CONTENTS

Unified Power Flow Controller Design based on Shuffled Frog Leaping Algorithm

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Abstract: This paper presents the application of Unified Power Flow Controller (UPFC) to enhance damping of Low Frequency Oscillations (LFO) at a Single-Machine Infinite-Bus (SMIB) power system installed with UPFC. Since UPFC is considered to mitigate LFO, therefore a supplementary damping controller based UPFC like power system stabilizer is designed to reach the defined purpose. Optimization methods such as Shuffled Frog Leaping algorithm (SLFA) and Genetic Algorithms (GA) are considered to design UPFC supplementary stabilizer controller. To show effectiveness and also comparing these two methods, the proposed methods are simulated under different operating conditions. Several linear time-domain simulation tests visibly show the validity of proposed methods in damping of power system oscillations. Also Simulation results emphasis on the better performance of SLFA in comparison with GA method.

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Keywords: Flexible AC Transmission Systems, Unified Power Flow Controller, Low Frequency Oscillations, Shuffled Frog Leaping algorithm, Genetic Algorithms.

1. Introduction

The rapid development of the high-power electronics industry has made Flexible AC Transmission System (FACTS) devices viable and attractive for utility applications. FACTS devices have been shown to be effective in controlling power flow and damping power system oscillations. In recent years, new types of FACTS devices have been investigated that may be used to increase power system operation flexibility and controllability, to enhance system stability and to achieve better utilization of existing power systems (Hingorani and Gyugyi 2000). UPFC is one of the most complex FACTS devices in a power system today. It is primarily used for independent control of real and reactive power in transmission lines for flexible, reliable and economic operation and loading of power systems. Until recently all three parameters that affect real and reactive power flows on the line, i.e., line impedance, voltage magnitudes at the terminals of the line, and power angle, were controlled separately using either mechanical or other FACTS devices. But UPFC allows simultaneous or independent control of all these three parameters, with possible switching from one control scheme to another in real time. Also, the UPFC can be used for voltage support and transient stability improvement by damping of low frequency power system oscillations (Faried and Billinton 2009; Jiang et al. 2010). Low Frequency Oscillations (LFO) in electric power system occur frequently due to disturbances such as changes in loading conditions or a loss of a transmission line or a generating unit. These oscillations need to be controlled to maintain system stability. Many in the past have presented lead-Lag type UPFC damping controllers (Guo and Crow 2009; Zarghami et al. 2010). They are designed for a specific operating condition using linear models. More advanced control schemes such as Particle-Swarm method, Fuzzy logic and genetic algorithms (Taher and Hematti 2008) offer better dynamic performances than fixed parameter controllers.

The objective of this paper is to investigate the ability of optimization methods such as Genetic Algorithms (GA) and Shuffled Frog Leaping algorithm (SLFA) for UPFC supplementary stabilizer controller design. A Sigel Machine Infinite Bus (SMIB) power system installed with a UPFC is considered as case study and a UPFC based stabilizer controller whose parameters are tuned using SLFA and GA is considered as power system stabilizer. Different load conditions are considered to show effectiveness of the proposed methods and also comparing the performance of these two methods. Simulation results show the validity of proposed methods in LFO damping.

2. System under Study

Fig. 1 shows a SMIB power system installed with UPFC (Hingorani and Gyugyi 2000). The UPFC

is installed in one of the two parallel transmission lines. This configuration (comprising two parallel transmission lines) permits to control of real and reactive power flow through a line. The static excitation system, model type IEEE – ST1A, has been considered. The UPFC is assumed to be based on Pulse Width Modulation (PWM) converters.

3. Dynamic model of the system

3.1. Linear dynamic model

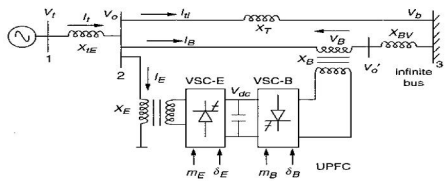


Figure 1. A Single Machine power system installed with UPFC in one of the lines

A non-linear dynamic model of the system is derived by disregarding the resistances of all components of the system (generator, transformers, transmission lines and converters) and the transients of the transmission lines and transformers of the UPFC (Wang, 2000). A linear dynamic model is obtained by linearizing the nonlinear dynamic model around nominal operating condition. The linear model of the system is given as (1).

$$\begin{cases} \Delta\dot{\delta} = w_0 \Delta w \\ \Delta\dot{\omega} = \left(-\Delta P_e - D\Delta\Delta\right)/M \\ \Delta\dot{E}'_q = (-\Delta E_q + \Delta E_{fd})/T'_{do} \\ \Delta\dot{E}_{fd} = -\dfrac{1}{T_A}\Delta E_{fd} - \dfrac{K_A}{T_A}\Delta V \\ \Delta\dot{v}_{dc} = K_7\Delta\delta + K_8\Delta E'_q - K_9\Delta v_{dc} + K_{ce}\Delta m_E + K_{c\delta\delta}\Delta\delta_E \\ \qquad + K_{cb}\Delta m_B + K_{c\delta\delta}\Delta\delta_B \end{cases} \quad (1)$$

Where

$$\Delta P_e = K_1\Delta\delta + K_2\Delta E'_q + K_{pd}\Delta v_{dc} + K_{pe}\Delta m_E + K_{p\delta\delta}\Delta\delta_E + K_{pb}\Delta m_B + K_{p\delta\delta}\Delta\delta_B$$

$$\Delta E_q = K_4\Delta\delta + K_3\Delta E'_q + K_{qd}\Delta v_{dc} + K_{qe}\Delta m_E + K_{q\delta\delta}\Delta\delta_E + K_{qb}\Delta m_B + K_{q\delta\delta}\Delta\delta_B$$

$$\Delta V_t = K_5\Delta\delta + K_6\Delta E'_q + K_{vd}\Delta v_{dc} + K_{ve}\Delta m_E + K_{v\delta\delta}\Delta\delta_E + K_{vb}\Delta m_B + K_{v\delta\delta}\Delta\delta_B$$

Fig. 2 shows the transfer function model of the system including UPFC. The model has numerous constants denoted by K_{ij}. These constants are function of the system parameters and the initial operating condition. Also the control vector U in Fig. 2 is defined as (2).

$$U = [\Delta m_E \quad \Delta\delta_E \quad \Delta m_B \quad \Delta\delta_B]^T \quad (2)$$

Where:

Δm_B: Deviation in pulse width modulation index m_B of series inverter. By controlling m_B, the magnitude of series- injected voltage can be controlled.

$\Delta\delta_B$: Deviation in phase angle of series injected voltage.

Δm_E : Deviation in pulse width modulation index m_E of shunt inverter. By controlling m_E, the output voltage of the shunt converter is controlled.

$\Delta\delta_E$: Deviation in phase angle of the shunt inverter voltage.

The series and shunt converters are controlled in a coordinated manner to ensure that the real power output of the shunt converter is equal to the power input to the series converter. The fact that the DC-voltage remains constant ensures that this equality is maintained.

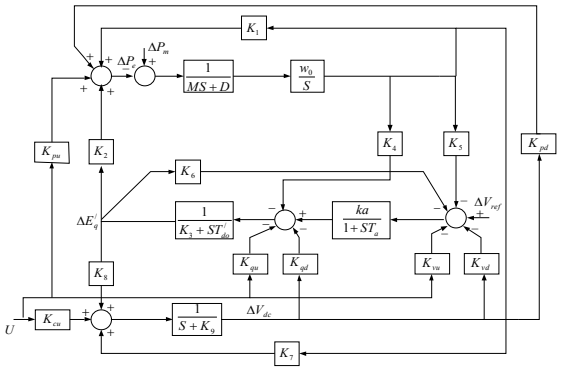


Figure 2. Transfer function model of the system including UPFC

It should be noted that K_{pu} , K_{qu} , K_{vu} and K_{cu} in Fig. 2 are the row vectors and defined as follow:

$$K_{pu} = [K_{pe} \quad K_{p\delta e} \quad K_{pb} \quad K_{p\delta b}]$$

$$K_{qu} = [K_{qe} \quad K_{q\delta e} \quad K_{qb} \quad K_{q\delta b}]$$

$$K_{vu} = [K_{ve} \quad K_{v\delta e} \quad K_{vb} \quad K_{v\delta b}]$$

$$K_{cu} = [K_{ce} \quad K_{c\delta e} \quad K_{cb} \quad K_{c\delta b}]$$

3.2. State-space model

The dynamic model of the system in state-space form is as (3).

$$\begin{bmatrix} \Delta\dot{\delta} \\ \Delta\dot{\omega} \\ \Delta\dot{E}'_q \\ \Delta\dot{E}_{fd} \\ \Delta\dot{V}_{dc} \end{bmatrix} = \begin{bmatrix} 0 & w_0 & 0 & 0 & 0 \\ -\frac{K_1}{M} & 0 & -\frac{K_2}{M} & 0 & -\frac{K_{pd}}{M} \\ -\frac{K_4}{T'_{do}} & 0 & -\frac{K_3}{T'_{do}} & \frac{1}{T'_{do}} & -\frac{K_{qd}}{T'_{do}} \\ -\frac{K_A K_5}{T_A} & 0 & -\frac{K_A K_6}{T_A} & -\frac{1}{T_A} & -\frac{K_A K_{vd}}{T_A} \\ K_7 & 0 & K_8 & 0 & -K_9 \end{bmatrix} \times \begin{bmatrix} \Delta\delta \\ \Delta\omega \\ \Delta E'_q \\ \Delta E_{fd} \\ \Delta v_{dc} \end{bmatrix} \quad (3)$$

$$+ \begin{bmatrix} 0 & 0 & 0 & 0 \\ -\frac{K_{pe}}{M} & -\frac{K_{p\delta e}}{M} & -\frac{K_{pb}}{M} & -\frac{K_{p\delta b}}{M} \\ -\frac{K_{qe}}{T'_{do}} & -\frac{K_{q\delta e}}{T'_{do}} & -\frac{K_{qb}}{T'_{do}} & -\frac{K_{q\delta b}}{T'_{do}} \\ -\frac{K_A K_{vc}}{T_A} & -\frac{K_A K_{v\delta e}}{T_A} & -\frac{K_A K_{vb}}{T_A} & -\frac{K_A K_{v\delta b}}{T_A} \\ Kce & K_{c\delta e} & K_{cb} & K_{c\delta b} \end{bmatrix} \times \begin{bmatrix} \Delta m_E \\ \Delta\delta_E \\ \Delta m_B \\ \Delta\delta_B \end{bmatrix}$$

In this research the power system oscillation-damping controller are considered for UPFC.

4. Analysis

For the nominal operating condition the eigenvalues of the system are obtained using state-space model of the system presented in (3) and these eigenvalues are shown in Table 1. It is clearly seen that the system is unstable and needs to power system stabilizer (damping controller) for stability.

Stabilizer controllers design themselves have been a topic of interest for decades, especially in form of Power System Stabilizers (PSS) (Taher and Hematti 2008; Guo and Crow 2009; Zarghami et al. 2010). But PSS cannot control power transmission and also cannot support power system stability under large disturbances like 3-phase fault at terminals of generator (Mahran et al. 1992). For these problems, in this paper a stabilizer controller based UPFC is provided to mitigate power system oscillations. Two optimization methods such as SLFA and GA are considered for tuning stabilizer controller parameters. In the next section an introduction about SLFA is presented.

Table 1. Eigen-values of the closed-loop system without damping controller

-15.3583
-5.9138
0.7542 + 3.3055i
0.7542 - 3.3055i
-0.7669

5. SFLA Overview

Over the last decades there has been a growing concern in algorithms inspired by the observation of natural phenomenon. It has been shown by many researches that these algorithms are good alternative tools to solve complex computational problems.

The SFLA is a meta-heuristic optimization method inspired from the memetic evolution of a group of frogs when searching for food (Huynh 2008). SFLA, originally developed in determining the optimal discrete pipe sizes for new pipe networks and for existing network expansions. Due to the advantages of the SFLA, it is being researched and utilized in different subjects by researchers around the world, since 2003 (Ebrahimi et al. 2011).

The SFL algorithm is a memetic meta-heuristic method that is derived from a virtual population of frogs in which individual frogs represent a set of possible solutions. Each frog is distributed to a different subset of the whole population described as memeplexes. The different memeplexes are considered as different culture of frogs that are located at different places in the solution space (i.e. global search). Each culture of frogs performs simultaneously an independent deep local search using a particle swarm optimization like method. To ensure global exploration, after a defined number of memeplex evolution steps (i.e. local search iterations), information is passed between memeplexes in a shuffling process. Shuffling improves frog ideas quality after being infected by the frogs from different memeplexes, ensure that the cultural evolution towards any particular interest is free from bias. In addition, to improved information, random virtual frogs are generated and substituted in the population if the local search cannot find better solutions. After this, local search and shuffling processes (global relocation) continue until defined convergence criteria are satisfied. The flowchart of the SFLA is illustrated in Fig. 3.

The SFLA begins with an initial population of "P" frogs $F=\{X_1,X_2,...,X_n\}$ created randomly within the feasible space Ω. For S-dimensional problems (S variables), the position of the i^{th} frog is represented as $X_i=[x_{i1},x_{i2},...,x_{is}]^T$. A fitness function is defined to evaluate the frog's position. Afterward the performance of each frog is computed based on its position. The frogs are sorted in a descending order according to their fitness. Then, the entire population is divided into m memeplexes, each of which consisting of n frogs (i.e. $P=n\times m$). The division is done with the first frog goes to the first memeplex, the second frog goes to the second memeplex, frog m goes to the m^{th} memeplex, and the $(m+1)^{th}$ frog back to the first memeplex, and so on. The local search block of Fig. 3 is shown in Fig. 4.

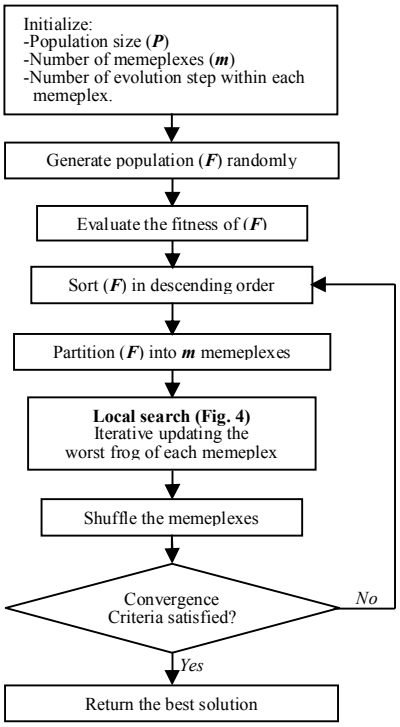


Figure 3. General principle of SFLA (Ebrahimi et al., 2011)

According to Fig. 4., during memeplex evolution, the position of frog i^{th} (D_i) is adjusted according to the different between the frog with the worst fitness (X_w) and the frog with the best fitness (X_b) as shown in (4). Then, the worst frog X_w leaps toward the best frog X_b and the position of the worst frog is updated based on the leaping rule, as shown in (5).

$$\text{Position change } (D_i) = rand() \times (X_b - X_w) \quad (4)$$

$$X_w(new) = X_w + D, (\|D\| < D_{\max}) \quad (5)$$

where *rand ()* is a random number in the rang [0,1] and D_{max} is the maximum allowed change of frog's position in one jump. If this repositioning process produces a frog with better fitness, it replaces the worst frog, otherwise, the calculation in (4) and (5) are repeated with respect to the global best frog (X_g), (i.e. X_g replaces X_b). If no improvement becomes possible in this case, then a new frog within the feasible space is randomly generated to replace the worst frog. Based on Fig. 3., the evolution process is continued until the termination criterion is met. The termination criterion could be the number of iterations or when a frog of optimum fitness is found (Huynh 2008).

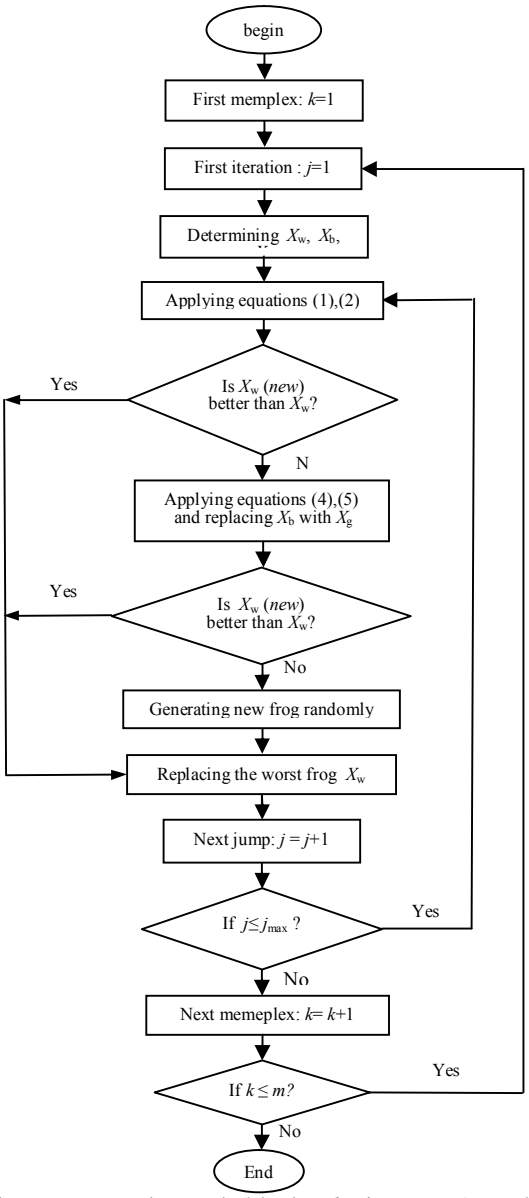


Figure 4. Local search block of Figure 3 (Huynh, 2008).

To compute the fitness value for each frog, firstly, the values of the I_{pi} variables are extracted by decoding the frog information. In this study the fitness index is considered as (6). In fact, the performance index is the Integral of the Time multiplied Absolute value of the Error (*ITAE*).

$$\text{ITAE} = \int_0^t t|\Delta\omega|dt \quad (6)$$

Where, $\Delta\omega$ is the frequency deviation, ΔV_{DC} is the deviation of DC voltage and parameter "t" in ITAE is the simulation time.

Based on Fig. 3 the local search and shuffling processes (global relocation) continue until the last iteration is met. In this paper, the number of iteration is set to be 50.

6. Stabilizer controller design using SLFA

In this section the parameters of the proposed stabilizer controller are tuned using SLFA. Four control parameters of the UPFC (m_E, δ_E, m_B and δ_B) can be modulated in order to produce the damping torque. The parameter m_E is modulated to output of damping controller and speed deviation $\Delta\omega$ is also considered as input of damping controller. The parameters of supplementary stabilizer controller are as follow:

K_{DC}: the damping controller gain

T_W: the parameter of washout block

T_1 and T_2: the parameters of compensation block

The optimum values of T_1 and T_2 which minimize an array of different performance indexes are accurately computed using SLFA and T_W is considered equal to 10.

To compute the optimum parameter values, a 0.1 step change in mechanical torque (ΔTm) is assumed and the performance index is minimized using SLFA.

The first step to implement the SFL is generating the initial population (*N* frogs) where *N* is considered to be 20. The number of memeplex is considered to be 2 and the number of evaluation for local search is set to 2. Also D_{max} is chosen as *inf*. To find the best value for the solution, the algorithms are run for 10 independent runs under different random seeds. The optimum values of T_1 and T_2, resulting from minimizing the performance index is presented in Table 2. Also in order to show effectiveness of SLFA, the parameters of stabilizer controller are tuned using the other optimization method, GA. In GA case, the performance index is considered as SLFA case and the optimal parameters of stabilizer controller are obtained as shown in Table 3.

Table 2. Optimum values of stabilizer controller parameters using SLFA

T_1	0.2187
T_2	0.01

Table 3. Optimum values of stabilizer controller parameters using GA

T_1	0.251
T_2	0.1

7. Simulation results

In this section, the designed SLFA and GA based stabilizer controllers are applied to damping LFO in the under study system. In order to study and analysis system performance under system uncertainties (controller robustness), two operating conditions are considered as follow:

Case 1: Nominal operating condition

Case 2: Heavy operating condition

SLFA and GA stabilizer controllers have been designed for the nominal operating condition. In order to demonstrate the robustness performance of the proposed method, The *ITAE* is calculated following 10% step change in the reference mechanical torque (ΔP_m) at all operating conditions (Nominal and Heavy) and results are shown at Table 4. Following step change, the SLFA based stabilizer has better performance than the GA based stabilizer at all operating conditions.

Also for case 1 the simulation result is shown in Fig. 5. The simulation result shows that applying the supplementary control signal greatly enhances the damping of the generator angle oscillations and therefore the system becomes more stable. The SLFA stabilizer performs better than the GA controller. For case 2, the simulation result is shown in Fig. 6. Under this condition, while the performance of GA supplementary controller becomes poor, the SLFA controller has a stable and robust performance. It can be concluded that the SLFA supplementary controller have suitable parameter adaptation in comparing with the GA supplementary controller when operating condition changes.

Table 4. The *ITAE* following 10% step change in the reference mechanical torque (ΔP_m) at all operating conditions

	The calculated ITAE	
	SLFA Stabilizer	GA Stabilizer
Nominal operating condition	0.0016	0.0020
Heavy operating condition	0.0018	0.0022

8. Conclusions

In this paper Genetic Algorithms and Shuffled Frog Leaping algorithm have been successfully applied to design stabilizer controller based UPFC. A Single Machine Infinite Bus power system installed with a UPFC with various load conditions has been assumed to demonstrate the methods. Simulation results demonstrated that the designed controllers capable to guarantee the robust stability and robust performance under a different load conditions. Also, simulation results show that the SLFA has an excellent capability in power system

oscillations damping and power system stability enhancement under small disturbances in comparison with GA method.

