Life Science Journal

Life Science Journal   2012   Volume 9,   Number 4,   Part2   ISSN:1097-8135

Marsland Press

PO Box 180432, Richmond Hill, New York 11418, USA

Website:
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ISSN 1097-8135

9 771097 813002

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Multidisciplinary Academic Journal Publisher

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I

Seyyed Mehdi Hashemi

Evaluation of Hepatotoxicity of Valproic acid in albino mice, Histological and Histoistochemical studies

Ibrahim,M. A

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Abstract: Background: Epilepsy is an abnormal functional state of the central nervous system that is characterized by uncontrolled nerve cell activity and clinicaly by convulsive seizure with or without loss of consciousness. Valproic acid is widely used antiepileptic medication and has sever toxic effect on liver , this study aimed to investigate the histopathological and histochemical changes due to the effects of therapeutic dose (25mg/kg b.w.) of antiepileptic drug (Valproic acid) in albino mice. **Material and methods:** Forty mice were used, they were divided into four groups, one group serves as control group and the other three groups (A, B and C) administered the drug as follow: Group A received Valproic acid for 15 days, group B received Valproic acid for 30 days and group C, the recovery group (animals were administrated with drugs for 30 days then administration was stopped for another 10 days). The animals were sectioned 24 hours after the last dose, liver was taken for histopathological and histochemical studies. **Results**: The drug induces toxic effects on liver tissue which showed vacuolar degenerative changes, hypertrophied nucleus with fragmented chromatine, inflammatory cells aggregates and congested vasculature.**Conclusion:** Valproic acid has sever toxic effects on liver tissue and the toxicity was time related. Sort of recovery was recorded after discontinuation of the drug so its effect was reversible.
 [Ibrahim,M. A. **Evaluation of Hepatotoxicity of Valproic acid in albino mice, Histological and Histoistochemical studies**. *Life Sci J* 2012;9(4):153-159] (ISSN:1097-8135). http://www.lifesciencesite.com. 23

Key words: liver- tissue- valproic acid- hepatotoxicity.

1. Introduction

Epilepsy affects 5 -10 per 1000 of the general population. It is due to sudden, excessive depolarization of some or all cerebral neurons. This may remain localized (focal seizure) or may remain generalized seizure, it may also affects all cortical neurons. Bromide (1857) was the first treatment of epilepsy, but it use now obsolete **(Bennett *et al*., 2003).**

Valproic acid is a branched –chain carboxylic acid similar in structure to endogenous fatty acid **(Bruni and Albright, 1993).** It was approved for treatment of epilepsy either as monotherapy or in combination with other anticonvulsant drugs **(Beatalden *et al*., 1979).** It is also used in the treatment of a variety of neuropsychiatric illnesses as mania, bipolar affective disorder, migraine, headache, prophylaxis and several anxiety disorders **(Owens and Nemeroff, 2003).** It elevates the whole brain gamma amino butyric acid (GABA) a major inhibitory neurotransmitter in the brain, and inhibits GABA degradation. **(DeVane, 2003).** Some severe side effect associated with Valproic acid treatment as encephalopathy which can be accompanied by hyperammonemia**, (Schmidt, 1984)** other side effects as Hepatotoxicity, thrombocytopenia, platelet aggregation and pancreatitis **(Kis *et al*., 1999).**

In **(1991) Siemes and Nau** found that the sever hepatotoxicity associated with Valproic acid is due to depletion of β-oxidation with increased synthesis of toxic unsaturated Valproic acid derivates. Another hypothesis lies in the possible Valproic acid induced depression of free radical scavenging enzyme activities.

Raza *et al*. (2000) studied the effect of Valproic acid on mouse liver, they found that this drug caused marked alteration in liver cell morphology, which was proportional to the period of treatment. Valproic acid induces fatty degeneration of hepatocyte, swelling and increased number of kupfer cells. Prolonged time of administration produce inflammation of portal tract, albumious degeneration and necrosis of septa, .precirrhotic condition, cirrhosis, degeneration of hepatocyte and glassy eosinophilic homogenous cytoplasm. When the time of administration increase the portal tract invaded by small, rounded inflammatory cell, hepatocytes were swollen with large nuclei and increased amount of condensed chromatin.

It was found that steatosis and necrosis of hepatocyte are the main histopathological changes in liver of albino rat induced by toxic dose of Valproic acid**. (khan *et al,* 2005).**

EL-Deeb, (2006) studied the effect of Valproic acid on the exocrine part of pancrease when used alone and when given concomitantly with L-cartnitine. When animals treated with Valproic acid alone the result obtained, showed variable degree of acinar degeneration and cellular infiltration between the acinar. The collagen fibers around the blood vessels were increased. Ultrastucturally, there were dilation of rough endoplasmic reticulum and Golgi appretus, focal destruction of mitochondria, increased number of secondary lysosome, decreased or even depletion of

zymogene granules and nuclear changes. Fat droplets in the basal part of acinar cells, cytoplasmic vacuoles as well as DNA damage were also noticed. On the other hand, pancreatic section of animals that were treated with Valproic acid and L- carnitine showed marked reduction of the pervious cellular change and cytoplasmic organelles were slightly affected. So L–carnitine minimizes the adverse effects of Valproic acid on the exocrine part of pancrease.

According to **Khera (2005),** the teratogenic effects of Valproic acid on rats, was in form of abortions, reduction in the number of live fetuses and defects of the tail, rib phalanx, cytotrophoblasts and suppressed proliferation of fetal capillaries, reduced diameter nearing obliteration of umbilical vessels with or without karyorrhexis of embryonic tissue .the lesion in the placental labyrinth were specific but in the embryonic tissues they were generalized. It was postulated that the vascular lesion in the labyrinth and umbilicus may have influenced embryonic development by reducing maternoembryonic gaseous and nutritional exchange.

As Epilepsy is an abnormal functional state of the central nervous system that is characterized by uncontrolled nerve cell activity and clinicaly by convulsive seizure with or without loss of consciousness. Valproic acid is widely used antiepileptic medication, the aim of this work is to investigate the toxicity of Valproic acid on liver tissue .The drug was stopped for a period to study the recovery possibility of this toxicity.

2. Materials and methods:
Drug used:
Valproic acid is a white hydroscopic, crystalline powder very soluble in water. Each 100 ml depakine syrup contains 5.7 gm sodium Valproate. Produced by Global Napi pharmaceuticals Egypt. The experimental dose used in this study is the therapeutic dose of Valproic acid (25) mg/kg b.w. This dose was converted using conversion equation between different species **(Paget and Bernes, 1964).**

Experimental animals:
Forty adult female albino mice, each weighing 25-35 gm. were kept in the laboratory under standerdized condition of temperature and were maintained on a standerd diet and water *ad libtium* for at least one week before the expermintal study. They were divided into four groups as follow:

Group 1: The control group consists of ten mice.

Group 2: Ten animals were treated intraperitoneal with Valproic acid for 15 days.

Group 3: Ten animals were treated intraperitoneal with Valproic acid for 30 days.

Group 4: Ten animals were treated intraperitoneal with Valproic acid for 30 days then left to recover for another 10 days.

Methods:
1- Histopathological preparations:
Animals were sacrified by cervical dislocation, dissected and small pieces of liver were fixed, processed for light microscopic examination, sections were stained with Ehrlish H&E **(Bancroft and Gamble, 2002).** The cytoplasm appeared pink and nuclei acquire a blue color.

2- Histochemical preparation:
a- PAS-Reaction
The Periodic acid reaction (PAS), **(Hotchkiss and Marmu, 1954)** as applied for carbohydrate demonstration Fixation was carried out in 10% neutral buffered formalin, Paraffin section (5um thick) were brought down to water then placed in 1% periodic acid for 5 minutes, washed for minutes in running water, rinsed in distilled water and treated with Schiff's reagent for 20 minutes Section were then transferred through freshly prepared 0.5 % sodium bisulfate for 3 changes, followed by 5 minutes in running tap water, dehydrated, cleared in xylol and mounted in DPX. The positive materials appeared pink.

b-Total proteins:-
The total proteins were demonstrated by using the mercuric bromophenol blue method (BPhb) **(Mazia *et al*., 1953).** Small pieces of tissue were fixed in neutral 10 % buffered formalin. Staining was carried out in mercuric bromphenol blue stain and differentiated in 0.5% acetic acid. Tertiary butyl alcohol was used for dehydration the sections were then cleared in xylol and mounted in DPX Proteinic substance acquired deep blue color.

3. Results
1-Histopathological results
Control group:
A cross section of normal liver of mice shows that the hepatocytes are arranged in cords or plates, one or two cell thick, forming the normal liver cords radiating from the central vein. The space lying between the hepatic cords constitute hepatic sinusoid, which are lined with flattened endothelial cells and few phagocytic cells namely Von Kupffer cells. The nuclei of the later are spindle shape. The cytoplasm of the hepatic cells is pink in color with scattered basophilic granules. The nuclei of the hepatic cell are rounded in shape with granular chromatin material, some hepatocytes are binucleated. (Fig.1).

Treated groups:-
Histopathological examination of liver section in tissue of animals treated with Valproic acid for 15 days, revealed partial distortion of liver architecture, accompanied with vacuolar degenerative changes seen focally in hepatocytes.

Focal areas of necrosis could be also detected (Fig.2) scattered focal aggregates of inflammatory cells seen in portal areas(Fig.2&3) and between hepatocytes. (Fig. 4). Congested portal vein (Fig.5) with enlarged atypical cells could be recognized in areas. (Fig.6)

When animals were treated with Valproic acid for 30 days section in liver tissue showed, marked distorted hepatic architecture, In addition to scattered multifocal necrotic areas also were detected. Majority of hepatocyte showed vaculation, (Fig.7) accompanied with variation in nucleus regarding to size and shape, hypertrophied nuclei with fragmented chromatin could be also seen.(Fig.8) normal mitotic figure were occasionally seen (Fig.9)

Sections in liver of mice treated with Valproic acid for 30 days then left for 10 days to recover showed mild improvement where normal lobular architecture could be identified in areas, hepatocytes were intact with vesicular nuclei. However mild focal necrosis, with congested dilated central vein was observed in areas, in addition to hepatocellular vaculation. (Fig.10)

2-Histochemical Studies
1- Polysaccharide:-
Control:-

In the liver section obtained from control mice, a markedly strong PAS reaction was clearly illustrated indicating their richness in polysaccharide. The reaction product was demonstrated in the form of intensely red color particles condensed together into massive patches located exclusively in the cytoplasm of the individual hepatocytes, since the nuclei have not acquired any PAS positive reactivity. Such reactivity was comparatively stronger in the peripheral than the inner (centrilobular) areas. (Fig.11).

Treated groups:-

Inspection of the polysaccharide content in the liver tissue of Valproic acid treated group for (15 days) revealed mild to moderate loss of their content, where polysaccharide content appeared as small aggregates of fine granules dispersed in the cytoplasm of hepatocytes. Longer duration of Valproic acid administration (30 days) produced complete depletion of polysaccharide content. After 10 days recovery, revealed no significant restoration of polysaccharide content, however with differences within liver areas, where focal areas of hepatocytes appears with traces of polysaccharide.(Fig.12&13&14).

2-Total protein:-
 Control group:-

Using the mercuric bromophenol blue method, the protein content of liver cells of normal mice was demonstrated as numerous irregular blue particles against a lightly stained cytoplasm. These particles were scattered in all the cytoplasmic

regions. The cells were limited by intensely stained plasma membranes. The nuclear envelops, the nuclei as well as some chromatin element were also positively stained. (Fig.15).

Treated groups:-

In Valproic acid treated mice, the liver manifest, mild to moderate loss in the protein content in their hepatocyte the loss was time dependent, where 30 days treatment induce further loss than 15 days treated time, whereas the group of animal treated for 30 days, followed by 10 days recovery showed restoration to great extant, in 15 days group, the cells membrane, nuclear membrane and nuclei attain high staining affinity, while some hepatocytes showed mild diffuse portentous material or few deeply stained large particles, meanwhile in 30 days moderate stain affinity were noticed in cell membrane, nuclear membrane and nuclei. (Fig. 16 & 17 & 18).
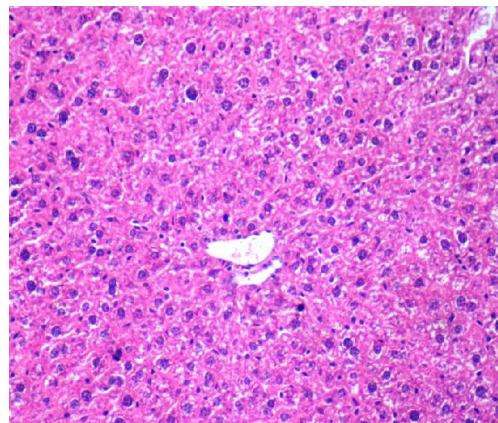
Fig(1):A photomicrograph of liver section in control animal showing normal lobular architecture (H&E). (X200)
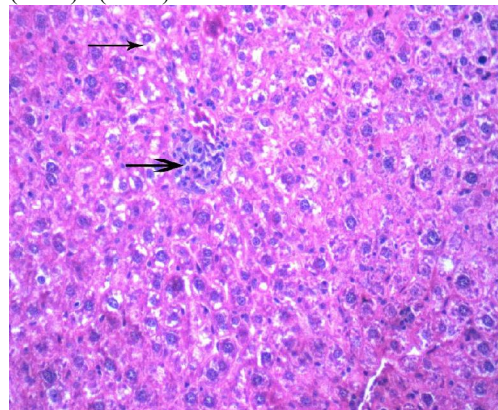
Fig(2): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing vacuolated hepatocyte (thin arrow) focal necrotic area (thick arrow) partial distortion of liver architecture (H&E). (X200)
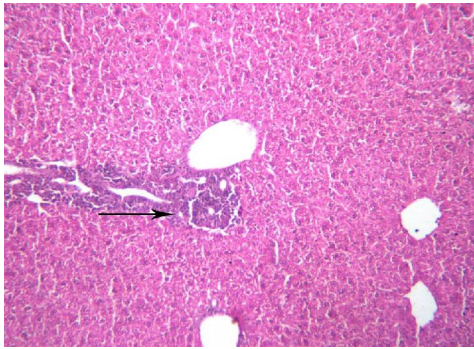
Fig(3): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing focal inflammatory cell aggregates in portalarea(arrow) (H&E).   (X100)

Fig(4): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing focal inflammatory cell aggregates between hepatocytes area (arrow) (H&E). (X200)

Fig(5): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing dilated congested portal vein (arrow) (H&E). (X400) (X400)

Fig(6): A photomicrograph of liver section in animal treated with Valproic acid for 15 days showing enlarged atypical hepatocytes (arrow) (H&E). (X400)

Fig(7): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing focal necrotic area (thin arrow), vacuolated hepatocytes (thick arrow) and marked distortion of liver architecture (H&E). (X400)

Fig(8): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing variation in nuclear size and shape,(thin arrow) enlarged nucleus with fragmented chromatin (thick arrow) (H&E). (X400)
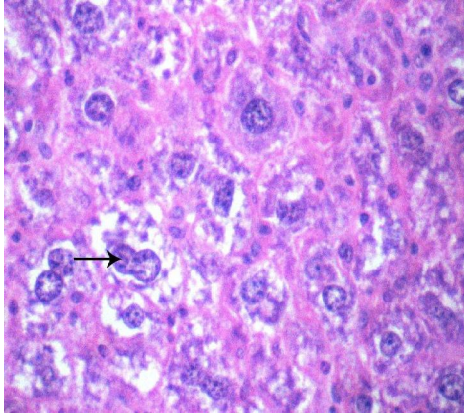
Fig(9): A photomicrograph of liver section in animal treated with Valproic acid for 30 days showing normal mitotic figure (thin arrow)(H&E). (X400)

Fig(10): A photomicrograph of liver section in animal treated with Valproic acid for 30 days then left to recover for 10 days showing normal liver architecture, mild necrosis (thin arrow), dilated congested central vein (thick arrow) and vacuolated hepatocytes (arrow head)(H&E). (X400)

Fig(11): Liver section of control animal showing normal polysaccharide content in hepatocytes (PAS)   (X400)

Fig(12): Liver section of animal treated with Valproic acid for 15 days showing moderate loss in polysaccharide content in many hepatocytes. (PAS) (X400)

Fig(13): Liver section of animal treated with Valproic acid for 30 days showing sever loss in polysaccharide content in liver hepatocytes (PAS) (X400)
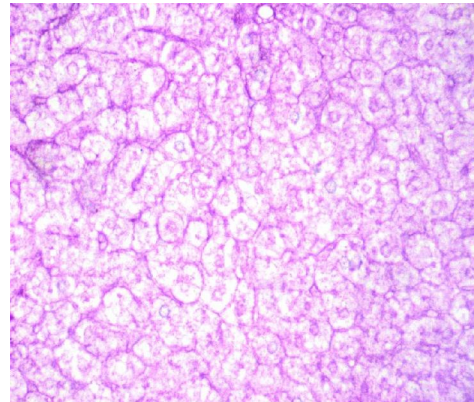
Fig(14): Liver section of animal treated with Valproic acid for 30 days then left for 10 days to recover showing sever depletion in polysaccharide content in most of hepatocytes (PAS)   (X400)
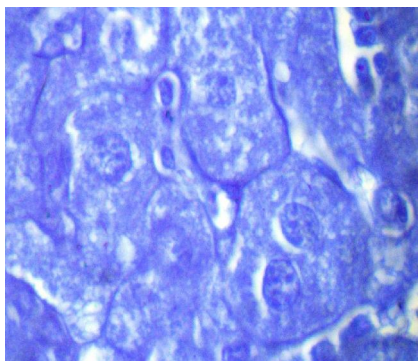
Fig(15): Liver section of control animal showing normal protein content in hepatocytes(Bphb). (X1000)
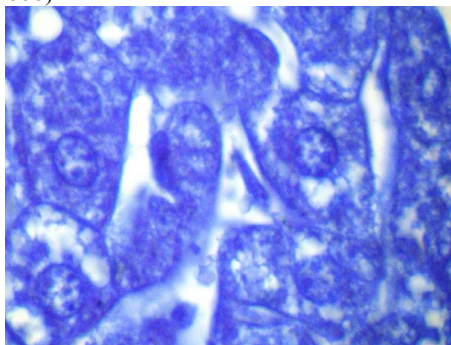
Fig(16): Liver section of animal treated with Valproic acid for 15 days showing mild loss in protein content In hepatocytes (Bphb). (X1000)
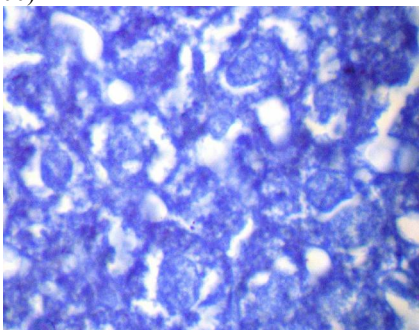
Fig(17): Liver section of animal treated with Valproic acid for 30 days showing moderate loss in protein content in hepatocytes (Bphb) (X1000)
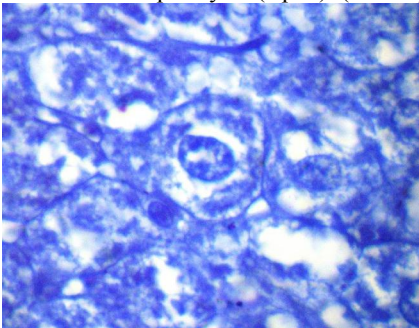
Fig(18): Liver section of animal treated with Valproic acid for 30 days then left for 10 days to recover showing mild loss in protein content in hepatocytes (Bphb). (X1000)

4. Discussion:
1. Histological studies

The liver tissue of Valproic acid treated mice showed histopathological changes which include partial to sever distortion of liver architecture, congested vasculature, scattered to multifocal necrotic areas with focal aggregates of inflammatory cells, vacuolar degenerative changes, enlarged atypical hepatocytes, also variation in nucleus regarding to size and shape and hypertrophied nucleus with fragmented chromatin. However after a period of recovery mild improvement, where normal lobular architecture could be seen, with massive vacuolated hepatocytes.

These results were in agreement with the finding of Khan *et al.*, 2005who reported the histopathological changes of toxic dose of Valproic acid in liver of albino rats, these changes represented in partly distorted in the lobular architecture, in addition to foci of inflammatory cell infiltrate focal necrosis and congestion in the portal areas. It was postulated that Valproic acid aberrant metabolites or mediation of lipid per-oxidation might be the underlying mechanism of serious hepatic reaction.

Vacuolar degenerative changes and enlarged nucleus with condensed clumped chromatin material in hepatocytes due to administration of Valproic acid in our study have also been reported by Raza *et al.*, 2000who found that when mice given sodium Valproate 0.71% weight / volume in drinking water for 7, 14, and 21 days caused alteration in liver cell morphology, in the form of degeneration of hepatocytes and glassy eosinophilic homogenous cytoplasm, also hepatocytes were swollen ,with large nuclei and increased amount of condensed chromatin. These changes were proportional to the period of treatment, so prolonged use of this drug should be carefully assessed.

After 30 days administration of Valproic acid, hepatocytes revealed variation in shapes and size of nucleus. This may be confirmed by Isharwal *et al.,* 2009 who reported that Valproic acid treatment caused significant nuclear alterations in normal drug-filtering organs (liver and kidney tissue), this due to the fact of Valproic act as Histone deacetylase inhibitors and promising anticancer agents that change the acetylation status of histones and loosen the chromatin structure.

Wiegand *et al.*, (2009) reported that Valproic acid may cause impairments in fatty acid metabolism and disrupt the urea cycle leading to hyperammonemia which is considerd as marker of liver disease.

2-Histochemical Studies
1-polysacchride:-

In the liver tissues, carbohydrate inclusions have been proved to be almost formed of glycogen.

These inclusions were markedly reduced as a consequence of Valproic acid treatment, being more pronounced with increase time of administration, after recovery period there were mild restoration of carbohydrate content in the liver tissue.

These results were in agreement with the results of (Turnbull *et al*., 1983), who found that Valproate administration inhibit gluconeogenesis by 30-50% in isolated rat hepatocytes lead to decrease glucose deposition in hepatocytes, this was explained in terms of the accumulation of Valproyl –CO A and its further metabolites in the matrix of hepatic mitochondria .

Similar results were obtained by Thurstone *et al*., 1985, who reported that even a single dose of Valproate in the therapeutic range for man caused significantly reduction of the plasma glucose concentration.

The present results were agreed to great extent with the result of Kesterson *et al*. (1984) who found that PAS positive material was not observed in the hepatocytes of the rats treated with Valproic acid and its metabolites.

2-Total protein:-

The present study indicated that protein content was depleted significantly in liver tissue and this depletion was directly proportional with duration, but after recovery period there was restoration of protein content.

The pervious changes in the liver tissue may be coincide to great extent with the result of Thurstone *et al*., 1985 who observed that concentration of aspartate, glutamate and glutamine (amino acids) were reduced. All of this changes induced by valproate metabolite which can be explained by decrease of acetyl CO-A due to the accumulation of acid –soluble (non-acteyl) CO-A esters which would limit the activities of one or more enzymes in the pathway of fatty acid oxidation and the Krebs citric acid cycle. Decreases in acetyl Co-A would limit both ketogenesis and gluconeognesis.

It can be concluded that the administration of Valproic acid produce hepatic injury. The results suggest that histopathological and histochemical changes are dependent upon the duration of exposure to the drug so prolonged use of these drugs should carefully assessed.

All described changes induced by Valproic acid, were partially reversible, so kind of recovery could be detected after discontinuation of these drugs

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9/5/2012

Optimal operation of a Microgrid in the Power Market Environment by PSO Algorithm

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Abstract- In this paper for optimal operation of a microgrid a model is represented including wind turbine, photo voltaic, generator diesel, battery bank, converter, critical load and controllable load. This Microgrid Management System (MMS) generates an optimum operation plan for a microgrid on next day. Modeled microgrid has ability of converting electric energy to main grid. At proposal model, uncertainty in predicting wind velocity is considered. Operation of this microgrid with purpose of reduction of cost is optimized. In this paper, the Particle Swarm Optimization Algorithm (PSO) is used for optimization. At the end of a model example for applying the results of the proposed model will be examined and analyzes the results. Results show that the model is an appropriate method for the operation of this microgrid.

Keywords: Microgrid, Optimal Operation, Reduction of Cost, Uncertainty, Power Market, PSO Algorithm

Introduction

Interconnection of small, modular generation and energy storage to low or medium voltage distribution systems forms a new type of power system, the microgrid (MG) [1]. Microgrids can be connected to the main power network or be operated autonomously, similar to power systems of physical islands. In other words, the microgrid concept assumes an aggregation of loads and micro sources (<500 kW) operating as a single system providing both power and heat.

Currently, power in the entire world leaded to construct changes in order to reach to power grid simultaneously and also use of Distributed Generation (DG) dispersed. Many years ago, electricity was produced in large power plant and generation, transmission, distribution and providing electricity were done at there. But today production and consume are in a competitive space and equipments of this industry should move to its functions and activities in order that electricity industry has efficacy commercially. As a result this power market expelled from exclusion of few counties and thousands producers will substitute it If they accomplish this, the consumer will have the opportunity to choose their electricity supplier. Main reasons of different countries refer to production references of DG include higher efficacy, little pollution, flexibility at consuming fuel and reduction of need to development of transmission and distribution system. Distributed Generation (DG) has

been available, such as solar systems, wind turbines fuel cell, small-scale gas turbine units. DG use local energy source which are Renewable Energy Sources (RES), for electricity production. Amount of converted energy compared with produced values from large central power plant are little. So they are consumed at its installment place. Most of percent of electrical energy is consumed at place of production, as energy is consumed or microgrid is generated. Integration of little and modular productions and energy store at low-voltage systems, from a new type of power system called microgrid [1]. At recent years, different researches about shapes and sizes of micrgrid were done [2, 3]. Also a lot of proposals about fundamental relations of exploitation between different sources at microgrid for solving the problem of consumption optimization for fuel are available in papers [4]. In this paper a microgrid which includes wind turbine and photovoltaic unit and a diesel generator and battery and a converter and critical and controllable loads was modeled. Also this microgrid according uncertainty of power wind unit was made. Optimization of proposed model in this paper was done by means of Particle Swarm Optimization (PSO).

In the section 2 of this paper, we'll explain the structure and the direction of energy current in a suggestive model. In section 3 it is presented the uncertainty in forecasting wind energy production. In section 4, we'll explain suggestive model modeling and the extension of model by using the PSO algorithm. After that, a realistic case study is

presented in section 5. Finally, concluding remarks are given in section 6.

Structure and direction of energy current in suggestive model

The suggestive model of the microgrid is like to fig. 1 in this paper.
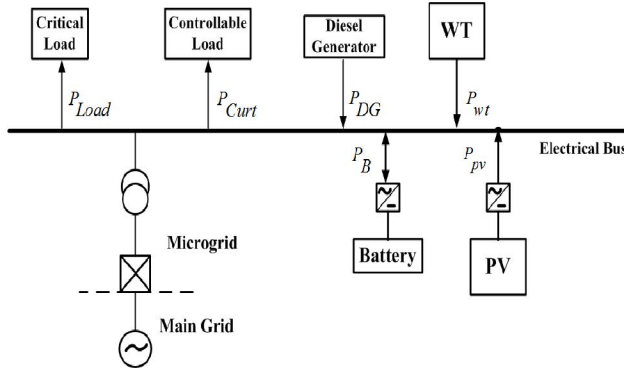
Fig. 1. Structure and direction of energy current

As it is observed in fig. 1, in this microgrid is a wind turbine (P_{wt}), photovoltaic system (P_{pv}), a diesel generator (P_{DG}), a battery bank (P_B) and converter DC/AC. In this microgrid output power from wind unit (P_{wt}), output power from photovoltaic (P_{pv}) as well as requested power provision are stored at batteries. In this model, batteries power (P_B) and diesel generator (P_{DG}) and requested power from controllable electrical loads (P_{curt}) covers emergency situation. Sizing this model was done by homer software and amounts and capacity of each unit, has been accounted. As we can see from fig.1, photovoltaic, wind unit and diesel generator provide power for critical (P_{load}) and controllable (P_{curt}) loads. In this microgrid is used a battery bank in order to store output energy of wind and photovoltaic. Microgrid with a main grid exchanges the power. Also microgrid has ability of selling and buying the electrical energy.

Wind turbine model

In order to account and amount of wind turbine output power according to different velocities of wind during 24 hours and Cut-in and Cut-out velocity of wind turbine we can use Eq (1) [5].

$$P_{wt}(t) = \begin{cases} a.V^3(t) - b.P_R & V_{c_i} < V < V_r \\ P_R & V_r < V < V_{co} \\ 0 & V < V_{c_i} \end{cases} \quad (1)$$

Which for a, b we have:

$$a = P_r / (V_r^3 - V_{ci}^3) \quad (2)$$

$$b = V_{ci}^3 / (V_r^3 - V_{ci}^3) \quad (3)$$

At above equation, P_r is indicator of generator rated power, V_{ci} is cut-in velocity, V_{co} is cut-out and V_r is turbine rated velocity.

Curvy of power-velocity of wind turbine is shown at fig.2.
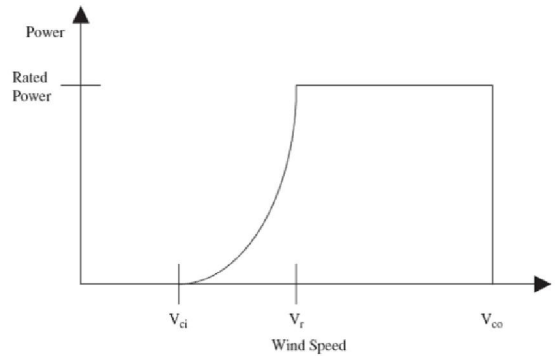
Fig. 2. Curvy of power-velocity of wind turbine

Model of photovoltaic panels

Output power of photovoltaic panel is accounted by their relation between amount of sunlight on the panels and environment temperature.

Output power from a photovoltaic panel is accounted by Eq(4).

$$P_{pv} = P_{stc} \frac{G_{ING}}{G_{STC}} (1 + k(T_c - T_r)) \quad (4)$$

P_{pv} is output power from photovoltaic panels, P_{STC} is maximum power of panel at STG, G_{ING} is amount of sun radiations, G_{STC} is sun radiation at standard state ($1000 \frac{w}{m^2}$), K is thermal coefficient, T_C is temperature of solar cell and T_r is environment temperature.

Model ling battery bank

For charging and de charging the batteries in a microgrid, amount of being charged of a battery is accounted by Eq (5) [5].

$$soc(t + \Delta t) = soc(t) + \eta_{bat} \left(P_B(t) \Big/ U_{bus} \right) \Delta t \quad (5)$$

Δt is interval time or interval of two successive times at one hour, η_{bat} is efficiency of being charged in a battery and this value reach to 100% for de charge, u_{bus} is DC bus voltage of microgrid, $P_B(t)$ is batteries power, $SOC(t)$ is amount of charge in a battery at time (t) which its unit is ampere hour (Ah).

$$SOC_{min} = (1 - DOD).Soc_{max} \quad (6)$$

Eq (6) show how a battery is de charged and SOC_{min}, Soc_{max} is respective low limit and high limit of battery charge state. DOD is percent of battery charge.

Diesel generator model

In order to account the diesel generator cost, two important costs are considered [6].

1. Fuel cost, 2. Maintain and repairmen cost

$$C_{D_G}(t) = C_{o\&m}(t) + C_{fuel}(t) \quad (7)$$

$$C_{fuel}(t) = P_{r_{fuel}}.(A + B.P_{DG}(t) + C.P_{DG}^2(t)) \quad (8)$$

$C_{D_G}(t)$ is diesel generator ($\notin$), $C_{o\&m}(t)$ is maintain and repairmen cost of diesel generator ($\notin$), $C_{fuel}(t)$ is fuel cost at diesel generator ($\notin$), $P_{r,fuel}$ is fuel price ($\frac{\notin}{L}$), $P_{DG}(t)$ is output power of diesel generator (Kw) and coefficients A,B,C are respective diesel generator at account part and given numerical results.

Uncertainty in forecasting wind energy production

Uncertainty in wind energy production is the characteristic of this natural energy, so when the utilization of networks, including the type of units is considering the issue of uncertainty is essential. If the location of wind units are geographically dispersed set of output changes greatly reduced the units and of course still has the problem of uncertainty considered. Fluctuations in power output of wind turbines in wind speed changes to come there, not completely random and are not completely predictable. For a long period activity of a wind farm, the prediction error is similar to a normal distribution function. In order for risk prediction error at issue reduced the utilization of the combined system, we can predict the errors at different levels, we calculated reliability. Prediction error of wind energy production, known as risk. For example, 95% said the level of probability that the prediction error is greater than the amounts of production risk is less than 5%. This method of production for the proposed wind energy to a specific level of reliability in production planning gains. Wind energy minus the predicted rate of production risk that can issue operating system should be used. Since the operator more willing to overestimate the production of wind generators is therefore a unilateral distribution curve is considered as normal distribution curve. The operator to determine the issue of risk needs to determine the production of a certain confidence level. The following equation estimates the high error level unilateral normal distribution curve with confidence level (α-100) % [7].

$$P[e - \mu_e \geq Z_\alpha \sigma_e] = \frac{\alpha}{100} \quad (9)$$

$$\tilde{e} = \mu_e + Z_\alpha \sigma_e \quad (10)$$

$\tilde{e}$ represents the high-risk relationships, μ_e producing an average forecast wind error standard deviation and σ_e are the wind forecasting error. Below a graphic expression of these equations with the confidence is level (α-100) %.

$$P\left(\frac{e - \mu_e}{\sigma_e} \leq z_\alpha \right) = P(e < \tilde{e}) = (100 - \alpha)\%$$
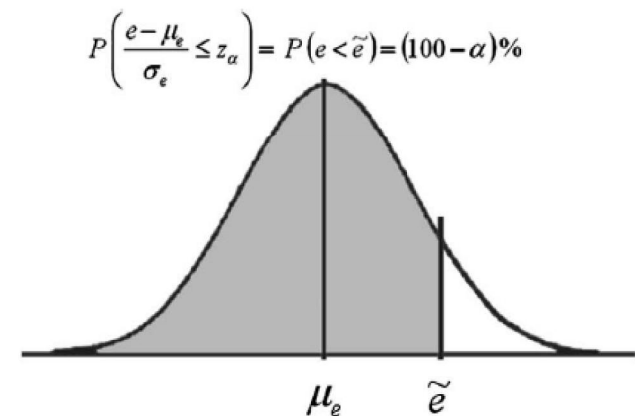
Fig. 3. Level of confidence level on a normal distribution curve

$\widetilde{e}$ is some probability that states that the prediction error is higher than $\widetilde{e}$ is less than% α.

$$P(e < \widetilde{e}) = \alpha\% \qquad (11)$$

100% of the total area under the curve and the area eaten part hachure% (α-100) are. Z_α Value for the reliability levels of 90%, 95% and 99% in Table I is given.

TABLE I

Z_α VALUE FOR DIFFERENT CONFIDENCE LEVELS

Z_α	$P[e - \mu_e \geq Z_\alpha \sigma_e]$
1.285	90%
1.645	95%
2.329	99%

Mean error Mean error as a historical period is calculated. We have For N observation:

$$\mu_e = \frac{1}{N}\sum_{i=1}^{N} e_i \qquad (12)$$

$$\sigma_e = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(e_i - \mu_e)^2} \qquad (13)$$

The e_i prediction error rate in sample i, μ_e and σ_e mean wind forecast error standard deviation error of prediction are wind. To enter risk production in the utilization plan, planners should first determine what level of confidence they need. The mean error and standard deviation can be recorded from the previous authentication information and relationships (12) and (13) come get. Values using the error mean value, standard deviation, and risk production value of Z_α ($\widetilde{e}$) relation (10) is calculated. Value obtained for ($\widetilde{e}$) of the predicted value is low and this number as the output of wind generators will enter the optimization problem. Considering the above mentioned wind turbine output matching relationship (14) is considered.

$$P_{wt} = (1-\widetilde{e}).P_{wt}^{forcs} \qquad (14)$$

The term P_{wt}^{forcs} represents an equivalent wind turbine production forecast and $\widetilde{e}$ is the production risk.

modeling for optimal operation from microgrid

Objective function of microgrid with regard to uncertainty at velocity prediction is defined as Eq (15) to least cost.

$$\min\left\{\sum_{t=1}^{24} Cost(t)_i - Income(t)_i\right\} \quad (15)$$

i is available units at grid.

$$\min\left\{\sum_{t=1}^{24} C_{pv}(t) + C_{wt}(t) + C_{bat}(t) + \right.$$
$$\left. C_{DG}(t) + C(P_{curt}(t)) \pm P_{exch}(t).\rho_{exch}(t)\right\}$$
$$(16)$$

At this objective function each parameter is defined as following:

$C_{pv}(t)$ is photovoltaic unit cost ($¢$), $C_{wt}(t)$ wind turbine unit cost ($¢$), $C_{bat}(t)$ is batteries bank cost($¢$), $C_{DG}(t)$ is diesel generator unit cost ($¢$), $C(P_{curt}(t))$ is cost of cut strategy at controllable loads ($¢$), $P_{exch}(t)$ is amount of bought or sold power (kw) and $\rho_{exch}(t)$ is price of bought or sold power ($\frac{¢}{kw}$).

Account of photovoltaic unit cost

Photovoltaic unit cost is accounted according to Eq (17):

$$C_{pv}(t) = C_{T,pv}(t) + C_{m,pv}(t) \qquad (17)$$

At above equation, $C_{T,PV}(t)$ is sum of panels buy cost and their installment cost at an hour ($¢$), $C_{T,PV}(t)$ is account by means of Eq (18).

$$C_{T,pv}(t) = \frac{C_\circ(1+\alpha)^n}{20\times365\times20} \qquad (18)$$

At above, $C_\circ$ is cost of buy and installment of panels at beginning of the project, α is interest rate and n is age of project.

$C_{m,PV}(t)$ is repairmen and maintain of photovoltaic panels at interval of one hour ($¢$) and is accounted according to Eq (19) [5].

$$C_{m,pv}(t) = \frac{(\frac{C_0}{100}) \times 20}{20 \times 365 \times 24 \times 20(kw)} \times P_{pv}(t)$$
(19)

Account of wind turbine unit cost

Wind unit cost is accounted according to Eq (20).
$$C_{wt}(t) = C_{T,wt}(t) + C_{m,wt}(t) \qquad (20)$$

At above equation, $C_{T,wt}(t)$ is sum of wind turbine sell cost and its installment cost at an hour ($\not\in$) and $C_{m,wt}(t)$ is repairman and maintain an hour ($\not\in$) of wind turbine, value of $C_{T,wt}(t)$ is accounted by Eq (21) [5].

$$C_{T,wt}(t) = \frac{C_\circ(1+\alpha)^n}{20 \times 365 \times 24} \qquad (21)$$

$C_\circ$ is buy and installment cost of wind turbine at beginning the project, α is interest rate and n is age of project.

Cost of wind turbine maintains and repairmen are accounted by Eq (22).

$$C_{m,wt}(t) = \frac{(\frac{C_0}{100}) \times 20}{20 \times 365 \times 24 \times 300(kw)} \times P_{wt}(t)$$
(22)

Account of battery bank cost

Cost of battery is considered by Eq (23).
$$C_{bat}(t) = C_{T,Bat}(t) + C_{m,Bat}(t) \qquad (23)$$

$C_{T,Bat}(t)$ is cost of battery bank buy and its installment at a hour ($\not\in$) and $C_{m,bat}(t)$ is buy and exchange cost.

$C_{T,Bat}(t)$ is accounted by Eq (24).

$$C_{T,bat}(t) = \frac{C_\circ(1+\alpha)^n}{20 \times 365 \times 24} \qquad (24)$$

Cost of charge and de charge is accounted by Eq (25).

$$C_{m,wt}(t) = \frac{(\frac{C_{\circ bat}}{100}) \times 20}{20 \times 365 \times 24 \times 228(kw)} \times P_B(t)$$

(25) $C_\circ$ is buy and installment cost of batteries at

beginning the project, α is interest rate and n is age of project.

Cost of cut strategy in controllable loads

Cut strategy cost at controllable loads is accounted according to Eq (26) [9].

$$C(p_{curt}(t)) = \beta.P_{curt} + \gamma.P_{curt}^2 \quad (\not\in) \qquad (26)$$

β, γ are cost coefficients at cut strategy at controllable loads. $P_{curt}(t)$ is cutting power at controllable loads (Kw).

Constraints of objective function for optimal operation from microgrid

Constraints of objective function for optimal operation from microgrid are following:

1. Constraint related to battery bank: charge state of batteries bank between SOC_{min}, SOC_{max} is changing [9].

$$SOC_{min} \leq SOC(t) \leq SOC_{max} \qquad (27)$$

2. Sum of photovoltaic unit power and wind turbine and exchange power with main grid and diesel generator power and batteries bank should be able to provide requested power from critical and controllable loads.

$$(P_{pv}(t) + P_B(t)) \times \eta_{con} + P_{wt}(t) + P_{exch}(t) + P_{DG}(t)$$
$$= P_{LOAD}(t) + P_{curt}(t) \qquad (28)$$

$P_{pv}(t)$ is productive power by photovoltaic panels (Kw), $P_{wt}(t)$ is productive power by wind turbine (Kw), $P_{exch}(t)$ is bought or sold power amount (Kw), $P_{LOAD}(t)$ is requested power by critical loads (Kw), $P_{curt}(t)$ is requested power by controllable loads (Kw), η_{con} is converters outputs.

If $P_B(t) < 0$, batteries are charging and if $P_B(t) > 0$, batteries are de charging.

3. Limitations of maximum and minimum capacities of productive power of wind turbine unit and photovoltaic unit and diesel generator are the following, which:

$$P_{pv,min} \leq P_{pv} \leq P_{pv,max} \qquad (29)$$

$$P_{DG\,min} \leq P_{DG} \leq P_{DG\,max} \qquad (30)$$

$$P_{wt,\min} \le P_{wt} \le P_{wt,\max} \qquad (31)$$

$P_{pv,\min}$ and $P_{pv,\max}$ are low high limits of photovoltaic unit productive power. $P_{wt,\min}$ and $P_{wt,\max}$ are low and high limits of wind turbine unit productive power. $P_{DG,\min}$ and $P_{DG,\max}$ are low and high limits of diesel generator productive power.

4. Maximum limitation of microgrid power exchang with main grid is accounted by Eq (32) [10].

$$P_{exch}(t) \le P_{exch}^{\max} \qquad : \forall t = 1:24 \qquad (32)$$

5. High and low limits of critical and controllable loads are accounted by Eq (33) [1].

$$0 \le P_{curt}(t) \le P_{curt}^{\max} \qquad (33)$$

The software development of suggestive model

According to objective functions and mentioned constraints for optimization as well as prediction of wind velocity, we need power plant units' features at microgrid, requested power amount, price of electricity buy and sell and bought and sold power amount.

At fig.4 flowchart of optimization program was shown.

As we can see at fig.4, at the first step according to wind speed and uncertainty at win production power and by environment temperature and sun radiation amount after the determination of initial input parameters and maximum production power of each source during 24 hours, after selecting the powers alternatively in each source, the cost function will be accounted and program is repeated until the best output power of each source be determined and the least cost be received.

Optimization of suggestive model by using of PSO algorithm

In this paper, the optimization of suggestive model is accepted by using PSO algorithm.

PSO algorithm is applied for optimization:

The first step:

1) The production of initial population of articles, all of the articles accidentally are produced in limit which provides the bridles.

2) Set the repetition number equal to 1.
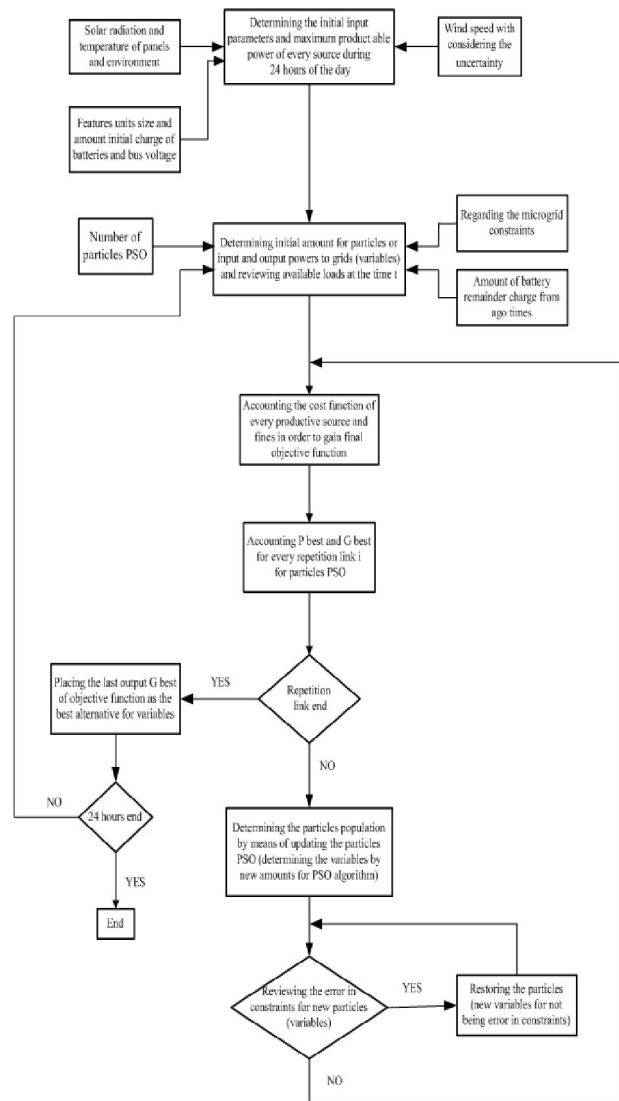
Fig 4. Flow chart optimization program

The second step:

1) Calculation of the value of objective function.

2) Calculation of the amount of sufficiency.

Third step: producing the new articles.

Fourth step:

1) Consideration the constraints: if constraints aren't provided by an article, that part of article, which over step from the free bridle is produced accidently than that constraint will be produce finally.

2) edit the repetition number: If the repetition number is smaller than its maximum, it goes to second step, if not, goes to fifth step.

Fifth step: the result of optimization.

Regarding to the explained stages, the flowchart of optimization by using PSO algorithm is as fig. 5[11].
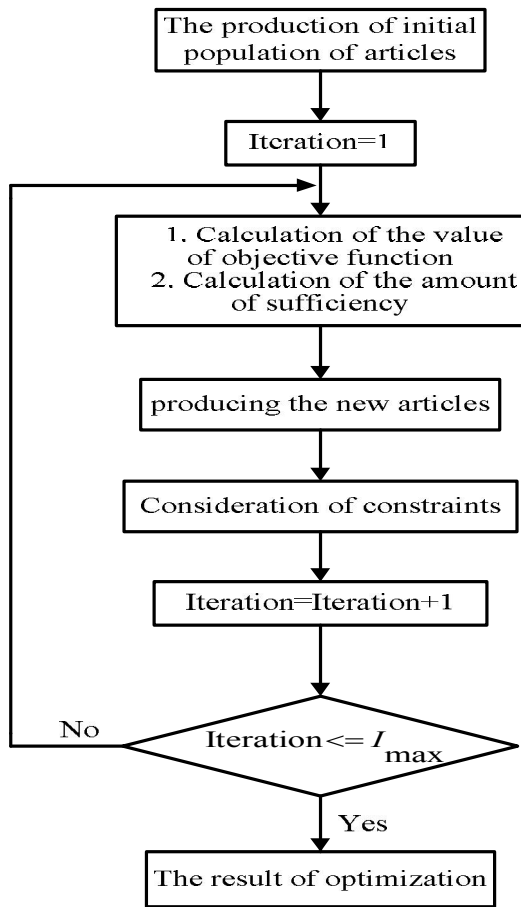
Fig. 5. Flowchart of optimization by using of PSO algorithm

CASE STUDY

In this section the optimum operation microgrid of suggestive model on a sample model is done for different parameters and the results of optimization are studied.

Also in this section, input information related to available units at microgrid and necessary amount for optimization according to objective function of Eq (16) and constraints of Eq (26) to (33) and with regard to uncertainty at prediction wind velocity in order to receive the least cost, is represented.

Input Data

In table II to V and fig. 6 to 12, the internal data are brought for program performance.

The used wind turbine in this model has the power 2MW, which it used 6 wind turbine 2MW that have the same features. The values of parameters of used wind turbine in this paper are brought in table II.

TABLE II. THE VALUES OF PARAMETERS WIND TURBINE

Parameter	Value
Rated power	200 Kw
V_r	13.8 (m/s)
V_{c_i}	3.1 (m/s)
V_{c_i}	25 (m/s)
$C_{\circ wt}$	190000 €

Predicted wind velocity at 24 hours is as fig. 6.
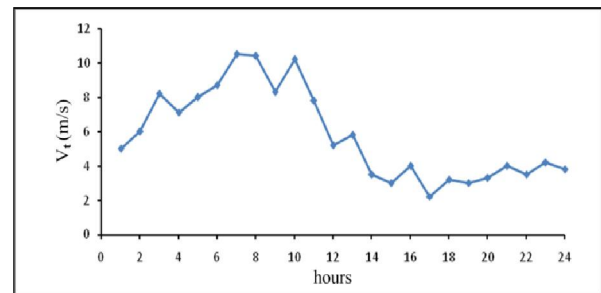
Fig. 6. The scenarios of predicted wind speed in 24 hours of market day

The uncertainty in forecasting wind energy production, the average error in predicting the 10% and standard deviation of the predicted 5% to be considered. Referencing Table (1) the amount of Z_α will be obtained, then using relation (10) the amount of production risk ($\widetilde{e}$) will be obtained. Values of these parameters in Table III are given.

TABLE III
VALUES FOR THE PARAMETERS GIVEN THE UNCERTAINTY IN FORECASTING WIND ENERGY

σ_e	μ_e	Z_α	$P[e - \mu_e \geq Z_\alpha \sigma_e]$	$\widetilde{e}$
5%	10%	1.645	95%	0.1822

Considering the amount of production risk ($\widetilde{e}$) in

Table III, and using relationship (14) the amount of wind power production capacity with considering the uncertainty in predicting energy Wind is calculated.

Fig. 7 shows production power amount of wind power plant according to wind turbine parameters amount and uncertainty at predicting the wind energy.
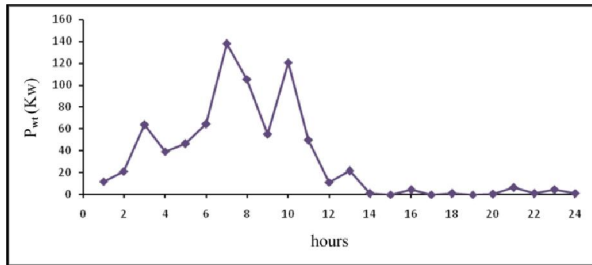
Fig. 7. Wind turbine output power in 24 hours

In order to account the photovoltaic panels output power amount by Eq (4), sun radiations and environment temperature at every hour of the day, is required [10]. The radiations amount and environment temperature shown at fig. 8 and 9. So received power amount by photovoltaic cells is according to fig. 8.
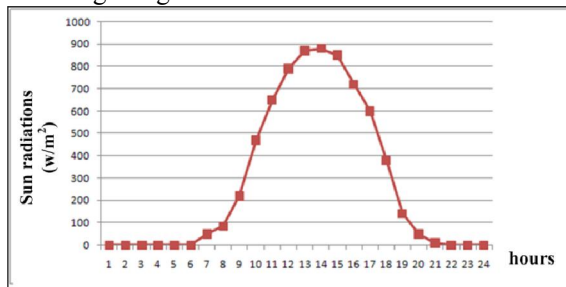
Fig. 8. Amount of sun radiations during 24 hours of the day
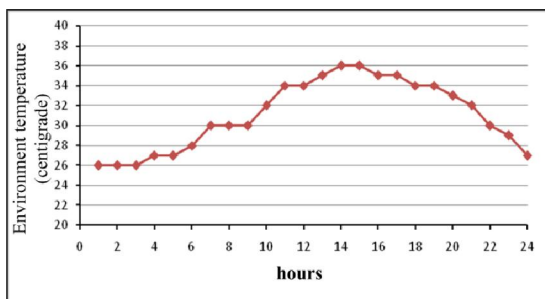
Fig. 9. Amount of environment temperature during 24 hours of the day
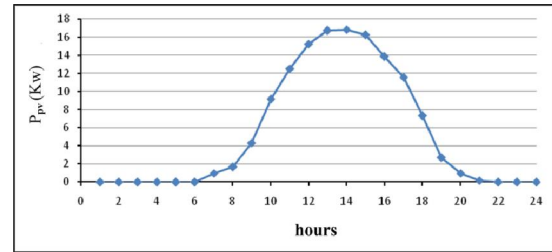
Fig. 10. Photovoltaic unit output power in 24 hours

Also numerical amounts related to photovoltaic panels features at the microgrid are represented at table IV.

TABLE IV. THE VALUES OF PARAMETERS PHOTOVOLTAIC UNIT

Parameter	Value
P_{STC}	20 Kw
G_{STC}	228 (w/m^2)
K	-0.004
T_r	25 ^{oc}
$C_{\circ pv}$	210000 €
(112 number)	

At this microgrid in order to store energy, 30 batteries were used and numerical amounts related to battery bank features at this microgrid was shown at table V [5].

TABLE V. THE VALUES OF PARAMETERS BATTERY BANK

Parameter	Value
Rated capacity of 30 battery	1900 Ah
Productive energy	228 Kwh
Voltage every battery	4V

Parameter	Value
DOD	80%
η_{bat}	80%
$C_{\circ bat}$	448000 €
(30 number)	

Amount of critical load for participants of microgrid during 24 hours of the day is as fig.11 [6].
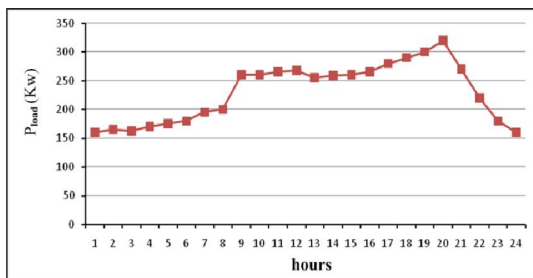
Fig. 11. Amount of critical load for participants of microgrid during
24 hours of the day

Amount of bought or sold electricity price with main grid (power market) during 24 hours of the day shown fig.12.
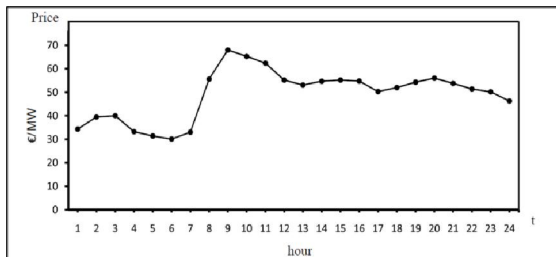
Fig. 12. Amount of bought or sold electricity price with main grid during
24 hours of the day

Features of controllable loads shown at table VI.

TABLE VI. THE VALUES OF PARAMETERS CONTROLLABLE LOADS

Parameter	Value
$P_{curt\ min}$	0
$P_{curt\ max}$	20 Kw

Parameter	Value
β	0.08
γ	0.001

Also amount of other parameters of model shown at table VII.

TABLE IV
THE VALUES OF THE OTHER PARAMETERS

Parameter	Value
$P_{pv,\min}\ (kw)$	0
$P_{pv,\max}\ (kw)$	20
$P_{wt,\min}\ (kw)$	0
$P_{wt,\max}\ (kw)$	300
$P_{DG,\min}\ (kw)$	0
$P_{DG,\max}\ (kw)$	400
$P_{exch}^{\max}\ (kw)$	30
η_{con}	80%
$n\ (years)$	20
α	0.06
A	0.3842
B	0.0466
C	0.0007

Price of diesel generator fuel is 0.9 €/Lit and cost of diesel generator repairmen and maintain is 0.2 € at an hour.

Applied to the parameter values for the PSO algorithm are in Table VII.

TABLE VII
THE PARAMETER VALUES FOR THE PSO ALGORITHM

Parameter	Value
Population size	350
Number of replications	1500
Acceleration coefficient C_1	2
Acceleration coefficient C_2	2
Retention Weight (W)	1

B. The program exit and analysis of results
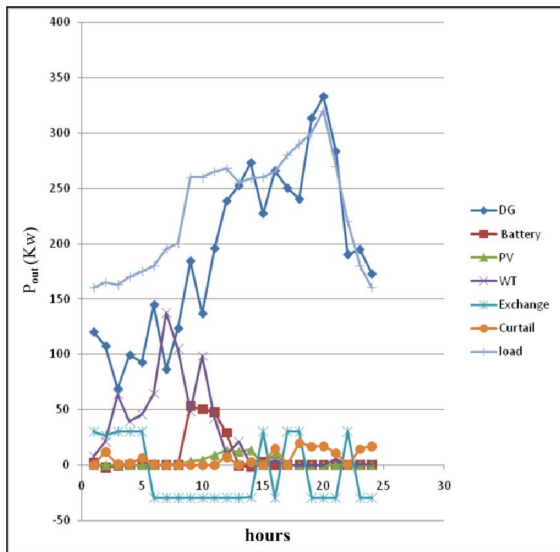
Fig.13. Programming of productive power from operation of microgrid

The results of microgrid optimization according to uncertainty at predicting the wind velocity for optimal operation of microgrid in order to receive the least cost are shown at following figures. Fig.13 shows the production power amounts of every unit and also amount of sale and buy of the power with main grid and amount of cutting load at critical loads and amount of charge and de charge of batteries during 24 hours of the day.

