescence, genetics and environment (Singh 1960).

The objective of the present study is comparing inflorescences characteristics, pollen viability, germination and dimensions of Tommy Atkins, Kent and Keitt cultivars grown under Nubaria conditions.

2. Materials and Methods

This study was carried out during two successive seasons (2007 and 2008) on three mango cultivars namely Tommy Atkins, Kent, and Keitt at a private orchard located in Nubariya at Alexandria at desert road (km. 140 Cairo – Alex), Beheira governorate, Egypt. The selected trees were about nine years old, budded on seedling rootstocks and planted at 3×5 meters apart, in sandy soil and irrigated by drip irrigation system.

Four branches, one year old on each tree, one towards each direction were chosen, and four

shoots from the current spring growth on each branch were labelled to carry out the following measurements:

Panicle characteristics
        Three panicles of each replicate for each cultivar were chosen at random at the full bloom time to carry out the following measurements:
1. Panicle length (cm.)
2. Panicle width (cm.)
3. Panicle thickness (cm.)
4. Number of branches per panicle

Flower Sexuality
1. Number of flowers per panicle
        Three panicles on each replicate for each cultivar were chosen at the full bloom time. Total number of flowers (male and perfect) per panicle was counted.

2. Percentage of perfect flowers per panicle
        The percentage of perfect flowers on the same panicles were calculated as the following equation.
$$\frac{\text{No. of perfect flowers}}{\text{Total No. of flowers}} \times 100$$

3. Percentage of male flowers per panicle
        The percentages of male flowers were calculated as the following equation:
$$\frac{\text{No. of male flowers}}{\text{Total No. of flower}} \times 100$$

4. Sex ratio (male/perfect flower)
        Sex ratio was calculated as the following equation:
$$\frac{\text{No. of male flowers}}{\text{No. of perfect flowers}} \times 100$$

Pollen viability, germination and dimensions
1. Pollen grain viability
        Pollen grain viability for each cv. was determined according to (Sharma and Bajpai, 1969).

2. Percentage of pollen grain germination
        The percentage of pollen grain germination for each cv. was calculated as follows according to Fernandez – Escobar *et al*. (1983).
$$\frac{\text{No. of germinated pollen grain}}{\text{Total No. of pollen grains}} \times 100$$

3. Pollen diameter (μm)
        Statistical analysis and comparison among means were made using L.S.D. test at 5% level according to Steel and Torrie (1980).

3. Results and Discussion

1. Panicle length
        Data in Table (1) show that panicle length varied significantly according to three cultivars grown Nubariya region conditions in 2007 and 2008 seasons. Keitt cv. had the maximum length of panicles (38.75 cm. in 2007 season and 37.22 cm. in 2008 season) while, Kent cv. had the least length of panicles (27.50 cm. In 2007 season and 27.74 cm. in 2008 season); whereas Tommy Atkins cv. had as intermediate value (28.54 cm. in 2007 season and 28.97 cm. in 2008 season).
        These results are in harmony with those obtained by Ahmed (2002), Ragab and Ahmed (2002), Hoda *et al*. (2003), Tawfik (2003) and Sweiden *et al*. (2007) who found that panicle length was varied according to different mango cvs.

2. Panicle width
        Data in Table (1) indicate that panicle width varied significantly according to three cultivars grown Nubariya region conditions in 2007 season and 2008 seasons.
        Keitt cv. had the maximum width of panicle (32.40 cm. in 2007 season and 30.88 cm. in 2008 season), while Tommy Atkins cv. had the minimum width of panicle (21.04 cm. in 2007 season and Kent cv. 21.05 cm. in 2008 season). The width of panicle in Kent cv. was in between.

Panicle thickness
        Data presented in Table (1) revealed that panicle thickness varied significantly according to three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Keitt cv. recorded the maximum values of panicle thickness (1.06 cm. in 2007 season and 1.05 cm. in 2008 season), while Kent cv. recorded the minimum values (0.95 in 2007 season and 0.87 cm. in 2008 season).

4. Number of side branches per panicle
        Data in Table (1) show that Number side branches per panicle varied significantly according to three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.
        Keitt cv. had the highest Number of branches per panicle (53.71 in 2007 season and 51.65 in 2008 season); meanwhile Kent cv. had the lowest values (48.69 in 2007 season and 48.58 in 2008 seasons).

Flower Sexuality
1. Number of flowers per panicle
        Data presented in Table (2) show the number of flowers per panicle of three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

There were significant differences among the studied cultivars in the averages number of flowers per panicle. The highest value of panicles flowers per panicle was detected in Tommy Atkins cv. in the two seasons (2205.00 in 2007 season and 2252.38 in 2008 season) followed by Keitt cv. (1628.48 in 2007 season and 1657.33 in 2008 season). Kent cv. registered the lowest value (1224.08 in 2007 season and 1279.08 in 2008 season).

The variation in total number of flowers per panicle among mango cvs. was announced by results of Shawky and Dahshan (1982), Ahmed (2002), Tawfik (2003) and Abd El-Hadi (2006) on various mango cvs. Many other workers reported that the total number of flowers per mango panicle varied according to many factors such as cultivar, year, time of blooming, genetics information and environment. (Singh 1960, Azzouz 1961, Chah 1964, El-Azzouni *et al*. 1965 and El-Kady 1973).

Table 1. Inflorescences characteristics of the three mango cultivars grown under Nubariya rgion conditions in 2007 and 2008 seasons.

Characteristics	Cultivars		
	Tommy Atkins	**Kent**	**Keitt**
2007 season			
Panicle length (cm.)	28.54 b	27.50 b	38.75 a
Panicle width (cm.)	21.04 b	21.40 b	32.40 a
Panicle thickness (cm.)	0.96 a	0.95 a	1.06 a
No. branches / panicle	49.66 b	48.69 b	53.71 a
2008 season			
Panicle length (cm.)	28.97 b	27.74 b	37.22 a
Panicle width (cm.)	2.94 b	21.05 b	30.88 a
Panicle thickness (cm.)	0.91 b	0.87 b	1.05 a
No. branches / panicle	50.55 a	48.85 b	51.65 a

Mean having the same letter (s) in each row are insignificantly different at 5% level.

Table 2. The number of flowers, Male flowers (%), perfect flowers (%) sex ratio and Malformed per tree (%) of three mango cultivar grown under Nubariya rgion conditions in 2007 and 2008 seasons

Characteristics	Cultivars		
	Tommy Atkins	Kent	**Keitt**
2007 season			
No . flower per panicle	2205.00 a	1224.08 c	1628.48 b
Male flowers (%)	56.76 c	64.85 a	62.83 b
Perfect flowers (%)	43.12 a	36.16 b	37.14 b
Sex ratio	1.32 c	1.86 a	1.69 b
Malformed panicle/ tree %	3.12 b	4.61 b	6.59 a
2008 season			
No . flower per panicle	2252.33 a	1279.08 c	1657.33 b
Male flowers (%)	57.81 b	62.55 a	63.42 a
Perfect flowers (%)	42.18 a	37.43 b	36.53 b
Sex ratio	1.36 b	1.66 a	1.73 a
Malformed panicle/ tree %	2.77 b	3.32 b	5.3 a

Mean having the same letter (s) in each row are insignificantly different at 5% level

2. Percentage of perfect flowers

Data presented in Table (2) show the percentage of perfect flowers of the three mango cultivars grown at Nubariya region conditions in 2007 and 2008 seasons. There were significant differences among the studied cultivars in the percentage of perfect flowers in the two seasons.

The highest percentage of perfect flowers was detected in Tommy Atkins cv. (43.12 in 2007 season and 42.18 % in 2008 season), followed by

Kent cv. (36.16 % in 2007 season and 37.43 % in 2008 season) and Keitt cv. (37.14 % in 2007 season and 36.53 % in 2008 season) with insignificant differences between them in the two seasons of study.

These results are in agreement with those found by Singh (1954), Azzouz (1961), Bastawrous (1977), Kalyanasundaram (1978), Shawky and Dahshan (1982), Salem (1993), El-Masry (2001), Ahmed (2002), Tawfik (2003) and Abd El-Hadi

(2006) where significant differences were among cultivars in perfect flower percentages per panicle.

3. Percentage of male Flowers

Data in Table (2) show the percentage of male flowers of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. There were significant differences among studied cultivars in the percentage of male flowers. The highest percentage of male flowers per panicle in 2007 season was detected in Kent cv. (64.85%). Followed by Keitt (62.83%) and Tommy Atkins (56.76%). In the second season, panicles of mango cvs. Keitt had the maximum percentage of male flower (63.42%), followed by Kent (62.55%) and Tommy Atkins (57.81%) cvs.

The minimum values were recorded in the panicle of mango cvs. Tommy Atkins in 2007 and 2008 seasons (56.76 and 57.81%).

These results are in agreement with those found by Shawky and Dahshan (1982), Salem (1993), Ahmed (2002) and Abd El-Hadi (2006) where significant differences were found among cultivars in percentage of male flowers.

4. Sex ratio (male/perfect flowers)

Data in Table (2) show the sex ratio in panicle of the studied three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons.

Sex ratio in the panicle was significantly varied among the three mango cvs. The significant lowest sex ratio (more perfect flowers) was shown in Tommy Akins cv. inflorescence in both seasons (1.32 and 1.36, respectevely). Followed in a decreasing order by Keitt and Kent inflorescence.

The differences in sex ratio among the various mango cvs was confirmed by the results of El-Azzouni et al. (1965), Hassan (1970), Dahshan (1971), Rao and Bhandary (1976), Shawky et al. (1977), Hussein et al. (1989), Sukhvibul et al. (2000), El-Masry (2001), Ahmed (2002), Tawfik (2003), Abd El-Hadi (2006) and Sweidan et al. (2007).

Percentage of malformed panicles

Data in Table (2) indicated that Percentage of malformed panicles per tree varied significantly according to three cultivars grown under Nubariya region conditions in 2007 and 2008.

Malformed panicles occurred in the three studied mango cultivars grown under Nubariya conditions. Keitt cv. recorded the maximum percentage of malformed panicles per tree (6.59% in 2007 season and 5.30% in 2008 season), while Tommy Atkins cv. recorded the significant minimum

percentage of malformed panicles per tree (3.12% in 2007 season and 2.77% in 2008 season).

Pollen viability, germination and dimensions
1. Pollen viability

Percentage of pollen viability determined by Acetocarmine dye-method showed significant differences among the three studied cultivars, i.e. Tommy Atkins, Kent and Keitt cvs. under Nubariya environments (Table 3) in the two studied seasons 2007 and 2008.

Data presented also show that the percentage of pollen grains viability indicated insignificantly differences among cultivars in 2007 season, while it significant in 2008 season.

Keitt cv. showed the highest percentage of pollen grains viability (71.67% in 2007 season and 70.00% in 2008 season) followed descendingly by Kent cv. (65.00% in 2007 season and 68.00% in 2008 season) then Tommy Atkins cv. (63.67% in 2007 season and 64.00% in 2008 season).

These results were in agreement with those found by Singh (1964), Dahshan (1971), El-Kady (1973), Deasi et al. (1986), El- Masry (2001), Tawfik (2003) and Abd El-Hadi (2006) that pollen grains viability percentages differed due to the cultivar.

2. Percentage of pollen germination

Data in Table (3) revealed that percentage of pollen germination varied insignificantly according to the three cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Kent cv. recorded the highest percentage of pollen germination (71.33% in 2007 season and 73.00% in 2008 season). Keitt and Tommy Atkins cvs. recorded the next percentage of pollen germination (70.33%) in 2007 season and Keitt cv. (69.67%) in 2008 season.

These results are in harmony with those obtained by Afify (1999), Afifi et al. (2000), Sukhvibul et al. (2000) who found that percentage of pollen germination was varied according to different mango cvs.

3. Pollen diameter

Data in Table (3) show pollen diameter of the three mango cultivars grown under Nubariya region conditions in 2007 and 2008 seasons. Pollen diameter varied insignificantly according to cultivars during the same seasons. Tommy Atkins and Keitt cvs. recorded higher pollen diameter (29 μm) in 2007 and 2008 seasons, than Kent cv. as it recorded insignificantly lower Pollen diameter (28 μm) in 2007 and 2008 seasons.

Table 3.The pollen grains viability (%), germination (%) and diameter (μm) of the three mango cultivar grown under Nubariya rgion conditions in 2007 and 2008 seasons .

Characteristics	Cultivars		
	Tommy Atkins	Kent	Keitt
2007 season			
Pollen viability (%)	63.67 a	65.00 a	71.67 a
Pollen germination (%)	70.33 a	71.33 a	70.33 a
Pollen diameter (μm)	29.00 a	28.0 a	29.00 a
2008 season			
Pollen viability (%)	64.00 b	68.00 a	70.00 a
Pollen germination (%)	71.33 a	73.00 a	69.67 a
Pollen diameter (μm)	29.00 a	28.00 a	29.00 a

Mean having the same letter (s) in each row are insignificantly different at 5% level.

The obtained results agree with those of Singh (1961) found that pollen diameter of 50 Indian cultivars ranged from 23.50 to 28.30 μm. Two cultivars had large pollen grains which were over (41 μm) in length and Tawfik (2003) who reported that pollen diameter of Keitt and Tommy Atkins cvs. was (30 μm) compared to Sediek and Ewais cvs. (40 μm).

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Moderating Influence of Gender on the Link of Spiritual and Emotional Intelligences with Mental Health among Adolescents

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Abstract: This study examined whether, Spiritual Intelligence (SI) and Emotional Intelligence (EI) can be considered as predictor for Mental Health (MH). Also, this study explored the moderating effects of gender on the link between SI and EI with MH among high school students. The participants in the study were 247 high school students, (124 male and 123 female, in the age range between 14-17 years old) at the Gorgan City, north of Iran. The research design was an ex post facto and tested the alternative hypotheses. Three valid and reliable instruments were used to assess SI, EI and MH. Descriptive statistics, multiple and moderated regression analysis were used to analyses the data. The result demonstrated that MH can be influence by SI and EI. In addition, the moderating effect of gender on the relationship of SI and EI with MH was not established.

1. Introduction

Adolescence is a critical developmental period characterized by biological, cognitive, and psychosocial changes in young people. This stage in a young person's life presents opportunities for positive psychosocial growth and healthy life choices and conversely, the potential for the development of psychological difficulties and engagement in precarious behaviors (Crockett & Petersen, 1993). Though mental, emotional, and behavioral challenges may emerge during adolescence (Kazdin, 1993) many, if not most, young people negotiate this life stage without serious difficulty (Petersen, 1988; Loh & Wragg, 2004). The literature, however, acknowledges an increase in negative social and psychological development trajectories (i.e. teen depression) for today's generation of adolescents (Small & Covalt, 2006), exemplifying the need for continued focus on the psychosocial well-being of this group. As current treatments for mental disorders in adolescence are costly (Ringel & Sturm, 2001), and underutilized (US Department of Health and Human Services [USDHHS], 1999), departing from the more prevalent pathology or deficit based model of examination (Loh & Wragg, 2004).

MH is essential to the overall health and well-being of adolescents (World Health Organization [WHO], 2004). The WHO conceptualized MH separate from mental ill-health and defined the concept as: a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her own community. (WHO, 2007, p. 1) Previous studies are clear on the influence of better MH versus mental ill-health for the individual and society. Individually, MH affects our expressive, cognitive, perceptive, relational, and coping abilities, undergirding our general health and wellbeing and capacity to integrate into and become productive members of society (Dwivedi & Harper, 2004). Better MH outcomes in adolescents are characterized by greater adaptation in family, school, and society environment, improved quality of life, and reduced symptoms of psychological disorders (Hoagwood et al., 1996; USDHHS, 1999). Positive MH is also linked to better physical health, increased pro-social behaviors, and participation in less adverse behaviors in adolescence (Resnick, 2000). On a societal level, MH is perceived as a positive source contributing to asset development individually, socially, and economically (WHO, 2004). Conversely, poor MH and well-being (i.e. depression, low self-esteem) during the adolescent years can lead to adolescent health risk behaviors, school failure, physical ill-health, suicide, involvement in juvenile and criminal justice systems, negative life choices, and mental disorders in adulthood (Lewinsohn et al., 1993; Canals, et al., 2002; Trzesniewski et al., 2006; Hjemdal et al., 2007).

There was some evidence that SI and EI development and spiritual and emotional experiences are helpful for health. At the same time, there is a significant relationship between awareness of spiritual and emotional experiences and health (Hay& Morisy,

1978; Hay, 1982; Ioannis & Ioannis, 2005). As whole, it seems spiritual and emotional functions including SI, EI and its components can be used as an instruments in relates with individual MH.

EI was originally recognized as having its roots in the concept of social intelligence (Thorndike, 1920; Salovey & Mayer, 1990; Goleman, 1995). Later, researches provided evidence that the two concepts actually represent interrelated components of the same construct (Salovey & Mayer, 1990;   Bar-On et al., 2003; Lane & McRae, 2004). Consequently, this broad construct was accurately referred to as 'emotional-social intelligence' (Bar-On, 2006). Based on historical reference, traits such as the capacity to navigate through and to adapt to one's own environment and the possession of social and emotional 'skills' are important not only to basic survival, but have implications in the areas of relationships, work, school, and emotional and MH (Goleman, 1995; Salovey & Mayer, 1990).

Today, there has been an increasing interest in how emotional reactions and experiences affect on mental health. For example, it has been claimed that negative emotional states are associated with unhealthy patterns of physiological functioning, whereas positive emotional states are associated with healthier patterns of response in both cardiovascular activity and immune system (BoothKewley & Friedman, 1987; Herbert & Choen, 1993).

The popularity of the concept for the past decades has led researchers to examine its potency in various areas of human functioning. Among the areas with the strongest connections to EI is developmental, educational, clinical and counselling, industrial and organizational psychology. Hence, characteristic or ability EI were related to life success (Bar-On, 2001; Goleman, 1995), life satisfaction and well-being (Martinez-Pons, 1997; Bar-On, 2002; Palmer et al, 2002), physical and mental health (Ioannis & Ioannis, 2005), interpersonal relationships (Fitness, 2001; Flury & Ickes, 2001), academic achievement (Van der Zee et al., 2002; Parker et al., 2004), and more.