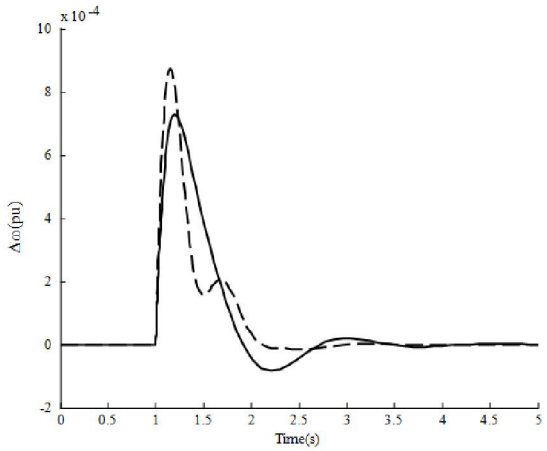
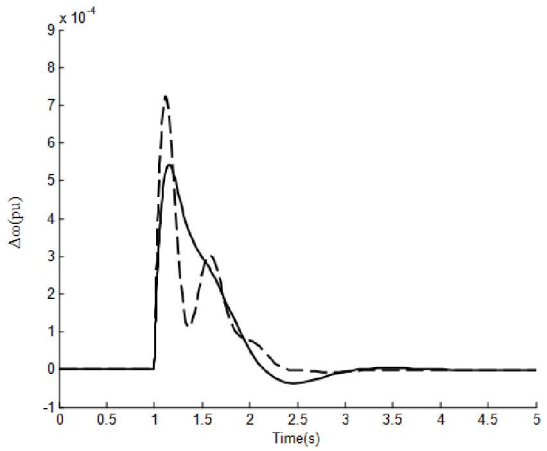


Figure 5. Dynamic response Δω for case 1



Fig**ure** 6. Dynamic response Δω for case 2

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5/27/2012

Investigation on New Eco-Core Metal Matrix Composite Sandwich Structure

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Abstract: The introduction of the eco-core sandwich panel composite is contributing a new approach to the designer to achieve high performance and light weight. These advanced natural fibre reinforced composite materials are increasingly being used in many applications including structural, aerospace, and defense and household appliances. However, the practical application for commercial use is not so widespread. High manufacturing cost can be justified if the product life cycle of the component is increased. Efforts have therefore been directed in recent years towards the development of suitable light-weight materials for many engineering applications and polymer matrix composites (PMCs) with phenolic or aluminium foam laminated composite have shown great promise in order to fulfill the current demands for structural applications. In this research project, the new kenaf eco-core sandwich panel will be developed and then laminated with aluminium for the development of new advanced composite with the aim to investigate the effects of sandwich eco-core and variable metal faces on the properties of developed composites. The final goal is to find the optimum eco-core metal matrix composite sandwich structure with maximum mechanical properties such as stiffness and buckling.
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Keywords: Composite Sandwich Structure, Kenaf, Eco-core, Aluminium, Metal Matrix Composites

1. Introduction

Natural fiber composites are gaining more and more attention due to their eco friendliness, biodegradibility and cost effectiveness. Such natural or bio fibres can generally be classified into three types [1]. "Bast" fibres, such as flax, hemp, jute and kenaf, are noted for being fairly stiff when used as a composite reinforcement. Leaf fibres, including sisal, henequen, pineapple and banana, are noted for improving composite toughness with somewhat lower structural contribution. Finally, seed or fruit fibres — cotton, kapok and coir (from coconut husks) — demonstrate elastomeric type toughness, but are not structural. Within the last five years several studies [2,3,4] have been conducted using such natural materials, like jute, kenaf, balsa, coir etc. Most if not all, of such studies have typically focused on material characterization and morphological knowldge. Now with the ever increasing necessity to go green and utilize natural materials in typical structural and engineering application, focused efforts are required to study viable application of such natural materials.

In this regard, industry incentives especially automotive industry has indeed imparted much attention and attraction to the use of such eco friendly materials on account of their properties like, light weight, eco friendly and lower cost. Now it is required to undertake exhaustive research for natural fiber materials in general so that it could be best exploited for its properties against the required performace parameters in a more cost effective manner. All such efforts are therefore directed towards exploring new alternatives to replace existing synthetic materials with natural fibres having higher strength, lower cost and environmentally friendly attributes and greater sustainability [5].

The present work would be therefore of immense valuable original addition for the greater interest to the application of new eco-core metal matrix composite sandwich structure for automotive bottom part and to the scientific community as a whole. The mechanical performance such as tensile strength and compressive strength of the composites will be reported and validated with journal [1].

2 . Materials and methods

2.1 Materials

In this research, the sandwich panel is fabricated by using layer by layer method (hand lay-up). The materials need for this construction of sandwich panel are Aluminium for its skin, natural fiber Kenaf for its core and epoxy resin with hardener, EP-B125 Hardener, EP-A125 Epoxy as the adhesive to bond the core and the skin. The length of Kenaf with long fibre is 10cm and 5cm for the Kenaf with short fibre.

2.2 Sandwich Panel preparation

The composite core materials consisted of Kenaf/epoxy and Kenaf/ epoxy with rubber having a total thickness of 10mm. Specimens with dimensions of L= 610mm, w= 305mm, 2h = 3.4mm were used.

2.3 Mechanical testing

The final products of composites were cut in accordance to the American Society for Testing Materials (ASTM) standards for the mechanical testing of polymer composite materials. The flatwise tensile tests were conducted in accordance to ASTM C297 by using Materials Testing Machine. Compression tests were carried out in accordance to ASTM 365 using a Shimadzu Universal Testing Machine equipped with 25000N load cell with the cross-head speed of 1mm/min. The test results were typically the average of the six specimens of each test.

3. Results and Discussion

3.1 Tensile properties

In this research, tensile test were carried out on the eco-core composite sandwich structure. The samples are Kenaf long fiber with rubber, Kenaf long fiber and Kenaf short fiber without rubber. Tensile strength is determined at relatively high deformations where any weakness is magnified, reducing in the process of stress transfer. From Fig. 1, long fibre with rubber will give better result compare to others. This is due to the increase in the inherent ductility of the matrix can often only be achieved at the expense of other important properties of the adhesive. The stress that obtained from this test is 31.5MPa for long fibre with rubber, then 27.6MPa for long fibre and 27.5MPa for short fibre.

The tensile strength of Kenaf long fiber is slightly increased by 0.2% compared to Kenaf short fiber. This small increment occurred because of epoxy resin is not well distributed on Kenaf fiber (core). When epoxy is not well distributed it will caused weak interface between core and aluminium plate. A weak interface results in low stiffness and low strength but high resistance to fracture. Another factor causing this problem is by using nonwoven fibers, where the Kenaf fibers will not be distributed uniformly.

The highest tensile strength was obtained for the long fiber with rubber modification. This was due to it possessed long and continuous fibers to resist and transfer the tensile force whereas the short fiber contained short, broken and discontinuous fibers. The distribution of Kenaf fibers plays an important factor to obtain good mechanical properties of sandwich panel. When the stress applied to the matrix, it will be transferred to the fibers across the adhesive. The skin and the Kenaf fiber will experience different tensile strains because of their different modulus. In the region of the short fibers ends the strain in the fiber is less and this result the fibers is stressed in tension.

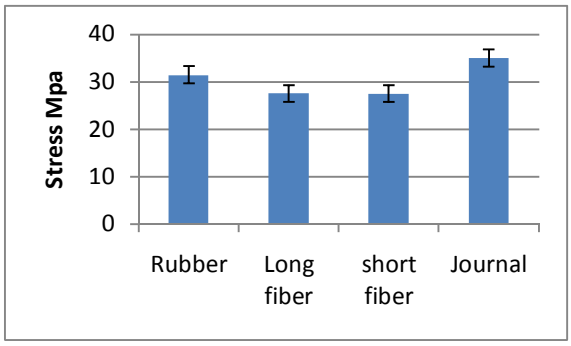

Figure 1. Comparison of the tensile strength for three different types of eco-core with Journal

3.2 Compressive properties

The compressive loading is applied to the specimen until the failure of occurs on Aluminium sandwich panel. The compression tests were performed to find different in strength of the sandwich panel when compressive loading was applied to the samples surface. The specimens of flatwise compression test have dimension 150 mm x 30 mm x 10 mm. The compression loading was applied to the specimen until the failure of core occurs. The test result from the Fig. 2 shows that the value of long fibre reinforced with rubber giving the best result compare to long fibre and short fibre without rubber. The increment by 0.4% of Kenaf long fibers with rubber compared to Kenaf long fibers without rubber is due to rubber toughening. It is expected that rubber contributing to toughening by initial yielding in the surrounding adhesive which is the conventional mechanism of epoxy rubber toughening. Rubber plays an important role in order to increase their toughness without significantly impairing the other desirable engineering properties. Thus, such rubber-toughened epoxy adhesives can be used in applications where a very high impact resistance is required for the adhesively-bonded joint.

From the result also, there are some factor may be contributed to the reading. The first factor may be due to fibre criteria. During construction, fibre is sieved manually by hand. This is possibly the reasons for the length of fibre that obtained is inconsistent. In order to get good result, Kenaf length should be measured precisely especially for long fibre and for short kenaf fibre should undergo sieve by machine. In this test, it covers the determination of compressive strength and modulus of sandwich core. These properties are usually determined for design purposes in the direction normal to the plane of facings as the core should be placed in a structural sandwich construction.

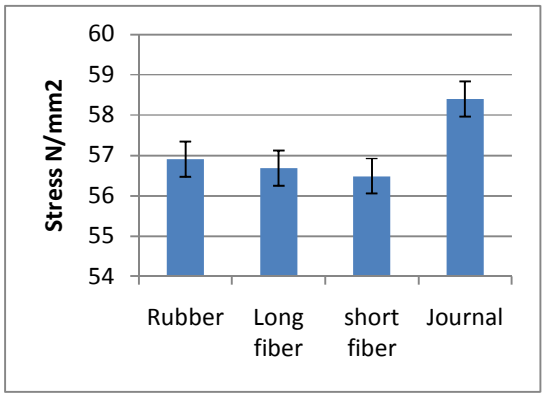

Figure 2. Compressive strength for three different types of eco-core

4. Conclusion

Kenaf long fiber with rubber showed great improvement in tensile strength and compressive strength. In addition, by combination of porosity and light weight inner core with the fibrous outer bast, kenaf core will produce strong yet light material. The factors which determined properties of composite materials are the chemical strength characteristics of the interface between the fibers and the matrix is particularly important in determining the properties of the composite [8]. The interfacial bond strength has to be sufficient for load to be transferred from the matrix to the fibers if the composite to be strong. Besides, the shape, size orientation and distribution of the fibers and matrix significantly affect the properties of the composite. Ideally, a composite should be homogeneous. Maintaining a uniform distribution of Kenaf is an important factor to improve the properties of sandwich structure. The orientation of the reinforcement within the matrix affects the isotropy of the sandwich panel. Furthermore, rubber modified epoxy resin help to improve mechanical properties of the sandwich panel.

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5/27/2012

Mechanical Characterization, Fabrication and FTIR Spectroscopic Analysis of Fish Scale Reinforced Epoxy Composites

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Abstract: This paper describes the processing and characterization of a new class of epoxy matrix composites reinforced with short fibers obtained from the scales of a fresh water fish (Labeo rohita). The functional groups involved in the formation of the resulting composite are identified. Fourier Transform Infrared (FTIR) spectroscopic analysis shows that the formation of hydrogen bonds occurring at the fiber-matrix interface between the oxygen atom of the epoxy and hydrogen atom of the polypeptide chain of fish scale is responsible for the formation of this new class of composites. These composites possess improved micro-hardness and exhibit tensile and flexural strengths marginally different from those of neat epoxy. These composites are expected to find applications as potential materials for conveyor belt rollers, pipes carrying pulverized coal in power plants, pump and impeller blades and also as low cost housing materials.
[Reza Atefi, Ali Razmavar, Farhad Teimoori, Farshad Teimoori. **Mechanical Characterization, Fabrication and FTIR Spectroscopic Analysis of Fish Scale Reinforced Epoxy Composites.** Life Sci J 2012;9(2):1080-1082] (ISSN:1097-8135). http://www.lifesciencesite.com. 160

Keywords: Polymer composites, Bio fibers, Fish scale, FTIR

1 Introduction

Recently the critical discussion about the preservation of natural resources and recycling has led to the renewed interest concerning biomaterials. Because of increasing environmental consciousness and demands of legislative authorities, use and removal of traditional composite structures, usually made of glass, carbon or aramid fibers being reinforced with epoxy, unsaturated polyester or phenolics, are considered critically [1]. Over the years, many researchers have also reported on the performances due to the poor interfacial bonding between the hydrophilic natural fibers such as sisal, jute and palm fibers and the hydrophobic polymers [2]. But in spite of this, there has been a growing global attention on natural fibers primarily because they are environment friendly and are of very low cost. Bio-fibers like animal whiskers and poultry feather are also recently drawing attention of researchers [3]. But the potential use of fish scale fiber in composite making has not been explored so far.

Fish is one of the most abundantly available aqueous species and its scale, regarded as a waste material, can be gainfully converted to value added products. Although some earlier works studied fish body scales to strengthen their role in fish taxonomy [4], the available reports on fish scale research are relatively less. Ikoma et al. [5] have studied the micro structural, mechanical, and bio-mimetic properties of fish scales from Pagrus major. However, reports available on composites using fish scale are rare. Against this background, the present investigation is undertaken to study the possible utilization of fish scales in polymer composites.

2 Experimental Details

2.1 Materials

Epoxy LY 556, chemically belonging to the 'epoxide' family is used as the matrix material. The mature fish scales of Labeo rohita are washed in water to remove adhering dust and soluble surface impurities. The scales are allowed to dry in sunshine for two days and are then kept in an oven at 70°C till they become crispy. The dried scales are then cut into short flakes of dimension, approximately 6-8mm in length and 1mm in width. These flakes are used as the reinforcing phase.

2.2 Composite Fabrication and Characterization

The low temperature curing epoxy resin and corresponding hardener (HY951) are mixed in a ratio of 10:1 by weight as recommended. The dried flakes of fish scales in four pre-determined weight proportions (0, 5, 10 and 15 wt %) are reinforced with random orientation into the epoxy resin. The castings are put under load for about 24 hours for proper curing at room temperature. Specimens of suitable dimension are cut using a diamond cutter for physical and mechanical characterization. Micro-hardness measurement is done using a Leitz micro-hardness tester. The tensile test is performed in the universal testing machine (UTM) Instron 1195. 3-point bend test is performed on these samples to evaluate the flexural strength as per ASTM standard (D2344-84).

2.3 FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is an analysis technique which detects characteristic functional groups in molecules of any matter [6]. On interaction of an infrared light with the matter, chemical bonds stretch, contract and bend and as a result, each functional group tends to absorb IR radiation in a specific wavelength range regardless of the structure of the rest of the molecule. Based on this, functional groups present in the composite are identified. In the present work, spectroscopy is performed using a FTIR spectrophotometer interfaced with IR microscope operated in reflectance mode (Perkin Elmer Spectrum RX-1).

3.1 Mechanical Properties

The tensile strengths of the composites with fiber content of 5 wt%, 10 wt% and 15 wt% are recorded as 66 MPa, 63.65 MPa and 62.36 MPa respectively where as that of neat epoxy is about 70 MPa. However, the incorporation of flakes results in only marginal improvement in the flexural strength. The hardness values of the composites with fiber content of 5 wt%, 10 wt% and 15 wt% are recorded as 42.4 Hv, 42.95 Hv and 43.75 Hv respectively. For hardened neat epoxy, this value is found to be 41.75 Hv. It is thus seen that the hardness is improved by the incorporation of fish scale short-fibers.

3 Results and Discussion

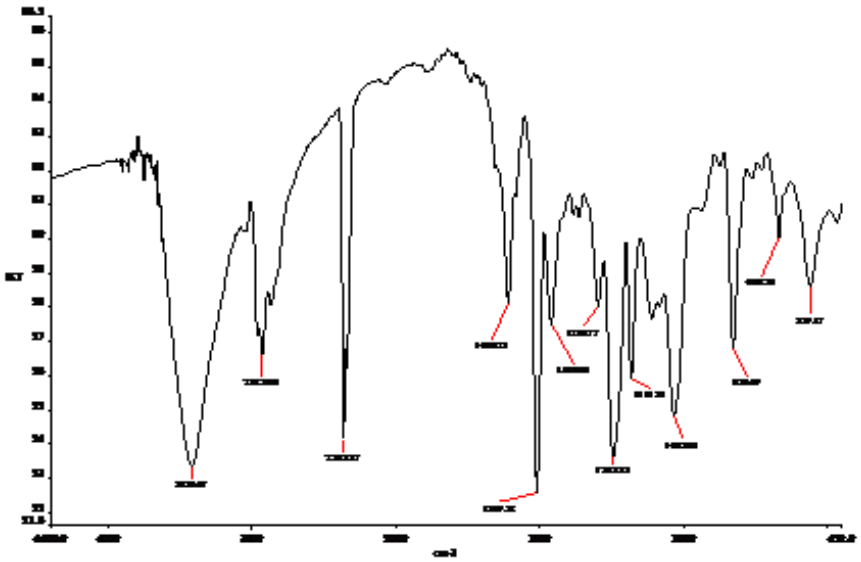

Figure 1. FTIR spectra of epoxy resin

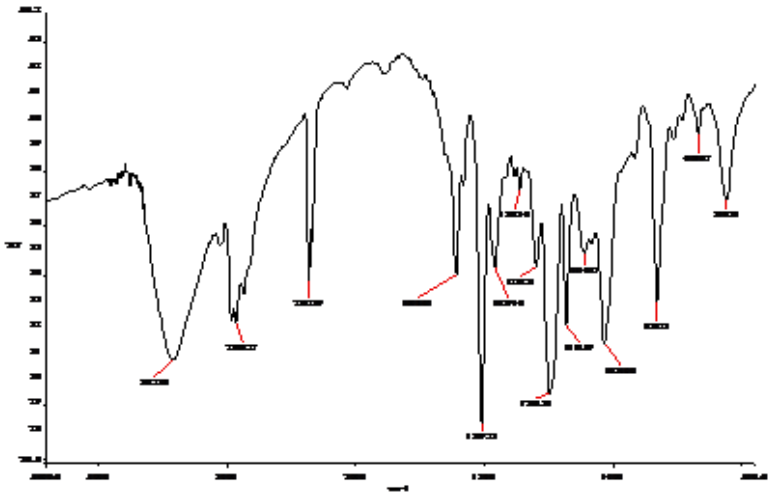

Figure 2. FTIR spectra of epoxy- fish scale composite

functional groups present in the raw fish scale as obtained from the FTIR spectroscopic analysis are identified as C – H in methyl, N – H in amine, C – N in amide and H – bonding in amide linkage. Similarly, Figure (1) presents the spectra of epoxy resin which reveal the presence of different functional groups such as benzene, ether, epoxide, alcohol etc. The IR spectra for the fish scale reinforced epoxy composite are given in Figure (2). This gives us an idea of the rearrangement of the functional groups during composite formation. On comparing Figures (1) and (2), it can be seen that the IR peaks are almost similar but in case of Figure (2), these are laterally shifted towards lower frequency region. Moreover the peak at 3422 cm^{-1} is relatively shorter and wider in Figure (2) as compared to Figure (1). This leads to the conclusion that the hydrogen bonds are formed between the oxygen atom of the epoxy and hydrogen atom of the polypeptide chain of fish scale. This is schematically illustrated in Figure (3).

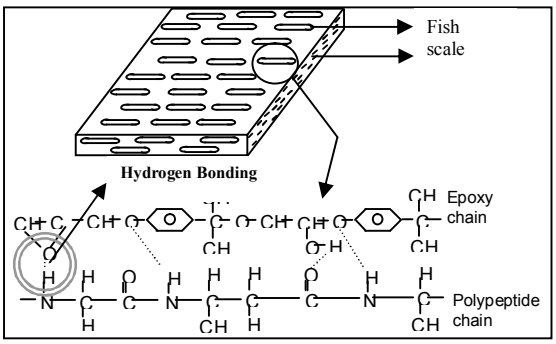

Figure 3. Chemical bonding between elements of epoxy matrix and the poly-peptide chain of the fish scale in the composite

The newly formed hydrogen bonds between O---H in the composite leads to weakening of all other bonds present in epoxy due to stretching of bonds. This phenomenon is supported by the lateral shifting of respective IR peaks in the composite. Since all the hydrogen atoms of polypeptide chain cannot take part in this hydrogen bond formation with oxygen atoms in epoxy chain, the interface between the epoxy body and the scale does not exhibit high strength.

4 Conclusions

Successful fabrication of epoxy matrix composites reinforced with flakes of fish (Labeo rohita) scale is possible. The FTIR spectroscopic analysis shows that the formation of hydrogen bonds occurring at the fiber-matrix interface between the oxygen atom of the epoxy and hydrogen atom of the polypeptide chain of fish scale is responsible for the formation of this new class of composites. This study opens up a new avenue for utilization of a bio-waste like fish scale.

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Investigation on Weldability of new ferritic stainless steel for exhaust manifold application

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Abstract: In the current context of fossil energy scarcity, car manufacturers have to optimize vehicles energy efficiency. This global and continuous improvement includes a change of the exhaust manifold design. Usually in cast iron, exhaust manifolds tend to be mechanically welded in order to fit new constraints such as lightness, durability, efficiency and small size. To achieve such requirements, ferritic stainless steels with high chromium content (19%) and molybdenum (2%) are developed. For the welding, the use of existing filler wire does not satisfy fully the application requirements. This leads to oxidation problems and / or thermal fatigue strength that drastically reduces assembly lifetime. New flux cored wires are developed in the context of this study in order to provide molten zone characteristics close to those of the base metal. Different chemical compositions are tested in order to highlight the influence of stabilizing element on microstructure. Welding tests revealed the major influence of titanium on the grain refinement in the molten zone. A minimum Ti content of 0.45 weight % in the filler wire is required to be efficient as grain refiner.
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Keywords: ferritic stainless steel, automotive exhaust, stabilization, columnar to equiaxed transition, welding.

1 Introduction

In an environment of industrial competition, ecological and economic arguments are crucial today, especially in the automotive sector. The manufacturers are adopting a global and continuous improvement approach, in particular to optimize the energy efficiency of their vehicles. Weight gain and increased motor efficiency, resulting in an increase in temperature of exhaust gas, are thus two major explored ways. The exhaust system is a set of complex parts, and the manifold is the part closest to the engine, so the hottest and most critical. Conventional cast iron design has evolved to manifolds with welded thinner steel sheets more appropriate for current constraints, lighter, with better resistance to service conditions (corrosion, thermal cycling, mechanical stress, and high temperature service). This evolution involves the development of new materials and assembly solutions adapted to these new technical and economic constraints. Among the various materials for such applications, ferritic stainless steels are certainly the most promising because they combine good corrosion resistance and advantageous properties at high temperature, for moderate cost compared to austenitic stainless steels.