As shown at fig.13, amount of charging and de charging the batteries didn't become less than 20% maximum charge. At first the full charge was assumed in the batteries. Negative amounts at exchange power show that microgrid is selling power to main grid and positive amounts show that microgrid is buying the power from main grid.
At fig.14 the cost amount of every production unit for production power of fig.13 and penalty of cutting for controllable loads and buy and sale of microgrid power is represented.
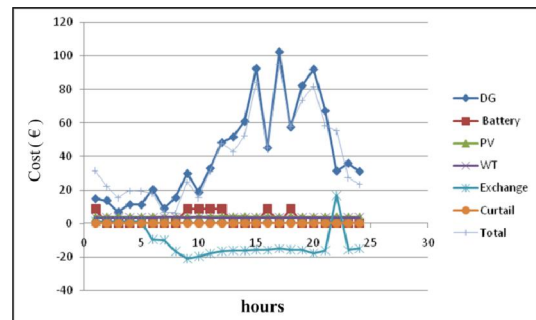
Fig.14. the cost amount of every production unit and cost of microgrid in 24 hours

At fig.14 the least operation cost of microgrid during 24 hours is represented. Negative amounts of fig.14 related to power exchange with main grid are for sale and regarded profit.

CONCLUSIONS

In this paper a model from microgrid is represented including wind power production units, photovoltaic unit, diesel generator, battery storage units, critical and controllable loads. In this microgrid, exchange with main grid is executed. In this model, the objective function expresses least cost of microgrid operation according to uncertainty at wind energy. Also for solving the optimization problem, different constraints were used such as constraints related to productive units, constraints related to microgrid and constraints related to critical and controllable loads. Using wind turbine units and photovoltaic units because of least cost at operation can be effective in microgrid and other pointes of power system. Diesel generator system is used as support in order to provide remainder power.

Also optimization was done by means of PSO algorithm. The results show efficiency of PSO algorithm.

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9/5/2012

A Hybrid Intelligent Information System for the Administration of Massive Mass of Hajjis

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Abstract: In this paper the development of a hybrid intelligent information system for the administration of massive Hajjis crow has been described. The main objective is to design an information system to manage the crowd during one of Hajj rituals, "Nafra" so as to avoid crowding disasters. The developed system incorporates data acquisition and processing via several thermal cameras deployed as sensors at strategic points on "Nafra" access roads. The sensors are linked to an analysis module, which in turn measures crowd flow and density in real time. The analysis results are fed into a fuzzy logic module to determine the priority of roads with respect to their widths and lengths. Then, the integrated decision support system generates decisions to the controllers in order for them to take the appropriate actions. Hybridization is done by integrating fuzzy logic, operations research and decision support to produce alternate decisions to the system controllers enable them to control the movement of the huge crowd. The paper illustrates different system components. It also describes the architecture of each component as well as the architecture of the entire system. A complete case study is illustrated with real snap shots of system screens in order to prove the system methodology. The results show solid decisions that help the authorities manage the huge pilgrim crowds. The system can contribute to provide complete safety for crowds during the "Nafra" event that attracts millions each year.
[Mohamed Osama Khozium, **A Hybrid Intelligent Information System for the Administration of Massive Mass of Hajjis**] Life Science Journal 2012; 9(4):171-180]. (ISSN: 1097-8135). http://www.lifesciencesite.com.

1. Introduction

According to the Islamic faith, every able Muslim who can afford to do so must make the pilgrimage to Mecca, the holy city in Saudi Arabia, at least once in his or her lifetime. Known as the Hajj, the pilgrimage involves a number of sacred rituals, and represents a profound personal and spiritual journey for Muslims. This annual Hajj occasion brings around 3.0 Million Muslims of all countries, colors, and races in a unique opportunity of worshipping Allah collectively in large gathering at one place.

Over the years, overcrowding and difficulties in crowd control have resulted in a number of accidents and fatalities during the Hajj. Despite many efforts and improvements for roads and footbridges, ensuring the safety of pilgrims continues to challenge especially with the annual increase of the number of pilgrims. The challenge has attracted many researchers who provided several methodologies for crowd monitoring and estimation of its density. However, even with the crowd control techniques, there are still many incidents during the Hajj, as pilgrims are trampled in a crush, or ramps collapse under the weight, causing hundreds of deaths.

The main objective of this paper is to provide an integrated intelligent crowd management system allows for close monitoring and control of crowd movements of pilgrims and protection against accidents caused by overcrowding, and preserving a level of comfort during the movement to keep the sanctity of emotions at its best.

The presented system incorporates data acquisition through the use of several thermal cameras deployed at critical points on the target route. The thermal videos will be fed into an analyzer to calculate the density of the crowd in each road in the target route. The crowd analysis will be fed into a fuzzy logic module along with pre-stored information about roads geometry to devise the status of each road individually. Then, the intelligent decision support system is triggered. It incorporates an operations research module that determines the pilgrims mass per minutes for each available road due to road parameters and possible time remains. It also incorporates a capacity weighted approach based on roads priority. The decision support system will generate different alternatives showing the closed roads, road priorities, and which group should move through which road. The alternatives will be used by the authorities to take the necessary actions.

The system architecture is illustrated along with the information flow diagram as well as a detailed description of different system components. A complete case study is illustrated that traces the systems with a real example. The results show solid decisions that help the authorities manage the huge pilgrim crowds.

This paper is divided into six sections. The first section is this introduction. The second section defines the problem under discussion. The third section surveys

some related work. The fourth section illustrates different system components. The fifth section, discusses a complete case study. And the last section concludes the work and discusses the future of the research.

Problem Definition

After the sunset of the ninth day of "Dhul-Hijjah" pilgrims go from "Arafat" to "Muzdalifa" during "Nafra". The "Nafra" process includes the movement of 3.0 Million Hajjis before sunrise using certain limited roads. Khozium *et al.* (2012) showed a map with plotted pedestrians' roads on the "Nafra" route. Figure 1 shows a view of pilgrims during the "Nafra".

Figure 1: A View of Hajjis During "Nafra"

Ben-Mahmoud *et al.* (2010) have shown that the number of pilgrims will dramatically increase in the next few years to reach almost 3.75 million Muslims. Moreover, managing millions of people gathered from diverse countries around the globe is not only a matter of placement them in the correct route. The gathered troops are different in nationalities and so in customs. Pilgrims coming from Gulf area prefer to move by cars or buses; pilgrims coming from India, Pakistan and Bangladesh prefer to move by walking together, and pilgrims that belong to Shi'a also prefer to stay together. This makes the challenge more difficult in order to manage the moving of different troops together.

To control pilgrims, the Kingdom Ministry of Hajj has established six establishments to provide services for pilgrims plus GCC and interior establishment. These six establishments are:

- National "Tawafa" Establishment for South Asian Pilgrims
- National "Tawafa" Establishment for Pilgrims of the Non-Arab African countries
- National "Tawafa" Establishment for South East Asian Pilgrims

- National "Tawafa" Establishment for Pilgrims of the Arabian Countries
- National "Tawafa" Establishment for Pilgrims of Iran
- National "Tawafa" Establishment for Pilgrims of Turkey and Muslims of Europe, Americas and Australia.

Each establishment has around 100 offices; each office is responsible for around 5000 hajj and consists of a manager and a group of personnel. These establishments are responsible to provide accommodation, transportation and other services to pilgrims. These service providers are the main stakeholders of the intended system; they will be the controllers and users. Each establishment is responsible for half million hajj divides them into one hundred groups according to nationality, race and religious group. The establishment is responsible for moving these groups through different roads in the "Nafra" route.

In the following text the author assumes that each establishment is responsible for twenty groups of Hajjis over one hundred who will move by walking using the pedestrians' "Nafra" roads. Each one has 4000 Hajjis; total will be 80,000 Hajjis. Each of these groups is going to move as one block of Hajjis.

Related Work

Generically, crowd can be defined as a large number of people gathered together with or without orderly arrangement. Crowd management is defined as the systematic planning for, and supervision of, the orderly movement and assembly of people. Crowd management involves the assessment of the people handling capabilities of a space prior to use (Fruin, 1993).

Most major crowd disasters can be prevented by simple crowd management strategies. The primary crowd management objectives are the avoidance of critical crowd densities and the triggering of rapid group movement (Fruin, 1993). Helbing *et al.* (2007) showed that as crowd density rose, they identified the start of stop-and-go waves similar to those found in road traffic jams. This was followed by transition to a much more chaotic state, with outbreaks of panic as individuals lost control. This phenomenon – known as crowd turbulence – can trigger disasters. In the following paragraphs, some crowd control and management systems have been studied.

Sirmacek and Reinartz (2011) have introduced a novel approach to detect crowded areas automatically from very high resolution satellite images. Although resolutions of those images are not enough to see each person with sharp details, they can still notice a change of color components in the place where a person exists. Therefore, they developed an algorithm which is based on local feature extraction from input images. They have

tested their algorithm on panchromatic Worldview-2 satellite image dataset, and also compared with an algorithm result obtained from an airborne image of the same test area. The presented results indicate possible usage of the algorithm in real-life events.

Deshpande and Gupta (2010) have proposed a computer based system combining fuzzy logic and Graphical Information System (G.I.S) to monitor and avoid the crowding disasters. They have proposed two-step mode. The first step is pre-disaster planning incorporating the determination of sensitive locations and space management, evacuation paths using (G.I.S) and management related arrangements. The second step is real time analysis of crowds to detect a possible emergency. Their system contains two modules. The first is a fuzzy inference system to determine crowding situations and plan of action. The fuzzy interface depends on the number of pixels and shape of objects to determine the crowd density. It also uses object characterization from the image to determine the speed of the crowd. The second is the determination of the shortest evacuation path for the current area under surveillance. The shortest path is determined with the help of G.I.S. and the overall crowding situation. Their proposed system follows certain steps: acquiring basic information, formation of evacuation network, and calculation and decision.

Al-Habaibeh *et al.* (2009) have described a novel application of low-cost infrared system for estimating people's density using infrared thermography. They have conducted their experiments inside Madina Mosque in Saudi Arabia. They designed a fusion of three sensors low-cost infrared sensor, light intensity sensor and temperature sensor. By taking one shot every one minute they have been able to predict the people density by plotting the number of warm infrared pixels which are found to be very representative to the density of people in the mosque. Because of the air condition inside the mosque, the temperature sensor has no use in their experiment which leaves a question about its role in the fusion system. In addition, the change in light intensity has caused significant source of error in their experiments. The obtained results did not give an indication about the critical crowd density level; however they only showed that in different prayer times, people are increasing in the mosque.

The use of still images has been extended to analyzing the work from multiple cameras using information fusion. Andersson *et al.*   (2009) have presented a useful tool for estimating behaviors of a crowd derived from distributed and heterogeneous sensors. Their tool doesn't need to identify specific persons or decide their exact positions in the scene but it aims to become aware of that something abnormal has occurred. The concept is used for automatically alerting operators when abnormal behaviors occur, or are about to occur. Yang *et al.* (2003) estimated the number of people directly from groups of image sensors. For each sensor, foreground objects are segmented from the background, and the resulting silhouettes are combined over the sensor network. A geometric algorithm is then introduced to limit the number and possible locations of people using silhouettes extracted by each sensor.

Mahalingam *et al.* (2009) have presented a simplified method for tracking people in crowded scene from a video sequence. The method is based on computing the Minimum Mean Square Error (MMSE) between frames of the video sequence to identify people in subsequent frames. They have succeeded to handle small occlusions, varied lighting conditions and camera motions. Pini *et al.* (2004) have presented a system for crowd detection from a moving platform. The system uses slices in the spatiotemporal domain to detect inward motion as well as intersections between multiple moving objects. The system calculates probability distribution functions for left and right inward motion and uses these probability distribution functions to deduce a decision about inward motion or crowd detection. The system has succeeded to automatically detect scenes that contain crowd consisting of multiple pedestrians moving in opposite directions, even at a large distance

Schubert and *et al.* (2008) presented a decision support system for crowd control. Decision support is provided by suggesting a control strategy needed to control a specific disturbance situation. Control strategies consist of deployment of several police barriers with specific positions and strengths needed to control the disturbance. The control strategies are derived for a set of pre-stored example situations by using genetic algorithms where successive trial strategies are evaluated using stochastic agent-based simulation. The optimal control strategy for the current situation is constructed by the best linear combination of pre-stored example situations. The optimal strategy is given as the same linear combination of associated strategies. So, their system is using a decision making algorithm where a current situation is compared to all simulated situations. A linear combination of control strategies, whose corresponding weighted superposition of simulated situations most closely resembles the current situation, is given as the required decision.

System Components

Figure 2 shows the information flow between different system components. The flow goes with the arrow's direction through thermal cameras, video sequence analyzer, road selection module, fuzzy module, operations research (OR) module, decision support system module, and establishment status module.
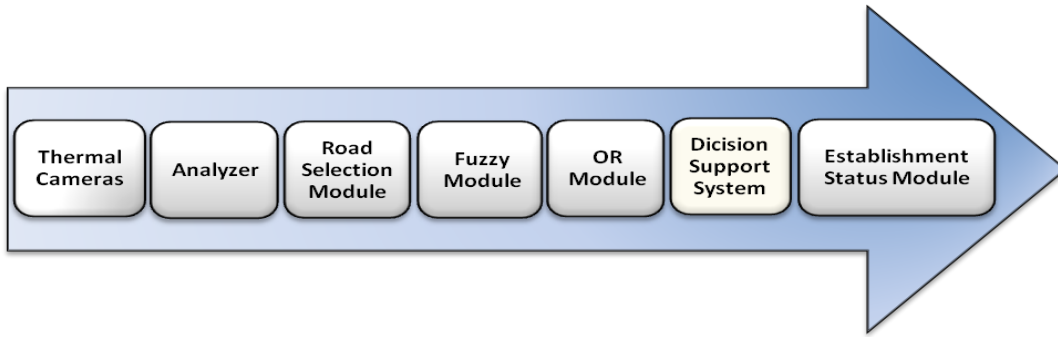
Figure 2: Information Flow Diagram

The thermography module (Abuarafah *et al.* 2012) uses a set of FLIR E60bx thermal camera deployed over an elevation about 10m above each pedestrian road in the route. The cameras will be connected to a controller. The controller collects different video sequences about each road in certain period of time and feeds the second module which is the video sequence analyzer with the collected video sequences and the necessary calibration information such as human temperature. The video sequence analyzer module calculates the crowd density in real-time in pre-defined steps; every step includes a specific number of frames pre-defined in system configuration. The less number of frames in the step will result in increasing the accuracy of calculating the average crowd ratio. The module will indicate the crowd density percentage in different colors. In addition the module infers the movement behavior from whether it is accelerating or decelerating. The road selection module will reject the closed roads, and marks other roads with critical or open road according to the crowd density that comes from the analysis module. The module then feeds the output to the next module.

The next module is the fuzzy logic module (Ross, 2010). This module manipulates the output of the analyzer in integration with road parameters such as road length and width to formulate the status of each road individually. The fuzzy logic module prioritizes the roads according to three parameters the crowd density on the road, the road length, and the road width. So, suppose that the crowd density is normalized among several roads; then the priority of them is arranged according to the shortest and widest one. The roads will be assigned a discrete number from 0 (lowest) to 10 (highest) to describe its priority.

Table 1summarizes the fuzzy module.

Table1: Fuzzy Module Rules

Input	Density 0 - 100%		**Fuzzy Rules** 1. If (density is empty) and (length is short) and (width is thick) then (priority is high) 2. If (density is full) and (length is long) and (width is thin) then (priority is low) 3. If (density is medium) and (length is short) and (width is thick) then (priority is high) 4. If (density is empty) then (priority is high) 5. If (density is full) then (priority is low) 6. If (density is medium) and (length is medium) and (width is medium) then (priority is average) 7. If (density is medium) and (length is long) and (width is thin) then (priority is low)
	Length 5.0 – 7.0 km		
	Width 20 - 35 m		
Output	Priority 0 - 10		

The output of the fuzzy module is fed to the operations research module which determines pilgrims mass per minute for each available road due to road parameters and possible time remains.

The decision support component along with establishment status module works in a new approach which can be named capacity weighted using priority approach (CWP).This new technique uses the capacity of the establishment which depends on the remaining number of Hajjis and the priority of the roads for each establishment to achieve balance among the remaining Hajjis in the entire establishments. The approach is done in the following steps.

1. A matrix is constructed with its columns represent the available establishments and its roads represent the available roads in the "Nafra" route;

2. The matrix is initialized with the roads sorted according to their distance from the corresponding establishment, the closer the road from the establishment the higher rank it takes in the matrix.

3. Table 1 shows an initial matrix assuming that there are six available establishments and four available roads in the "Nafra" route. For example, road number 4 is the nearest road to establishment number 1 then road number 3, road number 2, and road number 1 is the farthest one;

4. From the road analysis module, the closed roads are excluded from the matrix. For illustration, road number four is assumed to be closed;

5. From the fuzzy logic module, roads with priority less than 5 are excluded from the matrix. For illustration, road number three is assumed to be excluded.

6. Table 2 shows the matrix after the exclusion of the roads Rd_4 because it is closed and Rd_3 because it has the low priority;

7. The weight WX for the capacity or remaining groups of each establishment is generated with respect to the priority of the roads as follows:

$$(W_x)_R = G_x \times \left(1 - \left((R - 1) \times C\right)\right)$$

Where: W is the weight, x is the establishment number, G_x is the number of remaining groups in the establishment x, R is the row index in the matrix, and C is a coefficient factor = 0.2. For example to calculate the weight for establishment number three in row number 2, the weight is equal to

$$(W_3)_2 = G_3 \times \left(1 - \left((2 - 1) \times 0.2\right)\right) = 0.8 G_2$$

Table 3 shows the calculated weights associated with roads in the matrix;

8. The maximum weight for all the cells is selected. This means that the road associated to this weight will be assigned to the corresponding establishment. Then the next maximum weight in matrix is selected and the road associated to this number is assigned to the corresponding establishment, and so on until all groups are done. If two or more equal weights are found the left most one will go first.

Table 1: Establishment/ Road Initial Matrix

Est$_1$	Est$_2$	Est$_3$	Est$_4$	Est$_5$	Est$_6$
Rd$_4$	Rd$_4$	Rd$_3$	Rd$_3$	Rd$_1$	Rd$_3$
Rd$_3$	Rd$_3$	Rd$_2$	Rd$_2$	Rd$_2$	Rd$_2$
Rd$_2$	Rd$_2$	Rd$_1$	Rd$_1$	Rd$_3$	Rd$_1$
Rd$_1$	Rd$_1$	Rd$_4$	Rd$_4$	Rd$_4$	Rd$_4$

Table 2: Establishment/ Road Matrix after Removing Closed and Low Priority Roads

Est$_1$	Est$_2$	Est$_3$	Est$_4$	Est$_5$	Est$_6$
				Rd$_1$	
		Rd$_2$	Rd$_2$	Rd$_2$	Rd$_2$
Rd$_2$	Rd$_2$	Rd$_1$	Rd$_1$		Rd$_1$
Rd$_1$	Rd$_1$				

Table 3: Calculated Weight for each Road

Est$_1$	Est$_2$	Est$_3$	Est$_4$	Est$_5$	Est$_6$
				(1.0G$_5$)Rd$_1$	
		(0.8G$_3$)Rd$_2$	(0.8G$_4$)Rd$_2$	(0.8G$_5$)Rd$_2$	(0.8G$_6$)Rd$_2$
(0.6G$_1$)Rd$_2$	(0.6G$_2$)Rd$_2$	(0.6G$_3$)Rd$_1$	(0.6G$_4$)Rd$_1$		(0.6G$_6$)Rd$_1$
(0.4G$_1$)Rd$_1$	(0.4G$_2$)Rd$_1$				

Case Study

In order to prove that the system generates the correct decisions to move Hajj groups, this section discusses a full case study with real numbers and snap shots of the system during its run. The following is the case study assumptions:

The establishment under the study will be establishment number three i.e. x = 3;

Period of time will be 10 minutes which is quite enough to pass easily one group (4 thousand persons) through the neck of each road. So, the time interval between different thermal video shots will be 10 minutes;

There are four pedestrians' roads Rd1, Rd2, Rd3 and Rd4 under study;

Due to the location of establishment (3), the roads are sorted nearest to farthest as Rd3, Rd2, Rd1 and Rd4.

Figure 3 shows a snapshot of the system including position of the four cameras and the position of the six establishments with 20 croups for each one. The right panel shows a video for each road and over each road its density is written in the main panel.
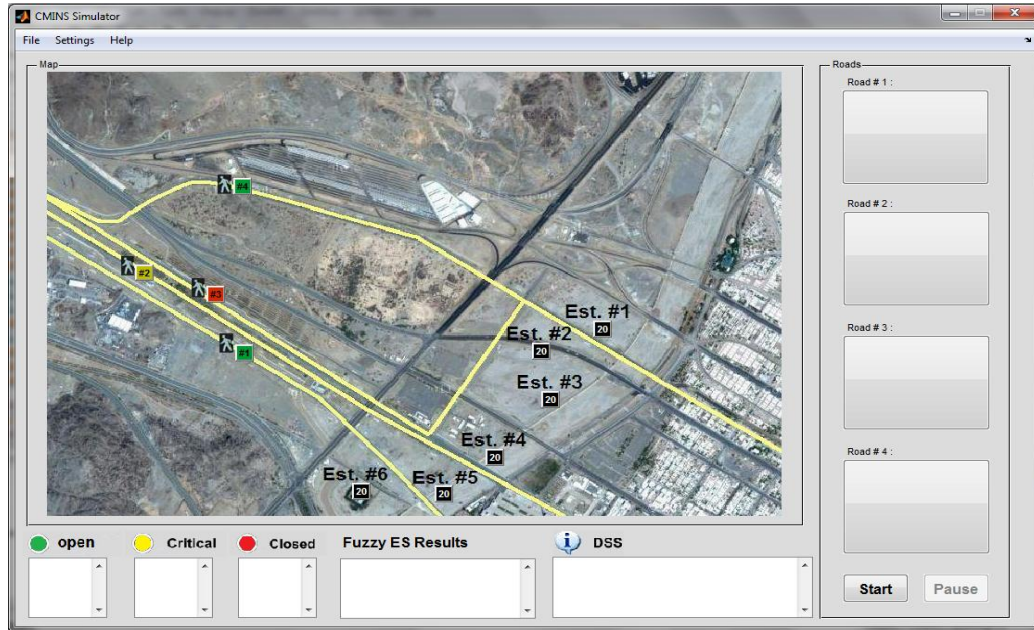
Figure 3: Initial System Screen

Figure 4 shows the system screen after the first period (10 minutes):
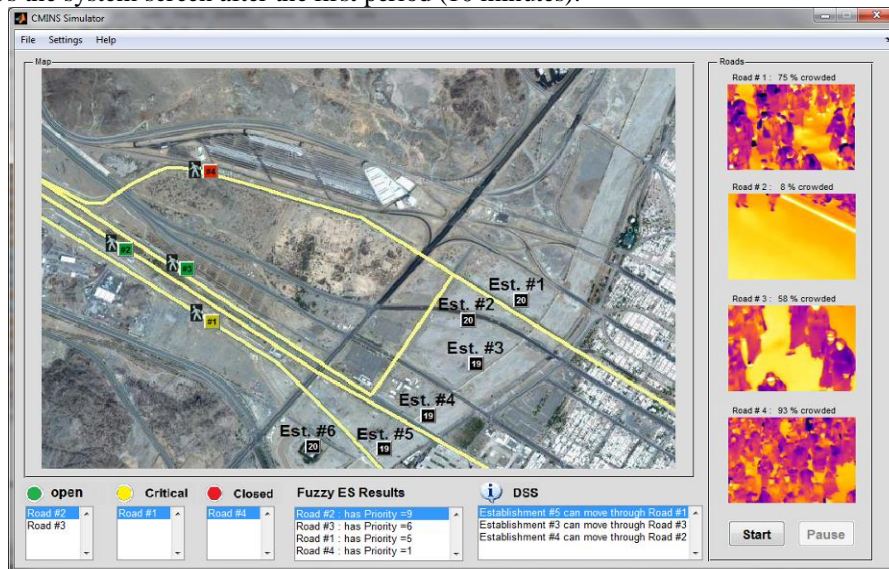
Figure 4: System Screen after the First (10 Minutes) Period

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Video sequences from the thermal camera are generated for each road;

Crowd density of each road is calculated through the analyzer which equals to 75%, 8%, 58%, and 93% for roads Rd1, Rd2, Rd3 and Rd4 respectively;

From the road selection module, the roads Rd2, Rd3 are open, Rd1 is critical and Rd4 is rejected because it is closed;

From the fuzzy module, roads Rd1, Rd2 and Rd3 have priority greater than or equal to 5;

For establishment number three the roads can be sorted nearest to farthest as Rd3, Rd2, Rd1 and Rd4;

From the OR module, the valid roads for establishment number three are sorted from the best to

the worst by assigning weight for each road considering the location and the remaining groups. The result will be this order Rd2, Rd3 and Rd1;

From the decision support system, Rd3 will be assigned for establishment 3 after analyzing all roads for all establishments;

One group from establishment 3 will move through Rd3, the remaining group will be 19.

Figure 5 shows the system screen during the second period the system has directed establishment 3 to move through road 2 instead of road 3. The remaining groups will be 18 groups.
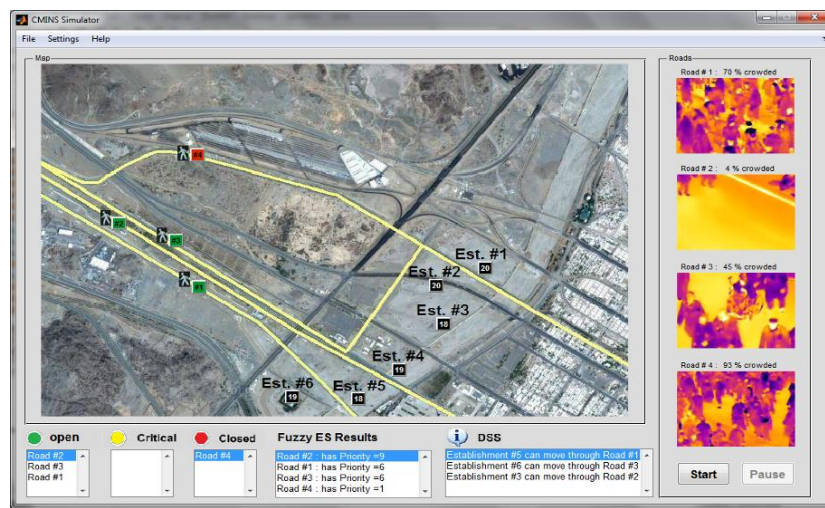
Figure 5: System Screen after the Second (10 Minutes) Period

In the third period no suitable road is available for establishment 3. Then the remaining groups are still 18 groups. Figure 6 shows a snap shot of the system after the third period. Notice that, DSS generates no decision for establishment 3.
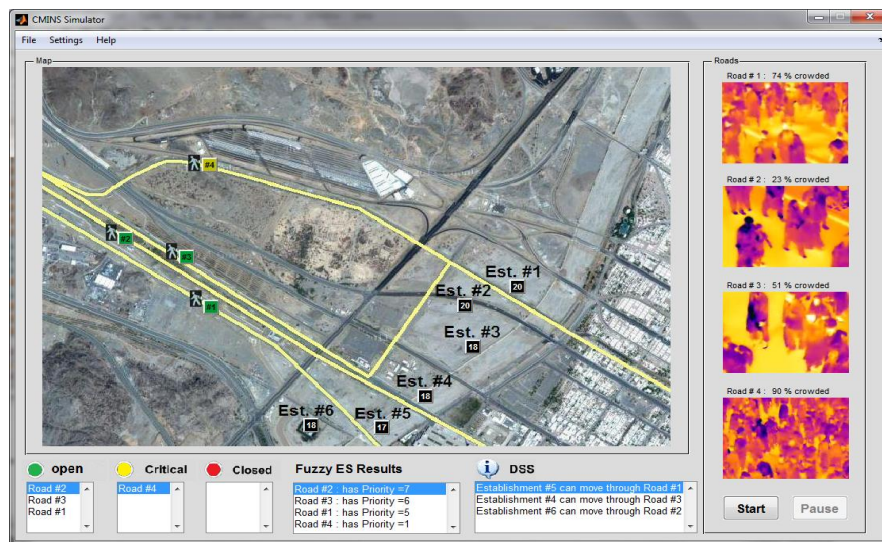
Figure 6: System Screen after the Third (10 Minutes) Period

In the fourth period the DSS has directed establishment 3 to move through road 3, then the groups has been reduced to 17 groups. Figure 7 shows DSS for establishment 3 and number of groups becomes 17.
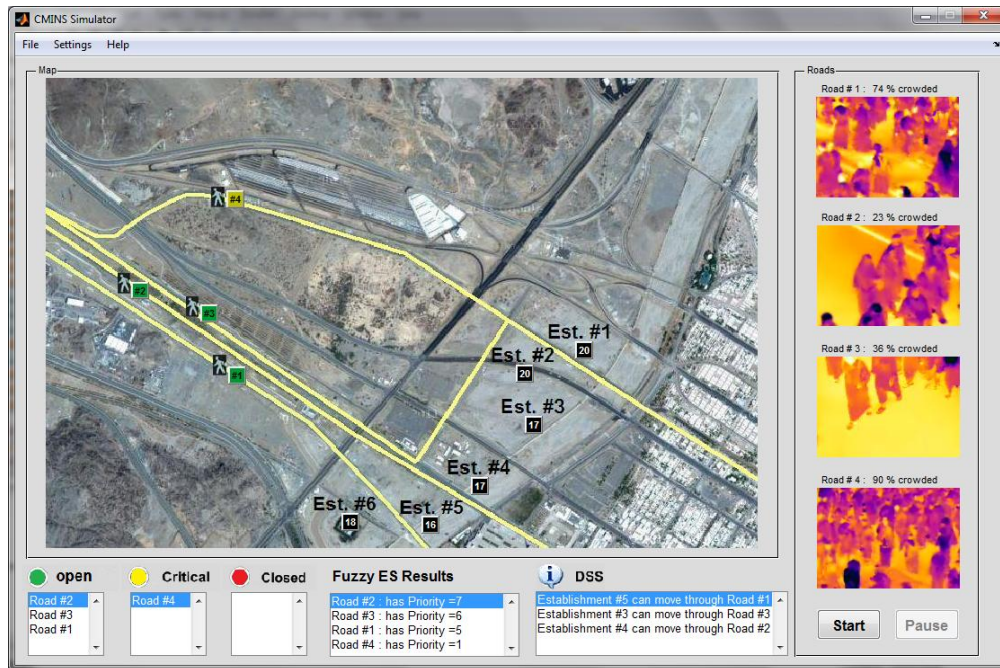
Figure 7: System Screen after the Fourth (10 Minutes) Period

In the last period the groups of establishment 3 has been reduced to be zero. See Figure 8.
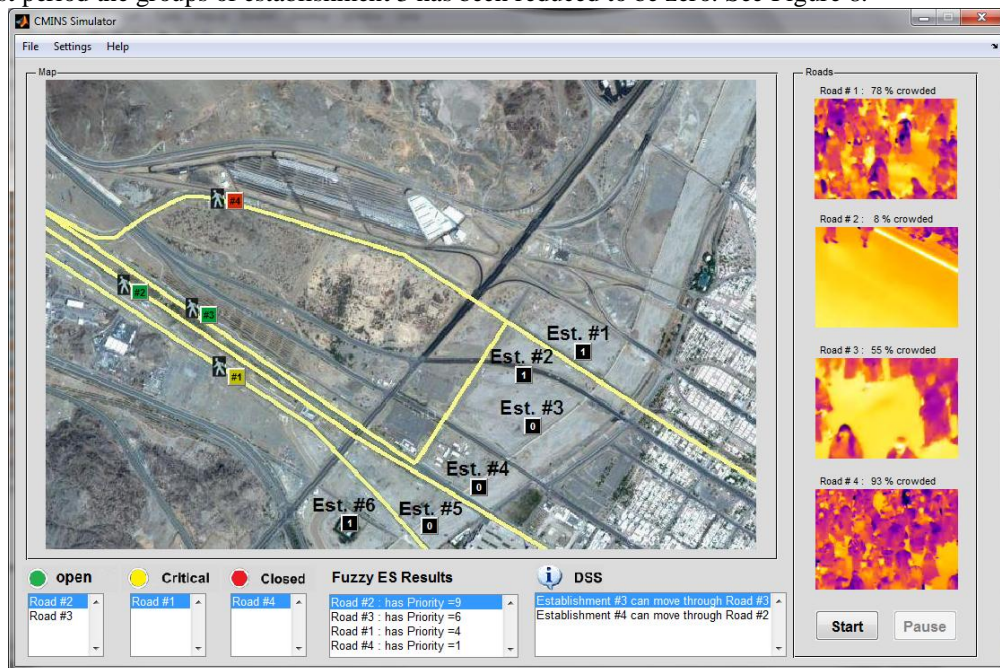
Figure 8: System Screen after the Last (10 Minutes) Period

Table 5 shows a summary of the case study. Four periods are shown in the main columns in addition to the last period. Different steps during the running of the system are shown on different rows; the steps follow the information flow direction as illustrated above.

Table 4: A Summary of the Case Study

Period = 10 Minutes	First period				Second period				Third period				Fourth period				Last period			
Roads Density %	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
	75	8	58	93	70	4	45	93	74	23	51	90	74	23	36	90	76	8	55	93
Roads selected by density <= 90	R2	R3		R1(c)	R2		R3	R1	R2	R3	R1	R4 (c)	R2	R3	R1	R4 (c)	R2		R3	R1 (c)
Fuzzy priority	R1 5	R2 9	R3 6	R4 1	R1 6	R2 9	R3 6	R4 1	R1 5	R2 7	R3 6	R4 1	R1 5	R2 7	R3 6	R4 1	R1 4	R2 9	R3 6	R4 1
Roads priority due to fuzzy =<5	R2	R3		R1	R2	R1		R3	R2		R3	R1	R2		R3	R1	R2		R3	
Roads priority for Est.3 setup	R3	R2	R1	R4	R3	R2	R1	R4	R3	R2	R1	R4	R3	R2	R1	R4	R3	R2	R1	R4
Roads selected by DSS For Est.3	R3				R2				-------				R3				R3			
Groups remained in Est.3	19				18				18				17				0			

The case study shows solid decisions generated by the system. For example, Rd4 is rejected in the first period because it is closed by the massive crowd. Rd$_3$ is assigned to establishment number 3 after sorting the remaining roads according to their priorities. Also, the case study shows accurate assignments of roads to other establishments. By noticing the remaining roads in the last period, almost all establishments have drained their groups through the decided roads by the system. Thus, this can ensure safe moving of the massive mass of Hajjis as well as balancing of their distribution so that nobody waits too long time. In addition, by moving groups as solid units, the system ensures that different customs among Hajjis are respected.

Conclusion

In this paper, a hybrid intelligent decision support system for the administration of massive mass of Hajjis has been introduced. Hybridization is done by integrating fuzzy logic, operations research and decision support to produce alternate decision to the system controllers enable them to administrate the movement of the massive mass of Hajjis in a way that ensures safety with compromised time of moving of the groups as well as respect the customs among Hajjis.

New tech. has been proposed named capacity weighted priority. The technique shows solid generations of decisions during the movement of Hajjis. The technique is based on matrix calculations which keep its running cost minimal. A complete case study is discussed that proves the use of the CWP approach. Solid decisions have been generated by the system which will help authorities to administrate and control the massive crowd during the "Nafra" event.

For future research the decisions that are generated by the system need to be measured with respect to their success to move different Hajj troupes. An adaptation module needs to be added to system to avoid decisions that have low success ranks. Adaptability will improve the decisions generated by the system and increase its usefulness to the controllers.

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Dr. khozium has published many articles in international journals and conferences in the area of electronic warfare, expert systems, information security and software engineering, he participated in organizing many international conferences, he is an active reviewer for numerous international.

Dr.khozium has been awarded "doing duties honestly and faithfully award" and "excellent duty medal from the first level" from the president of Arab Republic of Egypt, 1996, 2006 respectfully.

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Clinical and Laboratory Examinations of Diarrhea and Dehydration in Newborn Friesian Calves with Special Reference to Therapy with Hypertonic and Isotonic Solution

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Abstract: This   study was carried on 61 calves from different farms at Sharkia governorate. From these calves 25(healthy control calves) and 36 diarrheic dehydrated calves were used for this study. The calves had normal body temperature. The clinical signs of the diseased calves included a whitisg to yellowish diarrhea with foetid odour and highly moisture percent of faeces. Moderate and severe dehydration were recorded including varying degree of dryness of skin, sunked eyes, recumbency, sometimes coma and death occurred. 18 calves showed hyperpnea with standing position but need help to stand (moderate degree of dehydration and clinical acidosis). Other 18 calves showed lrecombency, a decrease of heart beats and respiratory rates (severe degree of dehydration and clinical acidosis). **Blood analysis of diseased calves** showed acidemia as well as a significant increase in erythrocytic count, haemoglobin content and packed cell volume with a non significant variation in Leucocytic count. **Serum analysis** showed an increase of the total protein, total solids, a significant increase of serum urea nitrogen and creatinine as well as hyponatremia, hypocholoremia and hyperkalamia. **Thereapeutic trials:** Antidiarrheal drugs as well as isotonic solution in moderate dehydration and hypertonic together with isotonic in severe dehydration were used for treatment. It could be concluded that diarrhea and dehydration in calves has a harmful effect on general health condition, gastrointestinal tract, body fluid, blood, serum and faecal contents. Hypertonic together with isotonic solution and antidiarrheic drugs safe the animal life and control this problem.
[Abdel Khalek R. El-sheikh, Hayam M. Samy Morsy, Tarek H. Allam Abbas and Wafaa M. Abdelrazik. **Clinical and Laboratory Examinations of Diarrhea and Dehydration in Newborn Friesian Calves with Special Reference to Therapy with Hypertonic and Isotonic Solution.** *Life Sci J* 2012; 9(4):181-184]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 26

Key words: Calves, Diarrhoea, Dehydration, blood, serum, hypertonic, isotonic solution.

1. Introduction

Diarrhea and enteritis syndromes represent the most serious digestive problems among the newborn calves causing economic losses to producers due to high morbidity and mortality rate, moreover, the causes of diarrhea are multifactorial included interaction between: calf, environment, nutrition and infectious agents (Don Hudson & Gene White, 2009). It causes varying degree of dehydration, gastroenteritis, body fluid loss and various body fluid changes (Roy1980, El-Sheikh 1987, , El-Sheikh *et al.,* 2004, Radostitis *et al.,* 2007and Smith, 2009*)* so that this work was designed to evaluate the effect of diarrhea and dehydration on general health condition, body fluids, haematological picture and serum contents with special reference to fluid therapy with hypertonic and isotoic solution.

2. Material And Methods

This study was carried on 61 calves from different farms at Sharkia governorate. The calves aged from birth to 45 days and weighted from 24-50 kg. They were with normal body temperature and free from parasite. From these calves 25(healthy control calves) and 36 diarrheic calves with different Degrees of dehydration (Table 1) were used for this study.

Blood analysis: Total erythrocyte and leucocytic count, packed cell volume (PCV %), haemoglobin content (g/100 ml) were done according to Coles (1986). Blood pH was done by digital pH meter model 201.

Serum analysis: Determination of serum total protein (Henry, 1974), urea nitrogen (Tietz, 1990), creatinine (Henry, 1974) and chlorides (Kaplan and Pesce, 1996) were determined. Also, Serum sodium and potassium were determined by using flame photometer according method described by Hawk (1965).For determination of total serum solids: Serum sample (W1) was dessicated in glass dish at 107 oc till obtaining constant weight giving Dry matter DM (W2), then moisture content=(W1-W2), The DM%=(W2 divided by W1)x 100, The moisture %=100-DM%. Also, The DM and Moisture % of faeces was done by the previous method but usig faecal sample instead of seum sample.

Lines of treatment: Avoid suckling in the first day then gradually introduced, in addition to Kaopectin[®] (antidiarrheal drug produced by Idpco Animal Health. It contains kaolin, pectin, peppermint oil, lemon oil, electrolytes and others, Each diseased calf received 100 ml of kaopectin[®] daily for three successive days or till recovery).

Fluid therapy for moderated diarrheic dehydrated calves included: Normal saline 0.9 %, Glucose 5 %, sodium bicarbonate 2.6%: 500 ml of each intravenous, may be repeated every 12 hour when necessary.

Fluid therapy for severe diarrhoeic dehydrated calves included: Glucose 5 % (500-1000ml), saline solution 7% (25 ml), sodium bicarbonate 8.2% (25 ml) injected slowly intravenous for each calf, may be repeated every 12 hour when necessary.

Statistical analysis:

The obtained data were analyzed by univariate analysis of variance according to *Snedecor and Cochran (1969)*. For Mean separations, Least Significant Differences (LSD) and Duncan Multiple Range Test were used. Probability $\leq$ 0.05 considered significant.

3. Results:

Clinical findings: The diseased calves were suffered from diarrhea which varied from whitish to yellowish in color, offensive to foetid or unpleaseant odour, soft to watery in consistency with highly moisture percent. The faces which soiled the buttock and tail may be tinged with mucous with or without tenesemus. The temperature was normal, while pulse and respiration rates were increased (Table 1).

Dehydration and clinical metabolic acidosis were represented with a moderate to a severe degree. They were the most serious complication of diarrhea.

Dehydration was manifested by varying degree of dryness of skin, sunken eyes, loss of body weight, recumbency, sometimes coma and death occurred in non treated cases. While acidosis were manifested clinically by increased respiration, loss of condition and weakness which varied from standing position with dullness, depression and inappetence to complete anorexia.

Eighteen calves were found in standing position or need assistance to stand. These calves were dull, depressed, decreased or no appetite, increased respiratory rate, slight degree of sunken eyes and dryness of skin which persist for 5-10 seconds (Moderate degree of dehydration). Other18 calves were found recumbant with complete loss of suckling reflex, increase of respiratory rate as well as clear sunken eyes and dryness of skin which persist for 10-15 seconds *(Severe degree of dehydration).*

Blood examinations of diseased calves revealed a significant increases of RBCs. count, PCV%, Hb content with non significant increases of WBCs.

Serum of the diseased calves showed a significant increase in the level of serum total protein and serum total solids, a highly significant increase of SUN and a significantly increases of serum creatinine.

Serum electrolyte of diseased calves reveals a highly significant decrease of serum sodium, a significant decrease of chloride meanwhile the serum potassium level was significantly increased.

From all examined calves three of diseased calves were dead after severe dehydration, coma, circulatory failure, subnormal body temperature as well as delayed and incorrect therapy.

Table (1): Clinical findings of different degree of dehydrated diarrheic calves

Items	Control(25 Cases)	Moderate (18 Cases)	Severe (18 Cases)
Skin elasticity/seconds	Less than 2 Sec	4-6 Seconds	More than 10 Sec
Eye ball appearance	Normal	Moderate sunked	Deeply sunked
Capillary refilling time	Less than 2 Sec	2-6 Sec	2-6 Sec
Distention of jugular vein	Good	Good	Collapse
Scleral blood vessel	Filled with blood	Filled	Empty
Conguctival muc. membran	Bright red colour	Pale	Pale & dry
Rectal body temperature	38.5-40	39-40.5	36-38
Extremities temperature	Normal	Warm	Cold extremities
Heart rates beat/minute	85-100	120-140	50-88 bradycardia(Weak, irregular)
Respiratory rates/min	25-36	33-53	40-70 irregular
Corneal & anal reflexes	Good	Sluggish	Very Sluggish or absent
Suckling reflexes	Normal	Weak	Weak or absent
Dry matter % of faeces	More 28.53+-0.27[c]	11.07+-0.04[b]	7.13+-0.13[a]
Moisture % of faeces	Less than71.97	88.98	92.87
Calf demeanur	Stand	Stand with assistant	Sternal or lateral recumbency
Mental condition	Normal	Lethargy	Coma & or Convulsion
Body condition	Good	Good to moderate	Poor

Table (2): Blood and serum contents of 15 healthy control calves and calves suffering from moderate (15 cases) and severe (15 cases) dehydration and dietetic diarrhea, before and after treatment.

Items	Control calves	Moderate diarrhoeic dehydrated calves		Severe diarrhoeic dehydrated calves	
		Before treatment	After treatment	Before treatment	After treatment
RBCs million/Cu mm	7.44±.03[a]	7.99±.03[b]	7.54±.03[a]	10.15±26[c]	7.7±.04[ab]
WBCs Thousand/Cu mm	7.64±.05[a]	7.91±.03[b]	7.60±.02[a]	7.96±.03[b]	7.64±.03[a]
Hb gm/dl	9.77±.17[a]	10.7±.20[b]	9.57±.11[a]	10.90±.29[b]	9.60±.10[a]
PCV %	33.53±.60[a]	43.07±.64[c]	36.40±.59[b]	51.73±.76[d]	36.73±.63[b]
Blood pH	7.41±.007[c]	7.24±.01[b]	7.38±.007[c]	7.13±.03[a]	7.41±.003[c]
Total serum protein g/dl	6.33±.06[a]	6.8±.05[b]	6.4±.02[a]	8.12±.06[c]	6.76±.05[b]
Total serum solid g/dl	3.14±.06[a]	5.20±.07[b]	3.25±.03[a]	6.17±.07[c]	3.31±.04[a]
Serum urea nitrogen mg/dl	30.27±.83[a]	37.10±.36[c]	33.80±.38[b]	37.60±.41[c]	34.87±.24[b]
Serum creatinine mg/dl	1.64±.03[a]	2.64±.08[b]	1.62±.03[a]	3.00±.04[b]	1.63±.02[a]
Serum sodium m.Eq./L.	134.07±.41[c]	117.67±.55[a]	128.33±.45[b]	116.87±.27[a]	129.00±.48[b]
Serum potassium m.Eq./L.	5.44±.03[a]	6.43±.05[c]	5.69±.03[b]	7.21±.06[d]	5.70±.03[b]
Serum chloride m.Eq./L.	90.60±.65[c]	71.93±.358[b]	91.00±.218[c]	69.13±.60[a]	90.00±.49[c]

4. Discussion

The observed clinical signs of diarrhea may be attributed to dietetic errors, suckling managements, fermentation of milk and formation of lactic acidosis causing irritation of the mucosa of the gastrointestinal tract leading to maldigestion, malabsorption, hyperperistaltic and rapid passage of gastrointestinal contents resulting in a significant decreases of DM% and a Significant increases of moisture % of the faeces (Table 1), Moreover, the increased respiratory rates with labored respiration may be attributed to the decrease of blood pH(Table 2) that stimulated respiratory centers in the medulla oblongata, leading to increase in the depth and rate of respiration (hyperpnoea) to eliminate the excess of carbon dioxide(El-Sheikh 1987, Radostitis *et al.,* 2000) Elsheikh *et al.,* 2004. Radostits *et al.,* 2007, and Smith, 2009). They attributed dehydration and acidosis to loss of body fluids and electrolytes included bicarbonate, sodium, potassium and chlorides in faeces. The acidosis was manifested clinically by hyperventilation and increase the respiratory rates.

The recorded tachycardia at the early stage of diarrhea(with moderate dehydration) and bradycardia with cardiac arrythemia at severe diarrhea and dehydration agree with Radostitis *et al.* (2000). They attributed tachycardia at the early stage to an increase in the heart rate to maintain the circulation, meanwhile at late stage the higher concentration of potassium in blood resulted in bradycardia and cardiac arrythemia or even death.

The increases of Hb, PCVand serum total solids (Table 2) may be attributed to dehydration and the reduction of water content in the vascular space (Coles, 1986, Radostitis *et al.,* 2000).

The recorded metabolic acidosis (Table 2) attributed by El-Sheikh (1987) to: 1.Excessive loss of bicarbonate in faeces. 2.Excessive production of lactic acid in body tissue by anaerobic glycolysis. 3, Organic acid production by abnormal gut flora. 4.Limitation of renal excretion of hydrogen ion.

The slight increase in serum total protein and the significant increases of total serum solids may be attributed to the excessive loss of body fluids and concentration of some blood component. However, the protein loss by catabolism or by leakage into intestinal lumen was lowered in diseased calf *(*Scott *et al.*, 2004*)*

The elevation in both serum urea nitrogen and creatinine levels in enteric calves was previously reported by El sheikh(1987) and Deshpande *et al.* (1993). Furthermore, the serum urea nitrogen and creatinine levels were increased in severe than moderate dehydrated diarrheic calves. This increase may be attributed to hypovolemia, reduced renal perfusion rate and function as well as increasing the catabolism of protein by increasing the degree of dehydration (Schlerka and Baumgartner, 1995).

The gradual decreases of serum sodium and chloride with gradual increases of serum potassium levels in diarrheic calves are supported by Kaneko *et al.*(1997) *and* Radostitis *et al.* (2000)*. They* attributed this changs to the loss of sodium and chloride with intestinal secretion which associated with diarrhea. Tasker (1971) also added that the serum chlorides level usually follows sodium level because chloride was usually found in the form of sodium chloride. The increase of serum potassium level in diarrheic calves was attributed by Coles, 1986; Radostitis *et al.* (2000) and Scott *et al.* (2004) to:-1, Excessive excretion of K in scouring faeces17.5 times than normal. 2,Decrease renal tubular excreton of K. 3,The body made

compensatory mechanism by moving hydrogen ions in cases of metabolic acidosis and during catabolism into the intracellular fluids, this movement of hydrogen ions into cells would force the potassium ions to extracellular fluids resulted in hyperkalemia

An improvement of general health condition and most determined data in diseased calves was occured after treatment as a results of improved plasma expansion and restoration of normal fluid balance (Constable, 2002 and Smith, 2009):

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8/12/2012

Seroepidemiology evaluation of Toxoplasma IgG values in women at their marriage age
and pathogenesis factors

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Abstract: Protozoan Toxoplasma gondi is an important cause of serious infections spreading among the human population and domestic animals in the world and the chronic phase in animals is after asymptomaticsyndromes. What's disease, acute infection, asymptomatic? In allcases of acute infection due to the persistence of parasite cysts observed, the disease is caused by the reactivation of cysts. Married women exposed to the disease could predispose them to disease, This study aimed at determining the level of IgG antibodies at the time of their marriage counseling has been done in Zahedan. During this descriptive – analytical study, 280 women referring to the Razmjoo moghaddam central laboratory of Zahedan from February 2009- to December 2009 were measured for IgG by ELISA method. Then blood samples were taken from them and the level of blood IgG antibody was measured by ELISA method and the results with obtained IgM measurement and their relationship with demographic issues, including those of residence, education level, were noted. Home cat care and pet at home, and how the meat is cooked were surveyed.28.2% of patients with type IgG antibodies were having significant positive correlation with the maintenance of home cats and how the meat was cooked. In other words, women who kept cats in their home and had half-cooked or raw meat to eat, showed increased positive antibody. The results of these studies indicated that a high percentage of susceptible women of childbearing in Zahedan city, were without a note of antibodies against Toxoplasma and thus they are susceptible to infection. Thus in order to prevent infection of susceptible pregnant women and prevent problems that may arise during pregnancy, awareness and control of pathogens associated with disease, education and health care practice, are recommended.
[Hossein Ali khazaei, Mohammad Bokaeian, Mani Javadimehr , Gholam Reza Soleimani , Amin Khazaei , Behzad Narouie , Mohammad Jafari Modrak and Agheel Miri. **Seroepidemiology evaluation of Toxoplasma IgG values in women at their marriage age and pathogenesis factors.** Life Sci J 2012; 9(4):185-190]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 27
Keywords: Antibody, Toxoplasma gondi, ELISA

Introduction:
Toxoplasma gondi was found for the first time in 1908 at the Pasteur Institute in Tunis by Nicolle and Manceaux in a North African rodent, called Clendactylus gondi and was named after it(1). The condition of the intracellular protozoan is one of the main causes of serious infections in humans and in domestic animals with worldwide distribution.

Remember this is a chronic disease after disappearance of the acute phase of

infection and also because of the persistence of the parasite to form cysts is observed and hence the reactivation of the disease is seen (2, 3).That cats are definite veintraepithelial host and protect the sexual cycle of the parasite. Other contaminated domestic animals have non-sexual cycle outside intestine, and are also considered as secondary hosts (4, 5). This disease

transmits in different ways such as gastrointestinal, placenta to the fetus during pregnancy, transmit randomly to laboratory workers, organ transplantation, through skin abrasions and conjunctivae tissue wounds and blood transfusion (6, 7).Contamination, especially in women during pregnancy can cause health problems. Their fetus is contaminated and susceptible to infection and miscarriage, premature birth and congenital anomalies, and the disease such as corioretinitis is caused (8, 9, and 10). There is fetal risk of congenital infection, depending on the time of mother's pregnancy. If it happens at the last period of pregnancy, most of the symptoms of disease occurred in child are seen as subclinical (11, 12).In order to prevent the effects of disease before pregnancy, giving counseling before marriage about awareness of routine laboratory tests can benefit them. Whether they are susceptible to acute or chronic infection therapeutic measures can belinked in the treatment so that non-immune individual can be benefited from special health care facilities before pregnancy (13).

Methods:
Women referred to Central Laboratory for marriage consultation during October 2010 –January 2011 were studied by descriptive and cross-sectional study.
Given the prevalence of disease with 35 percent, obtained by studies conducted in different parts of the country and with regard to statistical formulae, the accuracy of samples as0. 07, and with 95%confidence interval, 280samples were determined. Demographic data collected through questionnaires were completed by each personandthen2mlof peripheral blood samples was collected with heparin and IgG levels were measured by ELISA method, using commercial kits. Results obtained were analyzed and evaluated by using SPSS 17 statistical software and chi-square Test was used to find out the significant relationship among results.

Results:
The average age of women who were referred to University Central Laboratory, for marriage counseling was 21.73 years, and the highest age frequency calculated was 20 years. Education level of most of them (41.1%)was high school degree, and the least had primary school qualifications, if not uneducated, that no statistical significant relationship between antibody reactivity with the educational level was observed(P=0.65).Serum test results, of them determined showed that 28.2 % individuals, had IgG antibody and71.8% of them had no antibodies.
Therefore, the prevalence of IgG antibody positivity among women who are going to be married was estimated as 28.2%.Those who (50%) kept catsin their home, have had positive IgG. The statistical analysis revealed statistically significant positive relationship between the two variables. This means that the group that was dealing with cats, the likelihood of positive IgG was approximately 3.5 times equal to the groups that had no dealings with cats (P= 0.014) (Table and Figure1).While18.1%ofpeople who have had their home domestic animals, were IgG positive that showed no significant statistical correlation between the maintenance of petat home and test IgG responses(P=0.93))(Figure2).In addition,57.1% of people, who ate half-cooked meat, were IgG positive with the positive relationship between these two variables was statistically significant.(P=0.002)(Figure3).

Discussion:
Toxoplasma infection is relatively common parasitic disease between humans and warm-blooded animals. This is a wide spread disease worldwide and its prevalence in different parts of Iran is different, and the exposure to different people, especially women, the possibility of miscarriage, premature delivery and congenital malformations in children who are born is imminent. It is necessary to obtain a level of awareness for safety of women and girls susceptible and non-immune to Toxoplasmosis at the age of marriage. Using this method, it was found that 28.2% of them have anti-Toxoplasma IgG antibodies have confirmed that this is the point, that 28.2% of women in Zahedan already "been infected with Toxoplasma and there is no need to re-evaluate and follow up them during pregnancy.
In addition,71.8%of these are such individuals, who were not safe at the age of marriage, and are not safe during pregnancy, stand for the chances of acute toxoplasmosis infection .If these women decide to marry after getting their test results and within short time after they are pregnant, they have to take urgent action to prevent pregnancy complications to make sure that their baby remain immune to congenital toxoplasmosis. In a research similar to that of marriage on women in different areas of country, the reports vary according to geographical and environmental conditions, and predisposing environmental factors provided the results of these investigations, and these results are consistent.
In a similar study conducted on women during marriage in different areas of Iran, the reports provided varied, according to geographical and environmental conditions, and predisposing environmental factors, and these results are in consistent with our present study. In many countries, studies conducted on pregnant women ,no global study was done on IgG-positive cases prior to marriage, and Globally, only one study is available through Medline, Mr. Hashemi and his colleagues study conducted on Ghazvin unmarried women , in 2010 , showed that34%of them have had positive IgG(15) and

in studies similar to these results, in women at the age of marriage in Ardabel City 3.42%, and in high school girls in Julfa 21.8% (17), in women of reproductive age 25% in Ahvaz City(18), in girls at Ahvaz University of Medical Sciences 4.6%(19), in girls at girls high school in the six areas City of Isfahan18.4%(20),In unmarried women of the total population of Isfahan Province 41.4% (21),girls among different regions of area 10to 21percentpro rata to age, dietary habits, and exposure to domestic cats(22) ,this rate in Mazandaran women introduced to laboratory before marriage, 74.6%(23),and girl students in different fields of Kerman University of Medical Sciences, 16% (24),have been reported, and these results suggest that differences are due to climatic conditions, socio-economic status and literacy levels and other environmental predisposing factors such as , living with cat sand pets, and how to cook meat are some of data involved in the study of the researchers are consistent with the present study results. In this study the relationship between IgG positive IgM results obtained in previous measurements in the study population was found(14) and it was shown that 10 people who were IgM positive, had IgG positive titers.(Table 1).

In other words, one third of study population who have had positive IgM, were IgG negative, that this suggests that in recent weeks they have been affected by disease, and if pregnancy occurs during this time follow up and further investigations are needed and may be based on reports of researchers, about 50% of untreated pregnant women, can spread infection through the placenta to their fetus (2, 20)

In the study of Mr. Baillargeon JP similar findings in women referred Health Center in Urmia City this level was 32% for IgG and 6% for IgM (25).