Also, considering Gardner's theory, existential intelligence can be define as an ability to find and realize meaning in life (Halama & Strizenec 2004). Based on this definition, Halama & Strizenec (2004) suggested that the ability to find and realize meaning in life is an important element of SI. Since SI involves a set of abilities that draw on spiritual resources, it can be concluded that existential and SI is non-identical but mutually related and overlapping construct (Halama & Strizenec 2004). Drawing on Gardner's definition of

intelligence, Emmons (2000b) argued that spirituality can be viewed as a form of intelligence because it predicts functioning and adaptation and offers capabilities that enable people to solve problems and attain goals. Earlier, Emmons (1999) defined spirituality as the search for, and the experience of elements of sacred meaning, higher consciousness, and transcendence, SI entails the abilities that draw on such spiritual themes to predict functioning and adaptation and to produce valuable products or outcomes. Zohar & Marshall (2000) stress the utility of SI in solving problems of meaning, value, and those of an existential nature, concurring with Vaughan (2002) and Wolman (2001). Looking at spirituality through the lens of intelligence, Emmons (1999) writes, "SI is a framework for identifying and organizing skills and abilities needed for the adaptive use of spirituality" (p. 163). Hence, SI can be differentiated from spirituality in general, spiritual experience, (e.g. a unitary state), or spiritual belief, (e.g. a belief in God), (Amram, 2007). However, the theory and research of the spirituality and SI were well reviewed by many authors and researchers (Emmons, 1999; MacHovec, 2002; Mark, 2004; Schuller, 2005; Sisk & Torrance 2001; Wolman, 2001; Zohar & Marshall, 2002; Nasel, 2004; Amram, 2009).

However, many authors claimed and reported that there existed a significant relationship between EI and MH (Goleman, 1995; Salovey & Mayer, 1990, Bar-On, 2006; Ioannis & Ioannis, 2005), and SI and MH (Emmons 2000; Nobel, 2000). Also, spiritual functions including SI and its components can be used as a possible instrument to increase individual's MH (West, 2004). Therefore, the aim of this study was to investigate the relationship between SI and EI with MH. In addition, the current study aimed to explore the effects of moderator variable (gender) on the relationships between the independent (SI & EI) and dependent (MH) variables.

2. Material and Methods

2.1. Sample

Two hundred and forty seven Iranian high school students in Gorgan city, north of Iran (124 females & 123 males age range between 14 – 18 years) were recruited as participant in this study. They were recruited at random sampling, and their participation was voluntary and anonymously.

2.2. Procedure

Data were collected by means of structured questionnaires and by taking class as a unit. Based on verbal agreements of the training lecturers and participants, the questionnaires forms were distributed to the 247 high school students. Participants were asked to complete the questionnaires simultaneously at the start of a core lecture and return them to their lecturer on the spot. All completed questionnaires were passed on to the researchers. All participants were informed that participation was voluntary and anonymous.

2.3. Measures

All participants responded to an Iranian translation of the three instruments in this study include:

2.3.1. The Integrated Spiritual Intelligence Scale (ISIS, Amram & Dryer, 2008).

Amram & Dryer's Integrated Spiritual Intelligence Scale (ISIS) reviewed and chosen for measure of SI due to its comprehensive nature and strong psychometric properties (Amram & Dryer, 2008). ISIS is an 83-item long form, and a 45-item short form, self-report and observer-rated instrument containing 22 subscales assessing separate capabilities that are grouped into five main domain scales of spiritual intelligence. Responses are answered a six-point scale ranging from "never or almost never" to "always or almost always". For this study a 45-item short form, self-report and the simple Likert method (1–2–3–4–5–6) was chosen. The measure yields an overall SI score (range 0–270). The scale has a Cronbach alpha of 0.76.

2.3.2. Emotional Intelligence Inventory, Youth Version (EQ-i YV, Bar-On & Parker, 2000).

Utilized to measure emotional intelligence, the Bar-On Emotional Quotient Inventory: Youth Version (EQ-i: YV) was developed by Reuven Bar-On, Ph.D. and James D.A. Parker, Ph.D., and published by Multi-Health Systems, Inc., in 2000. The EQ-i: YV was developed to measure emotional intelligence in adolescent populations, based on the theoretical basis of the Bar-On model of social and emotional intelligence. This 60-item inventory is a self-report instrument designed to measure emotional intelligence in young people aged 7 to 18 years. The instrument measures a cross-section of abilities and competencies that constitute the core features of emotional intelligence. Responses are invited on a four-point scale ranging from "very seldom true of me" to "very often true of me". For this study the simple Likert method (1–2–3–4) was chosen. The measure yields an overall EI score (range 0–240). The scale has a Cronbach alpha of 0.74.

2.3.3. General Health Questionnaire (GHQ 28, Goldberg, 1972; Goldberg & Williams, 1998).

In 1972, Goldberg developed a simple questionnaire, the General Health Questionnaire (GHQ), which is the most widely used instrument for detecting non-psychotic psychiatric "Cases". The GHQ is a self-administered screening questionnaire used to diagnose psychiatric disorders both in primary care and in the community. The main benefits of GHQ are that it is easy to administer, brief, and objective. Several versions of GHQ are available: there is a 60-item version, and shorter versions (comprising 30, 28 and 12 items). The 28-item version (GHQ-28) developed by Goldberg and Hillier (1979) is constructed on a different basis when compared with the other versions. Responses are responded on a four-point scale ranging from "less than usual", to "much more than usual". Of the four possible ways of scoring this instrument (Goldberg & Williams, 1998), for this study the simple Likert method (0–1–2–3) was chosen. The measure yields an overall health score (range 0–84) and is composed of four subscales described as somatic symptoms, anxiety and insomnia, social dysfunction and depression. High scores indicate high levels of psychological strain. The measure was found to have an acceptable level of internal consistency reliability (alpha = 0.92). High score on this scale indicate poor general health.

3. Results

To carry out the main objective of the present study, the obtained data were subjected to a number of statistical analyses by using statistical package for social sciences (SPSS 17.0). Besides, descriptive statistics, multiple and moderated regression analysis were also used in this study.

3.1. Descriptive statistics;

Table 1 presents the mean and standard deviations of all the observed variables. Descriptive statistics is worked out to know the pattern of score distribution. A perusal of table 1 reveals that the mean scores on SI is 3.93 with the SD of .36, EI is 2.90 with the SD of .29, and on MH the mean scores was .91 with the SD of .43. (See table 1)

3.2. Multiple Regression Analysis (MRA);

MRA was computed to assess the strength of relationship between dependent and independent

variables. MRA provides an opportunity with little ambiguity to assess the importance of each of the predictors to the overall relationship. The results of regression analysis for the dependent variable (MH) are presented in table 2. It is clear from the results that the regression analysis accepted both the variables (SI & EI) as a significant predictor of MH. In overall both the predictors contributed Multiple R of .640. The F ratio computed for the significance of multiple {F (2,244) = 48.98, P < .05}.

3.3. Moderated Multiple Regression (MMR);

Moderated Multiple Regression (MMR) was employed in examining the effects of moderator variable (gender) on the relationships between the independent (SI & EI) and dependent (MH) variables.

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

Table 3 shows that for model 1, R = .640, R^2 = .409, adjusted R^2 = 404 and {F (2,244) = 48.98 P < .05}. This R^2 means that 40.9% of the variance in MH increase is explained by SI and EI. Model 1 does not include the product term and, thus, ignores a possible moderating effect of gender. To find out whether the potential moderating effect of gender on the SI and EI with MH relationship, we need to interpret the model 2 in table 3.

Model 2 incorporates the product term into the prediction equation. As shown in table 3, the addition of the product term resulted in an R^2 change of .009, F change (1,243) = 3.636, 'Sig. F' change = .058 with a P <.05. This result does not support presence of a moderating effect. In other words, the moderating effect of gender explains .9% of variance in MH above and beyond the variance explained by SI and EI. The result suggests that the gender is not important moderating the relationships of SI and EI with MH.

4. Discussion

The results in this study found EI was significantly and negatively correlated with MH scores. This finding is in line with (Bar-On, 2002; Palmer et al, 2002; Ioannis & Ioannis, 2005), Also between SI and

MH scores, the finding of this study provides evidence to the claims of the previous researchers (Hay & Morisy, 1990; Emmonce 2000; Nobel, 2000; Zohar & Marshall 2000; West, 2004). The results of the Multiple Regression Analyses (MRA) revealed the overall score of the SI and EI are statistically significant predictors of MH in the study. EI was found to be the strongest predictor followed by SI for MH scores. So, the findings of this study supported a positive effect of SI and EI on students' MH. The overall regression model was successful in explaining approximately 40.9% of the proportion variance explained in MH scores. This study also supports that gender is not significant moderate for the relationship between SI and EI with MH.

5. Conclusion

The main purpose of the present study is conducted to explain the role of SI and EI on MH (somatic symptom, anxiety, social dysfunction and depression) of high school students. The present investigation also was to test the moderating effects of gender on the relationship of SI and EI with MH. In this research, we found that student's MH can be predicted by SI and EI. In other words, The R-squared of .409 implies that the two predictor variables (SI & EI) explain about 40.9% of the variance in the MH (dependent variable). Also, this study does not support the presence of a moderating effect of gender on link of SI and EI with MH. In other words, the moderating effect of gender explains .09% of variance in MH above and beyond the variance explained by SI and EI. The result suggests that the gender is not important moderating factors on relationship between SI and EI with MH. These findings suggest that SI and EI are important and should be encouraged in school and students MH life. By combining the concept of SI and EI in the analyses of multiple regression and moderated regression, a new understanding emerged in this area of psychology. Therefore, this information will be valuable to community counsellors, teachers, school counsellors, and parents, all of whom are concerned with SI and EI development and MH of the high school students, especially those of Iranian population.

Table I: Descriptive Statistics of the Independent & Dependent Variables

Variables	N	Minimum	Maximum	Mean	Std. Deviation
Spiritual Intelligence (SI)	247	3.02	4.87	3.9340	.35637
Emotional Intelligence (EI)	247	2.15	3.67	2.9028	.29031
Total Mental Health (MH)	247	.04	2.04	.9110	.42770

Table II: Result of Multiple Regression Analysis

Variables	Summary of Regression	Un-std Coefficient B	Un-std Coefficient Std. Error	Std. Coefficient Beta	t	Sig. Value
(constant)		4.063	.248			
Spiritual intelligence		-.352	.076	-.293	- 4.638	.000
Emotional intelligence		-.609	.093	-.413	- 6.533	.000
Multiple R	.640					
R Square	.409					
Adjusted R Square	.404					
F-Statistics	84.504					

Note. Predictor: SI & EI. Dependent Variable: Total Mental Health, * $p < .05$.

Table III: Result of MMR Analysis for the Moderated Effect of Gender on the Relationship between SI & EI with MH

Model	R	R Square	Adjusted R Square	Std. Error of the estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.640a	.409	.404	.33009	.409	84.504	2	244	.000
2	.646b	.418	.411	.32832	.009	3.636	1	243	.058

Note. Predictors step 1: SI & EI; step 2: SI & EI, Students Gender, * $p < .05$.

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Double CSF to Areolar Connective Tissue Shunting. An Efficient and Minor Procedure in Idiopathic Intracranial Hypertension. A Prospective Comparative Study.

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Abstract: Study Design: A prospective study to evaluate the outcomes of 2 complimentary minor surgical procedures for shunting CSF to loose areolar connective tissues in both the eye and the scalp as an ensured, effective and definitive treatment to idiopathic intracranial hypertension.

Objective: To explore a more effective and less invasive technique without extensive tissue injury to idiopathic intracranial hypertension (IIH).

Summary of Background Data: The optic nerve sheath decompression (ONSD) is an established minimally invasive operation for IIH. The latter operation usually fails to completely eliminate headache as its effect is largely potent as regard the opthalmological manifestations of IIH. Thus a shunt operation is frequently needed thereafter. In the other hand; the traditional neurosurgical operation subtemporal decompression proved itself for decades as an effective surgery for resistant cases of IIH, however, it was plagued by the temporal-subtemporal extensive tissue injury. We found the newly revived minimally, invasive ventriculo-subgaleal shunt (VSS) that proved effective in our Kasr El Aini hospital and actually divert the CSF to the scalp areolar connective tissues, a minimally invasive alternative to the old style operation subtempotal decompression and a very beneficial and effective operation that compliment the ONSD.

Methods: This prospective study included 18 consecutive patients, 8 patients underwent ONSD and the rest ten patients needed in addition VSS to effectively manage all the manifestations of IIH.

Results: The evaluation of the manifestations of IIH; the headache, papillaedema-visual field defects and the abducent nerve paresis were performed before surgery and after surgery. Both maneuvers had an excellent-to-fair operative result, with a low incidence of complications. Thus according to our data the double CSF shunting is a minimally invasion technique with a high efficiency and ensure a very satisfactory result.

Conclusion: Our novel operation of double CSF to areolar connective tissue shunting is a minimally invasive and exceptionally effective operative management to IIH and can be a standard procedure for the surgical treatment of this insidious and dangerous vision threatening disease.

1. Introduction:

Idiopathic intracranial hypertension (IIH) remains an enigmatic diagnosis of exclusion. It is a vision-threatening disease of unknown aetiology that affects predominantly obese women of childbearing age. Prompt diagnosis, thorough evaluation and efficient treatment are crucial for improving the presenting symptoms. IIH is a syndrome of increased intracranial pressure (ICP) without hydrocephalus or mass lesion and with normal chemical and cellular CSF composition. It is usually presented by headache (75%), papilledema (95%), and sixth nerve palsy (25%) with otherwise normal neurological examination.

To start with; Medical therapies should be tried which include:

Weight control and non-ketotic diet.

Discontinuation of contributing medications.

Serial lumbar punctures.

Carbonic anhydrase inhibitors and diuretics.

Steroids, and certain analgesics.

Some authorities believed in digoxin.

Neurosurgical procedures previously used to alleviate ICP include subtemporal decompression, lumboperitoneal shunts, parietal flaps, and recently the venous sinus stenting trials. Lumboperitoneal shunt is considered the traditional method for providing prompt reduction of ICP. However, other surgical option is optic nerve sheath decompression ONSD,

which has an established role for reversal of visual loss. It is a rather simple procedure without great morbidity and it has a long term stability of the result without such complications as shunt obstruction, kinking or recurrence of IIH. It actually addresses the nearest subarachnoid tissue that lacks any bony coverage. It simply involves cutting slits in the dura surrounding the optic nerve; which allows CSF flow into the orbital areolar fat to be absorbed into venous circulation. Previous researchers showed that neither headache is reliably relieved by this approach nor aggressive papilledema. We herein in this paper assessed and tested its combination with another simple surgical and minor manoeuvre that recently showed efficiency in Kasr El Aini neurosurgical department Cairo University and promise after its revival in the past two decades, the ventriculo-subgaleal shunt VSS; as to manage successfully all the complaints of the patients including headache and aggressive papilledema. This latter surgery adds only about 15 minutes to the surgical time without any drawbacks. THE HYPOTHESIS WAS CLEAR patients failing medical therapy have ONSD performed if visual deterioration is the only morbidity. Adding VSS procedure is considered if headache is a major symptom, failure of ONSD and/or the papilledema is aggressive.

Aim of work

Assessment of the outcome of patients with idiopathic intracranial hypertension comparing patients adopted and persisted on medical therapy to those who underwent either optic nerve sheath decompression alone or combined with VSS.

2. Methodology
Study design:

A prospective (longitudinal) comparative study on therapeutic modalities for IIH.

It included 29 patients; they were followed up for at least 18 months.

Criteria for diagnosis:

Presence of papilledema and headaches, documented raised CSF pressure (>250 mm/CSF), with normal CSF analysis.

Exclusionary criteria:
(1) Ventriculomegaly or an intracranial mass on imaging.
(2) Evidence of venous sinus thrombosis.
(3) Abnormal CSF constituents.
(4) Patients with irregular follow-up.

Patients were considered irregular when two follow-up visits were missed.

Initial Evaluation:

Initially 43 patients were included, (14 were *irregular*) and were not subjected to any surgical intervention.
All the other 29 patients were submitted to:
Neurological Assessment:

History taking [headache characters and intensity, visual symptoms including transient visual obscurations (TVOs) and double vision].

Funduscopic assessment, visual acuity (VA), pupillary reactions, ocular motility, other cranial nerves, and signs of lateralization.
BMI was calculated.

Ophthalmological Assessment:
 58 eyes were evaluated for:
Degree of papilledema with fundus photographs.
Intraocular pressure measurement.
Visual Acuity.
Pupillary reaction.
Clinical assessment of field of vision.

According to severity of papilledema:
Early papilledema.
Acute fully developed papilledema.
Chronic papilledema.
Atrophic papilledema: divided into:
    Atrophic on top of chronic papilledema.
    Atrophic on top of acute papilledema.
Post-papilledemic optic atrophy.

Visual Field Testing.:
The test strategy: full threshold test.
It evaluates the mean deviation of retinal sensitivity (MD) in each eye, and the size of blind spot.
The mean deviation (MD) was used to evaluate the deterioration and improvement, taking (-/+ 2) of MD as a significant worsening or improvement (Spoor and McHenry).

Radiological Assessment:
All patients had had brain CT, MRI and MRV.

Laboratory Tests:

Include CBC, fasting and post-prandial blood sugar, hormonal profile when clinically indicated, coagulation profile and parallel blood sugar level just before spinal tap. For patients receiving diuretics, serum K+ level was periodically checked.
Spinal tap.
Medical Management.
Modest dieting and a low-salt regimen.
Carbonic anhydrase inhibitor [acetazolamide] (4 weeks).
Repeating of spinal tap for 9 patients.

If no clinical improvement steroids were added, in addition to furosemide as a 2nd line agent (for 8 weeks).

Thereafter, patients were reassessed neurologically and ophthalmologically, and by using visual field testing.

Surgical management:

1-ONSD

▪Operative procedure: a single incision over the insertion of the medial rectus, extending from the upper to the lower fornices without release incisions was done.

▪Disinsertion of the medial rectus muscle with a double armed 6-0 Vicryl leaving a muscle stump of about 1mm for the intrascleral suture.

▪Traction on the globe to obtain adequate exposure; anterior traction then lateral traction on the globe was done by vertical recti silk sutures looped beneath the insertion of the muscle and tied. This was combined with intrascleral traction suture (5-0 non-absorbable), (which is a suture passed through the scleral stump of the medial rectus muscle taking partial thickness of the sclera). This suture was mainly used to obtain lateral traction of the globe. The traction on the globe was limited to 30 to 60 seconds and the interval between traction periods was 15 to 30 seconds. Because of the frequent cutting of the suture through the muscles stump, one suture is passed through the stump until the middle and another suture is passed from the other end of the muscle stump to meet the other suture in the middle. Now we have 4 points of traction instead of only two points. A retractor was used to retract the medial rectus and the medial orbital contents medially. The intrascleral course of the long posterior ciliary artery was used as an easy and constant guide for orientation in finding the nerve sheath. The operating microscope was focused along this line to reach the sheath.