In order to provide a solution to car manufacturers, ARCELOR MITTAL has developed a ferritic stainless steel, designated thereafter K44X. This material, produced in the form of thin sheets (0.6 to 2.5 mm), has a special composition (Table 1) enables to guarantee specifications of the application, especially for the corrosion resistance and mechanical strength at high temperature. However, commercial welding filler materials which could correspond to the listed constraints do not exist.

Table 1. K44X chemical composition.

K44X	Composition [weight %]												
	C	Si	Mn	P	S	N	Cr	Cu	V	Ni	Mo	Ti	Nb
	<0.022	0.6	0.3	<0.03	<0.05	<0.025	19	<0.3	0.1	<0.025	1.9	<0.01	0.6

Ideally, the filler material and the welding process should allow obtaining welding with similar properties than the base material. However, the welding operation generally modifies the composition and the microstructure. For instance, nitrogen and carbon, that can be dissolved in liquid metal during welding, have very low solubility in the ferritic matrix, resulting in hardening and embrittling effects. Then, chromium carbides can be formed at grain boundaries, creating a deficiency of chromium in the matrix, and promoting intergranular corrosion. To prevent this phenomenon, low contents of niobium and/or titanium are generally added, to promote Ti or Nb carbide and nitride formation

instead of chromium carbide [1]. If the niobium excess remaining in solid solution in ferritic matrix can improve the mechanical strength at high temperature, the titanium excess has generally a detrimental effect on the toughness, so its content must be adapted to the C and N content in the material [2]. To help to the determination of titanium and niobium contents required for a complete stabilization, some authors proposed "practical formula", more suited than stoechiometric formula [3-6]. The addition of Ti and Nb can also limit grain growth during the welding cycle, which is an important problem for ferritic stainless steels, by pinning effect thanks to the formation of precipitates at grain boundaries [6]. Finally, large columnar grains can be formed in the molten zone [1], having a detrimental effect on the mechanical properties of the assembly, especially on the fatigue behavior. With a titanium addition it is possible to obtain a molten zone with fine equiaxed-grains, due to the precipitation of titanium nitride particles in the liquid metal, acting as nucleation sites for equiaxed grains during solidification [7].

The goal of this study is to develop and test various metal cored wires (MCW), intended for ensuring mechanical properties, especially thermal fatigue and corrosion resistance, of assemblies of grade K44X welded with processes currently used in the automotive industry. In this paper we show the first results concerning the effect of the composition of the filler wire and the welding cycle on the grain structure of the molten zone.

2 Metal core wire development and welding tests

The composition of the welded zone has to be as homogeneous as possible as the base metal, so the filler metal must contain similar contents of chromium, molybdenum and niobium to insure good corrosion resistance and high temperature strength, and to limit grains growth at high temperature. In addition, it has to contain titanium to promote equiaxed grains nucleation.

To adjust the composition, the choice of a metal cored filler wire is convenient because the additional elements can be introduced as powder mixture in the core. The external foil is an AISI 409 stainless steel, containing 12% Cr and 0.2% Ti. Cr, Mo, Nb and Ti additional contents are then adjusted in the powder mixture of the core, to obtain the desired composition in the weld metal (Table 2). Here we must distinguish the compositions of the filler wire, of the deposited metal, and of the molten zones obtained after welding. The composition of deposited metal is so different from the filler wire, because some elements suffer losses by vaporization or reaction during the phenomenon of chemical transfer in the arc. Similarly, the weld metal has a different composition to the deposited metal, due to dilution with the base metal. Because of these phenomena, which depend on welding energy, the relationship between the filler wire composition and the desired molten zone composition is not direct.

First analyses on deposited metals have revealed nitrogen contents between 0.012 % and 0.023 % and carbon contents between 0.021 % and 0.037 %. The titanium and niobium contents required to stabilize the steel in the molten zone have been chosen from these values using practical stabilization formula, which are more common than stoechiometric formula [3-6]:

$$\%Nb = 0.2 + 7\ (\%C+\%N) \quad (1)$$

$$\%Ti = 0.15 + 4\ (\%C+\%N) \quad (2)$$

$$0.2 + 4(\%C+\%N) < \%Nb+\%Ti < 0.8 \quad (3)$$

From these data, a calculation of stabilizing elements contents to be added in the filler wire was made taking into account the dilution of base metal in the molten zone, and the transfer rate of the elements in the arc. Finally, seven welding wires have been realized with different contents of titanium and niobium, in order to allow us to study the effect of these elements.

Table 2. Desired composition of deposited metal.

Composition (weight %)												
C	Si	Mn	P	S	N	Cr	Cu	V	Ni	Mo	Ti	Nb
0.01	0.6	0.3	<0.03	<0.005	0.01	19	<0.3	0.1	<0.25	1.9	**0 to 0.6**	**0 to 0.8**

A Gas Metal Arc Welding (GMAW) process using a DIGIWAVE® generator has been used for all the welding tests. The welding gas is a mixture of Ar with 2% CO_2 (AIR LIQUIDE ARCAL 12), usually used for welding stainless steel in automotive industry. Two set of welding parameters have been determined for each filler wire, corresponding to different transfer modes, pulsed or short-circuit. To have a good transfer, electrical parameters (voltage and intensity) have been adjusted for each filler wire, so the electric power is slightly different for each one. However, for each filler metal, the electric power and the welding speed always are higher in pulsed mode than in short-circuit mode.

Table 3. Welding parameters

Transfer mode	Electric power [W]	Welding speed [cm.min^{-1}]	Linear welding energy [J.cm^{-1}]
Short-circuit	1620-1840	50	1945-2205
Pulsed	2540-2675	70	2180-2290

3 Results: morphology of welded joints and grains in the molten zone

The results presented below are focused on microstructure observations and chemical analysis associated.

For all the compositions of filler wires, stable pulsed and short-circuiting transfers produce regular welded joint. The macrographs performed on the specimens allow us to calculate the dilution rate, which affect the composition of the molten zones. Dilution rates are lower in short-circuiting mode (between 19 and 37%) than in pulsed mode (40 to 50%), that could partly explain the lower content of titanium measured in the molten zone for each filler wire in pulsed mode.

The macrographs also highlight the geometric characteristics of the welds (Fig. 1). The beads obtained in pulsed welding show a better wetting than those obtained by short-circuiting. Based on the results of chemical analysis, we observe a detrimental effect of titanium content in the wetting of the molten zone for a given transfer mode, has show the comparison between Fig. 1A and 1C.

Micrographic observations show that depending on the thermal conditions (transfer mode) and on the filler wire composition, it is possible to obtain three types of molten zone structures (Fig. 1):

- Columnar grain structure with growth direction perpendicular to the welding direction (Fig 1 A);
- Equiaxed fine-grained structure (Fig. 1 C);
- Mixed structure comprising an equiaxed fine-grained and columnar grain zones (Fig 1 B).

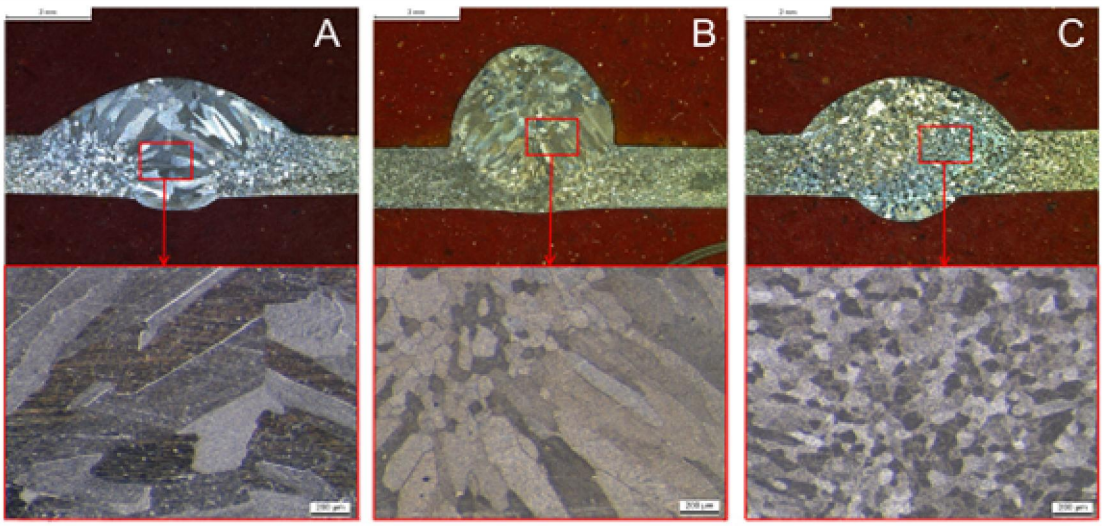


Figure 1. macrographs and micrographs of the molten zone: A-pulsed transfer, 0.10%Ti, 0.021%N, 0.036%C; B-short-circuiting transfer, 0.11%Ti, 0.0205%N, 0.035%C; C-pulsed transfer, 0.15%Ti, 0.0231%N, 0.037%C.

According to all these results, it seems there is a minimum Ti content of about 0.12 % required in the molten zone to produce grain refinement. Because of losses due to the transfer and to the dilution, which depend on energy parameters and transfer mode, the amount of Ti contained in the filler wire must be higher, typically about 0.45%.

3 Discussion

Depending on the different parameters (chemical and thermal), the solidified zone can take two morphologies, columnar or equiaxed [7]. This microstructural change, commonly called Columnar to Equiaxed Transition or CET, has an important influence on the mechanical properties of the molten zone, a fine equiaxed grain structure conferring a better fatigue behaviour than a large columnar structure.

Basically, the occurrence of a CET requires two conditions [8]:

- Potential nuclei or embryos must exist ahead of the advancing columnar front (heterogeneous solidification);
- Favourable thermal conditions must allow embryos to grow and block columnar growth.

In stainless steels, titanium has a major effect on the CET, because titanium nitride particles act as nuclei for ferritic equiaxed grains nucleation, whereas we haven't seen any effect of the niobium, probably because niobium nitride or carbides are formed after the beginning of the ferrite solidification.

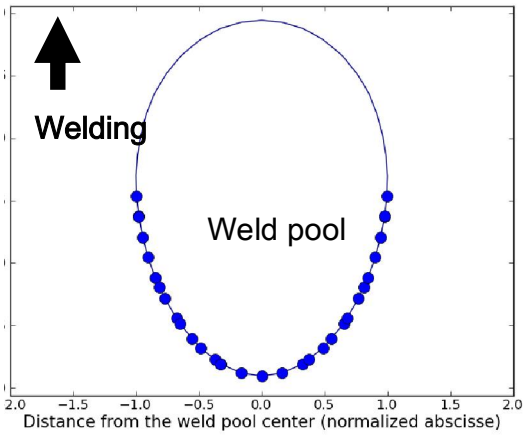
Considering only the thermal aspect, columnar growth is favoured by a high temperature gradient and a rather low growth rate of columnar grains, depending on welding speed. However, in welding, both growth rate and temperature gradient change along the solidification front at the rear of the weld pool (Figure 2 A). A numerical modelling of the thermal phenomenon during welding allows us to evaluate these parameters along the border of the weld pool. Figure 2 B shows the evolution of both parameters from the edge of the weld pool to the centre. As well known, the temperature gradient is maximal on the edge and continuously decreases down to a minimal value in the centreline of the weld. At the opposite the growth rate is close to zero at the edges of the weld, and increases up to the welding speed in the centreline. Then, columnar growth is favoured in the edges, but if the solidification rate becomes too high relatively to the temperature gradient, a critical supercooling region appears before the columnar dendrite tip, and equiaxed grains can be formed, leading to a CET.

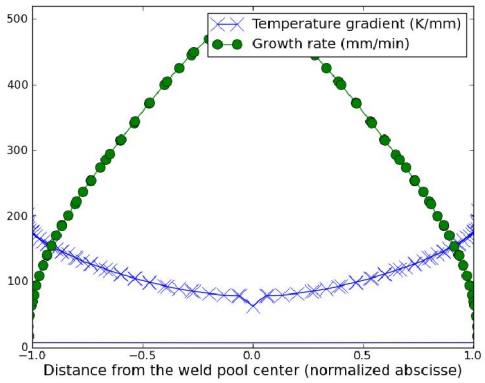
For heterogeneous nucleation of equiaxed grains, solid particles, here titanium nitride, must be present in the supercooling region. Supposing all these solid particles have the same size, we can consider that all equiaxed grains instantaneously nucleate for the same supercooling range ΔTn [10].

Then the volumic fraction of equiaxed grains is controlled both by their growth rate, and by the advancing rate of the columnar front. Supposing the growth rate of equiaxed grains is proportional to ΔT^2, where ΔT is the supercooling range, i.e. the difference between the liquidus temperature of the steel and the columnar dendrite tip temperature [10], then we can estimate the volumic fraction of equiaxed grains f_{eg} by the Eq. 4:

$$f_{eg} = n_g \frac{4\pi}{81} \frac{A^3}{(GV_g)^3} \left(\left(\frac{V_g}{A} \right)^{3/2} - \Delta Tn^3 \right)^3 \quad (4)$$

where n_g is the grains density (corresponding to the density of TiN particles in the supercooling region), G the temperature gradient, V_g the growth rate of columnar dendrites, and A is a constant.





A B

Figure 2: Temperature gradient and growth rate of columnar grains (B) along the rear border of the weld pool, shown in top view (A)

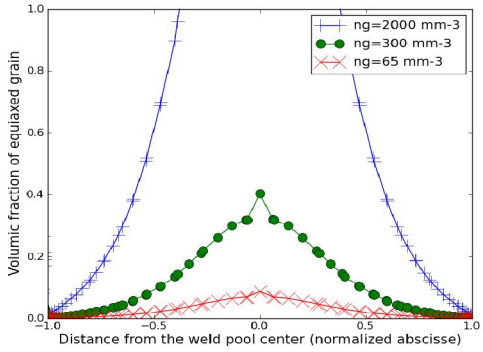


Figure 3. Evolution of volumic fraction of equiaxed grains for 3 grains densities corresponding to the micrographs A (n_g=65 mm^{-3}), B (n_g=300 mm^{-3}) and C (n_g=2000 mm^{-3})

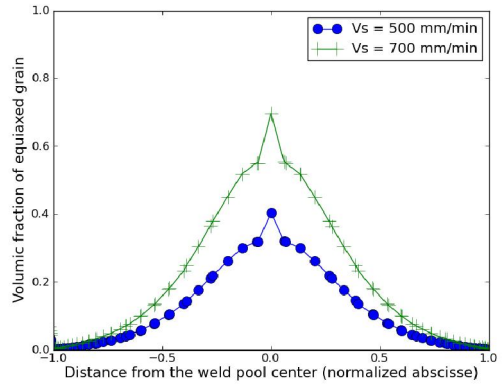


Figure 4. Evolution of volumic fraction of equiaxed grains for a grain density of 300 mm^{-3} and for the two welding speeds

Fig. 3 shows the evolution of the equiaxed grains fraction f_{eg} for the 3 grains densities deduced from the micrographs (Fig 1). The results obtained with this simple relation predict a completely equiaxed structure, except on the edges, for case C, a medium fraction of equiaxed grains (40%) only on the centreline for case B, and a negligible fraction for case A, that is well correlated to the micrographs of figure 1. In Fig. 4, the effect of the welding speed on f_{eg} is presented according to Eq. (4), showing that for a similar density of TiN particles, an increase of welding speed promotes equiaxed structure. However, the real situation is more complex because a change in welding parameters simultaneously affects the temperature field around the weld pool and the content of transferred Ti, and then the number of nucleation sites.

4 Conclusion

The aim of this study was to propose a welding solution for the K44X ferritic stainless steel by the development of homogeneous filler metal and the associated welding parameters. Several metal cored filler wires were tested, revealing the titanium content has a very important effect on the grain morphology of the molten zone. To allow the formation of a fine equiaxed grain structure, the Ti content in the molten zone must be higher than 0.12 weight %, that require Ti content in the filler wire of about 0.45 wt% due to the dilution with the base metal and elements losses associate to the transfer. Thermal numerical modelling of a welding operation allows predicting qualitatively the microstructure type, columnar or equiaxed, and shows the number of nuclei in the supercooling region is very important. A slight increase of Ti (and N) should be then increase the number of nuclei, and then produce a better grain refinement. However, structure obtained depends also on the welding energy parameters, that control the dilution, the elements losses during transfer, and the thermal cycles acting on the TiN precipitation and on the supercooling range.

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Tissue Expression of PCNA and Caspase3 in the Liver in Patients with Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma

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Abstract: Our aim is to evaluate the role of nuclear proliferative activity mainly PCNA and apoptosis mainly caspase3 in cases of chronic hepatitis, liver cirrhosis and HCC. Methods: This is a prospective study, ninety patients with chronic liver disease and HCC were subjected to clinical examination, laboratory investigations for hepatitis C and liver function tests and to abdominal ultrasonography. Liver biopsy was performed for histopathological examination. They were 3 groups: chronic hepatitis (35), liver cirrhosis (25) and HCC (30) and ten control patients with negative serological markers for hepatitis (C&B). Immunohistochemical staining for PCNA and Caspase 3 was done. Results: The highest PCNA expression was in the HCC group and the lowest was in the control group. The PCNA level increased gradually from the control group to the CH group, LC group to reach its highest level in the HCC group. This denotes that cellular proliferation in LC and HCC groups was higher than in the CH and Control groups. CH, LC and HCC groups showed a statistical significant difference relative to control group at a $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). PCNA expression difference in CH and LC groups is statistically significant relative to the HCC group and to each other at a $p < 0.01$. There is statistically significant difference in PCNA expression levels between HCC grades I and II at $p < 0.05$, and HCC grade III with a statistical significant difference relevant to grades I and II at a $p < 0.01$. The highest caspase3 expression was in the LC group and the lowest was in the control group. The caspase3 level increased from the control group to the CH and LC groups and decreased in the HCC group. The CH, LC and HCC groups showed a statistical significant difference relative to the control group at a $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). Caspase 3 expression in CH and LC groups is statistically significantly higher relative to the HCC group and relative to each other at a $p < 0.01$. There is statistically significant difference in Caspase3 expression levels between HCC grades I and II at $p < 0.05$, and low Caspase3 expression level in HCC grade III with a statistical significant difference relevant to grades I and II at $p < 0.01$& $p < 0.05$, respectively. Conclusion: Increased PCNA expression in hepatocytes indicating recent replication is increased in HCV chronic liver disease and in HCC. Development of HCC in patients with cirrhosis can to be predicted by liver cell proliferation. Reduced caspase3 expression in liver cirrhosis may indicate malignancy and a higher grade in HCC. Research directed to suppress nuclear proliferation and to enhance apoptosis may be of therapeutic value to control HCC development in cirrhosis.
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Key words: PCNA, Caspase 3, IHC, Hepatitis C, HCC

1. Introduction

The recently released Egyptian Demographic Health Survey (EDHS) tested a representative sample of the entire country for HCV antibody. The overall prevalence positive for antibody to HCV was 14.7%. Not everyone remains infected but EDHS reported that 9.8% continue to have HCV-RNA [(1)]. It is observed that the prevalence of HBV- and HCV-associated chronic liver diseases in liver biopsy material over the last decade showed a steady rise of HCV-associated diseases and a decline in HBV-associated ones [(2)]. Chronicity of HCV infection leads to chronic hepatitis and liver cirrhosis much more frequently than chronic infection by other hepatitis viruses [(3)], and shows a higher degree of association with hepatocellular carcinoma in several parts of the world [(4)]. Hepatocellular carcinoma is one of the most prevalent malignancies worldwide, being the third largest cause of cancer deaths. HCC is considered a multistage disease whose occurrence is caused by the interaction between genetic and environmental factors [(5)]. Usually, HCC arises from an adenomatous hyperplasia in an already diseased liver and progresses from a well-differentiated stage to less-differentiated forms [(6)]. While the sequential progression of chronic hepatitis to cirrhosis and ultimately to cancer seems to be well established, the exact mechanism of viral hepato-carcinogenesis is yet to be clearly defined [(7).] It has been suggested that the most important factor for the development of HCC is not the integration of viral DNA, but possibly the persistent liver cell necrosis and the resultant irregular regeneration [(8).] Therefore, the

proliferative rate of regenerating hepatocytes may be an important pathogenetic and prognostic factor in chronic liver disease [9].

Inoue *et al.* [10] found that the proliferation capacity of hepatocytes estimated immunohistochemically by proliferating cell nuclear antigen staining was markedly increased at early stage of cirrhosis development. However it was gradually decreased thereafter and suppressed substantially at the time of cirrhosis manifestation, the proliferative capability of hepatocytes is exhausted during continuous hepatic damage, this is considered to be a main cause of the development of liver cirrhosis.

A number of markers have been used in the assessment of the proliferative status of cells, like PCNA. The proliferating cell nuclear antigen (PCNA) is a 36 kDa protein that interacts with multiple proteins that play a key role in DNA synthesis and repair, cell cycle regulation, chromatin remodelling and apoptosis [11-12]. PCNA is one of the best markers for evaluating cell proliferation in studies on retrospective material, since the antigen can be localized in routine formalin-fixed paraffin-embedded tissue [13] . The apoptotic process appears to be a host defense mechanism against viral infections and tumourgenesis. However, many viral genomes encode proteins, which repress apoptosis so as to escape from immune attack by the host. Therefore, virus-host interactions may determine viral persistence, extent and severity of liver inflammation and possibly viral hepato-carcinogenesis. Apoptosis of liver cells may play a significant role in the pathogenesis of hepatitis C. It may represent a mechanism for viral shedding rather than for viral elimination, thereby raising the concept that inhibition of apoptosis could ameliorate hepatitis C. Apoptosis may occur in the absence of significant transaminase elevation, thereby explaining the lack of correlation between biochemical activity and liver cell histological injury. Monitoring caspase activation might provide a reliable tool to estimate the efficacy of HCV therapy, and might open challenging therapeutic strategies in HCV infection [14].