In Oormiey city these levels on women on the verge of marriage were reported as 32.8% for IgG and 4% for IgM (26) and among unmarried women aged 35-14 years in Kashan, 96.4% for IgG and 3.6% for IgM were positive, and of those infected with the parasite were detected (27).Girls in Ardebili referred to Ardabil laboratory health centers for tests before marriage, in 1381 , 34% were reported to have a positive IgM and 4% of them have anti-Toxoplasma IgG positive for Gondi ,that these differences in the reports are relating to different methods of disease transmission, climate, cultural traditions and food, and laboratory methods (28).

In this study the age range of study subjects between 13-43 years with a mean age of21.73 was achieved among the positive IgG levels, and age relationship was not statistically significant. Because the people may be infected by parasites in the body after two to three weeks, in the chronic form, cysts formed for a long time even to end of life in the heart, brain and other tissues, including muscle are likely to gain strength. In addition

to among the factors of educational level and the residency status due to which IgG was positive, significant relationship was not observed. Non-significant relationship of amount of IgG with demographic factors dealing with the toxoplasma epidemiology has been reported by many studies.(16-18,21,25,28).This in some studies a significant relationship with age, occupation, education level, place of residence is reported that with increasing age, contamination has been common. Hashemi and colleagues study the highest risk to people without jobs (38.3%) and lowest (22.6%) were allocated to High School students (15).In an another study carried out in the city of Ahvaz, the lowest IgG level (6.4%) was observed in educated girls with high health behavior (19).Similar findings, among high school girls aged 14-19yearsfromdifferent parts of the city of Isfahan were observed. This rate increased with age and the results were proportionally variable to urban defined range. So in some areas of town IgG positive rate was up to the border of 27.5% and the lowest was 14.5% (20).The situation among girls of different areas of the city the age varied proportionally from 10 to 21 percent (21) and in different cities of Mazandaran Province this amount, was 74.6% that infection rate increased with increasing age and it was more in illiterate women, and major difference among the rate of positive cases in rural and urban residents, and also in employed and unemployed was not seen(23). In girl students of different subjects at Kerman University of Medical Sciences, Kerman, a positive serologic test for significant relationship between living and not living in dormitories was observed (24).

The above results obtained are similar to this study results. The survey on predisposing factors of pollution in the study cases show 28.2%ofwomenin Zahedan, have been infected maybe due to factors such as stray cats in the city because of this reason city is contaminated by pollution and to have a need for further investigation on population and rate of contamination with parasite and contact with cats.

In this study 50% of the subjects were keeping cats in their home. Similar findings in women referred to the city of Urmia health centers (25) and high school girls in Julfa were also observed (17).While this relationship has not been established in other studies(18,19).Tourkan and colleagues study (30) done over rate of infection in owners of domestic cats and others without contact with cats showed some of both groups had a positive IgG, it shows that they were previously infected with this parasite, and none of them were negative for IgM and significant interface between Toxoplas main fection and a history of keeping cats at home was not observed. Thus we can say that somehow infected cats often frequented their homes and the excreted acolytes by cat led to pollution of water, vegetables, cooking utensils

and food in their home and individuals in our study had a similar situation. Differences resulting from various studies conducted in Iran indicate that climatic condition, socioeconomic status and educational level and abundance of cats and how to live well, are associated with them. So through train in gand raising their awareness we can prevent them from getting infected.

About people who ate raw vegetables or vegetables were not properly cleaned and disinfected, possible contamination of cat oocytes was increased (29), and in the city of Zahedan, according to its desert condition improper growing of vegetables and imported ones, are possible. The vegetables were imported from neighboring provinces or vegetables were not well disinfected and cleaned, contamination was possible by means of cat oocytes. The researchalsofoundthat18.1% of subjects had domestic animals like sheep and cats, at their home, with positive IgG, which is similar to studies made by other researchers(16-19, 25,28) and in all studies, no statistical significant relationship between maintenance of domestic pet animals at home and level of IgG positive was seen. Although it was possible that these animals had no direct role in pollution and because of the supply of water and forage contaminated accidentally by soil contaminated with oocytes, the disease has spread.

In this study it was found that 57.1% of people who consumed half-cooked meat had IgG positive with as statistically significant relationship between these two variables. These results are similar to other similar studies that have been reported (16, 20, 22, 24, 25, and 27). While other studies that have been conducted (17-19, 24), did not reveal such relationship.

The difference in these reports may be related to several demographic factors including low levels of awareness about the way they spread disease, buy contaminated meat from itinerant people, lack of hand washing and be cyst attached to it and eat it when contact with contaminated hands, lack of sufficient heat to cooking a meal and consuming half cooked and get used to tasting the food before cooking is complete. Thus, adopting practices that promote health and raising the level of awareness about the occurrence and prevention of infection are recommended. Further studies on predisposing factors of disease are under way. Screening the women during marriage for infection should be given serious health priorities.

Conclusion:

The results of this study indicate that a large number of Zahedanian women during marriage did not show a note of antibodies against Toxoplasma and as result, they are susceptible to infection during pregnancy. Therefore, by adopting correct and improved practices we can improve their living conditions, and prevent infection and awareness and control of pathogens associated with disease is recommended. Education and health care should be exercised in them.

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10/03/2012

Table 1 - Relationship between Anti-Toxoplasma IgG and IgM levels in the population studied.

Total	negative	positive	IgG \ IgM
15	5	10	positive
245	186	59	negative
20	10	10	suspicious
280	201	79	population
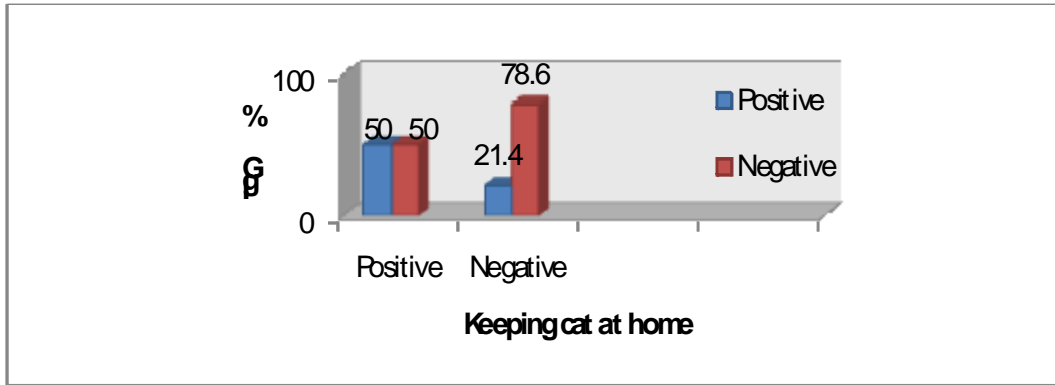
Figure 1: Relationship between positive and negative for IgGand maintenance of cats at home
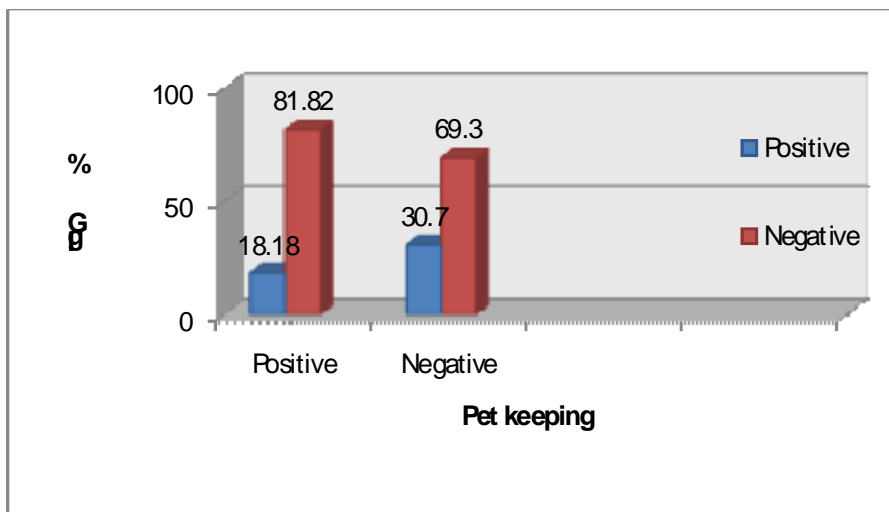
Figure 2 : Relationship between the rate of positive and negative IgG and individuals who maintain domestic pet animals at home
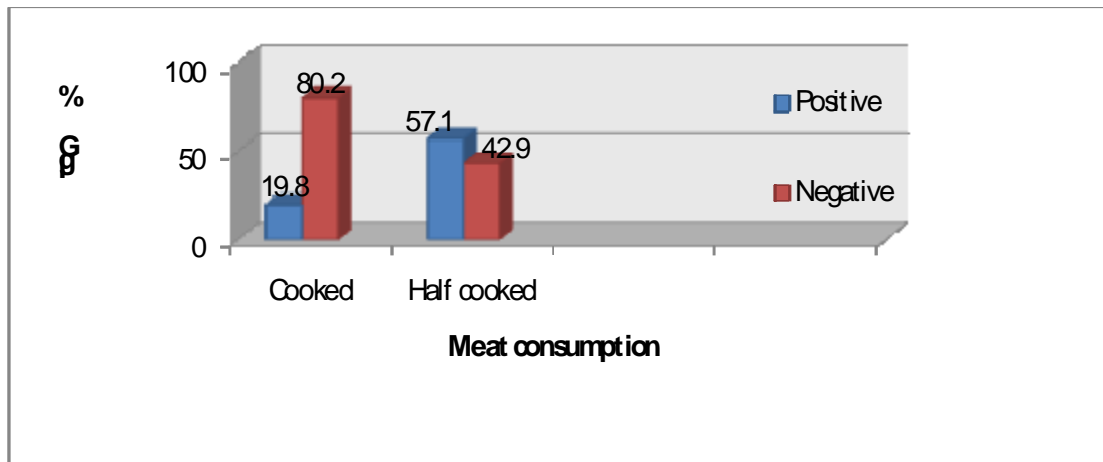
Figure 3 : Relationship between positive and negative IgG and method of meat consumption

The Condition of Hydrogen and Cell Battery in Iran

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Abstract: Today, the consequences of human intervention in the environment are more evident than any time before. Development has been synonymous with the nature and environment protection and in economic indexes of national accounts, such as gross domestic product, taking natural and environmental resources into account is so important. Energy is a basic need for economic development and providing welfare and comport for human life. Regarding what was said, the only way to human is using new clean green and renewable energies. Therefore in this study, in addition to a brief introduction on hydrogen energy and cell battery, the condition of these kinds of renewable energies in Iran will be discussed.

1- Introduction

In the 1980s, scientific evidence revealed that green house gases produced by human activities created some dangers for the global climate. Therefore public opinion sensed the necessity of establishing periodic international conferences and forming a treaty to solve this problem. In 1997, climate changes convention with the goal of fixing greenhouse gases concentration in the atmosphere up to the level that prevents dangerous intervention of human activities in climate wrote "Kyoto protocol". Under this protocol, industrial countries were obliged to reduce greenhouse gases [1, 2].

The ultimate objective of this convention was fixing greenhouse gases concentration in the atmosphere up to the level that prevents the intervention of human dangerous activities in climate. Such a level must be achieved in the determined time frame work so that ecosystems naturally adapt themselves with weather changes and it is assured that food safety is not threatened and economic development is not hampered. Thus, renewable energies are getting more and more share in global energy provision system. Renewable energy resources are alternately available and are not transportable or savable. For this reason they cannot be used as a fuel specially in transportation. So far the most common fuel for transportation in many countries in the world has been gasoline and diesel. Cars consuming gasoline or diesel generate harmful materials and pollutants with complicated chemical compounds. Although different measures have been taken to reduce them in the developed countries, they haven't helped much to the important issue of pollutants decrease. When fossil fuels are incompletely burnt with different hydro carbons, carbon monoxide, a poisonous material, is produced. Some unburned carbon atoms in fuel compounds and solid carbon particles are accumulated and exited as smoke and when subjected to sunlight, it is mixed with nitrogen monoxide compounds resulted from combustion and produce azoth [4].

Clean fuels have natural physical and chemical properties that make them cleaner than gasoline. These alternative fuels produce less unburned hydro carbons while combusting and the resultant materials produced by their burn have less chemical activities to produce other poisonous materials. Moreover, using alternative fuels reduce the formation and accumulation of carbon dioxide. Noticing technical-economic aspects of alternative fuels and their wide availability in Iran as well as increased consumption of hydro carbon liquid fuels in Iran which has caused a lot of damage to public budget and the environment has turned attention to introducing alternative fuels and studying about possibility of using them.

2- Hydrogen fuels features

Hydrogen is an abundant element on earth. It dose not exit in its pure form in the nature. So it is obtained from other elements by different methods. Hydrogen is the most important alternative as the new energy. This material compared to other fuels can turn other energies by much cleaner combustion

and higher efficiency. Nowadays using hydrogen in different industries is an urgent need. Hydrogen is widely used in chemical, food, metal and mineral industries, with the industrial development in the world and increased demand for energy, the world has encountered two important problems, namely in creased environmental pollution and the limitation of fossil fuels reserves. Since today one of the biggest problems of the world is pollutants caused by fossil fuels, hydrogen possesses some advantages in this regard. There fore noticing positive environmental, economic aspects of hydrogen, the world is driven toward steady development using hydrogen energy [1, 2].

Among advantages differentiating hydrogen from other fuel alternatives, abundance in consumption, insignificant production of pollutants, returnable production cycle and reduction of greenhouse effects can be named.

At present different countries in the world are investing in this section. Energy production seems one of the biggest challenges of the future. Thus using hydrogen to produce electricity seems essential. For this reason many countries are working on turning generators fuels into hydrogen fuel. Using hydrogen along with fuel cell is promising a bright perspective for the future. Hydrogen energy system, due to its independence from initial energy resources is also a permanent renewable infinite energy. Also it is predicted that in a near future, hydrogen production and consumption as an energy resource will spread all over the economic world; however, it must not be expected that hydrogen, at the beginning of its introduction to the world can compete with other energies in terms of price. On the other hand the role and share of gas fuel in the future is increasing with the movement toward the simultaneous production of heat and electricity in smaller power plants [1, 2].

In the future hydrogen and fuel cell can play a key role in controlling pollution in cities. Electric engines and fuel cell are suitable alternatives for combustible engines. In fact if hydrogen is provided from fossil fuels, fuel cell automobiles can result in zero pollutants in air. Total decrease in pollutants depends on the fact that if hydrogen is produced by fossil fuels or wind power or water – electricity power. Hydrogen as the best alternative and the most economical fuel cell automobiles has received a suitable position. Hydrogen is the simplest fuel to be used in fuel battery cars and increases their efficiency, with regard to producing hydrogen from natural gas (CNG) resources, its price is less than diesel and gasoline. Due to CNG resources available in the world and CNG distribution infrastructures in most countries as well as high efficiency of CNG transformer and its low environmental – natural damage, the use of CNG and changing it into hydrogen has attracted a lot of attention. The most important advantage of using hydrogen as a fuel is the fact that it produces water vapor and nitrogen oxide after combustion [1, 2].

3- Hydrogen production technology

Hydrogen is obtained from different sources including initial energy resources (Finite resources like petroleum), secondary energy resources (produced by initial energy sources such as gasoline) and renewable resources (produced naturally without human intervention like wind, such, water). Scientists call hydrogen the final fuel. At present hydrogen produced in industry is used as a chemical product. Hydrogen sale is less than %10 of its production in the world. In other words, %90 of the produced hydrogen is consumed in place.

Today hydrogen can be obtained by processes such as water electrolyses, CNG reforming and fossil fuel oxidation. At present more than %90 of the total produced hydrogen in the world is gained by fossil fuels and it is mostly used in oil industry and refinement.

Hydrogen is mostly used as a by product and now a small amount of the produced hydrogen is used as energy. Further study and research is required in order to optimize and diversify hydrogen production methods. Advanced methods are required to separate pollutants in order to decrease hydrogen price and increase its efficiency. Hydrogen is produced in big refineries, industrial are as and can be distributed easily to rural are as and customers homes [1, 2].

4- Fuel cell – hydrogen technology

Currently noticing energy crisis, fossil fuels restriction and problems caused by fossil fuels pollution, using new energy resources has attracted much more attention. For this reason a new viewpoint for using hydrogen due to its unique features has gained ground.

Using hydrogen as a fuel can decrease environmental pollutants and remove sulfur and carbon oxides caused by fossil fuels. The above – mentioned factors have put fuel cell cars in the center of attention. This technology, due to non – production of pollutants like nitrogen oxide, carbon monoxide and unburned hydrocarbons is a unique one.

Fuel batteries are kinds of energy trans formers that turn chemical energy directly into electrical energy. Fuel batteries act as a battery but unlike batteries, they keep working as long as they receive fuel and don't need recharge and their by products are water and heat.

Hydrogen needed for fuel batteries can be provided by different resources such as hydro carbon resources like petroleum, CNG, coal,… and renewable resources like wind, sun.

Common classifications for fuel battery based on type of their electrolyte are as follows:

- Polymer Fuel Cell (PEMFC)
- Alcoholic Fuel Cell (AFC)
- Phosphoric Acid Fuel Cell (PAFC)
- Melted Carbonate Fuel Cell (MCFC)
- Solid Oxide Fuel Cell (SFC)
- Metabolic Fuel Cell(DMFC)

Fuel cell consists of 2 electrodes and 1 electrolyte between them. Oxygen moves on cathode and hydrogen moves on Anode and produces electricity, water and heat.

Today these are enough attempts for the presence of Fuel cell in power plant industries, Transportation, and commercial stage.

Efficiency and the spreading Of Fuel cell cars are proper than common cars.

They also provide the decentralized production of energy as the 4th generation of power plants in future.

The possible uses of Fossil Fuel like Methanol and or natural gas in Fuel cell are other advantages of their system.

Today all car producers invest on commercial production of Fuel cell cars, these Fuel cell can be posed as a generator of energy for buses, boots, planes and even bicycles. In a small scale, they can be used in mobiles, phones and portable computers.

Fuel cell production technology like any other technology at the beginning might be quite expensive, but later in mass production and high rate of demand and technological development, its price will considerably decrease.

Fuel cell generally has many advantages as below:

High productivities, less chemical thermal and Audio, flexible installation, high safety, low Maintenance during and cost; division of administrative load, flexible in consumption of fuel, and possible thermal recovery. They have also other potential advantages. It produces carbon dioxide and less Nitrogen oxide against per KW produced Electricity and lack of presence of many moving parts Fuel cell can apply different gases such as natural gas, propane, gases produced from waste materials, Methanol and hydrogen as fuel. They even can be used for emergency light, housing electricity.

Iran has faced with two problems one is high Fuel consumption and another problem is lack of proper development in technology at car industries and power plant. And lack of attention to problems caused by environmental pollutions has increased this problem.

There for, producing Fuel cell can be a proper remedy for continuous development.

With certain consideration to this technology different research centers in Iran including Iran new energy organization has performed many projects.

5- Projects in Iran

5-1- energy pilot independent from Network based on solar Hydrogen and fuel cell

- Year start 1996
- Year and 2005
- place of implementation Taleghan sit Independent pilot from network with converting solar energy to electrical energy will provide production and hydrogen saving as energy carrier. This carrier can be converted to electrical energy and fulfill the ultimate user [3, 5].

Basic elements of this pilot are as follows:

Photolytic 10 KW capacity (Figure 1)

Electrolysis with 5 kw and Nominal production capacity 1 normal m/hr hydrogen (Figure 2)

Hydrogen tank (Figure 3)

Fuel cell system with 1.2 KW capacity (polymer – like) (Figure 4)

At present this pilot has lunched in Talegan energy site and is at data obtaining process.

Trained results:

- Practical feasibilities of photovoltaic system connection to hydrogen production systems.
- Presentation of independent energy system from network based on solar energy, hydrogen and Fuel cell.
- Analyzing application of solar hydrogen system independent firm network.
- Analyzing performing hydrogen as energy carrier.

Figure 1, Photolytic 10 kw capacity Power Plant

Figure 2, Electrolysis with 5 kw and Nominal production capacity 1 normal m/hr hydrogen

Figure 3, Hydrogen tank

Figure 4, Fuel cell system with 1.2 KW capacity

5-2- pilot installation of Hydrogen semi – industry with 200 kw capacity and purchasing and installation of Electrolyze device 30 normal per m (150 kw) and liquid making Equipment and other spare gas Analyzing systems.

Data started: 2001
Forecasting of date ended
Place Taleghan
Project goals:
- Hydrogen production with:
- Hydrogen storage with capacity of 20 m and 100 bar force
- Production of electricity with using of Fuel cell systems.
- Lunching a unit for hydrogen filling

Project description
The design of energy pilot accustomed with ecology which is performing in Taleghan energy site, is done with the aim of hydrogen Energy and providing conditions of recognizing bassinets of this energy carrier.

This pilot is consisted of production units (water electrolyzed), storage (gas – like under high pressure), transferring (hose line) and hydrogen consumption (Fuel cell), which hydrogen production unit consists of 2 electrolyzing device with 2 capacity 30 Nm/hr and 40 nm/hr and receive the needed electricity from worldwide network. The hydrogen produced in this stage, is prepared for entering to liquidation cycle (with capacity of 20 lit/hr) after purification processes with 99/95 purity, produced hydrogen, first will be stored in a 20 m tank under operative pressure of 10 Bar. And then will be saved in 20 m tank under operative pressure of 10 Bar [5].

Hydrogen, in this stage is prepared for Application in Fuel cell (25 Kw) and lastly for ultimate users. In case for transferring of (H) we can replace some parts of hydrogen in the mentioned tank with special cylinders.

5-3 performing feasibilities studies – Analyzing Fuel cell attraction in Iran

Year started – 2002
Year ended – 2004
Place – Tehran
Project description
Guiding committee of new energies organization has defined the feasibility studies – Fuel cell attraction and strategies for technological development in the country in order to respond to substance trap questions and strategic of Fuel cell technology area [5].

For this reason guiding committee of Fuel cell has separated into 3 substantial stages:

1. Knowing the Fuel cell technology and its related bases
2. Collection of technological development strategy of Fuel cell in the country.
3. Determination of Approaches of Fuel cell technological development strategy.

Project results
- Obtaining the suitable energy generator
- Developing Fuel cell Technology in transportation industries, power plants, and countries portable strategy
 - Determination of Fuel cell Technology place for comparing with other competitors

5-4- Studies about saei peik power plants in the country by Fuel cell

Year started 2004
Year ended 2004
Place Tehran
Project Description
In this research the electricity of off – peak will produce by use electrolyzed and hydrogen.
Hydrogen will be kept in Hydrogen tank and during peak hour, this hydrogen will be converted by Fuel cell to electricity and enter the network [5].
Obtained results:
Application of Fuel cell for energy preservation during peak – off in power plants.

5-5 Marking planets of z – polar Thermoplastic polymer for polymeric Fuel cell
Year started 2005
Year ended 2006
Place: Esfahan
Project Description
Fuel cell is made is made of 2 – polar plates elements and the performance procedure in Fuel cell has a direct effects on efficiency and exist system. There are different ways to make 2-polars plate and based on the selection of certain way, different materials will be used [5].
In this project the aim is attain the technology of making Thermoplastic polymeric 2-polar plates.
In this procedure, thermoplastic polymer will mix with smoke particles and or Graphite and then in pressed molding device be molded to specific desired form.
Obtain results:
Attaining the Technology of making Thermoplastic polymeric z-polar plates for polymeric Fuel cell

5-6 Providing 25 KW Fuel cell
Year started 2006
Year ended 2007
Place: Taleghan site
The used Fuel cell system consists of 2 stack with capacity of 25 Kw, the durability of this system has estimated around 5000 hrs. The output electricity of these system is around 36 – 57 w DC and produced water in this system is 80 ml/min. figure 5 and 6 show the implements of this project [5].
Obtain results:
- To obtain experience of electricity to network.
- Exploiting and data gathering for studying the problems and finding the solution.
- Knowing the procedure of performance of Fuel cell system for applying in Fuel cell making.

Figure 5 Implements of 25 KW Fuel cell project

Figure 6 Implements of 25 KW Fuel cell project

5-7 Hydrogen – burn of peykan engine
Project Description
The application of Hydrogen gas different advantages such as, burning without co, coz and unburned hydrocarbons flam, low energy needed for flaming, high rate of flam high penetration speed, Approaching to Auto cycle suitable thermal value and thermal efficiency [5].
- In this project the following steps for hydrogenising paykan engine are considered:
1- Essential changes on Peykan Engine
2- Designing and making mixer of air and (H)
3- Improving sparkling system
4- Improving timing of
Obtain results
The transferring of technology of using hydrogen fuel in automobiles
Localized the referred technology

Figure 7 Implement used in 7 Hydrogen – burn of peykan engine project

6- Conclusion

According to the referred materials there is a serious effort in Iran to use fuel cell and hydrogen energy .and the policies for development and completion of plants are searched and under exploration and we hope in the coming future we witness a multilateral improvement in this field through hydrogen planets and fuel cell's in Iran.

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11/4/2012

Rapid Detection of Bcr-Abl Fusion Proteins by Immunobead Assay Flow Cytometry in Leukemia Patients

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Abstract: Philidalphia(Ph) chromosome [t(9;22)(q34;q11)] which results in the production of BCR-ABL fusion protein, with deregulated tyrosine kinase activity, is a hallmark of chronic myelod leukemia(CML) and present in a high risk group of acute lymphoblastic leukemia(ALL). This BCR-ABL tyrosine kinase has been specifically targeted by tyrosine kinase inhibitors (TKI) which have profoundly modified the treatment and prognosis of the diseases harboring this genetic abnormality. Consequently, the rapid and accurate detection of BCR-ABL is of utmost importance in the diagnosis and optimal management of leukemias. Currently applied techniques are RT-PCR and cytogenetic analysis, which are costly, time-consuming, and require specialized laboratories. We utilized a recently developed immunoassay that qualitatively identifies the presence of BCR-ABL proteins in the leukemic cell lysate. The BCR-ABL is captured and detected by a cytobead assay and analysed by flow cytometry. We aimed at evaluating the effectiveness of this technique in detecting BCR-ABL in ALL patients and identifying CML cases among CML-suspected patients. The assay was conducted on 100 peripheral blood and bone marrow samples of 20 healthy controls, 55 patients suspected of having CML and 25 ALL patients. Results were compared to those obtained by conventional karyotyping and fluorescence in situ hybridization (FISH). BCR-ABL protein was positive in 35/55(63.6%) of CML suspected cases and 15/25(60%) ALL cases with 100% concordance with cytogenetic analysis data. The procedure was simple and feasible and proved to be reliable in rapidly identifying CML cases and Ph+ALL allowing for their prompt management.

Key words: BCR/ABL, flow cytometry, acute leukemia.

1-Introduction

The molecular analysis of recurrent chromosomal abnormalities (e.g. rearrangements, deletions) has provided valuable insight into the pathogenesis of hematological malignancies. A paradigm is Philidalephia (Ph) chromosome, caused by the reciprocal translocation between chromosome 9 and 22, t(9;22)(q34;q11), resulting in the BCR-ABL fusion gene which encodes a cytoplasmic protein with deregulated tyrosine kinase activity responsible for leukemogenesis.[1,2] Three fusion proteins of different sizes may be produced (p190, p210 and p230) depending on the breakpoint site within the BCR gene. The oncoprotein product phosphorylates many cellular targets, leading to the activation of intracellular signaling pathways such as Ras[3] ,Jak/STAT[4] and Akt/PI-3kinase[5] pathways, which induce abnormal proliferation, resistance to apoptosis, modification of cellular adhesion and genetic instability.

The Ph chromosome represents a hallmark of chronic myeloid leukemia (CML) as it can be found in almost all cases (95%). Furthermore, the introduction of the use of BCR-ABL tyrosine kinase inhibitor (TKI) (imatinib mesylate), that specifically targets the ATP-binding site of ABL kinase domain, in the treatment of CML has modified the natural history of the disease.[6,7]

As far as acute lymphoblastic leukemia (ALL) is concerned, the new 2008 WHO classification now recognizes B-ALL with t(9;22)(q34;q11) as one of seven genetic entities.[8] It can be identified in 2-5% of childhood, in 25-30% of adult and about 40% of older ALL patients. It is incorporated into risk stratification as its presence is associated with a very unfavorable prognosis[9,10] ,as well as risk- adapted therapy as TKI, in combination with chemotherapy or alone, is nowadays utilized upfront as first-line therapy for Ph+ALL.[11-14]

Therefore, it is of utmost importance in the diagnosis of both CML and ALL to demonstrate the presence of Ph chromosome or its transcripts. Molecular techniques, by real time qualitative polymerase chain reaction (RT-PCR) and cytogenetic analysis by conventional karyotype and fluorescence in situ hybridization (FISH) to identify BCR-ABL oncoproteins and Ph+ translocation respectively, represent the methods currently used.[15] These techniques, although reliable, are costly, time consuming and require specialized staff; criteria which are usually confined to specialized regional laboratories. A novel method for the detection of BCR-ABL fusion proteins using bead-based flow cytometric immunoassay has been produced and is available for research use only. The method utilizes a principle very similar to a classical ELISA test, where

the BCR-ABL proteins are immunocaptured on bead coupled with anti-BCR capture antibody, subsequently detected using a secondary anti-ABL1 detection antibody, producing a Sandwich complex comprised of both capture bead and detection fluorophore, to be read out by flow cytometry. The assay was performed on a cell lysate to allow for the detection of the intra-cellular proteins.[16]

The rapid-turnaround time, ease and specificity of this assay, coupled with the availability of flow cytometry in many laboratories and its established role as a cornerstone in the diagnosis and classification of hematologic malignancies, render this method very attractive for evaluation as it can be run in parallel to routine immunophenotyping. On the basis of the above considerations, we aimed, in the present study, at testing the effectiveness of the BCR-ABL proteins immunobead-based flow cytometric assay to rapidly and reliably identify Ph+ ALL cases among ALL patients and CML patients among CML-suspected cases.

2. Subjects and Methods

The presence of BCR-ABL protein was investigated in 100 freshly obtained peripheral blood (PB) or bone marrow (BM) samples. Those consisted of 20 healthy controls, 55 patients representing with neutrophilia and/or thrombocytosis suspected of having CML (age range=35-65 years) and 25 newly diagnosed ALL cases (17 adults and 8 children) attending Hematology/Oncology Units of Armed Forces Hospitals, Eastern Province, KSA over a period of 24 months.

All patients were subjected to thorough history taking, complete physical examination and radiological investigations (U/S and CT-scanning). Peripheral blood and/or bone marrow samples were drawn from the 55 patients suspected of CML for complete blood count (CBC), peripheral smear and BM aspirate microscopic examination, neutrophil alkaline phosphatase (NAP) scoring (Sigma, St Louis, USA), followed by detection of Ph chromosome by conventional G-banding and FISH analysis and the flow cytometric immunobead assay for the presence of BCR-ABL proteins.

Diagnosis of the 25 denovo ALL patients was established by morphological, cytochemical and immunological criteria according to FAB and WHO classifications. PB and BM aspirate samples were used for CBC, microscopic smears examinations and cytochemical staining. Immunophenotypic analysis was performed on Coulter Epics-XL flow cytometer (Coulter electronics, Hielaeh, FL, USA) by staining BM/PB cells with various combinations of fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin cyanin 5 (PC5) labelled monoclonal antibodies against the following antigens: CD2, CD3,

CD5, CD7, CD10, CD13, CD14, CD19, CD20, CD33, CD34, CD45, HLADR and MPO (Coulter electronics, Hielaeh, FL,USA).[18] Conventional G-banding and FISH analysis were used to detect t(9;22)(q43;q11), followed by immunobead assay for BCR-ABL proteins.

It should be noted that care was taken to include all the cases proved to be Ph+ by cytogenetic analysis in the study.

Detection of BCR-ABL oncogene or its transcript using:

I. Cytogenetic analysis by: A) conventional G-banding, which was done by classic techniques and interpreted according to International System of Human Cytogenetic Nomenclature (ISCN19) using chromoscan, applied imaging system (CytoVision). B) FISH analysis, using fluorophore-labeled single stranded DNA sequence probe homologous to t(9;22)(q34;q11) (TelVysion).[17]

II. Flow cytometric immunobead assay (CBA): was done utilizing the BCR-ABL protein kit (BD-Biosciences) which qualitatively identifies the presence of BCR-ABL fusion proteins in the cell lysate of examined sample. By lysis of leukemic cells , the oncogene proteins are released and are recognized by anti- BCR antibody coupled to a bead and a PE-labeled anti-ABL antibody, to be detected as the bead population mean fluorescence intensity (MFI) using a Coulter Epics-XL flow cytometer.

The manufacturer's instructions were followed:

1) Preparation of mononuclear cells concentrate (MNCs) using ficole-hypaque (in cases of suspected CML) or whole blood/marrow specimen lysate using lysing solution (in cases of ALL) containing at least 25×10^6 cell/mL.

2) Performing of cell pretreatment: 250 µL of pretreatment buffer (obtained by dilution of1X Stock pretreatment A and Stock pretreatment B) were added to each sample, incubated on ice for 10 minutes and washed once using phosphate buffer saline (PBS).

3) Lysing the leucocytes (to release the intra-cellular BCR-ABL protein): 100µL of lysing solution (prepared by diluting 50X Stock BD lysate treatment reagent in BD pharmagin cell lysis buffer) were added to each sample, incubated for 15 minutes and centrifuged at20,000g for 10 minutes at 4°C. The supernatant (cell lysate) was not discarded.

4) Performing the bead immunoassay: 50µL of cell lysate were combined with 50µL of capture beads (ant- BCR antibody coupled) and 50µL of detector reagent (PE-labeled anti-ABL antibody), incubated for 2 hours in the dark at room temperature with shaking sufficient to maintain

constant agitation. Washing was performed with CBA wash buffer and samples were resuspended in 300µL of the wash buffer.

5) Acquiring the data: on Coulter Epics XL flow cytometer after performing the cytometer set up according to the manufacturer's guide lines. The average MFI from normal healthy subjects was calculated, and a sample was considered positive for the presence of BCR-ABL fusion protein if its MFI was >2SD of PE-MFI of normal peripheral leucocytes.

3. Results

The results of the present study are presented in Tables 1-2 and figure 1.

This study was conducted on 100 subjects divided as follows: 20 healthy controls, 55 patients suspected of having CML and 25 denovo ALL cases.

The 55 patients suspicious of CML were 34 males and 31 females (M: F ratio=1.6:1) with age ranging from 35-65 years. Their clinical data are shown in (Table1). Complete clinical, radiological and laboratory investigations proved them to be 20 non-CML and 35 CML patients. According to clinical and laboratory criteria, the CML patients were further subdivided into 31(88.6%) chronic phase (CP) and 4 (11.4%) accelerated phase (AP) cases. The presence of AP cases accounts for the high upper level of blast count ranges noted in the PB and BM of CML cases. All 35 CML cases were Ph+ by conventional karyotyping and FISH analysis.

Regarding the 25 denovo ALL patients, they were 17 adults and 8 children with age ranging from2-52 years and M: F ratio of 1.5:1. In the samples collected from these patients, the leucocyte count (TLC) ranged from 18.2-60.8 x10^9/L with leukemic blast cells constituting 19-88% in the PB (absolute blast count=6.7-48.9 x10^9/L) and 45-95% in the BM (Table 1). Immunophenotypically, only one patient was T-ALL and the remaining 24 were B-ALL, which were further subdivided into 13 common ALL (cALL), 5 pre-B ALL and 6 pro-B ALL. By

cytogenetic analysis (G-banding and FISH analysis) 15 cases were Ph+ (14 adults and 1 child). Of note, 2 of the adult B-ALL cases were proved to be originally CML cases in blast crisis (BC).

As previously mentioned, care was taken to recruit all CML and ALL cases established to be Ph+ in this study.

The 20 healthy control subjects were cytogenetically examined for Ph+ chromosome by G-banding and FISH analysis and were proved negative (Table 2). The binding of the peripheral leucocytes of those 20 healthy subjects to the immunocapture beads was used to determine the analytical detection limit of the presence of BCR-ABL oncoproteins by flow cytometric immunobead assay (CBA). The MFI value from the normal cellular samples was 12.2±3.4, giving a cut-off value between positive and negative set at 24.3±2.2, as calculated by mean±2SD (Figure 1A).

When the 55 patients suspected of CML were tested by CBA for BCR-ABL fusion proteins, the results were 100% concordant with those of cytogenetic analysis, being negative in the 20 non-CML cases and positive in all 35 CML cases (Table 2). The CML cases expressed varying MFI, 21 with low MFI and 14 with high MFI, the highest being noted in the accelerated phase (AP) cases and the newly diagnosed patients (Figure 1B and 1C).

Similarly, fully concordant cytogenetic analysis and CBA results were observed in the 25 ALL cases included in the study (Table 2). 15 of the 25 (60%) studied cases proved to express Ph+ by G-banding and FISH, as well as being positive for BCR-ABL fusion protein by CBA, all with high MFI, including the B-ALL blast crisis of CML (Figure 1D). The CBA positive cases were 14/17 (82.3%) adults, and only 1/8 (12.5%) childhood cases. Regarding immunophenotypic distribution, 10/13 (77%) cALL, 2/5 (40%) pre-B ALL and 3/6 (50%) pro-B ALL expressed the BCR oncoprotein, while the single T-ALL case studied was negative for it (Table 2).

Table (1): Clinical and laboratory data of CML-suspected cases and ALL patient

	Non-CML (n=20)	CML (n=35)*	ALL (n=25)
Age (years)	35-58	38-65	2-52
Sex (M: F)	14:8 (1.5:1)	22:13 (1.7:1)	15:10 (1.5:1)
TLC (x10^9/L)	30.1-63.0	35.2-230.4	18.2-60.8
Hb (g/dL)	9.8-14.9	8.4-12.6	8.5-12.1
Platelets (x10^9/L)	263-845	108-634	18-201
%Blasts in PB	0	1-18**	19-88
%Blasts in BM	0-1	5-21***	45-95
Absolute blast count (x10^9/L)			6.7-48.9
NAP score	11-201	0-56	-
IPT: B-ALL		2 (BC)	24
T-ALL			1
Ph+ FISH	0	35	15

*: CML cases were 31 CP (chronic phase); 4 AP (accelerated phase) which account for the high blast count in PB
(**) and BM (***).

Table (2): Results of flow cytometric immunobead assay (CBA) compared to Ph+ by FISH analysis in all studied samples

		Ph chromosome (FISH)		BCR-ABL (CBA)		
		Negative	Positive	Negative	Positive	
					Low MFI	High MFI
Healthy controls(n=20)		20	0	20	0	0
Non-CML (n=20)		20	0	20	0	0
CML (n=35)	CP (n=31)	0	31	0	21	10
	AP (n=4)	0	4	0	0	4
ALL (n=25)	cALL (n=13)	3	10	3	0	10
	Pre-B ALL (n=5)	3	2	3	0	2
	Pro-B ALL (n=6)	3	3	3	0	3
	T-ALL (n=1)	1	0	1	0	0

A.
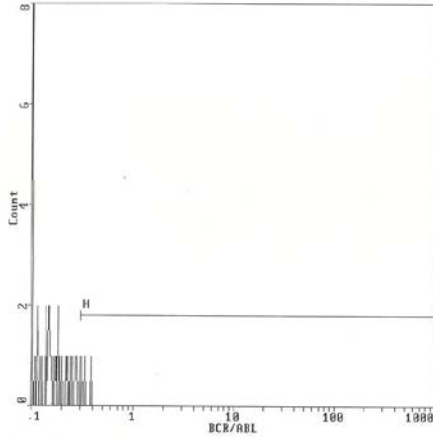
B.
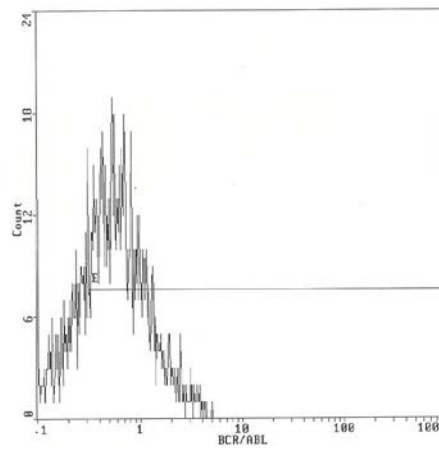
C.
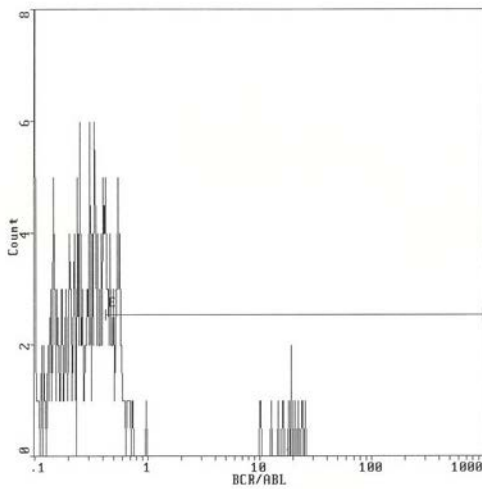
D.
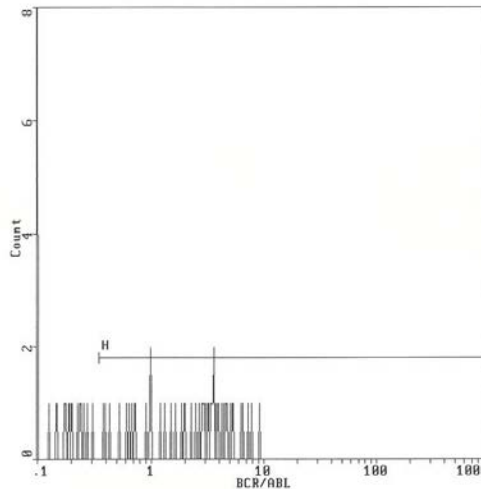
Figure (1): Representative data of BCR-ABL protein flow cytometric immunobead assay in healthy controls (1A); low MFI CML case (1B); high MFI CML case (1C) and B-ALL case (1D)

4. Discussion

Philadelphia (Ph) chromosome or t(9;22)(q34;q11) results in the BCR-ABL fusion gene, which encodes cytoplasmic proteins with constitutive tyrosine kinase activity. It is present in 95% of patients with CML and in a high risk subset of patients with ALL (2-5% of childhood ALL and 25-30% of adult ALL).[18,19] The BCR-ABL fusion protein has been successfully targeted for therapy by tyrosine kinase inhibitor (TKI), imatinib mesylate, which selectively induces growth inhibition and apoptosis of BCR-ABL positive cells.[20] Consequently, the detection of BCR-ABL Aberration is of utmost importance for the diagnosis, classification and treatment of leukemia patients.

Conventional approaches to BCR-ABL detection are limited to karyotyping and FISH analysis or RT-PCR techniques, all of which are time consuming and require specialized facilities.

A novel simple flow cytometric immunobead assay for the detection of BCR-ABL fusion proteins in cell lysates has been developed, which utilizes a bead bound anti-BCR catching antibody and a fluorophore conjugated anti-ABL detection antibody.[16] We aimed at evaluating this technique in detection of BCR-ABL protein in ALL patients and its differentiating power among CML suspected cases by comparing its results with those of conventional karyotyping and FISH analysis.

As far as CML is concerned, Ph+ is a hallmark of the disease, and its definitive diagnosis requires t(9;22)(q34;q11) demonstration. In the present study, 55 patients suffering from neutrophilia and/or thrombocytosis suspected of having CML were investigated. 35/55 of those patients were cytogenetically proved to be Ph+, and expressed BCR-ABL proteins positivity by flow cytometric immunobead assay (CBA), displaying 100% concordant results between the two techniques (35/35 cases). Our data are similar to those obtained by **Weerkamp et al.** [16], who demonstrated the positivity of BCR-ABL proteins in 19/19 CML cases studied by CBA and RT-PCR, and those of **Lucas et al.** [21], who studied 110 suspected CML cases and 70/110 were positive for BCR-ABL proteins by CBA and RT-PCR (70/70 cases, 100% concordance).

Pandey et al.[22], were able to detect BCR-ABL by CBA in 65 CML patients out of 75 cases proved Ph+ by FISH analysis, (65/75) with 88% concordance value. This difference in resullts could be due to the fact that in cell samples containing high frequencies of mature myeloid cells, protein instability problems may be encountered as a result of protease activity.[21] In an attempt to reduce this proteolytic activity present within mature neutrophils which could influence the CBA assay sensitivity, we chose to use MNCs of CML patients rather than lysate. Another step to decrease this interference could be the addition of protease inhibitors at several steps of the assay.[16]

We noticed a difference in the levels of MFI of BCR-ABL protein detected by CBA in the CML patients included in our study; 14 expressed high MFI while 21 expressed lower MFI. This variability in the degree of BCR-ABL expression is also present in previous studies.[16,21] Of interest, this high positivity was noted in all 4 AP cases and newly diagnosed CML patients, which could be attributed to the fact that the transcript and protein levels of BCR-ABL are elevated in primitive CML progenitors relative to more mature cells.[23] Furthermore, it has previously been shown that the degree of decrease in BCR-ABL transcript level within the first 3 months of TKI treatment can predict subsequent clinical outcome.[24] Therefore, this observation regarding the BCR-ABL MFI variability needs further evaluation, as it might pave the way to the use of a feasible, rapid and readily available test for monitoring disease course, response to therapy and prediction of clinical outcome.

Fifteen (14 adults and 1 child) of the 25 ALL studied cases proved positive for BCR-ABL by both cytogenetic analysis and CBA, with 100% concordance. Similar results were described by **Weerkamp et al.**[16] However, **Raponi et al.** [25] demonstrated lower sensitivity of the CBA, as they were unable to detect the fusion protein (proved positive by RT-PCR) in 2 steroid treated patients due to the very low leukemic cell count (blast cells constituting 2-2.3% of total cell count). Practically, this should not be a hindrance to the denovo diagnosis and classification of ALL, as virtually, no ALL patients at diagnosis has very low number of marrow or blood leukemic cells.

Therefore, in the study at hand, we have documented an absolute correlation between the expression of BCR-ABL protein using CBA and Ph chromosome detection by cytogenetic analysis. CBA successfully differentiated CML patients from cases with neutrophilia and/or thrombocytosis suspected of having CML. Since most patients with neutrophilia and/or thrombocytosis do not have CML, a rapid and simple screening test for BCR-ABL protein could be clinically useful. In ALL cases, CBA was able to identify the higher risk group of Ph+ ALL patients. The assay was reliable, applicable, relatively rapid, being successfully completed within 4 hours. It could easily be run in parallel to routine immunophenotyping.The availability of such a method capable of detecting the presence of BCR-ABL protein has important implications as it can document the effective transduction of the molecular transcript.

Furthermore, the BCR-ABL antibody was developed against a non homologous region of ~80 amino acids, encoded by exon-1 in order to detect all known BCR-variants (p190, p210 and p230) irrespective of the breakpoint in BCR-gene.[16]

The development of specifically targeted TKI against BCR-ABL kinase has had profound impact on the management of diseases harboring this genetic aberration. Indeed, TKIs have modified the natural history of CML.[6,7] Moreover, recent studies were able to induce hematologic remission with oral TKI, no chemotherapy and partly at home in ALL patients with Ph+.[11-14] This therapeutic approach to CML and Ph+ ALL patients requires the accurate and rapid identification of BCR-ABL fusion proteins in order to allow timely implementation of a targeted therapeutic strategy. Criteria currently unmet in many places, as the already utilized molecular techniques are expensive and require specialized staff. Furthermore, FISH largely depends on the availability of intact and informative interphase nucleii, and RT-PCR is hindered by the inherent variability in amplification and standardization of quantitation.[26] On the other hand, flow cytometry has long been established as a cornerstone in the diagnosis and classification of hematologic malignancies. Therefore, the possibility of utilizing such a rapid, simple, reliable and readily available technique opens the way to offering much required approach for diagnostic and therapeutic causes.[27]

In conclusion, the flow cytometric immunobead assay is a fast, easy and reliable technique for the specific detection of BCR-ABL protein in leukemic cells, with highly concordant results with currently used molecular methods. It can be used as a rapid and simple screening test for CML suspected cases, which could economize time and cost, as most patients will be negative and will not require further detailed investigation. In acute leukemia, it could contribute to its faster classification. Therefore, CBA could allow, in both diseases, CML and Ph+ ALL, for earlier diagnosis, prompt clinical and therapeutic management. It awaits further evaluation regarding its possible role in monitoring of clinical outcome and minimum residual disease.

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8/18/2012

Self Esteem, Job Satisfaction and Organizational Commitment of Faculty Members of Secondary Level Teacher Training Programme in Mysore (India)

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Abstract: The purpose of this investigation was to examine the demographic variables of length of teaching experience and age, affects self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore (India). In addition, the study aimed to find out the relationship between the three variables i.e. self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore. This research has been designed with a descriptive model. The study was carried out on all the faculty members who are working in educational colleges, affiliated to the University of Mysore in Mysore. The sample for the present study was drawn using stratified random sampling technique. About 165 survey questionnaires were distributed in 2012.Multiple follow ups yielded 134 statistically usable questionnaires. For collecting data, three standard measures of Rosenberg self esteem scale (1965), a job satisfaction scale developed by Singh and Sharma (1999) and organizational commitment questionnaire (OCQ) developed by Mowday, Steers, and Porter (1979) were used to confirming the research hypotheses; and for analyzing the obtained data, the statistical methods of one way ANOVA, Pearson's Correlation Coefficient and DMRT (Duncan's Multiple Range Test). The findings of the study indicated a significant difference between length of teaching experience and age of faculty members with reference to their self esteem. But with regard to job satisfaction and organizational commitment there was no significant differences. Test statistics revealed that teacher's self esteem, job satisfaction and organizational commitment were mutually related. Based on the results of this study, self esteem was found to be negatively related to job satisfaction and organizational commitment. These findings are important since previous research has not tested these variables together.
[Cherabin M, Praveena K.B, Mohammad Azimi H, Qadimi A, Shahani Shalmani R. **Self Esteem, Job Satisfaction and Organizational Commitment of Faculty Members of Secondary Level Teacher Training Programme in Mysore (India).** *Life Sci J* 2012; 9(4):204-214] (ISSN:1097-8135). http://www.lifesciencesite.com. 30

Keywords: Self esteem; job satisfaction; organizational commitment; educational college; faculty member

1. Introduction

Universities are the most important centers of research and educational activities in every country and faculty members at Universities are among the most significant players in the educational system and the most principled elements of development in all countries. Also, training expert human resources is achievable through reinforcement of academic education. Therefore, we must try to identify different motivating and stimulating factors and also variants that form these factors and their effects, and study and analyze them.

This research studies the self esteem, job satisfaction and organizational commitment among faculty members of secondary level teacher training program in Mysore.

One of the elements that are considered for the survival of organizations by managers or organizational heads is Human Resource. In a general view, success in any organization depends on efforts and job satisfaction of organization at staff. The 20th century has been called the century of anxiety and psychological disorders and one of the duties of managers; is considering their staff/employees' mental health. Managers should always consider self-esteem and job satisfaction as two important elements in the mental hygiene of the staff.

The relationship between job satisfaction and self-esteem and job satisfaction and organizational commitment has been pondered over for decades by many different professionals. These groups of professionals include Psychologists, Sociologists, academic professors, and people from the business community. Organizational commitment and job satisfaction are widely studied factors in management literature which are the precursors of employee's

performance. These factors are even more important to study in academic institutions, especially universities and colleges which are the sources of human resources and solely responsible for educating the intellect of nations. Teacher is the central element in educational systems holding various important responsibilities. The overall performance of universities depends upon the teachers and ultimately their level of commitment and job satisfaction. Thus understanding their behavior and attitudes needs more attention in organizations (Tsui & Cheng, 1999).

Faculty members are the main structure of every university. Colleges and Universities need experienced teachers as one of the principles for education in order to the raise of scientific level of students. Job dissatisfaction and low self esteem among faculty members could threaten their physical and mental health and life quality and could prevent achieving individual and social development. Faculty member's self esteem, job satisfaction and organizational commitment in higher education have been given little attention by researchers. The study presented here aims to be an incentive to the development of more studies in this thematic area.

2. Literature Review

2.1 Self Esteem

Self-esteem has long been considered an essential component of good mental health and has drawn many researchers' attention in recent years. In the field of psychology, a positive attitude toward the self has been accepted historically as a marker of healthy psychological functioning (Neff, 2003). Self-esteem is composed of person's self assessment and a combination of his/her self- concept of characteristics and abilities (Flouri, 2006; Osborn, 1997; Pope & McHale, 1988).The concept of self-esteem is one that is difficult to clearly define. To some it is confidence in our ability to think; confidence in our ability to cope with basic challenges of life; confidence in our right to be successful and happy; feelings of being worthy, deserving, entitled to assert our needs and wants, achieve our values, and enjoy the benefits of our efforts (Hooks, 2003). Self-esteem is generally defined as "a personal judgment of worthiness that is expressed in the attitudes the individual holds towards himself" (Coopersmith, 1967).

In basic terms, self-esteem is an internal belief system that an individual possesses about one's self. The concept of self-esteem has been researched by several social scientists. Self-esteem means truly loving and valuing oneself and is a personal assessment of worthiness. People with high self-esteem appear poised and confident and are less influenced by others. This is different from being self-centered, conceited, or obnoxious. Building

self-esteem is an ongoing process. It reaffirms that you have accepted yourself as you are, but continue to work on capitalizing on your strengths (Robins, Gosling, & Trzesniewski, 2002).

An individual with high self-esteem feels good about him/her and can face the challenges of life more effectively. High self-esteem provides the basis for success and coping with daily life in a rapidly changing environment. Self-esteem affects the way we relate to ourselves, to others, and to life in general. It affects the way we learn, work, and build relationships. Our personal success or failure lies in our self-esteem. If we believe we can, we do. If we believe we can't, we don't even try. If you have high self-esteem, you are willing to try new things, develop closer relationships, maintain self-confidence, and remain flexible (Potter, Gosling, & Trezesniewski, 2001). Orth, Meier, and Robins (2009) show that individuals with low self-esteem are prone to depression because they lack sufficient coping resources, whereas those with high self-esteem are able to cope effectively and consequently avoid spiraling downward into depression.

Self-esteem is often divided into "global" self-esteem and "specific" self-esteem; global self-esteem refers to the degree to which people like themselves as a whole, while specific self-esteem refers to the degree to which people like a specific part of themselves (Sanford & Donovan, 1984).College effectiveness is largely determined by the teachers in a particular college. Even in this era of technological progress, computers, televisions and videos have not replaced the teacher. Teachers who have a high level of self-esteem manifest themselves in the classroom as confident, relaxed and have a respectful attitude towards students. Teachers who have a high level of self-esteem are more likely to be flexible and exploratory in their approach to teaching.

2.2 Job Satisfaction

Job satisfaction does not have only one description. It has been defined in several ways by many authors and researchers. According to Spector (1986) job satisfaction is an attitude that illustrates the quality of people's feelings in connection with their job, generally or in connection with its different aspects. Weiss (2002) defined job satisfaction as the emotional reactions concerning the duties of employees. According to Ivancevich and Matteson (2005), job satisfaction stems from various aspect of the job such as pay, promotion opportunities, supervisors, and co-workers. Other dimensions such as policies and procedures, work group affiliation, working conditions and fringe benefits were found to be part of the five core dimension.

Lester (1982) defined teacher job satisfaction as the extent to which a teacher perceives and values

various factors such as evaluation, collegiality, responsibility and recognition. Teacher job satisfaction refers to a teacher's affective relation to his or her teaching role and is a function of the perceived relationship between what one wants from teaching and what one perceives it is offering to a teacher (Zembylas & Papanastasiou, 2004).

Woods and Weasmer (2002) suggested that when teachers are satisfied, the rate of attrition is reduced, collegiality is enhanced, and job performance improves. When teachers perceive a lack of support for their work, they are not motivated to do their best in the classroom, and that when teachers are not satisfied with their working conditions; they are more likely to change schools or to leave the profession together. Some other variables have significant interactions with teacher's job satisfaction, namely; gender, age, experience and position.

2.3 Organizational Commitment

There is a relationship between the perceptions and behavior of individuals. Individuals are influenced by their qualities, by the features of the people, the events which are perceived and by the atmosphere by which the processes are realized.

The study of commitment to the organization is important, because organizational commitment can influence employee's creativity, innovativeness, adaptation, and reduces withdrawal behaviors, such as delay and turnover (Clugston, 2000; Riketta, 2002).Organizations can achieve a competitive advantage through committed employees. Communication plays an influential role in the degree to which an employee is committed to organizational goals and values. Research indicates that employees who accurately receive information from their supervisors about their work environment, are better informed, feel an integral part of the organization, therefore the higher their commitment to the organization (Sias, 2005).

For enhancing quality of education, organizational commitment provides a suitable background and is a vital factor in educational environments. Organizational commitment has been a difficult multidimensional phenomenon to define (Meyer & Herscovitch, 2001) and it relates to constructs of productivity, organizational performance and service quality (Oshagbemi, 2000). Organizational commitment is a strong desire to maintain membership in the organization (Hackett & Lapierre, 2001; Mowday, Porter, & Steers, 1982).Organizational commitment is a psychological stabilizing or obliging force that binds an individual to courses of action relevant to the target of the organization (Bentein, Vandenberghe, & Florence, 2005; Meyer & Herscovitch, 2001) and it is a form of loyalty to the organization (Lambert, 2004).

One of the key factors that affect organizational commitment is human resources management practices. Herrbach (2009) states that training opportunities, assignment to new roles and provision of flexible working conditions are associated with organizational commitment and more specifically, these practices are strongly associated with the willingness to remain in the organization.

3. Research Methodology

3.1 Purpose of the study

The purpose of this investigation is to examine how certain demographic variables of length of teaching experience and age, affect self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore. In addition, the study aimed to find out the relationship between self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore.