▪Multiple incision technique was used in 19 surgeries and 18 eyes by the fine microvitreo-retinal (MVR) blade, starting from 2mm behind the globe and extending for about 5mm, not exceeding 8mm behind the globe making 3 incisions. An initial CSF gush was seen in most of the cases, also secondary gushes or leaks were noticed with the second and sometimes the third incisions. Window removal technique was used in 4 surgeries. The window removed was always rectangular, and in the least vascular area of the nerve sheath. It measured 2 x 4 mm.

▪The medial rectus was reattached with closure of the conjunctiva. Subconjunctival getamicin and decadron were given in the lower fornix in all cases. The operation is minor, easy and straightforward with minimal manipulations. The following figures show crucial steps in ONSD; in a respective manner:

▪The fornix based conjunctival incision.

▪Lateral traction on the globe using the single continuous (base ball) suture.

▪Clear view of the optic nerve sheath with the lateral traction on the globe and with the retractor in place.
(notice the intrascleral course of the long posterior ciliary artery)
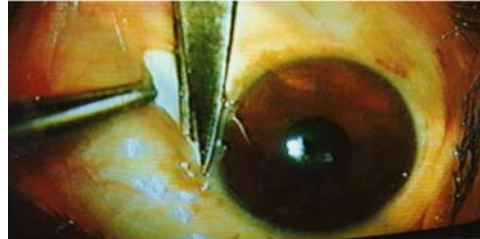
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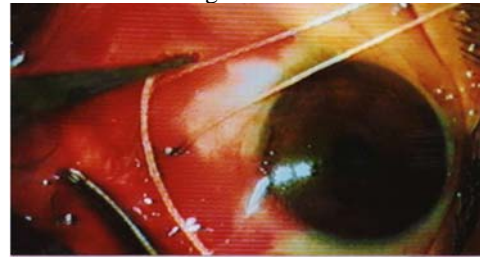
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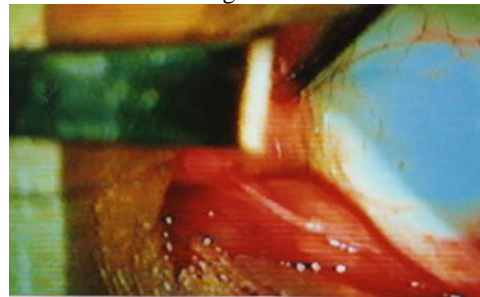
Figure 3.

▪Figure 1. The fornix based conjunctival incision.

▪Figure 2. Lateral traction on the globe using the single continuous (base ball) suture.

▪Figure 3.Clear view of the optic nerve sheath with the lateral traction on the globe and with the retractor in place.
(notice the intrascleral course of the long posterior ciliary artery)

2-VSS

It is actually a very simple procedure that can be done even on urgent basis. All that is needed is a Nelaton catheter (size 10) (that was prepared with multiple small elliptical pores done using the scissore), and through a burr hole this tube is used to tap the ventricle. The other end of the catheter is placed in the subgaleal space after its dissection in the areolar connective tissue layer i.e. separation of the galeal layer from the periosteal layer.

The patient is put in the supine position with the head 30 degree flexed and 45 degree tilted to the left side. Linear skin incision of about 3.5 cm is done in the right frontal region, 3 cm from the midline and one cm in front to the coronal suture and is held in its position by a self retaining retractor. Dissection of the sub-galeal space is performed for a circle of a 7 cm diameter. Perfect hemostasis is achieved usually easily. The frontal burr hole is done. Dura opened and cauterized. The lateral ventricle is tapped via a brain canula. A Nelaton catheter size N 10 (that was prepared with multiple small elliptical pores done using the scissors) is introduced using a suitable stillet after removal of the brain canula and in the same tract. Sudden gush of the CSF is avoided to prevent the occurrence of subdural hematoma. The Nelaton catheter is then fixed to the surrounding galea. The distal end is inserted in a smooth curve in the subgaleal space to prevent kinking. The direction of the shunt in the sugaleal space should be posteroinferior. Closure in two layers in a water tight manner is mandatory.

Follow up Assessment:
Patients broadly fell into three categories according to *(Higgins et al)*:
Asymptomatic: complete resolution of headache and visual impairment.
Improved with some residual not requiring further intervention.
No change or worsening of headache intensity or visual impairment.
Two weeks Post-operatively Assessment included:
Headache evaluation (change in frequency and severity).
Visual symptoms and visual field test.
Fundus photography.

Long-term Follow-up.
Every 3 months for at least 18 months: patients were evaluated for visual field, detailed headache assessment, and full neurological and ophthalmological examination.

3. Results:
Mean Age: 29.07 + 4.89 yrs (Range: 5 – 43 yrs).
Initial presentations: headache and visual symptoms.
Duration of symptoms: Mean: 5.5 + 2.63 months (Range: 2 – 13 months).
Asymmetric visual affection was detected in 40%.
Automated Field Evaluation:
Bilateral enlargement of blind spot in all patients.
Baseline mean deviation (MD) of retinal sensitivity were:

> OD: 9.31 + 3.77 (range 4.9 – 21.7).
> OS: 8.59 + 3.19 (range 4.8 – 23.1).

The Mean CSF Pressure: 362.5 (range 290 – 470 mm/CSF).

Clinical Presentation:
11 TVO.
8 Blurring and sparkling.
5 Diplopia [6th nerve palsy].
4 VA deterioration, and 3 relative afferent pupillary defect (RAPD).
1 Trigeminal neuralgic – like pains.
Maximum medical therapy was advocated for all patients, and they were re-assessed after 12 weeks:
Responder Group (11/29).
Non-Responder Group (18/29).

Characteristics of Responders:
Shorter duration of illness.
Milder degree of papilledema, lesser affection of field of vision.
Lower basic CSF pressure.
Drugs tolerability.
Less frequently needed repeated spinal tap.
Lower BMI.

Post-operative Assessment [2 weeks].
Measurable improvement was detected regarding visual symptoms and the MD of retinal sensitivity in both surgical groups.
After ONSD, Eight patients underwent ONSD, one patient (1/8) experienced marked improvement of headache intensity (reduction of acetazolamide dose), other 7 patients received the same doses of diuretics.
After ONSD+VSS , Ten patients underwent ONSD+ VSS (none was on diuretics post operative) all patients had major headache improvement.
All patients with preoperative transient visual obscurations TVOs experienced complete recovery of this condition after surgery (ONSD or ONSD+VSS).
Three eyes had RAPD; those patients were subjected to surgery. One of them improved after ONSD surgery, and the remaining two improved after ONSD + VSS with normal pupillary reactions.
In ONSD, the unoperated eye showed improvement in response to surgery in the fellow eye.
All eyes with atrophic papilledema that improved after surgery had residual field defects. No complications were encountered in short-term follow up period.
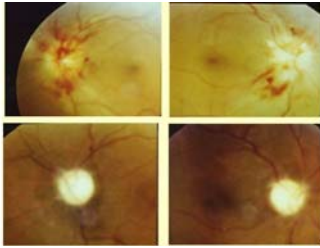
Figure4. The pre- (top) and post- operative (bottom) fundus pictures of patient N 9 where atrophic papilledema on top of acute papilledema in the preoperative fundus with rapid diminution of vision to HM, was replaced in the post operative (after ONSD+VSS) fundus with increased disc pallor despite functional improvement to V.A. of 6/36 and 6/60
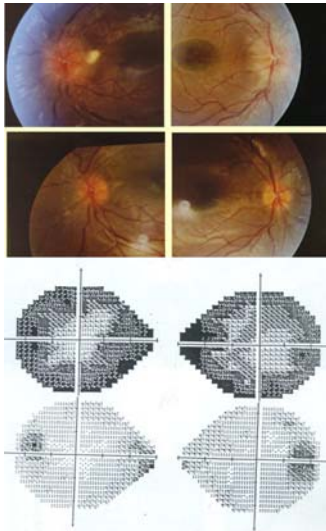
Figure 5. The preoperative (top) and the postoperative (bottom) fundus picture and visual fields of the patient n12 with preoperative acute papilledema that was resoluted in both eyes after ONSD+VSS, also the visual field showed marked improvement.

During 18 months Follow-up:

One patient who was on medical therapy has recurrence of symptoms associated with increase in CSF pressure [non-compliance]; and she was subjected to ONSD+VSS.

One patient who underwent shunting procedure had the shunt kinked and not working and it was revised once.

Doses of acetazolamide were reduced in 4 patients out of the responders to 750 mg/d after one year of stabilization.

All patients with ONSD+VSS markedly improved without the need of any therapy.

Two patients of ONSD were on reduced doses of medical treatment.

4. Discussion:

It was proven in the literature that visual loss can happen with a functioning VP shunting procedure (27). Optic nerve sheath decompression (ONSD) alone has very good potential for the reversal of visual loss according to many reports (1,5,35). The procedure is simple, without great morbidity, with a very low rate of complication, and a long term stability of the results (11, 12). However, headache is frequently not improved denoting that the bouts of increased intracranial tension in some patients are still present. Combining the former operation with (VSS) completely resolved all the complaints of the patients and the fundus examination as well as the field of vision follow-up showed marvelous results.

The female dominance was noted in this study as well as in other similar studies. The two children in this study were males, and this may be due the fact that IIH has nearly equal sex incidence in children (9, 38).

Headache was present in all cases in this study; however, visual loss may occur in IIH patients even if medical therapy succeeded in relieving headache (38). ONSD alone is not a surgery for headache relief. Headache relief should be viewed as a bonus on top of visual recovery (25). Thus we combined VSS to it and gave marvellous results.

A very important rule is that in absence of papilledema there is no potential for visual loss. On the other hand, in cases of atrophic papilledema, effective surgery is indicated without waiting for the results of medical therapy because this may jeopardize the remaining vision of the patient. It is very important to know that a white flat disc is not hopeless because inside this atrophic disc there may be viable fibers suffering only axoplasmic flow obstruction and their number may not be sufficient to cause ophthalmoscopically visible disc swelling (35,37).

In acute fully developed papilledema, the visual loss was observed to follow all or none rule. Visual loss is either severe acute and not responsive to medical therapy or there is no or mild visual loss. If visual deterioration is detected, an emergency ONSD +VSS is a must. If visual functions are stable, with or without medical therapy, follow up of cases is indicated. In our cases, as well as in the literature, acute fully developed papilledema and chronic papilledema have the best prognosis of visual recovery after surgery (1, 35).

All the eyes in this work had improved or stabilized V.A. except one eye with transient outer layer retinal ischemia as a complication of ONSD surgery. However, there are other 2 cases with V.A. deterioration after initial improvement. In one case, the V.A. regressed to the preoperative level. However, after a secondary ONSD+VSS, the V.A. improved, but

less than the level reached after the first surgery. The other eye was improved after few days because of the resolution of macular edema after VSS. The results of this novel work are very satisfactory and in harmony with most of the literature about the successful and more sophisticated manoeuvres.

Preoperative V.A. does not always predict the final postoperative V.A. If we draw a line at the level of 6\60 vision, acute cases even with optic atrophy have the greatest chance of crossing this line of legal blindness. Atrophic chronic cases without disc elevation usually improve, but usually few cases cross this line.

During this work, two problems were especially related to IIH patients going for field examination. The first problem was headache. Field examination should not be done while the patient has a headache because this usually will lead to an unreliable result and time wasting due to repetition.

The second problem is TVOs which can obscure vision for seconds and lead to false negative errors and fixation losses. Field-testing should be done during a TVO-free period. Medical therapy may be intensified before field examination in a trial to decrease both headache and TVOs.

In the results of this work, all eyes improved after ONSD alone or combined with VSS except 2 eyes, which had deterioration of visual field. VSS also resolved the problem of the headache and improvement of all aspects were detected. Thus a higher incidence of eyes had no residual field defects and only 2 eyes had a mild postoperative field defects. This is because of the relatively delayed presentation in our cases.

The effect of ONSD on visual field is far beyond any doubt as proved in this work and many other similar studies. The role of the visual field as the major indicator for surgery is also the cornerstone of any management decision in every patient with IIH and visual deterioration (17, 20, 21).We found in addition that when ONSD was combined with VSS, prompt and excellent solution to all the manifestations were satisfactorily gained and assured.

Important points and modification of the ONSD technique:

The medial transconjuctival approach, as most of the recent literature states, is much easier, time saving, and without significant difficulty in the nerve sheath exposure, which is the most important goal of the orbitotomy (27, 32). We also found that changing the direction of traction from anterolateral to lateral will be more suitable. As the anterior traction on the globe will consume the redundancy of the optic nerve and the globe will be tethered during the trial to rotate it laterally. This will make lateral traction more difficult and will expose all the vascular structures attached to the nerve sheath to maximum stretch. Conversely, if we used all the redundancy for lateral traction only, the result will be easy rotation of the globe laterally and, also, of the optic nerve that becomes suitably medial to it so as to be readily visible at the surgical site. ONSD is actually a filtration surgery and opening the sheath with the least manipulation, traction and instrument introduction is crucial to surgical success. It is believed that cotton tipped applicators should not be used for exposure because this may provoke further reaction and fibrosis with more risk of surgical failure. If it is a must, an alternative like the surgical microsponges is a much better choice. Using the mentioned modifications, we needed only 2 tractions each of 60 seconds interval to open the sheath by multiple slits.

Important points and modification of the VSS:

As regard the neurosurgical VSS, we think that it is a refined and renewed operative procedure of the same category and idea as the old style operation subtemporal decompression that for many decades was the only available surgical procedure for IIH and proved itself in the old classical literature. Actually; the absorptive surfaces in the subtemporal decompression operation is the areolar connective tissue in the surrounding area. The areoloar connective tissue of the subgaleal layer of the scalp is magnificently loose, capacious and extensible. The simple separation of the galea layer of the scalp from the periosteum is fast, easy and almost bloodless. In the same time, the heroic blood supply to the scalp actually denotes and reflects our results of efficient CSF absorption in this scalp layer. We preferred the right frontal burr hole and found it suitable; also we found separation of the galea from the periosteum for only 7-8 cm is enough. However the operation is extensible and can satisfy and cope with whatever high is the intracranial pressure by increasing the size of the absorptive surfaces if needed. It should be noted that the key of success of VSS is to keep open the CSF filled gap between the periosteum and the galea, thus a suitable surface area of the areolar connective tissue layer should be freshened and prepared; if larger than enough; reapposition of the galea upon the periosteum will abolish the absorptive ability of the operation. Fixation of the 12-14 cm shunt tube should be properly done to the galea to prevent the shunt tube segment from being driven inside the ventricles which is a complication that occurred in one patient. Water-tight closure in two layers should be considered mandatory. When combined with ONSD the surgeon felt satisfied, and the results were excellent.

Ophthalmologically wise; we consider papilledema is the mirror image of glaucoma, and the

value of ONSD equals the value of trabeculectomy in the glaucoma patient. This subject deserves much more attention because visual loss from papilledema due IIH can be disastrous. Comparing ONSD with the VSS technique of CSF diversion; the former is limited to the orbital area and the intracranial pressure secondarily improve, however headache was frequently unchanged denoting less than optimal management of ICT; yet we found combining both manoeuvres had an assured and long-term satisfactory efficacy.

5. Conclusion:

IIH is a chronic disease that requires long-term maintenance on medical therapy to prevent recurrence. Medical therapy is usually considered satisfactory as long as it is maintained effective and tolerated. ONSD is the surgery of choice for visual deterioration or gradual visual loss in IIH patients. It is not an alternative to a neurosurgical shunting procedure. We found VSS to be effective, easily done, and was indicated mainly for headache and aggressive papilledema. Visual field testing is the main tool for surgical decision and follow-up of cases. Lower surgical manipulations are much correlated to success of surgery. In order to get the best visual results, surgery should not be delayed.

ONSD and VSS were found effective surgical means to reduce the pressure in patients who were intolerant or not responsive to medical therapy or when clinical deterioration was detected. The choice between ONSD or ONSD + VSS is clear and resolved, patients failing medical therapy have ONSD performed if visual deterioration is the only morbidity. Adding VSS procedure is considered if headache is a major symptom, failure of ONSD and/or the papilledema is aggressive; in few cases optic nerve fenestration was required then followed by VSS in another operative setting to satisfy the patient complaint and completely save the optic nerve.

The three modalities (medical therapy, ONSD and VSS) are complementary. A neurosurgeon and an ophthalmologist should work together to provide the optimal and rational decisions in the care of patients with IIH.

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Calculated equation and specific absorption rate roots (*SAR*) for the nutrient

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Abstract: The experiment conducted in Islamic Azad University research farm of Ahwaz city (IRAN). Average of rain was 256mm based on split plot design and along with complete random block pillar, two factors and four repetitions. After measuring the parameters of root morphology and nutrient elements specific absorption rate (*SAR*), which is an indicator of the efficiency of root uptake based on the calculated parameters were measured, using the following formula based on root weight (***R_W***) and mg unit g/g root dry matter per day was calculated:

$$\bar{A} = (M_2 - M_1) / (t_2 - t_1) / (LnR_2 - LnR_1) / R_2 - R_1$$

$\bar{A}$: Average speed of absorption of mineral nutrients ($mgg^{-1}day^{-1}$),
R: root dry weight (g)
M: Dry weight root element value (mg)
t: sampling time (day)
Specific absorption rate (*SAR*) four elements: nitrogen, phosphorus, potassium and sodium were measured. Numeric value to attract the highest speed with the amount of potassium element 0.19 mg / g. day in the severe stress and was calculated with the lowest amount of sodium was 0.001 mg/g day. Although in mild stress, nitrogen value increased to 0.21 mg / g. day, which are because of osmotic pressure phenomenon, but the element phosphorus absorption to the wilting point plant (PWP) element did not decrease. *SAR* of nutrient nitrogen, phosphorus, in comparison to control with full irrigation decreased the N *SAR* decreased even with the incidence of mild water stress found, although in severe stress treatment, water, SAR value of N increased, but severe water stress treatment, *SAR* showed a significant reduction of nitrogen. SAR of P only to stress that in the permanent wilting point (PWP) reduced, did not show decreasing to PWP. *SAR* of K^+ applying different levels of water stress than the control group showed an increasing trend, especially between these two elements, *SAR* K^+ increases much higher than the sodium was almost *SAR* sodium potassium approximately 55 times the most stress treatments water respectively. Conditions without water stress, the highest *SAR*-related elements nitrogen phosphorus and potassium elements was then that water stress, especially severe water stress, the highest *SAR* related to the element potassium phosphate, nitrogen and sodium were. Nitrogen absorption rate equations shows that reducing the amount of water absorption of this element also severely reduce ($Y_N=0.016X+0.046$). But the element phosphorus, the absorption process does not stop until the point wilt ($Y_P=0.02X+0.002$), Potassium absorption process according to the equation is increasing ($Y_K=0.016X+0.030$), Element in the situation is different sodium absorption rate of this element is extremely low ($Y_{Na}=0.006X+0.003$).
[Tayeb Saki Nejad. Calculated equation and specific absorption rate roots (*SAR*) for the nutrient. Life Science Journal. 2011;8(1):121-126] (ISSN:1097–8135). http://www.lifesciencesite.com.