The aim of the work is to evaluate the role of nuclear proliferative activity (PCNA) and apoptosis (caspase3) in cases of chronic hepatitis, liver cirrhosis and HCC.

2- Patients and Methods:

Patients

Ninety patients (72 males and 18 females; mean age 40.3 ± 2.4, range 25-65 years) were the subject of this study. Patients were admitted to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute, Giza, Egypt. They included 35 cases of chronic hepatitis C virus infection (CH), 25 cases with liver cirrhosis (LC) and 30 cases of HCC, all with HCV infection. Of the HCC patients (7) patients were grade I, (14) were grade II; and (9) were grade III. The presence of HCV-RNA in patient's sera was detected by real-time polymerase chain reaction. They were subjected to thorough clinical examination, urine and stool analysis, liver function tests, ultrasonography and liver biopsy for histo-pathologic and immuno-histochemical studies. The study protocol was approved by the Ethics Committee of TBRI according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland [15].

Ten control liver biopsies were taken from individuals subjected to laparoscopic cholecystectomy after their consent. They were 4 males and 6 females with a mean age of 48.3 ± 2.3 years. Their liver function tests were normal and had no serologic evidence of hepatitis B or C viruses.

Liver biopsies were fixed in 10% buffered formalin for 24 hours, and then processed in ascending grades of ethyl alcohol, xylene, wax and paraffin blocks. Sections (4µm) were cut on albuminized glass slides and stained with Hematoxylin & Eosin and Masson trichrome stains. All sections were subjected to light microscopic examination for evaluating the histopathological and basic classification of cases. Five histological features have been observed to be relatively characteristic of (although not pathognomonic for) chronic hepatitis: (1) lymphoid aggregates in portal tracts, (2) degenerative injury-type changes of bile ducts, (3) large droplet steatosis, (4) Mallory body-like material within injured hepatocytes, and (5) lymphocytic aggregates within the lobules [16]. They were evaluated on a five point scale, using 20 random fields at x100 and x400 magnification per slide. Architectural changes, fibrosis and cirrhosis were evaluated on a seven point scale according to Knodell score system [17]. HCC cases were classified into 3 grades (I,II,III)well-, moderately-, and poorly differentiated) tumors [18]. Other liver sections (4µm) were cut on slides, which were treated with TESPA (3-aminopropyl-triethoxysilane, Sigma) for immune-histochemistry (IHC).

Immuno-histochemistry for Detection of tissue PCNA and Caspase 3 antigens:

Immuno-histochemical reaction was performed using an avidin biotin complex (ABC) immune-peroxidase technique according to Hsu and Raine 1981[19] using anti human PCNA and Caspase3 on paraffin sections, which were de-waxed in xylene and hydrated in descending grades of ethanol. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in 100% methanol for 20 min. Antigen retrieval was performed by microwaving the sections in citrate buffer (PH 6.0) for 15 min at 700 W. Sections were incubated overnight at 4°C with the anti-human primary antibodies against PCNA, (purchased

from Dako, Demark), and Caspase3 (purchased from Santa Cruz Biotechnology Inc.; Santa Cruz, USA) monoclonal antibody, diluted at 1:50,150 respectively in BPS. Next day, after thorough washing in PBS, the sections were incubated with streptavidin-biotin-peroxidase preformed complex and evidentiated using a peroxidase/DAB (diaminobenzidine) enzymatic reaction for PCNA & Caspase3. Staining is completed by 5-10 minutes incubation with 3, 3'-diaminobenzidine (DAB) + substrate - chromogen which results in a brown-colored precipitate at the antigen site for PCNA & Caspase 3 (cytoplasmic stain). Slides were washed in PBS for 5 minutes. Slides were placed in 70%, 95% and then 100% alcohol each for 5 minutes. The cell nuclei were counterstained with Mayer's hematoxylin. The cover slips were mounted using Dpx.

Positive and negative control slides for each marker were included within each session. As a negative control, liver tissue section was processed in the above mentioned sequences but the omission of the primary antibody and PBS was replaced.

Immuno-histochemical scoring of PCNA

Sections were examined under Zeiss light microscopy at x40, the proliferation index (P.I) is the number of positive nuclei stained by PCNA in 1000 hepatocytes then the mean of them calculated, and the mean for each group is taken according to Akyol *et al.*[20]. Zero% was given to unstained sections

Immuno-histochemical scoring of Caspase3:

Expression of caspase3 was cytoplasmic, sections were examined under Zeiss light microscopy at x40, in ten microscopic fields. The mean of the percentage of the positively stained cells was calculated from their mean according to Shen *et al.* [21].

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) for Windows (version 10) computer program was used for statistical analysis. For comparison of more than 3 group's means, one-way ANOVA test, Post Hoc test was used. Comparison between percent positive cases was calculated by Chi-square test. A P value < 0.05 was considered statistically significant.

3- Results:

Subjects were 66 males (73.3 %) and 24 females (26.7 %), their age ranged (23-72 years) with a mean of 46.6±4.5 years, as well as 10 patients with normal liver as control group. They were 4 males and 6 females, their age ranged (30-45 years) with a mean of 39.4±3.7years. Some important demographic data & lab investigations are showmen in table I.

Table 1. Liver biochemical profile of studied groups

Variables	CH (n=35) Mean ± SD	LC(n=25) Mean ± SD	HCC(n=30) Mean ± SD	Control (n=10) Mean ± SD
ALT (0-41) U/L	53.95 ± 29.16 [a',b]	45.8 ± 31.91 [a']	36.87 ± 21.5	19.8 ± 5.59
AST (0-38) U/L	38.3 ± 17.33 [b]	69.8 ± 16.15 [a',c]	72.9 ± 34.75 [a']	20.2 ± 5.87
Tot. protein (6-8) gm/dl	7.76 ± 1.17	8.02 ± 0.64 [a]	7.74 ± 1.14	7.24 ± 0.39
S. albumin (3.5-5) gm/dl	4.46 ± 0.4 [b]	3.62 ± 0.22 [a,b,c]	3.28 ± 0.54 [a]	4.22 ± 0.18
Total bilirubin (0.1-1.2)mg/dl	0.9 ± 0.5 [b]	1.2 ± 0.45 [b]	1.7 ± 1.02 [a]	0.9 ± 0.15
Direct bilirubin (0-0.25)mg/dl	0.46 ± 0.35 [a]	0.55 ± 0.5 [a]	1.09 ± 0.81 [a]	0.21 ± 0.06
Prothrombin Concentration (70-100)%	90.2 ± 10.43 [b]	83.2 ± 10.6 [a,b,c]	76.0 ± 11.29 [a]	98.8 ± 2.53

[a]: p value <0.05 relative to the control group
[a']: p value <0.001 relative to the control group
[b]: p value <0.05 relative to the HCC group
[c]: p value <0.001 relative to the CH group

The highest PCNA expression was in the HCC group and the lowest was in the control group. The PCNA level increased from CH to the LC group to reach its highest level in the HCC group. The CH, LC and HCC groups show a statistical significant difference relative to control group at $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). PCNA expression in LC group is statistically significantly is higher than CH at $p < 0.01$ and in HCC relative to LC at $p < 0.01$ (Table 2, Figure 1A,B,C, D).

There is statistically significant difference in PCNA expression levels between HCC grades I and II at $p < 0.05$, and there is a high PCNA expression level in HCC grade III with a statistical significant difference relevant to grades I and II at $p < 0.01$ (Table 3).

The highest caspase3 expression was in the LC group and the lowest was in the control group. The caspase3 level increased from the control group to the CH group, LC group and decreased in the HCC group compared to LC. The CH, LC and HCC groups show a statistical significant difference relative to control group at $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). There is a statistically significant difference in CH& LC groups relative to the HCC group and relative to each other at $p < 0.01$. The HCC group showing a statistically significant difference $p < 0.01$ higher compared to CH group& lower than LC group compared to LC group (Table 4, Figure 2A,B,C,D).

Table 2. PCNA scoring (Proliferating index) in studied groups

Variable	CH (n=35) Mean ± SD	LC (n=25) Mean ± SD	HCC (n=30) Mean ± SD	Control (n=10) Mean ± SD
PCNA scoring	8.5 ± 2.9 *,^	20.7 ± 3.4 **,^,$	46.4 ± 12.7**	0.3 ±0.1

*: p value <0.05 relative to the control group **: p value <0.01 relative to the control group
^: p value <0.01 relative to the HCC group $: p value <0.01 relative to the CH group

Table 3. PCNA expression levels versus different grades of HCC in studied HCC group.

Variable	Grade I (n=7)	Grade II (n=14)	Grade III (n=9)
PCNA Scoring	27.2 ± 8.54	47.9 ± 12.92*	63.0 ± 10.5 **,^

* p value <0.05 relative to grade I **: p value <0.01 relative to grade I
^: p value <0.01 relative to grade II

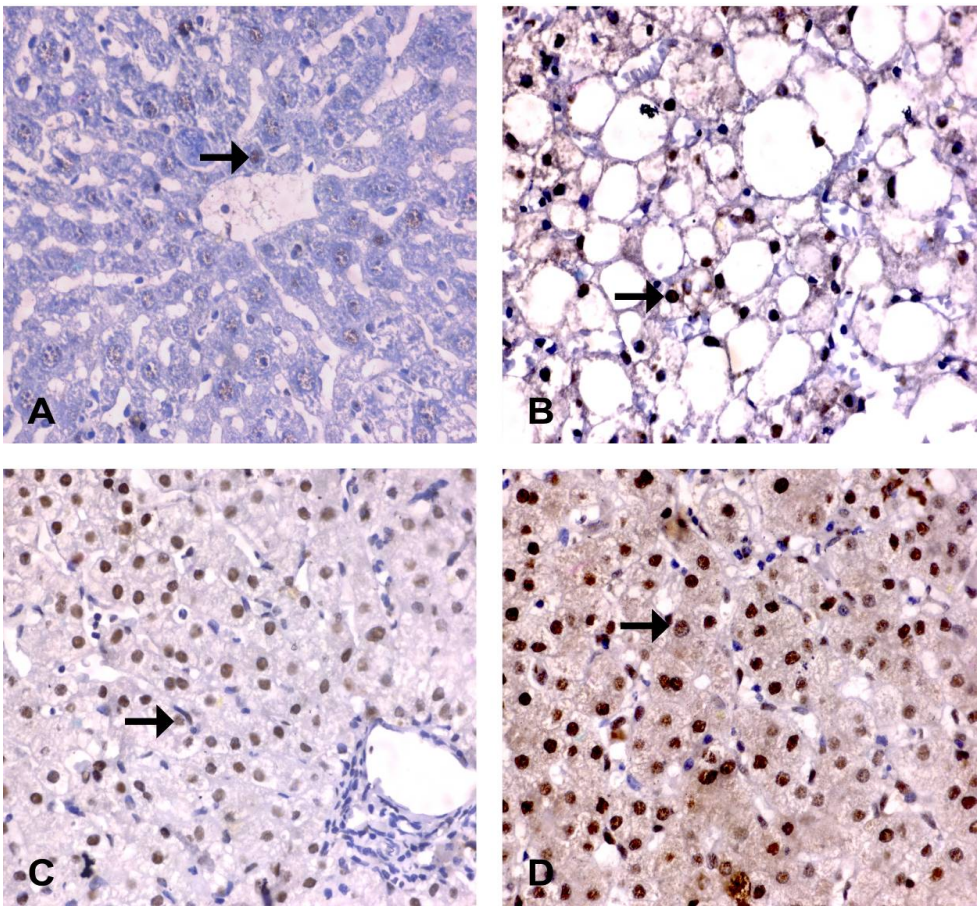

Figure (1): A) A control case showing scattered hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) **B)** A case of chronic hepatitis, showing few positive nuclei for PCNA monoclonal antibody (arrow) **C)** A case of liver cirrhosis showing moderate number pf hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) **D)** A case of HCC showing many showing hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) (Immunohistochemistry, DAB, x 200).

Table 4. Expression of Caspase3 in studied groups

Variable	CH (n=35) Mean ± SD	LC (n=25) Mean ± SD	HCC (n=30) Mean ± SD	Control (n=10) Mean ± SD
Caspase 3	17.5 ± 4.5 *,^	48.5 ± 3.7 **,^,$	32.5 ± 11.9**	1.2 ±0.2

*: p value <0.05 relative to the control group
**: p value <0.01 relative to the control group
^: p value <0.01 relative to the HCC group
$: p value <0.01 relative to the CH group

Table 5. Expression of Caspase3, versus different grades of HCC in studied HCC group.

Variable	Grade I (n=7)	Grade II (n=14)	Grade III (n=9)
Caspase3	47.6 ± 9.22	35.4 ± 7.92*	24.0 ± 3.5 **,^

*: p value <0.05 relative to grade I
**: p value <0.01 relative to grade I
^: p value <0.05 relative to grade II

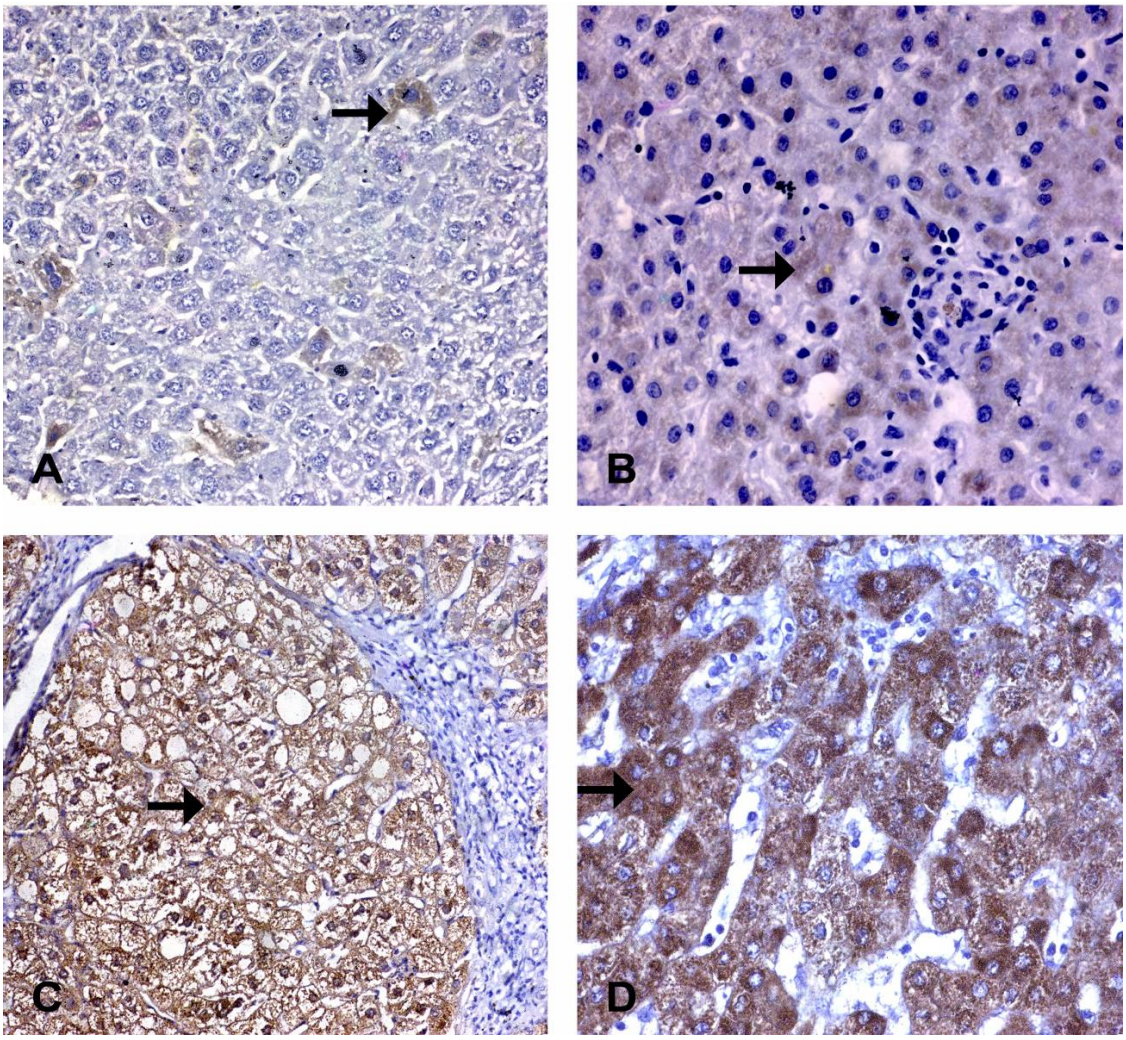

Figure (2): **A)** A control case showing scattered hepatocytes positive for Caspase3 monoclonal antibody. (as cytoplasmic brownish stain) (arrow) **B)** A case of chronic hepatitis, showing few positive hepatocytes for Caspase3 monoclonal antibody (arrow) **C)** A case of liver cirrhosis showing moderate number of hepatocytes nuclei positive for Caspase3 monoclonal antibody(arrow). **D)** A case of HCC showing many hepatocytes positive for Caspase3 monoclonal antibody (arrow). (Immunohistochemistry, DAB, x 200).

Discussion:

In this study we found that PCNA level increased gradually from the control group to the CH group, LC group to reach its highest level in the HCC group. Our data are consistent with other reports [22]. Most normal hepatocytes are in a state of proliferative quiescence

(Go phase) in the adult. This correlates with the low PCNA labeling index (PCNA-LI) observed in normal livers [23].

Lake-Bakaar [24] reported that the fraction of replicating or PCNA-staining cells in chronic HCV liver disease and in HCV-related hepatocellular cancer is increased compared to that in controls. Approximately 20% of hepatocytes in chronic HCV liver disease with or without cirrhosis showed evidence of recent cell division. In contrast, less than 1% of liver cells in controls had recently divided.

PCNA labeled fractions (PLFs) in chronic hepatitis and cirrhosis were no different in the material of Abdul Sathar et al. [25]. Virtually identical PCNA-labeling indices have been reported in chronic viral hepatitis and cirrhosis, though seperately, in some earlier reports [12, 18]. On the other hand, hepatocyte proliferative activity has been reported to decrease at the stage of cirrhosis from a higher rate in active chronic hepatitis. Compared to chronic persistent hepatitis, chronic active hepatitis, particularly of the more severe types, has been reported to show higher replicative rates [26].

Donato et al. [27] reported that the mean PCNA-LI ranged from 0.1% for patients with minimal changes to 3.6% for those with cirrhosis and hepatocellular carcinoma. Both HCV or HBV cirrhotics had similar liver cell proliferation rate but those with HBV had higher prevalence of liver cell dysplasia with respect to those with HCV. They concluded that PCNA-LI was a reliable assay for assessing liver cell proliferation rate in patients with chronic viral hepatitis and correlated with liver disease severity.

As in the study by Nakamura et al. [9] Abdul Sathar [25] did not find significant difference between PCNA-LI in HBV- and HCV-associated diseases, though there appeared to be a somewhat higher rate in the latter. Also, in terms of location, PCNA-labeling did not necessarily occur in the vicinity of piecemeal necrosis, fibrosis or portal inflammation. If this excess of PCNA-LI in HCV-associated chronic liver disease is confirmed by correcting the limitation of small numbers, it may partly explain the intriguing phenomena of some biological features of chronic HCV infection [28] regarding the more frequent association with cirrhosis and quicker progression to hepatocellular carcinoma when compared to those in chronic HBV infection.

The relationship between hepatocyte proliferation and liver functional reserve in cirrhotic patients was analyzed by Delhaye et al. [29] and showed that the PCNA-LI value declines with worsening Child class and is closely associated with serum albumin, a marker of protein synthesis in the liver, whatever the cause of the disease. An interesting aspect of their study resides in the observation that hepatocyte proliferative activity seems to reflect the liver functional reserve in human cirrhosis.

Evaluation of liver cell proliferative activity in the tumoral and non-cancerous surrounding tissue, expressed as PCNA positive cells demonstrated a significantly increased proliferation in tumoral as compared to peri-tumoral tissue [30]. In tumoral tissue, a high PCNA score is associated with a bad prognosis in HCC [31-32].

Hepatocellular proliferation rates observed in our material were generally similar to those reported by others in chronic hepatitis and cirrhosis [12, 18, and 33]. Ojanguren and colleagues [33] found no PCNA labeling in more than half (7/13) of biopsies showing cirrhosis, while Ballardini et al[13] reported absence of strong labeling indicative of pre-mitotic phase in cirrhotic livers that did or did not develop HCC during follow-up. Since replicating cells are always randomly distributed, non-detection of labeling may merely be due to relatively small tissue samples obtained by biopsies, particularly on needle aspiration.

Venturi et al. [34] found that in none of cirrhotic tissues was a PCNA basic spot was detected. These data show that PCNA is differently expressed in HCC and cirrhotic tissues, in terms of structure, isoforms and post-translational modifications, strongly implicating functional alterations in PCNA in the hepato-cancerogenetic process. The PCNA basic isoforms, exclusively detected in hepatic malignant samples, may represent a new signature for neoplastic liver cells compared to cirrhotic tissues. The several PCNA isoforms detected in neoplastic samples compared to non-cancerous tissues might help explain the PCNA over-expression in HCC detected at immune-labelling and immune-staining analysis [35].

In our study, we found that there is statistically significant difference in PCNA expression levels between HCC grades I and II, and there is a high PCNA expression level in HCC grade III with a statistical significant difference relevant to grades I and II. This is in accordance with previously published literature [36-38]. PCNA positively stained cells were 10% in low-grade HCC specimens. In moderately differentiated malignancies, PCNA staining was detected in approximately 30% of the nuclei. High-grade malignancies showed 70% of tumor cells positive to PCNA.

A progressive increase in the PCNA-LI from regenerative to dysplastic nodules to HCC has been observed [39]. Additionally, an increase in the DNA index was correlated with an increase in PCNA labeling, and both were correlated with pathological changes in HCC tumors [40].

Only HCC had a significantly increased PCNA LI compared to benign categories. Thus is probably related to the malignant nature of HCC and may reflect

the uncontrolled proliferation of the neoplastic hepatocytes [41].