3.2 Hypotheses

For the purpose of the study, the following research hypotheses were directed:

1. There is significant difference between the following categories of teachers with reference to their self esteem (A) Teachers of different length of teaching experience (B) Teachers of different age groups.

2. There is significant difference between the following categories of teachers with reference to their job satisfaction (A) Teachers of different length of teaching experience (B) Teachers of different age groups.

3. There is significant difference between the following categories of teachers with reference to their organizational commitment (A) Teachers of different length of teaching experience (B) Teachers of different age groups.

4. There is significant relationship among job satisfaction, self esteem and organizational commitment of teachers.

3.3 Sample and sampling design

Statistical range of population for this research consists of all 343 faculty members from 36 colleges of education, affiliated to the University of Mysore, Mysore.

In this research, Stratified Random Sampling Method was used. Sample size was calculated according to the table provided by Morgan and Corgis. Regarding the statistical range which is 343 number of sample is 186. Among 186 faculty members 73 were male and 61 were female teachers.

3.4 Measures used

In this research the following three tools were used:

3.4.1 Self Esteem Scale

Rosenberg Self-Esteem Scale (RSES) was used to measure self-esteem. The RSES is a 10-item

self report measure of self-esteem based upon satisfaction of one's self and life. The instrument consists of five positive items and five negative items and need to be scored accordingly by reversing the value of either the positive or negative item responses. Typically, each item is answered on a four point Likert scale ranging from "strongly disagree" to "strongly agree". In this study the Cronbach's alpha was found to be 0.81.

3.4.2 Job Satisfaction Scale

This scale developed by Singh and Sharma (1999) has been used for measuring job satisfaction. The scale incorporating 30 items of both, intrinsic and extrinsic of the job. (1) Job intrinsic statements (factors lying in the job itself.i.e.job concrete and job abstract factors) (2) job Extrinsic statements (factors lying outside the job i.e. Psycho-social, Economic and Community/ National growth). The scale has both positive and negative statements. The positive statements carry a weightage of 4, 3, 2, 1, and 0 and the negative one a weightage of 0,1, 2, 3 and 4.The total score gives a quick measure of satisfaction/dissatisfaction of a worker towards his/her job. In this study the Cronbach's alpha was found to be 0.84.

3.4.3 Questionnaire of Organizational Commitment

In this research organizational commitment was measured using the organizational commitment questionnaire, developed by Mowday, Steers, and Porter (1979).According to Mowday, Steers, and Porter (1979), the OCQ provides a fairly consistent indicator of employee commitment levels for most working populations. It is the most frequently used instrument for the measurement of organizational commitment. Responses to the 15-item OCQ are measured on a 7-point Likert-like scale which ranged between 1="strongly disagree" and 7="strongly agree". In this study the Cronbach's alpha was found to be 0.86.

3.5 Procedure

The researcher selected the required number of education colleges as per the sample, to collect the necessary data. The researcher visited and administered the questionnaires personally to faculty members. Further classifications were given for the question and doubts raised by them. The responses of the faculty members on all the three tools were scored as specified in the tools.

3.6 Data Analysis

The data was analyzed with the help of computer software SPSS (Statistical Package for Social Sciences). The data analysis here presented in different tables. In analysis of data one way ANOVA, Pearson's Correlation Coefficient and DMRT (Duncan's Multiple Range Test) were used.

4. Results

The following results have been observed regarding each hypothesis of the study:

Hypothesis 1: There is significant difference between the following categories of teachers with reference to their self esteem

H1a: Teachers of different length of teaching experience

Table 4.1 Mean scores and standard deviation with respect to self esteem of teachers with different length of teaching experience

Experience in years	N	Mean	S.D	F Valve	P Value
1-10 years	51	21.16	2.61		
11-20 years	58	21.10	3.01		
21-30 years	18	23.17	2.50	2.830	.041 (S)
above 30 years	7	21.86	2.54		
Total	134	21.44	2.84		

Note: S-Significant at 0.05 level.

One way ANOVA revealed a significant difference in the mean self esteem scores of teachers with different lengths of teaching experience. F value of 2.830 was found to be significant at .041 level. The mean values obtained for teachers with 1-10, 11-20, 21-30 and above 30 years are 21.16, 21.10, 23.17 and 21.86 respectively. Further, DMRT (Duncan's Multiple Range Test) indicated that teachers with experience of 21-30 years had maximum self esteem than teachers with experience of 1-10, 11-20 and above 30 years. In this context, the hypothesis that there is significant difference between teachers of different length of teaching experience with reference to their self esteem is accepted.

Figure 4.1 Mean scores with respect to self esteem of teachers with different length of teaching experience

H1b: Teachers of different age groups

Table 4.2 Mean scores and standard deviation with respect to self esteem of teachers of different age groups

Age group (in years)	N	Mean	S.D	F valve	P value
less than 30 years	19	21.42	2.12		
31-44 years	72	20.63	2.93		
45-54 years	34	22.53	2.33	6.646	.000
above 55 years	9	23.89	2.71		
Total	134	21.44	2.84		

Note: S-Significant at 0.05 level.

One way ANOVA revealed a significant difference in the mean self esteem scores of teachers with different age groups. F value of 6.646 was found to be significant at .000 level. The mean self esteem scores obtained for teachers with <30, 31-44, 45-54 and above 55 years are 21.42, 20.63, 22.53 and 23.89 respectively. Further, DMRT (Duncan's Multiple Range Test) indicated that teachers with above 55 years had maximum self esteem than teachers in the age groups of 30, 31-44, and 45-54 years. In this context, the hypothesis that there is significant difference between teachers of different age groups with reference to their self esteem is accepted.

Figure 4.2 Mean scores with respect to self esteem of teachers of different age group

Hypothesis 2: There is significant difference between the following categories of teachers with reference to their job satisfaction

H2a: Teachers of different length of teaching experience

Table 4.3 Mean scores and standard deviation with respect to job satisfaction of teachers with different length o f teaching experience

Length of teaching experience (in years)	N	Mean	S.D	F Valve	P Value
1-10 years	51	76.20	8.47		
11-20 years	58	75.28	11.27		
21-30 years	18	79.00	13.60	1.450	.231 (NS)
above 30 years	7	83.14	14.99		
Total	134	76.54	10.91		

Note: NS-Not significant at 0.05 level.

Length of teaching experience of teachers did not have significant influence over their job satisfaction as the

ANOVA revealed a non-significant difference among teachers with different length of teaching experience in their mean job satisfaction scores (F=1.450; =.231). The mean job satisfaction scores of teachers with 1-10, 11-20, 21-30 and above 30 years are 76.20, 75.28. 79.00, and 83.14 respectively, which were statistically same, contributed for the non-significant differences. In this context, the hypothesis that there is significant difference between teachers of different length of teaching experience with reference to their job satisfaction is rejected.

H2b: Teachers of different age groups

Table 4.4 Mean scores and standard deviation with respect to job satisfaction of teachers with reference to age groups

Age groups (in years)	N	Mean	S.D	F	Sig.
less than 30 years	19	76.21	10.53		
31-44 years	72	75.96	8.60		
45-54 years	34	77.65	11.67	.219	.883 (NS)
above 55 years	9	77.67	22.39		
Total	134	76.54	10.91		

Note: NS-Not significant at 0.05 level.

One-way ANOVA revealed a non-significant difference among teachers belonging to different age groups (F=0.219; P=.883). The mean job satisfaction scores of teachers belonging to <30 years, 31-44, 45-54 and above 55 years are 76.21, 75.96, 77.65 and 77.67 respectively, which were statistically same, contributed to the non-significant difference. In this context, the hypothesis that there is significant difference between teachers of different age groups with reference to their job satisfaction is rejected.

Hypothesis 3: There is significant difference between the following categories of teachers with reference to their organizational commitment

H3a: Teachers of different length of teaching experience

Table 4.5 Mean scores and standard deviation with respect to organizational commitment of teachers with different length of teaching experience

Experience (in years)	N	Mean	S.D	F Valve	P Value
1-10 years	51	78.80	16.40		
11-20 years	58	78.76	13.68		
21-30 years	18	76.89	16.95	.416	.742
above 30 years	7	84.43	12.82		
Total	134	78.82	15.09		

Note: NS-Non-significant at 0.05 level.

Length of teaching experience of teachers did not have significant influence over their organizational commitment scores as the ANOVA revealed a non-significant difference between teachers with different length of teaching experience in their mean job satisfaction scores (F=0.416; =.742). The mean organizational commitment scores of teachers with 1-10, 11-20, and 21-30 and above 30 years are 78.80, 78.76, 76.89 and 84.43 respectively, which were statistically same, contributed for the non-significant difference.   In this context, the hypothesis that there is significant difference between teachers of different length of teaching experience with reference to their organizational commitment is rejected.
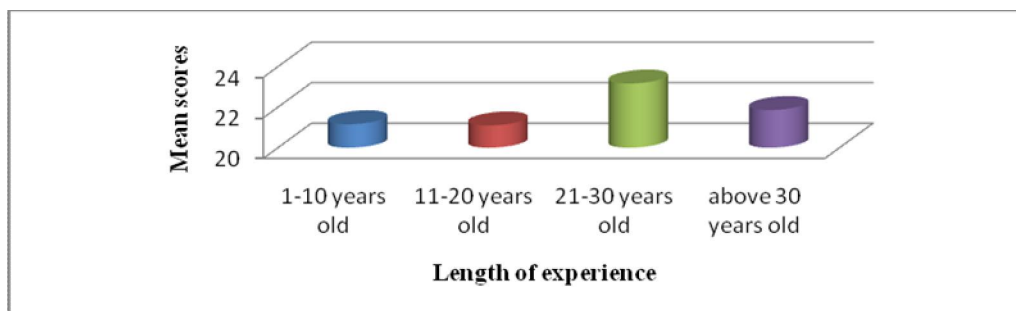
H3b*:* Teachers of different age groups

Table 4.6 Mean scores and standard deviation with respect to organizational commitment of teachers with different age group

Age group (in years)	N	Mean	S.D	F valve	P value
less than 30 years	19	80.16	22.94		
31-44 years old	72	78.58	11.84		
45-54 years old	34	77.91	14.65	.177	.912 (NS)
above 55 years	9	81.33	21.33		
Total	134	78.82	15.09		

Note: NS-Non-significant at 0.05 level.

One-way ANOVA revealed a non-significant difference among teachers belonging to different age groups (F=0.177; P=.912) in their organizational commitment scores. The mean job satisfaction scores of teachers belonging to <30 years, 31-44, 45-54 and above 55 years are 80.16, 78.58, 77.91 and 81.33 respectively, which were statistically same contributed for the non-significant difference**.** In this context, the hypothesis that there is significant difference between teachers of different age groups with reference to their organizational commitment is rejected.
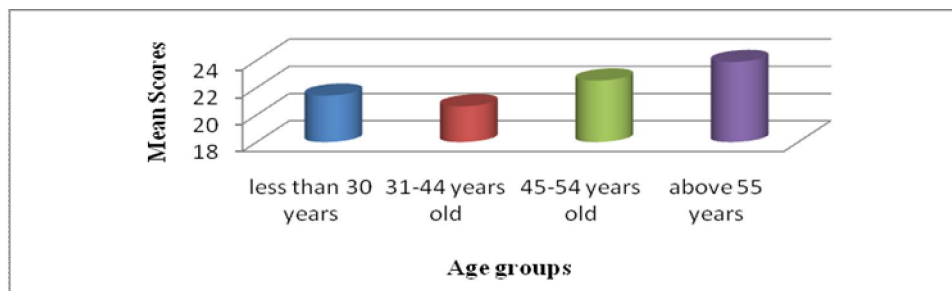
Hypothesis 4: There is significant relationship among job satisfaction, self esteem and organizational commitment of teachers

Table 4.7 Correlation coefficients between job satisfaction, self esteem and organizational commitment

Variable 1	Variable 2	Correlation coefficient	df	Significance
Job Satisfaction	Self Esteem	.083	132	.341
Job Satisfaction	Organizational Commitment	.388	132	**.000
Self Esteem	Organizational Commitment	-.127	132	.143

Note: **Correlation is significant at the 0.01 level

When correlation coefficients were calculated for teachers, it was found that only job satisfaction was significantly and positively correlated with organizational commitment (r=.388; P=.000), however, self esteem was found to be independent of both job satisfaction and organizational commitment.

5. Discussion

The purpose of this investigation was to examine how certain demographic variables (teachers of different length of teaching experience and teachers of different age groups) affect self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore. In addition, the study aimed to find out the relationship between self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore.

This study revealed that there is significant difference between teachers of different length of teaching experience and self esteem. The finding of this study is consistent with the findings of (Lee, 1992). Results of this study also showed that there is significant difference between ages of faculty members with reference to their self esteem. As age increased, self esteem of the teachers increased enhanced. This finding is consistent with previous studies (Alasker & Olweus, 1992).However; the

findings of the current study do not support the previous researches done by (Erdwins, Mellinger, & Tyer, 1981; Trimakas & Nicolay, 1974).

Test statistics revealed that teachers of different length of teaching experience did not influence job satisfaction. Bishay (1996) indicates that in many professions, increased length of teaching experience may lead to boredom and dissatisfaction with an occupation. About differences between length of teaching experience and job satisfaction, according to Cetin (2006) and Mathew (2007), non significant difference has been found in job satisfaction based on the experience variable. However, the findings of this study contradicts the findings of (Bishay, 1996; Blood et al., 2002; Crossman & Harris, 2006; Lambert et al., 2001; Poppleton & Risborough, 1991; Reyes, 2001).

One-way ANOVA revealed a non-significant difference among teachers belonging to different age groups (F=0.219; P=.883). The mean job satisfaction scores of teachers belonging to 30 years, 31-44, 45-54 and above 55 years are 76.21, 75.96, 77.65 and 77.67 respectively, which statistically contributed for the non-significant difference. The result of this study supports the findings of (Leafy et al., 2005; Steers, 1977). Contradicting these findings are several of researches. Some studies such as (Angle & Perry, 1981; Ross & Reskin, 1992) revealed that increasing

in age have been found to increase job satisfaction.

One way ANOVA testing revealed that there is no significant difference between teachers of different length of teaching experience with reference to their organizational commitment. About differences between work experience and organizational commitment, the findings of this study is in agreement with the findings of some studies like (Cetin, 2006; Singh & Shifflette, 1996; Sharma, 1994) which revealed teachers with varied experience were not different in their organizational commitment. However findings of the study contracts with the findings of a study by (Bashir et al., 2011; Kumar & Pathaik, 2004; Reyes, 2001) which revealed that there are significant difference between organizational commitment and length of teaching experiences.

From the data collected we found that there is no significant difference between ages of faculty members with reference to their organizational commitment. These findings are in agreement with previous studies (Cohen, 1992; Demiray, Curabay, & Curabay, 2008) who reported that there are no significant differences between age and organizational commitment. Findings of the study contrasts with the findings of (Cramer, 1993; Dunham, Grube, & Castaneda, 1994; Harrison & Hubbard, 1998; Hellman, 1997; Kacmar, Carlson, & Brymer, 1999; Lok & Crawford, 1999; Loscocco, 1990; Mathieu & Zajac, 1990; Meyer & Allen, 1997; Mowday et al., 1982; Sekaran, 2000).

Test statistics revealed that teachers self esteem, job satisfaction and organizational commitment were mutually related. The relationship between job satisfaction and organizational commitment is very crucial now-a-days, because people these days do not prefer to stay with the same organization for long. Employers normally expect that people with higher levels of job satisfaction will have higher levels of organizational commitment. The reason why satisfaction will lead to the commitment is that a higher level of job satisfaction may lead to good work life and reduction in stress. Similarly, if employees are highly satisfied with their work, co-workers, pay, and supervision and derive high level of overall job satisfaction with their jobs, they are more likely to be committed to the organization than if they are not satisfied. The focus on these two key concepts cannot be over stated because job satisfaction and commitment are primary determinants of employee turnover, performance, and productivity (Okpara, 2004). Kalleberg and Mastekaasa (2001) found that previous research on the relationship between job satisfaction and organizational commitment has not shown any consistent and easily reconcilable findings, the majority of research investigating this relationship indicates that there is a

significant relationship between job satisfaction and organizational commitment (Aranya, Lachman, & Amernic, 1982; Boshoff & Mels, 1995; Harrison & Hubbard, 1998; Johnston et al., 1990; Knoop, 1995; Kreitner & Kinicki, 1992; Morrison, 1997; Norris & Niebuhr, 1984; Ting, 1997).The results of this study support the findings of researchers mentioned above.

Based on the results of this study, self esteem was found to be a negatively related with job satisfaction and organizational commitment. The findings of this study are consistent with the findings of (Lerner et al., 2011).Findings of the study contrasts with the findings of (Alavi & Askaripur, 2003; Lopez & Greenhaus, 1978). Alavi and Askaripur (2003) state that a decrease in job satisfaction may be due to a decrease in self-esteem, and thus, organizations should increase their employees' self-esteem, which, in turn, will increase their job satisfaction (Alavi & Askaripur, 2003). According to this study, one of the best methods for increasing self-esteem in personnel is to increase their job satisfaction in all its dimensions.

6. Conclusion

The purpose of this investigation was to examine how the demographic variables of length of teaching experience and age, affect self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore (India). In addition, the study aimed to find out the relationship between self esteem, job satisfaction and organizational commitment of faculty members of educational colleges in Mysore.

From the above results we can conclude that there were significant differences between age and length of teaching experience of faculty members with reference to their self esteem.

From the above results we can conclude that non significant differences were found between length of teaching experience and age of faculty members with reference to their job satisfaction. It was also found out that there were no significant differences between length of teaching experience and age of faculty members with reference to their organizational commitment.

7. Educational Implications

1. Findings can improve organizational performance, quality of education and development of human resources.
2. Finding of this study can play an illuminating role in opening the eyes of policy as well as decision makers on possible disadvantages of their system, i.e. regarding facilities, quality, etc., and on how they could be minimized.
3. The findings of the present research could be employed as a trigger by heads of Universities and colleges to pay more attention to the nature of interaction they have with the faculty.

4. The findings in the present dissertation could also give the faculty a better idea of themselves, etc., and they could be encouraged to take measures that would diminish their weakness.

5. Theoretically, the findings will motivate theoreticians to pay more attention to variations among different countries when introducing theories, as well as instruments regarding the variables analyzed in this research.

8. Recommendations

The following recommendations were made on the basis of the results of the study:

1. The differences between faculties job satisfaction, self esteem and organizational commitment in urban, suburban and rural colleges should be explored.

2. Since the data of this study were collected from one city in Mysore, the generalization of the findings is limited. Therefore, it is necessary to expand the data sources to include the larger populations, specially a national sample, in India. It would be interesting to see whether the same variables will have an impact on faculty member's self esteem, job satisfaction and organizational commitment of other cities and other levels of the educational system.

3. Because there wasn't more research about faculty members self esteem job satisfaction and organizational commitment, this research revealed that job satisfaction and organizational commitment are significantly and positively related. So, another research is needed for study about relationship between faculty member's job satisfaction, self esteem and organizational commitment.

4. Research as well as scientific cooperation and collaboration with different countries, particularly those which are labeled as developed, as a means of identifying weaknesses in each country, should be conducted.

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8/27/2012

The Comparison of Intracoronary Versus Intravenous Eptifibatide Administration during Primary Percutaneous Coronary Intervention of Acute ST-Segment Elevation Myocardial Infarction

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Abstract: Background: Administration of the glycoprotein IIb/IIIa inhibitors, including eptifibatide is an effective adjunctive treatment strategy during primary percutaneous coronary intervention (PPCI) for ST-segment elevation myocardial infarction. Recent data suggest that the intracoronary administration of these drugs during PPCI may increase the efficacy of them. **Methods:** A total of 40 ST-segment elevation myocardial infarction patients undergoing PPCI within 12 hours of symptom onset were randomized to either intracoronary or intravenous two boluses of eptifibatide (0.180 µg/kg) each 10 minutes. The primary endpoints of the trial were enzymatic infarct size, myocardial reperfusion measured as ST-segment resolution (STR), and post-procedural Thrombolysis in Myocardial Infarction (TIMI) grade flow of infarct related artery. The secondary endpoints were intra-procedural adverse effect (arrhythmia) and no-reflow phenomenon, in-hospital mortality, reinfarction , hemorrhage and post-procedural global systolic function. **Results:** Post-procedural TIMI grade 3 flow was achieved in 95% and 90% of the intracoronary (IC) and intravenous (IV) groups (P=0.61). The enzymatic infarct size assessed by the area under the curve of creatine phosphokinase-mb (CPK-mb) in the first 48 hours after PPCI ($\mu mol.L^{-1}.h^{-1}$) was similar in the IC and IV groups with 7206 (IQR, 5346.75 to 10384.50) versus 7294 (IQR , 10384.50 to 10384.50), P=0.87. Complete STR was achieved in 55% and 40% of the IC and IV groups (P=0.27). No deaths, urgent revascularizations, reinfarctions, or TIMI major bleeding events were observed among the both groups. **Conclusion:** Although, the IC administration of eptifibatide is safe, but does not add a benefit in comparison to the standard IV route.
[Morteza Safi MD, Mohammad Hasan Namazi MD, Hosein Vakili MD, Habibollah Saadat MD, Ramin Khameneh Bagheri MD, Javad Ramezani MD, Mostafa Ahmadi MD, Amin Sahebi MD. **The Comparison of Intracoronary Versus Intravenous Eptifibatide Administration during Primary Percutaneous Coronary Intervention of Acute ST-Segment Elevation Myocardial Infarction.** *Life Sci J* 2012;9(4):215-222]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 31

Keywords: Primary percutaneous coronary intervention, ST elevation myocardial infarction, Eptifibatide, Intracoronary, Intravenous.

Introduction

Primary percutaneous coronary intervention (PCI) is the treatment of choice in the management of acute ST-segment elevation myocardial infarction (STEMI). It has been constantly observed that, despite restoring good epicardial flow with PCI, myocardial perfusion at the cellular level remains impaired in nearly 50 % of STEMI patients.[1] This is attributable to embolisation of the coronary thrombus into the distal vasculature, producing microvascular plugging, vasospasm, interstitial oedema and cellular injury. With Doppler guidewire technology, it was estimated that an average of 25 embolic events occurred during primary PCI for ST-segment elevation myocardial infarction. [2,3,4] There is consequently less salvage of infarct size, reduced left ventricular function and poorer clinical outcomes. There have been efforts to identify mechanical and pharmacological strategies to improve myocardial perfusion after primary PCI. Compared with systemic administration of intravenous pharmacotherapies, highly localized administration of intracoronary pharmacotherapy may be associated with a several-hundred-fold increase in the local concentration of an agent in the epicardial artery and microcirculation. A number of pharmacotherapies, including adenosine[5,6], calcium channel blockers[7], vasodilators[8,9],

antithrombotics[10,11], and antiplatelet[12-14] agents have been used to treat microvascular dysfunction.

This led to the development of a new class of antiplatelet drugs, termed glycoprotein IIb/IIIa inhibitors (GPIs), which are the most potent inhibitors of platelet aggregation and have been repeatedly shown to improve clinical outcomes in acute STEMI when administered intravenously during primary PCI. [15]

Platelet receptor occupancy studies have demonstrated that if there are fewer GPIIb/IIIa receptors free and available for cross-linking with fibrinogen, then myocardial perfusion is improved. [16] In recent years, randomized trials have demonstrated that glycoprotein inhibitors administered by the intracoronary route are safe and effective in reducing infarct size and providing better clinical outcomes than when given intravenously, without a significant increase in major bleeding. [14, 15]There were no adverse events during its administration. The

intracoronary strategy was not associated with any significant delay in revascularization compared with the intravenous route. [14]

The absolute number of GPIIb/IIIa receptors available for cross-linking is reduced among patients with successful restoration of myocardial perfusion and ST-segment resolution in an STEMI population. [17] Thus, the hypothesized mechanistic basis for intracoronary administration of GPIIb/ IIIa inhibitors is that high local concentrations of the drug would lead to fewer GPIIb/IIIa receptors being available for cross-linking with fibrinogen in the coronary microcirculation and therefore promote clot disaggregation with a minimal increase in systemic drug concentrations. This greater blockade of GPIIb/IIIa receptors would in turn reduce the incidence of microcirculatory thrombosis, improve myocardial perfusion, and ultimately improve clinical outcomes. [14, 16]

Table 1. Baseline Characteristics of the 40 Patients Randomized To Intracoronary or Intravenous Administration Of Eptifibatide.

Patients' baseline characteristics	Intracoronary Group (n=20)	Intravenous Group (n=20)	P
Age (year)	53.9±10.6	58.60±7.0	NS
Male gender, n (%)	17 (34.7)	17 (34.7)	NS
Cardiovascular risk factors, n (%)			
Hypertension, n (%)	4 (20)	5 (25)	NS
Hypercholesterolemia, n (%)	5 (25)	6 (30)	NS
Diabetes mellitus, n (%)	9 (45)	6 (30)	NS
Family History, n (%)	4 (25)	2 (10)	NS
Smoking, n (%)	11 (55)	11 (55)	NS
Ischemic time(min)			NS
Angiographic, n (%)			
No. of diseased vessels			
1	8 (40)	8 (40)	NS
2	7 (35)	5 (25)	NS
3	5 (25)	7 (35)	NS
Infarct-related artery			
LAD	14 (70)	11 (55)	NS
LCX	1 (5)	0	NS
RCA	5 (25)	9 (45)	NS
TIMI flow grade, n (%)			
0	18 (90)	17 (85)	NS
1	2 (10)	2 (10)	NS
2	0	1 (5)	NS
3	0	0	NS
Thrombus present, n (%)	18 (90)	19 (95)	NS
Procedural, n (%)			
Thrombus aspiration	15 (75)	13 (65)	NS
Balloon predilatation	10 (50)	9 (45)	NS
Postdilatation	13 (65)	12 (60)	NS
DES	12 (60)	14 (70)	NS

DES: Drug Eluting Stent

We hypothesized that intracoronary administration of eptifibatide during primary PCI for ST-elevation myocardial infarction would be safe and would be associated with higher rates of myocardial reperfusion and smaller myocardial infarct size.

Patients and Methods

Study Design and Population

In the present study, the investigators randomized 40 STEMI patients, presenting within 12 hours of symptom onset, to either intracoronary or intravenous two boluses of eptifibatide. STEMI was defined as chest pain suggestive of myocardial ischemia for at least 30 minutes before hospital admission, and an ECG with new ST-segment elevation in 2 or more contiguous leads of 0.2 mV or more in leads V2 to V3 and/or 0.1 mV or more in other leads. The use of this drug was strongly encouraged based on the existing guidelines.

The exclusion criteria were as below:

1- Patients presenting with STEMI after 12 hours from symptoms onset.

2- Patients presenting with vasospastic angina (determined by resolution of ST segment elevation, and relief of symptoms after intravenous administration of nitroglycerin).

3 -Patients presenting with non-STEMI.

4-Contraindications for antiplatelets such as bleeding disorder including gastrointestinal bleeding, hematuria, or known any bleeding tendency either inherited or acquired.

5- Thrombocytopenia (Platelet count<100.000/cm3).

6- Recent (<6 months) Stroke.

7- Intracranial hemorrhage at any time in patient's life or any known intracranial malformation.

8- Cardiogenic shock.

9- LBBB change in ECG.

Patients' baseline characteristics are listed in Table 1.

Treatment

All patients received 325 mg of acetylsalicylic acid orally and a 600-mg loading dose of clopidogrel 30- 60 minutes before PCI. And a maintenance dose of 75 mg/day for 4 weeks to one year were administered after PCI. A bolus and a maintenance dose of unfractionated heparin were administered and titrated to achieve an activated clotting time 250 seconds.

Two boluses (each 180 μg/kg) of eptifibatide were administered in both groups. Coronary guiding catheters were used to administer intracoronary eptifibatide. When the wire had crossed the occlusion, the first dose was administered, and after 10 minutes the patient was received the second dose. Thrombus aspiration was performed using Export catheter, if it was necessary. Stenting was performed for all of the patients. Intra-aortic balloon pump (IABP) was not used for any patient.

End Points

The primary endpoints of the trial were enzymatic infarct size, myocardial reperfusion measured as ST-segment resolution (STR), and post-procedural TIMI grade flow the infarct-related artery. The secondary endpoints were intra-procedural adverse effect (arrhythmia) and no-reflow phenomenon, in-hospital mortality, reinfarction and hemorrhage (major or minor) using TIMI criteria, and post-procedural global left ventricular systolic function, using global ejection fraction.

ECG Analysis

For evaluation of the ECG end points, a 12-lead ECG was acquired at the time of presentation and at 90 minutes after primary PCI. STR was assessed by comparing the ST-segment elevation in the infarct-related area on the ECG after PCI with the ECG at presentation.STR indirectly indicates the myocardial reperfusion. In our study, STR was categorized as complete (≥70%), partial (30% to <70%), or absent (<30%).[18]

Infarct Size

The infarct size was measured indirectly by the area under the curve of the cardiac creatine kinase-mb (CPK-mb) release, derived from measurements 0, 6, 24 and 48 hours after PCI.

Angiographic Analysis

"TIMI Grade Flow" is a scoring system from 0-3 referring to levels of coronary blood flow assessed during <u>percutaneous coronary angioplasty</u>:

- TIMI 0 flow (no perfusion) refers to the absence of any antegrade flow beyond a coronary occlusion.
- TIMI 1 flow (penetration without perfusion) is faint antegrade coronary flow beyond the occlusion, with incomplete filling of the distal coronary bed.
- TIMI 2 flow (partial reperfusion) is delayed or sluggish antegrade flow with complete filling of the distal territory
- TIMI 3 flow (complete perfusion) is normal flow which fills the distal coronary bed completely. [19]

A single observer reviewed all angiographic and ECG data.

In-hospital Clinical Course

In-hospital clinical course was obtained from the central personal records database, hospital records, and interviews with the patients and/or their general practitioners. Mortality was considered cardiac unless an unequivocal noncardiac cause of death was established. Reinfarction was defined as recurrent symptoms suggestive of ischemia with new ST-segment elevation and/or elevation of the levels of cardiac markers. [20]

Major or minor hemorrhage was determined using Thrombolysis In Myocardial Infarction (TIMI) criteria, including:

A) Major criteria: intracranial hemorrhage, or clinical bleeding associated with loss of >5 mg% of hemoglobin (or hematocrit decrease by > 15 points or by 10-15 points with clinical bleeding).
B) Minor criteria: loss of > 3gm% of hemoglobin (or hematocrit decrease by < 10 points) with clinical bleeding or loss of > 4 mg% of hemoglobin (or hematocrit decrease by 10- 15 points) with no clinical bleeding.

Clinical bleeding will be defined as: large hematoma, gastrointestinal blood loss, retroperitoneal bleeding.

All patients prior to discharge will undergo a conventional transthoracic echocardiographic examination (TTE), to assess the global left ventricle systolic function (LVEF) by 2D eyeballing approach.

Statistical Analysis

Due to the lack of normal distribution of data and small sample size in each group, quantitative data were described by median and inter-quartile range and compared by Mann-Whitney U test. Categorical data were described by frequencies and percentages and analyzed by chi square and Fisher exact test. AUC was calculated by trapezoidal rule. Significance level was determined at $P<0.05$. Statistical analyses were done by SPSS version 20.

Results

Angiographically apparent thrombus was noted in 95 (IV group) and 90 percent (IC group) of cases. More than 95% of arteries had a closed artery (TIMI grade 0/1 flow) before PCI, as noted in table 1. No deaths, urgent revascularizations, or reinfarctions were observed among the 40 patients of both groups. There were no TIMI major bleeding events. One TIMI minor bleeding events were noted in whom were treated with intracoronary eptifibatide. No adverse events including arrhythmias were noted during intracoronary eptifibatide administration.

Postprocedural TIMI grade 3 flow was achieved in 95% and 90% of the intracoronary and intravenous groups, respectively (P=0.61). (Table 2)

Table 2. Post-procedural TIMI flow grade of infarct related artery.

Post-procedural TIMI flow grade	Intracoronary Group (n=20)	Intravenous Group (n=20)	P
3, n(%)	19 (95)	18 (90)	0.61
2, n(%)	1 (5)	2 (10)	0.85

The enzymatic infarct size assessed by the area under the curve of CPK-mb in the first 48 hours after primary PCI ($\mu mol.L^{-1}.h^{-1}$) was similar

in the intracoronary and intravenous groups with 7206 (IQR, 5346.75 to 10384.50) versus 7294 (IQR , 3797.25 to 11803.50), P=0.87. Also, the other enzymatic criteria were similar in the IC and IV groups: peak CPK-MB (U/L) was 277 (IQR, 187.75 to 498.75) versus 257.5 (IQR, 115 to 420), P=0.48; and time to peak CPK-MB (hour) was 6 (IQR, 6 to 16.5) and 6 (IQR, 6 to 12), P=0.84. (Table 3 and figure 1, 2, 3)

Median STR was 71% (inter-quartile range 40 to 72 %) in the intracoronary group versus 63.5% (inter-quartile range, 50 to 75%) in the intravenous group; (P=0.75). The primary end point of complete STR was achieved in 55% of the intracoronary group and 40% of the intravenous group (P=0.27). Therefore myocardial reperfusion was similar in both groups. (Table 4, and figure 4)

Global LV systolic function which assessed by ejection fraction (2D eyeballing approach) in transthoracic echocardiographic examination before discharge, was 45 % (inter-quartile range35 to 47.25 %) in the IC group versus 40 % (inter-quartile range, 38.5 to 45 %) in IV group; (P=0.21). (Table 5)

Table 3. Enzymatic Infarct Size in both groups.

	Intracoronary Group (n=20)	Intravenous Group (n=20)	P
Time to peak CPK-MB, h	6 (6 to 16.5)	6 (6 to 12)	0.84
Peak CPK-MB, U/L	277.0 (187.75 to 498.75)	257.5 (115 to 420)	0.48
AUC48 CPK-MB, $\mu mol.L^{-1}.h^{-1}$	7206 (5346.75 to 10384.50)	7294 (3797.25 to 11803.50)	0.87

AUC: Area under the Curve
CPK-mb: Creatine PhosphoKinase-mb

Figure 1. Time to peak CPK-mb (hour)

CPK-mb: Creatine Phosphkinase-mb

Figure 2. Infarct size according to Area under Curve (AUC) of CPK-mb during the first 48 hours after PCI
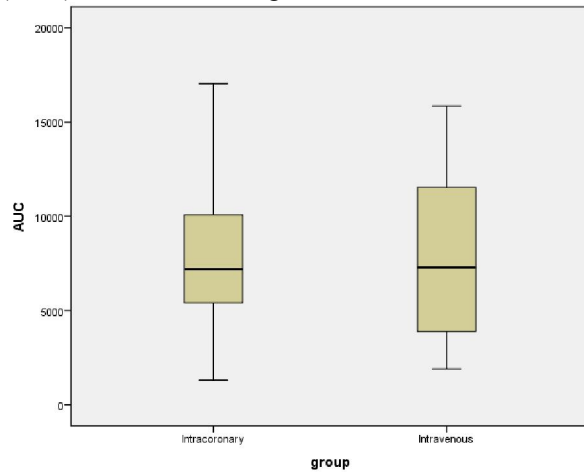
Figure 3. CPK-mb serum level changes the first 48 hours after PCI.

CPK-mb: Creatine Phosphokinase-mb
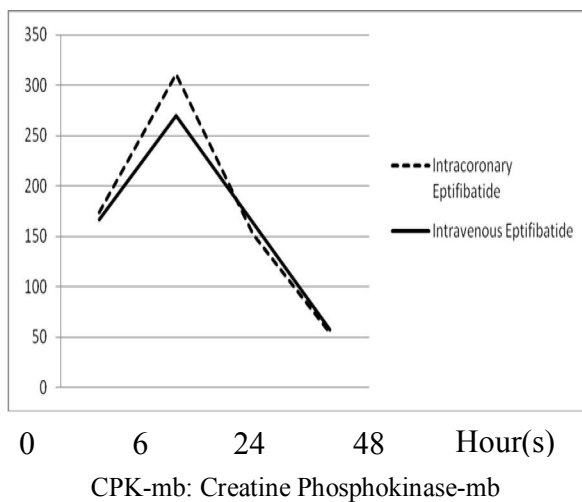
Table 4. Distribution of myocardial reperfusion as assessed by ST-segment resolution (STR)

Post-PCI STR	Intracoronary Group (n=20)	Intravenous Group (n=20)	P
Median	71 (40-72)	63.5 (50-75)	0.75
Mean ± SD	55.95± 25.7	61.35± 15.25	
Absent (<30%), n (%)	3 (15)	1 (5)	
Partial (30- <70 %), n (%)	6 (30)	11 (55)	
Complete (≥70 %), n (%)	11 (55)	8 (40)	0.27

STR: ST resolution

Figure 4. Distribution ST-segment resolution (STR)
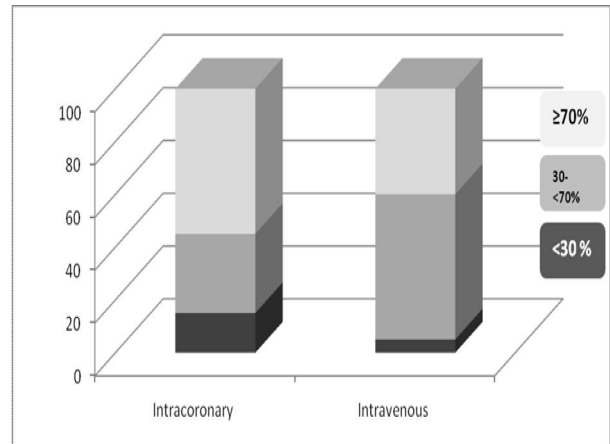
Table 5. Ejection fraction (EF) in both groups before discharge (P=0.21)

	Intracoronary Group (n=20)	Intravenous Group (n=20)
Median EF (%)	45 (35.0- 47.25)	40 (38.5- 45)
Mean EF (%)	40.80± 9.39	42.00± 5.876

EF: Ejection Fraction

Discussion

Mechanisms underlying impaired myocardial perfusion after restoration of epicardial blood flow are likely to be multifactorial such as oxygen free radicals, cellular and interstitial edema, endothelial dysfunction, vasoconstriction, and thromboembolism. [21,22] The increased concentration of Gp IIb/IIIa inhibitors like Abciximab, Eptifibatide, Tirofiban are shown to improve the outcomes of PCI safely and efficaciously in terms of reduction in infarct size, peri-procedural MI and improved TIMI flow. [16]

Depending on the relation of inflow and outflow and the size of the ischemic area, the local eptifibatid concentration can vary substantially. However, even in situations with restitution of normal flow and perfusion in the infarct-related artery, intracoronary drug bolus administration will result in very high local concentrations, which might be much higher than the usual intravenous application that might more reduces platelet aggregation. [23] Another potential mechanism of high local concentration benefits might be related to the anti-inflammatory properties of GP IIb/IIIa inhibitors. These considerations are supported by experimental data showing a dose-dependent platelet disaggregation. Concentrations that produced complete platelet disaggregation also induced partial displacement of platelet-bound fibrinogen, which might play a role in the clinical setting. [25]

The angiographic and electrocardiographic end points are well established for the assessment of perfusion at the epicardial and microvascular levels. The sensitive ECG assessment at a later measurement reflected improved tissue perfusion. [26]

In our study, the STEMI patients undergoing primary PCI, intracoronary administration of eptifibatide is not superior to intravenous administration, with respect to the primary endpoints: improving myocardial reperfusion as assessed by STR, the enzymatic infarct size measured by the area under the curve of CPK-mb in the first 48 hours after primary PCI, and post-procedural TIMI grade flow of infarct related artery.

The other findings of the present investigation were associated without significant differences in moderate and/or severe bleeding complications, deaths, urgent revascularizations, or reinfarctions, and adverse events (during intracoronary eptifibatide administration) including arrhythmias, between two groups.

Also we recognized that if balloon postdilation was necessary, it could be performed safely, with minimal risk of no-reflow phenomenon.

Recently the use of intracoronary versus intravenous was compared in some trials and studies; which had different results. For example, in CICERO (The Comparison of Intracoronary Versus Intravenous Abciximab Administration during Emergency Reperfusion of ST-Segment Elevation Myocardial Infarction) Trial, 534 patients of STEMI undergoing PPCI with thrombus aspiration within 12 hours of symptom onset, were randomized to either intracoronary or intravenous bolus of abciximab (0.25 mg/kg).No difference was noted in ST resolution between intracoronary versus intravenous groups. However, intracoronary administration was ssociated with improved myocardial perfusion assessed by myocardial blush grade and a smaller enzymatic infarct size. [18]

Similarly, in the Intracoronary Eptifibatide (ICE) Trial, the intracoronary bolus administration of eptifibatide during PCI in patients with acute coronary syndromes resulted in higher local platelet glycoprotein IIb/IIIa receptor occupancy, which was associated with improved micro-vascular perfusion demonstrated by an improved Corrected TIMI Frame Count. [27]

The Randomized Leipzig Immediate Percutaneous Coronary Intervention Abciximab IV Versus IC in ST-Elevation Myocardial Infarction Trial showed that intracoronary bolus administration of abciximab in primary PCI is superior to standard intravenous treatment with respect to infarct perfusion (according to ST-segment resolution at 90 minutes). In this study, each group contained 77 patients. [28]

In the RELAX-AMI Trial, the angiographic perfusion grade after PCI was similar between the intravenous abciximab group and previously published results, whereas the intracoronary abciximab group had nonstatistically significant higher perfusion grades. [29]

A retrospective analysis of angiographic and clinical outcomes among 59 patients who received intracoronary eptifibatide as part of clinical management of primary PCI for STEMI between January 2001 and March 2005 showed intracoronary eptifibatide can be administered safely during primary PCI and is associated with few adverse events. [14]

Intracoronary bolus application of tirofiban was not associated with reduction in MACE rates, and enzymatic infarct size compared to intravenous administration in patients with STEMI who underwent primary PCI. At six months the incidence of MACE was 6.25% in IV group and 11.1% in IC group (p=0.45). Peak creatine phosphokinase (CPK) levels between IV and IC groups were also statistically non significant (2657±2181 U/L in IV group and 2529±1929 U/L in IC group) (p=0.92). [30]

For the AIDA STEMI trial, 2065 STEMI patients undergoing PCI, from July 2008 to April 2011, were randomized to receive abciximab by an IV infusion or directly into the blocked coronary artery for 12 hours. Intracoronary bolus administration of abciximab does not add a benefit in comparison to the standard IV bolus, with respect to the combined primary study endpoint consisting of death, reinfarction or new congestive HF within 90 days. Although previous research suggested intracoronary dose during PCI could boost concentration of abciximab at the treatment site, limit heart tissue destruction and improve blood flow, the AIDA STEMI researchers did not find a difference in blood flow or infarct size (assessed by AUC CK-Release, P=0.74), early ST resolution (P=0.37) between the two routes. The IC route might be only related to reduce rates of new congestive heart failure. [31]

As seen, the results of the comparison between intracoronary and intravenous GP IIa/IIIb inhibitor administration during primary PCI in STEMI patients, diffusely vary in many studies and trials. This is, might be, the reason that the AHA and the ACC in the updated 2011 guidelines for PCI, noted that in pateint undergoing primary PCI with abciximab, it may be reasonable to administer intracoronary abciximab (class IIb). But it is not emphasized for other GP inhibitor drugs. [32]

Although in our study, all angiographic, and ECG measurements were blinded, interventionalists were aware of the group assignment. Thus, a potential investigator bias cannot be ruled out entirely. Another important problem was the inadequate number of patient in each group, which was due to wide range of exclusion criteria.

Confirmation of the results with respect to clarification of the long-term effects on infarct size, myocardial reperfusion, ventricular size and function and, more important, on clinical outcome requires a larger trial.

Acknowledgments

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8/2/2012

Effects of Inducing Plant Growth on performance and performance Components of Corn (*Zea mays* L.)

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In order to examine the effect of spraying plant growth inducers on performance and performance components of corn in the double-cross 704 corn, a test in the form of a randomized complete block design under condition of a farm with eight replications and six treatments of plant growth inducers, Marmarine, HB_101, auxin, cycocel, ethephon and control was performed. Analysis of variance of data showed that characteristics of the number of rows of grain, number of grains per row, number of grains per maize, grain weight per maize, weight of 1000 grains, cob weight, grain performance and harvest index were significant at the 5% level. The Marmaryn , HB-101 and auxin treatment led to increase in the number of rows of grain, number of grains per row, number of grains per maize, grain weight per maize, weight of 1000 grains, cob weight, grain performance and harvest index. So that the greatest value was related to Marmarine treatment, then HB_101 treatment and eventually auxin treatment. Also, Cycocel did not indicate any significant effect on the performance and performance components of corn except for reducing the weight of 1000 grains. Ethephon decreased grain number per row, number of grains per maize, weight of 1000 grains and cob weight, but its impact on the performance was not statistically significant.

Key words: performance components, inducing plant growth substances, and corn.

Introduction

Corn (zea mays L.) has the maximum production among grains, and in terms of area under cultivation has the third place after wheat and rice (Tajbakhsh, 1375). Corn is one of the cheapest and purest sources of organic material for industrial use. In starch factories, animal feed, sugar syrup and oil are extracted from the corn starch. Today, more than 500 types of product of the second degree are obtained from corn (Noormohammadi et al, 1380). Performance in the corn is about grain production per unit area and grain weight, from which grain weight is more stable, and large difference in performance is usually a result of change in number of grains. On the other hand, Ten percent of the total dry substance of the grain is provided by remobilization of stored substance in the vegetative organs, although this value is low, but regardless of daily fluctuations in photosynthesis, this photosynthetic substance is really essential in keep the grain dry substance constant during the stage of filling of the grain (Duncan et al, 1965). Also, the cereal grain only includes some parts of the produced biomass. High performance in a variety of new products is in relation to their biological function, provided that the biomass of produced organs, and their distribution is for grain production, which in turn is depended on the high development of photosynthetic apparatus and roots in the vegetative phase and rapid increase in dry matter production in reproductive phase (Kuchaki & Banayan, 1373), and the absorption rate of nutrients in maize is reduced after flowering (Fathi and Ismailpoor, 1379). Experimental results show that the mobility of stem reserves, which includes the surplus products of photosynthesis before the grain filling stage, has contributed largely to the grain performance. This is very important particularly in the occurrence of tensions such as dryness and high temperatures in the stage of grain filling (Austin at el, 1980 at el, 1976 and Gallagher).

Due to what mentioned above, using inducing produce plant growth substances may provide these conditions for high performance and production. Plant growth regulators which are used on financial products have a direct or indirect influence on final fruiting, product quality or both (Mojabi, 1373). Indole Aceto Nytryl is a hormone first extracted from the leaves and stems of organic plants of Cruciferae family (Jones et al, 1952). The Auxin term is derived from a Greek word means the growth, and along with longitudinal growth, may also affect other processes. But its impact on the longitudinal growth is of more concern (Fathi, 1378). The plant response to auxin is changing from the

effect on cell metabolism to coordination for physical structure of plants including falling leaves and the plant aging, and its cellular effects includs an increase in proton exchange, membrane load, and potassium absorption (Marre, 1977) and the effect is on phytochrome with red lights (Kuchaki and Sarmadnya, 1377). Also, it is transported through the phloem, and has an important role in the formation of ectopic root and fruit development and tillering of plants (Shabestari and Mojtahedi 1369; Artec et al, 1980). Research reports show that the application of cycocel can change number of tillers, and increase in number of tillers is done through the response to the daylong, preventing from the biosynthesis, and auxin transport or change in Osylylate or nutrents (Lungw at el, 1982). In response of wheat to leaf consumption of cycocel, despite the general reduction in plant height and lodging rates, no significant increase in grain performance is observed (Sarkar et al, 2002). Cycocel increases the production of short inter-nodes and the vascular bundles (Prakash and Ramachandran, 2000), and by affecting the performance factors, increases the grain performance, and fertilizer capacity of the plant (Fabebri et al, 1986). In cereals, it increases the number of spikes per area unit, spike length and grains per spike (Shabestari and Mojtahedi 1369; Ma and Smith, 1992), causes lag in aging of lower canopy leaves (Green, 1985), and by creating thicker and darker leaves, increases photosynthesis (Myhre et al., 1973). Ethephon increased tillering in cereals, and since these tillers are competing on photosynthetic materials and minerals absorbed, the number of sterile spikelets are increased, and cycocel treatment of cereals, increases the number of grains per spike, but often decreases the grain size (Shokoufa and Imam, 1384). Ethephon by-releasing Ethylene significantly affects plant growth and development (Davis, 1988), stimulates seed germination (Khajehpour, 1377), is effective in fruiting, increases tillering and rooting in cereals (Foster et al, 1992), and has a direct impact on performance factors (Farrahi Ashtiani et al, 1378; Bulman and Smith 1993). Marmarine is substance induces natural growth and is extracted from marine algae (Ascuphyllum nodusom), and contains more than sixty kinds of food elements, enzymes, organic acids and plant growth inducer (Dadkhahipoor, 1386). In the United Kingdom, the algae are used as fertilizer and growth inducer in various products such as potatoes and cabbages, in tomato increases the period of flowering and fruiting, and makes the earth free from aphids (Dawes, 1981). The existence of hormone compounds like auxin, gibberellin, phenyl acetic acid and citokenin in brown algae has been proved. The amount of corn, rice, tomatoes, potatoes, peppers, oranges, and pineapples is significantly increased by using this type of growth inducers. Also, the production of rice is increased by increasing algae. Especially in the agricultural environment Syanvfys·h the power of the flooding and the amount of rice to 20 percent increase. (Blunden .1972).

Materials and Methods

In this experiment, corn grain (Double Cross 704) with the effect of five growth inducers including ethephon, indole acetic acid, cycocel, H. B. _101, and Marmarine, and control group (without use of growth inducers) formed the experimental treatments.

The method of testing: testing was performed with 6 treatments and 8 replications in the form of randomized complete block design under field conditions, in the agricultural research stations in Urmia Sa'atlou, in 1387.

Farm operations: land where the experiment was performed was tillaged deeply by the moldboard plow in the Mid April 2008, and after surface rotivator, the farm was plotted. Plots area was with dimensions of 4×3 m and plot number was 48. The inner plots were ploughed by the shovel. The seeds were cultured in plots in four rows and with row spacing of 60×22, and with 75 thousand plants per hectare and depth of 7×5 cm. In order to ensure germination and a nearly complete count of sprigs in each plot, three seeds were placed in each planting hole, and after emergence, and removing the risk of Agrevits, in each hole, the two plants was eliminated and a plant was kept safe. Fertilizers were used according to soil analysis results. 360 kg per hectare of urea in three stages (one-third before cultivation, one third in the stage of having three to four leaves, and one third in the phase of tasseling), and 100 kg per hectare of di-ammonium phosphate and 200 kg per hectare of potassium sulfate were used evenly in all treatments before cultivation. Combating the weeds was performed regularly during the growing season, and in order to prevent drought stress in plants, watering was done regularly and accurately between once in 7-10 days during the whole growing season. According to the test plan, one week before and after pollination, the growth inducer substances treatment was performed twice and in solution form sprayed to leaves and the tested characteristics were measured and noted after treatment till the harvest. Inducer substance and plant growth regulators (treatments) concentration was under field conditions and was as follows: Indole acetic acid 20 ppm, cycocel 100 ppm, ethephon 33 ppm, Marmarine 1.5 liters per thousand liters of water and HB _101 100

cc per thousand liters of water (according to the instructions on the manufacturer and its use on products crops and cereals). Acquired data were analyzed by the Mstat_c software, and variance and mean were compared with LSD test at the five percent level of probability.

Discussion and Result

Results of data analysis (Table 1) showed that application of plant growth inducer substances on the performance and performance components is effective in the corn. The characteristics were rows of grains, grains in rows, number of grains per maize, grain weight per maize, weight of 1000 grains, cob weight, and grain performance, and harvest index was significant at the 5 % level.

The number for rows of grains in maize

Plant growth inducer treatment on the characteristic of the number for rows of grains in maize indicated highly significant statistical difference in (p < 1 %). Maximum increase was related to Marmarine, and then HB_ 101 and finally auxin treatment (Figure 1). Usually the number of rows per maize under genetic control mechanisms was always even and usually between 8, 16 and 24 (NoorMohammadi et al, 1380). This characteristic is under genetic control, and less control factors affect it significantly (Kuchaki and Banayan, 1373).
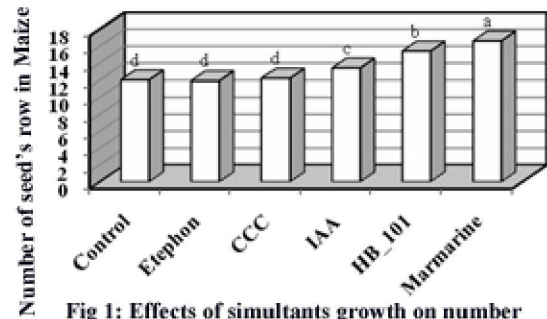
Fig 1: Effects of simultants growth on number of seed's rows in Maize

Plant growth inducer treatment on the characteristic of number of grains in rows per maize, and number of grains per maize indicated highly significant statistical difference (p < 1 %). Maximum increase in number of grains in rows per maize was related to Marmarine, and then HB_ 101 treatment. But this characteristic was decreasing dramatically in ethephon treatment, and cicocel decreased it more, but it was not statistically significant (Figure 2). The same results with a minor change were observed in the characteristic of the number of grains per maize. So that Marmarine treatment caused the maximum increase, and Etephone decreased it significantly, and cicocel decreased it insignificantly (Figure 3). Growth inducers do not cause the accumulation of dry matter in grain, but affect the division pattern of dry matter, which depending on environmental conditions may have positive or negative effects on the grain performance (Ma and Smith, 1992).

Table 1. Analysis of randomization complate blocks experiment under field conditions.

S.O.V	df	Number of rows in Maize	Number of grains in row of Maize	Number of grains in Maize	Length of Maize wood	Weight of Maize wood	Weight of grain in Maize	Weight of thousands seed	Grain performance	Harvest Index
						Mean of squares				
Replication (R)	٢	٣٠٦/١ns	٨٧٨/١٩ns	٠٩٨/٩١٧٥ns	٢٢١/٠ns	٣٥١/١٦ns	٣٠٤/٩٣٩ns	١٧٢/٣٢٦ns	٥١٢٦٦٦٥٤ns	٤٠٧٥*
Treatment (T)	٥	٩٩٢/٣٠**	٩٢/٥٩٥**	٥٨٤/٢٩٥٥٠٠**	٢٢١/٧٧٥**	٢٨١/٣١٧**	٢٨٩/٣٢٦١٥**	٠٤٤/٥٤٠٩**	١٨٤١٠٠٢٨٤٥**	٢٧٧/٤٤٤**
Error	٣٥	٤١/١	٢٢٧/١٣	٧٩٤/٩٤٣٩	١٩٤/١	٨٩٧/٨	٢٧٨/١٠٣٩	١٧٦/٦٠٨	٠٠٧٥٦٦٦٨٠٠	٨٨٥/١٣
% C.V		٨٢/٨	٥٩/٩	٩٤/٥	٠٦/٥	٢٨/١٠	٣٠/١٨	١٥/٩	٨٤/٢١	٩/١٣

ns, * and ** : Not significant, significant at ٥٪ and ١٪ probability levels of probaibility, respectively.
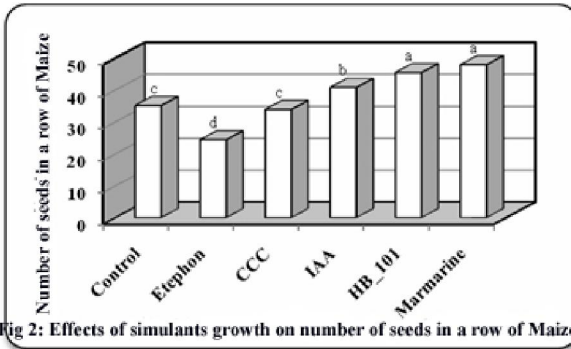
Fig 2: Effects of simulants growth on number of seeds in a row of Maize
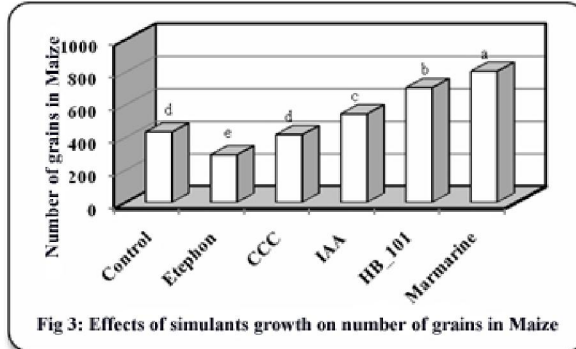
Fig 3: Effects of simulants growth on number of grains in Maize

Any increased duration of grain filling, increases the grain performance and has a large correlation with grain performance. Deficiency in maize pollen is rarely responsible for the lack of fertile eggs, but the main cause is usually late tasseling because of shortages of water, food elements, moving carbohydrates and nitrogenous substances (Kuchaki and Banayan, 1373). The use of algal growth inducing substances causes increased flowering and fruiting period in the tomato (Davies, 1988). Number of spikelets per spike is increased by the use of Indole Acetic Acid in cereals (Shabestari and Mojtahedi, 1369; Sarkar et al, 2002). A factor that negatively affects production of maize in flowering time is reduction in net assimilation rate at

the time of flowering till grain filling, and possibly lack of food or water and very high density will exacerbate the negative impact (Kuchaki and Banayan, 1373).