Key words: specific absorption rate roots (SAR), nutritional elements and corn

1. Introduction

Absorption capacity of a root system depends on the permeability or conduction is. And is expressed as follows:

$$LR = LP + AR$$

In this connection LR absorption capacity or root system guidance, LP Hydraulic conductivity and permeability or AR root surface (cm^2).

Different physical and chemical factors in rhizosphere, plant-like properties, responsible for absorption of minerals by the roots are. According to Zhong and Klasn (1998) the important factors are:
1 - **Chemical composition of food**, their concentration in soil solution and small trip, pH and ventilation.

2 - **Position proportional to the distance element in soil plant roots.**
3 - **Moving element with a mass flow and diffusion**, which implies the ability to create plants gradient potential of concentration and water is traveling.
4 - **Growth, distribution and physiological roots** of form and strength to move on and earn uptake.
5 - **Secretion of organic compounds** with low molecular mass of roots, such as amino acid, gelatinous material with high molecular mass (Mucilage) and peel and repair cells and tissues may cause mobility minerals either directly or indirectly that their energy for the microbial activity in the rhizosphere provides makes (29).

Although the intensity of absorption of water and nutrients or ions is different from single solution, as soluble materials into the root are the same directions with water flow follow. When plant roots completely in the solution with uniform concentration is to be established in most cases concentration of pipes Vascular much more than the concentration in the external solution. Ions during physical movement (mass flow or diffusion) to the exterior space root, during more than anions cautions and thus the frequency of negative charges is at the level of cell walls.

Total cautions and anions into roots are often not equal with the name of ion exit from the root to be out of balance. Amount of food that solutions with fixed concentration into the roots are, depending on the status of plants and salt has transpiration rate (metabolic demand).

Figure 1. Corn root (32)
http://www.emc.maricopa.edu/faculty/farabee/biobK/rootts.gif
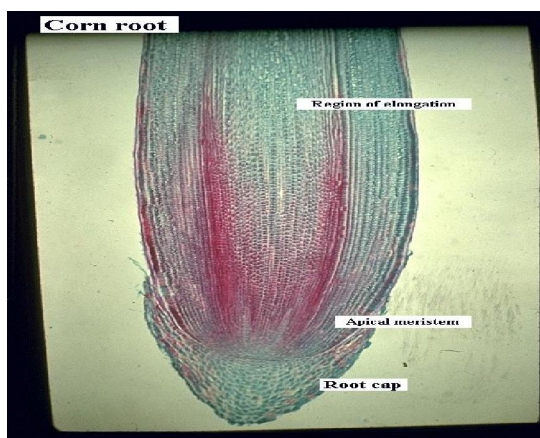
Relationship between fluorescence and absorption of root ion (e.g. iron absorption by soybean) by Dyer and Brown (1998) is shown. Ions into the roots as active or inactive come to move on. Active movement and energy metabolism to act, usually by breathing comes depends on moving ions and including a region with low to high electrochemical potential on both sides of the membrane and concentration gradient is the opposite. Physical process by moving ions may cause active transport ions move opposite to be the concentration gradient. (Transmission disabled). These two types of transmission reflection absorption materials by roots intact when the concentration of foreign substances low (mechanism I: characteristics of a process of active displays) or be much (Mechanism II: characteristics of a process to disable shows.) Many minerals as ions are released into the roots, but there are also exceptions, such as urea or elements. Describe the flow of elements in the root level, the term; root

absorbed (Flux); is discussed as the ratio of elements in entry-level unit (X) can be expressed.

Zhong and Klasn (1998) flow to the roots of phosphorus and potassium as a function of concentration as; saturation; and using equation Adjusted infected (Nielsen) of these were described (29):

$$I_n = \frac{I_{max}(C - C_{min})}{(K_m + (C - C_{min}))}$$

This formula in net flow of food, I_{MAX} maximum current intensity, K_m is a constant Michelins mentum (1998) affinity nutrient uptake of a show. Minimum concentration of ionic plant that is able to absorb it. Tibet and colleagues (1895), the rate of magnesium uptake by plants using a change in Mg concentration in solution and the net weight change roots outreach calculated that income to the following equation:

$$I_m = \frac{(M_2 - M_1 / WR_2 - WR_1) \times 1n(WR_2 / WR_1)}{(t_2 - t_1)}$$

In the absorption intensity of fresh root weight, M the total amount of material Skives long (leaves and roots), WR root fresh weight and t is the time of day.

Models absorption properties of materials by plant roots like other root expansion rate, average root radius, the average hair length and density fatal are included. Some other authors as well as environmental factors such as temperature, pH, and soil moisture also consider effective models washmen (1998), for example, models of different plants to accumulation of sodium ions from the show which can plant requires ion Na + pointed out that a completely can replace potassium and increased growth will be like: sugar beet or plants that absorb sodium done but could not quite substitution ions K +, the growth amount of minor by ion Na + is observed, such as: cabbage, cotton, chickpea, cotton, wheat and spinach, but plants a small amount of ions Na + uptake that these amounts replacement ion K + is, like: barley, rice, oats, tomato, potato and other plants Ryegrass model in which substitution by Na + K + ion is not observed and growth by sodium in these plants does not make sense, such as: corn, rye, soybean, common bean and Timothy(27).

Salisbury and colleagues (1995) response of dry weight of C3 and C4 in the treatment of sodium and without the presence of these ions studied were, they stated that in the presence of ions Na +, dry matter accumulation per plant C4 increases, which This due to increased concentration of acid pyrovat in cells

Mesophyll these plants is that increased uptake CO2 than is, but in plants C3 change in dry matter by ion Na + report did not, of course, this experiment plants C3 and C4 relatively resistant to ion Na + was used. When the ions K + sufficient and accessible, the condition that the amount of ions of potassium and the percentage of moisture is the result of corn absorbed K + completely done and the substitution K+ by Na$^+$ very minor and insignificant, the coursein the absence of K$^+$ and moisture content low, ions Na + and its absorption more was that the effect of salinity on corn shows, comparing the size of ion hydrated 0.331 = K + and 0.353 = Na + nm, absorption of ions of K+ moisture adequate and optimal concentration of potassium, most evident under conditions of salinity absorbed K + by Na + limit is the ratio of the vacuoles in the amount of low salinity to medium (200 mmol) is higher in salinity above this ratio sharply decreased, which due This replacement of Na+ ions instead of K+ ions are. The ratio of stem less, indicates the mechanism of resistance in the way of absorbing ions Na + and transfer of low sodium shoots are possible, due to layer multiple is possible that the wall radial cells, the six-way its four funds and Sabrina Caspar ring that acts selectively share Simplest move towards apoplast increases, whatever the contribution increases than decreases, the plant has resistance against the entry of salt of course, mechanisms resistance, such as another exit from the leaves and roots of salt, ionic regulation in shoots and reduced plant transpiration in this regard has been reported(20).
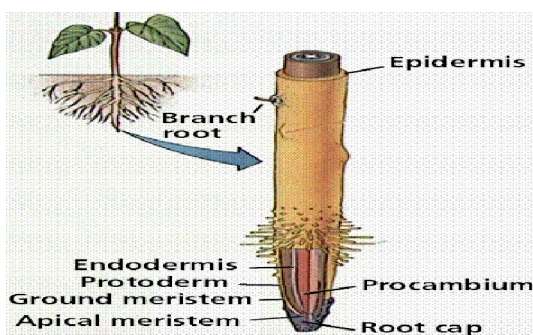
2. Materials and methods

The experiment conducted in Ahwaz Azad

Figure 2. Corn root (32)

[http://www.iowacorn.org/cornuse/cornuse_3.html Usage of Iowa and U.S. Corn Crop]

university research farm 3 Km to south of Ahwaz city during in 2006-7 year. A split plot design experiment in randomized complete block design (treatments main plot: different amounts of irrigation (I) and sub-plots: plant growth phases(S)) was performed with four replications

After measuring the parameters of root morphology and nutrient elements specific absorption rate (SAR), which is an indicator of the efficiency of root uptake based on the calculated parameters were measured, using the following formula based on root weight (RW) and mg unit g/g root dry matter per day was calculated:

$$\bar{A} = (M_2 - M_1)/(t_2 - t_1)/(LnR_2 - LnR_1)/R_2 - R_1$$

$\bar{A}$: Average speed of absorption of mineral nutrients (mgg-1day-1),

R: root dry weight (g)

M: Dry weight root element value (mg)

t: sampling time (day)

3. Result

Numeric value to attract the highest speed with the amount of potassium element 0.19 mg / g. day in the severe stress and was calculated with the lowest amount of sodium was 0.001 mg / g. day. Although in mild stress, nitrogen value increased to 0.21 mg / g. day, which are because of osmotic pressure phenomenon, but the element phosphorus absorption to the wilting point plant (PWP) element did not decrease. *SAR* of nutrient nitrogen, phosphorus, in comparison to control with full irrigation decreased the N *SAR* decreased even with the incidence of mild water stress found, although in severe stress treatment, water, SAR value of N increased, but severe water stress treatment, *SAR* showed a significant reduction of nitrogen. SAR P only to stress that the permanent wilting point plant conditions (PWP) contract, did not show decreasing trend. *SAR* K applying different levels of water stress than the control group showed an increasing trend, especially between these two elements, *SAR* K$^+$ increases much higher than the sodium was almost *SAR* sodium potassium approximately 55 times the most stress treatments water respectively. Conditions without water stress, the highest *SAR*-related elements nitrogen phosphorus and potassium elements was then that water stress, especially severe water stress, the highest *SAR* related to the element potassium phosphate, nitrogen and sodium were shown in Table 1.

Table1: Rated SAR values of nutrient elements during growth (mg / g. day)

111	99	87	75	63	51	39	27	15	Days after sowing
0.13	0.17	0.19	0.21	0.17	0.14	0.08	0.05	0.04	N
0.15	0.16	0.162	0.15	0.13	0.097	0.057	0.021	0.014	P
0.16	0.175	0.18	0.19	0.19	0.14	0.067	0.04	0.03	K
0.001	0.0017	0.002	0.003	0.0035	0.004	0.005	0.0014	0.001	Na
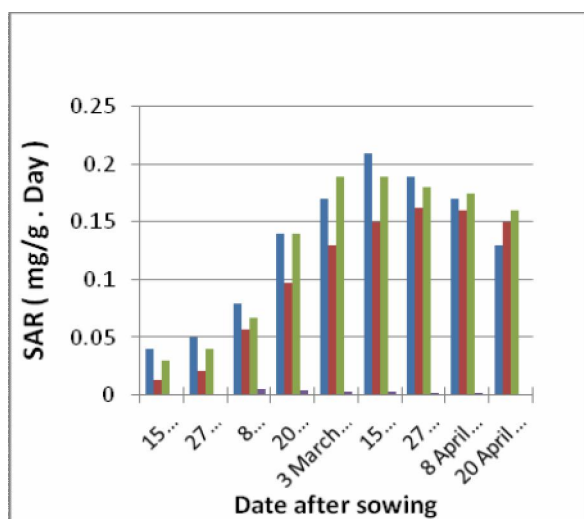
Fig 3. SAR nutrients in during seasonal growth

Nitrogen absorption rate equations shows that reducing the amount of water absorption of this element also severely reduce ($Y_N=0.016X+0.046$).

But the element phosphorus, the absorption process does not stop until the point wilt ($Y_P=0.02X+0.002$), Potassium absorption process according to the equation is increasing ($Y_K=0.016X+0.030$), Element in the situation is different sodium absorption rate of this element is extremely low ($Y_{Na}=0.006X+0.003$) (Table2).

Table 2. Calculation SAR equations

Nutrients	SAR Equation	R^2
N	Y=0.016X+0.046	0.561
P	Y=0.02X+0.002	0.871
K	Y=0.016X+0.030	0.690
Na	Y=0.006X+0.003	0.037

4. Discussion

The study of specific nutrient uptake rate (SAR) in the process of accumulation of root nitrogen, phosphorus, potassium is clear that by applying different levels of water stress, especially nutrient uptake rate (SAR) of nitrogen and phosphorus can be decreased. The decreasing trend of N SAR even mild water stress occurrence found, although more severe treatments water stress (treatment I2), SAR value of N increased, but treatment I3, SAR nitrogen reduction can be significant. Mild stress applied before the point of many changes in the SAR PWP plant phosphorus in comparison to control without water stress showed. The cause of this problem, not the element phosphorus uptake in plant roots when treated in the most severe water stress occurred in the plant was at the point of PWP and phosphorus in this SAR conditions suddenly decreased. SAR phosphorus treatment to I3, a significant decreasing trend did not, but that this treatment plant water stress conditions, permanent wilting (PWP) was, SAR phosphorus reduction was significant increase in these elements under drought stress conditions cause high accumulation of the elements sodium and potassium in plant roots were. Particular element of potassium sodium SAR 55 times its estimated water stress conditions, the highest SAR-related elements nitrogen phosphorus and potassium elements was then that water stress, especially severe water stress, the highest SAR related to the element K phosphorus, nitrogen was sodium, sodium SAR Although water stress conditions, but it increases the size of this increase nothing SAR K is not even comparable to that limitation of this nutrient by the plant showed.

Because plants need nitrogen and potassium elements in intervals beginning of growth can be seen that the two SAR element in this interval than SAR phosphorus and sodium, but after a while the growth (40 days after planting) plants that need SAR element phosphorus can increase the process of this element increased SAR and K to the end of seed maturity to process high SAR won all four elements nitrogen, phosphorus, potassium growth during the period of growth And at the highest S 2 S1, which spread root weight and the number is not complete, showed the lowest amounts of nitrogen and potassium SAR period of 35-30 days after sowing of the SAR and sodium phosphate was much higher than the 40 days after planting increased considerably accelerated by phosphorus SAR will amount to close before the flowering period of SAR values was potassium N, N SAR maximum flowering period was obtained after this step, SAR decreased nitrogen, but potassium SAR 15 days before their pea K flowering period of the course Receipt K, grain filling in some samples even up to grain maturity K, SAR K range was from their

pea K, SAR phosphorus during flowering horse grain had the maximum value, the maximum SAR sodium after double ring appearance was achieved what every age plant will increase the amount of sodium SAR showed downtrend. In other words, specific absorption rate of Sodium in the older plant vegetative period and then declined rapidly due to water stress conditions, changes in the behavior of root absorption, SAR elements nitrogen and phosphorus were dramatically decreased, which due to restriction of root growth and components solubility of these two elements are slightly (Mashner 1989)(6). But the SAR, especially potassium and sodium potassium SAR can be increased and due to high accumulation of potassium in plants to increase the SAR element. Root morph physiological changes such delusion rhizosphere water status of drought, especially absorption rate (SAR) nutrient nitrogen, phosphorus, potassium and sodium remarkable changes in comparison to control (without water stress), presented, as well as in each growth period, the rate of absorption of this nutrient, especially, showed different values.

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[http://www.howtocooKcornonthecob.com How to cook corn on the cob]
[http://www.milpa.nl Maize of Guatemala]

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Providing a Supervised Map of Olive Orchards by IRS Satellite Images

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Abstract: Due to some changes which are created above time in olive cultivated surface preparing updated map of olive is one of the most important requirements in the management and region agricultural planning. In this research, surveying of olive orchards investigated using IRS Satellite images in the region including some sector of Roudbar, Manjil, Loshan and Abbar, Guilan, Iran. Two methods evaluated to images controlled classification in order to separate olive orchards spectrum reflex from the other surface covers which include: 1.classifcation using spectrum reflex statistics and slicing and 2. classification with Minimum Distance method. The results indicate that in classification of images with spectrum reflex statistics, more than 60% of training points had again olive class in the olive orchards classified map.

1. Introduction

Much efforts had been carried out to simplify automatic preparation process of land covering map, which and refer to use Normalized dressing vegetation index (NDVI). In this index the curves of vegetation radiation are unique. To from this index, in addition to identify vegetation can refer to identify the region under volcano burst. NDSI index had been obtained using bands 2 and5 data of TM. This index is effective to distinct snow from upper soil, vegetation, features and cloud. NDBI index which is the fraction differential of two hands 4 and 5 to sum of this two bands, is very effective to prepare residential regions map (Zha and Gao, 2003; Chen et al., 2006).

In order to investigate the changes vegetation on soil erosion rate, Essa (2004) used remote sensing and GIS. In this line, the estimation of soil erosion had been carried out revised universal soil loss equation (RUSLE) in GIS medium. He obtained landuse map from MSS land sat (1972) and TM land sat (1992) digital images and calculated the vegetation changes during past 20 years. The results indicate lost of the soil in the region in 1972 to 1992 as a result of land use changes.

Increased destruction during two past decades had been because of reducing vegetation. Rembold et al. (2000) investigated land cover changes in a 22 years period at Lakes region in south of Ethiopia by aerial photographs (1972) and classifying TM land sat images (1994). The analyses indicate that cultivated surface had been increased and more erosion had been occurred in new cultivated lands.