Gramantieri et al. [42] suggested that in HCC PCNA participates both in DNA synthesis & repair and that highly proliferating HCC may display a sustained DNA repair .Also PCNA LI was significantly higher in cancer & correlate with tumor size [43].

Recent, study [44] demonstrated an association between small cell dysplasia, but not large cell dysplasia, and an increased HCC risk in patients with HCV related cirrhosis. Interestingly, liver specimens showing small cell dysplasia changes also had a higher proliferative rate by PCNA and a lower apoptotic rate compared with samples with large cell dysplasia. PCNA assay and small cell dysplasia seemed to be more predictive in the context of HCV patients [44]. The finding of PCNA–positive liver cells clustered near areas of spotty, confluent, or piecemeal necrosis in patients with acute and chronic viral hepatitis [42] provides further support to a possible correlation between increased liver cell proliferation rate and high hepatic inflammation scores in patients with chronic liver diseases [45]. An inverse correlation between liver cell proliferation and survival was shown. differences in survival between patients with high versus low PCNA-LI values points to a link between liver cell proliferation, HCC and liver-related mortality in patients with compensated cirrhosis [46].

Venturi et al. [34] concluded that human HCC express specific PCNA isoforms compared to those found in cirrhosis, implicating a role for PCNA functional alteration in hepatocarcinogenesis.

Proliferating activity of tumor defined by PCNA immune-histochemical study has been reported to be related to metastatic potential, recurrence and overall prognosis [47]. In our study, the PCNA LI was significantly higher in more poorly differentiated HCCs, which is consistent with previous reports [48], that cell proliferative activity of HCC cells correlates with their degree of dedifferentiation.

In our study, the caspase3 level increased from the control group to the chronic hepatitis group, liver cirrhosis group and decreased in the HCC group. Farinati et al. [45] reported that cyto-proliferation is more pronounced in chronic HCV-related hepatitis, while apoptosis is not significantly higher than in other types of liver damage, suggesting an imbalance between the two. Apoptosis and cyto-proliferative index are directly related to the extent of liver damage.

Arzberger et al. [49] found an inverse correlation between the strength of an apoptotic stimulus and the infectivity of the virus particles released: the more potent the apoptotic stimulus, the higher the ratio of non-enveloped capsids to virions and the lower their infectivity.

Recent studies provide evidence for variable degrees of liver cell apoptosis in the liver of patients with chronic hepatitis C. Apoptosis does not correlate with transaminase levels, viral load or genotype [50-51]. The lack of correlation of caspase activation to viremia or serum transaminase levels may be related to different types of cell death. *In vivo* studies have shown that the apoptotic process in hepatocytes is accompanied by increased transaminase levels, but the release of transaminases is lower in apoptosis than in necrosis. Therefore, relative differences in the occurrence of apoptosis and necrosis could explain why transaminase levels and caspase activation are not correlated [52].

In our study, Caspase 3 was highest in LC group and was lower in HCC group. In biopsy specimens with low activity (grade 0), 7.7% of the hepatocytes have caspase3 activation, whereas 20.9% of the cells stain positive in grade 3. Kinetic analysis of viral turnover in patients indicates that HCV infection is a highly dynamic process with a short half-life of viral particles and HCV-infected cells [53]. It is estimated that the daily turnover of HCV-infected cells may be as high as 13-25%. Assuming that in patients with chronic HCV infection approximately 50% of liver cells are infected, this would mean that between 6.5 and 12.5% of hepatocytes are killed daily [54]. It is notable that this number is very similar to the amount of cells with active caspases ranging from 7 to 20%, thereby suggesting that the daily liver cell death might occur via apoptosis [54].

We found that there was statistically significant difference in Caspase3 expression levels between HCC grades I and II at $p < 0.05$, and a low Caspase3 expression level in HCC grade III with a statistical significant difference. Sun et al. [55] reported that 53.8% cases of HCC were found to express caspase3 transcripts, while 46.2% of HCC failed to express it. The expression of caspase3 was correlated with HCC differentiation, as 72.2% (13/18) of moderately to well differentiated HCC showed positive caspase3 transcripts, while only 38.1% of poorly differentiated HCC was caspase3 positive. No relationship was found between caspase3 and tumor size or grade or metastasis, although 62.5% (5/8) of HCC with metastasis was caspase3 positive but it did not differ significantly from that without metastasis. Expression of caspase3 alone did not affect the apoptosis index (AI) of HCC. Sun et al. [55] concluded that caspase expression may not be related to cell apoptosis in HCC. In contrast, a previous report had reported reduced expression of caspase-3 by immune-histochemistry in HCCs compared to non-tumor liver tissue [56]. These differences may be due to differences in methodology including use of different primary antibodies. Caspase3 over- expression was not associated with histology or prognosis. However, caspase3 expression has been associated with histological type and grade of tumor, and with prognosis in other tumor types [57].

Conclusion,

Increased PCNA in hepatocytes indicating recent replication is increased in HCV-related chronic liver disease and in HCC. These findings have potential implications for linking chronic HCV infection to cirrhosis and HCC. Development of HCC in patients with cirrhosis can to be predicted by liver cell proliferation status. Reduced caspase3 expression in liver cirrhosis may indicate malignancy and higher grade in HCC. Research directed to suppress nuclear proliferation and enhance apoptosis may be of therapeutic value to control HCC development in cirrhosis.

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Clinical Utility of Serum Chemerin as a Novel Marker of Metabolic Syndrome and Type 2 Diabetes Mellitus

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Abstract: Background: Chemerin is a novel adipokine which has dual roles in adipose tissue metabolism and regulation of immune response. Its concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo and suggested to be involved in insulin resistance in obesity and type 2 diabetes. **Objective:** was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions. **Subjects and Methods**: This study was conducted on 20 patients with metabolic syndrome (group I) and 20 patients with type2 DM (group II), in addition to 15 healthy control subjects. All individuals included in the study were subjected to full history taking, thorough clinical examination including waist circumference and blood pressure measurement, laboratory investigations including: fasting and post-prandial blood glucose, fasting serum insulin, lipid profile, serum chemerin level assessment by ELISA technique. Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated. **Results:** Serum chemerin levels were significantly higher in group I (metabolic syndrome group) when compared to group II (type 2 diabetes mellitus) and control group ($p<0.001$, respectively). In addition, it was significantly higher in group II compared to control group ($p<0.001$). Serum chemerin levels negatively correlated with HDL-C and CAD-risk and positively correlated with systolic blood pressure, diastolic blood pressure, waist circumference, fasting blood insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in both groups ($p<0.05$, respectively). Multivariate analysis showed that serum chemerin, waist circumference and total triglycerides were the most significant predictors for metabolic syndrome with F value=7.68 ($p <0.05$). ROC curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77%. The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively. **Conclusion:** In conclusion, the association of high serum chemerin levels with components of metabolic syndrome and type 2 DM indicates that this adipokine represents a novel marker of these derangements and could be considered one of the metabolic risk factors leading to insulin resistance in type 2 DM as well as metabolic syndrome. Moreover, its assessment could be beneficial in early detection of these pathological states and prevention of their unfavorable consequences especially the cardiovascular complications and atherosclerosis.

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Key words: Chemerin; metabolic syndrome; type 2 diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus is a group of disorders characterized by hyperglycemia and associated with microvascular and macrovascular complications. Hyperglycemia results from lack of endogenous insulin or resistance to the action of insulin in muscle, fat and liver in addition to an inadequate response by the pancreatic beta cells ***(Wolfs et al., 2009).***

The metabolic syndrome is a cluster of coronary heart disease (CHD) risk factors including high blood pressure, dyslipidemia, hyperglycemia and central obesity that are associated with decreased ability of insulin to stimulate glucose disposal on peripheral target tissues, referred to as insulin resistance ***(Olufadi and Byrne, 2008).***

Insulin resistance is determined by impaired sensitivity of insulin to its main target organs i.e. adipose tissue, liver and muscle. Obesity, particularly central obesity, is the prominent risk factor for insulin resistance and results in type 2 diabetes and metabolic syndrome ***(Zeyda and Stulnig, 2009).***

Adipose tissue represents an active endocrine organ that releases a large number of bioactive mediators (adipokines) that signal to organs of metabolic importance including brain, liver, skeletal muscle, and the immune system thereby modulating hemostasis, blood pressure, lipid and glucose metabolism, inflammation and atherosclerosis. These adipokines include adiponectin, leptin, omentin, resistin, retinol binding protein-4, tumor necrosis factor-α, interleukin-6, vaspin, visfatin and chemerin ***(Yan et al., 2012).***

Chemerin is an adipokine that has been reported to modulate immune system function through its binding to the chemerin receptor. It is secreted as an

18kDa inactive pro-protein and undergoes extracellular serine protease cleavage of the C-terminal portion of the protein to generate the 16kDa active chemerin which is present in plasma and serum ***(Stejskal et al., 2008).*** It is secreted by the mature adipocytes and can regulate adipogenesis and adipocyte differentiation. Chemerin serves as a chemoattractant for pro-inflammatory cells of the immune system such as macrophages and immature dendritic cells that express the cognate receptor chemokine-like receptor-1 (CMKLR1) ***(Bozaoglu et al., 2010*** and ***Iannone and Lapadula, 2011)*** Furthermore, chemerin and its receptor/ChemR23 are expressed abundantly in adipose tissue, suggesting its function in autocrine/ paracrine fashion ***(Ernst et al., 2012).***

Chemerin serum concentrations are elevated in obese, insulin-resistant, and inflammatory states *in vivo* and suggested to be an obvious cause of insulin resistance ***(Hart and Greaves, 2010)***: Obesity induces inflammation in adipose tissue and since chemerin is a pro-inflammatory cytokine that recruits and activates immune cells and contributes to inflammation by promoting macrophage adhesion to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, it may link obesity and inflammation ***(Ouchi et al., 2011).*** Muscle insulin resistance is a major risk factor for the pathogenesis of type 2 diabetes. Therefore, a possible relation of chemerin to inflammatory proteins and insulin resistance in obesity and type2 diabetes is suggested. Chemerin is also proved to be associated with components of metabolic syndrome and it may play a role in the pathophysiology of this condition ***(Lambernd et al., 2012)***

Aim of the work: The aim of the present study was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions.

2. Subjects and Methods:

A- Subjects:

1- Patients' Group (n = 40):

This group included forty patients attending to Ain Shams University Hospitals outpatients Cardiology and Internal Medicine Clinics for medical investigations. They were 17 males and 23 females. They were further divided into two groups:

(a) Group I; Patients with metabolic syndrome (n=20):

This group included twenty non-diabetic patients. They were 10 males and 10 females whose ages ranged from 32 to 69 years (Mean 51.3 ± 11.4). Clinical identification of the metabolic syndrome was done according to the **National Cholesterol Education Program Adult Treatment Panel III (2002) (NCEP ATP III**) guidelines, as described by ***(Lin et al., 2009)*:**

(i) Hypertension was defined as a systolic blood pressure of ≥130 mmHg and/or diastolic blood pressure of ≥ 85 mmHg on repeated measurements, or the patient receiving antihypertensive treatment.

(ii) Abdominal obesity was defined as waist circumference exceeding 102 cm and 88 cm in men and women, respectively.

(iii) Fasting serum triglycerides more than 150 mg/dL (1.7mmol/L) or patient receiving treatment for this lipid abnormality.

(iv) Fasting serum HDL-C less than 40 mg/dL (1.0 mmol/L) and 50mg/dL (1.3 mmol/L) in men and women, respectively or patient receiving treatment for this lipid abnormality.

(v) Fasting plasma glucose ≥ 110 mg/dL (6.1 mmol/L)

Metabolic syndrome was diagnosed by the presence of three or more of the previous criteria ***(American Heart Association, 2004)***

(b) Group II; Patients with type 2 diabetes mellitus (n=20):

This group included twenty patients, 7 males and 13 females, whose ages ranged from 35 to 74 years (Mean 55.3 ± 9.6). Patients were diagnosed according to the Report of the **Expert Committee on the Diagnosis and Classification of Diabetes Mellitus in 1997** and the **follow-up reports in 2003**. Type 2 diabetes mellitus was identified by one of the following criteria:

i) Symptoms of diabetes mellitus plus random plasma glucose concentration ≥ 200 mg/dL.

ii) Fasting plasma glucose (FPG) ≥ 126 mg/dL on more than one occasion.

iii) 2 hours post load plasma glucose concentration ≥ 200 mg/dL during oral glucose tolerance test.

2- Control Group (n = 15):

This group included 15 healthy subjects. They were 9 males and 6 females whose ages ranged from 27 to 69 years (49.1 ± 10.2).

Exclusion criteria: A number of clinical conditions, which were known to be associated with increased serum chemerin levels, were excluded e.g. chronic liver disease, hepatitis B or hepatitis C virus infection, liver cell failure ***(Marra and Bertolani, 2009)***, chronic kidney disease and renal failure ***(Hu and Feng, 2011).***

All individuals included in this study were subjected to:

-Full history taking: focusing on family history of type 2 diabetes mellitus, hypertension, smoking and physical activity.

-Thorough clinical examination including waist circumference and blood pressure measurement.

-Mean arterial blood pressure (MAP) was also calculated. MAP = [(2 x diastolic) +systolic] / 3 -

Laboratory investigations that included: fasting blood

glucose, post-prandial blood glucose, fasting insulin, lipid profile and serum chemerin level.

The Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated.

B- Sampling:

Five milliliters of venous blood were collected after 12-14 hours fasting under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 xg for 15 minutes). The separated serum was divided into three aliquots. One was designated for the immediate assay of fasting glucose and lipid profile. The other two aliquots were stored at -20ºC for subsequent assay of insulin and chemerin. Hemolysed samples were discarded. Repeated freezing and thawing was avoided.

C- Methods:

1- Analytical Methods:

(a) Serum glucose level:

The analysis was done using Synchron CX-9 (Instruments Inc.; Scienfitic Instruments Division, Fillerton, CA 92634, 3100, USA.) system auto-analyzer applying enzymatic colorimetric method ***(James et al., 1970)***.

(b) Total cholesterol (TC):

Total cholesterol was assayed on the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method ***(Dietschy et al., 1976)***.

(c) Triglycerides (TG):

The analysis of TG was done using the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method ***(McGowan et al., 1983)***.

(d) High density lipoprotein cholesterol (HDL-C):

The HDL-C was assayed on the Synchron CX-9 system auto-analyzer after precipitation of LDL and VLDL by dextran sulfate and magnesium in the separating reagent. The LDL and VLDL portions were then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant was assayed with an enzymatic timed endpoint method ***(Assman et al., 1983).***

(e) Low density lipoprotein cholesterol (LDL-C):

LDL-C value was calculated according to "Friedewald equation":

LDL-C = Total cholesterol-(HDL-C+TG/5)

This equation was applied provided that serum TG level is <400 mg/dL ***(Friedwald et al., 1972*** and ***Warnick et al., 1990).***

(f) CAD-risk percentage:

For the assessment of cardiovascular risk, HDL-C/TC % was calculated ***(Carl et al., 2006)***.

(g) Insulin:

Insulin was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxSYM (Abbott Ireland, Diagnostic Division-Lisnamuck, Longford Co. Longford, Ireland +353-43-31000) for the quantitative determination of insulin in human serum or plasma. The used method based on the Micro-particle Enzyme Immunoassay (MEIA) technology. In this assay, sample and reagents required for one test, were pipetted by the sampling probe into various wells of a reaction vessel (RV). The RV was immediately transferred into the processing center. Sample, anti-insulin (mouse, monoclonal) coated micro-particles and assay buffer were pipetted into one well of the reaction vessel. During the incubation of this reaction mixture, the insulin in the specimen binds to the anti-insulin coated micro-particles forming an antibody-antigen complex. An aliquot of the reaction mixture was transferred to the matrix cells. The micro-particles bind irreversibly to the glass fiber matrix. The matrix cell was washed to remove unbound materials. The anti-insulin (mouse, monoclonal) alkaline phosphatase conjugate was dispended onto the matrix cell and binds to the antibody-antigen complex. The matrix cell was washed again to remove unbound materials. The substrate, 4-Methylumbellifryl Phosphate was added to the matrix cell and the rate of fluorescent product formation was measured by the MEIA optical assembly ***(Jennifer et al., 1999)***:

(h) The homeostasis model assessment-insulin resistance index (HOMA-IR): It was calculated using the equation:

HOMA-IR =

$$\frac{\text{fasting glucose}(\text{mg/dL}) \times \text{fasting insulin}(\mu\text{U/mL})}{405}$$

The cutoff point to define insulin resistance corresponds to HOMA-IR ≥ 3.8 ***(Shirai, 2004)***:

(i) Chemerin:

Chemerin concentrations were measured using a commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by BioVendor Laboratory Medicine, Inc (Biovender GmbH, Im Neuenheimer Feld 583, D-69120 Heidelberg, Germany). Surface of the wells in microtitration plate was coated with polyclonal anti-human chemerin specific antibody. Standards, quality controls (QC) and diluted samples were pipetted into the wells. After a 60 minutes incubation followed by washing, biotin labeled polyclonal anti-human chemerin antibody was added and incubated with the captured chemerin for 60 minutes. After another washing step, streptavidin-HRP conjugate was added. After 30 minutes incubation and last washing step, the remaining conjugate was allowed to react with the substrate solution. The reaction was stopped by addition of acidic solution (0.2M H_2SO_4). Absorbance of the resulting product was measured spectrophotometrically at 450nm. The absorbance is proportional to the concentration of chemerin. A standard curve was constructed by plotting absorbance

values versus chemerin concentrations of standards. Concentrations of the unknown diluted samples were determined using this standard curve and then multiplied by the dilution factor to get the actual amount of chemerin in the original serum ***(Bozaoglu et al., 2007)***.

2- Statistical Methods:

Statistical analysis was done using SPSS software, version 9.02, 1998, Echosoft Corporation, USA. The goodness of fit test was done to determine the distribution of data. Descriptive statistics in the form of mean±SD were calculated for parametric data. On the other hand, non parametric data were expressed as median and interquartile range. Between-group comparisons were done using the Student's t test in case of normally distributed data, and Wilcoxon's rank sum test in case of skewed data. Regarding the correlation analysis, Spearman's rank correlation (r_s) was done to evaluate the degree of correlation between two variables if one of them or both had skewed data. p values <0.05 were considered significant, whereas p values <0.01 were considered highly significant. Receiver operating characteristic (ROC) curve analysis was applied to evaluate of the diagnostic utility of chemerin.

3. Results:

The results obtained in the present study are presented in tables 1-3 and figures1 and 2. Systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p<0.001, respectively). Moreover, the waist circumference was significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and <0.001, respectively) (Table 2).

Fasting blood sugar, 2-hs post prandial blood sugar and HOMA-IR were significantly higher in group II (type 2 diabetes mellitus) when compared to group I (metabolic syndrome) and control group (p <0.001, respectively) (Table 2). In addition, HOMA-IR levels were significantly higher in group I (metabolic syndrome) when compared to control group, p <0.001. Fasting insulin levels were significantly higher in group I (metabolic syndrome) and group II (type 2 diabetes mellitus) when compared to control group (p <0.001, respectively). However, there was no significant difference between group I (metabolic syndrome) and group II (type 2 diabetes mellitus) concerning fasting insulin levels (p >0.05) (Table 2).

Serum HDL-C was significantly lower in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and p <0.001, respectively). In addition, total cholesterol and LDL-C levels were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and p <0.01, respectively). Moreover, Serum triglycerides were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). However, the difference between group II (type 2 diabetes mellitus) and control group regarding serum HDL-C, total cholesterol and triglycerides did not reach statistical significance, p >0.05; respectively (Table 2).

CAD-risk percentage was significantly lower in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). CAD-risk percentage was also significantly lower in group II (type 2 diabetes mellitus group) compared to control group, p <0.05 (Table 2).

Serum chemerin levels were significantly higher in group I (metabolic syndrome group) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). In addition, it was significantly higher in group II (type 2 diabetes mellitus) compared to control group (p <0.001) (Table 2).

Our correlation study between serum chemerin level and other studied parameters revealed a significant negative correlation between serum chemerin level and both HDL-C and CAD-risk in metabolic syndrome and type 2 DM (p <0.05, respectively). Moreover, a significant positive correlation was found between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, waist circumference, fasting blood insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in both groups (p<0.05, respectively). However, a non-significant correlation was observed between serum chemerin and each of fasting blood sugar and post prandial blood sugar, p >0.05 (Table 3).

Multivariate analysis was done for the measured variables to test them as predictors of metabolic syndrome. It showed that serum chemerin, waist circumference and total triglycerides were the most significant predictors with F value=7.68 (p <0.05).

Receiver operating characteristic (ROC) curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77%, with an AUC = 0.786 (Figure 2). The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively.