Weight and length of the cob

Weight and length of the cob indicated highly significant statistical difference in plant growth inducer treatment (p < 1 %). Weight and length of the cob in cicocel treatment did not indicate significant difference, but Ethephone caused significant difference in these two characteristics. The maximum increase was related to Marmarine, and HB_101 treatment, and then Indole Acetic Acid treatment (figures 4 and 5).
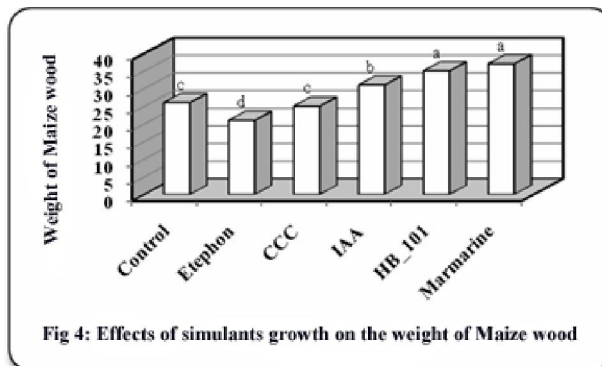
Fig 4: Effects of simulants growth on the weight of Maize wood
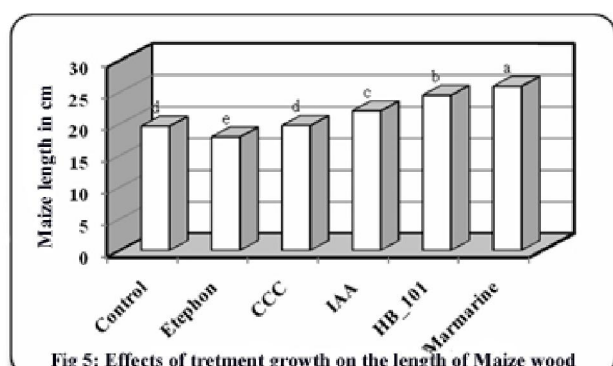
Fig 5: Effects of tretment growth on the length of Maize wood

Cycocel and different levels of nitrogen makes a significant increase in performance of wheat grain for the increase in the number of grains per spike, increase in the length of spikes, and increas ein the number of spikes per area unit (Shokoufa and Imam, 1384). Ethephon increased tillering in cereals and for the competition on photosynthetic substances and mineral absorption, the number of sterile spikelets is increased (Shabestari and Mojtahedi, 1369). Lack of carbohydrates, proteins, and possibly hormonal factors associated with dryness, lack of light in dense environments, cloudy days or nitrogen deficiency causes growth stop and death of fertilized

eggs at the tip of the maize, On the other hand, photosynthesis with water absorption and assimilation of food, is provider of energy supply for the performance (Kuchaki and Banayan, 1373). It appears that Marmarine treatment by affecting duration of pollination period and the effective pollination, by enriching the plant by the nitrogen substances, hormones and vitamins, increases the number of grains per row and maize, and the length and weight of cob. Also the impact of HB_101 treatment can be justified because of its effect on increasing the speed of nutrient transport.

Grain weight per maize and weight of 1000 grain

Grain weight per maize and weight of 1000 grains indicated highly significant statistical difference in plant growth inducer treatment (p < 1 %). Grain weight was increased respectively by Marmarine, HB_101 and indole acetic acid treatment, and dencrease by ethephon and cycocel. Also,

increase in weight of 1000 grains by Marmarine, HB_101 and auxin treatment, and increase in this characteristic by cycocel treatment, and no significant difference by applying ethephone, expalins largely the effect of grain growth inducers used in this study (Figures 6 and 7).
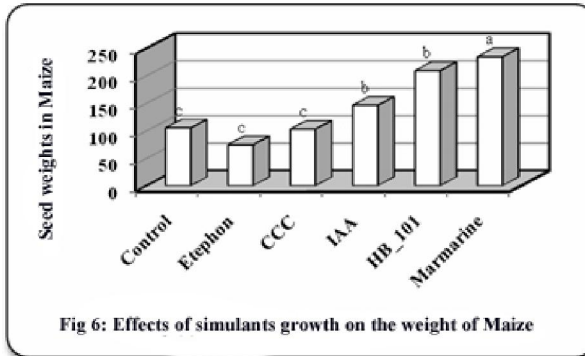
Fig 6: Effects of simulants growth on the weight of Maize
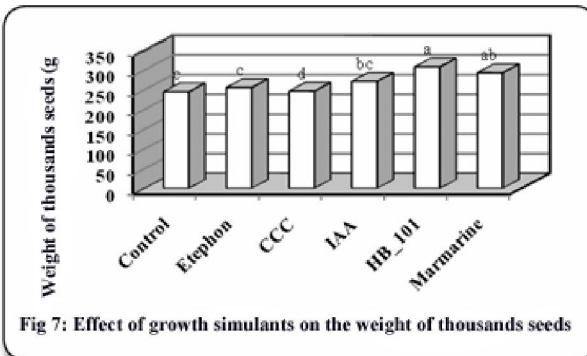
Fig 7: Effect of growth simulants on the weight of thousands seeds

The harvest index and grain performance

The harvest index and grain performance indicated highly significant statistical difference in plant growth inducer treatment (p < 1 %). The maximum grain performance increase (economic performance) was related to Marmarine, and then HB_101, and finally indole acetic acid. Other treatments decreased the performance insignificantly.

Also, the harvest index indicated the same results as the grain performance (Figures 8 and 9).

Performance in the corn is about grain production per unit area and grain weight, from which grain weight is more stable, and large difference in performance is usually a result of change in number of grains Fathi and Ismailpoor, 1379).
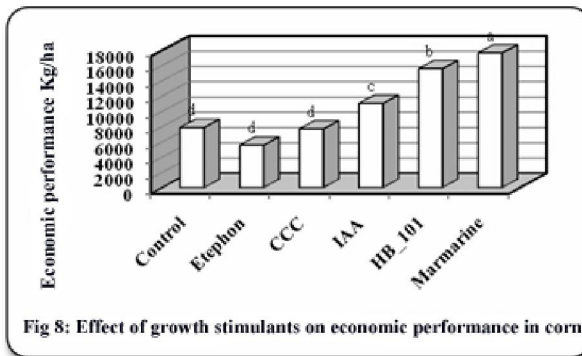
Fig 8: Effect of growth stimulants on economic performance in corn
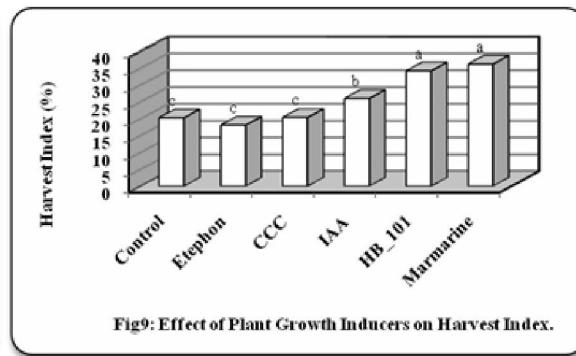
Fig9: Effect of Plant Growth Inducers on Harvest Index.

The number of grians formed, compared with the weight of 1000 grains, increases or decreases the grain performance mostly, and grain filling by photosynthesis substances in maize begins when the grain number is determined. Therefore, the number and size of grains per maize is severely affected by water, nutrients and light (Kuchaki and Banayan, 1373). In the condition that the leaves have a high amount of sucrose, the vascular sheath cells have a higher osmotic potential, and makes the loading easier. It seems that the intensity of photosynthesis is reduced by reducing the rate of acceptance by the target, and allocation of capacity consumed by photosynthetic substances, access to substances and

photosynthetic rate substance in this area is controlled. The hormones by affecting the formation, development and the loss of flowers and seeds, have an important influence on the relations between source and destination of plants. And may have effects on speed of transfer, and by having impact on the target requirements (demand) indirectly affect the transfer speed (Kuchaki and Banayan, 1373). Indole acetic acid treatment of cereals causeslateral buds to defeat, therefore reduces the production of tillers, improves the growth of main stem, and finally increases the number and weight of grain in cereals (Sarkar et al, 2002). Environmental stresses such as food shortages or radiation, especially 10-14 days

before pollination, significantly reduce the number of grains per maize (Kuchaki and Banayan, 1373), on the other hand, the factors that control the transfer to destination assimilation have also control on the distribution of photosynthetic substances. The effect of hormones on enzyme activity and flexibility of the target cells have a great impact on the distribution of photosynthetic substances (Kuchaki and Sarmadnia, 1377). Having large amounts of nitrogen and mineral elements and the presence of growth hormones such as auxin, jiberlin, phenyl acetic and citokenin in the algae such as Ascuphyllum nodusom is now proved, and by using them, the production of various products, including corn, potatoes, peppers, tomatoes, pineapple and orange has significantly increased (Blunden, 1972). The test of three year effect of etephone on grain performance in barley indicated that the number of grains in spike is increased, but their size is often reduced (Shabestari and Mojtahedi, 1369). Also with the increase in cycocel consumption, the weight of 1000 grain is reduced (Lungw et al, 1982). Mobilization of stem reserves (including surplus products of photosynthesis before the grain filling stage), is largely involved in grain performance in grain filling stage (Austin et al, 1980; Gllangher et al, 1976). The harvest index indicates the ratio of grain performance and biological function, and is acquired from the ratio of grain performance to the biological function (Kuchaki and Sarmadnia, 1377), so it is rarely influenced by the environment (Fathi, 1378), on the other hand, ninety percent of the corn grain dry matter is provided through photosynthesis during the grain filling, and is depended on leaf area index, leaves long-term activities (particularly upper leaves), the high rate of photosynthesis and photosynthetic substances that is transported to the beads (Kuchaki and Sarmadnia, 1377). The remained ten percent of the corn grain dry matter is provided through remobilization of stored substances in the vegetative organs; although this value is low, but regardless of daily fluctuations in photosynthesis, these photosynthetic substances are vital to keep grain dry matter increase during the grain filling stage (Duncan et al, 1965). According to the results obtained, it appears that the to increase production and higher grain performance in maize, plant growth regulators and growth inducers can be used a week before and after pollination, so that Marmarine by increasing pollination period, insemination and duration of grain filling, and more and faster transfer of photosynthetic substances to the grain, leads to increased number of grains per row and maize length, weight of 1000 grain and eventually grain performance in the corn. HB_101 also increases the grain performance by increasing the number of grains, maize length and weight of

1000 grain. Increase in photosynthesis in the leaves for the rapid absorption of nutrients in the stage before and after pollination, even grain filling by Calcium and sodium ions, which in turn increases the storage of sugar in the plant, and increased speed and duration of grain loading and unloading, can be considered as factors of this effect. Indole acetic acid also increases the effective transfer of photosynthetic substances to the grain, and so increases the relative number of grains and weight of 1000 grains in the corn. Undesirable effect of cycocel and ethephon on grain performance in corn can be caused by plant type and the time of their application. However, for widespread use of these substances, further research is required. The valuable note in this study is increased performance in an important crop such as corn by application of natural growth inducers (Marmarine and HB_101), which in addition to achieving the goals of sustainable agriculture, can be a starting pint for new researches about other important crops.

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8/3/2012

Rule of Rectal Manometry in Late Management of Fecal Incontinence in Patients Treated By Posterior Sagital Anorectoplasty

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Abstract: Eleven patients with proximal types of imperforated anus (mean age 15 Y) were evaluated clinically by a single examiner and by rectal manometric studies. All were males with problems related to fecal continence. It was found that local examination alone is enough to put a plan for further management with no need for Manometric evaluation.
[Jamal S. Kamal. **Rule of Rectal Manometry in Late Management of Fecal Incontinence in Patients Treated By Posterior Sagital Anorectoplasty**. *Life Sci J* 2012; 9(4):230-233]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 33.

Key words: long term management – local examination

1. Introduction

Patients underwent repair of imperforate anus may suffer from variable degree of fecal incontinence depending on the type of anomaly and the operative approach. Low anomalies treated via the Pena's posterior sagittal approach usually had better continence. In normal children, voluntary urinary and fecal control achieved after training, by the age of 3-4 years, therefore assessment patients treated for their imperforate anus, advisable to be done beyond this age[1]. Post operative assessment is directed towards assessing the strength of sphincter muscles, site of new anus and quality of life. The capacity of the sphincters and its distribution are evaluated by rectal manometry,electromyography, endosonography and magnetic resonance imaging,[2] while quality of life assessed by direct interviewing. Although not universally agreed, several scoring systems were described to clinically evaluate the results of treatment like; the Kelly's and the pena's (Krikcenbeck) scoring system.[3] In this study we reviewed a group of patients with proximal types, treated by Pena's approach and had difficulties in fecal control.

Aim of the study

To determine the efficiency of clinical examination alone and wither there is role of ano-rectal manometry in the management of fecal incontinence in patients with proximal types of imperforate anus treated by posterior sagittal ano-recoplasty (PSARP).

2. Material & Methods

Eleven male patients with different proximal types of imperforate anus, treated by Pena's PSARP and having defecation problems were evaluated by clinical examination and rectal manometry, their mean ages15 years (range 7.5 to 28 years).The PSARP was done primarily in 9 cases, and as a secondary procedure in 2 patients. (Initially treated via abdomino-perineal approach and had obvious mislocation of the rectum), 4 patients had recto-vesical fistula(R-V), 4 with recto-bulbar urethral fistula (R-U) and 3 cases had supra levator imperforate anus without fistula. The clinical examination was done by a single examiner, at least 2 hours from last the cleaning. It includes; the site of the neo-anus, presence of soiling and digital rectal assessment of the sphincters. A score was given to each item; 1 when the neo-anus is central, 0 if mislocated, a score of 1 for absence of soiling and 0 when soiling present. On Digital rectal examination; voluntary squeeze by the patient is given a score of 1 when it is tight grip, 0.5 when it is weak and 0 when there is the absence of griping. Rectal manometry was done with the catheter introduced 5 cm from muco-cutaneous junction. The resting pressure (RP), squeeze pressure(sq p) in mmHg are measured(The squeeze pressure measured twice and the average was taken),then the Recto Anal Inhibitory reflex (RAIR) recorded after inflating the balloon up to 25 ml of water. The manometric evaluation was given a score of 1 to each positive Resting rectal pressure, rise of squeeze pressure and presence of Recto Anal Inhibitory Reflex (RAIR) and a score of 0, in their absence. Table (1)

3. Results

Seven patients had obvious soiling, four with mislocated neo-anus. Voluntary anal grip was good in 2, weak in 7 and no voluntary finger grip in 2 patients. All patients had positive resting anal pressure of 27.4 mmHg (range 23-39.9) and positive rise of squeeze pressure of 52.4 mmHg (range 31- 72) with positive Recto Anal inhibitory Reflex(RAIR) in 2 patients (18%). In the 2 patients with no voluntary grip RP was 24.5 &39.9 mmHg and their sq P 65 & 72 mmHg. In both patients the soiling was due to impacted feces, one with recto-vesical and the other had high imperforate anus without fistula.

No relation seen between the type of imperforate anus, clinical assessment and,manometric studies. When our score applied, The best results was seen in cases with recto-urethral fistula but there was no relation between the clinical and manometric score. (Table 2).

Table(1): Summary of the Clinical & Manometric Evaluation.

Demographic data				Clinical findings				Manometric findings			
NO	Sex	Age	Type	Soiling	R site	Vol grip	S	R P mmHg	Sq P mmHg	RAIR	S
1	M	8.5 y	R- v	+ 0	Ecc lt 0	Weak 0.5	0.5	26.5 1	37 1	− 0	2
2	M	9.5 y	R-v	+ 0	Ecc dn 0	Non 0	0	24.5 1	65 1	+ 1	3
3	M	8 y	R-v	+ 0	Cent 1	Weak 0.5	1.5	19.2 1	46.7 1	− 0	2
4	M	9y	R-u	+ 0	Cent 1	Weak 0.5	1.5	30.9 1	50 1	− 0	2
5	M	7.5y	R-u	− 1	Cent 1	Weak 0.5	2.5	15.3 1	40 1	− 0	2
6	M	11y	H,no F	+ 0	Cent 1	Weak 0.5	1.5	36.8 1	50.3 1	+ 1	3
7	M	17 y	H,no f	− 1	Ecc up 0	Good 1	2	39.1 1	49 1	− 0	2
8	M	21y	R-u	+ 0	Cent 1	Weak 0.5	1.5	25.4 1	70.6 1	− 0	2
9	M	19y	H,no f	+ 0	Cent 1	Non 0	1	39.9 1	72 1	− 0	2
10	M	26y	R-u	− 1	Ecc up 0	Good 1	2	21.3 1	31 1	− 0	2
11	M	28y	R-v	− 1	Cent 1	Weak 0.5	2.5	23 1	65 1	− 0	2

R site : Rectal site, Vol grip ; Voluntary grip, RP : Resting pressure, Sq P : Squeeze Pressure, S: score, RAIR : Recto Anal Inhibitory Reflex, R-V Recto vesical fistula, R-U : Recto urethral fistula, H no f : High without fistula, Ecc lt : Eccentric to left of patient, ECC dn ; Eccentric downward, Ecc up : Eccentric upward, Cent : central

Table (2) The Score results of Clinical & Manometric evaluation.

Type	NO. of cases	Mean clinical score	Mean Manometric score
R-U	4	1.87	2
R-V	4	1.125	2.25
H.no fist	3	1.5	2.3

R-U: recto Urethral Fistula, R-V: Recto Vesical Fistula, H.no Fist : High type without Fistula

4. Discussion

Imperforate anus is a form of ano-rectal malformations, it occur in 2-2.5 per 10,000 live births,with significant variations in the prevalence between regions throughout the world.[4] It occur in a range of spectrum and classified into different types (Krikinbeck classification).[5] Due to lack of national registry of birth defects, the incidence in our society is not known but it has been reported in association with other anomalies.[6-8] The management of these abnormalities starts from the neonatal period in a single or multiple stages. The principal stage is the rectal pull through, with the widely accepted approach is the posterior sagittal, described by Pena' in 1982.[9-11] Although several techniques described, fecal continence and voluntary bowel control, markedly improved after the introduction of this approach. [12,13]

Complications like; constipation, fecal soiling, mucosal prolapse and mislocationof the neo-anus are known to occur, particularly in high types. [14,15]The presence of sacral anomalies may further enhance these complications.[16,17] Fecal soiling could be either a true incontinence due to sphincter muscle hypoplasia, or false incontinence as a result of constipation, the

difference between the two, can be done through local examination(impacted feces), contrast enema(hypo motile colon), endosonography and rectal manometry[18-20] Quality of life and social acceptance are highly affected by disturbance in fecal control. Different scoring systems were described for assessment of defecation problems and quality of life, like the Kelly's & Krinckenbeck (Pena's score) for small children and the Bai score for age 8-16 Y. The scores on these systems are based mainly on patients and parents councelling.The Kelly's score includes digital assessment of the sphincter muscles strength and rectal manometry, included in Holschneider scoring system. local examination and the site of the neo- anus was not included in these systems. The functional outcome score can be considered as an index of management of defecation disorders in children with anorectal malformation.[3,21]

Colonic and rectal manometry is done for evaluation of patients with chronic constipation, it measures endo colonic and rectal pressure.[22] Patients with ano-rectal malformation are known to have motility disorders which is segmental in low anomalies and more generalized in the high types.[23,24]

Early post operative evaluation by ano- rectal manometry may give realistic information about the future continence so that a plan for further management can be started.[25-27] The long-term functional outcome in children with anorectal malformations is significantly lower than normal controls, however it improves at adolescence with proper management of constipation that's why regular follow-ups are required for proper care.[21,28,29]

In our patients, the resting and squeeze pressure were in the highest score according to Holschneider scoring system in spite of having defecation problems. Their age and having a common problem, would allow easy Personal counseling and local examination, this would eliminate the need for further assessment by a other methods, this was also suggested by Tsuji et al.[30]

5. Conclusion

Local Clinical examination, by a single examiner may be adequate in evaluating and putting a plan for further management in patients with complications following PSARP.

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9/1/2012

The Impact of Selected Aerobic Aquatic Exercises on the Depression and Happiness Levels of Patients with Multiple Sclerosis (M.S)

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Abstract: Introduction: Multiple sclerosis (M.S) is one of the most common chronic diseases of the central nervous system. The chronic nature of the disease, lack of prognosis, lack of any definitive cure and affecting the individual in the young age will cause several mental disorders in patients, including depression and low rate of happiness. **Objective:** Evaluation the effect of aquatic exercise programs on depression and happiness levels of the patients with multiple sclerosis (M.S). **Methodology:** In this quasi-experimental research, 40 patients with M.S. with grades 1 to 4 were randomly divided into two experimental and control groups. The exercise program was performed for the experimental group for eight weeks, three sessions per week with 50-60 percent of the maximum heart rate. The depression and happiness rates were evaluated using the Beck Depression questionnaire and the Oxford Happiness questionnaire at intervals before and after the exercise. Analysis of the data was performed by independent t test, and the data normalization was evaluated using the Kolmogorov-Smirnov test. **Results:** The test group, regarding the depression level ($p < 0.001$) and happiness level ($p < 0.002$) after exercise had significant changes compared to the pre-exercise time. Thus, the mean differences for depressions and happiness levels of these two groups were obtained respectively as 4.65 and -7.80. **Conclusion:** Regular aquatic exercise provides more productive emotional communications and also causes the improvement of depression and increased happiness in M.S. patients. Hence, doing these exercises is recommended as an effective non-pharmacologic therapy method in addition to drug therapy.
[Mohammad Bagher Toorang. **Evaluation of Golshan Shabestari and Raz mashahed's characteristics.** *Life Sci J* 2012;9(4):234-240] (ISSN:1097-8135). http://www.lifesciencesite.com. 34

Key words: Multiple sclerosis, Aquatic exercise, Depression, Happiness

Introduction

Multiple sclerosis (M.S) is one of the most frequent chronic and progressive diseases of the central nervous system that is considered as the third cause of neurological disabilities. In this disease, the immune system of the patient's body shows inflammatory reaction against his nerve tissue and causes demyelinating of the tissue (1). The prevalence of M.S. disease is geographically different and increases more from the equator towards the hemispheres (North and South). The disease has infected more than 3.5 million people worldwide. In Iran, 57 individuals out of every 100 thousands people are affected by this disease (2).

The most common involving age of this disease is between 20 and 40 years old; right the years in which the individual has the most family and social responsibilities, and actually in the reproductive age. As a result, the productive forces of the society are harmed and the damage is related to all people who live in the community (3).

The disease prognosis is unclear and the patients experience a variety of physical and mental disorders due to disease complications; such disorders strongly affect the patient's daily functioning, social and family life, functional independence and the individual's program planning for the future and as a whole, will severely impairs the person's well-being feeling.

About 80% of patients will develop some degree of disability, and only the condition of 1 out of every 5 patients remains constant and the does not lead towards disability. It can be concluded from the mentioned items that the disease can cause symptoms and mood and mental disorders due to the chronic nature of the disease in the patient (4).

Given the high levels of depression and low rate of happiness in M.S. patients, using the diagnostic tests and treatment methods other than common medicines therapy to reduce these symptoms seems to be necessary (5), since the common drug treatments are not effective in all patients with multiple sclerosis, and on the other hand, the medicines have many side effects such as fatigue and psychological imbalances, for which there is no known cure completely effective to overcome such complications (6).

The findings in conjunction with recovery of the damages show that the observed damages in M.S. patients can be due to reduced physical activity levels in these patients compared to the time they were completely well and healthy. This is not yet clear that how much these damages in M.S. patients can be recovered. The damages resulting from the progress in the disease process will not likely to be reversed by exercising.

Meanwhile, the injuries that have progressed as a result of deactivation are likely to be reversible. According to all the research conducted on highly improving in patients with M.S. after physical exercising, it appears that the major part of damages occur as a result of inactivity rather than the irreversibility of the damaged tissue. Therefore, exercising is a vital tool for recovery of the patients with M. S. (7).

American Physical Therapy Association (APTA) has suggested a foundation of exercising methods for the patients that also include the patients with M.S. One of these proposed strategies is the aquatic therapy program (8).

Given the prevalence of balancing- motor problems in patients with M.S., water will provide the adequate support for performing movements that are difficult to be done on the ground (9). Buoyancy and viscosity features of the water can help in motion and cause increased muscular strength (8).

Increased body temperature is also one of the main problems of M.S. patients during physical activity, and it is believed that water can partly prevent the increase in body temperature, and hence, the patient will exercise and work better (10). Of water activities, aquatic exercise training can be mentioned.

Given the prevalence of this disease in Iran and the positive effect of physical activities on the mood of M.S. patients, in this study, the researchers are to investigate the changes in depression and happiness of patients with M.S during a period of 8 weeks aquatic exercising.

Methodology

This is a quasi-experimental type study. The statistical population consists of a hundred of women with M.S. who have been diagnosed by M.S. by a neurologist physician; they have been all under pharmaceutical treatment and have medical files in a valid specialized clinic.

Among the statistical population, 40 people were randomly selected as the research sample; they were divided into two groups, including the test group (20 subjects) and the control group (20 subjects) based on the selecting inclusion criteria with features such as disease grade based on Expanded Disability Status Scale (EDSS) of 1 to 4, the mean duration of the disease as 6.5 years and the age range between 20 and 50 years old.

Patient inclusion criteria for participation in this study were as:
1. Definite M.S. diagnosis approved by a neurologist physician
2. Having a measure of Expanded Disability Status Scale (EDSS) from1 to 4
3. Age range from 20 to 50 years old
4. Affected by no other diseases (epilepsy, cardiovascular, respiratory, skin, etc.)
5. Not pregnant
6. The ability to participate in exercise training regularly

Exclusion criteria for project were as the following:
1. Not performing at least two thirds of the number of exercise sessions
2. Relapse of M.S. during intervention
3. Becoming pregnant
4. Changing the patient's medicines during the 8 weeks intervention

Procedure

To perform the study, the patients gathered in the exercise place one day before starting the exercise program, and after the descriptions about the way of training, both test and control groups participated in the pre-test. At this stage, the Expanded Disability Status Scale test was measure and recorded by a specialist neurologist using the Krutz Physical Disability Scale, which determines a score between 0 and 10 for M.S. patients (depending on the amount of damage to the CNS). Also, both experimental and control groups completed the depression and happiness questionnaires.

Depression

Depression levels were measured in this research using the Beck Depression Inventory.

The reliability coefficients test-retest based on intervals between performing times and the population type were in the range of 48/0 to 86/0 (Beck et al., 1988).

This questionnaire includes 21 items. Each item has 4 options that are scored based on 0 to 3. It determines different degrees of depression from mild to severe. The maximum and minimum scores on this test are respectively as 63 and 0.

Happiness

Happiness measurement in this study was performed using the Oxford Happiness questionnaire. Argayel et al. (2002) have reported the reliability of the Oxford questionnaire using the Cronbach's alpha coefficient as 0.90 and the test-retest reliability over a 7-week period as 0.78. The test consists of 29 four-option statements. Thus, the highest score that a subject can get on the

scale is 87, which represents the highest level of happiness and the lowest score on this scale is zero that suggests the subject's dissatisfaction of life and the person's depression. The normal score in this test is between 40 and 42.

The attribute of the selected aquatic training program

A. The kind of exercise trainings and their duration

The patients in the experimental group performed the aquatic exercise training for 8 weeks as 3 sessions per week at 28 ° C, while the control group patients did not participate in any training program during this period, and were only similar to the test group regarding the social and group activities. Meanwhile, these training programs were leading and guiding by aquatic sports and swimming teachers.

B. Aquatic exercise sessions schedule

Warming up

Each session was beginning with warming-up, including walking in the water (the correct way had been instructed to the patient) and performing gentle stretching movements for 10 minutes within the water.

The main activity (each session objective)

The basic trainings related to each session consisted of kinetic and stretching exercises and movement within the water in the first eight sessions for 40 minutes, in the second 8 sessions for 50 minutes and in the third 8 sessions for 60 minutes were provided to the patients. During the training period, with making the movements more difficult and using the combined movements and shortening the resting time interval between periods, the exercise pressure was applied (the over-load principle).

Cooling

At the end of each training session, for 5 to 10 minutes, the subjects were performing the stretching movements to return to the initial state by slow walking and doing simple movements with low intensity.

C. The exercise intensity

The subjects were performing the aquatic selected aerobic training program with the intensity of 50 to 60% of maximum heart rate, which had been obtained in the preliminary test. The exercise intensity was measured during each session by Polar heart rate monitor.

Statistical methods

After completion of the exercise period, the subjects completed the depression and happiness questionnaires as the post-test.

Immediately after the preparation of the questionnaires results, the obtained and required data was extracted form the related sheets and was entered into the SPSS, v.16 software table for statistical analysis.

The percentages, means, standard deviation and the graphs drawing were used to show the results. The normalizing of data was performed by Kolmogorov-Smirnov test.

The independent t test at alpha level of 5% was used to compare the difference between the pre-tests and the post-tests of the two groups.

Results

The descriptive results of the subjects are presented described in Table 1. The findings obtained from both groups in pre-test and post-test in conjunction with depression and happiness are provided respectively in Tables and Figures 1 and 2 is:

Table 1: Descriptive results

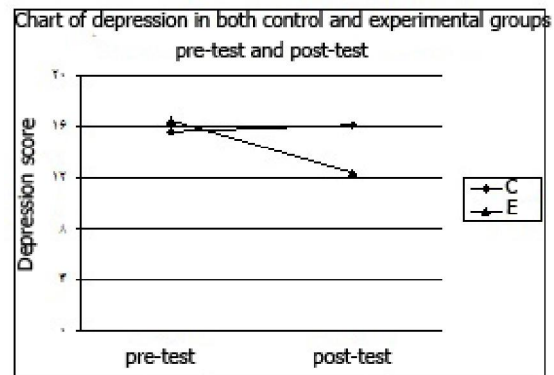| Variable	Group	
	Control	Test
The individuals mean age	30.4	35.45
The mean age of disease onset	35.80	30.05
The mean duration of M.S.	5.45	7

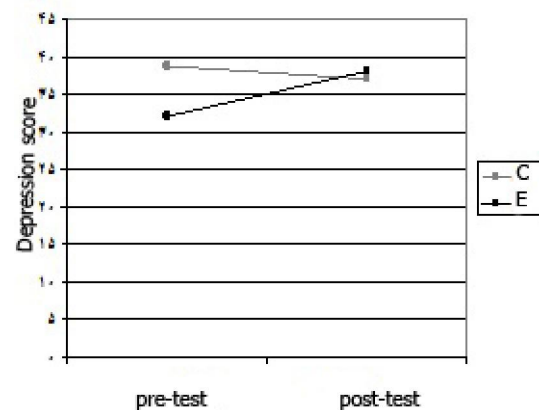Figure 1: The comparative depression test of the two groups of subjects

Figure 2: The comparative happiness test of the two groups of subjects

Table 2: The comparative depression test of the two groups of subjects

Variable	Group	Stage	Mean and standard deviation	Means difference	T value	P value
Depression	Control	Pre-test	15.55 ± 2.394	4.65	1.597	0.08
		Post-test	16.1 ± 2.552			
	Test	Pre-test	16.45 ± 2.327		20.107	0.001
		Post-test	12.35 ± 2.183			

Table 3: The comparative happiness test of the two groups of subjects

Variable	Group	Stage	Mean and standard deviation	Means difference	T value	P value
Happiness	Control	Pre-test	38.90 ± 11.07	7.80	1.671	0.111
		Post-test	37.10 ± 12.94			
	Test	Pre-test	32.15 ± 15.73		0.002	-3.64
		Post-test	38.15 ± 17.63			

In general, the most common symptoms and problems caused by M.S. are mental psychological and social complications, which are created due to the primary or secondary symptoms and problems, meaning the symptoms that are caused directly due to the loss of myelin sheath in special nerves such as visual disturbances as well as complications caused by early symptoms such as paralysis, inactivity, etc. (2). On the other hand, the disease is a mental - physical experience and can be a stress- causing event in patients' lives. The chronic disease implies on the separation between past and present and changes the image of the person from himself and creates an unfamiliar situation. The women with a chronic illness may face with some problems in combining and matching with the new identity (due to the illness) (11).

Since the disease has no definitive cure, the patients should rely on methods that only reduce the disease symptoms. Exercise meanwhile increasing the behavioral reinforcement through participation the individual in group activities will strengthen the individual's positive behaviors and prevents him from disappointment, frustration and depression and help him to be a healthy and self- thriving person regarding the psychological aspects.

In conducted research on patients with M.S. that have been fully described by Charcot since 1877, the mood (mental) disorders have been always described associated with the disease (12).
In 1996, Petajan et al. performed a study entitled as "Effects of Aerobic Exercise on the Health of Patients with M.S" (with EDSS score from 1 to 6).
They reported the significant improvement of mental health aspects in their own results.
The scores of depression, anger and fatigue of the participants in the exercise have significantly reduced (13).

Sutherland et al. (2001) by conducting a 10-week (30 sessions) of aquatic exercise on patients with M.S. evaluated the effect of this exercise training course on the life quality of these patients. The results indicated increase energy and reduced fatigue and pain (14). The results of these studies are consistent with the present research.

Oken et al. (2004) conducted a research in the United States to investigate the influence of Yoga and aerobic exercise on the mood of patients with M. S.

In this study, the subjects have been definitely diagnosed with M.S. (EDSS $\leq$ 6/0) and have been randomly divided into three groups until the end of 6 months.
1. Weekly yoga and exercise at home group
2. Weekly training by use of fixed bicycle associated with exercise at home group
3. Control group

No significant change was seen in mood related to the yoga exercise (15).
Freeman et al. (2004) in their research entitled as "group exercise classed in patients with M.S." performed a guiding study on 10 volunteers covered by M.S. Society in London. Holding group exercise training classes in a short term had the greatest impact on the health outcomes. Although the psychological aspects had been also improved, but they were in lower range (P = 0.48) (16).

Reiberg et al. (2005) reviewed the impact of exercise therapy in patients with M.S. on daily activities and health-related quality of life in a case study. The results showed that exercise therapy had a significant impact on the power of the muscles functioning, exercise tolerance functions and activities related to mobility and as well as a partially effect on mood. No harmful effects due to exercise have been reported in the conducted studies (17).

The results of these three studies are in disagreement with this research. One of the reasons may be the different types of exercise training. Thus, in this study, the aquatic exercise followed by the soothing advantage of the water has been used.

Tedman (2005) performed a research in the UK entitled as "study the depression in patients with motor neurological diseases and other diseases due to neurological disabilities on 40 patients with motor neurological diseases and 92 patients with multiple sclerosis. He found that the depression was associated more significantly with multiple sclerosis than other motor neurological diseases (P = 0.001) (2).

Researchers at the University of Utah (2006) studied on patients with M.S. in a different way. Having knowledge on the physical and mental benefits of exercise, they decided to find out how the patients with M.S. respond to exercise. After 15 weeks of aerobic training, their study showed that some changes have occurred in some of the factors in M.S. patients, including more positive attitudes and less depression (18).

In their study, Rampello et al. (2007) reviewed and compared the effects of tow kinds of aerobic exercise training and neurological rehabilitation programs for 8 weeks on the life quality of people with M.S. They used the 54-question specific life quality questionnaire to measure the quality of life in patients before and after the training period.

The results of this study showed that both training programs have caused improved quality of life in patients, although the improved aspects of the life quality were different in both groups. The aerobic training showed a significant increase in understanding of public health, energy and vitality. The training program of neurological rehabilitation had a contradictory impact and had reduced the vitality due to its threatening for mental health (19).

Macually et al. (2007) conducted a research on effects of persistent physical activities and their relationship with health and happiness on patients with M. S. The results indicated that the individuals participating in persistent exercises were working harder and doing the practices made them feel better compared to the people who had no exercise training and increased their happiness rate (20).

Since doing physical activity has increased the patients' happiness rate in this study, their results are consistent with each other.
Ghaffari et al. (2008) conducted a quasi-experimental study aiming to determine the effects of muscular progressive relaxation technique on depression, anxiety and stress in patients with M.S. in Tehran.

The method in this study included a session for making the samples familiar with the objectives and method of intervention and 63 sessions of performing the technique by the test group during two months, while no intervention was done for the control group.

Data collection tools included the demographic data questionnaire, depression-anxiety-stress-21 (DAS -21) questionnaire and self-reporting checklists.

66 subjects participated (33 subjects in the control group and 33 subjects in the test group) in this study. The mean scores of depression, anxiety and stress showed no significant differences before the intervention; however, one month and two months after the intervention, the difference between the experimental and control groups in the anxiety and stress scores became statistically significant (P <0.05). Also, no significant differences were found in the depression and anxiety scores in three times of measurements between the two groups, but this difference was significant in mean scores of stress in three times of measurements between the two groups (P <0.008). Consequently, implementation of this technique could lead to decreased depression, anxiety and stress in patients with M.S. (21).

Mccullagh et al. (2008) evaluated the effect of aerobic exercise on depression in M.S. patients with mild disabilities in a study.

Thirty patients diagnosed with M.S. with the ability to move without help were studied. The patients were influence by the exercise trainings for 3 months twice in a week. The control group was reviewed monthly and their program was unchanged. 24 subjects completed the program, and according to the three-month changes, the test group had more improvement in decreased depression rate. Then, a three-month training program improved the quality of life and depression in participants (22).

George Jeling et al. (2009) concluded that the exercise had a positive effect on the M.S. patients' immune system, maintaining their strength, balance and functioning as well as maintaining their readiness in mental health and social work. They also showed that exercise affected on reduced depression due to its contravention with immune system cytokine in M.S. (23).

Strod et al. (2009) conducted a research about the effect of regular exercise programs on depression in patients with M.S.
The purpose of this study was to compare depression in the patients having regular exercise trainings with those did not participate in such activities.

121 patients with M.S. participated in this study with the age range of 25-65 years old that lived in Australia. The depression levels were measured using the Beck Depression Inventory.
52 participants 2 times a week and at least for 30 minutes per session and 69 subjects participated in normal physical activities.

People who were exercising, reported less depression, which was compared with the people

having no activity; the results showed that physical activity had benefits for patients while the people without exercise were excluded such advantages (24). George Jeling (2010) wrote in a book entitled as "Overcoming M.S.: The aerobic exercises are very useful in breaking the cycle of fatigue and depression in M.S. patients, but they require a discipline. It has been observed recently that these people used to exercise less than other individuals. Other research suggests that exercising regularly will reduce the prolonged incapability by increasing the neuronal shielding through production of neuronal growth factor by fluctuation in cytokines and stress hormones changes (25).

Soltani et al. (2010) performed a study entitled as "Reviewing the impact of a course of aerobic activity in water on the quality of life in patients with M.S. In this research, some exercises as three times a week for 8 weeks were performed, while the control group had no exercise.

It was concluded that doing aerobic exercises in the water significantly improved the quality of life in M.S. patients (2).

Kargarfard et al. (2010) reviewed the changes in quality of life and fatigue in patients with M.S. after 8 weeks of aquatic exercises in their study. The findings showed the statistically significant effect of exercise in water (aquatic exercises) in improving the mental status ($P = 0.01$), vitality ($P = 0.000$) and energy ($P = 0.03$). Since this research includes aquatic aerobic exercises for 8 weeks and studies the happiness rate, which changes will cause improved mental state and increased patients' vitality, it seems that its results are consistent with their research results (26).

Motl Robert et al. (2010) conducted a research on clinical, psychological, and physical activity manifestations among adults with recurrent reducing M.S. (RRMS).

In this study, the relationship between clinical and mental manifestations such as depression and pain rate and exercise training was studied. There were 269 participants in this project. The research instruments consisted of a questionnaire and an accelerometer.

It was concluded that there is an indirect relationship between exercise training and clinical and psychological manifestations such as depression in RRMS patients that means increased physical activity reduces depression in these patients (27). Although in most conducted studies the training program has been other than the aquatic exercises, but in many studies, the effects of aquatic exercises on patents' mood and enhanced happiness levels in people in different age ranges of other patients (other than M.S.) have been studied that all have supported the ability of aquatic exercises in increasing the life satisfaction.

Based on the results of this study, it appears that aquatic exercises have the ability to reduce the depression level and increase the happiness rate in patients with M.S. They also demonstrated more ductility level than other exercises.

It seems that improvement in patients' mental- psychological status is affected by the followings during aquatic exercises program:

1. Getting out of the uniform environment at home and engaging in a happy environment that makes the subjects to forget the feeling of being sick for a while.
2. Communicating more with others and performing group exercise, which improves their social functioning.
3. The effect of water relaxation factor
4. Learning aquatic exercises and the ability to float in water that makes the participants experiencing the power sense.
5. Increased endurance, improved functionality and ability to perform more activities will increase their self-confidence.

Conclusion

In general, this study will reveal that the depression levels of patients with M.S. have decreased at the end of aquatic exercises program and their happiness rate has increased. Thus, it is recommended to the relevant experts to use such exercises as a complementary method besides the drug therapies to improve the M.S. patients' situation.

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8/8/2012

Distribution of Radionuclides in the Leachates for Several Rock Types at Different Time Intervals

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Abstract: In the present study, the behavior of the different isotopes activity concentrations for three different rock samples (siltstone, shale and sandy dolomite) as well as in the different leachates of these rocks were studied at different time intervals, using a HP-Ge spectrometer. The activity concentration of ^{238}U for the leachates gradually increases while that for ^{235}U is nearly constant. The results revealed that the solubility gradually decrease from ^{235}U to ^{234}U, ^{238}U and ^{226}Ra respectively. The reason for the higher ^{234}U concentrations in leachates is due to α- recoil process which enhances the mobilization and solubility of the decay product of ^{234}U relative to the parent (^{238}U).Also the^{238}U activity concentration percent in leachates to that in origin samples increases towards equilibrium state due to the growth of the ^{234}Th. It is noticed that the^{238}U% of siltstone and shale are very close and higher compared to dolomite which is much less in spite of the higher activity of the dolomite rock sample. The ratio (^{234}U/^{238}U) for the first measurement of the leachates was considered at zero time as 1.09±0.17, 1.4±0.21 and 1.6±0.18 for siltstone, shale and sandy dolomite respectively. The enrichment of ^{234}U is accordingly related to the crystal damage and leaching, which are the main mechanisms for the (^{234}U/^{238}U) disequilibrium. The variations in the activity ratio (^{238}U/^{235}U) are related to the increase in the activity concentration of ^{238}U with time and also to very little variations in ^{235}U.The solubility for Ra, Th, and K are very low and mainly remain in the residuals.

Key Words: Gamma-spectrometry; Leachates of rock types; Uranium activity ratios.

1. Introduction

Uranium, the heaviest naturally occurring element on the earth, plays an important role in daily life because of its use in nuclear power plants. It comprises three natural isotopes (^{234}U, ^{235}U, and ^{238}U) with relative isotopic abundances of 0.0054%, 0.720% and 99.275%, respectively [1]. These values may have small variations depending on the geographic origin of rocks due to natural isotopic fractionation, nuclear reactions or anthropogenic contamination. Precise measurement of the isotopic composition of uranium is of importance for the nuclear industry as well as in environmental and earth sciences. In earth sciences, interest is centered on the observation of the parent/daughter pairs in the U and Th decay series, which are present in natural samples in radioactive equilibrium. In case of environmental sciences, soil and sediments are the most suitable materials that preserve any remains of anthropogenically altered uranium [1].

^{238}U/^{235}U isotope ratio has changed dramatically during earth history from about 3.3 to the present-day value of 137.88 [2, 3and 4].The natural variations, observed on earth today, must have been produced by chemical reactions that fractionate isotopes [5, 6, and 7].

Knowledge of the ^{234}U/^{238}U activity ratio in the ecosystem provides information about the mechanisms and processes of uranium transport and origin. It is estimated to be greater than one. Some factors are suggested to influence the uranium isotope ratio; the age of rocks, rock types and climate differences. In rocks older than a few million years, ^{234}U/^{238}U activity ratio should be in secular equilibrium [8].

However, the highly energetic alpha-decay of ^{238}U damages a mineral's crystalline lattice and allows ^{234}U to be more mobile during weathering of rocks by meteoric waters. Increased physical weathering and the resulting decrease in grain size increase the mineral surface area (per unit volume). This increases the number of alpha recoil fractures that are exposed to the surface of the grains, which in turn allows for an increased rate of ^{234}U removal [9]. The rate of this removal is expected to decline with time as the more labile uranium is removed first, leaving the less- mobile uranium in the mineral crystalline lattices [10]. Rock type determines rock weathering rates. Where dolomite weather the most rapidly one is followed by shale and sandstones. Climate differences have been hypothesized to affect the ^{234}U/^{238}U activity ratio [11, 12].

The separation of radionuclides from any ore material passes through two steps. The first step is the leaching or dissolution of radionuclides by either acid or alkaline solution or then separation (filtration) of the solution (leachate) from the solid (residual).The second step is the precipitation of radionuclides from the leachate.

In the present study, the radionuclides were measured for the original rock samples (shale,

siltstone, and sandy dolomite) and their leachates (solution after leaching processes) by HP-Ge detector at three time intervals starting directly after leaching processes (zero time) to follow-up their radionuclides distribution. Siltstones are usually characterized by strong to very strong radioactivity due to their composition and can be more radioactive than shale. The high radioactivity for sandy dolomite may be due to the presence of phosphate or organic matter[13] Thus, the main aims of this study is to determine the concentration of dissolved ^{234}U, ^{238}U, ^{235}U isotopes and the ratios(^{238}U/^{235}U and ^{234}U/^{238}U) with their possible variations .

2. Samples Preparation

Different rock samples were collected from different sedimentary rocks, at west of WadiNaseib area, Southwestern Sinai, Egypt. 20 rock samples of each type were chosen, crushed and grounded to mesh 60 and then quartered to obtain representative sample of each rock type, they were packed in 200 ml marinelli beakers and sealed for measuring, after 28 days. These samples were prepared for gamma-ray spectrometric analyses by HP-Ge spectrometer.

Leaching processes were carried out on 100 g from each sample, using 30% H_2SO_4 and the leachates were separated (filtered) and packed in 200 ml marinelli and prepared for measuring the activity concentration (Bq/l) for different radionuclides

Gamma-ray Spectrometric Analysis

The gamma spectrometer was used to measure the activity concentrations for the different uranium isotopes in three different rock samples. In addition the behavior of the isotopes concentrations in the different leachates of these rocks was studied at different time intervals.

The work was carried out, using a closed-end coaxial gamma-ray detector (n-type) made of high purity germanium (HP-Ge) in a vertical configuration (Pop- Top cryostat configuration) cooled with liquid nitrogen. The used HP-Ge (EG&G Ortec Model GMX60P4) has a resolution of 1.10 keV at the 5.9 keV gamma transition of ^{55}Fe and 2.3 keV at the 1.33 MeV gamma transition of ^{60}Co. The detector has a photo-peak relative efficiency of about 60 % of the 3" × 3" NaI(Tl) crystal efficiency. The spectrometer has a peak-to-Compton ratio of about 56 to 1 at the 1.33 MeV peak transition of ^{60}Co. Energy calibration of the detector was performed, using standard point sources.

The efficiency calibration was performed by using threewell-known reference materials obtained from the International Atomic Energy Agency for U, Th and K activity measurements: RGK-1, RGU-1 and RGTH-1[14, 15]. The IAEA reference materials are similar to the examined rock samples Absolute efficiency calibration of the gamma spectrometry

system was carried out, using the radionuclide specific efficiency method in order to reduce the uncertainty in gamma-ray intensities, as well as the influence of coincidence summation and self-absorption effects of the emitting gamma photons [16]. The sample containers were placed on top of the detector for counting.

The ^{238}U concentration was determined by 63.3 keV and confirmed by 1001 KeV,then the same geometry and size were used for both the samples and the reference materials [17]. The different concentrations of each rock sample were measured for about one day. The first measurement of the leachates was considered at a time (t = 0) and was measured directly after the leachates were prepared Each leachate was measured for an accumulation time between 2 &3 days and the measurement was repeated at different time intervals to study the behavior of the isotopes concentration with time. An empty cylindrical plastic container (polyethylene marinelli beaker) was measured, for a counting period of 3 days in order to collect the background count rates.

Uranium-238 activity was determined indirectly from the gamma-rays emitted by its daughter products (^{234}Th and ^{234m}Pa) whose activities are determined from the 63.3 and 1001 keV photo-peaks, respectively [18]. The uranium-235 activity was determined directly by its gamma- ray peaks; 143.8, 163.4, 185.7, and 205.3 keV[19, 20, and 21]. The ^{234}U activity was determined directly from the gamma-rays emitted from this nuclide at energy of 53.2 keV[22, 23].

The specific activity of ^{40}K was measured directly by its own gamma-ray at 1460.8 keV. The specific activity of ^{214}Pb was measured, using the 295.2 keV and 351.9 keV, while the specific activity of ^{214}Bi was measured, using the 609.3 keV. The mean specific activity of ^{232}Th was measured, using decay products in secular equilibrium; the 338.4 keV&911.2 keV from ^{228}Ac and 583 keV& 2614.4 keV from ^{208}Tl.

3. Results and Discussions

The activity concentrations of the individual radionuclides were measured for three rock samples and at different time intervals starting directly after the leachates were prepared. The results are presented in Tables 1, 2 and 3. The activity concentration of ^{238}U for the leachates gradually increases from 1143±22.54 to 1971±23.34 Bq/l for siltstone, while that of shale it increases from 1079±21.42 to 1921±30.39 Bq/l and for sandy dolomite, it increases from 1506±30.45 to 2924±78.96Bq/l. The activity concentrations of ^{235}U for the different samples are nearly constant with time. The reason for the higher ^{234}U concentration value is due to alpha-recoil

process, which enhances the mobilization and solubility of the decay product (^{234}U) relative to the parent (^{238}U). After the alpha-particle has been emitted, the uranium is often stabilized in solution [24, 25].Fig. (1) indicates that there is an increase in the activity towards secular equilibrium with time for ^{238}U and^{234}U.

Table (4) indicates the solubility (%) of different radionuclides for rock types measured directly after the leachates were done at time (t = 0). It is clear that, the solubility of ^{235}U is greater than that of ^{234}U, ^{238}U and ^{226}Ra. Fig (2) illustrates these solubility variations in the radionuclides for the selected rock types. It is clear that the activity of ^{238}U (%) = [(^{238}U activity concentration in leachate / ^{238}U activity concentration in rock sample) *100] for different rock types. In leachates it increases with time towards equilibrium state due to growth of ^{234}Th (Table 5). The activity (%) in siltstone & shale is very close when compared to sandy dolomite. Solubility in sandy dolomite is much less than that of siltstone & shale in spite of the high activity in the original sandy dolomite sample (Fig.3).

^{234}U/^{238}U activity ratios in the original samples were 0.85±0.15, 0.94±0.16 & 1.03±0.01 for siltstone, shale and sandy dolomite respectively.The ratios for

first measurement of the leachates were considered at t = 0 for siltstone, shale and sandy dolomite samples as 1.09±0.17, 1.4±0.21, and 1.6±0.18 respectively. The enrichment in ^{234}U is accordingly related to the crystal damage and leaching, which are the main mechanisms for the ^{234}U/^{238}U disequilibrium [12, 26].

The activity ratio of ^{238}U/^{235}U is assumed to be nearly constant (21.7).This is because that the uranium was thought to be too heavy to undergo significant isotope fractionation. The ratios between ^{238}U and ^{235}U for siltstone, shale and sandy dolomite in leachate samples are shown in Table (6) and Fig. (4). The differences in these ratios were interpreted as a result of the preferential leaching, oxidation and reduction (redox) processes .This variation is related to the increase in the activity concentration of ^{238}U with time and very little variation in ^{235}U. In case of siltstone, activity reaches almost secular equilibrium for ^{234}Th in about two half –life times. This may be attributed to the fact that the solubility of ^{235}U is more than that of ^{238}U as shown in Table (4). In addition, it is higher in siltstone & shale than that of sandy dolomite rock. It is noticed in this table that, the Ra, Th and K solubility are in low percent (5.77%, 2.22 % &1.11%) respectively. This is expected that the major remain activities will may be in the residual.