Unal et al. (2004) rendered to classify cultivated land and separation of pistachio garden and orchard from the other vegetation in Gaziantep province of Turkish. Ramos et al. (2007) tried to measure and identify of soil movement in various gradient using GPS, GIS and DEM. Also, Moschen et al. (2001) tried to separate agricultural area from non-agricultural area using controlled classification of integrated images of TM5 with IRS IC PAN land sat and ERS 2 radar by maximum likelihood method. In addition to this separation, they tried to separated wheat, maize farm and rangelands. Using AIF (adaptive image fusion) index, Fletcher (2005) used high resolution QuickBird satellite images to recognize citrus with black mold (capnodium citri) in Texas region of America and indentified it as a suitable method. Das et al. (2009) tried to prepare map for regions with reducing citrus production capacity in Meghalaya region of India using IRS satellite images. The map of regions where citrus production capacity had been reduced was prepared using soil erosion information, vegetation condition and humidity tension.

Classification of enhanced images from SBI, NDWI and NDVI performed with maximum likelihood method to recognize the regions where citrus culture had been reduced. The results of their study was the identification of 29 villages with humidity tension from heavy soils in steep stop which because of lacking nutrients balance, be followed by reducing citrus production.

Due to some changes which are created above time in olive cultivated surface preparing updated

map of olive is one of the most important requirements in the management and region agricultural planning. With regard to this that land surveying required high cost and time and also preparing the map through aerial photographs is required to prepare aerial photograph which still along with high cost, use of satellite data along with remote sensing technique may be employed as a useful and effective tool to estimate crop area.

2. Materials and Methods

The study area is located between eastern longitudes of 48°55′48″ and 49°52′54″; and northern latitudes of 36°31′19″ and 36°59′57″ that the region area is 4590 km^2. Administrative boundary of the study area includes Roodbar Township along southern portion of Guilan province, Iran. Different image processing techniques are usually available to highlight a certain land use. In present research, two techniques were employed to highlight olive orchards from other land covers which are going to be described in following: 1. by spectral reflectance stochastic (DN[1]) of different land covers and slicing and 2. by Minimum Distance method.

IRS images of July 2006 were used to map olive farming area and software ILWIS 3.3 Academic was used for processing data. Field views (248 points) were done to determine accurate positions of land covers including: 1. Olive, 2. Hard wood forest, 3. Soft wood forest, 4. Cultivation lands (paddy), 5. Bare lands, 6. Non olive-plant covers, 7. Water area and 8. Urban regions. A point map of training and auxiliary point of different land covers was prepared to overlay on a sample set of color composite (bands 1, 2 and 3). The mean and standard deviation of training and auxiliary pixels of olive orchards was calculated. Upper and lower limits of DN-olive orchards were distinguished by the adding standard deviation to mean or diminishing of that ($\overline{X}$ (B_1 , B_2 , B_3) $\pm$ 2 $S.d$).

After rounding the upper/lower limits of Olive spectrum reflexes, 22-26, 51-78 and 90-115 of spectrum reflexes limits had been considered for bands 1, 2 and 3 with olive class. In each band, Olive limits introduced to software and slicing method used to prepare Olive map. Final map of Olive obtained from crossing of these three maps. The olive orchards map has been crossed by training point map to calculate the accuracy of method.

In another way, taking into consideration training and auxiliary points of different land covers, the supervised classification of IRS images was done by Minimum Distance method. Supervised algorithm

were tested by changing bias and threshold values in order to select the best boundaries in the spectral space beyond that a pixel has such a low probability of inclusion in a given class that it is excluded from that class. In the supervised classification methods, we differentiated dense olive orchards of its low dense orchards. Figure 1 shows the provided map of Olive orchards by minimum distance method with search radius 10 m. Finally, the olive orchards map has been crossed by training point map to calculate the accuracy of method. The classification accuracy was assessed on the entire study area by estimating the overall, producer's and user's accuracies and Cohen's Kappa coefficient (Congalton and Green, 1999) derived from the error matrix that is the core of accuracy assessment of a classified map (Foody, 2002; Liu et al., 2007).

The overall accuracy incorporates the major diagonal and gives the crude percentage of pixels correctly allocated. Producer's and user's accuracies detail the omission and commission errors, respectively. Kappa coefficient includes off-diagonal elements also taking into account the commission and omission errors. K, by including also information on these errors, represents a more realistic and reliable indication about the probability that a pixel classified on the map actually represents that category on the ground.

3. Results

Table 1 indicates mean, standard deviation and upper/lower limits of training pixels spectrum reflexes-Olive in order to image slicing in bands 1, 2 and 3. As we can see, in band 1, there is a shared DN between Olive spectrum reflexes and the other surface covers including barren land, hard wood and soft wood forests, non-olive vegetation and even urban regions. In band 2 of IRS satellite image, the greatest spectrum reflexes interference with olive class in non-olive vegetation and then soft wood forests and agricultural and paddle land in found. In this band, in $\overline{X}$ (B_1 , B_2 , B_3) $\pm$ 2 $S.d$, spectrum reflexes interference of hard wood forest, bare lands and urban regions had been lasted, but still DN interference of water zones with Olive is seen. Also, in band 3, there is interference between broad leaf wood, non-olive vegetation, agricultural lands, residential and industrial regions with Olive, but soft wood DN interference and water zones with Olive had been lusted.

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[1] Digital Number
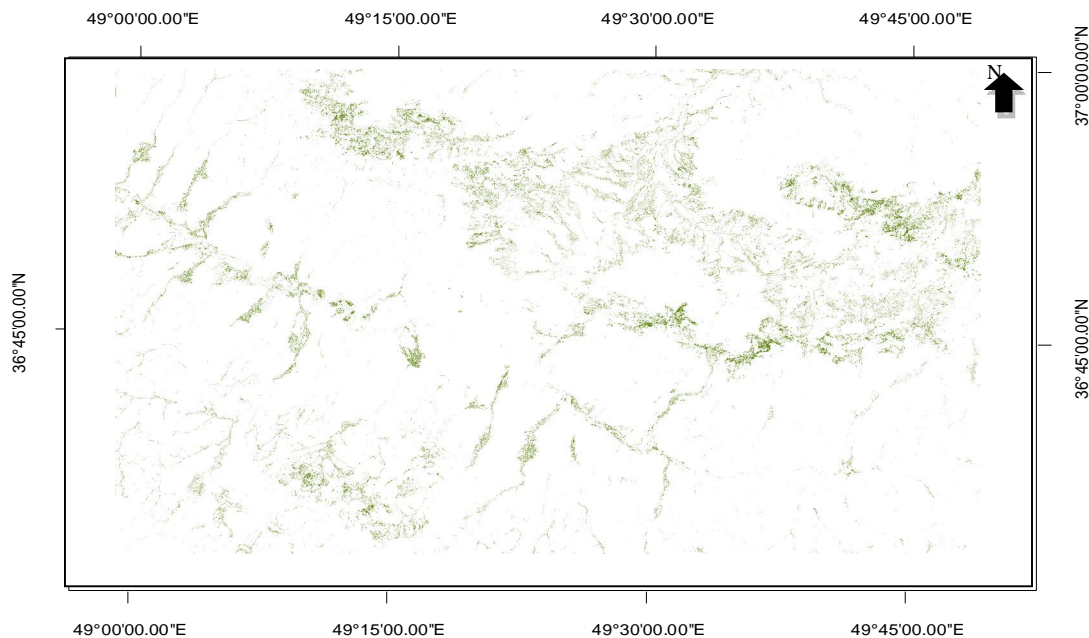
Figure 1. The provided map of Olive orchards by minimum distance method with search radius 10 m

Table 2 indicated the crossing result of training points map with Olive map. According to results, more than 60% of training points in classified Olive map recognized as olive class. Only 0.89% of training points of broad leaf forest class in classified map of Olive by slicing method had olive class which is negligible. Also, no one training points soft wood forest were not placed in classified map of olive class. Also in surface cover classes of barren lands, urban and water, none of the training pixels classified in olive class. Spectrum reflexes interference of agricultural and paddle lands with Olive had been found, so that, 17.7% of training pixels of agricultural lands in the classified map had olive class.

Confused matrix of minimum distance with search radius 1m (Tabel 3) indicate that this method have user's accuracy 67% in preparing Olive map. Table 4 indicated the confused matrix of minimum distance with search radius of 10m. User's accuracy in the classification of condensed Olive is 55.0%, in classification of low is 51.6% and in classification of total Olive orchards is 60.3%. 27.7% of training points of condensed Olive after classification had been classified in non-olive vegetation class and 11.4% in agricultural land class. Also, 17.3% of

training point of low-dense Olive had been introduced as barren land after the classification .with regard to table 4 relating to confused matrix of minimum distance with search radius of 50m, increasing the radius from 10 to 50 m cause not change the accuracy of surface cover classes.

Confused matrix of minimum distance with search radius of 100 m (Table 5) indicates that user's accuracy in classification of dense Olive is 57.1%. 23.2 and 11.6% of training point of dense Olive class had been placed in non-olive vegetation class and agriculture and paddle land after the classification, respectively. Also, in low-dense Olive, 23.6% of training point had been placed in non-olive vegetation, agriculture and paddle land class, after the classification. In sum 64.3% of training point of Olive class had been placed in this class, after the classification.

Table 6 indicates the overall accuracy and Kappa coefficient in various search radiuses. The largest overall accuracy and Kappa coefficient is related to search radius 1 m, but this point is important that in 1 m search radius, only 0.1% of area had been classified (Table 7).

Table 1. The Mean, Standard Deviation and Upper and Lower Limits of spectral reflectances in training and auxiliary points of olive orchards class in the bands of IRS satellite images for image slicing

Band Number	$\overline{X}\,*$			$S.d\,**$			$\overline{X}-2S.d$			$\overline{X}+2S.d$		
	1	2	3	1	2	3	1	2	3	1	2	3
	24.1	64.6	102.3	0.8	6.7	6.2	22.5	51.3	90.0	25.7	77.9	114.7

*   Digital Numbers Mean of Training Points in Olive Orchards Class
**   Digital Numbers Standard Deviation of Training Points in Olive Orchards Class

Table 2. Crossing classified map of olive orchards by training points map

Surface cover class	N_t*	$N_{t-o}**$	N_{t-o}/N_t***
Olive	2016	1416	70.23
Hard wood forest	293	270	92.15
Soft wood forest	10884	97	0.89
Non olive-plant covers	208	-	-
Bare lands	1213	215	17.72
Cultivation Lands (paddy)	38734	-	-
Water area	1134	-	-
Urban	8597	-	-

* Total numbers of training points
** Numbers of olive class-pixels after crossing classified map of olive with training points map
*** olive class-pixels/total pixels ratio (%)

Table 3. Confused matrix of minimum distance method with search radius 1m

Surface cover class	O	O-l,d	F	F-s	P	R	C	W	U	Total number	Users Accuracy (%)
O	2	1	-	-	-	-	-	-	-	3	67.0
O-l,d	-	-	-	-	-	-	-	-	-	-	0
F	-	-	93	-	-	-	2	-	-	95	97.9
F-s	-	-	-	1	-	-	-	-	-	1	100
P	1	-	-	-	-	-	-	-	-	1	0
R	-	-	-	-	-	46	-	-	23	69	66.7
C	-	-	-	-	-	-	4	-	-	4	100
W	-	-	-	-	-	-	-	51	-	51	100
U	-	-	-	-	-	-	-	-	-	-	0
Total number	3	1	93	1	-	46	6	51	23	224	
Producer Accuracy (%)	66.7	0	100	100	-	100	66.7	100	0		

O Olive, O-l,d Low dense forest F Hard wood forest, F-s Soft wood forest, P Cultivation lands (paddy), R Bare lands, C Non olive-plant covers, W Water area and U Urban regions

Table 4. Confused matrix of minimum distance method with search radius 10 and 50 m

Surface cover class	O	O-l,d	F	F-s	P	R	C	W	U	Total number	Users Accuracy (%)
O	780	82	-	-	393	2	162	-	-	1419	55.0
O-l,d	18	146	-	-	44	49	19	-	7	283	51.6
F	29	3	8889	-	13	-	205	-	-	9139	97.3
F-s	-	-	-	208	-	-	-	-	-	208	100
P	113	11	-	-	110	1	9	-	1	245	44.9
R	-	-	-	88	-	18792	-	-	14221	33101	56.8
C	74	-	66	-	14	-	879	-	-	1033	85.1

	O	O-l,d	F	F-s	P	R	C	W	U	Total number	Users Accuracy (%)
W	-	-	-	7	-	-	-	6758	-	6765	99.9
U	-	1	-	-	-	263	-	-	327	591	55.3
Total number	1014	243	8955	303	574	19107	1274	6758	14556	52784	
Producer Accuracy (%)	76.9	60.9	99.3	68.6	19.2	98.3	69.0	100	2.2		

Table 5. Confused matrix of minimum distance method with search radius 100 m

Surface cover class	O	O-l,d	F	F-s	P	R	C	W	U	Total number	Users Accuracy (%)
O	1220	141	17	6	496	3	249	-	3	2135	57.1
O-l,d	18	305	22	-	66	2	48	-	20	481	63.4
F	38	5	17036	180	23	-	5984	-	-	23266	73.2
F-s	-	-	-	208	-	-	-	-	-	208	100
P	121	23	10	-	131	1	21	-	4	311	42.1
R	4	13	-	1853	4	30092	-	17	30080	62063	48.5
C	242	2	267	3	15	-	973	-	-	1502	64.8
W	-	-	-	26	-	-	-	8565	-	8591	99.7
U	4	10	-	39	5	385	-	-	826	1269	65.1
Total number	1647	499	17352	2315	740	30483	7275	8582	30933	99826	
Producer Accuracy (%)	74.1	61.1	98.1	9.0	17.7	98.7	13.4	99.8	97.2		

Table 6. Overall Accuracy and Kappa Coefficient of minimum distance method in the classification of different lands covers

search radius (m)	1	10, 50	100
Overall Accuracy	87.9	69.8	59.4
Kappa Coefficient	82.8	58.3	46.4

Table 7. The classified area of different lands covers as compared with basin area in different methods of supervised classification.

Kind of Lands Cover	Search Radius (m)		
	1	50 and 10	100
Olive	14.9	5887.3	19723.5
Low Dense Olive	4.8	6019.1	17535.5
Hard wood forest	205.1	26352.9	47334.4
Soft wood forest	1.1	1753.9	27916.7
Non olive-plant covers	9.2	5239.8	10610.3
Bare lands	206.0	78781.2	11523.7
Cultivation Lands (paddy)	12.8	7879.4	44607.8
Water area	10.7	2357.0	3272.0
Urban	0.0	78997.4	172134.1
Total Area (ha)	464.6	213268.0	354658.0
Unknown area of Basin (ha)	458534.9	245731.5	104341.5
Classified Area Ratio to Total Area of Basin (%)	0.1	46.5	77.3

4. Discussion

The aim of current study was to separate the olive orchards regions from the other surface area, so as the condensed of olive canopy cover impact on spectrum reflections, olive orchards have been considered in two condensed and low condensed categories. When there is a low condensed olive orchard, it is natural that in one pixel, spectrum reflections is influencing on olive green canopy cover and soil zone of the lands between olive trees. In various regions, the type of surface phenomenon impact on map accuracy from classification,

intensively. For example, separating of water zones in IRS 3-bands images from surface phenomenon maybe possible, simply which in turn have its own certain condition , so, when the issue of separation one vegetation from the other vegetation is consider the possible of separating is most difficult. In this research, olive consider as one class and the other vegetation including orchards, woodlands , garden and etc had been considered in another class by the title of non-olive vegetation .Also , the vegetation of broad-leaf and conifer each consider in a separate class.

Minimum distance method was not a suitable method to prepare olive map. In this method, when search radius rate was 1 m, only 0.1% of area classified as identified pixels or in other words, nearly all area of unknown domain classified. In this method also in higher search radius, more part of area classified as unknown area. As it could find, spectrum reflections interference of olive and non-olive vegetation cause to hesitate in using minimum distance method. When the spectrum classes are close to each other, this classification method is not so good (Alavi panah, 2003). Out of minimum distance methods, classification method with 100 m search radius because of 77.3% cover area and 64.37% user's accuracy in classification of olive orchards was relatively better than the other rate of search radius in this method.

It must be consider that the overall accuracy of this method was about 60%, but Kappa coefficient was less than 50% and 46.4%, so as the goal is to prepare olive orchards map, the main judge criteria is user's accuracy in classification of olive, since it consider the Kappa coefficient, correct classified pixels and error pixels of all surface vegetation classes. Cuneo (2009) provided a map of African Olive distribution was produced from the image analysis and checked for accuracy at 337 random locations using ground observation and comparison with existing vegetation maps. Results indicated that a total area of 1907 ha of dense African Olive infestation was identified, with an omission error of 7.5% and a commission error of 5.4%. Sepulcre-Canto (2009) monitored a total of 1076 olive orchards in area in southern Spain, gathering the field location, field area, tree density, and whether the field was drip irrigated or rainfed by. An approach based on a cumulative index using temperature and the normalized difference vegetation index (NDVI) information for the 6-year ASTER time-series was capable of detecting differences between irrigated and rainfed open-canopy orchards, obtaining 80% success on field-to-field assessments. The method considered that irrigated orchards with equal vegetation cover would yield lower temperature and

NDVI than rainfed orchards; an overall accuracy of 75% and a kappa (kappa) of 0.34 was obtained with a supervised classification method using visible, near infrared and temperature information for the 6-year ASTER imagery series.

Overall accuracy indicates the efficiency of a method in classification of various surface covers, but it is possible in an overall accuracy that user's accuracy be less in classification of olive. Therefore, as the goal is to classify olive, user's accuracy is enjoying from the most importance in classification of this olive orchards. Ahadnejad Reveshty (2003) in a research concluded that PCA analysis is the most effective method to increase discrimination factor among different classes. Color composites of PCA1 PCA2, PCA3, consisting the majority of information were used for training area selection. He employed maximum likelihood classifier to highlight olive farming area that olive area estimated around 3843 ha.

In classification of condensed olive orchards, as there is high error in classification of olive, non-olive vegetation and agricultural lands, so the increase of kappa coefficient indicating less error and more capacity of this method in classification of surface covers and olive. In classification of less-condensed olive orchards, because of spectrum wave interference of olive green canopy cover and the soil zone between the canopy cover, the interference of digital number of low-condensed olive observed not only with the other vegetation cover but also with bare lands. There was this issue even for wave interference of low-condensed olive with urban and residential regions as some part of olive located in urban and residential regions and one pixel digital number can be an average of reradiating wave of olive canopy cover and urban and residential region. So, some true pixels of low-condensed olive had been classified as residential region or vice versa.