Table 1: Descriptive statistics of the studied parameters in the different studied groups

Group / Parameter	Group I (Metabolic Syndrome) [n=20] $\bar{X}$ ±s/ Median (IR) *	Group II (Type 2 Diabetes Mellitus) [n=20] $\bar{X}$ ±s/ Median (IR) *	Control Group [n=15] $\bar{X}$ ±s/ Median (IR) *
Systolic Blood Pressure (mm Hg)	140±16.9	122±8.9	112±9.7
Diastolic Blood Pressure (mm Hg)	93±10.9	83±6.9	70±7.2
Mean Arterial Blood Pressure (mmHg)	111±9.2	91 ±7.2	85 ±8.2
Waist Circumference (cm) in males	110±12.4	101 ±11.8	80±8.8
Waist Circumference (cm) in females	103±8.7	92 ±9.8	76±7.5
Fasting Blood Sugar (mg/dL)	92 (89 – 99) *	144 (130 – 175)*	75 (67 – 84)*
2 Hours Postprandial Blood Sugar (mg/dL)	111 (100 – 121)*	173 (137 – 197)*	101 (90 – 114)*
Fasting Blood Insulin (µU/mL)	8.7 (7.2 – 12.3)*	9 (6.1 – 13.7)*	4.3 (2.7 – 5.5)*
HOMA-IR	2.1 (1.6 – 3.2)*	3.2 (2.3 – 5.3)*	0.8 (0.4 – 1.2)*
HDL-C (mg/dL)	39±8.7	48±9.4	53±7.4
LDL-C (mg/dL)	133 (110 – 185)*	115 (97 – 137)*	109 (84 – 120)*
Total Cholesterol (mg/dL)	221 (182 – 242)*	192 (157 – 204)*	179 (147 – 198)*
Triglycerides (mg/dL)	170 (123 – 213)*	116 (75 – 130)*	108 (70 – 112)*
CAD-risk (%)	18 (15 – 22)*	26 (25– 30)*	34 (27 – 36)*
Serum Chemerin (ng/mL)	455 (275 – 725)*	112 (80 – 173)*	50 (40 – 90)*

Table (2): Statistical Comparison between Various Groups Regarding Different Studied Parameters

Group / Parameter	Group I (Metabolic syndrome) Vs Controls		Group II (Type 2 Diabetes) Vs Controls		Group I (Metabolic syndrome) Vs Group II (Type 2 Diabetes)	
	t or z*	***p***	**t or z***	***p***	**t or z***	***p***
Systolic Blood Pressure (mm Hg)	6.1	<0.001	2.5	<0.05	4.3	<0.001
Diastolic Blood Pressure (mm Hg)	5.8	<0.001	2.6	<0.05	3.6	<0.001
Mean Arterial Blood Pressure (mm Hg)	5.3	<0.001	2.5	<0.05	3.5	<0.001
Waist Circumference (cm) in male	8.3	<0.001	3.7	<0.001	2.6	<0.05
Waist Circumference (cm) in female	5.3	<0.001	3.5	<0.001	2.5	<0.05
Fasting Blood Sugar (mg/dL)	3.8*	<0.001	4.9*	<0.001	4.9*	<0.001
2 Hours Postprandial Blood Sugar (mg/dL)	1.6*	>0.05	4..0 *	<0.001	3.8 *	<0.001
Fasting Blood Insulin (µU/mL)	3.9*	<0.001	3.6*	<0.001	0.1*	>0.05
HOMA-IR	4.2*	<0.001	4.8*	<0.001	2.7*	<0.001
HDL-C (mg/dL)	5.2	<0.001	1.9	>0.05	2.6	<0.05
LDL-C (mg/dL)	3.1*	<0.001	1.2*	>0.05	2.1*	<0.05
Total Cholesterol (mg/dL)	3.5*	<0.001	1.3*	>0.05	2.5*	<0.05
Triglycerides (mg/dL)	4.6*	<0.001	1.5*	>0.05	3.6*	<0.001
CAD-risk (%)	4.8*	<0.001	2.8*	<0.05	4.3*	<0.001
Serum Chemerin (ng/mL)	5.0*	<0.001	3.1*	<0.001	4.8*	<0.001

t: Student's t test; z*: Wilcoxon's rank sum test (P<0.01): highly significant difference; (P <0.05): significant difference; (P >0.05): non significant difference

Table 3: Correlation analysis between serum chemerin level and other studied parameters in metabolic syndrome and type 2 DM groups using Spearman's rank correlation coefficient

Parameter		Group I(Metabolic syndrome)	Group II(Type 2 Diabetes)
Systolic Blood Pressure	r_s p	0.578 <0.05	0.362 <0.05
Diastolic Blood Pressure	r_s p	0.542 <0.05	0.421 <0.05
Waist Circumference	r_s p	0.642 <0.05	0.532 <0.05
Fasting Blood Sugar (mg/dl)	r_s p	0.087 >0.05	0.196 >0.05
2 Hours Postprandial Blood Sugar (mg/dl)	r_s p	0.127 >0.05	0.207 >0.05
Fasting Blood Insulin (µU/mL)	r_s p	0.367 <0.05	0.284 <0.05
HOMA-IR	r_s p	0.286 <0.05	0.295 <0.05
Total Cholesterol	r_s p	0.556 <0.05	0.461 <0.05
CAD-risk (%)	r_s p	-0.699 <0.05	-0.321 <0.05
HDL-C	r_s p	-0.374 <0.05	-0.311 <0.05
LDL-C	r_s p	0.445 <0.05	0.352 <0.05
Triglycerides	r_s p	0.542 <0.05	0.332 <0.05

(P <0.05): significant correlation; (P >0.05): non significant correlation

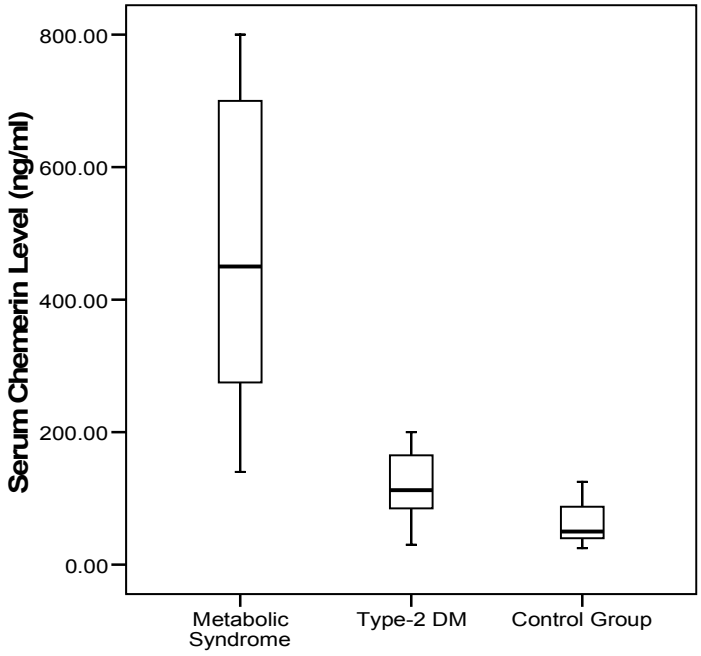

Figure 1: Box-Plot Chart showing difference between study groups concerning serum chemerin level

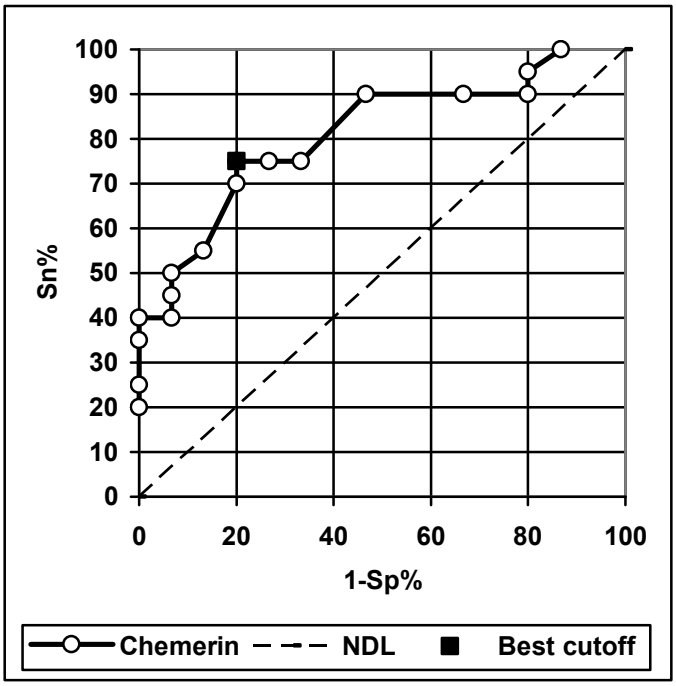

Figure 2: ROC curve analysis showing the diagnostic performance of chemerin in patients with type 2 DM

4. Discussion:

Increased adipose tissue mass, especially in the visceral compartment, is characterized by altered metabolic and endocrine function leading to an increased secretion of pro-inflammatory adipokines ***(Suzuki et al., 2010)***. Chemerin is a recently described adipokine which has dual roles in adipose tissue metabolism and regulation of immune response. Chemerin serum concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo and suggested to be an obvious cause of insulin resistance in obesity ***(Parlee et al., 2010*** and ***Ferraccioli and Gremese, 2011)***. Chemerin is also suggested to be linked to obesity-induced insulin resistance in type 2 diabetes ***(Hu and Feng, 2011).***

In the view of the previous studies, the aim of our work was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions.

The results of the present study revealed that the systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, waist circumference, fasting serum insulin, fasting serum glucose, HOMA-IR, total cholesterol, LDL-C, triglycerides and serum chemerin levels were significantly higher in metabolic syndrome group when compared to control group. In addition, serum chemerin correlated positively with each of systolic blood pressure, diastolic blood pressure, waist circumference, fasting serum insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in metabolic syndrome patients.

These findings were in agreement with those of ***Stejskal et al. (2008), Bozaoglu et al. (2009)*** and ***Dong et al. (2011)***. They proved that subjects with metabolic syndrome had significantly higher serum chemerin levels compared with healthy controls. In addition, they reported that higher serum chemerin concentrations have a strong and independent association with aspects of the metabolic syndrome including serum glucose, waist circumference, fasting serum insulin and lipid profile independent of age and sex in non-diabetic subjects.

Our results also go hand in hand with ***Shin et al. (2011), Yoo et al. (2012)*** and ***Yan et al. (2012)***. They proved that serum chemerin levels were significantly increased in obese individuals compared with lean controls and circulating chemerin levels had a significant positive correlation with the body mass index, waist circumference, abdominal visceral fat area, blood pressure, fasting serum insulin, HOMA-IR, TC, LDL-C and TG levels in obese individuals. In addition, ***Blüher et al. (2012)*** proved that insulin, triglycerides and chemerin are biomarkers whose dynamics tightly correspond to changes in body weight, with the trend to go to the opposite direction during the weight loss phase. Moreover, ***Ernst et al. (2012)*** stated that CMKLR1(-/-) mice had lower food consumption, total body mass, and percent body fat compared with wild-type controls. These findings suggested that chemerin may play a role in the pathophysiology of obesity and metabolic syndrome.

Sell et al. (2009) explained these findings by the fact that human adipocytes express chemerin and

chemokine-like receptor-1 and chemerin release is correlated with adipocyte volume. Furthermore, higher chemerin release is associated with insulin resistance at the level of lipogenesis by its reversible binding to the extracellular domain of insulin receptor-tyrosine kinase in peripheral tissues and decreasing the rate of auto-phosphorylation and subsequent downstream intracellular signaling cascades. Chemerin also inhibits glycogen synthase kinase phosphorylation, an enzyme necessary for glycogen synthesis and storage, and thus inhibits glucose uptake. In addition, chemerin activates extracellular signal-regulated kinase (ERK). Inhibition of ERK prevents chemerin-induced insulin resistance, pointing to participation of this pathway in chemerin action.

Takahashi et al. (2008) disagreed with this suggestion and postulated that, in adipocytes chemerin has the opposite effect, where it increases insulin-stimulated glucose uptake, and so, it stimulates insulin sensitivity. Hence, the increase in the levels of circulating chemerin is a compensatory mechanism in patients with insulin resistance. Thus, chemerin may exert different actions in endocrine and paracrine/autocrine ways. Moreover, ***Takahashi et al. (2011)*** showed that chemerin-deficient mice are glucose intolerant and glucose intolerance was mainly due to increased hepatic glucose production and impaired insulin secretion. They suggested that chemerin and its receptor were expressed in β-cell and chemerin regulates β-cell function and plays an important role in glucose homeostasis in a tissue-dependent manner.

Our results concerning blood pressure were in agreement with studies done by ***Stejskal et al. (2008)*** and ***Bozaoglu et al. (2009)***. They revealed that chemerin serum levels correlated positively with systolic and diastolic blood pressure. Chemerin may also be a novel regulator of blood pressure because of good correlations with both systolic and diastolic pressure. This hypothesis is supported by the fact that chemerin is highly expressed in the kidney, a key site of blood pressure regulation. Chemerin is structurally related to other circulating factors, as kininogens, whose proteolytic product is the vasoactive peptide bradykinin.

Our results revealed that HDL-C and CAD-risk percentage were significantly lower in metabolic syndrome group compared to control group. Moreover, a significant negative correlation between serum chemerin levels and both HDL-C and CAD-risk were proved in metabolic syndrome patients. Our results agreed with ***Wang et al.(2009) , Dong et al.(2011) , Yoo et al. (2012)*** and ***Yan et al.(2012)*** who revealed that serum chemerin levels were elevated in subjects with metabolic syndrome and were associated with several cardiovascular risk factors. Serum chemerin levels were significantly elevated in metabolic syndrome patients with CAD compared to those without CAD and healthy subjects and negatively correlated with HDL-C levels. Serum levels of chemerin could be considered as an independent predictive marker of the presence of atherosclerosis and CAD in patients with metabolic syndrome.

McCarthy et al.(2008) supported our findings by stating that chemerin expression by endothelial cells in inflamed tissue and its ability for chemotactic recruitment for macrophages and dendritic cells expressing CMKLR1, as well as its ability to promote cholesterol uptake and foam cell formation, suggests a role of chemerin in inflammatory states and possibly atherosclerosis. In addition, ***Lehrke et al. (2009)*** proved that chemerin is strongly related to markers of inflammation as tumor necrosis factor-α, interlukin-6 and CRP. Thus, it is conceivable that chemerin may be up-regulated in states of inflammation such as obesity, metabolic syndrome and atherosclerosis to dampen inflammatory processes and to improve metabolic regulation.

Our study also compared between insulin resistant type 2 DM and insulin sensitive control group. We reported that fasting blood sugar, 2 hours postprandial blood sugar, fasting serum insulin, HOMA-IR, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and serum chemerin levels were significantly higher in type 2 DM compared to control group. In addition, CAD-risk values were significantly lower in type 2 DM compared to control group. A significant positive correlation between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, LDL-C, triglycerides, total cholesterol and waist circumference and a significant negative correlation between serum chemerin levels and both HDL-C and CAD-risk in type 2 DM group were proved.

Our findings were in agreement with those of ***Wang et al. (2009)*** and ***Yang et al. (2010)*** who stated that plasma chemerin levels were found to be markedly increased in patients with type 2 diabetes mellitus with hypertension compared with normal controls. Our results are also supported by a study done by ***Ernst et al. (2010)*** who revealed that recombinant chemerin administration exacerbated glucose intolerance in obese and diabetic mice. This study provided evidence that serum chemerin levels are elevated in obesity and diabetes and that chemerin exacerbates glucose intolerance in these models by decreasing serum insulin levels and glucose uptake in liver tissue.

Our results go hand in hand with ***El-Mesallamy et al.(2011)*** and ***Hu and Feng (2011)*** who proved that serum chemerin levels were significantly increased in patients with type 2 diabetes and in patients with type 2 diabetes with ischaemic heart disease compared with healthy control subjects. Interestingly, chemerin levels

were found to be significantly correlated with BMI and SBP. Moreover, ***Chakaroun et al. (2011)*** proved that chemerin mRNA is significantly expressed in adipose tissue of patients with type 2 diabetes mellitus and correlates with circulating chemerin, BMI, percentage body fat and HOMA-IR. Obesity surgery-induced weight loss causes a significant reduction on subcutaneous chemerin expression. Decreased chemerin serum concentrations significantly correlate with improved glucose infusion rate independently of changes in BMI.

In contrast to our findings, ***Bozaoglu et al. (2007)*** describes important findings that circulating chemerin levels in type 2 diabetes human subjects were not significantly higher than those in normal control subjects, given that quite probably a proportion of their type 2 diabetic study subjects may have been taking anti-diabetic drugs. ***Tan et al. (2009)*** reported for the first time that metformin (an oral hypoglycemic drug employed by most physicians in both developing and developed countries) significantly decreases circulating chemerin levels with a concomitant decrease in insulin resistance in diabetic subjects.

The lack of significant correlation between serum chemerin levels and each of fasting blood sugar and 2 hours postprandial blood sugar in our study could be attributed to the anti-diabetic drugs taken by the diabetic patients' group beside that the cases of metabolic syndrome group are selected carefully to be non-diabetic with normal fasting glucose levels. In addition, it is conceivable that other factors such as the level of glycemic control, medication history, duration of diabetes, and the presence of complications such as renal disease may have an impact on the relationship between circulating chemerin levels and blood glucose levels ***(Bozaoglu et al., 2009).***

In the present study, multivariate analysis was done to choose the best predictors of the metabolic syndrome. It revealed that serum chemerin, waist circumference and total triglycerides were the most significant predictors with F value=7.68 (p<0.05) which supports the hypothesis that serum chemerin can be added to the conventional parameters as a new marker for the metabolic syndrome.

These results are in agreement with ***Bozaoglu et al.(2007)*** who proved that after adjusting age and sex, chemerin levels were significantly associated with measures of WC and metabolic syndrome components (fasting glucose, fasting insulin, triglycerides and blood pressure) in non-diabetic subjects. After further adjustment for WC, serum chemerin levels were still independently associated with metabolic syndrome components, including systolic blood pressure and plasma triglycerides. When the data were adjusted for age, sex, WC and triglycerides, chemerin levels were only associated with systolic and diastolic blood pressure. These results clearly demonstrate that circulating chemerin levels are associated with components of metabolic syndrome.

Lastly, the ROC curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77% with an AUC = 0.786. The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively.

Our results were in accordance with those of ***Stejskal et al.(2008)*** who found in a study done to evaluate serum chemerin as a marker for the metabolic syndrome, that serum chemerin levels had a sensitivity of 75 % and specificity of 67 % with an AUC = 0.75 at a chemerin cutoff concentration = 240 ng/mL. Their study proposed that serum chemerin should be added up to the profile used regularly to diagnose metabolic syndrome.

In conclusion, the association of high serum chemerin levels with components of metabolic syndrome and type 2 DM indicates that this adipokine represents a novel marker of these derangements and could be considered one of the metabolic risk factors leading to insulin resistance in type 2 DM as well as metabolic syndrome. Moreover, its assessment could be beneficial in early detection of these pathological states and prevention of their unfavorable consequences especially the cardiovascular complications and atherosclerosis.

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Inflammatory and Nutritional Biomarkers: Role as Non -Traditional Risk Factors for Cardiovascular Morbidity in Patients with Chronic Kidney Disease

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Abstract: ***Introduction:*** Chronic kidney disease (CKD) patients have an increased cardiovascular (CV) risk that cannot be explained completely by traditional cardiovascular risk factors (CVRFs). Chronic low grade inflammation is common among patients with renal disease and probably contributes to cardiovascular disease (CVD). Moreover, the prevalence of protein energy wasting (PEW) among patients with CKD is high and is associated with a proinflammatory state. Malnutrition, inflammation, and atherosclerosis often coexist among patients with CKD, and each of these risk factors independently predicts outcome in these patients. ***Aim of the work:*** This study was designed to assess inflammatory and nutritional biomarkers in conjunction with echocardiographic assessment in CKD patients on hemodialysis and on conservative treatment in order to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in these patients.***Patients and methods:*** This study was carried out on 70 subjects: 30 CKD patients on regular hemodialysis, 20 CKD patients on conservative treatment and 20 healthy subjects. All cases were subjected to history taking, full clinical examination, ECG, echocardiography and carotid duplex. Hemoglobin level and serum level of creatinine, urea, albumin, cholesterol, triglycerides, HDL, LDL, iron, ferritin, CRP, IL-1ß and IL-18 were measured. ***Results:*** There was a significant increase in the level of inflammatory markers: CRP, IL-1ß and IL-18; and a significant decrease in the level of nutritional factors: albumin, iron and ferritin in both patient groups versus the controls and in group I versus group II. Regarding the echocardiographic data, there was a statistically significant difference in fractional shortening (FS), ejection fraction (EF), posterior wall thickness (PWT), inter ventricular septum thickness (IVST) and intima-media thickness (IMT) in both patient groups compared to the control group.A positive correlation was found between inflammatory factors (CRP,ILß and IL18) and urea and creatinine while there was a negative correlation between nutritional factors(albumin, iron, ferritin and hemoglobin) with urea and creatinine with a negative correlation between inflammatory factors and nutritional factors .***Conclusion:*** CKD patients especially those on HD, should be considered at high risk for developing CVD. The elevated levels of proinflammatory markers are associated with CV morbidity and may contribute to the deterioration of nutritional status in end stage renal disease (ESRD). Thus, it could be speculated that suppression of the vicious cycle of malnutrition, inflammation and atherosclerosis would improve survival in dialysis patients.

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1. Introduction

Chronic kidney disease (CKD) patients have an increased cardiovascular (CV) risk that cannot be explained completely by traditional cardiovascular risk factors (CVRFs). Non-traditional CVRFs such as oxidative stress, abnormal calcium and phosphate metabolism, hyperhomocysteinaemia, malnutrition and "inflammation syndrome", represent novel therapeutic targets for clinical interventions in this patient population[1].

Chronic low grade inflammation is common among patients with renal disease and probably contributes to cardiovascular disease (CVD)[2]. Moreover, the prevalence of protein energy wasting (PEW) among patients with CKD is high and is associated with a proinflammatory state[3-5]. Malnutrition, inflammation, and atherosclerosis often coexist among patients with CKD, and each of these risk factors independently predicts outcome in these patients[6].

The causes of inflammation in CKD are definitely multifactorial[7]. Low- grade infection, repeated exposure to dialysis filters and auto-oxidation products are considered as likely inciting factors in these patients[8]. Furthermore, a variety of traditional and non-traditional risk factors such as sympathetic hyperactivity, dyslipidemia, hyperphosphatemia/ hyperparathyroidism, diabetes and smoking may activate and/or amplify the inflammatory process in end-stage renal disease (ESRD)[9]. Available data suggest that pro-inflammatory cytokines may play a central role in the genesis of the metabolic syndrome[7].

Atherosclerosis is the main cause of morbidity and mortality in patients with ESRD and there is consistent evidence that C-reactive protein (CRP)

and proinflammatory cytokines such as interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α) are risk factors for atherosclerotic cardiovascular outcomes in these patients[10-11].

CRP, a nonspecific marker of inflammation, is the most important factor in the inflammatory syndrome, and its production is controlled by several inflammatory mediators including IL–6 which is the main cytokine in the acute phase of inflammation. CRP and serum albumin levels can predict mal-nutrition, arthrosclerosis, cardiac diseases, and death in ESRD patients[12].