Table (1): Activity concentrations (Bq/l) ofradionuclides for siltstone leachate at different time intervals

Radionuclides	Original sample	Zero Time	After21 days	After10 days	After7 days
238U	5373±63.14	1143±22.54	1643±35.95	1832±33.34	1971±23.34
235U	245±4.69	91±1.29	96±1.66	94±1.60	98±1.32
234U	4544±785.12	1235±194.17	1891±344.98	1804±316.78	2158±197.45
226Ra	6824±18.01	394±3.41	245±3.14	294±2.88	270±2.05
214Pb	4958±6.92	5.5±0.26	45±0.92	48±0.84	46±0.65
214Bi	5011±5.62	5.4±0.22	38±0.75	41±0.72	42±0.48
232Th	50±2.36	1.1±0.11	1.3±0.12	1.7±0.13	3.4±0.22
40K	315±6.12	3.5±0.19	3.7±0.27	3.6±0.22	N.D
238U/235U	21.9±0.42	12.6±0.31	17.1±0.48	19.5±0.49	20.1±0.36
234U/238U	0.85±0.15	1.09±0.17	1.15±0.21	0.98±0.17	1.09±0.10

Table (2): Activity concentrations (Bq/l) ofradionuclides for shale leachate at different timeintervals

Radionuclides	Original sample	Zero Time	After18 days	After11 days	After6 days
238U	5843±61.64	1079±21.42	1671±29.62	1868±22.65	1921±30.39
235U	268±0.66	97±1.24	97±1.39	98±1.30	98±1.85
234U	5496±916.58	1522±227.62	1537±372.74	1788±220.23	1920±259.88
226Ra	6297±14.34	487±3.71	515±4.12	547±2.94	540±3.88
214Pb	4891±20.86	22.6±0.46	173±1.66	231±1.30	236±1.74
214Bi	4976±27.64	22.6±0.46	161±1.27	207±1.01	213±1.34
232Th	35.8±1.27	1.7±0.16	1.1±0.10	1.5±0.09	2.3±0.19
40K	213±5.11	4.2±0.20	4.4±0.26	3.8±0.18	4.1±0.26
238U/235U	21.8±0.24	11.1±0.26	17.05±0.26	19.06±0.34	19.6±0.48
234U/238U	0.94±0.16	1.4±0.21	0.92±0.22	0.96±0.12	1±0.14

Table (3): Activity concentrations (Bq/l) ofradionuclides for sandy dolomite leachate at different time intervals

Radionuclides	Original sample	Zero Time	After 18 days	After9 days	After6 days
238U	20417±167.01	1506±30.45	2047±45.00	2796±44.45	2924±78.96
235U	941±13.09	172.8±2.13	157±2.46	168±2.85	167±3.65
234U	20998±2453.70	2489±273.56	2903±489.62	2659±392.21	3544±829.55
226Ra	47299±50.53	1064±6.57	713±5.95	1217±6.85	1421±11.15
214Pb	38503±18.23	71±0.88	352±2.97	455±2.64	494±4.78
214Bi	38913±14.66	61±0.88	351±2.33	429±2.17	511±4.39
232Th	22.2±2.06	1.2±0.11	0.06±0.01	0.25±0.03	0.41±0.08
40K	112.8±11.09	1.3±0.14	N.D	N.D	6.6±0.63
238U/235U	21.7±0.35	8.7±0.21	13±0.35	16.6±0.39	17.5±0.61
234U/238U	1.03±0.01	1.6±0.18	1.4±0.24	0.95±0.14	1.2±0.29

Table (4): Solubility (%) of radionuclides for rock types measured directly after theleachates was prepared at (t = 0)

Radionuclides	Siltstone (%)	Shale (%)	Sandy dolomite (%)
238U	21.27±0.49	18.47±0.42	7.37±0.16
235U	37.14±0.88	36.19±0.05	18.36±0.34
234U	27.18±6.35	27.69±6.20	11.85±1.90
226Ra	5.77±0.05	7.73±0.06	2.25±0.01
214Pb	0.111±0.01	0.46±0.01	0.184±0.00
214Bi	0.108±0.00	0.45±0.01	0.157±0.00
232Th	2.2±0.24	4.75±0.48	5.41±0.70
40K	1.11±0.06	1.97±0.11	1.15±0.17

Table (5): ^{238}U%of different rock types at different time intervals [(^{238}U% = ^{238}U activity concentration in leachate / ^{238}U activity concentration in rock sample)*100]

Accumulated time in days	siltstone	shale	sandy dolomite
0	21.27±0.49	18.47±0.42	7.37±0.01
21	30.58±0.76	28.6±0.59	10.02±0.02
32	34.1±0.02	31.97±0.51	13.69±0.02
39	36.68±0.61	32.88±0.63	14.32±0.04

Table (6): The activity ratio (^{238}U/^{235}U) for siltstone, shale and sandy dolomite of leachates at different periods

Accumulatedtime in days	^{238}U/^{235}U		
	Siltstone	Shale	Sandy dolomite
0	12.6±0.31	11.1±0.26	8.7±0.21
21	17.1±0.48	17.05±0.26	13±0.35
32	19.5±0.49	19.06±0.34	16.6±0.39
39	20.1±0.36	19.6±0.48	17.5±0.61
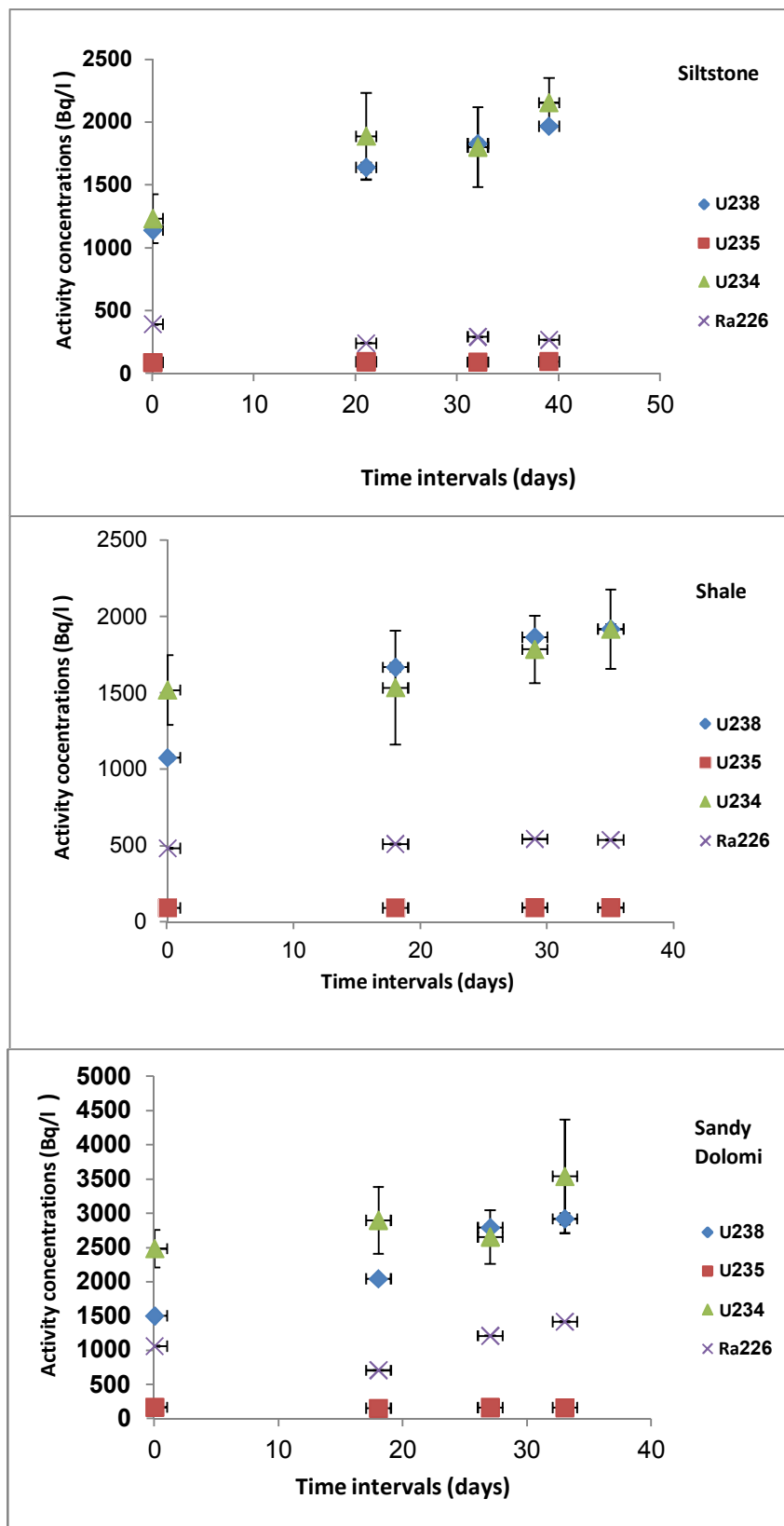
Fig. (1): The activity concentrations (Bq/l) for different rock samples at three timeintervals
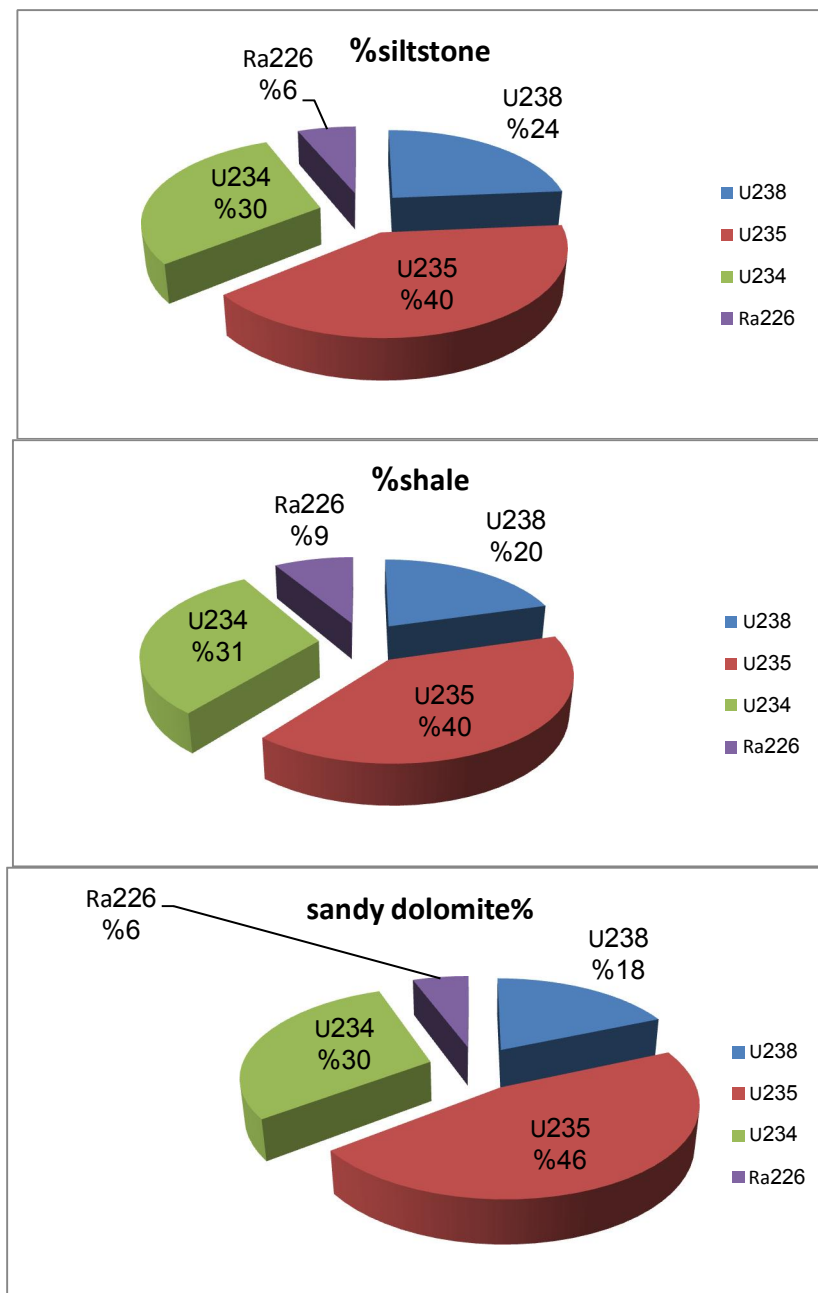
Fig. (2): The solubility (%) for different radionuclides of different rock types starting afterleaching process was prepared.
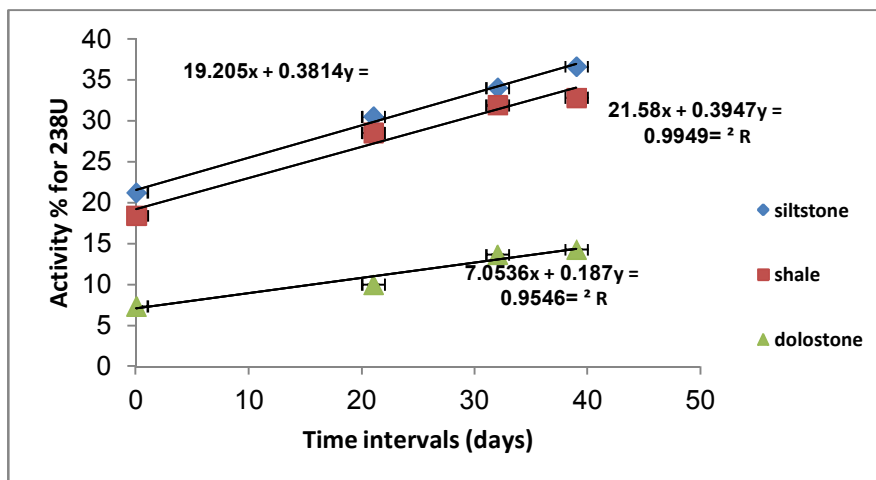
Fig. (3): Increasingofactivity (%) for ^{238}U with time for different rock types
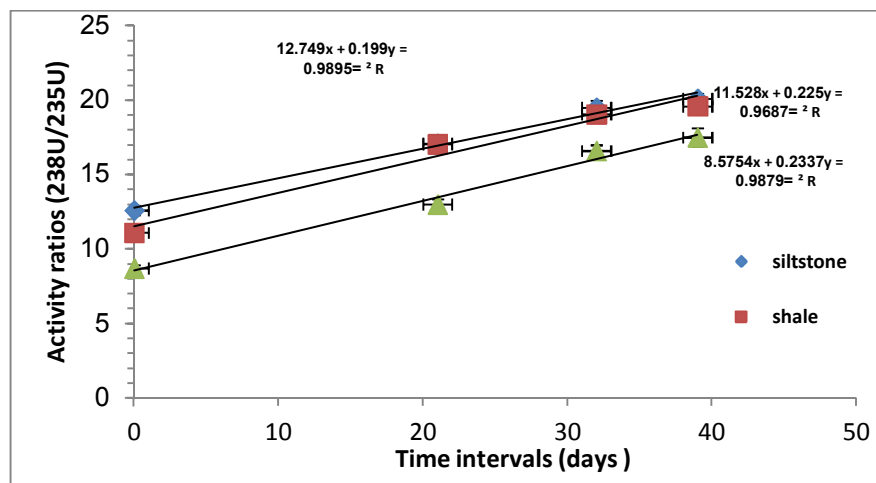
Fig (4): The activity ratio (^{238}U/^{235}U) for siltstone, shale and sandy dolomite at three time intervals

Conclusions

The radionuclides concentration was determined by gamma-spectrometry for three different rock samples and in their leachates at three time intervals to follow-up their distributions. The absolute efficiency calibration was performed by using threewell-known reference materials.

The activity concentrations for^{238}U and^{234}U increase towards secular equilibrium with time, while that of ^{235}U, it is nearly constant. The activity of ^{238}U (%) in siltstone & shale is very close when compared to sandy dolomite. Solubility in sandy dolomite is much less than that of siltstone & shale in spite of the high activity in the original sandy dolomite sample.

^{234}U/^{238}U ratios were obtained where most samples exhibited isotopic ratios are higher than one in leachates. This is related to the crystal damage and leaching, which are the main mechanisms for the ^{234}U/^{238}U disequilibrium. In this study, the deviation of ^{238}U/^{235}U activity ratio has been observed .This variation is related to the increase in the activity concentration of ^{238}U with time with very little variation in ^{235}U. In case of siltstone, activity reaches almost secular equilibrium for ^{234}Th in about two half –life times. Ra, Th and K solubility are in low percent. This is expected that the major remain activities are in the residual.

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9/1/2012

The use of different laboratory methods in diagnosis of *Helicobacter pylori* infection; a comparative study

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Abstract: Background: *Helicobacter pylori* are well recognized as a major cause of gastrointestinal illnesses and gastric cancers. Therefore, the current study aimed to assess different methods for detection of *H. pylori* in the oral cavity (saliva and dental plaque) and in gastric biopsy among patients with gastric affection, as well as, detection of *H. pylori* antigen in stool, moreover, to evaluate the antibiotic susceptibility testing of the isolated strains.
Methods: Specimens were obtained from Endoscopy Unit, Internal Medicine Department, Faculty of Medicine, Beni Suef University Hospital, Egypt. Thirty patients were subjected to detailed history and different sampling; gastric biopsy, oral and stool samples. The oral and gastric samples were processed and cultured. Thereafter, microscopic examination and rapid urease tests (RUTs) were conducted. *H. pylori* antigen detection was carried out in the stool samples, as well as, susceptibility testing to several antibiotics for all isolates identified.
Results: The selected patients had a mean age of 36.23 ± 6.317 years. They included 17 males (56.7%) and 13 females (43.3%). 90% of the cases were found positive by culture of the gastric biopsies, while, 96.7% were positive in oral cultures. 92.5% of the gastric samples showed positive results by microscopic examination, however, RUTs were positive in 63.3% of the gastric samples and in 73.3% of the oral samples, meanwhile, 66.7% of patients were found positive by testing their stool for *H. pylori* antigens. The prevalence of resistance among gastric and oral isolates to Amoxycillin, Amoxycillin/Clavulinic acid, Ampicillin/Sulbactam, Clarithromycin , Tetracycillin and Metronidazole were; (3.7 and 17.2 %), (11.1 and 24.1 %), (11.1 and 20.7 %), (11.1 and 24.1 %), (25.9 and 37.9 %) and (96.3 and 100 %) respectively.
Conclusion: There is an evidenced association between gastric affection and oral *H. pylori* recognition that, even exceeds stool detection of *H. pylori* antigen. Moreover, continuous evaluation of antibiotic susceptibility should be carried out and clinicians should be aware about it to select the appropriate empiric regimen for *H. pylori* eradication.

1. Introduction

Helicobacter pylori is a gram-negative bacterium that colonizes the gastric mucosa, and is associated with chronic gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma. Investigators found that chronic *H. pylori* infection is in approximately one-half of the world's population and is etiologically linked to 63% of all stomach cancer or approximately 5.5% of the global cancer burden, and approximately 25% of cancers associated with infectious etiology. They also suggested it would nearly double between the years 2005 and 2050 if no sufficient measures were taken to eradicate such infection *(Megraud et al., 1993, NIH Consensus Development Panel, 1994, Mbulaiteye et al., 2009 and Ford and Axon, 2010).*

Around the world, the prevalence of *H. pylori* infection ranges from 20% to over 90% in adult populations. Infection rates average at about 30% in Western populations *(Breckan et al., 2009 and*

Sykora et al., 2009), while, infection rates in Asian countries and in developing countries are higher and range from 60% to 90% *(Prinz et al., 2005).* Moreover, **Mohammad et al. 2008** found that, the overall prevalence of *H. pylori* infection was 72.38% among Egyptian school children.

Although *H. pylori* was first isolated nearly 30 years ago *(Marshall and Warren, 1983)*, the process of infection, reinfection or human transmission remains unclear and there are few reports of its prevalence in the oral cavity of individuals with periodontal disease and gastric diseases *(Riggio and Lennon, 1999).* Many researchers have assumed that the primary extragastric reservoir for *H. pylori* is the oral cavity and may be the source of infection and transmission. The first documentation of the presence of *H. pylori* in the oral cavity was reported in 1989, when the bacterium was cultured from the dental plaque of one of 29 patients with *H. pylori* associated gastric disease *(Krajden et al., 1989).* Since then, some

reports indicated that *Helicobacter* may be present in oral cavity which can serve as a reservoir for bacteria and a source of gastric reinfection. *H. pylori* have also been detected by culture and PCR in both dental plaques and saliva *(Veerman et al., 1997, Alm et al., 2000, Fritscher et al., 2004 and Sayed et al., 2011).*

Several diagnostic strategies for *H. pylori* are available. They can be divided into two groups: invasive and noninvasive tests. All invasive test methods are based on endoscopic examination during which biopsy specimens are obtained for direct (histological analysis, isolation) or indirect (urease test) diagnosis of *H. pylori* infection. Noninvasive methods reveal the presence of *H. pylori* by measuring the activity of urease (urea breath test), then by confirming the presence of antibodies in the serum. Meanwhile, other noninvasive tests were also evaluated in several studies including, detection of *H. pylori* antigens in stool and presence of *H. pylori* in the saliva *(Ozdemir et al., 2001, Viara et al., 2002 [a] and Viara et al., 2002 [b]).*

However, culture and identifying *H. pylori* in gastric biopsy, still, the most definite method for identification. Nevertheless, it requires experience, accuracy and dexterity, as identification and culturing may sometimes be difficult and tedious process. In addition, the erratic distribution of *H. pylori* could also lead to flawed results. Microscopy and RUT can be highly specific if strictly performed, but they are based on biopsy specimens and thus are theoretically prone to sampling error, as in the case of culture *(Yoshida et al., 1998).*

On the other hand*,* noninvasive tests for *H. pylori* are important in primary care, both for initial diagnosis of *H. pylori* infection and for confirmation of eradication. Current guidelines recommend noninvasive testing for diagnosis and treatment follow up of young dyspeptic patients without alarming symptoms as a primary care setting by using low-cost noninvasive tests *(Vaira et al., 2002 [a])*, including oral cavity bacterial isolation and identification. Therefore, determining the type of strain prevalent in the oral cavity or saliva, it could be easy to diagnose the strain colonizing the gastric mucosa as reported by *Li et al., 1996* who demonstrated that same strain was present in both niches. Consequently, saliva specimens may potentially be reliable, and could serve as an effective and valuable noninvasive specimen to diagnose and monitor the efficacy of eradication therapy *(Tiwari et al., 2005 and Sayed et al., 2011)***.**

Resistant strains of *H. pylori* are now widely prevalent in the United States and Europe, and eradication therapy with current regimens fails in 10% to 20% of patients *(Vakil et al., 1998 and Megraud et al., 2012)*. *In vitro* antibiotic sensitivity for *H. pylori* should be done to guide the selection of antibiotic and to predict the clinical response to treatment aiming at successful eradication *(Hunt et al., 2000)*.

Therefore, the current study aimed to assess different methods of detection of *H. pylori* in saliva and dental plaque of the oral cavity and in gastric biopsy among Egyptian patients with gastric affection, as well as, detection of *H. pylori* antigen in stool. Moreover, the investigation also evaluated the antibiotic susceptibility testing of the isolated strains in the present study.

2. Subjects and Methods

In the present study, specimens were obtained from Endoscopy Unit, Internal Medicine Department, Faculty of Medicine, Beni Suef University Hospital, Egypt, during the period from December 2008 to April 2009. Detailed personal history (including age, sex, occupation, smoking, etc.), patients complaints, family history, past history (especially any previous gastric complaint) as well as blood group type were recorded for each patient.

1- Selection criteria

The selected group of this study consisted of 30 patients referred for gastroscopy with upper GIT symptoms at the endoscopy unit. All subjects were more than 21 years of age and had received no previous treatment for ulcer or gastritis. Individuals who had received antibiotic treatment in the last 2 months, with history of alcohol abuse, having chronic debilitating disease, pregnant and lactating females were excluded from the study. The individuals who were included in the study showed good oral hygiene, although small amounts of supra-gingival plaque could be visually detected at the time of sampling, and proven to be with gastric affection in the form of ulcer, erosion or gastritis shown by gastroscopy. All individuals signed an informed consent in order to be included in the study group. Scientific Research Ethical Committee of Beni Suef Hospital approved the study. Control group of 20 (13 males and 7 females) individuals were included in the present study. They were free from any upper GIT symptoms, did not take any treatment for gastritis or ulcer before and had no antibiotics for the previous 2 months.

2-Sampling:

a- Oral specimens (saliva and dental plaque) were collected from each volunteer before undergoing an upper gastric endoscopy. The samples were collected from the patients and the control group, in sterile containers for performing culture for *H. pylori*. The dental plaque sampling was conducted using cotton swab and placed in a tube containing the saliva collected and treated as a single specimen. Unstimulated salivary flow was collected in amount of 1-2 ml in long tubes that can accommodate the dental plaque swabs *(Tiwari et al., 2005)***.** After centrifugation

(14,000 x g) for 10 min, the supernatant was transferred into a fresh microtube and stored at 70ºC for future analysis *(Kignel et al., 2005).*

b- Gastric biopsy: two biopsies of the gastric antrum were collected, during upper gastric endoscopy, under complete aseptic condition in 2 sterile cups sized 2x2 cm^3 containing 1 ml of sterile saline *(Bayerdorffer et al., 1989).* One specimen was used for doing rapid urease test (RUT) and the other was used for direct Gram staining and culture of *H. pylori*. During endoscopic examination, patients were evaluated for the presence of gastritis, gastric ulcers or erosion to undergo the current investigation.

c- Stool samples were collected for performing stool antigen of *H. pylori* from the selected group as well as the control group.

3- Microbiological examinations:

The microbiological part of this work was performed at the Medical Microbiology & Immunology Department, Faculty of Medicine, Beni Suef University.

a- Rapid urease test (RUT):

This test was performed directly on the unprocessed gastric biopsies and oral samples, using urea soft agar tube, by dipping the sample into the soft agar. The agar was inspected at 30 minutes, 1, 2 and 24 hours for a colour change from yellow to pink indicating a positive result *(Logan and Walker, 2001)*.

b- Stool antigen detection:

This was done for stool samples using "The One Step *H. pylori* Antigen Test Device (feces)", which is a qualitative test using immunoassay for detection of *H. pylori* antigens in human feces specimens providing results in 10 minutes.

c- Gastric biopsy processing:

Processing was done for biopsy specimens used for culture of *H. pylori*. A sterile automated mechanical homogenizer (house developed) with a speed of about 60-100 run/minute was used for gastric biopsy specimens processing for 2-3 minutes to mince the biopsy into very small pieces. This was followed by centrifugation at 6000 x g for 20 minutes *(Logan and Walker, 2001)*.

i. **Supernatants from the homogenized tissues**: were used for direct Gram stain

ii. **Deposits from the homogenized tissues:** were used for culture of *H. pylori*, freshly prepared blood agar plates (containing citrated sheep blood and made selective by addition of Dent supplement ‹Oxoid; Basingstoke, Hampshire, England›) were used to perform the culture of *H. pylori* and were incubated at 37ºC for 3-7 days under microaerophilic conditions. Colonies were identified as *H. pylori* by its colonial morphology, typical appearance on Gram stain as well as catalase, oxidase and urease tests.

iii- **Cultures were performed on saliva and dental plaque specimens:** Culture and identification of *H. pylori* were performed as mentioned above.

iv- **Antimicrobial susceptibility testing for *H. pylori:*** Antibiotic sensitivity of the isolates was determined using the Kirby-Bauer antibiotic testing (KB testing or disk diffusion method) as recommended by the Clinical and Laboratory Standards Institute (CLSI, previously called NCCLS) (2006 guidelines). In this method, a microbial suspension equal to 4 McFarland turbidity (12×10^8 CFU/ml) was prepared and cultivated on Muller-Hinton agar (Merck, Germany) supplemented with 10% sheep blood *(Mishara et al., 2006)* , using the following antibiotics; Amoxycillin (AML), Amoxycillin/Clavulinic acid (AMC), Unasyn (Ampicillin/Sulbactam) (SAM), Clarithromycin (CLR), Tetracyclin (TE) and Metronidazole (MTZ).

4- Statistical analysis:

Data were collected and analyzed statistically using Statistical Package for Social Sciences program (SPSS v16). The following tests were used in this study: mean, standard deviation, T test for independent samples, ANOVA test (analysis of variance). Significance levels: $P>0.05$ insignificant, $P<0.05$ significant and $P<0.001$ highly significant.

3. Results

The current investigation included 30 patients their ages ranged between 20 and 60 years, with mean age of 36.23 ± 6.317 years. The highest prevalence of *H. pylori* infection was noted among 31-40 Yrs age group as shown in table 1. The tested cases included 17 males (56.7%) and 13 females (43.3%). Moreover, 20 volunteers were enrolled as the control group, their ages ranged between 21 and 68 with mean age of 41.1 ± 13.1 Yrs, including 13 (65%) males and 7 (35%) females.

Table (1): Comparison between different age groups among the studied cases:

Age group (Yrs)	No	Percent
21-30	7	23.3
31-40	16	53.3
41-50	6	20
51-60	1	3.3
Total	30	100

Risk factors were assessed in the current study among the 30 patients with gastric affection including blood groups, smoking habit and occupational stress. All patients had occupational stresses; however, 50% of them were smokers. Regarding blood groups, 50% of the tested group was from the O group type, followed by A group

(26.7%) while, the B group patients represented 23.3% and none were from AB group blood type (Figure 1).
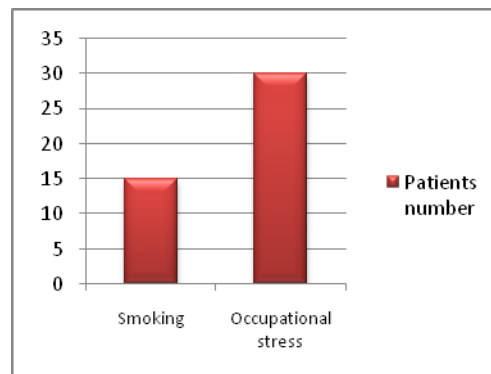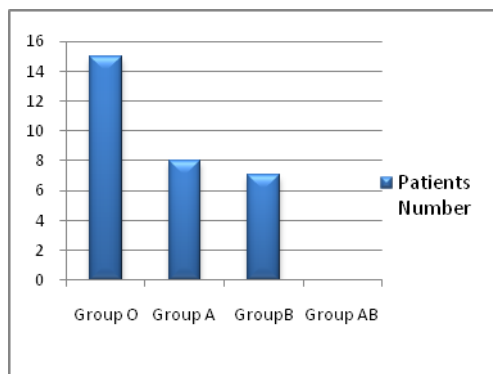
Figure 1: The distribution of risk factors among the tested group.

Out of the 30 patients examined, 27 (90%) were found positive by culture of the gastric biopsy. On the other hand, oral cultures were positive in 29 cases representing 96.7%. The correlation between the culture results is shown in table 2.

On comparing the culture results of the oral samples with the control group, it showed high significance ($p \leq 0.001$); as the cultures of the oral samples of the control group were positive in only 40% of the specimens (Figure 4). Specificity and sensitivity of the oral culture results compared with gastric cultures were 50% and 96.4% respectively.

Table (2): Results of culture of *H. pylori* in different specimens:

Oral specimens' culture	Gastric biopsies' culture		Total
	Positive	Negative	
Positive	26 (96.3%)	3 (100%)	29(96.7%)
Negative	1 (3.7%)	0 (0%)	1 (3.3%)
Total	27 (90%)	3 (10%)	30 (100%)

Direct microscopic examination was performed on the gastric biopsies showing positive results in 25/ 27 culture positive specimens representing 92.5%. Specificity and sensitivity of

the results compared with gastric culture were 100% and 93.1% respectively.

Rapid urease test was performed on the gastric biopsy samples as well as oral specimens; it was found positive in 63.3% of the gastric samples and in 73.3% of the oral samples, meanwhile, represented 70.3% out of the 27 positive gastric cultures, and 75.8% out the positive 29 dental and saliva samples (table 3, Figure 2). The control group showed positive results of RUT in their saliva and dental samples in only 40% of the volunteers, which, when compared with the cases showed evident statistical significance representing $p \leq 0.01$ (Figure 4). Specificity and sensitivity of the RUT of gastric biopsies results compared with gastric culture were 100% and 77.1% respectively, nevertheless, RUT of oral samples when compared with oral culture, they showed specificity and sensitivity of 100% and 80.5% respectively.

Table (3): Results of Rapid Urease Test (RUT):

RUT of oral Specimens	Gastric biopsy RUT		Total
	Positive	Negative	
Positive	15 (78.9%)	7 (63.6%)	22(73.3%)
Negative	4 (21.1%)	4 (36.4%)	8 (26.7%)
Total	19 (63.3%)	11(36.7)	30 (100%)

Figure 2: The results of RUTs *versus* culture results of gastric and oral samples.

The *H .pylori* isolates from gastric and oral samples were subjected to oxidase, urease and catalase tests. Among the isolates from gastric biopsies oxidase test was found positive in 25/27 (92.6%) gastric isolates, while, 28/ 29 (96.7%) oral yields were found positive by the test.

Urease test showed positive results in all gastric yields; however, it only gave positive results in 26 /29 (89.7%) isolates taken from oral samples. In addition, catalase test was found reactive in 23/27 (85.2%) gastric isolates, similarly in 25/29 (86.2%) oral *H .pylori* isolates (Figure 3).
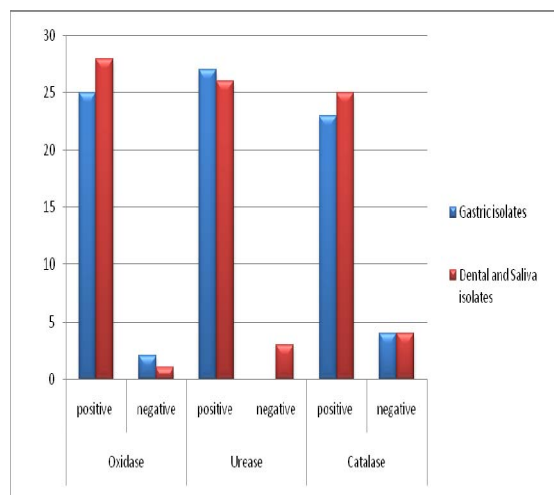
Figure 3: The results of biochemical reactions among the tested isolates.

Twenty patients were found positive by testing their stool for *H. pylori* antigens representing 66.7%. On comparing the cases with the control group it showed high significance ($p \leq 0.001$) (Figure 4). The correlation between the stool antigen results and gastric culture is shown in Table 4. Specificity and sensitivity of the stool antigen compared with gastric culture were 60% and 71% respectively.

Table (4): Results of stool antigen detection compared with gastric biopsy culture:

Gastric biopsy results	Results of stool antigen		Total
	positive	Negative	
Positive	18 (90%)	9 (90%)	27 (90%)
Negative	2 (10%)	1(10%)	3 (10%)
Total	20 (**66.7**)	10 (33.3)	30 (100%)
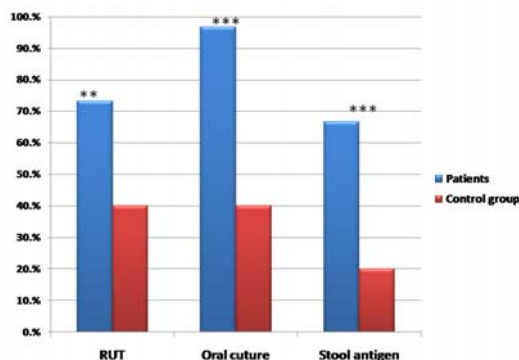
Figure 4: The correlation between the positive results of patients' specimens and the control group samples. The values are expressed in % of positive results. ***P < 0.001**P < 0.01.

All the isolates from gastric biopsy and oral specimens were subjected to antibiotic sensitivity testing on Muller Hinton sheep blood agar to the following antibiotics: amoxycillin, amoxycillin clavulinic, ampicillin salbactam, clarithromycin, tetracycline and metronidazole.

The isolated strains of gastric biopsy samples showed high sensitivity to amoxicillin followed by clatithromycin, while, showed least sensitivity to metronidazole (3.7%).
Meanwhile, the oral isolates showed the highest sensitivity to amoxicillin, amoxicillin clavulinic and clatithromycin. The strains were completely resistant to metronidazole (Table 5, Figure 5). Antimicrobial resistance was not statistically significantly associated with sex or age ($p >0.05$).

Table (5): Antibiotic susceptibility patterns of gastric and oral isolates:

	Gastric strains (Isolates number=27)						Oral strains (Isolates number=29)					
	Sensitive		Resistant		Intermediate		Sensitive		Resistant		Intermediate	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
AML	26	96.3	1	3.7	0	0	24	82.8	5	17.2	0	0
AMC	15	55.6	3	11.1	9	33.3	15	51.7	7	24.1	7	24.2
SAM	14	51.9	3	11.1	10	37	11	37.9	6	20.7	12	41.4
CLR	16	59.3	3	11.1	8	29.6	13	44.8	7	24.1	9	31.1
TE	13	48.2	7	25.9	7	25.9	11	37.9	11	37.9	7	24.2
MET	1	3.7	26	96.3	0	0	0	0	29	100	0	0

AML: Amoxycillin, AMC: Amoxycillin clavulinic, SAM: Ampicillin salbactam, CLR: Clarithromycin, TE: Tetracycline, MET: Metronidazole.

A.   B.
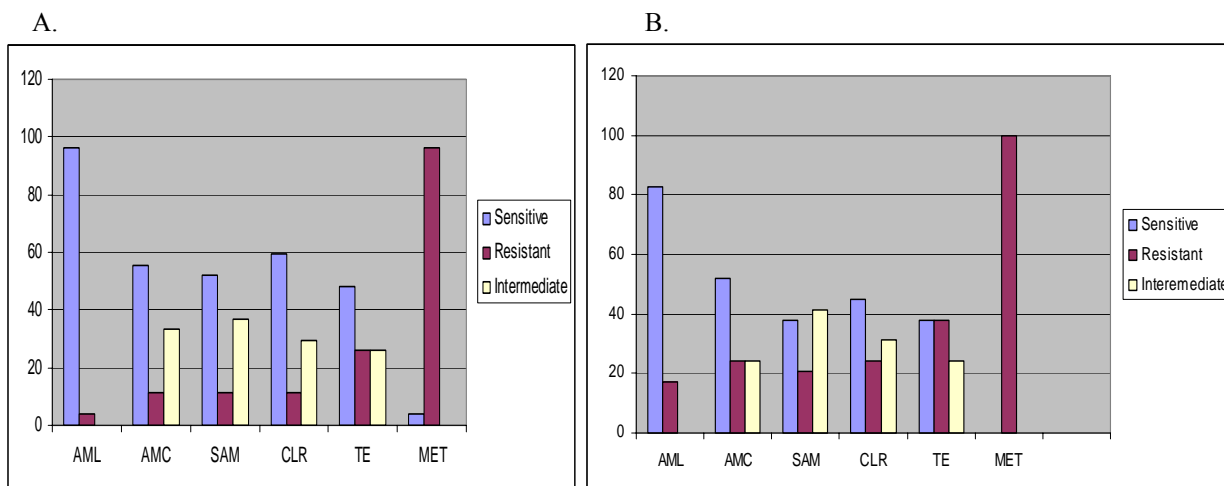
Figure (5): A. Antibiotic sensitivity pattern of the gastric isolates, **B.** antibiotic sensitivity pattern of the oral strains

On comparing the oral and gastric isolates, 17 (65.3%) showed good matching regarding their colony morphology, biochemical reactions and antibiogram. However, 5 (19.2%) yields showed minor differences in 2 or 3 of the tested antibiotics, meanwhile, 4 (15.3%) of them were completely different in 4 or more of the tested reactions, antibiogram and/ or colony morphological characters.

4. Discussion

H. pylori have been designated as key organisms in the etiology of chronic gastritis, peptic ulcers *(Megraud, 1993)* and gastric cancer *(NIH Consensus Development Panel, 1994 and Mbulaiteye et al., 2009)* and their suppression and elimination has been considered the gold standard therapy for infectious gastric diseases.

The current study included 30 patients with upper GIT symptoms proven to be with gastric affection in the form of ulcer, erosion or gastritis shown by gastroscopy, subjected to Outpatient Clinic of Internal Medicine and Endoscopy Unit, Faculty of Medicine, Beni Suef University.

Ages of the study participants ranged between 20 and 60 years, with mean age of 36.23 ± 6.317 years. There was association between 31-40 Yrs age group and infection density, though it did not reach statistical significance (Table 1). These results were comparable to European studies which reported correlation between age and gastric affection *(Breckan et al., 2009 and Jackson et al., 2009)*; however, these studies found increased prevalence of gastritis and *H. pylori* colonization with increasing age.

A higher level of gastric affection and *H. pylori* isolation was observed among male patients (56.7%) rather than females (43.3%). This may be due to the poor oral hygienic conditions and tobacco smoking. This was in agreement with *Lindella et al., 1991* who stated that there is an epidemiological evidence of an association between cigarette smoking and gastritis. Our results also agreed with those reported by *Khulusi et al. 1995* who assessed the same risk factors (sex and smoking), nevertheless, many studies contradicted these findings and found no such relation *(Roma et al., 2009 and Zhang et al., 2009).*

In the present study, 50% of the tested group was from the O blood group, followed by A group (26.7%) while, the B group patients represented 23.3%. None of the patients were from the AB group (Figure 1). Though the highest prevalence was among the O group patients, it did not reach statistical significance among the patients or in relation to *H. pylori* infection. The same finding was reported by *Demir et al., 2009 and Petrovic et al., 2011* who stated that different ABO blood groups may show insignificant rates in the colonization numbers of the bacteria (p value > 0.05). However, this contradicted with *Bhuiyan et al., 2009* who stated that children with blood group "A" were more susceptible to *H. pylori* infection than those with other ABO blood groups. In the current study, the cause of higher incidence among the O group patients were not clear and the etiology of gastric affection is unknown, nevertheless, some studies have justified these findings by similarities between blood cell surface proteins and surface proteins on bacteria that live in the intestines of normal humans. Patients with blood type O have antibodies against A and B antigens, so, they have antibodies against these surface proteins. *Helicobacter* and other bacteria in the gut have type A and B surface proteins. Therefore, antibodies that react strongly with *Helicobacter* cause more swelling and inflammation, more stomach pain, more belching and more ulcers *(Araya et al., 2000 and Alkout et al., 2000).*

It was observed in our study that psychological, occupational stress and smoking were considered as a risk factor of peptic diseases caused by *H. pylori* infection. This was consistent with the findings of *Levenstein, 1998* who reported that psychological

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stress and smoking probably functions most often as a cofactor with *H. pylori*. In addition, four studies conducted in European populations among adults in the United Kingdom revealed that, male gender, increasing age, tobacco use and lower socioeconomic status were all significantly associated with positive *H. pylori* colonization (*Jackson et al., 2009*). Therefore, *H. pylori* may be inadequate as a sole explanation for peptic ulcers.

Different diagnostic measures were performed in the current investigation and compared with the mostly used method for diagnosis; culture of gastric antral biopsy on selective media. They included RUT, microscopic examination, and culture of oral (dental plaque, saliva) samples, biochemical reactions for the positive isolates as well as stool antigen detection.

The present findings showed high positivity rate of *H. pylori* colonization in both gastric and oral samples in the examined group, in a percentage of 90% and 96.7% respectively (Table 2). On comparing the culture results of the oral samples with the control group, it showed high significance ($p \leq 0.001$) (Figure 4). Specificity and sensitivity of the oral results compared with gastric culture were 50% and 96.4% respectively. These results may justify the question raised by many studies, whether the oral cavity is a reservoir for gastric *H. pylori* infection or not (*Loste et al., 2006 and Bürgers et al., 2008*). Our results were comparable to those of another study done in Egypt as well; it revealed 100% prevalence in patients with gastric affection and showed specificity and sensitivity of 50% and 100% respectively (*Sayed et al., 2011*). This same study found also high significance when compared patients with gastric pathology with another group with no gastric affection ($p \leq 0.0017$). However, many other studies showed much lower rates of *H. pylori* colonization among dyspeptic patients, patients with gastric pathology and even among the control group with no gastric complaints (*Souto and Colombo, 2008 and Silva et al., 2009*).

These contradictions in the current findings from other investigations may be explained by local environmental factors which influence the establishment and composition of the plaque needed for microbial adhesion to tooth surfaces. Environmental conditions are not uniform and the microbial composition at the site depends on the outcome of a variety of host-microbial and microbial-microbial interactions (*Scheie, 1994*).

The higher prevalence of oral *H. pylori* in rural community, such as presented in the current investigation, may be explained by the characteristics of the patients investigated, little access to standard medical and dental care, close contact, overcrowding, poor sanitation, low level of education and low level of socio-economic status (*Al Asqah et al., 2009*) or may be due to iatrogenic

transmission during the poor dental care service (*Madinier et al., 1997*).

For patients undergoing upper gastrointestinal endoscopy, RUT is considered to be a cheap and a reliable test that allows screening of *H .pylori* infection (*Perri, 2003*). In the present study, 19 isolates (70.3%) out of the 27 positive gastric yields were found reactive by RUT, meanwhile, it was positive in 22 (75.8%) out the positive 29 dental and saliva samples (Table 3, Figure 2). On comparing the culture results with the control group, it was statistically significant ($p \leq 0.01$) (Figure 4). Specificity and sensitivity of the RUT of gastric biopsies results compared with gastric culture were 100% and 77.1% respectively, nevertheless, RUT of oral samples when compared with the oral culture, they showed specificity and sensitivity of 100% and 80.5% respectively. These results were comparable to those of *Pajares et al.(1998)* who found that the sensitivity and specificity of the rapid urease test ranged between 80 and 90%. Negative RUTs in our study may be explained that patients were in acute phase of ulcer bleeding (*Schilling et al., 2003*), or patients were on acid reducing drugs since recent use of the latter can cause false negative results. RUTs should be carried out 4 weeks after the completion of therapy (*Yakoob et al., 2008).

In the present study, *H. pylori* yields from gastric biopsies as well as oral samples had undergone several biochemical reactions; urease, oxidase and catalase tests. The results of oxidase test were positive in 92.6% of the gastric biopsy samples and in 96.7% of oral samples. While, urease test were 100% positive in gastric biopsy samples and 89.7% in oral samples. However, catalase test was 85.2% positive in gastric biopsy samples and 86.2% in the oral samples (Figure 3).

The results of "The One Step *H.pylori* Antigen Test Device (Feces)" revealed that 20 out of the 30 patients were found positive by testing their stool for *H. pylori* antigens, representing 66.7%. On comparing the cases with the control group it showed high significance ($p \leq 0.001$) (Figure 4). Specificity and sensitivity of the stool antigen compared with gastric culture were 60% and 71% respectively. This was different from the findings of *Forné et al.(2000*), *Manes et al.(2001)and Krausse et al.(2008)* who stated that *H. pylori* stool antigen represents a sensitive test and suitable for detecting *H. pylori* infection with sensitivity similar to that obtained with other standard tests. Also these results were different from those of *Vaira et al., 2002 [b]* who reported that the stool antigen detection of *H. pylori* had a sensitivity of 94% and a specificity of 97% before and after treatment. However, *Gulcan et al., 2005* found similar results with a percentage of 61% of the selected group; nevertheless, the specificity and sensitivity were higher than the current findings. These discrepancies in the results may be due to the variability in the tests used and/or

following certain antibiotic treatments that the patients did not reveal, which may cause the level of *H. pylori* antigens to decrease to the concentration below the minimum detection level of the test *(Soll. 1990, Anand et al.1996 and Cutler. 1996).*

Treatment of *H. pylori* infection is becoming a very relevant problem especially in the developing countries. Although different therapeutic regimens are currently available, treatment failure remains a growing problem in daily medical practice. Several factors could play a role in the eradication failure, but the most relevant are antibiotic resistance and patient's compliance*.* In our study, it was found that *H. pylori* strains were almost completely resistant to metronidazole in both gastric biopsy and oral isolates. This came in accordance with *Torres et al. (2001)* who reported that resistance to mertonidazole is even more prevalent in the developing countries, where up to 95% of isolates may be resistant. Resistance to metronidazole may be explained by the frequent use of nitonidazole derivatives (metronidazole, ornidazole) in treatment of amebiasis and giardia infections which renders the bacteria resistant against the drugs *(Zwet et al 1994).* Regardless of the reason, it is now clear that metronidazole should not be included in treatment regimens for *H. pylori* in Egypt *(Sherif et al., 2004).*

In the present study, the resistant rate was the least among isolates treated with amoxicillin among gastric yields (3.7%), while, it was higher among oral strains (17.2%). Whereas *Kato (2002)* reported 0% resistance to amoxicillin, higher resistance rates were reported in a study done in Iran (11.6 %) *(Milani et al., 2012).* On the other hand, resistance to clarithromycin was found to be 11.1% in gastric biopsy samples and 24.1% in oral samples, these findings agreed with the result reported by *Fijen (2003)* who stated 9.1% resistance rate among subjects of African descent. Resistance to clarithromycin is becoming more prevalent in some European countries, where the prevalence may be as high as 17% *(Romano and Cuomo, 2004 and Megraud et al., 2012)*.

Moreover, it was found that *H. pylori* were less susceptible to tetracycline, about 48.2% sensitivity rate in gastric biopsy samples and 44.8% in oral samples. This susceptibility to tetracycline was in a disagreement with *Kim et al (2003)* findings. They reported that *H. pylori* resistance to tetracycline was only 6.8% of the isolated strains. However, higher resistance rates have been reported in other studies from Iran *(Milani et al., 2012).* Interestingly, in the work of *Van der Wouden et al., 1999* isolates exhibited cross-resistance to metronidazole, and the cross-resistance could be transferred to tetracycline-susceptible *H. pylori* strains, which may be comparable with our findings*.*

Amoxicillin clavulinic and ampicillin salbactam showed moderate sensitivity rates among the isolates with better susceptibility among the gastric isolates rather than oral yields in the following order; 55.6, 51.9% in gastric strains and 51.7, 37.9% in oral isolates. Antimicrobial resistance showed no statistical significance in association with certain sex or age groups, which agreed with the work of *Milani et al., 2012* who concluded the same results in their study.

Many characteristics were different between the gastric and oral isolates regarding colony morphology, biochemical reaction and it was even clearer in their antibiotic susceptibility patterns. This may be explained by the environmental inconsistency between the oral and gastric habitats and colonization and biofilm formation. This variability may affect the genetic presentation of the bacteria or their gene expression as suggested by many studies *(Cole et al., 2004, Pattiyathanee et al., 2009 and Andersen and Rasmussen, 2009).*

5. Conclusion and Recommendations

In conclusion, there is no one non invasive test enough to diagnose *H. pylori* infection and subsequently follow up of treatment outcomes, nevertheless, saliva and dental plaque culture propose a good prediction tool associated with gastric affection caused by *H. pylori* infection, with fairly good specificity and sensitivity, that was found better than that of stool antigen detection in the current study. Moreover, appropriate eradication of *H. pylori* is necessary as many gastric cancers could be prevented by elimination of *H. pylori.* Antibiotic susceptibility patterns vary widely in different time and place, therefore, continuous evaluation should be carried out and clinicians should be aware about it to select the appropriate empiric regimen for *H. pylori* therapy.

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8/9/2012

New Binary PSO based Method for finding best thresholds in association rule mining

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Abstract: One of the important data mining techniques is association rule finding. Apriori is the most famous algorithm based on this technique. But it has a major weakness which cannot calculate the minimal value of support and confidence and these parameters is estimating intuitively by the user and this has an important effect on the algorithm performance. Main goal of this paper is to presenting an optimal method to find suitable values of minimum threshold for support and confidence by means of Binary Particle Swarm Optimization. Data used for the paper is a 4000 random records sample from Foodmart 2000 Database. Implementation of the proposed method has been done using R2010b version of MATLAB software. Proposed algorithm improves the performance of association rule mining by automatically setting suitable values for minimum support and confidence thresholds..
[Abdoljabbar Asadi, Mehdi Afzali ,Azad Shojaei, Sadegh Sulaimani , **New Binary PSO based Method for finding best thresholds in association rule mining.** *Life Sci J* 2012;9(4):260-264] (ISSN:1097-8135). http://www.lifesciencesite.com. 37

Keywords: data mining, association rule mining, minimal support, minimal confidence, particle swarm optimization

1. Introduction

Regard to information technology development many databases have been developed to store the related data. Analyses of these databases to mine hidden rules have incremental importance [1]. One of the noticeable techniques help managers to make good decisions is data mining. This technique makes great tools available for users during current decay to extract meaningful information and useful patterns from databases [2], and this knowledge should be exact, readable and easy to understand [3]. But in spite of vast area of applications for data mining it still needs some manual operations, not automatic, to complete [3]. One of the important data mining techniques is association rule finding. It can extract hidden rules and dependent properties which have important role in decision making [1]. Apriorri Algorithm is the most famous one to extract association rule mining. But this algorithm has a major weakness which cannot calculate the minimal value of support and confidence and these parameters is estimating intuitively [3]. There are several algorithms to improve performance and accuracy of Apriorri. In traditional algorithms of association rule mining both of support and confidence parameters minimal value is chosen by the user try and error and this has an important effect on algorithm performance [1]. This approach can also produce many rules in a large database, millions, which probably many of them are not useful; it can be implied that it doesn't have enough efficiency [20]. So we need a method to find best values of support and confidence parameters automatically specially in large databases. Main goal of this paper is to presenting an optimal method to find suitable values of minimum threshold for support and confidence efficiently. This aim is achieved using particle swarm optimization (PSO) algorithm. PSO as an optimization method [5, 6] can be used for optimization of association rule mining [4]. A special type of PSO, named Binary PSO, is used for our work regard to its efficiency for local and large interval domains [21].

2. Literature Review

Because of long runtime of Apriori algorithm to find association rules, its operational efficiency has a considerable importance. Several papers have presented different association rule mining algorithms to improve Apriori algorithm. Savasere et al. [8] developed Partition Algorithm for association rule discovery which is basically different form classic algorithm. This algorithm first scans the database to find strong item sets. Then support value is calculated for all item sets. Validity hint of Partition algorithm is that any strong item set appear in a section at least one time. Park et al. [22] introduced DHP at 1995. DHP is a derivative of Apriori plus some extra controls. It uses hash table to restrict candidates. DHP has two main properties: effective make of item sets and efficient reduction of database size by dropping adverse attributes. Toivonen et al. [23] presented sampling algorithm at 1996. This algorithm is about finding association

rules according to reduce database operations. DIC algorithm by Brin et al. at 1997 [9] splits database into some parts called start point. It determines support value for item sets belong to each start point and so extracts the patterns and rules. Bender search algorithm [10] by Lin et al. developed at 1998 can discover rules from most frequent itemsets. Yang et al. offered an efficient hash based method named HMFS which combines DHP and Bender search algorithms results in reduction of database scan and filtering repeated itemsets to find greatest repeated itemset [11]. This can shorten overall computation time for finding greatest repeated itemset. Genetics algorithm has been applied to association rule mining during recent years. [12] Utilizes weighted items to distinguish unique itemsets. Value of different rules is determined using weighted items in fitness function. This algorithm can find suitable threshold value for association rule mining. Saggar [13] et al. presented a method for optimizing extracted rules, using genetics algorithm. The importance of the work is that it can predict rules with negative value.

Kuo et al. [1] in 2011 developed a PSO based method for automatic finding threshold value of minimal support. Their work shows that basic PSO can find values faster and better than genetics algorithm. Gupta [3] also offered a method at 2011 for automatic finding of threshold value using weighted PSO. His results show high efficiency of PSO for associative rule mining. This approach also can gain better values of threshold in comparison with previous ones.

3. Basics

3.1 Association rule mining

Agrawal et al. raised associative rule mining idea at 1993 [14]. A positive association rule presented as if $A \rightarrow B$ which A and B are subsets of *itemset(I)* and each itemset includes all of the items $\{i_1, i_2, ..., i_n\}$; It can be shown that in database $D = \{T1, T2, . . ., Tk\}$ a customer buys B product after buying A one if $A \cap B \neq \emptyset$. Association rule mining should be based on the following two parameters:

1. Minimum support: finding item sets with the value above threshold

$$Support(A \rightarrow B) = P(A \cup B) = \frac{A \cup B}{D} \quad (1)$$

2. Minimum Confidence: finding item sets with the value above threshold

$$Confidence(A \rightarrow B) = p(B|A) = \frac{A \cup B}{A} \quad (2)$$

Better rules have greater support and confidence value. Most famous algorithm for association rule mining is Apriori, offered by Agrawal et al. It repeatedly determines candidate itemsets using minimal support and confidence to filter itemsets for finding repeated ones with more frequency [1].

3.2 Particle Swarm Optimization Algorithm

PSO algorithm first developed at 1995 by James Kennedy, Russell C. Eberhart. It uses a simple mechanism inspiring from simultaneous motion of birds and fishes fly and their social life. This algorithm has successful applications recent years [5, 6]; mainly neural network weighting and control systems and everywhere that genetic algorithms can be use. PSO is not only a tool for optimization but also a tool for human social recognition representation. Some scientists believe that knowledge will optimize in effect of mutual social behaviors and thinking is not only a private action, indeed it is a social one. There are some entities in search space of the function which we are going to optimize it, namely particles [15]. PSO as an optimization algorithm provides a population based search which every particle change its position according to the time. Kendy in 1998 represented that each particle can be a possible answer that can move randomly in problem search space. Position change of each particle in search space is affected by experience and knowledge of itself and its neighbors [16]. Suppose we have a d dimension space and i'th particle from the swarm can be present with a velocity vector and position vector. Position change of each particle is possible by change in position structure and previous velocity. Position of each particle is x_i and it has information about best value which has reached yet, named *pbest*. This information is obtained from particles attempt to reach the best answer. Also any particle knows the best answer obtained for *pbest* from others in the swarm, named *gbest*. Each particle tries to change its position in order to reach the best solution using the following parameters:

x_i current situation, v_i the velocity, destination between the current position and *pbest*, destination between current position and *gbest*.

So the velocity of each particle changes as follows:

$$(pbest_i - x_i^k) + c_2 r_2 . \quad (3)$$
$$(gbest - x_i^k) V_i^{k+1} = w v_i^k + c_1 r_1 .$$

Which V_i^k is the velocity of each particle in k'th repeat, w is the inertia weight, c1 and c2 are learning coefficients, r_1 and r_2 are random variables in the $[0,1)$ interval with the unique distribution, x_i position of each particle i in the k'th repeat, $pbest_i$ which is *pbest* of i'th particle and *gbest which is gbest* of the group. Maximum of velocity (V_{max}) is to prevent velocity from increasing unlimitedly [17,18]. Position of each particle is determined as follows:

$$X_i^{k+1} = x_i^k + v_i^{k+1} \quad (4)$$

Equations 1 and 2 are form primitive version of PSO algorithm. PSO algorithm is so easy and has low computational, speed and memory load. It is using to solve continues problems while our work needs

discrete version of the PSO. One of the discrete versions is binary PSO which has developed by Kennedy and Eberhart at 1997 [6]. They did a small change on the algorithm to support discrete quantities also. Velocity is used as a probabilistic threshold value here and can be 0 or 1. X_j^i, value of *j'th* bit from binary vector, shows the *i'th* particle position. So the following describes Binary PSO function [7]:

$$X_j^i[t] = \begin{cases} 1 & , \sigma < s(v[t]) \\ 0 & , otherwise \end{cases} \quad (5)$$

Which σ is a random number with the uniform distribution in [0,1] interval. **s(.)** is also the Sigmoid function described as follows:

$$S(z) = \frac{1}{1 + exp(-z)} \quad (6)$$

Velocity change in Binary PSO is the same way as standard PSO.

4. Method

Research steps of this paper are as the Figure 1. This is composed of two main parts: Data Preprocessing (Steps 1 to 4) and Data Mining (Step 5 and 6). First we describe data collection (Step 2) and preprocess it (Steps 3 to 4) and then we use the algorithm to mine association rules (Steps 5 and 6). In the part one we prepare the database to apply the algorithm. First we preprocess it in order to make it ready to change to binary form. After converting it to the binary form, well form for using with binary PSO, we will generate primary population in order to find *gbest*. Then we apply PSO to find minimum threshold value of support and confidence using particle movement funded optimal values. Having the best threshold we can use it for association rule mining resulting into better results. Hardest part of the research is to collect data and prepare it. Data is the primitive thing used in data mining. So it is important for good data mining to access and implement suitable data [19]. Data used for this paper is from Foodmart 2000 Database in the format of Microsoft SQL Server 2000, *Sales_fact_1997* table [1,3,24].

There are 86837 records from sale activity of a supermarket. Each record contains 8 properties. Our sample includes of 4000 random records. We have used optimal binary PSO to improve positive and negative rule production. Each particle represents a positive rule; consist of a predecessor and a successor. Figure 2 shows a particle; orange color is predecessor and blue one is successor. Every box represents a field from database. Containment of the boxes presents the value of a field in the database in the binary format.
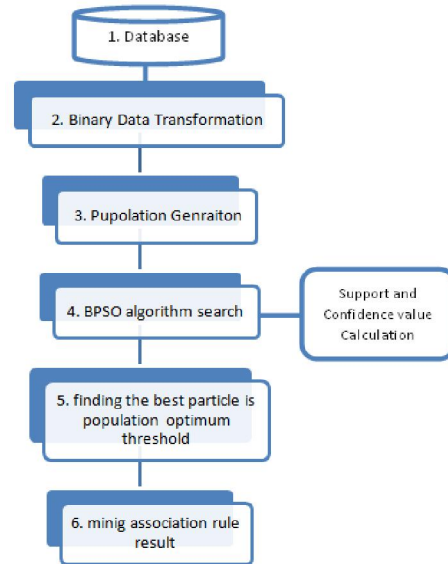
Fig1. Steps of the proposed method
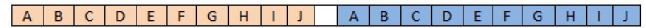
Fig2. Presentation of a particle

For example Fig. 3 shows a rule with the following specifications:
IF(product_id = 53   AND   store_id =1)→(customer_id = 3,   store_sales = 3)
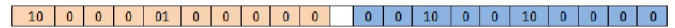
Fig3. Example of a particle in the database

Implementation of the proposed method has been done using *R2010b* version of MATLAB software. Movement representation of the particles toward the best goal is prepared form MATLAB also. Guiding a particle from the swarm population to an optimal answer is done by the fitness function. The particle with the greatest value of fitness usually supposed as the best particle [1] and [3]. In the proposed method *A* and *B* are collections of properties participating at predecessor and successor obtained from decoding respective particle according to what is explained. We calculate support and confidence values as follows:
In order to producing rule in the form of *if A → B*, two criteria form *cost(p)* function has been used to evaluate association rules quality.

$$Support = \frac{Supp(A \cup B)}{N} \quad (7)$$

$$Confidence = \frac{Supp(A \cup B)}{Supp(A)} \quad (8)$$

After sending the particles to the fitness function, particle with the greatest fitness level will be used to move other particles toward the most optimal rule. Fitness function is defined as follows:

Fitness= $α_1$*Support +$α_2$*Confidenss –$α_3$*NA

Which *NA* is the number of properties used in the rule and coefficients, $α_1,α_2,α_3$, is used to parametric control of fitness function and customized by the user. First and second parts of this function is related to support and confidence values. It is essential to take into account both parts simultaneously. Because only one of support or confidence values cannot be a criteria for quality assessment of produced rules. It is evident that the more the value of both factors simultaneously the better the quality of the rule. We know that long rules will probably result to low quality productions also. So we try to produce relatively short, readable rules with more concept and quality which has special importance in data mining [3].= First *n* particles are creating quite randomly, each one representing a rule. Then fitness value of each one will be evaluated using the function noticed before. Binary PSO search algorithm will run until reaching the end condition; i.e. the best particle has founded and support and confidence value of it can be used as minimal support and minimal confidence. So we can utilize them for mining better and more association rules.