5. Conclusion

To compare various classification methods to spectrum reflections statistic classification indicate that the classification based on spectrum reflections statistic while having accuracy same as the best image supervised classification, enjoying more simplicity. In this method, because of image classification, only focusing on olive spectrum reflexes statistic, the likelihood olive regions had been separated and preparing the maps is done with regard to goal, that is, olive and the other surface covers are not consider. As a whole, it seems that, if preparing the map of olive orchards is doing with the help of spectrum reflexes statistic in the regions with olive and non-olive vegetation, the separated area must indicated under the title of mixed olive land and non-olive vegetations. Also, it must be consider that

the commune spectrum reflexes found between the agriculture land and olive.

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Impact of an Educational Program on Nursing Care of Neonates with Congenital Hypothyrodism

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Abstract: Background: Unrecognized congenital hypothyroidism (CH) leads to mental retardation. Newborn screening and thyroid therapy started within two weeks of age and effective nursing intervention can normalize cognitive development. Initial dosage of 10 to 15 µg/kg Levothyroxine is recommended.
Objectives: this study aims to assess nurses' knowledge regarding CH, to evaluate nurses' intervention offered to CH neonates and to determine the impact of educational program on nurses' performance regarding care of CH neonates. Design: A quasi experimental design was used in this study. Subjects and Methods: This study included 60 nurses whom were recruited from 3 maternal and child health units (MCH) at Zagazig city. Two tools were used to collect the necessary data: a structured interview sheet and observational checklist were used to assess nurses' performance. Results: It was found that total nurses' complete knowledge and practice score about hypothyroidism was poor (100%) before program implementation and improved at post- test and this results was highly significant at 1%. Conclusion: The nurses' performance significantly improved after program implementation.
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Key wards: Congenital Hypothyroidism

1. Introduction:

Thyroid hormone is essential for normal brain development. Newborn who are born with congenital hypothyroidism (CH) and lacking for thyroid hormone during a circumscribed period of early development will be at risk of brain damage and mental retardation [1, 2].

Congenital hypothyroidism is the most prevalent endocrine disorder in the newborn and affects 1 in 3000 newborns. [3], while it was 1-1300in Bangladesh[4] and 1:370 neonates in Iran[5] Screening for CH is a major achievement of pediatricians because early diagnosis and treatment have resulted in normal development in early all cases[6].

The thyroid gland is responsible for controlling the rate of metabolism in the body through production of Thyroxine (T4) and tri-iodothyroxine (T3). If there are any defect in the thyroid gland it will lead to insufficient production of thyroid hormone(T3&T4)to meet the body metabolic needs which leads to physical and mental retardation if not start the treatment during or in the first month after delivery[7,8].

Congenital hypothyroidism is caused by an absent or under developed thyroid gland. Other causes may be hypothalamic or hypopituitary disorder in which there is insufficient thyroid stimulating hormone to stimulate the thyroid gland. In addition maternal intake of medications during pregnancy or exposure to radiation can cause transient or permanent hypothyroidism in the neonates [2,9].

Many researches suggested that the incidence of CH is expected to be elevated in the zone of moderate iodine deficient areas and parental consanguinity [10]

The neonates with CH present with cold extremities and hypothermia as well as slow pulse and respiratory rate. Neonates might have respiratory difficulty and prolonged jaundice [9,11]. In addition the CH neonates present with improper neurodevelopment mental state such as week reflexes and poor behavioral and orientational responses [12, 13].

Congenital hypothyroidism is usually diagnosed by neonates' thyroid screening and serological testing for T3&T4 which are low (below 5ug/dl) and increased thyroid stimulating hormone (TSH) above 6 uu/ml[7,8].

Therapeutic management of CH consists of lifelong thyroid hormone replacement usually in the form of levothyroxine; it is given as a single dose that varies with age. [14-16] moreover vitamin D supplement may also be given to prevent the development of rickets [1,2].

Nurses have a major role in caring for CH neonates such as assessing growth and development and ensuring compliance of mothers with medication. Nurses should instruct mothers how to count their neonates, pulse. She should also instruct

mothers regarding the signs and symptoms of hyperthyroidism (increase sweating, increase heart rate, diarrhea, loss of weight, increase body temperature and irritability) and signs of hypothyroidism (decreased heart rate, constipation, weight gain and hypothermia [9].

In addition, nurses should instruct parents about regular periodic measurement of thyroxine levels and follow up to ensure normal physical and mental development [9, 17].

So, establishing of an educational program for pediatric nurses is essential for their education and development, because the program would move nursing forward in the most effective way possible to improve their nursing intervention.

The aim of this study was to improve nurses' care of CH neonates.

The aim of the study was to:
Assess nurses' knowledge regarding congenital hypothyroidism, evaluate nurses' intervention offered to CH neonates, and to determine the impact of educational program on nursing care of neonates with CH.

2. Subjects and Methods

I- Research Design
A quasi experimental design was used in the present study.

II- Setting:
This study was conducted at three MCH at Zagazig city. On MCH from each sector of the city, which were chosen according to the highest number of nurses working at it and also the highest number of attendancy in it.

III- Subjects:
All nurses (60nurse) working in the above three mentioned setting were comprise the sample, 20 nurses from each one.

IV- Tools:
Two tools were developed by the researcher to collect the necessary data:

Tool I: A structured questionnaire interview sheet for nurses which include the following parts:
Part A: Biosocial characteristics of nurses such as age, qualification, years of experience and any previous educational program attended about CH.

Neurological & and behavioral assessment. Moreover, nurses' role regarding these neonates.

Scoring system of nurses' knowledge was developed by the researcher.
The total grad of knowledge equal 114 grads, each correct answer took 2 grads, while wrong one took zero.

The points were classified as following:
- Definition of CH took 2 grads
- Causes of CH took 14 grads
- Manifestations of neonates took 20 grads
- Manifestations of infancy took 22 grads
- Signs of over dose took 10 grads
- Signs of under dose took 8 grads
- Diagnosis of CH took 4 grads
- Treatment of CH took 2 grads
- Nurses' role of CH took 32 grads
The total knowledge score was classified as:
- Poor < 50% equal less than 57 grads.
- Fair 50 – 74% equal 57 –
- Good75% & more equal 85 –

Tool II: Observational checklist was developed to evaluate nurses' intervention offered to CH neonates, it includes anthropometric measures, and vital signs. Assessment of neonatal reflexes as well as behavioral and orientation responses which were adopted from Brazelton behavioral assessment scale was recorded. Moreover, health instructions which should be given to mothers was also recorded:
Scoring system of nurses' intervention was developed by the researcher.
The total grad of performance was 48 grades, each item done scored 2 grades and zero if not done.
The total score was classified as:
- Poor <50% equal less than 24 grads
- Fair 50-74% equal 24 –
- Good 75 % & more equal 36 –

Program development
Educational program was developed by the researcher that aimed to improve nurses' performance offered to CH neonates.
The program was applied in three sessions for nurses of each MCH.
- The first session took one hour and it include the assessment phase and the pre test using tool I and each nurse was observed separately using tool II.
- Second session was the program implementation and it took one hour. Various teaching methods were used as lectures, group discussion, demonstration on normal neonates & CH neonates & redemonestration were done. It includes complete information

about CH and post I test was done using tool I part B & tool II.

- Third session took 20 minutes. It was done after three months of program implementation using the same tools.

Evaluation of the educational program's success was based on the improvement of nurses' performance. And this evaluation was done before the program and immediately after the program and then after 3 months.

II- Methods

- Approval for data collection was obtained from the board of ministry of health.
- The tools were developed by the researcher based on reviewing literature.
- Jury was done to the tools by five experts (three professors of pediatric nursing, one professor of endocrinology, and one professor of statistics).
- All nurses were acquainted with the aims of the study.
- A pilot study was done on 5 nurses to test clarity and practicability of the tool, necessary modification was done
- Pre test was done for nurses of each MCH using tool I.
- Each nurse was observed separately to complete the observational checklist using tool II.
- The program was given and post I test was done immediately after the program implementation and also reassessment was done after 3 months by using the same tool.
- Data was collected over a period of 6 months starting from January to   June 2009.

Data analysis

The collected data were revised, coded and fed to a fox pro program data base. Data was then transferred to SPSS version 13 program for statistical analysis which included frequency, percentages, cross tabulation, mean, standard deviation, chi square and one way analysis of variance(ANOVA). The level of significant used was at $P \leq 0.01$.

3. Results

Regarding to biosocial characteristics of the studied nurses, it was found that the mean age of nurses was 28.05 years.

As regard to education, it was found that 80% of nurses had complete secondary nurses school, while 20% of them finished technical nursing education.

It was found also that 48.30%of studied nurses had 5.9 years of experience with mean 8.30 years. In addition 83.33% of studied nurses did not attend any previous educational program about congenital hypothyroidism.

Concerning to sources of nurses' knowledge about CH, it was found that 83.33% of them had gain their knowledge from the present educational program.

Nurses' complete knowledge about CH was illustrated in table I. It was found that 1.7% only of nurses had complete answers before program implementation about CH definition and this percentage increased to 98.3% at post I and slightly decreased to 68.3% at post II and the result was statistically significant.

Inborn error of thyroid hormone and prenatal iodine deficiency were mentioned by nurses as a cause of CH before implementing the program (46.7% &20% respectively), while some other causes of CH were reported by nurses at post I such as maternal intake of goiterogens (100%), geographic iodine deficiency (96.7%), agenesis of thyroid (90%) as well as Hypoplasia (78.3%) and Ectopic thyroid (70%).

Regarding to neonatal manifestations, it was found also at table I that nurses' knowledge was deficit before program implementation. On the other hand all nurses at post I reported increased birth weight, hypothermia, cold dry skin, poor feeding as well as hypotonia and constipation followed by prolonged physiological jaundice, abdominal distention and wide anterior fontanel (98.3% for each) as a neonates' manifestations.

Regarding to nurses' complete knowledge about infant's manifestations, it was found that no one of nurses knew the functional changes before program implementation except only 1.7% knew hypotonia. On the other hand all nurses at post I mentioned that diminish intestinal activity, hypotonia, abdominal distention as well as dry skin and physical changes were the functional changes among infant with CH.

Stunted height, over weight and delayed dental development were mentioned by all nurses at post I as a retardation of G&D compared to none before implementation of program the result was statistically significant between nurses' knowledge before the program and at post I and post II.

The result of nurses' knowledge was statistically significant between their knowledge before program implementation and at post I and post II.

Nurses' complete knowledge about signs of over and under dose was shown in table II. It was found that increase heart rate, diarrhea and loss of

weight were mentioned by 100% of nurses as a signs of over dose at post I followed by ↑ sweating and temperature(98.3% for each) compared to none before the program.

None of the studied nurses' knew the signs of under dose before the program. On the other hand all nurses at post I stated that decreased heart rate, increased body weight and decreased temperature were the signs of under dose followed by constipation (98.3).

The same table portrays that decreased T4, increased TSH were mentioned by 100%, 98.3% of nurses respectively at post I compared to 51.7% and 0.0% respectively before the program.

When the nurses were asked about the treatment of CH, 61.7% of them before the program answered that L-Thyroxine was the drug of choice compared to all nurses (100%) at post I&II. the result was statistically significant between the three phases of the program.

Table III illustrates that nurses' complete knowledge about their role regarding CH neonates. It was found that assessing physical growth as weight and height were mentioned by 76.7%&10% of nurses respectively before the program compared to 100% for each at post I.

Measure temperature, pulse and respiration were mentioned by all nurses at post I compared to 76.7%, 3.3% and 0% respectively before the program as physiological measures.

When the nurses was asked about assessing reflexes no one knew them before the program compared to 100% of nurses at post I reported rooting, sucking, grasping, dancing, moro and tonic neck reflexes followed by babiniski(80%).

The same table shows that all nurses knew auditory and visual response at post I as a behaviors response compared to none before the program. In addition 100% of nurses at post I stated inanimate auditory and visual were orientation response compared to none before the program. The result was statistically significant between the three phases of the program.

Nurses' performance toward CH neonates was illustrates in table IV. It was found that measure temperature. And assess weight was done by 80% & 78.3% of nurses respectively before the program compared to 100% at post I as well as all of them assess height and measure pulse and respiration.

When the nurses was observed before the program it was found that no one assess the reflexes compared to all nurses at post I except tonic neck reflex (98.3%) and babiniski (88.3%).

Moreover, When the nurses was observed before the program it was found that no one of nurses assess behavioral and orientational responses compared to 100% of them did it at post I, the result was statistically significant.

It was also found that all nurses did not give any instructions to parents before program implementation. On the other hand all nurses at post II instruct parents about periodic measures of T3 & T4, medication continuation, how to administer the drug as well as how to count pulse. In addition signs of over and under dose (98.3% for each), regular periodic follow up and signs of complication (71.7%for each), the result was statistically significant.

Total nurses' complete knowledge and practice score about hypothyroidism was shows in table V. Concerning to knowledge score, it was found that before program implementation 100% of nurses had poor knowledge score. On the other hand all nurses had good knowledge score at post II. One hundred percent of nurses had poor practice score before the program; while all of them had good practice score at post I as well as post II 56.70% of them had fair practice score. The result was highly significant.

Table VI portrays the relation between nurses' knowledge and practice score regarding CH. It was found that the nurses' knowledge is better than their practice as before program their mean knowledge score was 7.65 and mean practice score was 3.47.This mean score was improved at post I but still the knowledge better than practice as mean knowledge score was 111.7 compared to 46.9 mean practice score.

At post II the mean knowledge and practice score were slightly decreased to 75.85 and 33.77 respectively. The result was statistically significant. As ANOVA test was 9779.6 (P< 0.000) for total knowledge score and 4294.3(P < 0.000) for total practice score.

4. Discussion:

Thyroid hormones have been shown to be absolutely necessary for fetal brain development. Screening for thyroid hormone level in the first week of life is extremely important to identify infant with CH. World wide screening programs have been successful in decreasing childhood mental & physical retardation related to CH by early detection, treatment & proper nursing intervention [17].

Table I: Nurses Complete Knowledge about Definition, causes and manifestations of neonates and infants with CH:

Nurses' knowledge	Pre		Post I		Post II		X2
	No	%	No	%	No	%	
1- Definition of CH: Deficiency of thyroid hormone which present before birth	1	1.7	58	98.3	41	68.3	193.4**
2- Causes:*							
• Agenesis of thyroid	0	0	54	90	19	31.7	105.6**
• Hypoplasia	0	0	47	78.3	9	15	116**
• Ectopic thyroid	0	0	42	70	8	13.3	111.3**
• In born error of thyroid hormone	28	46.7	60	100	58	98.3	131.8**
• Prenatal iodine deficiency	12	20	60	100	55	91.7	151.9**
• Maternal intake of goiterogens	0	0	60	100	35	58.3	122.2**
• Geographic iodine deficiency	1	1.7	58	96.7	30	50	107.4**
3- Manifestations of neonates:*							
• ↑ birth weight	2	3.3	60	100	60	100	208.0**
• Hypothermia	5	8.3	60	100	60	100	199.5**
• Cold dry skin	2	3.3	60	100	27	45	115.0**
• Poor feeding	1	1.7	60	100	17	28.3	132.0**
• Prolonged physiological jaundice	0	0	58	96.7	10	16.7	150.8**
• Hypotonia	5	8.3	60	100	56	93.3	175.3**
• Abdominal distension	1	1.7	59	98.3	47	78.3	140.7**
• Constipation	1	1.7	60	100	52	86.7	166.4**
• Wide anterior fontanel	0	0	58	96.7	16	26.7	132.3**
• Open loop posterior fontanel	0	0	58	96.7	2	3.3	1181.3**
Manifestations of infancy:*							
A- Functional changes:							
• Poor peripheral circulation	0	0	54	90	18	30	107.8**
• Diminished intestinal activity	0	0	60	100	54	90	179.0**
• Hypotonia	1	1.7	60	100	58	96.7	198.3**
• Abdominal distension	0	0	60	100	46	76.7	145.7**
• Dry skin	0	0	60	100	13	21.7	145.2**
• Physical changes	0	0	60	100	52	86.7	169.3**
• Mental changes	0	0	59	98.3	47	78.3	143.6**
B- Retardation of G&D:* ☻							
• Stunted height	0	0	60	100	36	60	123.3**
• Over weight	0	0	60	100	59	98.3	207.4**
• Delayed osseous development	0	0	57	95	4	6.7	167.7**
• Delayed dental development	0	0	60	100	25	41.7	121.5**

*More than one answer
**Highly significant at P≤0.01
☻G&D= Growth and Development

Table II: Nurses' Complete Knowledge about Signs of Over and Under Dose, Diagnosis & Treatment of CH

Nurses' knowledge	Pre		Post I		Post II		X2
	No	%	No	%	No	%	
Signs of over dose*:							
• ↑ sweating	0	0	59	98.3	34	56.7	116.3**
• ↑ heart rate	0	0	60	100	32	53.3	119.9**
• Diarrhea	0	0	60	100	43	71.7	136.8**
• Loss of weight	0	0	60	100	60	100	213.9**
• ↑ temperature	0	0	59	98.3	54	90	173.1**
Signs of under dose*:							
• ↓ heart rate	0	0	60	100	19	31.7	130.1**
• Constipation	0	0	59	98.3	51	85.0	159.1**
• ↑body weight	0	0	60	100	59	98.3	207.4**
• ↓temperature	0	0	60	100	56	93.3	189.6
Diagnosis:							
• ↓T4,6<5 Mg/dl	0	0	60	100	60	100	140.0**
• ↑TSH>20Mμ/ML	0	0	59	98.3	34	56.7	116.3**
Treatment:							
• L- Thyroxine	037	61.7	60	100	60	100	129**

*More than one answer
**Highly significant at P≤0.01

Table III: Nurses' Complete Knowledge about their role regarding CH neonates

Nurses' knowledge	Pre		Post I		Post II		X2
	No	%	No	%	No	%	
Nurses' role:							
A- Assessing physical growth							
• weight	46	76.7	60	100	60	100	114.1**
• height	6	10	60	100	51	85	148.1**
B- Physiological measures							
• Measure temperature	46	76.7	60	100	60	100	114.1**
• Measure pulse	2	3.3	60	100	58	96.7	195.4**
• Measure respiration	0	0	60	100	28	46.7	119.7**
C- Assessing reflexes*							
• Rooting	0	0	60	100	60	100	213.9**
• Sucking	0	0	60	100	40	66.7	129.8**
• Grasping	0	0	60	100	55	91.7	184.2**
• Dancing	0	0	60	100	16	26.7	136.8**
• Moro	0	0	60	100	52	86.7	169.3**
• Tonic neck	0	0	60	100	5	8.3	175.4**
• Babiniski	0	0	48	80	0	0	160.6**
D- Assessing neonates behaviors							
1-Behavior response							
• Auditory response	0	0	60	100	52	86.7	169.3**
• Visual response	0	0	60	100	60	100	213.9**
2-Orientation response							
• Inanimate auditory	0	0	60	100	52	896.7	169.3**
• Inanimate visual	0	0	60	100	56	93.3	189.6**