IL-1ß, which is a major proinflammatory cytokine, may further amplify inflammation and lead to malnutrition, through inducing anorexia, and muscle wasting due to increased protein breakdown. Several clinical studies have shown that the circulating level of IL-1ß may affect nutritional status, especially body composition. Although a number of factors are related to malnutrition and wasting in ESRD, pro-inflammatory cytokines, such as IL-1ß, may play an important role.[13]

Whitman and his colleagues in 2002[14] pointed out that interleukin-18 (IL-18) is a novel proinflammatory cytokine that promotes atherosclerosis and plaque vulnerability in experimental models.

PEW is highly prevalent (34-65%) in CKD, and is considered among the strongest predictors of death in this patient population. Although many factors contribute to impaired nutritional status in CKD patients, inflammation is one of the most important factors. Furthermore, PEW and inflammation are strongly interrelated in the clinical setting[5,15-16]. Hypoalbuminemia, which is common in CKD patients and usually consequent to PEW, is strongly associated with inflammation. Many studies demonstrated that CRP was the primary predictor of serum albumin levels in hemodialysis (HD) patients. Increase in the levels of proinflammatory cytokines is related to high prevalence of PEW and hypoalbuminemia in CKD patients[17-19].

Although serum ferritin is an imperfect marker of inflammation, low levels have been shown to correlate well with iron deficiency and high levels are more likely to correlate with inflammation[20].

Inflammation is closely related to PEW in dialysis patients and the simultaneous combination of these two conditions, also referred to as 'malnutrition–inflammation complex syndrome' (MICS), is observed frequently in dialysis patients[21]. While MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinaemia and refractory anemia including erythropiotein hyporesponsiveness in these individuals[22]. It is not clear whether PEW alone or combined with inflammation in the form of MICS has a significant effect on serum ferritin in HD patients[23].

Aim of the work:

This study was designed to assess inflammatory and nutritional biomarkers simultaneously in patients with CKD, either on HD or conservative treatment, in conjunction with echocardiographic and carotid duplex assessment in order to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in CKD patients.

2. Patients and Methods:

This study was carried out on 70 subjects divided into three groups:

Group I: Including 30 patients with CKD on regular HD, 3 times per week in 4 hours sessions. They were 17 males (56.7%) and 13 females (43.3%).

Group II: Including 20 patients with CKD on conservative treatment. They were 10 males (50%) and 10 females (50%).

The etiology of renal failure was variable among the two studied patient groups.

Group III: Including 20 age and sex matched healthy subjects as a control group . They were 10 males (50%) and 10 females (50%).

Informed written consents were obtained from all patients according to the Declaration of Helsinki.

All patients and controls in this study were subjected to the following:

A. History taking: laying stress on symptoms of cardiac complications e.g. previous anginal episodes, thrombotic events, ECG documented arrhythmia etc.

B. Clinical examination: to confirm the diagnosis and to detect signs of CV complications, measurement of arterial blood pressure and pulse .

C- Electrocardiogram (ECG).

D-Echocardiography: Standard transthoracic M-mode, two dimensional, continuous and pulsed wave Doppler echocardiograms were obtained soon after a session of routine HD using 2.5 MHz transducer. Pulsed and continuous Doppler color coded duplex examination was used to evaluate both diastolic cardiac functions and the functional efficacy of the cardiac valves. M-mode measurements were used to evaluate the left ventricular posterior wall thickness and left ventricular internal dimensions both in systole and diastole aiming to calculate the fractional shortening (FS) and ejection fraction (EF).

E-Carotid Duplex: Ultrasonographic studies on common carotid arteries were performed by using a 7.5 MHz high resolution probe. The intima-media thickness (IMT) was defined as a low-level echo gray band that does not project into the arterial lumen and was measured during end-diastole as the distance from

the leading edge of the second echogenic line of the far walls of the distal segment of the common carotid artery, the carotid bifurication and the initial tract of internal carotid artery on both sides.

F- Laboratory Investigations: Blood sampling was performed after a 12-hrs fast. In HD group blood samples were obtained before the first session of the week. Ten ml venous blood was obtained by clean venipuncture from the antecubital vein and divided as follows: 2 ml into EDTA anticoagulated vacuum tube for complete blood picture and 8 ml into a plain vacuum tube, serum was separated after blood clotting by centrifugation. Part of freshly separated serum was used for routine chemistry done on the collection day namely: serum creatinine, urea, albumin, , serum cholesterol, triglycerides, high density lipoprotein (HDL),low density lipoprotein(LDL) cholesterol, calcium and phosphorus. These tests were performed using auto analyzer Beckman CX3 Delta analyzer.

The rest of the separated serum was stored at -20°C for further determination of inflammatory markers (CRP, IL-1ß and IL-18) and nutritional markers (iron and ferritin).

* **Serum CRP** determination was done using immunoturbidimetric assay (Randox laboratories, Ltd). Kon progress/specific.

* **Serum IL-1ß** levels were measured using the Quantikine High Sensitivity Human IL-1ß ELISA kit from R&D Systems, Inc, Minneopolis, Minnesota, USA (sandwich immunoassay).

* **Serum IL-18** levels were determined using the MBL International Corporation Human IL-18 ELISA kit from R&D Systems Inc, Minneopolis, Minnesota, USA. (a quantitative sandwich immunoassay technique).

* **Quantitative determination of serum iron** was performed by functional chromogenic assay using Stanbio Iron kit (Stanbio Laboratory Inc,USA).

* **Serum levels of ferritin** were determined by enzyme immunoassays using Pathozyme-Ferritin kit (Omega Diagnostics Limited, UK).

All assays were carried out according to manufacturers' instructions.

Statistical methods

Data were summarized as mean values± SD. The ANOVA test was employed for intergroup comparison. Association between variables was assessed by Pearson correlation coefficient. The threshold for significance was a *P*-value ≤ 0.05. Statistical analysis was performed with the aid of the SPSS computer program, version 16.

3. Results

The demographic and clinical data of patients and controls are shown in table 1. There was statistically significant increase in systolic blood pressure in both groups I and II compared to the control group.

Regarding the echocardiographic data, there was a statistically significant decrease in fractional shortening (FS) and ejection fraction (EF) and a significant increase in end systolic dimension (ESD), posterior wall thickness (PWT), inter- ventricular septum thickness (IVST) and intima-media thickness (IMT) in groups I and II in comparison to the control group (table 2).

The results of routine laboratory investigations are illustrated in table 3 while the results of inflammatory and nutritional markers are shown in table 4. The serum levels of CRP, IL-1ß and IL-18 showed statistically significant increase in group I and group II in comparison to the control group together with a statistically significant increase in group I compared to group II. However, there was a statistically significant decrease in serum iron, ferritin, hemoglobin and albumin in both groups (I&II) compared to the control group and also a statistically significant decrease in group I compared to group II.

The results of correlation analysis are shown in table 5 .

Table (1): Demographic and clinical data of studied groups. (mean values± SD)

	Group I (HD) (n=30)	Group II Conservative (n=20)	Group III (Controls) (n=20)
Age (years)	55.8±8.3	54.7±14.2	51.3±10.9
Gender M F	17(56.7%) 13(43.3%)	10(50%) 10(50%)	10(50%) 10(50%)
Systolic BP	132.6±21.3*	139.0±21.2*	118.3±8.1
Diastolic BP	83.0±11.4	87.5±12.5*	78.7±4.2
Pulse (beat/min)	84.3±7.7	84.8±13.2	79.2±10.2
Duration of dialysis (years)	5.6±3.5		

* Highly significant difference *vs* controls $p< 0.01$.

Table (2): The echocardiographic data of studied groups(mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
EDD (mm)	55.0±8.8	55.2±6.6	51.4±7.1
ESD (mm)	36.6±8.9*	36.9±9.0*	31.1±5.3
FS (%)	34.5±7.7*	34.4±5.6*	39.3 ±5.7
EF(%)	61.5±10.5**	58.8±14.7**	69.1±7.3
PWT (mm)	10.9±2.4**	10.7±3.0*	8.8±1.3
IVST (mm)	11.2±2.6**	9.9±2.4*	8.8±1.1
IMT (cm)	1.1±0.19**	1.0±0.22**	0.71±0.88

*Significant difference *vs* controls $p< 0.05$.
** Highly significant difference *vs* controls $p< 0.01$.
EDD: end diastolic dimension, ESD: end systolic dimension, FS: fraction shortening, EF: ejection fraction, PWT: posterior wall thickness, IVST: inter ventricular septum thickness, IMT: intima-media thickness.

\Table (3): Results of routine laboratory investigations in studied Groups (mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
Urea (mg/dl)	139.1 ±28.6*	125.5±47.3*	30.4±4.3
Creatinine (mg/dl)	8.0±1.9*#	3.6±2.0*	1.2±0.2
Ca (mg/dl)	9.1 ±1.2	9.0±1.1	9.6±0.6
PO_4 (mg/dl)	4.7±1.7*	5.3±1.2*	3.3±0.8
Cholesterol (mg/dl)	183.3 ±56.8	181.1±67.4	192.5±15.4
Triglycerides (mg/dl)	245.1±110.7*	202.2±109.5	153.1±13.8
HDL (mg/dl)	34.6 ±21.2*	34.6±11.6*	51.1±15.9
LDL (mg/dl)	105.1 ±51.7	106.0±58.3	110.7±28.2
Hemoglobin (g/dl)	8.5±0.45*#	10.3±0.4*	13.7±0.51

* Highly significant difference *vs* controls $p< 0.01$.
#Highly significant difference *vs* group II $p<0.01$.
Ca: calcium, PO_4: phosphorus, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol.

Table (4): Results of inflammatory and nutritional markers in studied groups(mean values± SD)

	Group I (HD)	Group II (Conservative)	Group III (Controls)
CRP (mg/l)	7.2±2.7*##	4.8±0.9*	1.8±0.4
IL-1ß (pg/ml)	42.5±8.8*##	25.5±5.9*	9.4±2.2
IL-18 (pg/ml)	502.2±99.2*##	307.9±67.7*	123.8±37.4
Iron (ug/dl)	34.8±4.7*##	57.6±16.05*	101.5±13.5
Ferritin(ng/ml)	40.6±6.2*##	57.2±13.5*	117.9±23.5
Albumin (g/dl)	2.9 ±0.5* #	3.3±0.4*	3.9±0.4

* Highly significant difference *vs* controls $p< 0.01$.
#Significant difference *vs* group II $p<0.05$.
##Highly significant difference *vs* group II $p<0.01$.
CRP: C-reactive protein, IL-1ß: interleukin-1beta, IL-18: interleukin-18.

Table (5): Correlation matrix between the parameters studied in patient groups.

	CRP		IL 1B		IL-18	
	r	*P*	r	*P*	r	*P*
Creat.	.69	.001	.73	.001	.60	.001
Iron	-.79	.001	-.81	.001	-.67	.001
Ferritin	-.63	.001	-.70	.001	-53	.01
Hemoglobin	-.74	.001	-.87	.001	-.55	.01
Albumin	-.16	NS	-.28	.01	-.037	NS

4. Discussion:

CKD is a major health problem. Patients with ESRD are characterized by higher mortality rates than the general population. The majority of deaths in these patients are due to CVD[1].

CKD patients have an increased cardiovascular risk that cannot be explained completely by traditional cardiovascular risk factors. Inflammation plays a life-threatening pivotal role in the initiation and progression of atherosclerosis, and is considered a major non-traditional risk factor for accelerated carotid intima thickening and plaque formation in dialysis patients[24-25].

Protein-energy wasting (PEW) is common in patients with CKD and is associated with an increased death risk from CVD. However, while even minor renal dysfunction is an independent predictor of adverse cardiovascular prognosis, PEW becomes clinically manifest at an advanced stage, early before or during the dialytic stage[26].

The present study was designed to assess inflammatory and nutritional biomarkers in conjunction with echocardiographic assessment and carotid Duplex in CKD patients on HD or conservative treatment in a trial to clarify the role of inflammatory and nutritional biomarkers as risk factors for CV complications in these patients.

The results obtained revealed significant increase in systolic blood pressure in patients on HD (group I) and patients on conservative treatment (group II) versus the control group ($P< 0.01$) . Also a significant increase in diastolic blood pressure was found in group II versus the control group ($P< 0.05$). In agreement with these results Foley and Agarwal, [27] stated that the pathogenesis of hypertension in renal failure is complex and arises from the interaction of hemodynamic and neuroendocrine factors.

Regarding echocardiographic findings in patients of this current study, they showed that the mean ejection fraction (EF) and fractional shortening (FS) were significantly lower in both diseased groups in comparison to the control group ($P < 0.01$ and $P < 0.05$ respectively). This was in accordance with findings of Zeng and his colleagues[28] in their study of the value of B-type natriuretic peptide in diagnosing left ventricular dysfunction in dialysis dependent patients. The detection of systolic dysfunction appears particularly relevant in asymptomatic individuals where myocardial disease may progress despite compensatory mechanisms involving the autonomic system, neurohormones, and changes in cardiac function and structure. In this regard, the study of Zoccali *et al.* [29] was the first showing that LV systolic function measured by EF and FS predict incident CV events in a large population of asymptomatic ESRD patients. The prediction power of these indicators was largely independent of traditional and novel risk factors in ESRD such as CRP.

Although, there was a significant increase in left ventricular ESD in both diseased groups in comparison to control group ($P < 0.05$), yet there was no significant difference in EDD between patients and controls. This was in agreement with findings of Fathi et al. [30] who found no significant difference in EDD between patients and controls. Also Lisowska and Musial, [31] stated that heart failure is highly prevalent in ESRD patients. Upon starting dialysis, 37% of their patients had a previous episode of heart failure, thus doubling the risk of death, both systolic and/or

diastolic function may be impaired, 15% of patients on dialysis therapy may have systolic dysfunction.

In the current study, LV wall thickness (IVST and PWT), were hypertrophic in both patient groups compared to control group. This was in agreement with Fathi et al.[30] and Zaslavsky et al.[32] who reported increased posterior wall thickness in ESRD and HD patients.

Carotid duplex revealed a significant increase in IMT in both patient groups versus the control group (P<0.01). This finding was in accordance with findings of Masho and Shigematsu[33], who demonstrated that CKD patients are well recognized to have advanced arteriosclerosis with vascular medial calcification and with high risk of cardiovascular death. It is extremely important in order to prevent vascular calcification to adjust serum phosphorus, serum calcium and parathyroid function within the suitable range. In addition, hyperphosphatemia is becoming the powerful risk factor for patients' survival. In the current study, there was a significant increase in serum phosphorus in both patient groups versus the control group (P< 0.01). This is in agreement with the study done by Spasovski [34] who stated that the abnormalities in bone and mineral metabolism in CKD patients regarding hypocalcemia and hyperphosphatemia are associated with an increased risk of fractures, vascular calcifications and CVD.

The inflammatory markers CRP, IL-1ß and IL-18 exhibited significant elevation in both patient groups versus the control group (P < 0.01 for CRP , IL1ß and IL18). Additionally, their levels were significantly higher in HD patients compared to patients on conservative treatment (P < 0.01 for CRP, IL-1ß and IL-18). In agreement with these results , Jeznach-Steinhagen et al.,[35] reported that there is evidence that CKD patients are in a state of chronic inflammation with activation of C-reactive protein and proinflammatory cytokines and is associated with increased oxidative stress and endothelial dysfunction.

Atherosclerosis is the main cause of morbidity and mortality in patients with ESRD and there is consistent evidence that C-reactive protein (CRP) and proinflammatory cytokines such as interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF-α) are risk factors for atherosclerotic cardiovascular outcomes in these patients[10-11]. Tripepi and his colleagues [9] stressed that the severity of inflammation, as assessed by CRP and IL-6, adds significant prognostic information above and beyond traditional and nontraditional cardiovascular risk factors in dialysis patients. The impact of inflammation is more prominent in HD patients due to additional sources of inflammation[5], probably due to bioincompatible membranes that may activate the complement cascade[36], nonsterile dialysate with small amounts of endotoxin and the use of prosthetic arteriovenous grafts or transcutaneous catheters and processing the dialyser for reuse[37].

The prospective study of Blankenberg and his colleagues [38], demonstrated a strong and independent association between IL-18 level and future coronary events. In addition, Tomasz and his colleagues [39] stated that the contribution of IL-18, but not IL-6, to arteriosclerosis occurrence in CKD patients is independent from CRP or other factors involved in the general inflammatory process. Numerous reports indicate that patients with CKD have elevated serum levels of IL-18[40] , likely related to a greater percentage of active circulating monocytes, the main cellular source of this cytokine[41]. IL-18 may also accelerate the arterial injury in CKD through the induction of lymphocyte differentiation towards T-helper (Th-1) cells, the primary source of Interferon-γ (IFN-γ)[42-43].

In the present study, there was a significant decrease in the level of serum albumin, iron and ferrtin in both patient groups versus the control group and also in I group in comparison to group II (P<0.01 except for albumin between group I and II, P<0.05)). Amaral et al. [44] found no increase in CV mortality associated with low serum albumin level; however, they detected a 45% lower risk of hospitalization for CV events in patients with serum albumin more than 4.0g/dl.

Hypoalbuminemia is the most commonly used surrogate of PEW in dialysis patients and has a strong association with increased mortality[45] and morbidity[46]. Hypoalbuminemia is associated with development of de novo and recurrent cardiac failure in HD and peritoneal dialysis patients[47]. The use of serum prealbumin has been advocated as a better surrogate of nutritional status than albumin in dialysis patients[48]. A confounding factor is that serum albumin and prealbumin are also negative acute phase reactants and their serum levels are profoundly affected by the presence of an inflammatory response[26]. In dialysis patients, hypoalbuminemia could also be favored by the loss of amino acids and/or protein during renal replacement therapy[49]. Therefore, it is not clear whether the negative clinical outcome in advanced CKD patients associated with hypoalbuminemia is a reflection of nutrition or of the inflammatory response or both.

During the acute phase response, inflammatory cytokines such as IL-1ß and TNF-α increase the synthesis of both H and L subunits of ferritin[50]. Hence, serum ferritin can be elevated in inflammation. MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinemia and refractory anaemia including EPO hyporesponsiveness in these individuals.[22] It is not clear whether protein–energy malnutrition alone or

combined with inflammation in the form of MICS has a significant effect on serum ferritin in HD patients.[23] Kalantar-Zadeh and his colleagues found that low serum ferritin has a high specificity to detect iron deficiency in dialysis patients receiving EPO[51]. Indeed, inflammation may not have an effect on serum ferritin, unless there is enough iron stores in the body so that serum ferritin is somewhat increased. IL-1β induces ferritin gene expression by translational control of its mRNA; however, this inflammatory induction of ferritin synthesis is different from iron-dependent ferritin gene expression. They showed that this inflammatory regulation of ferritin requires the background presence of cellular iron[50]. In other words, without adequate iron stores, serum ferritin is low and does not correlate with inflammation, but with enough iron, serum ferritin is a function of both iron and inflammation. This important bench-research finding is consistent with our current and previous clinical and epidemiological findings that, in the setting of absolute iron deficiency, serum ferritin is almost always low. However, once the minimal required iron is available, ferritin regulation also becomes a function of non-iron-dependent factors such as inflammation[23].

The present work revealed a significant decrease in hemoglobin level in both diseased groups versus the control group and in HD patients in comparison to patients on conservative treatment ($P<0.01$). In agreement with these results, Iseki [52] stated that anemia develops during the early stages of CKD and is common in patients with ESRD. Anemia is an important cause of left ventricular hypertrophy and congestive heart failure. Treatment by erythropoietin is expected to improve quality of life, survival, and prevent the CKD progression.

A positive correlation was found between inflammatory factors (CRP,IL1ß and IL18) and urea and creatinine, while there was a negative correlation between nutritional factors(iron, ferritin and hemoglobin) and urea and creatinine. Also there was a negative correlation between inflammatory factors and nutritional factors which was in harmony with Kalantar-Zadeh and his colleagues who found simultaneous, significant correlations between serum ferritin and both markers of inflammation and iron status independent of each other.[23]

In our study, there was a positive correlation between CRP , IL1ß and IL 18 with both PWT&IVS with a positive correlation between CRP and ESD. Also there was a negative correlation between FS &EF and CRP &IL-18 and a positive correlation between them and nutritional markers (ferritin , hemoglobin &albumin). In agreement of our results, Erten and his colleagues proved that proinflammatory cytokines have an association with LVH in hemodialysis patients [53].

Conclusion:

CKD patients especially those on hemodialysis should be considered at high risk for developing CVD. The elevated levels of CRP and proinflammatory cytokines IL-1ß and IL-18 are associated with increased cardiovascular morbidity and may contribute to the deterioration of nutritional status in ESRD. Thus, it could be speculated that suppression of the vicious cycle of malnutrition, inflammation and atherosclerosis would improve survival in dialysis patients. As there is not yet any recognized, or even proposed, treatment for ESRD patients with chronic inflammation, it would be of obvious interest to study the long-term effect of various anti-inflammatory treatment strategies.

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Expression of PRAME gene in Egyptian adult acute myeloid leukaemia and its correlation with clinical response

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Abstract: Preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen (CTA) belonging to the group of tumor associated antigens. Te PRAME gene expression is low or absent in almost all normal adult tissues. The PRAME transcript is highly expressed in acute myeloid leukemia patients and is usually associated with a favorable prognosis. The aim of this work is to assess the expression PRAME gene in Egyptian adult acute myeloid leukemia patients at diagnosis and to correlate its expression with the clinical response. PRAME transcript expression was studied in sixty adult acute myeloid leukemia patients using RT-PCR. PRAME m-RNA expression was detected in 33 (55%) of patients. No significant correlation was found between PRAME gene positivity and any of the clinical or hematological variables except for hepatomegaly. PRAME negative patients showed good response to treatment compared those who were PRAME positive. The rate of CR was 37.5% compared to 65.2% in PRAME positive and PRAME negative patients, respectively (p value = 0.043). It seems that there is an increase in the overall survival among the PRAME negative compared to the PRAME positive group although the difference was not significant (p value = 0.06). In conclusion, PRAME is an attractive tumor-associated antigen. Its expression was associated with poor prognosis. More studies should aim at detailed understanding of the mechanisms of PRAME action and its use in minimal residual disease detection and immunotherapy.

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Key words: Acute myeloid leukemia, PRAME

1. Introduction

Acute myeloid leukemia represents a group of clonal hematopoietic stem cell disorders in which failure to differentiate and over proliferation in the stem cell compartment result in accumulation of non-functional myeloblasts. Preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen (CTA) belonging to the group of tumor associated antigens. The PRAME gene maps on chromosome 22 at 22q11. It was first detected in a case of malignant melanoma [(1)].