5. Results and Discussion

Running proposed method on the sample noticed before, 4000 random samples from Sales_fact_1997 tables of Foodmart 2000 database, showed satisfactory results. We set the PSO algorithm parameters as follows:

Table 1. PSO Algorithm Parameters

Repeat Numbers	Learning rate of C_1,C_2	$α_3$	$α_2$	$α_1$
7	2	0.2	0.8	0.8

After running the algorithm for five population sizes, Table 2 was obtained:

We compared our results with those obtained from latest reference, reference [3]. Results

were so better. Fig. 4 compares confidence acquired by two algorithms and Fig. 5 compares acquired support. Red curve is our proposed algorithm while blue one is from [3]. X axis is the population size.

Obtained results form proposed algorithm will lead to better association rule mining, because of fine tuned minimum support and confidence. So we can conclude that proposed algorithm can improve the performance of association rule mining.
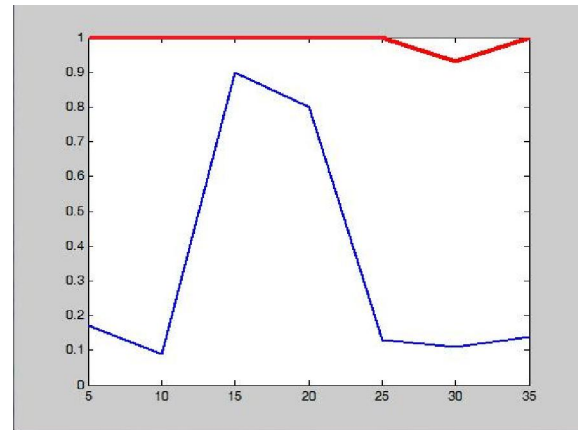
Fig. 4- Confidence compare of two algorithms
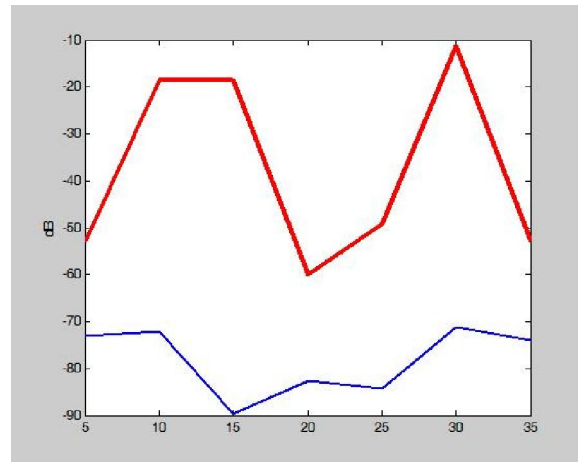
Fig. 5- Support compare of two algorithms

Table 2. Results of the proposed algorithm

Optimal Rule	State	Population Size	number of rules	discovered confidence	Discovered support	Runtime (sec)
a3=9642 --> a5=3	1	5	350	1	0.005	92
a8=1 --> a5=2	2	10	700	1	0.155	129
a8=1 --> a5=2	3	15	1050	1	0.155	220
a4=129 a6=4.9 a8=2 --> a5=2	4	20	1400	1	0.0025	314
a4=188 a8=2 --> a5=3	5	25	1750	1	0.0073	401
a8=3 --> a5=3	6	30	2100	0.93	0.32	471
a4=120--> a5=2	7	35	2450	1	0.005	562

6. Conclusion

Main disadvantage of association rule mining like Apriori is the intellectual computation of minimum support and confidence. Using the binary PSO algorithm, it is possible to compute both parameters quickly and efficiently. Results show improvements in comparison with previous methods. So we propose to add a prior computation to association rule mining before starting the task to determine minimum support and confidence. Future works can focus on improving PSO algorithm speed and considering negative rule mining in addition of positive rules. Because negative rules has the same importance of positive rules for managers. It is possible to suggest new methods to association mining of negative also.

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9/3/2012

Metabolic diseases: disease problems of Markhoz (Iranian Angora) goats

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Abstract: A metabolic disease technically refers to altered metabolism, but as used in this context refers to conditions which have their origin in nutrition or management as contrasted to infection. There are a number of such conditions which can occur with sheep and goats, but seldom represent a major constraint under commercial conditions. Included in these are acidosis, bloat, grass tetany, milk fever, posthitis, swelling disease (edema), urinary calculi, white muscle disease, etc,. Few of these actually cause serious troubles with goats under grazing conditions. [Farzad Abdollahzadeh, Tofiq Froghi. **Metabolic diseases: disease problems of Markhoz (Iranian Angora) goats.** *Life Sci J* 2012;9(4):265-269] (ISSN:1097-8135). http://www.lifesciencesite.com. 38

Keywords: Metabolic Diseases, Markhoz Goat, Iranian Angora

1. Introduction

In a broad interpretation the term "disease" includes anything which affects the well being of the animal., however, most people think of disease in a more restricted sense as those due to infection. The broader interpretation may include infectious and parasitic disease, as well as nutritional or metabolic disorders, toxic plants, and in the case of the Angora goat, a largely unique problem of "offshear" or freeze losses. The Angora often exhibit symptoms of "ill thrift" which is apparently predisposed by their high nutritional requirements. For this reason they may be slow to recover, even after disease or parasites are removed as a problem.

2. Acidosis

Refers to overloading on grain in an animal which is not adapted to this type of feed. The result is a high acidity of thedigestive system which can kill the animal, but more frequently cause the animal to go off feed for a variable period of time. There is little reason or logic to free choice feeding of goats on high grain rations, and thus little reason to incur this problem except for the case of accidental access to feed.

3. Bloat

Bloat is an extended rumen filled with gas which can result in death. It usually results from consuming fresh legume forage which also rarely occurs with the Angora under range conditions. Goats are less subject to bloat than some other species such as cattle, and they do not have a high dietary preference for or access to legumes such as alfalfas or clovers. There are two types of bloat which may have different origins. These are free gas bloat or frothy bloat. If observed early enough, some types of bloat can be treated by passing a stomach tube to release the gas. Prevention is more important and consists of altering the type of feed and/or by the use of anti-bloat agents in the feed. Rumensin which is often used with goats as a coccidiostat tends to reduce bloat. Other compounds in this group probably have similar properties. Poloxalene is a commonly used bloat retardant or can be used for treatment. It should be effective if taken regularly (daily), but the method of administration (i.e., in feed or salt) can present problems. It can be used to treat frothy bloat when given as a drench or directly into the rumen as an injection or by stomach tube.

4. Grass tetany

Grass tetany results from an electrolyte imbalance as a result of consuming fast growing highly succulent forage. The condition is rarely observed in Angora. An affected animal will die unless they are treated, but most can be saved by providing electrolytes in the form of glucose solutions (containing magnesium) in the vein.

5. Milk fever

Milk fever is a similar condition with the lactating animal which is producing a large volume of milk. Thus this problem appears to be rare in the Angora. It is caused by a rapid lowering of blood calcium as calcium is deposited in the udder following parturition. It can be treated by the use of electrolytes such as calcium gluconate in the vein or possibly under the skin or in the peritoneum. The author has not observed white muscle disease in the Angora, but it may well occur in areas which have low selenium levels in the soil. It is due to a deficiency of vitamin E or selenium or a combination of these. It can be treated or prevented by providing these as supplements in the feed, or injection. Selenium should only be used as directed, as it can be toxic.

6. Posthitis

Inflammation of the prepuce is a condition which will frequently be observed in goats. It is restricted to the male as it represents a scabby ulceration over the sheath or prepuce. It may result in an inability of the animal to extend the penis or urinate. The condition tends to result from one or more of three situations. One of these is a high protein feedstuff in which the excess nitrogen is excreted in the urine as urea which changes the pH of the urine causing irritation of the tissue of the prepuce or sheath. A second condition is that the animal spends a lot of time around barns or contaminated bed grounds resulting in unsanitary conditions which may predispose infection of this area. The third condition is the presence of a specific organism belonging to the *Corneybacterium* (*C. renale)* group which is often found in the urine or under unsanitary conditions. This condition may be found in either intact males or in wethers and can at times represent a high infection rate. Testosterone implants have been used in some countries to combat the problem as it causes the wethers to extend their penis and reduce the irritation from the urine or the threat of blockage. This material is not available to livestock producers in the United States. Another preventative is not to overload the animal on protein. However, under some grazing conditions a high protein intake can occur from natural vegetation. Antibiotics in the feed, such as terramyacin or aureomycin as used in feedlot rations, will tend to reduce the likelihood of infection and to reduce the problem. This solution is somewhat impractical for the grazing animal. Treatment is possible, but is time consuming and may be slow to yield results. It consists of cleaning the infected area and removing the scab if the animal is unable to urinate. A good antiseptic solution, such as iodine scrub or hydrogen peroxide or a mild chlorine solution can be used. The use of a medicated antibiotic ointment applied to the area and in some cases an injection of an antibiotic is sometimes advisable.

7. Urinary calculi

waterbelly or urolithiasis can also be a problem of Angora males. It can occur on the range, but under production conditions it tends to be restricted to potential breeding males which are receiving a high level feeding of formulated rations. It is also often seen in wether goats kept around the barns or in pens as lead animals. It is caused by formation of stones or calculus which blocks the urinary tract preventing urination and can cause rupture of the bladder. It can largely be prevented, but treatment has limited value. For young males to be fed in confinement, special rations should be

formulated. The problem often results from animals being fed high phosphorus feeds such as rations containing grains (sorghum) and oil meals (cottonseed meal) and alfalfa. This problem can largely be prevented by insuring that the calcium to phosphorus ratio is 2 to 2.5:1. This may require the addition of calcium supplements such as Calcium Carbonate to the ration. With valuable males it may also be advisable to include in the ration 0.5% ammonium chloride or 0.6% ammonium sulfate to acidify the urine. Potassium chloride may also have a similar effect, but would be preferable only if the ration tended to be low in potassium. Also salt (sodium chloride) should be included in the ration to insure that the animals drink plenty of water. The latter practice reduces the concentrations of chemicals in the urine and the tendency for calculus formation. It is important to insure that the animals have access to water (not frozen) on a continuous basis as calculi cases often show up during or after a period of water deprivation. The condition may be treated by surgical intervention, but this course of action has little value for goats since the cost would exceed the value of the animal. In some cases the initial blockage occurs in the urethral process (the small filiform appendage at the end of the penis). If this is the case it is possible to extend the penis and clip this off with no adverse effect to the animal. Any more drastic surgery would require veterinary assistance and likely leave the animal useless for breeding purposes. If detected in the very early stages some cases may be reversed by administration of urine acidifying agents. One of these is methionine hydrochloride, administered orally as a paste, or a solution of ammonium chloride used as a drench. The latter material could potentially be toxic or caustic and should be used with care. Relaxing drugs (such as Atropine) may be used to assist in passing stones. If treatment is to be effective, an early diagnosis must be made. The symptoms include dribbling of urine, sand or crystals around sheath, obvious signs of discomfort including biting or kicking at the sheath or belly.

8. Swelling disease

Waterbelly or ventral edema may sometimes be confused with urinary calculi, but it has totally different physiological explanation. It is largely due to anemia or fluid loss in the tissue with the excess fluid accumulating along the underline or in the legs (the lower part of the body). Affected animals do not normally appear sick, but the condition is usually evident to the observer. It is rarely fatal, but is certainly an indication that all is not well with the animal. This condition is almost totally restricted to reasonably high producing

Angora goats indicating that it is nutritional or physiologic in origin, and is associated with the high level of mohair production. It appears often to be increased or predisposed by parasitism, low protein intake or some type of stress such as shearing. Prevention should call for insuring that the animals are not suffering from a high level of parasitism and that they are receiving an adequate level of protein and to minimize stress. Hypoproteinemia may not necessarily result only from a low protein content in the ration, but also from a high protein (or a specific amino acid) requirement of the animal as a result of a high level of fiber production. An iron deficiency is sometimes considered to be a predisposing factor. The condition is often seen in young males being developed for breeding purposes, but since this condition is a strong clue that the animal may be "over bred or otherwise unsuited" for their environment, it may not be desirable to use this type of animal for breeding purposes at least as a stud animal, even if they recover. Treatment consists of removing the above mentioned predisposing conditions and in extreme cases treatment with a diuretic (such as lasix) to encourage the animal to pass the excess fluid accumulation. A complete or total explanation for this condition is not available at present.

9. Pregnancy toxemia

Ketosis, twin lamb or kid disease or pregnancy disease is often listed as a metabolic disease. It is due to incomplete breakdown of fat among pregnant animals requiring a great amount of energy (glucose) during late gestation. The writer has not observed this as a problem with Angoras but it presumably can occur. The small amount of fat on Angoras and their tendency to abort partially protect them from pregnancy toxemia. Prevention would consist of providing adequate energy during late pregnancy. Treatment might consist of oral administration of molasses or propylene glycol.

10. Abortion

Abortion occurs more frequently in Angora than other types of farm or ranch livestock. Goats in general and the Angora in particular, are highly predisposed to this problem. It is generally realized that this condition is related to nutrition, primarily a deficiency of size or energy or to stress which disrupts normal feeding. This problem was discussed more thoroughly in the chapter on reproduction.

Toxic Plants: Due to the nature of their grazing habits and conditions under which they are raised, toxic plants are one of the major problem areas for Angora goat producers. Discussing this problem is made more difficult by the realization that toxicity is a relative term in that most feedstuffs or forages can have adverse effects at some time or some place or in some amounts. For instance, some favorite feedstuffs can cause trouble. Alfalfa may cause bloat and a condition known as "red gut," and in some cases a high estrogen content interferes with reproduction. Small grain forages carry a threat of producing "swellhead" or photosensitization. Sorghum forages carry a threat of prussic acid poisoning. Overfeeding on grain carries the threat of acidosis or enterotoxemia. Cottonseed or cottonseed products have the potential, under some condition, of causing gossypol toxicity. Toxic plants are present on almost all rangelands, and the ability to use these lands is based on the premise that animals will not eat these plants or will not eat them in sufficient amounts to cause trouble. Many toxic plant problems result from overstocking (sometimes intentionally for control of noxious plants), forcing the animals to utilize plants which they might otherwise ignore. Additionally, goats may be included in grazing programs in the belief that they are less likely to graze toxic plants or are more resistant to them or that goats may be used in some causes to remove plants (leafy spurge) which constitute a problem with other livestock species. It is important for individual producers to know the potentially troublesome plants in their area, the likelihood of their causing problems and the symptoms they produce. There are a number of reviews of toxic plants (see James, *et al*., 1980, Sperry, Dollahite, Hoffman and Camp, 1977 and Rowell, undated) which collectively list approximately 100 plants which have the potential of causing problems, and still do not list some of those which can be a threat to goats. Many of the more common plants found on rangelands are listed as a threat under some conditions. These include most types of oaks, mesquite, white brush, black brush, a variety of milk weeds, and number of plants in the nightshade or Solanum group, etc. Fortunately the writer has had little experience with many of these potential problems. In many cases a good source of information may be obtained from producers who have had long experience running goats in a given area. Some of the plants have not been reported to cause trouble with goats, but they are listed in the belief that this may reflect the fact that goats are not routinely produced in areas where these plants are present. The current tendency for goats to move into new areas may result in losses in the future. Admittedly, this list does not contain all potentially toxic plants and tends to emphasize the plants found on the range lands of the Southwest. In addition to this listing, a few individual plants or conditions or groups of plants will be discussed in more detail.

Losses due to toxic plants are more likely to be observed on overstocked ranges, or when hungry animals are turned to pastures where toxic plants are a threat. A number of poisonous plants cause photosensitization or "swellhead" (sensitivity to light). These conditions are usually due to liver damage, but some plants result in this condition without evidence of liver damage. If caught in the early stages and removed to shady or protected areas some of the affected animals will recover, depending on the amount of liver damage. More importantly, if producers become proficient in identifying the early symptoms they may be able to take protective action (ie. move to pens or to new pastures) before heavy losses occur. A number of plants are nitrate accumulators (pigweed, carelessweed, kochia, etc.) and thus can be toxic. These tend to be those found around barns or pens where the soils have a high level of fertility, but the problem can also be encountered under field conditions. Care should be exercised in allowing hungry animals access to the areas or type of plants mentioned. Nitrates or their byproducts bind with hemoglobin in the blood to reduce the oxygen carrying capacity. There is little warning of toxicity and death may be the first evidence, but occasionally labored breathing, particularly after being stressed may be seen. Some plants are also selenium accumulators, but these are not common in areas where Angoras are normally produced.

11. Oak

Oak poisoning is rare in goats as many Texas ranges have some type of oak as an important forage for goats, and goats may be included in the range management program to control or utilize oaks. Problems are rare, but heavy losses are known to have occurred in cases where goats are forced to live almost exclusively on oak forage. Since most parts of the oak plant are low in feed value, losses may have been due to a combination of starvation and poisoning. Observed losses have been mostly with broadleaf type of oak such as scrub oak or blackjack oak. Early growth or buds of shin oak are known to be dangerous to cattle, and potentially goats as well. The problem with oak is tannins or tannic acid, and the feeding of an anti-acid such as calcium oxide (slaked lime) has been shown to be beneficial in preventing losses in cattle. It is usually administered in a salt or mineral mixture at 8 to 10%. In theory, animals such as the goat which rely heavily on the oak as a feed source might benefit from routine administration of calcium oxide. The writer has made repeated attempts to demonstrate such a benefit without success, but in those cases no death losses were encountered even in the control groups.

However, since the tannin in these plants interferes with protein digestion (and perhaps energy sources as well) a good response can usually be obtained by providing protein and energy to animals utilizing the oak plant extensively.

12. Hydrocyanic or **prussic acid**

Hydrocyanic or prussic acid poisoning represents a threat with certain groups of plants. The plants most commonly put into this category are the various forms of sorghum. However, other plant including chokecherry, plum, flax, mountain mahogany, and elder also fit into this category. These plants represent major threats after they are wilted or damaged by drought or freezing, and should not be used under these conditions without testing. Sodium thiosulfate or methylene blue are specific antidotes for hydrocyanic acid poisoning, but have little value because the time available for administration is short. Some specific plants with which producers in Texas should be familiar with before exposing goats to them are Guajillo, Coyotillo and Sacahuista. Guajillo is an important leguminous forage plant for goats, but animals existing almost exclusively on this plant develop "limberleg" or "wobbles" of the rear legs. Fortunately the condition is slow to develop and heavy losses can be prevented by close observation and removal to other areas or management system as soon as symptoms appear. Coyotillo also produces a condition sometimes called "limberleg", but the problem is much more acute than with Guajillo. Producers are able to use rangelands where this plant is present by developing a population of animals which either do not eat the plant or which have developed a tolerance for the plant, and exercising caution in introducing new or naive animals to these areas. The entire Sacahuista plant is toxic, but problems primarily occur when sheep or goats consume the blooms or seed heads. These plant parts are highly toxic, but are not a threat except at certain seasons of the year (summer or fall). This plant causes photosensitization or swellhead and animals exposed to the plant should be observed carefully at critical times in order that appropriate action can be taken. It is possible to run goats on pastures where Sacahuista is present depending on the stocking rate, amount of Sacahuista present, the amount and quality of alternate forage available and with careful management at critical times.

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9/3/2012

The effects of some agricultural By-products on blood metabolites, chewing behavior and physical characteristics of dairy cow diets

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Abstract: This study was carried out to evaluate the effects of feeding ensiled mixed tomato and apple pomace (EMTAP) on blood metabolites and chewing behavior of dairy cow. Six multiparous Holstein dairy cows in mid lactation were used in 3×3 Latin square design and fed alfalfa hay plus concentrate mixture with three levels replacement with EMTAP (0, 15, 30%) during 63 days. Results showed that, differences between treatments were significant. Feeding EMTAP resulted in higher glucose, cholesterol, BHBA, triglyceride, and total protein ($P<0.01$) concentrations then control diet. Data showed that, total eating time (hours per day) was not significantly ($P > 0.05$) affected by treatments, but time spent eating, ruminating and total chewing activity per daily intake (kg) of DM and NDF decreased significantly with increasing EMTAP in diet. It was concluded that, EMTAP can efficiently replace up to 30% alfalfa hay. The nutritive value of tomato and apple pomace could be improved when they are used together (50:50) in dairy cows diet.

Key words: blood metabolites; chewing behavior, agricultural By-products.

1. Introduction

Ruminants which are able in converting waste items in to useful products such as meat, milk and skin, offers a feasible solution of using by-products and preventing pollution (Oni et al., 2008). Tomato and apple pomace are two alternative by-products that obtained from tomato paste and apple juice industrial production, respectively. These by-products are produced in huge amount annually. The chemical composition of final pomace is linked to the morphology of the original feed stock and the extraction technique used. Although tomato and apple pomace are varying from nutrient density, effective processing can improve their nutritive value. According to NRC, (2001) apple pomace (AP) is very low in protein (6.4% protein on DM basis), it also serves as a useful energy source because of high content of soluble carbohydrate for ruminants. Researches conducted on AP (Rumsey, 1978; Fontenot et al., 1977), showed that AP supplemented with natural proteins was comparable to protein enriched corn silage. In contrast, Elloitt et al. (1981) demonstrated that, tomato pomace (TP) have the potential to be a good source of protein, however its energy source may be limited due to the high fiber content. Previous researches reported different results from feeding TP and AP. The complementary composition of AP (low protein concentration) (Alibes et al., 1984; NRC, 2001; Pirmohammadi et al., 2006) and TP (high protein content) (Fondevila et al., 1994; Del Valle et al., 2006; Weiss et al., 1997) suggest to use those by-products together. Our

previous observations (unpublished data) showed that processed TP with AP (ratio of 50:50) had more palatability and digestibility than processing with urea, wheat straw, NaCl and NaOH for sheep. The aim of the present study is to evaluate the effect of ensiled mixed tomato and apple pomace (EMTAP) on blood metabolites and chewing behavior of dairy cow.

2. Material and methods

Fresh experimental samples of tomato pomace (TP) and apple pomace (AP) were collected from several factories in Urmia city (Iran). TP and AP were mixed together (50:50 on DM basis) and ensiled without any additive in a trench silo on a concrete floor. The mixed TP and AP silage (EMTAP) was sealed for 55 days, next fed as TMR diets in three levels replacement of alfalfa hay. Chemical composition of TP, AP and EMTAP was determined using the method suggested by AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using method of Van Soest et al. (1991). Six multiparous dairy Holstein cows were used in a 3×3 Latin square experimental design with three 3-wk periods. They were kept in individual concrete tie-stalls and had free access to drinking water at all times. The daily TMR diets were given in two equal feeds at 08:00 and 20:00 h to provide approximately 10% feed refusal each day (as-fed basis). Feed refusal were removed and weighed before feed offered at 08:00 h. Body weight was recorded prior to morning feeding

on 2 consecutive days at the beginning and at finish of each period. The experimental periods lasted 21 d, including 14 d of adaptation and 7th d of sampling and data collection. During the last 7th d of the experimental period collection and sampling of TMR diets, feed refusal, rumen fluid, blood, feces and urine were performed. Normal herd management practices were followed during the experiment.

On the morning of last day in each sampling period, blood samples were taken from the jugular vein of each cow 3 h after feeding and placed into vacuum tubes. The blood samples gently kept in ice, and then were centrifuged at 1500 g for 15 min to separate the serum. The serums were transferred into storage pipe and labeled with data and animal identification and stored at 20°C until analysis. Concentration of plasma glucose, cholesterol, triglyceride, BHBA, albumin, urea, ammonia-N, total protein, calcium, phosphorus, were measured by using an auto analyzer Spectrophotometer, mark Unico, model S 2100 SUV, serial number 2165168, Japan. Sodium and potassium were measured by Flame photometer, model PFP7, Serial number 12377, Genewey factory, England.

Table 1. Ingredients and nutrient composition of experimental diets (DM basis)

	Diets (EMTAP levels)		
	1	**2**	**3**
Ingredients	0%EMTAP	15%EMTAP	30%EMTAP
Alfalfa hay	45.67	33.35	18.41
EMTAP‡	0	15	30
Soy bean meal	10.25	10.23	9.92
Barley	37.96	37.99	38.2
Fat (Oil plant)	0	0.57	0.99
Wheat bran	5.4	2.09	1.54
Caco3	0.22	0.27	0.44
Premix†	0.5	0.5	0.5
Nutrient compositions		**(% based DM)**	
DM	98	78.3	63.1
NEL (mcal/kg)	1.54	1.58	1.62
CP	15.4	15.5	15.5
NDF	35.4	35.2	35.1
ADF	21.4	23.1	24.3
Calcium	0.6	0.6	0.5
Phosphorus	0.4	0.4	0.4
Concentrate	54.33	51.65	51.59
Forage	45.67	48.35	48.41

‡EMTAP, ensiled mixed tomato and apple pomace; DM, dry matter; NEL, net energy for lactation; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber. †Premix supplied (on a concentrate DM basis): 400.000 IU of vitamin A/kg, 100.000 IU of vitamin D3/kg, 100 mg of vitamin E/kg, 219 mg/kg of Mn, 69 mg/kg of Zn, 116 mg/kg of Fe, 23 mg/kg of Cu, 1.8 mg/kg of I, 0.6 mg/kg of Co, and 0.46 mg/kg of Se.

The feces and urine pH were measured 3 h after morning feeding for two consecutive days on d 3 and 4 of each sampling period. Feces were collected and extracted through cheesecloth into a clean beaker; the urine samples were taken via vulva stimulation. Feces and urine pH was measured using a Schott Titrator Titroline easy pH-meter. Animal behaviors were monitored visually for 24 h period on day 5 of each sampling period. The assumption was that the particular chewing activity persisted for the entire 5-min period between each visual observation (Beauchemin et al., 2003). Chewing activities were expressed as total hours on 24 hr period per unit of DM and NDF intake by dividing minutes of eating or ruminating by the mean daily nutrient intake.

Collected data were statistically analyzed using the GLM procedure (SAS. 1998, Inst. Inc., Cary, NC). Level of significance was $\alpha = 0.05$, and the Tukey test was used to test for all pairwise

comparisons among means. The model used for this analyze was:

$$Y_{ijk}= \mu + T_i + C_j + P_k + \varepsilon_{ijk}$$

where Y is dependent variable, μ is the overall mean, T is treatment effect (i = 1, 2, 3 EMTPA levels), C is cow effect (j= 1 to 6), P is period effect (k= 1, 2, 3) and ε is random residual error term.

3. Results and discussion

Blood metabolite concentrations are shown in Table 2. Feeding EMTAP resulted in higher glucose, cholesterol, BHBA, triglyceride, and total protein (P<0.01) concentrations. In contrast, urea, albumin, calcium, phosphorous, sodium and potassium were not affected significantly (P > 0.05) by treatments. The results obtained are in good accordance with our expectations. Increasing the presence of soluble carbohydrates and digestible nutrients in AP (Rumsey, 1978) and high amount of protein (21.75) and fat (13.4) in TP resulted in higher concentration of blood metabolites. There was high correlation between rumen and blood ammonia-N, as

well the volume of rumen ammonia-N directly related to rumen degradability of diet protein. As above mentioned, TP has high content of not degradable proteins. In contrast with our results, Belibasakis (1995) reported that, replacing maize silage and soya bean meal with alone TP at 13% DM of dairy cows diet did not affect significantly same blood metabolites. While, in present study we saw significant differences. This inconsistency may be due to use of EMTAP in our study. Mean value of eating and ruminating times as well as particle size distributions for the diets are given in Table 3. Total chewing time (hours per day) varied from 13.58 to 12.09 with ruminating time varying from 7.30 to 6.13, for control and EMTAP diets, respectively. Data showed that, total eating time (hours per day) was not significantly (P > 0.05) affected by treatments, but time spent eating, ruminating and total chewing activity per daily intake (kg) of DM and NDF decreased significantly with increasing EMTAP in diet.

Table 2. Blood metabolites concentration of cows fed diets with different content of EMTAP.

Item	Diets (EMTAP Levels)				
	1 0% EMTAP	2 15% EMTAP	3 30% EMTAP	S.E.M	P value
Glucose, mg/dL	79.55[b]	90.89[b]	118.8[a]	4.76	<0.01
Ammonia-N, mg/dL	6.87	6.88	6.92	0.02	0.12
Urea, mg/dL	30	33.67	34	1.72	0.33
Cholesterol, mg/dL	86.95[b]	124.8[b]	139.8[a]	9.30	0.01
Triglycerides, mg/dL	9.69[c]	11.43[b]	13.2[a]	0.47	<0.01
BHBA, mg/dL	6.15[b]	8.25[b]	8.68[a]	0.31	<0.01
Calcium, mg/dL	8.44	8.68	8.96	0.53	0.48
Phosphorus, mg/dL	5.35	5.48	5.71	0.31	0.59
Sodium, mEq/L	136	136.33	136.5	1.57	0.98
Potassium, mEq/L	3.7	3.85	4.10	0.21	0.4
Total protein, g/dL	6.7[b]	8.5[b]	9.28[a]	0.37	<0.01
Albumin, g/dL	4.05	4.33	4.64	0.24	0.26

Diets; 1= control or 0% EMTAP; 2= 15% EMTAP; 3= 30% EMTAP; S.E.M.= standard error of mean; a,b,cMeans in the rows with different superscripts are significantly different (P<0.05).

Dietary fiber plays a fundamental role in ruminant DM intake and stimulates chewing activity and rumen fermentation. The fiber of by-products has different physical and chemical properties from forage NDF (Zhu et al., 1997), in particular their particles have smaller dimensions and higher density (Firkins et al., 1991). Chewing activity is usually a good indication of rumen health because chewing stimulates saliva secretion. The influence of the diet treatments resulted in linearly decrease in proportion of particles on the sieves (PSPS) with increasing

amount of EMTAP in the diets (Table 3). Lower ruminating and total chewing time for the diets containing EMTAP compared to the control, reflected their low peNDF and particle size distribution. According to our results, Beauchemin et al. (2003) reported that dietary peNDF were moderately associated with ruminating time but not with eating time and increasing ruminating time rather than increasing eating time may be a more efficient means of improving ruminal pH status. The pef value for the EMTAP containing diets (59 to 65,

15 and 30% EMTAP, respectively) was significantly (P<0.01) smaller than that of the control diet (pef: 73), because a large amount of these diets included concentrate and EMTAP, which almost entirely passed through the 19 and 8-mm sieve. Generally, total chewing time decreases as forage NDF (Beauchemin, 1991) or particle size (Grant et al.,

1990) in the diets decreases. According to our results, Chumpawadee and Pimpa (2009) reported that, the use of non forage fiber sources (such as TP) caused a decrease of chewing time due to the smaller particle size and lower peNDF.

Table 3. Particle size distribution and physical effectiveness factors of diets differing in ratio of EMTAP.

| Item | Diets (EMTAP Levels) | | | S.E.M | P value |
	1 0% EMTAP	2 15% EMTAP	3 30% EMTAP		
Eating activity					
h/d	6.28	6	5.96	0.11	0.16
Min/kg DM	17.73[a]	15.22[b]	14.63[b]	0.57	0.01
Min/kg NDF	50.11[a]	43.22[b]	41.68[b]	0.80	P<0.01
Ruminating time					
h/d	7.30[a]	6.47[b]	6.13[b]	0.23	0.02
Min/kg DM	20.61[a]	16.41[b]	15.05[c]	0.28	P<0.01
Min/kg NDF	58.24[a]	46.60[b]	42.87[c]	0.73	P<0.01
Total chewing time					
h/d	13.58[a]	12.47[b]	12.09[b]	0.19	P<0.01
Min/kg DM	38.34[a]	31.62[b]	29.68[c]	0.22	P<0.01
Min/kg NDF	108.35[a]	89.82[b]	84.55[c]	0.14	P<0.01
TMR offered, % DM retained on sieves.					
19 mm	35[a]	29[b]	26[c]	0.16	P<0.01
8 mm	38[a]	36[b]	33[c]	0.13	P<0.01
Pan	27[a]	35[b]	41[c]	0.07	P<0.01
pef †	73[a]	65[b]	59[c]	0.05	P<0.01
PeNDF, % of DM‡	25.84[a]	22.88[b]	20.71[c]	0.09	P<0.01

† Physical effectiveness factor determined as the proportion of dry matter retained by both sieves of thePenn State Particle Separator

‡peNDF measured as the NDF content of the TMR multiplied by the pef.

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9/3/2012

Metabolic Bone Disease in Children with Idiopathic Nephrotic Syndrome

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Abstract: Children with idiopathic nephrotic syndrome (INS) may be at risk for metabolic bone disease (MBD) because of biochemical derangements caused by the renal disease, as well as the corticosteroid effects on bone. We studied 70 children with INS for clinical, biochemical, and radiological evidence of MBD. These patients were divided into two groups: 55 frequent relapsers (FR; group I), and 15 infrequent relapsers (IFR; group II). Thirty healthy children matched for age and sex constituted the control group. Bone mineral density (BMD) of these children was evaluated by the Achilles Express Quantitative Ultrasound (QUS) device. Univariate and multivariate analyses were performed to analyze factors predictive of low BMD T- score. We observed that nephrotic children had significantly lower mean BMD T- scores compared with controls (-1.99 ± 0.74 versus -0.39 ± 0.87; $P = <0.0001$). Also, children in group I were found to have significantly lower mean BMD T- scores compared with group II (-2.1 ± 0.67 versus -1.31 ± 0.64; $P = <0.0001$). We also observed that 32.7% of group I had osteoporosis compared to none of group II ($P= 0.01$). Significantly higher doses of steroids over longer duration of therapy were administered to group I compared with group II ($P = < 0.0001$ and 0.0004 respectively). On multivariate analysis, the only factor found to be predictive of a low BMD T-score was greater cumulative steroid dose ($P= 0.02$). We concluded that children with INS are at risk for MBD, especially those receiving higher doses of steroids. Regular BMD evaluation and appropriate therapeutic interventions are recommended for these children. The role of prophylactic therapy in such patients needs to be further investigated.

1. Introduction

Children with idiopathic nephrotic syndrome (INS) may be at risk of metabolic bone disease (MBD) (*Gulati et al., 2003*). Patients with nephrotic syndrome and normal renal function frequently have abnormalities in calcium and vitamin D metabolism (*Grymonprez et al., 1995*).

Glucocorticoid (GCS) drugs, mainly prednisone, remain the sheet anchor of therapy in children with INS. These drugs are often prescribed on a long-term basis, and are associated with a number of side effects. The most common and serious, however, is bone loss leading to osteoporosis (*Cohen and Adachi, 2004*). In fact, they are considered the number-one cause of secondary osteoporosis (*Dore, 2010*). Bone loss occurs even with low-dose GCS therapy, but is most rapid and extensive at prednisone doses ≥ 5 mg per day or equivalent. Loss of bone mass is most rapid during the first year of GCS therapy and significant reductions can be seen as soon as 3 months after starting therapy (*McIlwain, 2003*). Up to 50% of chronic GCS users develop bone loss leading to fracture, especially of spine and neck of femur (*Saigal et al., 2006*).

Thus, children with INS are prone to MBD because of both biochemical derangements caused by the renal disease itself, in addition to GCS therapy (*Gulati et al., 2003*). However, the propensity of steroids to cause osteoporosis and fractures is often neglected (*Srinivasulu et al., 2010*).

The data that have been published on BMD in children with nephrotic syndrome (NS) who have been treated with GCS are equivocal and there is still conflicting evidence on the risk of low bone mass in these children. Whether nephrotic children are prone to MBD is of therapeutic significance because these children would merit prophylactic therapy with calcium and vitamin D. Therefore, we conducted this study to evaluate the prevalence of MBD in children with nephrotic syndrome with normal renal function, and to study the effect of different variables, including cumulative dose of received corticosteroids, on the BMD of these children.

2. Patients and methods

This retrospective cross sectional study was conducted at the Pediatric Nephrology Clinic, Sohag University Hospital, Sohag, Egypt. We recruited 70 children (47 males, 23 females), aged 4- 14 years

previously diagnosed to have INS according to the criteria of the International Study of Kidney Disease in Children (ISKDC, 1981). The following patients were excluded: (1) those with abnormal renal function defined as GFR less than 90 mL/min/1.73m^2 calculated by the Schwartz formula *(Schwartz et al., 1987)*. (2) those who have a regular follow up period less than 12 months. (3) those who received corticosteroid therapy for less than 6 months period, or in a dose less than 5mg prednisone per day. (4) those with clinical evidence of malnutrition or systemic diseases. (5) those receiving calcium and vit.D supplementation. In addition, 30 children matched for age and sex with no history of renal disease were taken as controls. Informed consent was obtained from parents of all children prior to their participation in the study.

All of the children with INS were treated according to the standard protocol according to Pais and Avner (2011). The initial therapy, consisted of daily prednisone 60 mg/ m^2 /day (80 mg daily max) for 4 weeks, followed by 40 mg/m^2/day given every other day as a single daily dose for at least 4 wks. The alternate-day dose was then slowly tapered and discontinued over the next 1-2 months. Relapses were treated with 60 mg/m^2/day in a single morning dose until the child entered remission (urine trace or negative for protein for 3 consecutive days). The prednisone dose was then changed to alternate-day dosing as noted with initial therapy, and gradually tapered over 4-8 wk. Patients who responded well to prednisone therapy but relapsed <4 times in a 12-mo period were considered infrequent relapsers (IFR; 15 patients), and those who relapsed ≥4 times in a 12-mo period were considered frequent relapsers (FR; 55 patients).

The medical charts of the patients were reviewed for date of diagnosis of NS, duration of the disease, duration of GCS therapy and cumulative dose of received steroids. A careful clinical history and physical examination were performed, and urine and blood samples were drawn from all children to measure serum albumin, calcium, phosphorus, alkaline phosphatase, calcitonin, 25(OH) vitamin D, intact parathyroid hormone (PTH), and urinary 24 hours proteins.

Bone scanning:

Although, the dual-energy x-ray absorptiometry (DXA) scan is considered the "gold standard" in measuring BMD, DXA scanning is not readily available to all patients. Quantitative ultrasound (QUS) is another bone scanning technique, introduced in the early 1990s. The Achilles Express Quantitative Ultrasound (QUS) device combines broadband ultrasound attenuation (BUA) and speed of sound (SOS) to reduce random measurement errors

and provide better precision in estimating fracture risk. Tissue attenuation is determined by the change in ultrasound intensity measured between 2 transducers (National Kidney Foundation, 2003). The Achilles Express combines BUA and SOS into a measure called the stiffness index (SI), which is then compared with those in age-matched controls to determine a "T-score equivalent " (Achilles Express Ultrasonometer, 2001). The advantages of this technique over DXA scanning are: it is less expensive, it does not involve irradiation exposure, and is relatively rapid (DeHart and Gonzalez, 2004). Because of their portability, the QUS device might be a more convenient and clinically useful screening and monitoring tool compared with DXA (Grabe *et al.,* 2006).

Bone densitometry in this study was estimated by The Achilles Express Quantitative Ultrasound (QUS) device (Lunar Corporation, USA, 2001). Each one of the patients and controls received QUS of each calcaneus (QUS- dominant and QUS-non dominant) using the Achilles Express. QUS measurements were performed in duplicate by the same investigator, with the child's heel positioned in the system as required, and the mean value was calculated. According to the WHO diagnostic guidelines (1994), normal bone mass was defined as a T-score >-1, low bone mass (osteopenia) was defined as a T-score <-1 and >-2.5, and osteoporosis was defined as a T-score <-2.5.

Statistical analysis:

Statistical analysis was performed using STATA intercooled version 9.0. Data were presented as mean ± SD or number (%) when appropriate. Results were analyzed for statistical significance using Student's test for continuous variables and chi-square test for discrete variables. The correlation of BMD T-score with other parameters was studied by using Pearson's correlation coefficient method. Subsequently, multivariate analysis was performed using multiple regression analysis to evaluate factors predictive of a low BMD T-score. *P* value less than 0.05 was considered statistically significant.

3. Results:

The patients under study comprised 23 girls and 47 boys, with the means of age and age at the onset of nephrotic syndrome being 8.73 ± (2.98) yrs and 3.73 8.73 ± 2.98 yrs and 3.73 ± 1.84 yrs respectively. Thirty healthy children constituted the control group. The demographic, clinical and biochemical characteristics of both groups are presented in (Table 1). Out of all studied parameters, serum albumin was significantly lower, while serum PTH, 24 hours urinary proteins and BMD T-score were significantly higher in nephrotic patients than controls. Other clinical and biochemical parameters were similar in both groups.

Table (1): Demographic, clinical, and biochemical parameters of cases and controls.

	Cases (n = 70)	Controls (n=30)	*P* value
Sex			
Female	23 (32.9%)	14 (46.7%)	0.19
Male	47 (67.1%)	16 (53.3%)	
Age (year)	8.73 ± 2.98	9.07 ± 2.99	0.60
Age of onset of NS (year)	3.73 ± 1.84		
Duration of disease (year)	5.03 ± 2.78		
Duration of therapy (year)	3.31 ± 2.19		
Cumulative dose of steroids (gm)	15.16 ±10.34		
S. calcium (mg/dl)	9.25 ± 0.66	9.45 ± 0.40	0.13
S. phosphorus (mg/dl)	4.67 ± 0.74	4.95 ± 0.48	0.06
S. alkaline phosphatase (IU/l)	176.89 ± 60.82	193.07 ± 60.08	0.22
S. 25(OH) vit. D (nmol/l)	68.1 ± (23.9	73.67 ± 29.54	0.32
S. parathormone (pg/ml)	53.01 ± 28.03	28.75 ± 14.60	**<0.0001***
S. calcitonin (pg/ml)	7.36 ± 2.11	7.41 ± 3.29	0.89
S. albumin (gm/dl)	3.95 ± 0.65	4.65 ± 0.55	**<0.0001***
24 hours urinary proteins (gm/24hrs)	0.57 ± 0.35	0.29 ± (0.15)	**0.0001***
T- score	-1.99 ± 0.74	-0.39 ± 0.87	**<0.0001***

Data are expressed as no (%) and mean ± SD

Of 70 nephrotic children, 55 children were frequent relapsers (FR; group I) and 15 were infrequent relapsers (IFR; group II). There were no differences between the two groups in regard to age, age of onset of NS, sex distribution, and duration of the disease. FR children received higher cumulative doses of steroids over longer duration of therapy (Table 2). Serum albumin was lower, while serum PTH and 24 hours urinary proteins were higher in group I. However, serum calcium, phosphorus, alkaline phosphatase, 25(OH) vit.D and calcitonin were similar in both groups. BMD T-score was greater in group I (mean -2.1) than group II (mean -1.3) *(P* = <0.0001). Eighteen of 55 patients in group I (32.7%) had osteoporosis compared to none of 15 patients in group II (*P*=0.01). Osteopenia was similarly observed in both groups.

Table (2): Demographic, clinical, and biochemical parameters of FR and IFR children.

	Group I (FR; n = 55)	Group II (IFR; n=15)	*P* value
Sex			
Female	17 (30.9%)	6 (40%)	0.51
Male	38 (69.1%)	9 (60%)	
Age (year)	8.85 ± 3.05	8.27 ± (2.76)	0.50
Age of onset of NS (year)	3.74 ± 1.90	3.63 ± (1.65)	0.93
Duration of disease (year)	5.15 ± 2.93	4.57 ± 2.10	0.48
Duration of therapy (year)	3.78 ± 2.19	1.60 ± 1.03	**0.0004***
Cumulative dose of steroids (gm)	17.75 ± 10.09	5.64 ± 3.16	**<0.0001***
S. calcium (mg/dl)	9.18 ± 0.68	9.51 ± 0.53	0.09
S. phosphorus (mg/dl)	4.65 ± 0.81	4.74 ± 0.39	0.66
S. alkaline phosphatase (IU/l)	175.09 ± 58.93	182.47 ± 62.61	0.67
S. 25(OH) vit. D (nmol/l)	69.94 ± 23.30	64.00 ± 21.76	0.38
S. parathormone (pg/ml)	58.36 ± 29.11	33.4 ± 8.99	**0.002***
S. calcitonin (pg/ml)	7.24 ± 2.16	7.66 ± 1.50	0.48
S. albumin (gm/dl)	3.73 ± 0.48	4.77 ± 0.50	**<0.0001***
24 hours urinary proteins(gm/24hrs)	0.65 ± 0.35	0.27 ± 0.14	**0.0001***
T- score	-2.1 ± 0.67	-1.31 ± 0.64	**<0.0001***
T-score < -2.5 (osteoporosis)	18 (32.7%)	0 (0%)	**0.01***
T-score <-1 and >-2.5 (osteopenia)	30 (54.5%)	8 (53.3%)	0.93

Data are expressed as no (%) and mean ± SD

Nephrotic children had significantly lower T-scores compared to the control children (-1.99 ± 0.74 versus -0.39 ± 0.87, *P*= <0.0001) (Table 1). Among the patients group, T-scores were significantly lower in FR (group I) than IFR (group II) (-2.1± 0.67 versus -1.31± 0.64, *P*= <0.0001) (Table 2). T-scores of the three studied groups are presented in Figure (1).

Multivariate analysis was performed to identify factors predictive of a low BMD T-score among our nephrotic children. We observed that the only factor found to be predictive of a low BMD T-score was greater cumulative steroid dose (*P*= 0.02) (Table 3). The correlation between T- score and cumulative dose of steroids is presented in (Figure 2).
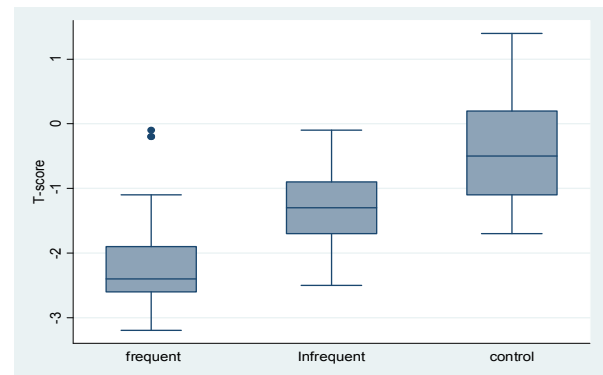

Figure (1): Comparison of T-scores in FR, IFR nephrotic children and controls.

Table (3): Multivariate Analysis for Factors Predictive of Low BMD T- Score

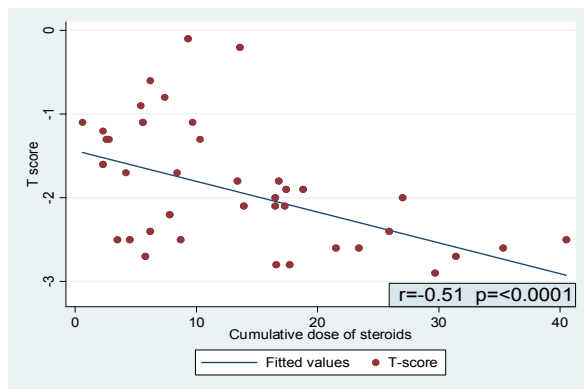| Predictor	Unstandardized Coefficient	t	P	[95% Conf. Interval]	
				lower	upper
Male sex	0.20	1.14	0.26	-0.15	0.54
Age	0.15	0.69	0.49	-0.28	0.57
Age at onset	-0.20	-0.89	0.37	-0.66	0.25
Duration of disease	-0.13	-0.59	0.56	-0.56	0.30
Duration of therapy	0.10	0.73	0.47	-0.17	0.37
Cumulative dose of steroids	-0.06	-2.49	**0.02***	-0.12	-0.01

Figure (2): Correlation between T-score and cumulative dose of steroids.

4. Discussion:

This study revealed that nephrotic children had significantly higher levels of PTH compared to the control group (*P*= <0.0001). Raised PTH levels were reported in adult nephrotic patients with normal renal function by Goldstein *et al.* (1977). In nephrotic children, Freundlich *et al.* (1986) reported modest hyperparathyroidism, but Grymonprez *et al.* (1995) found no difference in PTH levels between nephrotic children and controls. However these studies included

children with nephrotic syndrome not receiving GCS that have a proved effect on PTH. GCS suppress intestinal calcium absorption and decrease renal calcium reabsorption, resulting in the development of secondary hyperparathyroidism (Iwamoto *et al.* 2005). Among nephrotic children, FR patients were found to have significantly higher PTH levels than IFR patients which can be explained by the longer duration of disease and higher doses of GCS received by the FR children. This result is consistent with that observed by Mohamed and Abdel-Latif (2011), but contrasted to that reported by Gulati and colleagues (2003).

In this study, lower BMD T-scores were observed among nephrotic children compared to the controls (*P* = < 0.0001). This finding is supported by results of Lettgen *et al.* (1994), Fujita *et al.* (2000), Gulati *et al.* (2003) and Basiratnia *et al.* (2006). It has been shown that NS results in a number of biochemical and metabolic disturbances that may occur even with normal renal function and before GCS therapy (Grymonprez *et al.* 1995). Moreover, children treated with GCS are prone to the complex direct and indirect effects of these drugs on bone formation and resorption. These effects are much more complicated than was previously thought. GCS

reduce bone formation through decreasing osteoblast number and stimulation and decreasing synthesis of matrix constituents. They also increase bone resorption through decreasing serum calcium, osteoprotegerin and adrenal androgens and increasing PTH levels (Saigal *et al.*, 2006). Now, reduced bone formation rather than increased bone resorption is thought to be the predominant effect of glucocorticoids on bone turnover (Dore, 2010).

Our result contrasted with those of Polito *et al.* (1995), Morin *et al.* (1996) and Esbjorner *et al.* (2001). Although Esbjorner and coworkers found no difference in BMC between their patients and the control group, they observed reduced bone turnover and lower growth rate in nephrotic patients compared to the controls. Also, Leonard and associates (2004) reported that GCS therapy was not associated with bone loss at the lumbar area after correction for body mass index. However, they were in agreement with the effect of steroids on bone mineral and quality not captured by bone mineral content. This discrepancy might be explained by the fact that some of these studies included patients not receiving GCS at the time of investigation, and others included patients receiving calcium and vitamin D treatment which might have counteracted the demineralization effect of steroids.

Comparing BMD values in the two groups of nephrotic children, we observed that FR children had significantly lower T-scores compared to IFR children ($P = <0.0001$). Although mean duration of disease was similar in both groups, duration of treatment and cumulative dose of administered steroids were significantly higher in FR patients ($P = 0.0004$ and < 0.0001 respectively). Gulati *et al.* (2003) reported that FR nephrotic children had significantly lower z scores compared with IFR children. They also observed that osteoporosis affected 20 of 70 FR children (28.6%) compared to 6.7% of IFR children, a result which is consistent with ours.

On multivariate analysis, we observed that the only factor that was predictive of a low BMD score was the cumulative dose of steroids ($P = 0.02$). Patients' age, sex, age of onset of nephrotic syndrome, duration of disease and duration of steroid therapy were not correlated to BMD T-scores. This result is supported by that of Gulati and coworkers (2003) who reported that cumulative dose of steroids was predictive of a low BMD score. However, older age at onset of nephrotic syndrome was also a predictive factor for low score in their study. Our result is also consistent with Basiratnia and colleagues (2006) who found that higher cumulative steroid dose was associated with lower BMD in relapsing nephrotic children. The correlation of low

BMD score with steroid therapy has been also well documented in patients with asthma and rheumatoid arthritis (Bouvard *et al.*, 2010).

Our result is contrasted with those of Leonard (2007) who reported that intermittent treatment with high-dose GCS during growth was not associated and maturation in SSNS. GCS-induced obesity was associated with increased whole-body BMC and maintenance of spine BMC. It is also contrasted to the results of Mishra and associates (2009) who found that the majority of patients had normal BMD and was uninfluenced by cumulative dose of prednisolone, when other co-variants were adjusted.

Thus, we concluded that children with INS who are on steroid therapy are at risk for MBD. Children received higher doses of steroids had significantly higher PTH levels, and lower BMD T- scores. Greater cumulative steroid dose was the only factor observed to be predictive of low BMD T-scores ($P = 0.02$). Regular BMD evaluation and appropriate therapeutic interventions are recommended for these children. The role of prophylactic therapy in such patients needs to be further evaluated.

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9/3/2012

The Consequences of Divorce on Individual, Family and Society

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Abstract: Divorce is an effect of several complicated psychosocial causes. It is an obvious reason for the underlying conflicts, lacking the balance and harmony of a relationship, which lead couples through a decision making process to end up their marriage. In all cultures, divorce has not been welcome. Reviewing statistics and studies on its causes that strived to find solutions for its reduction, indicates the significance of divorce and the traces of its negative effects left on various aspects of the human society. Divorce, either directly or indirectly, affects the mental health of couples, children, relatives and friends. The lack of comprehensive and inclusive studies on this issue urged us to set the aim of this study to identify the consequences of divorce.

Keywords: Consequences, Divorce, Individual, Family, Society.

1. Introduction

Marriage is historically the most initial and globally widespread behavioral pattern of human social institution (1). Appropriate family relations are the building blocks of appropriate social relations. The more inmate ties are appropriate, the more family, and subsequently society, is firm and stable (2). If the family is the core social institution, then the roots of a healthy society lay in the healthy families. Divorce is one of the most important damages to a family. Experts believe that the process of divorce, in majority of the cases, includes: minor disagreements, serious arguments due to recurrence of such minor disagreements, separation or disinclination of one partner to the other, repeated long-term sheer offs and exhaustion caused by them, dispute, emotional divorce and ultimately legal divorce (3).One of the partners usually endure more emotional involvement and suffering during the process of divorce. It is also believed that effects of divorce are more damaging to women than men (4).

Divorce precedes neither easily nor quickly. In average, it takes at least two years for both partners to recover and regain the energy to found the bases of a new relationship on the ruins, remaining from the previous marriage (5). People try different ways like isolation, escape, oversleeping, work overload, time killing with friends, violence, ignorance and weeping to deal with the problems resulted from divorce during this two-year-period. Therefore, this key period determines the pathway of an individual's future that may lead to personal progress, development and perfection; or failure, adversity and collapse. Divorce is sometimes capable to transform love into hatred, trust into distrust, and support into aggression or vengeance (6).

2. Divorce in Iran

Divorce rate is increasing in Iran day by day; and there has never been such a tremendous collapse risk and its negative effects, threatening the core of the family as it exists today (7). The newest statistics regarding divorce rate in Iran indicate a growth (8). This growth has recently been more aggressive, so that in 2009, comparing to 2008, it is reported as a 13.8% growth (9). At the moment, Iran is placed in the fourth row of the of the divorce rate table among the countries of the world (10).

Passing through a divorce can be classified into three major stages: pre-separation, transition and restoration, and convalescence and renovation. People's experience of the first stage is diverse. Anger and loathing, frustration, surprise and perplexity are of those emotions that people may feel in this stage. Duration of this stage may likewise vary from person to person; and may take weeks, months or even years. The second stage—transition and restoration—for the majority of people, is the same experience during divorce, although its severity may differ from a person to another as well. The second stage—transition and restoration—for the majority of people, is the same as divorce experience, although different individuals, experience a degree of faint and sever. In this stage, couples can experience shock and damages in dealing with changes, grief of loss and unforeseen practical problems (11).

3. Different aspects of damages caused by divorce

According to Paul Bohannan, a number of major transitional stages in life style and attitude should be experienced, if a couple decides to divorce. He believes the divorce process consists of six overlapping stages or experiences, which may all cause difficulties and tensions, affecting couples,

children, relatives and friends. These six stages are the emotional, legal, economic, co-parental, community and psychic divorces.

3.1. The Emotional divorce

Official divorce statistics are not fully indicating couple's dissatisfaction of their marriage, for emotional divorce rates are greater than legal ones, which means some men and women lead cold, empty and silent lives together, but do not legally plea for divorce (12). In fact, some of the marriages, which does not end up with divorce, transform into marital relations empty of love, companionship and friendship that continue due to regular flow of time-consuming life (13). For Paul Bohannan, the emotional divorce, is the first stage of the divorce process and expresses the substitution of alienation emotion instead the marital relation, which is deteriorated. (14).Although a couple may continue the relation as a social group, but they are not attracted to each other and there is no trust left among them anymore. In the emotional divorce, the couple hurt one another due to feelings of sadness and despair that they experience (15). Emotional divorce implies the lack of trust, respect and love; couples instead of supporting each other tend to hurt their partner and decrease his or her self-esteem; and to exclude their partner, they look for any kind of excuse to prove a disadvantage or shortcoming of him or her (16). Emotional divorce describes a relationship, in which the partners seem good and intimate in social environments, but in private are not able to tolerate each other (17). So in emotional divorce, couples just live under the same roof, while their relationship is completely disconnected or is no longer desirable and satisfactory (18).

3.2. The Legal Divorce

According to common law of every country, dissolution of marital relationship in permanent marriage, either based on mutual consent or not, is plead by the couple's legal representative or by the court to be declared (19).

3.3.The Economic divorce

When a family is broken down, the assets and properties must be necessarily divided into two shares.

3.4.The Co-parental Divorce

Some decisions are made about the custody or visitation rights with children, their next meeting, and the financial or parental responsibilities of each parent toward children.

3.5.The community Divorce

It refers to some changes that occur in relationships with friends and acquaintances. This means that divorcee have to handle with individual reactions of those, who are aware of divorce.

3.6. The Mental Divorce

Through which individual breaks the emotional linkage and have to face the necessity of independent life. There is no empathy left, when the marriage is broken. None of the parents constitute a relation, because both of them feel lonely, which is a trauma for both of them.