*More than one answer
**Highly significant at P≤0.01

Table IV: Nurses' Performance toward CH neonates and about Health Instructions Given to Parents Having CH infants

Nurses' Performance	Pre		Post I		Post II		X2
	No	%	No	%	No	%	
- Assess weight	47	78.3	60	100	60	100	112.5**
- Assess height	7	11.7	60	100	48	80	133.6**
- Measure temperature	48	80	60	100	60	100	111.0**
- Measure pulse	2	33	60	100	60	100	208.0**
- Measure respiration	0	0	60	100	28	46.7	119.7**
Assessing reflexes*							
• Rooting	0	0	60	100	58	96.7	201.2**
• Sucking	0	0	60	100	47	78.3	149.1**
• Grasping	0	0	60	100	55	91.7	184.2**
• Dancing	0	0	60	100	10	16.7	155.2**
• Moro	0	0	60	100	50	83.3	160.6**
• Tonic neck	0	0	59	98.3	8	13.3	158.4**
• Babiniski	0	0	53	88.3	0	0	174.0**
Assessing neonates behaviors							
1-Behavior response							
• Auditory response	0	0	60	100	53	88.3	174.1**
• Visual response	0	0	60	100	60	100	213.9**
2-Orientation response							
• Inanimate auditory	0	0	60	100	35	58.3	122.2**
• Inanimate visual	0	0	60	100	57	95.0	195.3**
Health instructions*							
- Signs of over dose	0	0	59	98.3	56	93.3	183.6**
- Signs of under dose	0	0	59	98.3	56	93.3	183.6**
- Regular periodic follow up	0	0	43	71.7	10	16.7	104.7**
- Signs of complications	0	0	43	71.7	10	16.7	104.7**
- Periodic measures of T3 &T4	0	0	60	100	43	71.7	136.8**
- Importance of medication continuation	0	0	60	100	45	75	142.5**
- How to administer the drug	0	0	60	100	54	179.0	179.0**
- How to count pulse	0	0	60	100	34	121.2	121.2**

*More than one answer
**Highly significant at P≤0.01

Table V: Total Nurses' Complete Knowledge & Practice Score about Hypothyroidism

Score	Pre		Post I		Post II		X2
	No	%	No	%	No	%	
I- Knowledge							
Poor	60	100	0	0	0	0	348.347
Fair	0	0	0	0	58	96.700	P=0.000**
Good	0	0	60	0	2	3.30	
II- Performance							
Poor	60	100	0	0	0	0	251.163
Fair	0	0	0	0	34	56.70	P=0.000**
Good	0	0	60	100	26	43.30	

**Highly significant at P≤0.01

Table VI: Correlation between Total Nurses' Knowledge and Practice Score Regarding CH

Program phases	Knowledge score	Practice score
Pre program		
Mean	7.65	3.47
S.D	3.507	1.873
Post I		
Mean	111.7	46.9
S.D	3.679	1.581
Post II		
Mean	65.07	28.04
S.D	43.43	18.425
ANOVA Test		
F	9779.6	4294.3
P	0.000**	0.000**

**Highly significant at P≤0.01

The present study shows significant improvement in nurses' knowledge after implementation of educational program regarding CH. It was found that the majority of nurses mentioned that inborn error of thyroid hormone and prenatal iodine deficiency were the most common causes of CH, and this result goes with Kempers et al [19] whom found the effect of maternal Graves diseases on the fetal and neonatal thyroid gland and its function. Also supported by SaAYlam et al [20] and Saglam et al [21] whom found in their studies that increased incidence of CH in iodine deficiency area.

The result of the present study may be due to that these two causes were the most common causes of attendancy at Zagazig City as the data collected from rural area and these areas found to be deficient in iodine.

On the other hand other studies added that low birth weight was the cause of CH [22], while, Mcelduff et al [23] found that babies delivered by cesarean section were significantly more likely to have CH than those delivered vaginally. In addition, Veiga et al [24] fond in their study that there was seasonal variation as TSH collected in the summer were some what higher than those collected in the winter. While Mengreli et al [25] added that the cause of CH was maternal autoimmune thyroid disease.

As regards to manifestations of neonates, it was found that all nurses reported increase birth weight & hypothermia as a manifestation of CH followed by hypotonia and this result may be due to that these manifestations were obvious to nurses to see it, as it is routinely measured every visit. On the other hand the rest of manifestations were known by nurses after the program. These finding support the hypothesis that educational program related to health problems impact health knowledge and care [26].

Over weight as a manifestation of CH during infancy were reported by the majority of nurses weighing the infant routinely every visit, so it is obvious to her the increase in his body weight. This result clarified by the fact that the thyroid gland did not secrete its hormone which result in slow rate of metabolism thus leading to increase body weight [1,2,4].

When the nurses were asked about signs of over and under dose, no one of them knows it before the program rather than after the program. This result may be that nurses from their point of view considered that this is not their responsibility but it is the doctor's responsibility.

All nurses mentioned that decrease level of T4 was the main diagnosis and it is done on the third day after delivery and this result supported by Kempers et al [7] and Silva et al [8] who cited that screening test for CH should be done in the first week of life. On the other hand Ordookhani et al [10] recommended in his study done at Iran that screening test should be done since birth at delivery room.

L-thyroxine is the drug of choice which mentioned by all nurses and this result go with Rose et al [27] Lafranchi et al [28], Nakamizo et al and Yang et al [30] whom found that L- thyroxine treatment leads to normal or near normal neurocognitive outcome in infants with CH . In addition, Kato et al [31] reported normal growth and development of CH neonates whom treated with L- thyroxine. Treatment and diagnosis were mentioned by all nurses, and this result may be due to that screening program for CH is mandatory now in Egypt and is done in the first week of age.

Most neonates born with CH have normal appearance and no detectable physical signs. Hypothyroidism in the newborn period is almost always overlooked and delayed diagnosis leads to the

most sever outcome of CH as a mental & physical retardation [6]. So, the nurse had a great role toward assessing those neonates. The present study portrayed that the majority of nurses wee assessed physical & physiological measures, and thus may be due to that these measures were done routinely to the neonates every visit and also they consider that this was their role to do it. On the other hand the majority of them did not assess reflexes before program but they assess it at post I & post II, this may be clarified that they were considered this part of assessment was the responsibility of the pediatrician and after the program they know the importance of doing it because it is reflect the neurodevelopment of the neonates and this goes with the opinion of many researchers [9,12] as well as Brazelton [18] at its assessment in his scale.

Assessing behavior and orientation responses is another role of pediatric nurse as she can discover the progress of development of neonates with CH. The present study shows that no one of nurses did this assessment before program but the majority of them did it at post I & post II and this reflect the importance of educational program in guidance and development of nurses. This finding go with Joseph [32], Kempers et al [33] Hopfner et al [34] and Rovet [35] whom found that assessing cognitive and motor functioning is very important to know the developmental milestone of the neonate and discover any retardation as early as possible.

When the nurses were observed to evaluate their intervention after program implementation, it was found that their was significant improvement in their performance as they assessed growth and development, measure vital signs and assess reflexes as well as assess behavior and orientation responses. This finding may be due to the efficacy of training program, guidance & supervision. In addition, they did not know the importance of their role in preventing mental & physical impairment of CH neonates before the program but after the program they begin to know their role. This result supported by many studies [36,37]

As regards to health instructions given to parents , all of nurses did not gave any instructions before program but the majority of them gave it at post I& II. This finding may be due to shortage number of staff in relation to the number of cases as some nurses were busy by the administrative work as well as improper environment to gave the instructions (over crowding). In addition, there was no motivation, encouragement and supervision as well as guidance. So, when they attend the program and understand the importance of their role regarding

CH neonates, they became enthusiastic in their work. This result was supported by some researchers [36,37].

It was found that all of nurses had poor knowledge score before the program, while 60% had good score in post I and the majority had fair in post II. This result may be due to that the majority of nurses had secondary nursing school education and they did not study these subjects in their curriculum, and their source of information about CH from the present program. In addition, the majority of them did not attend any previous educational programs about CH before. This finding support the hypothesis of the importance of the educational program related to health problems on the nurses' performance [26].

As regards to nurses' performance it was found that all of them had poor practice score pre program, while the majority of them had fair & good practice score post I & II. This result reflect the impact of training course to enhance the nurses' technical knowledge and skills [37]

5. Conclusion:

Nurses' performance were improved after the educational program as all of them had poor knowledge & practice score before the program, while the majority of them had fair & good score after the program.

Based on the previous finding and results of the present study, the study recommended that: -
1- Educational program should be mandatory and continuous for nurses who are working at well baby clinic about CH.
2- Provide adequate head nurse for supervision, guidance and regular feed back to nurses about their performance.
3- Design clinical protocol and guidelines about CH to be exist in each MCH.

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Evaluation of the Role of Radiotherapy in Early Breast Cancer

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Abstract: Purpose: Retrospective study for evaluation of the role of radiotherapy in early breast cancer patients T1-2 M0 with (0-3 positive axillary LNs) as regard disease free survival (DFS) and over all survival (OS). Patients and methods: Between January 1999 to December 2004, ninety patients pathologically confirmed early breast cancer were classified into two groups, 50 patients in the first group were treated with Modified Radical Mastectomy (MRM) + adjuvant radiation therapy + systemic therapy (Chemotherapy and/or hormonal therapy) and 40 patients in the second group were treated with MRM + adjuvant systemic therapy. All patients entered in this study were monitored for treatment related events, toxicity, loco-regional and distant failure, time to death and survival. The (OS) rates and(DFS) rates depicted on Kaplan Meier plots. Results:Patients in the first group who received radiation therapy developed less loco-regional recurrence rate (8% vs 22.5%) than did those receiving no radiation therapy in the second group with no significances statistical, p=>0.05. According to the time gap since surgery to radiation therapy, twenty six percent of patients who received radiation therapy in period more than 3 months from the surgical interference had developed loco-regional failure vs no patients in population who received radiation therapy in period less than 3 months since surgical interference with statistical significant difference (P<0.005). Within 50 patients in the first group, skin desquamation was the most common acute complication of radiation therapy (20%) and hyperpigmentation was the most common late effect of radiation therapy (30%). There was statistical significant difference for the 5- year loco-regional recurrence free survival rates according to the treatment modalities in the first group versus those in the second group(88.24% vs 73.16 respectively ,P=0.0308). There was insignificant improvement in the 5-year (OS) rates and the 5-year (DFS) rates according to treatment modalities in the first group versus the second group, (70.62% vs 61.00%, p=0.2145) for (OS) rates and (51.54% vs 44.38%, p=0.111) for (DFS) rates respectively. Nodal and hormonal status was the only two prognostic factors that significantly influence (OS) and (DFS) rates. Bone was the most common site of distant failure in this retrospective study. Conclusions: Despite the improvement in loco-regional recurrence rates, loco-regional recurrence free survival rates, five-year (OS) and 5-year (DFS) rates for patients in the first group who received radiation therapy, still the value of routine use of radiation therapy in early breast cancer is unclear.
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Key words: Breast cancer- early stage disease ¨C surgery- radiation therapy-systemic therapy.

1. Introduction:

Breast cancer remains as a common disease through out the world. Early detection and treatment can often lead to cure. Cure is most likely in women whose breast cancer is confined to the breast. Substantial number of women with spread to the loco-regional lymph nodes can also be cured with appropriate therapy [1].

The prognosis of early breast cancer is generally favorable. There fore, early diagnosis and early treatment are essential for better results [2]. During the 30 years time period of DBCG (Danish Breast Cancer Cooperative Group) post-operative radiotherapy in breast cancer has moved from orthovoltage treatment based on direct clinical (set up) to megavoltage radiation with 2D and 3D treatment planning [3]. Through these studies, DBCG has contributed with important evidence about the role of postoperative radiotherapy in early breast cancer, and thereby also

supported the current paradigm that optimal loco-regional tumor control has influence on survival [4].

2. Patients and Methods

Between January 1999 and December 2004, ninety patients with early breast cancer (T1-2,0-3 +ve axillary lymph nodes, M0) to whom MRM was performed ,had been treated inclusively at Clinical Oncology Department, Tanta University. Exclusion criteria were including history of contralateral breast cancer, presence of serious nonmalignant diseases (eg, cardiovascular or pulmonary), presence of severe mental or physical disorder, presence of connective tissue disease, and pregnant women. Eligible patients were including, female patients with histologically or cytologically proven breast cancer, patients with clinical performance status (PS) grade 0-2 according to WHO,[5] patients with stage I-IIB disease, patients with tumor size < 5 cm, adequate negative surgical margin,

dissected lymph nodes at least 10 nodes, no evidence of distant metastasis on clinical or radiological examination at the beginning of treatment, patients with have adequate renal, hepatic, and bone marrow parameters at the time of presentation, patients compliance and geographic proximity that allow adequate follow up.

PRE_treatment assessment

The following data were obtained from the files of the patients that fulfilled the clinco-pathlogical criteria of early breast cancer (stage I and II) [6]: Historyphysical examination, investigations and histopathological examination.

Study Design and Treatment:

The patients were categorized into two treatment groups : The first group , included 50 patients with early breast cancer treated with surgical interference, modified radical mastectomy + adjuvant radiation therapy + systemic therapy (chemotherapy and/or hormonal therapy).The second group, included 40 patients with early breast cancer treated with modified radical mastectomy plus adjuvant systemic therapy (chemotherapy and\or hormonal therapy) i.e, did not received post operative radiotherapy

Modified radical mastectomy (MRM) was the surgical procedure performed for 90 patients (50 patients of them received postoperative radiation therapy due to high risk factors for local recurrence as microscopic residual, vascular invasion, lymphatic permiation , high grade and age less than 35years). Axillary dissection with clearance of at least 10 lymph nodes was done for all patients. All patients were planned through 2-D simulator- planning system using isocentric technique. Eligible patients received irradiation to the breast and chest wall through two tangential fields and the draining lymph nodes. Dose in the range of 5000 cGy was delivered in five weeks, 200 cGy, daily for five days weekly. Patients with risk factors like -ve hormonal receptors, +ve axillary lymph nodes, stage IIB, large tumor size or –ve nodal status with risk category like tumor size more than 2cm,-ve hormonal receptors, grade II-III and age less than 35 years were received chemotherapy regimens like CMF (cyclophosphamide   600mg/m^2,   methotrexate   40 mg/m^2, and 5FU 600mg/m^2)(10 patients), FAC(5FU 500   mg/m^2,   adriamycin   50   mg/m^2 and cyclophosphamide 500mg/m^2)(30 patients), FEC (5FU 500mg/m^2,   epirupcin   100   mg/m^2 and cyclophosphamide 500mg/m^2) (37 patients). All three chemotherapy regimens was administrated I.V. every 21 days for average 4-6 cycles $\pm$ Tamoxifen 10 mg twice daily according to ER and PR receptors status for 5 years (65 patients).Her-2 receptors (human epidermal

growth factor) were not available for all patients in this study and so were not evaluated in our results as a high risk factor.

Endpoints and statistical methodology:

All patients entered in this study were monitored for treatment related events and toxicity according to RTOG and EORTC Radiation Morbidity [7], loco-regional failure ,distant failure, time to death and survival. The overall survival and disease free survival rates depicted on Kaplan-Meier methodology[8].The influence of the various variables on overall survival and disease free survival rates was assessed by using the Log Rank test[9], Pearson Chi-Square test and Fisher's Exact tests were used whenever appropriates. Differences were considered significant when P-value was < or =0.05.Analysis was done using (SPSS) version 9.

3. Results:

Patients characteristics

This retrospective study included ninety patients with early breast cancer (T1-T2, 0-3 +ve LNs, M0) presented at Clinical Oncology Department, Tanta University Hospital, during the period from January 1999 to December 2004. Patients demographics and baseline characteristics were listed in table (1).The mean age was 45.62 years+/-9.56 years old (range 16-77 years). Forty seven percent were premenopausal, 86% with negative family history of breast cancer, infiltrating ductal carcinoma was 90%, seventy two percent had +ve hormonal receptors and fifty three percent presented with stage IIB. MRM was the surgical interference that had been done for all patients. Among the 50 patients in the first group who were assigned to receive radiation therapy, the loco-regional recurrence rate was 8% (4\50) vs 22.5% (9\40) for the second group with no significance difference, p=>0.05, table (2).

Table (3) shows that 26.7% (4\15) of patients within the first group who received radiation therapy developed loco-regional failure with time gap >3 months since surgery in comparison to zero percent (0\35) of patients within the same group with the time gap <3 months since surgery with significant p value =< 0.001.

The toxicity profile with radiation therapy was recorded according to RTOG and EORTC Radiation Morbidity (1995)[7]. Acute complications of the 50 patients in the first group who received radiation therapy were skin desquamation, radiation pneumonitis and radiation carditis (20% G3, 10% G3, 2%G2) respectively. While late complications were hyperpigmentation, radiation ulcer, lung fibrosis and

ischemic heart diseases (30% G1, 4%G4, 2% G3,4% G2) respectively, Table (4). Sites of distant failure in 90 patients with early breast cancer were summarized as followed bone, liver, lung and brain (5.6, 2.2, 4.4 and 2.2%) respectively, Table (5).

Survival:

At time of analysis ,the median duration of follow up was 72 months ,(range,12-90). On the basis of Kaplan-Meier estimates, the median overall survival (OS) for all patients in this study (n=90) was 74 months (range 12-92 months) (95% CI, 0.55-0.96) with the 5- year OS 67.15%, Fig (1). The median DFS was 56 months (range,10-90 months) ,(95% CI,0.59-0.86) with the 5- year DFS 48.55%, Fig, (2).

According to treatment modalities, the 5-year OS rates were (70.62 % vs 61.00%, p=0.2141) in the first and the second group respectively with no statistical significant difference, Fig (3).For 5-year DFS rates were (51.54% vs 44.38 %, p=0.111) respectively for the first to second groups with no statistical significant

differences, Fig,(4). Finally, there was statistical significant differences for the 5-year loco-regional recurrence free survival in the first and second group according to treatment modalities (88.24% vs 73.16%, p=0.03) respectively, Fig, (5).