4. Men and women after divorce

Mahnaz Akhavan Tafti study shows that women are more vulnerable to the social consequences of divorce. Economic problems have a similar impact on men and women, but women are more concerned about their economic future than men. Women, more than men, suffer from emotional and mental problems. Concern about the future of children, are more observed among mothers rather than fathers. Women show more concern on what people may think about their divorce than men do. Both men and women are weary of remarriage, but optimistic about the future. None of them show interest to consult to friends and are both reluctant in this case (20). Some consequences of divorce on the individual, family and social level briefly include:

4.1.Individual Level
4.1.1.The concerns of the future

After divorce lonesome is a feeling that women experience more than men. This concern is mostly about whether they will be able to find a partner again? Fear and despair of not being able to live without a husband, taking care of children single handedly and finding a job. These real concerns are the result of some realistic doubts about financial, labor market problems, raising children and change in personal and social life issues (21).

4.1.2.The Guilt

According to some experts, guilt is the most important feeling experienced by divorced of parents. The concerns about the happiness of children, in addition to their uncertain future, result various reactions that severs the guilt experienced by divorced parents (22).

4.1.3.The Loneliness

Divorced men and women may feel alone after a while, because they left the safe environment of the family, despite of all its problems and have to face the problems of life and divorce alone. Studies

show that loneliness after a divorce is experienced by women more than men.

4.1.4.Physical and psychological problems

The absence of a partner and loneliness after divorce, causes the majority of women suffer from different physical and mental disorders and discomforts after separation (23). The mental problems, of course, are far more harmful than physical ones. The severity emotional divorce is equal for parents, but other conditions can change the fact. Therefore the greater loss that is felt by divorced women is a result of socioeconomic inequality of two genders in the open society. Divorced women, due to the lack a certain social position, are dependent to their family: father or brothers. The opportunity to remarry of divorced men and women also depend on their different socioeconomic status. As a matter of fact "divorce" is considered a socioeconomic threat to women (24).

4.1.5.Duality of roles

During the divorce, women, regarding their relation with their children in addition to economic difficulties, are faced with the difficulty of fulfilling two roles. Divorced woman must act the both roles of parents, as a mother and a father. It is valid about the divorced man who has the custody right of the children (25).

4.1.6.Economic issues

Economic issues are more outstanding among divorced women. These issues affect women with low education and vocational skills severely. The reaction of other family members to divorce and separation is different. In some cases, they are worried about the economic dependence of divorcee to them. Divorce can affect socioeconomic status of a person such as his or her career. Many divorced people (whether male or female) lose their jobs after the divorce (26).

4.1.7.Social isolation and impaired social identity

Divorced parents are underprivileged by social isolation, impairment of social relations during marriage, lack of communication with external environment, loss of a confident and empathic person and lack of private place to live as their house. The attitude of community toward divorcee women, in some cases, is so negative that makes them feel that they entirely lost their social position. A divorced person is neither single nor married anymore. Therefore the community cannot define an appropriate position for divorced people and develop negative attitude toward them. They seem like people who do not want to face the realities of life; and has

preferably given priority to following their own desires rather than the interests of the institution of the family (27). In fact, a very negative assessment of divorce leads to a generalized negative assessment of a divorced couple (28).

4.1.8.Crime and delinquency

A divorced person is suddenly faced with lack of sexual gratification, since one of the functions of marriage is to meet such desires. Thus a probable increased sexual drive may lead to an unhealthy sexual relation, if the person does not have the opportunity to remarry. The probability of worsen circumstances especially increases when the divorced person is not capable to handle his or her own financial problems and ultimately he or she is driven to sexual and financial corruption (29).

4.2. Family Level

Children are the most vulnerable and defenseless members of the family who perceive their parents as their only supportive and protective guardians (30). Parent's argument is not only the cause to unbalance the secure environment of the family that affects children's physical and mental health, but also is considered as serious threat to the security of the society (31). Research results that are generally agreed by everyone indicate of long-term effects of divorce on mail children (32). Male children are vulnerable to psychological, social and educational impairments (33). Male children are more sensitive to their parent's relation and their divorce and usually react more disobedient and show destructive behavior, but female children quickly adapt themselves with the new situation and do not complain. The perhaps suppress their dissatisfaction (34). It usually takes two years of girls to find a compromise with the new environment, but boys need more time to adapt. Generally children express their disturbance through crying; however male children may show aggression, violence and decreasing educational performance and female children tend to be isolated and depressed. Parents apply more control over the children with the same gender. That means fathers do not take easy on their sons but are patient with daughters, while mothers are more kind to sons and are rigid with daughters. However, both boys and girls are equally in need of love and attention from their parents. But divorced parents generally deal with female children more affectionately. Therefore, their adaptation is slower than female children (35). Statistics confirm the point that the root of most delinquencies of children and teenagers lays in the disintegration of their warm family. The crime and suicide rates, among children of divorced parents, have dramatically increased (36).

These children are more vulnerable to sexual abuse and addiction is more prevalent among them. Studies on causes of delinquencies among children of divorced families indicates that the lack of mother figure within the family causes children to be unable to adapt, act deviant and finally escape from the family environment (36). However, existing of a family is helpful for children, when disputes and arguments within the family are not in a level that may affect children. Otherwise, the family itself turns to be a source for mental and psychological tensions and problems of the children living in that family.

Some studies show that divorce is not the cause for mental problems of the children, member to that family, but it is the constant disagreement of parents which creates a chaotic environment in the family prior to divorce, which ultimately leads to divorce, triggers misbehavior, mental or psychological disorder of the children. It is believed that divorce or separation, by itself, does not harm any member of the family; but underlying factors and problems that lead the family to the point of divorce are the main causes for mental and psychological problems children and even for parents (37).

4.2.1. Creating an urgent family

Divorce raises an urgent need for another family. One of the parents, mainly the mother of the family, due to financial problems, has no choice but to reside in her parents' house, tolerating any kind of circumstances after divorce. Although the majority of divorced women is literate and can have a job, but they steel feel to be a burden and dependent (38).

4.3. The community level

Divorce can increase the social damages like addiction, sexual abuse and other violent behaviors. On the other hand it is an effecting factor on increasing suicide rates. It shakes the very basic building blocks of the society that can ultimately lead to devaluation of the family and social values in a society (39). Studies show that unpleasant divorce experiences form negative radical and generalized attitudes among couples so that they feel that they must either avoid the opposite sex for the rest of their lives, or quickly jump to another relation escaping lonesome after divorce. Although both of these approaches seem to be normal reactions, but both of them are traumatic (40).

5. Conclusion

In the present study, the negative effects of divorce are pointed out on the individual, family and community levels. It must not be forgotten that divorce is not always a bad event. Divorce is a solution in some cases that continuation of a marriage either is harmful for the couples or at least does not meet the necessary requirements of a satisfactory mental, physical and social healthy relationship between them. Despite of the fact that divorce was considered very ethical, but today we all see that it is widely used as a solution and the new generation is no longer concerned about divorcing and it seems this social phenomenon is going to find its proper place in the society. Therefore increasing divorce rate is not totally a sign to derangement, but there are some times that it is positive. To cope with divorce, seeking help from mental health professionals and self-help groups consisting of divorced people can be helpful. Sometimes we see that, even when the couples are determined to divorce, counseling can change their decision. Some problem-solving techniques and conflict resolution skills can be useful to avoid effecting damages of divorce. For this mean it seems necessary that couples receive some consulting services that enable them to deal with the issue of divorce properly by reducing the pressure and tension between them, helping them forgetting the past, exonerating each other and break up the relation while they have both forgiven one another.

Both courts and consultants must remind them to avoid any hostile behavior toward each other after divorce.

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9/4/2012

HSE (Health, Safety and Environment) culture assessment via HSEMS (Health, Safety and Environmental Management System) (Case study in Arvandan Oil and Gas Company)

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Abstract: Nowadays it's believed that most of the accidents in industries are due to inconsideration and mistakes made by worker rather than errors in equipment and machines. So it seems that implanting an appropriate HSE culture would be a useful step toward a decrease in workplace accidents. Obviously developing an HSE culture first corrects individual behaviors then decreases human errors. Although implantation of an HSE culture deals with a number of factors, based on the results of the current study, management is the key factor. In fact values, incentives and behavioral patterns that show commitment to work methods and abilities of an HSE culture, are the main material which build the HSE culture. This study mainly discusses HSE cultures including commitment, leadership, policy, strategic objectives, organization, documentation, resources, risk assessment and management, planning, stability and revision and auditing. In this study, 175 employees of Arvandan Oil and Gas Company were chosen by Morgan table out of 320 employees then the questionnaires were distributed among them. Reliability and perpetuity of the questionnaire was calculated through Cronbach's alpha (0.971). Data from the questionnaire was transferred to data bank and statistical analyses were conducted by SPSS19 software. The desirability of the HSE culture was classified in a 5-point range using Likert's scale, ranging from "completely desirable" to "completely undesirable". Results of T and Friedman test showed that cultural elements of HSE are in a desirable level in Arvandan Oil and Gas Company.
[Almassi, Zia addin, RamazanMirzaei, Mahnaz nasrAbadi, Hedayat Allah Kalantari, Alireza Ghaseminejad. **HSE (Health, Safety and Environment) culture assessment via HSEMS (Health, Safety and Environmental Management System) (Case study in Arvandan Oil and Gas Company)**. *Life Sci J* 2012; 9(4):286-293]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 42

Key words: system, management, safety management, health and environment, culture, HSE culture

1-Introduction

Management system all around the world underwent great deal of change during this half century. These fundamental changes happened because of the limitations toward using natural resources and also because of the great cost of human resources, so that organizations tried to not only a responsible for their owner's profits but also gain a good situation in this competitive world of now. Traditional methods for increasing the efficiency concentrate on increasing production, profit and the result. In those methods the basis for production was the predictions on the market according to the current state of the market. These predictions always are accompanied by an inevitable error. Additionally, depreciation in equipment and machines affects production. So, big industries, which have a long life and different risks, are complicated to change (in their production technology) because of high fixed starting costs. On the other hand, after a while these equipment start to increase wastage and error risk. So regarding the human resources which is the inevitable part in dangers, to operate new equipment safety actions should be taken so that if managerial views changes risks could be lowered anyway.

2-Literature review

Safety has been defined differently by organizations and individuals. HSE culture was first used by International Nuclear safety Advisory Group in 1986 in IAEA's report on Chernobyl's incident. International Nuclear safety Advisory Group stated that "the term HSE culture refers to general topics, individual sacrifices and responsibility of all the people involved in any action affecting the safety in an atomic site".

Atomic Sites Monitoring Committee defines HSE culture as: "HSE culture is a direct result of values, views, perceptions, qualifications and group or individual behavioral samples that show commitment

to HSE and makes the manners and safety management's efficiency in an organization.

Cooper 2005 defined HSE culture as a result of objective interaction between individuals, tasks and organizations and a level of effort in which all the individuals try to improve safety[4].

In their researches, Cox, Lee, and Wilpert stated that HSE culture is the result of values, views, perceptions, efforts and behavioral patterns of those whose manners and commitment to health defines organizational health[10].

Also a number of scientists defined HSE culture as a collection of ideas, norms, views, roles and professional and social customs which are made of the efforts individuals do to prevent danger[13].

Richer et al defined it as "definition of experiences and interpretation of learning from work and safety which guides individual acts in the case of danger[19].

Regarding different definitions of HSE culture Wigman et al defined it this way: "HSE culture is the value or preference given to safety in any level and by any individual". HSE culture refers to the area in which individuals or groups commit to safety issues, efforts for continuous learning, consistency and correction and also learning form mistakes. Any organization trying to have a perfect HSE system should first implant an HSE culture which means safety should be a part of action from the designing phase until the end. Safety must be a routine. It could only happen if management truly believes in the importance of HSE and acts on the basis of this belief[21].

3. Studying views on HSE
3-1- Descriptive models
In this models viewer only observes the behavior and doesn't judge them. Description could be quality type (case study) or quantity type (statistical analyses). [1]

3-2- Prediction models
These models study the cause and effect relationships in organizations. In these models, different mathematical or statistical models are used which causes a better perception of the researcher about the cause and effect relationships. Generally not only prediction models aren't ahead of descriptive ones but also their made of descriptive models because first a good interpretation of what happened must occur then the causes can be identified. Since calculating all the variables affecting a behavior is hard, efficient prediction models are hard to find. Prediction models are usually resulted from repetitive observation but due to the unique properties of organizations are doubtful. An example of prediction models is Hathron's model. In this study the main

purpose was to find the cause and effect relationship between manners and efficiency[5]. They didn't find any result, in other words there was no relationship between manners and efficiency. Another problem with prediction models is that they seem unrealistic. For example take analyzing the effects of cash benefits on the HSE culture; if only employees' daily life is considered then the perfect relationship could not be found, because other organizational and individual factors affect this relationship. Additionally creating such a model requires observing the effects of all the variables. To overcome this problem some certain situations should be specified. So if the benefits are doubled or cut behaviors may change. But in real workplaces it's hard to change the benefits on a short term run just to change the safety behaviors.

3-3-Prescriptive models
These models (AKA normal models) prescribe a situation as the best situation and a behavior as the best behavior. In other words in a certain situation, prescriptive models tell the individual what to do. While the human relation theses say in which class to put the individual on the basis of what he/she does. Good prescriptive models come after the results of prediction and descriptive models. Prescriptive statements have a d good knowledge about the cause and effect relationships in any system.

Different prescriptive models
1. Cox model
Cox et al 1997 developed their model. In this model, organizational variables like management, policy, and HSE objectives affect environment and work procedures like danger assessment and this finally affects behaviors like responsibility. According to this model, managers and their actions are the main cause of any change in system.

2. Cooper model
In 2000 cooper developed a model which was similar to Geller's and its foundation was the 2-way certainty model by Albert and Androa. Regarding this model there is an interaction between mental, environmental and behavioral factors of HSE culture.

According to this model, organizational culture results from interactions between individuals (mental factor), tasks (behavioral factor) and organization (environmental factor). This model implies that individuals are affected forcibly by the environment and there is mutual effect between them. According to this model HSE culture could be assessed by questionnaire, just like behaviors can be assessed by behavior check lists and environment through monitoring and revision[2].

3. HSE culture's ladder model
The best way to understand an HSE culture is culture' ladder. Each step consists of unique properties

which is direction of last step. This ladder is like a map showing the situation form the HSE culture aspect and shows where the next step is headed. The 5 steps are as below:[6]

- Pathological
- Reactive
- Calculative
- Proactive
- Generative

1. Pathological: in this level no attention is paid to health, safety and environment and only rules are followed. HSE culture is not understood properly.

2. Reactive: in this level HSE culture is taken seriously but only in the time of danger. People say stuff like "it's dangers career, be careful" or "people who have the accident are the ones who were responsible for it".[8]

3. Calculative: organization has a system. The HSEMS is implanted successfully and HSE is taken seriously and the concentration is on numbers and statistics. Data are collected and analyzed and monitored. In this level there still are some casualties which make everyone surprised.[5,14]

4. Proactive: in this level previous incidents aren't the bases for decision. Not only previous accidents are prevented but also probable accidents in future are in the center of attention. In this level people are actually involved in HSE. People working in HSE are decreased and their only role is consulting. When the organization climbs the ladder trust and understanding is increased and people are more responsible.

5.Generative: generative organizations are of high standard organizations and try to act beyond the rules and regulations. These organizations have an honest act towards deficiency and use it for improvement not to blame. They don't expect prefect work they need better work. Management knows that employees trust it and are in touch with it[11].

4-HSEMS

In each system the thoughts determine the intervals after which a system will achieve the objectives. International companies found their experiences and background in HSE as the main factor in controlling dangers in the industries[20,18].

1. HSE is a part of management system in any organization which like any other system consists of planning, execution, controlling and correction. There are inputs like equipment, material, money, time, labor and facilities… processes like strategic planning, guiding and leading, managing, recognition and determining, analyzing and assessing, improving the behaviors… and they all lead to some outputs. But the output could be damage or danger if the system is not designed properly.

In 1974, OGP was established by companies cooperating in oil production and their members were introduced in the UN and EU[16].

Adding EMS to OHS and SMS, these two which only covered safety and health were completed and covered HSE.

OGP guidelines introduced some elements for HSE to implant and maintain this system. HSEMS consists of 7 elements[15]. Table (1) is shown in end of article.

1- Leadership and commitment

Senior management makes sure that the objectives are achieved through providing the resources needed for HSEMS. Management should make sure the necessity of HSE in understood for everybody and the actions are supported.

HSEMS must be supported according to the items below:

- Believing that the company wants to improve HSE
- Motivating the staff to improve their HSE actions
- Responsibility towards HSE
- All levels are involved in developing HSEMS
- Committing to an effective system in HSEMS[6]

2- Policy and strategic objectives

Management must define and document the objectives and policies. And make the followings happen:

- Consistency with the main company
- Consistency with productions and their effects on services and goods
- Consistency with other policies
- Having the same value as other objectives and policies
- Committing to rules and regulations
- If there no standard, setting a rational standard and applying it
- Committing to lower the risk involving in HSE to a minimum
- Committing to efforts that improve HSE performance

3- Organization, resources and documentation

This item contains the followings:

3-1-organizational structure and responsibilities:

Execution and performing a successful HSE program is amongst the responsibilities of organizations and all levels of management and leadership must get involved in it. This must be considered while designing the structure and allocation of the resources. To successfully implant HSE plans, organizational chart must contain all

responsibilities, tasks, authorities, and communications including:

- Providing labor and resources needed for HSEMS
- Making sure that every plan is consistent with HSE policy before even starting the plan'
- Gathering information about HSE topics and interpreting them
- Recognizing and recording corrective steps and improvement opportunities

3-2- resources

Senior management must make sure that there are adequate resources in order to achieve HSEMS goals. Resource allocation needs to be revised periodically.

3-3 documents

Following documents have got to be provided and controlled by the company:

- Policy, objectives and plan for HSE
- Determining and recording the responsibilities
- Explaining the HSEMS's elements and their interactions
- Connecting and explaining other documents related to the HSEMS
- Recording the results of risk and HSE assessment
- Developing ground rules related to HSE
- Procedures must be developed for special key actions
- Explaining reaction plans and responses in the cases of potential danger

4- Risk management and assessment

There is some risk in any human action. This section is devoted to:

Recognizing HSE risks and assessing them for every action, service or production and also developing risk lowering steps. Company must have practical methods to systematically identify risks and their effects and equipment needed in risks. Identification range must cover all actions from the beginning. Danger recognition must include the followings:

- In designing, building and developing stages (capital, activity improvement)
- In the normal and abnormal situations which involve emergency stops, repairing, and maintenance
- Potential incidents and situations which follow the following:
- Sabotaging the monitoring system
- Human factors including: destruction in HSEMS
- Potential risks and effects of theirs[9]

5- Planning and operation control

- This section is devoted to posture of planning the activities related to risk lowering (through assessment and management of risks). This part consists of programming for the new and current activities and managing the changes and developments needed to confront new situations. Company should embody HSE objectives in long-term programs. These programs include:
- Clarifying the objectives
- Clarifying the responsibilities in order to achieve the objectives in every level
- Incentive plans and motivating the staff to learn about HSE culture
- Processes in order to recognize good individual and group activities related to HSE
- Assessment and pursuit mechanisms[8]

6- Execution and perpetuity

This section explains about how the activities should be executed and continued. Tasks and activities must be clear before planning step. These activities in every level are as following

- Improving guideline objectives and planning senior section's activities ni accordance with HSE policies
- Providing and constituting procedures must be done by management and leadership

Management must be responsible for developing and approving tasks in accordance with procedures. Also management must make sure that controlling limitations aren't violated. Stabilizing the processes, management must guarantee the adequacy of HSE actions

7- Verification and revision

1-7 Verification

First the areas in need of verification should be recognized. Verification must include HSEMS and its range of action and its accordance with other executive actions. Verification must become a part of normal monitoring, so organization must consider the followings:

- Labor necessities and the properties of a verification team
- Monitors mustn't be involved in monitored actions in order to have a fair judgment
- The documentation and monitoring methods which could include using questionnaires, check lists, interviews or direct observance
- Accordance or non-accordance of HSEMS elements with necessities defined
- HSEMS's effectiveness in achieving executive norms

2-7 Revision

Senior management must revise the HSEMS regularly in order to make sure that its plans are still

effective. Revision must include the following but shouldn't be limited to these:

- Probable needs to change in policy and objectives and situations and permanent commitment to them
- Allocation of resources in order to implant and maintain HSEMS
- Places and sites according to risk assessment to confront emergencies[14]

5-Methodology

Form objective aspect this study can be categorized as: historical, descriptive and experimental. Also by its very nature it is: fundamental, theoretical and practical. As current study tries to improve practical knowledge in a certain area (organizational performance and happiness), in other words it's practical, this study is a practical type, and it's a descriptive type considering data gathering method and in descriptive type it can be categorized as padding because the main objective is to understand the best work situation in order to gain competitive advantages.

5-1-Hypotheses:

1) Commitment and leadership and HSE culture are in a good level in this organization
2) Policy and strategic objectives in HSE system are in a good level
3) Resource allocation and documentation and HSE culture are in a good level in this organization
4) Risk management and risk assessment and HSE culture are in a good level in this organization
5) Planning and HSE culture are in a good level in this organization
6) Execution and perpetuity and HSE culture are in a good level in this organization
7) Verification and revision and HSE culture are in a good level in this organization

6-Population and sampling

Topic territory: since the HSE matter is important both in private and governmental section, this study could be used in all organizations. All the organizations that share a property are a population. The population is all the staff in Arvandan Oil and Gas Company. The locality: this study was a case study in Arvandan Oil and Gas Company. The time territory: this study was conducted in 90-91. 175 people were chosen out of 320 according to the Morgan's table so that their staff number and names were taken and 175 people were chosen randomly. Questionnaires were distributed and 160 were completed properly by staff with different college degrees. The questions were about automation system governing the organization.

7-Reliability

One of the most important properties of measurement tools is reliability. Reliability shows the stability and consistency of the concept in question and helps users to judge the measurements. Reliability implies the extent to which the measurement tool results the same in the same situation. Cronbach's alpha is the most commonly used tool to assess the reliability.

Cronbach's alpha is weak below 0.6, acceptable on 0.7 and good above 0.8. The more it's closer to 1 the better it is (Danaeefard 1383, p489-490). Cronbach's alpha for a questionnaire is calculated by SPSS software.

According to the table below the Cronbach's alpha is 0.971 which is above 0.7 so it's acceptable.

Table(2): Reliability Statistics

Cronbach's Alpha	N of Items
.971	63

8- Analysis of the data form questionnaire

To analyze the hypotheses, H0 and H1 hypotheses are used which are stated as below:

H0: the element in question is in a desirable shape in HSE culture

H1: the element in question is not in a desirable shape in HSE culture

This study is done by one-sample test and test value equals 3 which means sig Is 3 and the mean is said to be above or below 3 that shows desirability and undesirability respectively. If sig is below 0.05 and both minimum and maximum are positive, then the element in question is above 3 and it's in a desirable level. If sig is above 0.05 element in question's mean equals 3 and it's undesirable so management should try to improve it. In the table (3) in end of article the means for HSEMS elements are calculated by SPSS software.

To analyze the hypotheses through one-sample test, the means of 7 elements of HSE is compared to the number 3 in table (4) that is shown in end of article.

Analyzing hypothesis no.1

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. Then it can be concluded that the leadership and commitment are in a desirable level in Arvandan Gas and Oil Company and senior management has developed appropriate procedures consistent with commitment in every level.

Analyzing hypothesis no.2

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. Then it can be concluded that the policy and strategic objectives are in a desirable level in Arvandan Gas and Oil Company. The objectives are firstly smart and secondly consistent with each other. Also the activities are in consistency with the policy and emphasize the permanent improvement.

Analyzing hypothesis no.3

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. This hypothesis shows that resource allocation and documentation which are a part of HSE culture are in a good level in Arvandan Gas and Oil Company. It shows that responsibilities and organizational structure are properly implanted. Also management's agents are doing great in managing HSEMS to improve HSE culture.

Analyzing hypothesis no.4

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. This hypothesis shows that risk management and risk assessment are in a good level in Arvandan Gas and Oil Company which means all potential dangers are recognized and all the risks are assessed for the activities, productions and services and also development plans are conducted to lower the risks. Also after assessing the risks all the people involved and then all the organization is told about it. This element is amongst the strong points in HSE culture.

Analyzing hypothesis no.5

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. This hypothesis shows that planning in HSE culture is in a good level in Arvandan Gas and Oil Company. This element shows that the organization has taken good steps towards reacting in the cases of emergency. The staffs are well trained through taking classes and participating in maneuvers. People are committed in the situations of danger. Amongst the strong points of planning in HSE culture is real integrity in capital, so that HSE is taken into account in every step, designing, buying and installing the equipment.

Analyzing hypothesis no.6

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. This means that execution and perpetuity in HSE culture are in a good level in Arvandan Gas and

Oil Company. Also correction is a part of the plan. Perpetuity is perfect in the company to make sure that plans are being continued just well. Identifying inconsistencies resulting from wrong decisions by the management or error in equipment or mistakes made by human resources, and the effort to correct them is being followed appropriately. Reports are done regularly and they are being taken care of systematically. Perpetuity of health in an organization shows that the organization cares about the human resources as the most valuable capitals.

Analyzing hypothesis no.7

As you can see in table 4, according to the sig that is below 0.05 this hypothesis is approved and this aspect is in a desirable level. Hypothesis H0 is approved. Revision and verification are in a desirable level in HSE culture meaning that periodic revision of the performance, effectiveness and consistency are in HSEMS. Procedures are regularly verified to guarantee permanent improvement.

Results and suggestions

Oil industry as the key industry in Iran (and also because of its complexity and, due to various dangers) needs a strong system which can identify and assess and control danger and also value the human resources as its main capital. In the Oil industry HSEMS is a necessity for all the Oil Companies which follows some objectives including: lowering casualties, profitability, social responsibility, and satisfactory. Achieving these objectives is impossible through classical management theories (like Taylor). It needs an organizational culture which is known as HSE culture. So it's necessary to understand and realize how important HSEMS is in improving HSE. Industrial environments, because of their very nature are in serious danger and the more technology grows the more dangers become more serious. In the year 1977, 250 million incidents happen all around the world, 350 thousand of which resulted in death. According to the reports of Universal Health Organization in 1994, 8 out of 10 labors were working in developing countries, 5-10 % of which had access to safety and health services. In that very year, 120 million incidents happened in these countries, 200 thousand of which resulting in death. In traditional safety engineering, safety in considered as preventing unsafe actions but new technologies has considered safety guards as naturally safe systems. Regarding the fact that human resources are the inseparable part of the system and also the main capital, and regarding that human resources aren't mistake free, human resources must be the focal point. Following suggestions are in direction with implanting a successful HSEMS:

- ✓ Resources must be allocated effectively in an organization (resources needed to maintain production equipment and resources needed to confront danger, verification and revision of the HSEMS, and improving new plans.
- ✓ Qualifications and competences are valued in an organization. People ought to be trained in accordance with their tasks.
- ✓ Adequate monitoring should be placed on contactors. They have to be assessed in the terms of consistency with HSE necessities in before, during and after contract.
- ✓ Communications within and beyond the organizations must be appropriate in the cases of danger
- ✓ There is enough documentation and there are documents for each and every action.
1.

Table (1): the elements of HSEMS

HSE elements	Definition
Leadership & commitment	Commitment in senior levels down to junior levels and proper organizational culture is the key to success
Policy & strategic objectives	Desires and would, principles and ideals regarding HSE aspects
Resource allocation & documentation	Organizing people, allocating the resources and documentation for a better HSE performance
Risk management & assessment	Identifying and assessing risk for productions, services and also steps towards improvement
Planning	Planning the steps and reactions toward emergencies
Execution & perpetuity	Execution and perpetuity and the state of correction steps
Verification a revision	Periodic verification and revision in systems performance and its effectiveness and consistency

Table (3): One-Sample Statistics for HSEMS

	N	Mean	Std. Deviation	Std. Error Mean
Commitment and leadership	160	3.2687	.83281	.06584
Policy and strategic objectives	160	3.6713	.62459	.04938
Organization, resources and documentation	160	3.3020	.64825	.05125
Assessment and risk management	159	3.4061	.80837	.06411
Planning	160	3.4896	.73864	.05839
Implementation an monitoring	160	3.7187	.63654	.05032
Review	160	3.6479	.66167	.05231

Table(4):One-Sample Test for HSEMS

	Test Value = 3					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Commitment and leadership	4.082	159	.000	.26875	.1387	.3988
Policy and strategic objectives	13.59	159	.000	.67125	.5737	.7688
Organization, resources and documentation	5.893	159	.000	.30200	.2008	.4032
Assessment and risk management	6.335	158	.000	.40611	.2795	.5327
Planning	8.384	159	.000	.48958	.3743	.6049
Implementation and Monitoring	14.28	159	.000	.71875	.6194	.8181
Review	12.38	159	.000	.64792	.5446	.7512

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9/7/2012

The Relationship between Locus of Control and Marital Satisfaction of Couples

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Abstract: The goal of the present research is to examine the relationship between locus of control and marital satisfaction of couples. The method of this research is descriptive one and of correlation kind. Statistical population includes the married students of Islamic Azad University of Sari. Research sample includes 62 couples who were selected by multi-step clustered sampling method. Research tools include locus of control (Rotter) and marital satisfaction (Enrich). Data was analyzed using of the methods of descriptive statistics and deductive statistics (The agreed correlation coefficient, X^2 square, and T-test). The findings showed that there is a significant relationship between internal locus of control and marital satisfaction of the couples. Also, results showed that there is no significant difference between the gender and the kind of locus of control, gender and level of marital satisfaction of the couples. Regarding the finding of the research, couples who suffer from decrease and dissatisfaction in marital relationship, have external locus of control, therefore, for improvement of marital relationship problems and having a coordinated, attractive and satisfactory marital relationship, it is suggested to replace internal control instead of external control.

Keywords: Locus of control, marital satisfaction of the couples

Introduction

One of the important goals of marriage is will attain the marital satisfaction .marital satisfaction is the most important issue in marital life which has the highest effect on the stability and failure of couples lives. Couples agree that they will attain their two main goals in the marriage having satisfactory marital relationship and mental agreement with the spouse. Although most individuals emphasize more on having marital relationship, satisfactory marital relationship and mental agreement are considered as two inseparable components in a successful marriage.

Therefore, a successful marital relationship is formed when satisfactory sexual relationship and mental agreement shall be created continually and interwoven to each other among the wife and husband (Ellis and Harper, 1995, page 45).

By starting the marriage and creating marital relationship, sexual satisfaction and marital satisfaction change into important variables in association with the marriage quality. Sexual satisfaction and marital satisfaction are regarded as one of the most important factors in affectionate stability of couples (Fisher & Nulty, 2008). marital relationship plays a fundamental role in intimate relationships resulted from the marriage and shared life (Crowe &Read Lee, 2000). Glasser and Glasser (2007) believe that marital relationship is a way by which the couples can really feel intimacy, sincerity and connection.

Multiple factors can be effective in formation of sincerity between the couples. Some of them are attachment, caring and sexual problems. Results of Faro jinn's investigations showed that attachment, caring and marital relationships had exclusive portion in intimacy levels of couples.

Closeness to the spouse is also assumed as the first necessary component to establish a romantic relationship. This finding that satisfaction with marital relationships had an exclusive portion in intimacy of romantic relationship is concordant and congruent with William et al. (2007) suggestion including the relationship between satisfaction with marital relationships and intimate relationships (Oliya et al, 2011).

Some regard marital satisfaction as the result of general satisfaction with the shared life, satisfaction with marital relationships and affectionate and exciting satisfaction (Shackel Ford, 2001, page27). Satisfactory marital relationship is the mandatory component of long- term marital life. The frequency or the

length of this relationship is not important, but it is important that the parties have satisfactory courtship. In an unsuccessful and unhappy marriage, it is possible marital relationship still exists but it is not satisfactory for both of them or the other partner (Glasser & Glasser, 2007, 111).

Also, other studies in respect of sexual relationship show that sexual satisfaction is highly related to marital satisfaction (Baron, 2006).

Hunt (1974) believes there is a relationship between general marital happiness and sexual satisfaction. Happy couples are more satisfied with their sexual life compared to unhappy wives and husbands and they have more sexual relationship.

Although happy wives and husbands have not stated satisfactory marital relationship as the best reason for their happiness, however, most of them are generally satisfied with their sexual relationship. %70 of them have expressed that they often agree with their spouses about sexual problems (Oliya et al, 2011 29). Also, the results of Morokoff and Gillillands investigations (1993) showed that there is a relationship between marital satisfaction and several aspects of sexual function. More importantly, sexual satisfaction of husband and frequency of marital relationship has a positive relationship with marital satisfaction. Generally, the more are negative exciting reaction and disasters faction with frequency of sexual intercourse, marital satisfaction will be lower. In view of Carlson and Dink Mayer (2002), sexual relationship of wife and husband is a barometer and shows ups and downs of marital relationship. Sexual relationship of wife and husband is the manifestation of their lives. However, marital relationship includes that part of marital life which misunderstanding is occurred about it very much. But the important problem is that how does a satisfactory marital relationship change into an unhappy and dissatisfied relationship which is often determined by bilateral disgust? For most couples, the reason for decrease in sexual relationship after several years is lieu of a closed secret. Public opinion of couples shows that the more time passes, sexual interactions will become lower and the most rapid decrease occurs during the first four years after marriage. This decrease in frequency of sexual interaction over time is also true about couples who live together but they have not married. Surprisingly, the investigations show that full- time working and part-time working

has no negative effect on sexual life of couples (Baron, 2006 p. 450). It is possible marital relationship has an undesired effect on their sexual satisfaction in different ways. Some of these effects are so delicate that there is an association between poor marital relationship and poor sexual relationship (Crowe and Reed lee, 2000, p. 35).

At the beginning of the shared life, the couples rarely experience sexuality problems. In continuing the relationship, it is possible external factors such as job and offspring create a disturbance in couple's relationship. In this case, wife and husband feel that they have physically less access to each other. If one of the partners has less tendency towards sexual relationship, the other may feel harm and attempts to conduct his/ her spouse towards sexual relationship. If lack of interaction continues, the couple's relationship becomes morepolar and the wife and husband will experience disappointment, unsuccessful, damage and harm. In this case, if one of the partners has more tendency, it is possible, he/she blames the other because of the lack of sexual intimacy. The outcome of this behavior can be lack of the couple inclination towards starting the marital relationship.

This incorrect cycle can be continued until they reach the crisis and seek for solving the problems, some couples continue the life with these same conditions (Roughan & Jennkins, 1990, 129).

In belief of Morokoff & Gillilland (1993), decrease in marital relationship of couples is often formed because of the existence of a problem in their affectionate relationship. Problematic affectionate relationships affect affectionate states of couples and healthy sexual performance is affected by it. Life events and conditions such as death of a close person, disease of a family member, occupational stress, baby birth or anxiety and problems, all can have an effect on starting sexual problems.

Also, some feelings such as treachery, rate, hostility, disturb banc, lack of self- confidence and envy can have negative effect on sexual relationship. In view of Crowe and Reedly (2000), factors which are effective on decrease in sexual relationship, include politeness and courtesy, continuous coarseness, wife and husband or marital relationship within the framework of <Parent-Child> or <Patient-nurse>, illegal relations, in balance in dominance and brevity, dispute on slight issues about lack of sexual sexuality tendency and

inability to close bedrooms door. Also, the results of Honar-parvaran research (2006). Showed that totally, %30 of women had complete satisfaction with sexual relationship, %4 of them had no satisfaction with it and %14 performed sexual activity just for the spouse satisfaction. The reasons for dissatisfaction included respectively disturbed family relationship, lack of paying attention to woman needs and inclinations, lack of sexual coordination and similarity, the effect of daily problems and difficulties, lack of sexual knowledge, poor affectionate relationship with the spouse, the existence of guilt feeling during sexual intercourse, negative attitude about sexual problems, lack of pre-caress, tormenting sexual demand, man's inability to satisfy the wife, fear from sexual dissatisfaction of the spouse, undesired sexual experiences before marriage.

In addition, in view of Glasser & Glasser (1998), the secret of decrease in sexual relationship is due to application of external locus of control by one couple or both couples against his/ her spouse.

No factor destroys sexual relationship faster and more crucial than seven destructive factors of external control (Criticism, blame, complaint, grumbling, threat, punishment and bribe). In view of them, couples who have decrease in sexual relationship and sexual dissatisfaction are involved in external control. The emergence and occurrence of it in marital relationship deteriorates sexual intimacy and marital satisfaction.

The assumption of external control is that if we feel dissatisfaction in sexual relationship, we are not responsible of such a feeling by ourselves but our spouse, the others, chance and out- of- control events are guilty. Also, in view of Glasser, for having a successful and satisfactory sexual relationship in marital life, exerting internal control (support, encouragement and assurance, listening, acceptance and friendship, respecting and negotiation) is necessary.

Regarding to theoretical patterns and findings of the present research, this general question is proposed that how should wife and husband behave to each other to achieve a satisfactory and consistent marital relationship?

The present research is in order to respond the above-mentioned question.

Research Hypotheses

1-There is a significant relationship between locus of control (internal-external) and level of marital satisfaction of couples.marital

2-There is a significant difference between the gender and level of marital satisfaction.

3- There is a significant difference between the gender and kind of locus of control.

Method and Material

Participants and Research design. The method of study is descriptive and of correlation kind. Statistical population of this research includes all married students who were studying in Islamic Azad University, Sari branch, provided that they have at least three years marital life up to 2011. Sample volume was selected based on Koch ran formula as 62 participants (31 women and 31 men) by multi-step clustered sampling method.

Instruments: For assessment of locus of control, Rooter's locus of control scale was used. Different researches have reported the validity of this scale between %70 to %81.

Also, for measurement of marital satisfaction, 47questions short from of Inrich's marital satisfaction questionnaire was used. Olson et al. have reported the validity of this questionnaire as 0.22 by calculation of alpha coefficient.

Performance approach: Referring to departments and official sectors of Islamic Azad University, Sari branch, two questionnaires of locus of control and marital satisfaction were available for 31 couples.

Each respondent answered the questionnaire individually and along with his / her spouse. For data analysis, descriptive statistical method (frequency, percentage, mean, standard deviation) and deductive statistical (the agreed correlation coefficient, X^2 khi–square and T–test) were used for comparison of independent means. For testing research hypotheses, significance level of at least %5 was selected.

Results

Statistical analysis of data by testing three hypotheses of research at confidence level of 95 percent was led to the following results.

First hypothesis: There is a significant relationship between level of marital satisfaction of couples with internal locus of control and external locus of control. (Table 1)

Table 1. Results obtained from the correlation between locus of control and marital satisfaction in couples.

	Locus	of control	Total
	Internal control	External control	
The observed frequency less then marital satisfaction mean	40	12	52
The expected frequency	46.2	5.8	52.0
The observed frequency	70	2	72
The expected frequency	63.8	8.2	72.0
The observed frequency	110	14	124
The expected frequency	110.0	14.0	124.0

Regarding to that the value of the obtained agreed correlation coefficient(x_2= 6.37, df=1, p<0.05) is more than the corresponding value in the table (3.84), therefore, zero hypothesis(Ho) is rejected. That is, there is a significant relationship between internal locus of control of couples and their marital satisfaction. Second Hypothesis: There is a significant difference between gender and locus of control. (Table 2)

Table 2. Results obtained from comparison of locus of control of couples by gender separation.

	Locus of control		Total
	Internal control	External control	
The observed frequency gender = woman	60	6	66
The expected frequency	58.6	7.4	66.0
The observed frequency	50	8	58
The expected frequency	51.4	6.6	58.0
The observed frequency	110	14	124
The expected frequency	110.0	14.0	124.0

As it is observed, the value of the observed X^2 square from the above mentioned table (p<0.05, df = 1, X^2 = 0.37) is less than the corresponding value in the table (3.84). Therefore, zero hypothesis (Ho) is not rejected. That is, there is no significant difference between two women and men groups in terms of locus of control. Third hypothesis: There is a significant difference between gender and level of sexual satisfaction of couples. (Table 3)

Table 3. T-test results for comparison of means of respondent's scores in marital satisfaction test.

Significance level of two ranges	T	Df	Standard Deviation	Mean	Number	Gender
			34.89	171.15	64	Female
0.106	1.64	60	24.4	183.83	60	Male

Regarding to the calculated T-value in the above mentioned table (T = 1.64, df/ = 60,

p<%5), the obtained significance level of 0.106 is more than %5.

Therefore, zero hypothesis (Ho) is not rejected. On the other hand, there is no significant difference between gender and marital satisfaction of couples.

Discussion

In this research, the relationship between functions of locus of control and marital satisfaction of couples was examined. Research findings show that there is a significant relationship between internal locus of control and marital satisfaction of couples. In expressing this finding, it can be said that couples with internal locus of control enjoy satisfactory marital relationship and subsequently, they are satisfied with their marital life, while couples with external locus of control suffer from decrease in marital relationship and marital dissatisfaction. And there are signs of criticism, blame, insult and humiliation, threat, punishment and in their marital relationship abundantly.

This finding is consistent and concordant with the studies and researches of Glasser &Glasser (1998, 2007), Ellis & Harper (1995), Baron (2006), Hunt (1974), Fisher & Nulty (2008), Morokoff & Gillilland (1993), as well as in domestic studies and investigations, with the researches of Honar–parvaran (2006), Oliya et al. (2011).

Generally, the results related to each of the two kinds of internal control and external control in this research represents this fact that except for marriage of two internal control, other marriages are not followed by appropriate and desired marital satisfaction and sexual satisfaction. While in marriage of two external control, it can be said that because of seven factors of internal control affection (support, appreciation, listening, acceptance,confidence, respect and negotiation) which the couples have in marital relationship, their sexual satisfaction and marital satisfaction will increase and stabilize and this is the best association and confirms the similarity in internal locus of control.

The second finding of this research showed that there is no significant difference between gender and level of marital satisfaction of couples. The previous studies on marital satisfaction generally show that the status of individual's gender doesn't predict marital satisfaction of couples (Garry, 1994, cited in Oliyaet al 2011).

But this feeling has a physical root in men. It should be considered that despite of what they want, women are emerged through physical manifestations as well as despite of what they claim, men stop at the level of physical relationship. Also, one of the other findings of this research is that there is no significant difference between locus of control and gender. Also, the third finding of this research represents that there is no significant difference between locus of control and gender.

It is recommended that more researches shall be performed in respect of this hypothesis.

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9/9/2012

Correlation between the Single Nucleotide Polymorphisms of CDH17gene and Gastric Carcinoma

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Abstract: Gastric cancer is one of the most frequently diagnosed malignancies in the world. The gene expression profile and molecular grouping of gastric cancer has been a challenging task due to its inherent complexity and variation among individuals.We sought to the correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.The method of the polymerase chain reaction - conformation polymorphism single-strand (PCR-SSCP) was used to detect genotype, combined with DNA sequencing was used to check the correctness of genotype.①The genotype frequencies of CDH17 in extron 6 A58G site: A/G type has 52 cases (76.47%), G/G type 11 patients (16.18%), A/A type 5 cases (7.35%). The allele frequencies of CDH17 in extron 6 A and G were 45.59%, 54.41% respectively.②The genotype frequencies and allele frequencies of CDH17 in extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma (P＞0.05). The genotype frequencies of CDH17 in extron 6 A58G site has correlation with lymph node metastasis and TNM grade of gastric carcinoma（P＜0.05).③The allele frequencies of CDH17 in extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type, differentiation, lymph node metastasis and TNM grade of gastric carcinoma(P＞0.05). The genotype frequencies and of CDH17 in extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma.

Keywords: Gastric Carcinoma; the CDH17 Gene; Single Nucleotide Polymorphism

1. Introduction

Gastric cancer (GC) is a world health burden, ranging as the second cause of cancer death worldwide[1]. Etiologically, GC arises not only from the combined effects of environmental factors and susceptible genetic variants but also from the accumulation of genetic and epigenetic alterations[2].In the world's wide gastric carcinoma's death rate only below lung cancer, colorectal and breast cancer. It's five years survival rate is below 20%[3]. Single nucleotide polymorphisms are the third generation of genetic markers, are the most important genetic change in humans. SNPs have characters of large quantity, stabilization and feasibility for detecting[4]. Many SNP sites have correlation ship with gastric carcinoma. CDH17 gene is a special member of cadherin superfamily. It is a gastric cancer related gene, which has its own characteristics in mediated cell adhesion[5]. We explored the correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.

2. Material and Methods

Clinical materials

The peripheral venous blood samples were collected from 68 unrelated patients with gastricl carcinoma in Henan province, China. The time of collecting samples were from December 2009 to April 2010. All patients with gastric carcinoma had treatments in the first affiliated hospital of Zhengzhou University. 1 mL Blood samples were collected from patients before surgery.

Method to identifying SNP genotypes

DNA extraction kits of spin column type was used to extract DNA. The method of the polymerase chain reaction - conformation polymorphism single-strand (PCR-SSCP) was used to detect genotype, combined with DNA sequencing was used to check the correctness of genotype.

Method of statistical analysis

SPSS17.0 statistical software package was used. About unordered categorical datas, analyse them by χ^2 test, Odds ratio(OR) and 95% confidence interval(95%CI) were used to express risk degree(OR

and 95%CI were computed by Logistic regression model). About ordered categorical datas, analyse them by rank sum test, the mean ranks were used to express risk degree. Using two-sides probability test when there were two kinds of side tests. Significant level was 0.05. Method of Bonferroni was used to mutiple comparision.
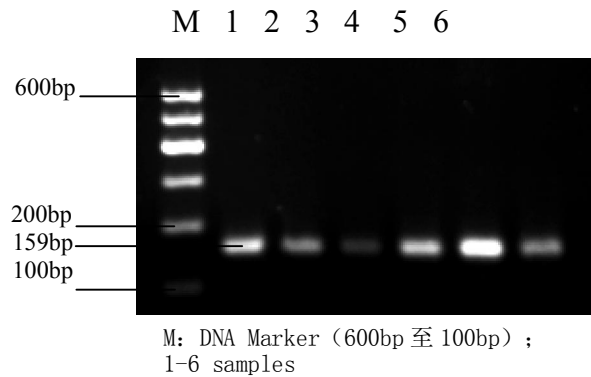
3. Results

The result of genomic DNA extracted

　Genomic DNA extracted by uv spectrophotometry identification evaluation, the A 0D260 of sample DNA is greater than 1.1 and A OD 260/ 280nm all in between 1.7-1.9.

The result of PCR

PCR products by 1.5% agarose gel electrophoresis, automatically gel imaging system observations. With DNA marker in the 159bp can saw a clear strip (Fig1).

M：DNA Marker（600bp 至 100bp）；
1-6 samples

The result of polyacrylamide gel electrophoresis

　PCR products after the thermal deformation by polyacrylamide gel electrophoresis, then silver stain the gel observaed .According to results observed to judge genotype (Fig 2)

M：DNA Marker（200bp 至 600bp）；1、2、3、4 samples
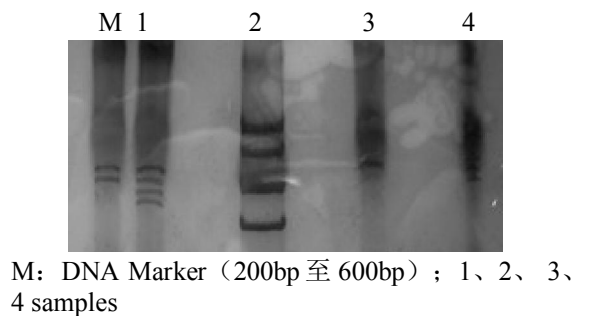
The result of DNA sequencing

　Select 10 cases have representative samples (including the belt type unexplained sample) for DNA sequencing. According to the result observed to judge genotype(Fig 3). Arrow signed for this single nucleotide polymorphisms (SNPs).
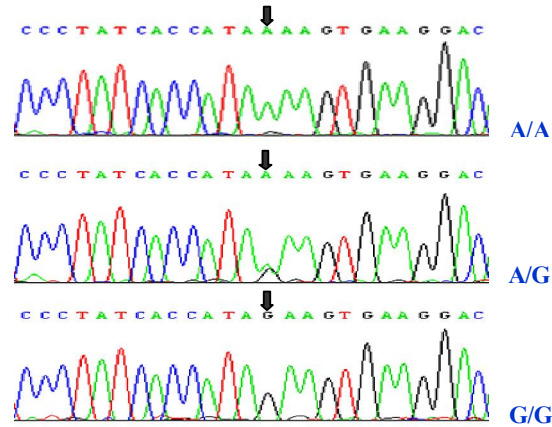
The result of statistical analysis

The correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.

　The genotype frequencies of CDH17 extron 6 A58G site: A/G type has 52 cases (76.47%), G/G type 11 patients (16.18%), A/A type five cases (7.35%). The allele frequencies of CDH17 exon 6 A and G were 45.59%, 54.41% respectively. he genotype frequencies and allele frequencies of CDH17 extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma ($P > 0.05$). The genotype frequencies of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade of gastric carcinoma（$P < 0.05$）(Table 1).

Table 1. The correlation between CDH17gene in extron 6A58Gsite and clinicopathologic parameters

Clinicopathologic parameters	n	G/ A	G/G	A/A	χ^2	P
Gender						
male	41	31	7		0.062	0.970
female	27	21	4			
Age (year)						
<50	11	8	2	1	0.111	0.946
≥ 50	57	44	9	4		
Tumor size(cm)						
<5	23	18	4	1	0.473	0.789
≥ 5	45	34	7	4		
Macroscopic appearance	12	9	2	1		
Protrude typ					1.214	0.786
Ulcera type	42	32	7	3		
Infiltrating type	14	11	2	1		
Histologic type adenocarcinoma	41	32	5	4		
adenocarcinoma and mucinous carcinoma	16	12	3	1	1.277	0.865

mucinous carcinoma	11	8	3	0		
Invasion depth						
≤muscular layer	26	21	2	3	0.207	0.902
＞muscular layer	42	31	9	2		
Differentiation degree						
well differentiated	7	4	2	1		
moderately differentiated	9	6	2	1	1.137	0.566
poorly differentiated	52	42	7	3		
lymph node metastasis						
no	31	21	9	1	1.957	0.036
yes	37	31	2	4		
TNM stage						
Ⅰstage	7	5	2	0		
Ⅱstage	28	23	4	1	4.053	0.032
Ⅲstage	21	16	4	1		
Ⅳstage	12	8	1	3		

4. Discussions

CDH17 namely cadherin 17, also known as LI - cadherin or liver - intestine cadherin located in 8q22.1[6]. Its a special member of cadherin superfamily, which has its own characteristics in mediated cell adhesion[7].Reserch has confirmed that LI - calcium sticky protein not through serial protein to connect with cytoskeleton actin , likely to be directly connected with the cytoskeleton .It has a important synergy effect on the adhesive attraction of the Classic calcium sticky protein. In normal tissue it take part in the integrity maintenance of the liver and intestinal epithelium cells[8] . Normally LI - calcium sticky protein expressed in liver cell and the intestinal mucosa ,in gastric mucosal surface is not expressed. Along with the progress of gastric cancer, LI - calcium sticky protein expression shows ascendant trend, in advanced intestinal-type gastric carcinoma LI – calcium expressed more than the early. LI - calcium sticky protein expression is more, the tumor is deeper infiltrated, the possibilities of lymph node metastasis is higher[8-13].

Nowtimes the study about the correlation between the SNPs of CDH17 gene and the carcinoma is not so many. Liu QS et al [14]results showed that a well-differentiated gastric cancer cell line had higher CDH17 expression. Down-regulation of CDH17 inhibited proliferation, adherence, and invasion of the poorly differentiated BGC823 gastric cancer cells in vitro, and induced cell cycle arrest.they confirmed that CDH17 silencing could obviously slow the growth of gastric cancer derived from BGC823 cells. Taken together,they have demonstrated that CDH17 maybe a positive regulator for proliferative, adhesive, and invasive behaviors of gastric cancer. Wang[15] et al research 164 patients with HCC patients 99 cases with cirrhosis of the liver and 293 example of healthy people found: LI - calcium glue CDH17 (651C > T protein. 35A > G) IVS6 + the T - G single figure is a genetic sensitive factor of hepatocellular carcinoma

in China crowds. Inoue M[16]et al study showed the grade of BilIN independently correlates with LI-cadherin expression in biliary intraepithelial lesions of ICC without hepatolithiasis.Lee NP[17], et al have reported aberrant expression of CDH17 in major gastrointestinal malignancies including hepatocellular carcinoma (HCC), stomach and colorectal cancers, and its clinical association with tumor metastasis and advanced tumor stages. Furthermore, alternative splice isoforms and genetic polymorphisms of CDH17 gene have been identified in HCC and linked to an increased risk of HCC. CDH17 is an attractive target for HCC therapy. Targeting CDH17 in HCC can inhibit tumor growth and inactivate Wnt signaling pathway in concomitance with activation of tumor suppressor genes. Weimann A et al[18] using immunohistochemistry detected LI-cadherin in esophageal adenocarcinoma (n = 16),.in adenocarcinoma, the expression of LI-cadherin was significantly weaker or absent.

Lee HJ,et al [19]reported expression of CDH17 was up-regulated in gastric cancer tissues. using immunohistochemistry showed that CDH17 was an independent prognostic factor in patients with stage I or node-negative disease. So CDH17 is a promising prognostic marker for early stage gastric cancer. Su MCet al[20] using immunohistochemistry showed that CDH17 Fewer than 1% of carcinomas outside the digestive system were positive for cadherin-17.the results show that cadherin-17 is a useful immunohistochemical marker for diagnosis of adenocarcinomas of the digestive system.Yasui W et al[21] expression of CDH17 were associated with an intestinal phenotype of gastric cancer. Ge Jet al[22] reported the expression of CDH17 is associated with the intestinal-type gastric carcinoma. Positive expression of CDH17 was significantly associated with the depth of gastric wall invasion, lymph node metastasis and stages of gastric carcinoma. The expression of CDH17 was significantly lower in diffuse-type carcinoma than intestinal- or mixed-type carcinoma. The patients with CDH17 expression associated with poor prognosis of gastric carcinoma,. The survival rate of patients with CDH17expression was the lowest. Dong WG et al[23] investigated expressions of Li-cadherin in gastric cancer by immunohistochemistry and semiquantitative polymerase chain reaction (PCR), and correlated this with clinicopathologic parameters in 91 cases of gastric cancer. the expression level of Li-cadherin mRNA was correlated to differentiation and lymph node metastasis, and the expression level of Galectin-3 was related to TNM staging, differentiation and lymph node metastasis. On Spearman correlation analysis, a definitive negative correlation was found

between the expression levels of Li-cadherin in gastric cancerous tissues.

Sakamoto N[24] analyzed gene expression profiles of HT-29 cells treated with EGFR ligands and identified 6 genes up-regulated by epidermal growth factor (EGF) and Transforming growth factor (TGF)-α treatment. Significant correlation was found between LI-cadherin expression and advanced T grade and N grade. Both EGFR and LI-cadherin expression was more frequently found in GC cases with an intestinal mucin phenotype than in cases with a gastric mucin phenotype. These results indicate that, in addition to the known intestinal transcription factor caudal type homeobox 2 (CDX2), EGFR activation induces LI-cadherin expression and participates in intestinal differentiation of GC.But we only finded G/G, G/A, A/A subtypes in gastrical carcinoma.

CONCLUSION:

The results suggest that the expression of CDH17 or CDX2 may be an important feature of gastric carcinoma. A combined detection of CDH17/CDX2 co-expression may benefit us in predicting the prognosis of gastric carcinoma.

For LI - calcium sticky protein gene explicit son 6A58G sites polymorphism and gastric carcinoma research did not see the relevance of reports. This experiment chose 68 cases of gastric cancer patients in Henan province as the research object, the results show: the genotype frequencies of CDH17 extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma . the genotype frequencies and of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma. This research conclution is agreed the findings of Ko[25] et al that LI - calcium sticky protein expression and gastric cancer pathological staging was associated with the lymph node metastasis. Gastric carcinoma lymph node metastasis and stomach TNM staging is closely related to the prognosis of patients with stomach cancer, and therefore may consider LI - calcium sticky protein as gastric lymph node potential transfer risk and the tumor 5-year survival rate judgment with gastric carcinoma patients.

Tan IB[26] et al have reported that intrinsic subtypes of GC, based on distinct patterns of expression, are associated with patient survival and response to chemotherapy. classification of GC based on intrinsic subtypes might be used to determine prognosis and customize therapy. Zhang J et al [27] reported Liver-intestine cadherin (CDH17) is a novel member of the cadherin superfamily implicated in gastric cancer progression. RNA interference mediated by recombinant lentivirus vectors expressing artificial CDH17 miRNA was applied to induce a long-lasting down-regulation of CDH17 gene expression in BGC823 cells. The expression levels of CDH17, tumor cell motility, migration potential, and pro-liferation were measured by flow cytometry, real-time RT-PCR, Western blot analysis, immunofluorescence staining, wound healing assay, and MTT assay, respectively. Results show that four recombinant plasmid expression vectors encoding pre-miRNA against CDH17, pcDNA-CDH17-miR-SR1, -SR2, -SR3, and -SR4 were constructed correctly and down-regulated the CDH17 mRNA levels by 5.5, 57, 91, and 98%, respectively, in BGC823 cells which had an overexpression of CDH17. They packaged the recombinant lentiviral vector for CDH17 RNA interference with pcDNA-CDH17-miR-SR4 which had the highest interfering efficiency and succeeded in construction of the stable transfectants. Of note, more than 90% knockdown of CDH17 expression in BGC823 cells was obtained by miRNA technique. The CDH17-miRNA-transfected cells showed significant decrease in cell proliferation, cell motility, and migration in comparison with the control cells. Thus, They proposed that CDH17 may be an oncogene up-regulating invasive features of gastric cancer cells and could be a hopeful target for the control of gastric cancer progression.

To sum up, the genotype frequencies and of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma. This will help to evaluate gastric carcinoma patients lymph node metastasis and 5-year survival rate.

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