Prognostic factors:

The 5-year OS rates were not significantly correlated with age (p=0.20), menstrual status (p=0.459), tumor site (p=0.9513), tumor size (p=0.89), pathological subtypes (p=0.202), tumor grades (p=0.636), stage (p=0.70), lines of treatment modalities (p=0.21) (Fig 4). The only two prognostic factors with statistical significance were, hormonal receptor status (p=0.02) Fig (6) and nodal status (p=0.04) Fig (7), Table (6).

For the 5- year DFS, there was statistical significant difference for the same two prognostic factors, hormonal receptors status (P=0.004), and nodal status (P=0.0042). There was no statistical significant difference for all other prognostic factors, table (7).

Table (1): Patients demographics and baseline disease characteristics in 90 patients with early breast cancer.

	Characteristics	No	%
Age in years	<35	28	31.1
	>35	62	68.9
Mean	45.62		
Median	45		
SD	9.56		
Menstrual status	Premenopause	43	47.8
	Postmenopause	47	52.2
Number of pregnancy	Nullipara	9	10.0
	Unipara	20	22.2
	Multipara	61	67.8
History of contraceptive pills	-ve history	28	31.1
	+ve history:	62	68.1
	> 2 years	52	57.9
	< 2 years	10	11.1
Family history	+ve family history	12	13.3
	-ve family history	78	86.8
Tumor site	Upper outer quadrant	55	61.1
	Upper inner quadrant	10	11.1
	Lower outer quadrant	14	15.6
	Lower inner quadrant	10	11.1
	Central	1	1.1
Tumor grade	Grade I & II	67	74.4
	Grade III	23	25.6
Pathological type	IDC	81	90
	Others	9	10
Hormone receptors status	HR +ve	65	72.2
	HR –ve	25	27.8
Nodal status	-ve	30	33.3
	+ve (1-3)	60	66.7
Tumor status	T1	7	7.8
	T2	83	92.2
Disease stage	I	2	2.2
	IIA	40	44.4
	IIB	48	53.4
Lines of treatments	MRM+Rth+ Systemic therapy	50	55.6
	MRM+Systemic therapy	40	44.4

Table (2): Loco-regional recurrence in all patients with early breast cancer according to treatment modality.

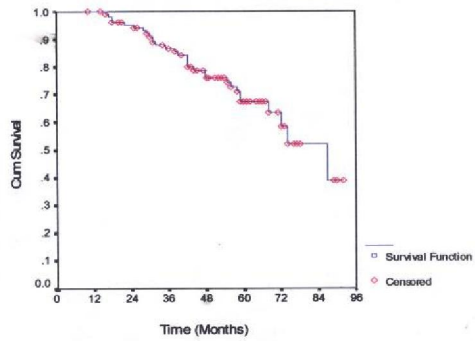| Treatment modality / Site of Recurrence	S+Rth+Systemic therapy (50)		S+ Systemic therapy (40)		Total	
	No.	%	No.	%	No.	%
Chest wall	0/50	0.0	5/40	12.5	5/90	5.6
Axillary LNs.	3/50	6	1/40	2.5	4/90	4.4
Supraclavicular LNs.	1/50	2	3/40	7.5	4/90	4.4
Total	4/50	8	9/40	22.5	13/90	14.4

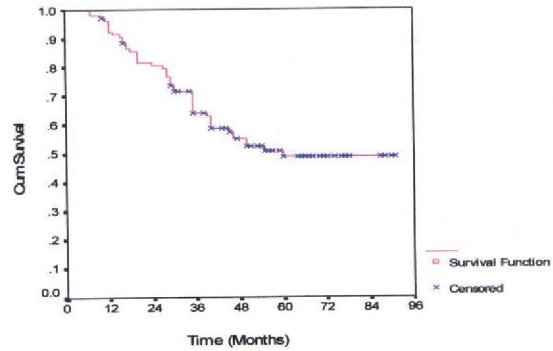**Fig. (1): Overall survival rate for the whole patients.**

Fig. (2): Disease free survival rate for the whole patients
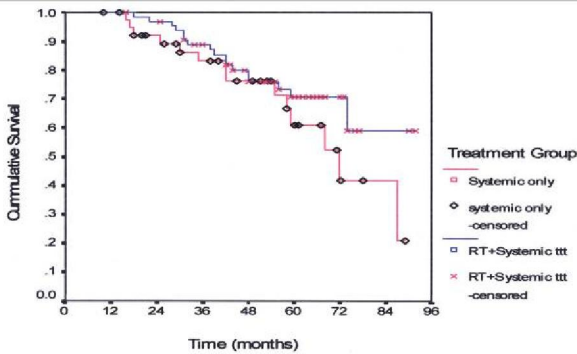

Fig, (3): Survival rate of all patients according to treatment modality
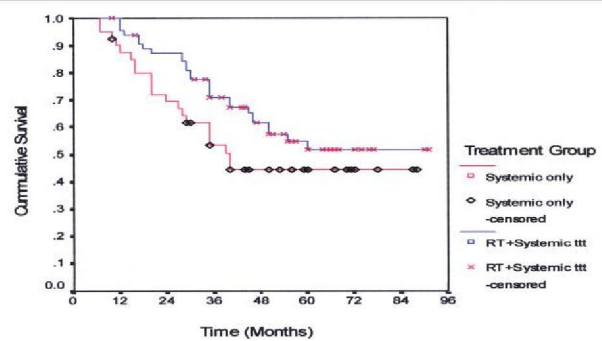

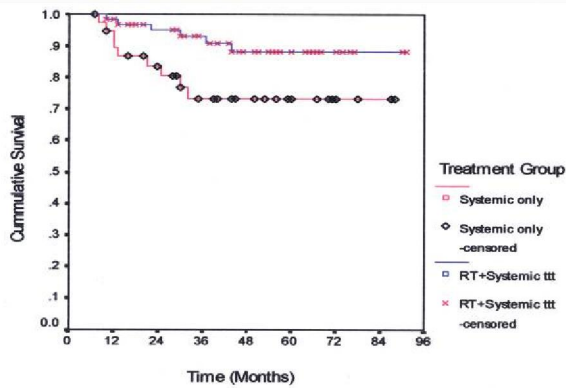**Fig. (4):Disease free survival rate of all patients according to treatment modality**

Fig, (5): Loco-regional recurrence free survival rates of all patients according to treatment modality.
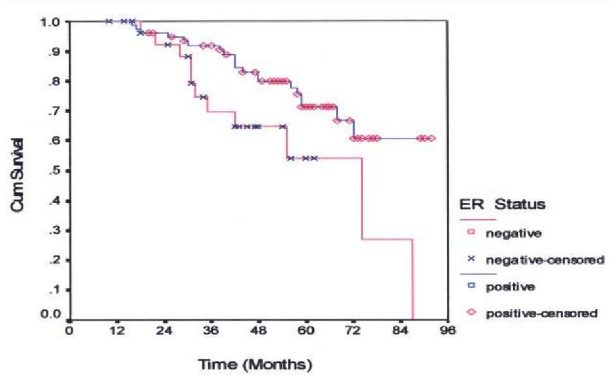

Fig, (6): survival rate of all patients according to hormonal receptor status
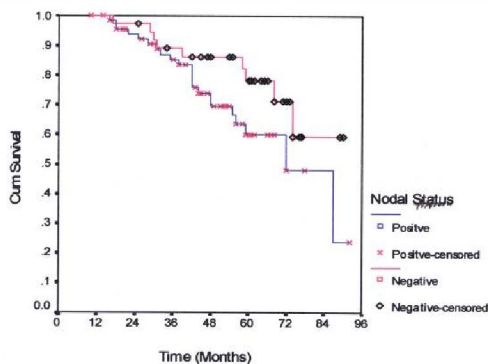
Fig, (7): Survival rate of all patients according to lymph nodes status.

Table (3): Frequency of loco-regional recurrence according to the time gap (surgery to radiation therapy) in 50 patients in the first group.

Time gap / Site of Recurrence	< 3 months		> 3 months	
	No.	%	No.	%
Chest wall	0/35	0	0/15	0.0
Axillary LNs.	0/35	0	3/15	20
Supraclavicular LNs.	0/35	0	1/15	6.7
Total	0/35	0	4/15	26.7

Table (4): Acute and late complications of radiation therapy in 50 patients received adjuvant radiotherapy.

	Complications	G1	G2	G3	G4	No.	%
Acute	Skin desquamation			-		10/50	20
	Radiation pneumonitis radiation			-		5/50	10
	carditis		-			1/50	2
Chronic	Hyper pigmentation	-				15/50	30
	Radiation ulcer				-	2/50	4
	Lung fibrosis			-		1/50	2
	Ischemic cardiac diseases		-			2/50	4

Table (5): Sites of distant metastases according to nodal status and tumor status.

	NO. of cases	Bone		Liver		Lung		Brain	
		No.	%	No.	%	No.	%	No.	%
Node –ve	30	2	6.7	0	0.0	0	0.0	0	0.0
Node +ve	60	3	5	2	3.3	4	6.7	2	3.3
T1	7	1	14.2	0	0	1	14.2	0	0.0
T2	83	4	4.8	2	2.4	3	3.6	2	2.4
Total	90	5	5.6	2	2.2	4	4.4	2	2.2

Table (6): The 5-year overall survival rates for the whole patients according to different prognostic factors

Prognostic factors	Number of cases	5-years survival rate %	Relative risk& 95% confidence interval	p-value
Age				
≤35	28	58.17	0.91(0.82 - 1.55)	0.2089
>35	62	74.63		
Menstrual status:				
Premenopause	43	62.97	0.93 (0.70-1.40)	0.4595
Postmenopause	47	71.66		
Tumor site:				
Upper O.Q.	55	63.48		
Upper I.Q.	10	77.78		
Lower O.Q.	14	70.91	0.81 (0.41-0.89)	0.9513
Lower I.Q.	10	68.18		
Central	1	53.33		
Tumor size:			0.76 (0.46-0.90)	
T1	7	72.19		0.8980

T2	83	60.43		
Pathological types:				
IDC	81	61.34	0.68 (0.44-0.74)	0.2027
Others	9	88.42		
Tumor grade:				
I & II	67	69.55	0.85 (0.60-1.52)	0.6369
III	23	61.22		
Nodal status:				
-ve	30	78.17	0.50 (0.52-0.81)	0.0438[#]
+ve (1-3)	60	59.98		
Disease stage:				
Stage I	2	-	1.20 (0.84-1.15)	
Stage IIA	40	67.65		0.7029
Stage IIB	48	66.26		
Line of treatment:				
Surgery+Rth+systemic therapy	50	70.62	0.49 (0.54-0.69)	0.2145
Surgery+systemic therapy	40	61.00		
Hormone receptors status:				
HR +ve	65	71.27%	0.66 (0.52-0.61)	0.0210[#]
HR –ve	25	53.81%		

Significant.

Table (7): The 5-year disease free survival rate for whole patients according to different prognostic factors

Prognostic factors	Number of cases	5-years DFS rate %	Relative risk & 95% confidence relative	p-value
Age:				
≤35	28	45.36	0.98 (0.72 - 1.32)	0.8043
>35	62	49.38		
Menstrual status:				
Premenopause	43	36.25	1.00 (0.74-1.35)	0.0579
Postmenopause	47	60.35		
Tumor site:				
Upper O.Q.	55	50.78		
Upper I.Q.	10	51.95		
Lower O.Q.	14	38.18	0.76 (0.45-0.99)	>0.05
Lower I.Q.	10	36.36		
Central	1	57.17		
Tumor size:				
T1	7	54.55	0.86 (0.71-0.93)	0.7522
T2	83	47.41		
Pathological types:				
IDC	81	47.50	0.48 (0.38-0.56)	0.3465
Others	9	55.58		
Tumor grade:				
I & II	67	51.82	0.75 (0.74-1.110)	0.5675
III	23	41.34		
Nodal status:				
-ve	30	61.67	0.58 (0.42-0.70)	0.0405[#]
+ve (1-3)	60	41.50		
Disease stage:				
Stage I	2	0	1.00 (0.74-1.35)	0.0873*
Stage IIA	40	60.40		0.1918**
Stage IIB	48	41.90		0.1069***
Line of treatment:				0.1110
Surgery+Rth+systemic therapy	50	51.54	0.48 (0.34-0.60)	
Surgery+systemic therapy	40	44.38		
Hormone receptors status:				
HR +ve	65	54.38	0.46 (0.43-0.57)	0.0042[#]
HR –ve	25	28.24		

Significant.* Stage I vs. IIA. ** Stage I vs. IIB.   ***Stage IIA vs. IIB.

4. Discussion:

Parallel to the development in radiotherapy with more effective high-energy equipment and new treatment techniques the results have clearly demonstrated the proof of principle that primary loco-regional tumor control has impact on survival even in patients who also receive an adjuvant systemic therapy. The first convincing results of adjuvant systemic therapy in early breast cancer appeared in the mid

seventies, especially premenopausal women with node positive diseases seemed to obtained a pronounced improvement in diseases –free survival [10].These results led to a paradigm shift in the treatment of early breast cancer towards a more multidisciplinary approach in high-risk patients [11].

In this study, the incidence of breast cancer accounted for 27.6% of all female malignancy and 13% of all cases registered in our department. These figures are slightly lower than other registry [12]. Carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 34% of National Cancer Institute cases.[13]

The median age of the patients in our study was 45 years and the mean age was 45.62 years (range: 16-77 years) [14], other study reported that the mean age of early breast cancer patients was 66 years [15, 16]. Concerning tumor size11.54% for T1 tumors, and 88.46% for T2 tumors, while in a series of 1234 cases, T1 tumors constituted 80% followed by T2 (20%) [17]. The lower incidence ofT1 tumors in Egypt may be explained by relatively late presentation due to lake of health education, screening programs and low socioeconomic factors leading to delay in seeking medical advise. In contrast, western countries programs of self breast examination and screening mammography are frequently carried out. Regarding the prognostic effect of the age, our results revealed that the younger the age, the worse the prognosis, as the five-year survival rates were 58.17% vs. 74.63% for the patients age group <35 years vs. >35 years respectively and the difference was statistically not significant,[15,18,19]. No, statistical significant difference for T1 versus T2 tumors for OS and DFS [20,21]. The axillary lymph nodes status and hormonal status were the most important factors in determining the prognosis, the 5-year survival rates for node negative patients were higher than that with 1-3 positive nodes (78.17% and 59.98% respectively) OS[22,23,24], where lymph node metastasis was a dependent factor for local failure, locoregional failure, distant failure, DFS and OS. As regard HR, (71.27% and 53.15%) OS For +ve HR versus –ve HR were respectively. ER (Estrogen reseptor)expression in the breast sample analyzed was associated with good prognostic factors, such as low histological and nuclear grade, smaller tumor size and fewer metastatic axillary lymph nodes. In general, ER expression was higher in patients in earlier clinical stages of the diseases. Determination of the presence of this receptor is fundamental, not only for its prognostic value but also for its unquestionable predictive value of response over time to sustained cancer – specific therapy. The same has not occurred with PR (progesterone receptor) which lost its independent predictive value during 1990s.[25]

As regard loco-regional recurrence, the incidence of loco-regional recurrence for whole patients was 14.44% (13\90), recurrence in chest wall was5.55%(5\90), reccurance in axillary lymph nodes was 4.44% (4\90) and supraclavicular lymph nodes was 4.44%(4\90).In previous clinical trial, loco-regional failure was 49%, chest wall recurrences was 23%, loco- regional nodes was 13%[26] and this conflict may be due to small number of patients in our study.

Twenty six percent of patients who received radiation therapy within more than 3 months from the surgical interference had developed loco-regional failure compared to no patients in the category who received radiation therapy within 3 months since the surgical treatment , and this difference was statistically significant, delays in initiation of radiation therapy more than 3 months were associated with higher loco-regional failure, overall mortality, cancer specific mortality and associated with poor survival[27].On the other hand , the 5-year local recurrence rate was significantly higher in patients treated with adjuvant radiotherapy more than 8 weeks after surgery than in those treated within 8weeks since surgery but there was little evidence about the impact of delay in radiotherapy on the risk of metastases or the probability of long term survival.[28]

Five-year OS rates were 70.62% vs 61.00% for the first versus the second treatment groups. Many authors had reported the treatment results for patients treated with modified radical mastectomy only without radiotherapy in early breast cancer, [29] (84%), [30] (64%),[31] (42%),[32,33] (49%),[34] (58%) and [35].

The five-year loco-regional recurrence free survival rates of all patients according to presence or absence of radiation therapy were 88.24% vs. 73.16% respectively and this difference was statistically significant (p=0.0308), adjuvant radiation therapy improve local control and provide a significant survival benefit for early breast cancer patients.[36]

In the present study the adverse events with radiation therapy were reported according to RTOG common toxicity criteria [7], skin desquamation was the most common acute side effect of radiation therapy (20%), moist desquamation was reported in 31.4% patients [37]. The incidence of radiation pneumonitis was very low (1.2%) [38] unlike pneumonitis represented by 10% among our patients, and this may be due to the difference in the physical and treatment consideration such as the conformal 3D-planning and treatment with linear accelerator with much exclusion of the lung volume included in the irradiated field. Ischemic heart diseases represented by 4%, in this trial may be due to inclusion of the anterior part of the heart which results in excess cardiac morbidity and mortality. Further, the standard adjuvant chemotherapy

in most patients now includes more cardiotoxic drugs, such as anthracyclins and Herceptin[39]. EORTC (22922\ 10925) was decided that in high risk ,irrespective of lumpectomy or mastectomy, the target should not include the internal mammary nodes (IMN) in patients with left-sided breast cancer whereas the target in patients with right-sided breast cancer if possible should include the IMN .The challenge for on-going trials in early breast cancer is to design more focused treatment planning in the individual patients with optimized tumor control and reduced radiation dose to critical tissues[40,41,42].

In conclusion, early stage breast cancer, radiation therapy is considered as cornerstone and the standard of care not only to decrease loco-regional failure but also to improve survival (DFS and 0S). It is the ambition to continue to do trials and answer questions regarding optimal radiotherapy in early breast cancers in Egypt. Systemic therapy should be added in early breast cancer patients guided by the developed prognostic and predictive knowledge to confer survival improvements (DFS and OS).

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