Te PRAME gene expression is low or absent in almost all normal adult tissues except for testis, adrenals, ovaries and endometrial tissues. This gene is expressed at a high level in a very large fraction of tumors, such as melanomas, non-small-cell lung carcinomas, sarcomas, head and neck tumors and renal carcinomas. But in contrast with most other tumor associated antigens, it is also expressed in leukemias [(2)]. In spite of the fact that the PRME antigen is recognized by autologous cytotoxic T cell-mediated immune responses, its expression is well retained. This suggests that expression of PRAME is addressed to be involved in the tumorigenic process [(3)].

The mRNA level of PRAME is used as a tumor marker due to its over expression in various malignancies. The PRAME transcript is highly expressed in AML patients and is usually associated with a favorable prognosis [(4-6)].

Retinoic acid (RA) induces proliferation arrest, differentiation, and apoptosis. Defects in retinoic acid receptor (RAR) signaling have been implicated in cancer. PRAME has been reported to function as a repressor of retinoic acid (RA) signaling through interactions with retinoic acid receptors (RARs) which was proposed as important contributory factor in AML disease progression [(3,7)].

The effect of PRAME on gene expression in leukemic cells remains controversial, while cell based reported the down-regulation of genes such as S100A4, RARb2, p21 and Hsp27 [(8)], another study reported lack of association between expression levels of these genes and PRAME expression in pediatric AML [(9)].

Quantification of PRAME transcript in acute myeloid leukemia could be used to monitor minimal residual disease for AML, patients with higher than normal levels and its increase over or persistently higher than normal range predict hematological relapse [(6,10)]. PRAME is also considered a good target for tumor immunotherapy [(5)].

The aim of this work is to assess the expression PRAME gene in Egyptian adult acute myeloid leukemia patients at diagnosis and to correlate its expression with the clinical response.

2. Material and Methods

Sixty adult acute myeloid leukemia patients attending the National Cancer Institute, Cairo

University between October 2008 and January 2010 and 10 healthy age and sex matched controls were included in this study. Patients' clinical characteristics are shown in Table (1). The study involved 33 females (55%) and 27 males (45%). The female to male ratio was 1.2: 1. The age of the studied patients ranged from 18-70 years with a median of 36 years and mean 37.7 years.

RNA extraction was done using QIAamp RNA blood Mini Kit. Reverse transcription was performed in 20ul reaction using random hexamer according to manufacturer's instructions (High capacity cDNA reverse transcription kit) (Applied Biosystems). PRAME and β-actin amplification were performed in two separate PCR reactions containing 1ug RNA, 1X buffer, 10 pmole of each primer: β-actin (5'-GTGGGGCGCCCCAGGCACCA-3') (5'-GTCCTT AAT GTC ACG CAC GAT TTC -3') [11] and PRAME: (5' -CTGTACTCATTTCCAGAGCCA-GA-3') (5'-TATTGAGAGAGGGTTTCCAAGGGGTT-3')[12], 1.5 mM Magnesium Chloride, 0.8 mM dNTPs (200 µM of each dNTP), 1.25 U Go Taq DNA Polymerase (Promega). The PCR conditions for B actin consisted of initial denaturation at 94ºC for 5 min, followed by 34 cycles of denaturation at 94ºC for 1min, annealing at 63ºC for 2min and extension at 72ºC for 3min. For PRAME, it consisted of Initial denaturation at 95ºC for 5 min, followed by 30 cycles of denaturation at 94ºC for 1min, annealing at 60ºC for 1min and extension at 72ºC for 2min. The PCR products were examined with gel electrophoresis using 1.5% agarose gel (Figures 1 and 2).

3. Results

PRAME m-RNA expression was detected in 33 (55%) of patients. None of the studied controls expressed PRAME m-RNA transcript. The difference between patients and controls was statistically significant (p=0.00).

No significant correlation was found between PRAME gene positivity and any of the clinical, hematological variables except for hepatomegaly (p=0.04) (Tables 1).

In our study out of 33 PRAME positive patients, fourteen patients were M2, seven were M1, five were M4, four were M3 and three were M5. PRAME expression was not correlated with immunophenotyping or stem cell marker CD34 expression on blast cells (Table 1).

Conventional cytogenetic study was done to 30 patients. Fifteen cases (50%) were of normal karyotype, five (16.6%) were positive to t(15:17), four patients (13.3%) were positive to inv(16) and two for t(8:21). While four cases show different cytogenetic abnormalities (-20, +21, del 11q23 and -14). Statistical analysis could not be done because of the small number of patients encountered in each cytogenetic abnormality.

In this study 10 out of 12 early death cases were PRAME positive. This group of patients shows a high leukocyte count and poor liver and kidney functions. In multivariate analysis the PRAME wasn't correlated with any of the laboratory or clinical criteria.

Complete remission was achieved in 27 out of 55 patients (49%). Five cases were missed in the follow up. Among those who were PRAME positive, 12/32 (37.5%) achieved CR compared to 15/23 (65.2%) of PRAME negative patients (Table 2). A statistical significant correlation was found between the response to treatment and PRAME gene expression with a (p value = 0.043). So PRAME negative patients showed a good response to treatment compared those who were PRAME positive.

After a follow up period of 16 months, the overall survival at one year was 37.9% with a median survival 4.2 month. In the group of patients with PRAME positive gene (32/55) the median survival was 1.5 month compared to 12.8 months in patients with PRAME negative gene (23/55). Although there is a bordered line statistical significant difference detected between the PRAME gene and the overall survival (p = 0.06), it seems that there is improvement in the overall survival among the PRAME negative compared to the PRAME positive group.

No statistical significant difference in median survival was found as regard age and sex with p =0.42 and 0.44 respectively. As regard the clinical findings, no statistical significant difference in median survival was found between patients with or without hepatomegaly, splenomegaly and lymphadenopathy with (p values = 0.91, 0.69, 0.99) respectively. As regard the hematological findings, no statistical significant difference in median survival was found regarding WBC and BM blasts.

Disease free survival (DFS) was 70% with a median duration of 13.1 months. No statistical significant difference in median survival was found between PRAME positive compared to PRAME negative group p value =0.4. No statistical significant difference in median survival was found as regard age, a statistical significant difference in median survival was found between males and females with p value =0.04. As regard the clinical findings, no statistical significant difference in median survival was found between patients with or without hepatomegaly and splenomegaly with a p = 0.81 and 0.64 respectively.

As regard the hematological findings, no statistical significant difference in median survival was found regarding WBC and BM blasts. Also no statistical significant difference in median survival was found regarding FAB subtype or immunophenotyping.

Table (1): Correlation of clinical and haematologic criteria with PRAME expression in AML patients

	Total	PRAME positive N(%)	PRAME negative N(%)	*p*- value
Age (y)				
<45		23(57.5)	17(42.5)	
≥45		10(50.0)	10(50.0)	0.58
Sex				
Female		17(51.5)	16(48.5)	0.55
Male		16(59.3)	11(40.7)	
Signs				
Hepatomegaly		20(69.0)	9(31.0)	0.04
Splenomegaly		13(72.2)	5(27.8)	0.08
Lymphadenopathy		3(60)	2(40)	1.0
Haematologic				
WBCs ($X10^9L$) ≥ 50		11(55)	9(45)	1.0
Immunophenotype				
Myeloid with monocytic		8(61.5)	5(38.5)	0.59
CD34 positive		15(53.6)	13(46.45)	0.84

Table (2): Complete remission in PRAME positive and PRAME negative groups

	PRAME positive N(%)	PRAME negative N(%)	*p*-value
Complete remission	12 (37.5)	15 (65.2)	0.04
No complete remission	20 (62.5)	8 (34.8)	

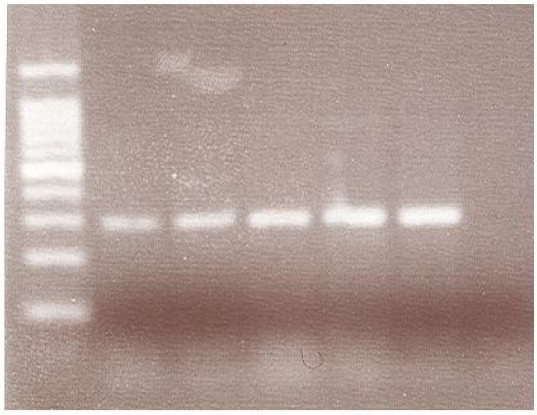

Figure (1): RT-PCR analysis showing β-actin expression
Lane 1: Molecular weight marker 1 kb (Fermentas).
Lanes 1- 5: β-actin positive cases.

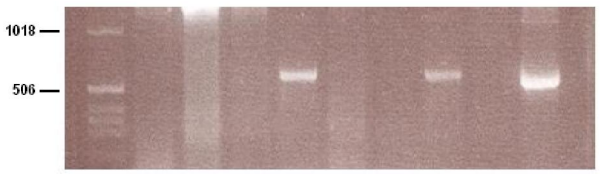

Figure (2): RT-PCR analysis showing PRAME expression.
Lane 1: Molecular weight marker 1 kb (Invitrogen).
Lanes 5,8,10: PRAME positive cases (556bp).
Lanes 2,3,4,6,7,9: PRAME negative cases.

4. Discussion

PRAME gene is highly expressed in AML patients and is usually associated with a favorable response to chemotherapy and prolonged survival [4,5]. It has been reported to function as a repressor of retinoic acid (RA) signaling through interactions with retinoic acid receptors (RARs). Expression of PRAME gene was assessed in adult acute myeloid leukemia patients at diagnosis and correlated with clinical outcome. Of sixty patients examined, thirty three patients (55%) expressed PRAME mRNA. This finding is approximately similar to that reported by Qin *et al* [13] in which PRAME was detected in 55.4% of AML patients and slightly lower than 64% reported by Greiner *et al.,* and Zhu *et al.,* [14,15]. On the other hand, PRAME expression was higher than was reported by Greiner *et al*, Zhou *et al.,* Tajeddine *et al.,* and Paydas *et al.,* [16,17,18,19] (47%, 42.9%, 40% and 30%, respectively). Ortmann *et al.,* [20] reported that PRAME expression has been observed in 30-64% of acute myeloid leukemia cases. In our study, PRAM mRNA was not detected in normal bone marrow cells. The difference between patients and control was statistically significant (*p*=0.00). Similar observation was demonstrated by Zhu *et al.,* and Zhou *et al.,* [15,17].

In this study, no significant correlation was found between PRAME expression and age, sex, white blood

cell count and the percentage of blasts in bone marrow at the diagnosis. These results are similar to that encountered by Paydas *et al.,* Zhou *et al.,* and Zhu *et al.,* [15,17,19].On the contrary, in a pediatric study done by Steinbach *et al.,* [4], he found that PRAME expression was negatively correlated to white blood cell count at diagnosis. PRAME expression was not correlated with immunophenotyping or stem cell marker CD34 expression on blast cells, this correspond to that reported by Paydas *et al.,* [19] who didn't find any important correlation between PRAME expression and cell surface antigens. PRAME expression mainly belongs to M2 FAB subtype but a valid significant statistics could not be done because of the small number of the cases included in different FAB groups. In a study carried Zhu *et al.,*[6], he reported that among the FAB subtypes, those with M1, M2, M3 and M4 had significantly higher level of PRAME transcripts than controls, however, those with M5 had similar level of PRAME transcripts as controls, also among cases with AML-M2, those with t(8;21) had significantly higher level of PRAME transcripts than those without[10].

As regard the clinical data, a significant correlation was found between PRAME expression and hepatomegaly (p=0.036), while no significant correlation was found with the splenomegaly and lymphadenopathy. This was different from what is reported by Paydas *et al.,* [19] who didn't find any correlation between PRAME expression and organomegaly or lymphadenopathy. However, our results can be explained by the high incidence of the hepatic bilharziasis in Egypt.

In this study a statistical significant correlation was detected between the response to therapy and PRAME expression where the number of CR is more in negative PRAME expression with a p value = 0.04. While, Zhou *et al.,* [17] and Zhu *et al.,* [6] found that PRAME is an indicator of favorable prognosis and can be a useful tool for monitoring minimal residual disease (MRD) in AML patients.

In this study 10 out of 12 early death cases were PRAME positive and this group of patients showed a high leukocyte count and poor liver and kidney functions which might lead to this result especially that in our multivariate analysis the PRAME wasn't correlated with any of the laboratory or clinical criteria.

Most studies on acute leukemia cases could not draw a clear association of PRAME expression as an independent prognostic [1,4]. Also in a study carried by Paydas *et al.,* [19], he didn't find any important correlation between PRAME expression and response to therapy. But various quantitative studies has shown that PRAME levels decrease in remission and increase in relapse suggesting its association to detect minimal residual disease [13,18,19].

On the contrary, high PRAME expression was found to be an independent prognostic marker of poor outcome in breast cancer and neuroblastoma [21,22]. Additionally, PRAME has been suggested as a predictive factor to determine the blastic phase in CML cases [23]. Also, it was found more frequently in mantle cell lymphoma as compared with other chronic lymphoproliferative disorders, thus making this antigen a marker for differentiation from other types of lymphoproliferative disorders [24].

As regard overall and disease free survival no statistical significant correlation was found with the PRAME expression. It seems that there is an increase in the overall survival among the PRAME negative compared to the PRAME positive group although the difference was not significant (p value = 0.06).This goes with Paydas *et al.,* [19] who didn't find correlation between PRAME expression and progression-free and overall survival. On the contrary, Greiner *et al.,* [5] found a significant correlation between high m-RNA levels of PRAME and longer overall survival. Also in a pediatric study, Steinbach *et al.,* [4], has found that the rates of overall and disease-free survival in the group of patients with high PRAME expression were higher than in patients with no or low expression.

In conclusion, PRAME is particularly an attractive tumor-associated antigen. Its expression was associated with poor prognosis. Its level of expression should be monitored during the course of the disease as a useful marker to predict remission or relapse. However, more studies should aim at detailed understanding of the mechanisms of PRAME actions and its use in minimal residual disease detection and in immunotherapy.

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Fibroblast Growth Factor 23 in Children With End Stage Renal Disease on Hemodialysis

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Abstract: Background: Fibroblast growth factor 23 (FGF-23) is a novel regulator of phosphate metabolism. In adults with chronic kidney disease (CKD) FGF-23 is increased; however, comparable studies in children are lacking. **Objective:** To investigate the level of FGF-23 in children with end stage renal disease (ESRD) on maintenance hemodialysis and its relation to serum phosphorus, Ca^{2+} and PTH. **Patients and Methods:** The serum level of FGF-23 was measured in twenty children with ESRD on maintenance hemodialysis and compared to their age- and sex matched healthy children. Biochemical parameters including serum urea, creatinine, hemoglobin, ALP, phosphorus, $Ca^{2+,}$ and PTH were measured in this study to unravel their relationship with circulating FGF-23. **Results:** Levels of FGF-23 were significantly higher in pediatric patients in comparison with healthy control group and positively correlated with PTH and phosphorus. Phosphorus level in the diseased group was significantly high in spite of increasing level of FGF-23. The blood urea, creatinine and PTH increased significantly, concomitant with significant decrease in hemoglobin level and insignificant alteration of calcium levels in the diseased pediatric patients, compared to the healthy control group. **Conclusion:** FGF-23 could represent a promising therapeutic target that might improve the fatal progression of dialysis in children with chronic kidney disease on maintenance hemodialysis.

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Keywords: FGF 23, PTH, CKD, hemodialysis

Abbreviations: FGF: Fibroblastic growth factor. **PTH:** Parathyroid hormone. **CKD:** Chronic Kidney Disease. **ESRD:** End stage renal disease

1. Introduction

Chronic kidney disease (CKD) is a growing public health hazard that is associated with a markedly increasing risk of cardiovascular and bone diseases. In healthy individuals, kidneys regulate calcium and phosphorus homeostasis through tubular reabsorption mechanism. Patients with CKD have seriously compromised homeostatic mechanism, giving rise to different adaptive changes in calcium(Ca^{2+}), phosphorus (Ph), parathyroid hormone (PTH) and fibroblast growth factor (FGF-23) levels ***(Mejia et al.,2001)***. Hyperphosphatemia is a risk factor for cardiovascular disease and mortality and thus a potential target for interventions to improve clinical outcomes in CKD ***(Isakova et al., 2009).***

Fibroblast growth factor (FGF-23) is a novel bone-derived hormone that inhibits phosphate reabsorption and calcitriol production by the kidney. This phosphaturic hormone, which is made predominately by osteocytes in bone, appears to have a physiologic role as a counter-regulator hormone for vitamin D ***(Stubbs et al., 2007).*** It achieves its cellular specificity in the kidney and parathyroid glands by binding to co-receptor Klotho which increases the affinity of FGF-23 for ubiquitously expressed FGF receptors ***(Urakawa et al., 2006).***

In patients with chronic kidney diseases, FGF-23 level is thought to increase as a compensatory response to maintain normal phosphate balance as the capacity for renal phosphorus excretion declines **(*Gutierrez et al., 2008)*.** As the main physiologic function of FGF-23 is the enhancement of renal phosphate excretion and elevation in FGF-23 precedes the rise of serum phosphate, FGF-23 level is found to be inversely related to renal function in patients with CKD. Thus, serum levels of FGF-23 might be a better prognostic biomarker than serum phosphate level for risk assessment in patient with CKD ***(Shigematsu et al., 2004).***

Control of bone and mineral homeostasis is considered essential in children with CKD to prevent skeletal complications, achieve adequate growth and maintain cardiovascular health ***(Claus and Otto, 2011).*** Thus, the aim of the present study was to assess the levels of FGF-23 and other biochemical variables of bone metabolism in children with chronic renal disease on hemodialysis.

2. Patients and Methods

Prior to initiation, this study received approval by the Ethical Committee of the Faculty of Medicine at Elazhar University. This study investigated twenty children with ESRD who had been on regular

maintenance hemodialysis. These patients were recruited from renal dialysis units at Al-Zahraa University Hospital. Informed consent was taken from the children and their parents. The ratio of male/female is 12/8. Their ages ranged from 10-18 years with mean (13.15 ± 2.91) years. Their mean weight and height was 21.79 ± 4.16 Kg and 120.85 ± 9.29 cm, respectively.. Twenty age and sex matched healthy subjects were enrolled in this study as a control group. Their mean age was 13.42 ± 2.78 years. Their mean weight and height were 32.17 ± 3.07 Kg and 135.83 ± 6.04 cm, respectively.

All patients and controls were subjected to full history taking, thorough clinical examinations, anthropometric measurement and only those children with end stage renal disease who had been on a regular dialysis were included in the study. Three hemodialysis sessions weekly (4 hours/session) were performed. Hemodialysis access was arteriovenous fistula and was performed using a bicarbonate buffered dialysate.

All patients and controls were exposed to laboratory investigations including complete blood count (CBC), renal function tests, Ca^{2+}, Ph, alkaline phosphatase (ALP), intact parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23).

Five mls of peripheral venous blood were withdrawn and divided into two parts. One ml was anticoagulated with EDTA for CBC performance, and from the remaining 4 mls serum was obtained by centrifugation at 4000 xg for 10 minutes. An aliquot of the serum was stored frozen at -20˚c for the assay of FGF-23. The remaining aliquot was used for assessment of renal function tests, Ca^{2} ,Ph, ALP and intact PTH.

CBC was done on Cell Dyn 1800 autoanalyzer **(**Abbott Cell Dyn 1800 Hematology Analyzer, USA). Renal function tests, Ca^{2+},Ph, and ALP were assayed photometrically on Hitachi 911Autoanalyzer using kits purchased from Roche-Diagnostic systems(F. Hoffmann-LaRoche Ltd., Basel, Switzerland)

Intact PTH was assayed by immulite 1000 systems (chemiluminescence method) using kit supplied by Seimens Health care Diagnostics products Ltd (United Kingdom). Intact PTH is a solid-phase, two site chemiluminesent enzyme-labeled immunometric assay. The solid phase was a polystyrene bead enclosed within a test unit containing the coated bead. An alkaline phosphatase conjugated to polyclonal goat anti intact PTH was also added to the test unit. After the wash and incubation steps, chemiluminescent substrate underwent hydrolysis in the presence of alkaline phosphatase. The photon output as measured by the luminometer is related to the presence of intact PTH in the sample ***(Babson, 1991).***

FGF-23 was assayed by ELISA immune assay kit (Glory Science Co., Ltd, USA) according to the manufacture instructions. The microtiter plate provided in this kit has been pre-coated with an antibody specific to FGF-23 ***(Larsson et al., 2003).*** Standards or samples were then added to the appropriate microtiter plate well with a biotin-conjugated antibody preparation specific to FGF-23 and streptavidin conjugated to Horseradish peroxidase (HRP) was added to each microplate well and incubated. Then achromogen solution (A and B) was added to each well. Only those wells that contained FGF-23, biotin-conjugated antibody and enzyme-conjugated streptavidin will exhibited a change in color. The reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at wave length of 450 nm. The concentration of FGF-23 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis:

Means ± SD of all basic and clinical variables were computed to identify sample characteristics. Differences in characteristics between participants were tested with t test for the normally distributed variables. The significance level was set at five percent. Univariate correlation analysis was done for variables that possibly associated with FGF using Pearson correlation. Statistical Package for Social Sciences Version 17.0 was used for these analyses.

3. Results

The clinical and laboratory data of the patients and their control counterparts are shown in table (1). Patients and their healthy control who are selected within the same age range showed a significant difference (P <0.001) regarding their mean weights and heights. The dialysed children showed a 32.26 % decrease in mean body weights and 11% in mean heights.

Statistical analysis of kidney function tests in the diseased group showed significant increase in serum levels of urea (5 times) and creatinine (10.4 times), compared to their control groups at (P<0.01).

The mean hemoglobin of patients was 9.09 ± 0.91 compared to 11.6 ± 0.67 among control subjects, showing 21.6 % decrease. Furthermore, the patients had significantly higher phosphorus and alkaline phosphatase levels (1.7 and 11 times, respectively at P< 0.00).

Concerning the serum calcium level, patients had lower mean level (8.48 ± 0.87 mg/dl), compared to control group(8.99 ± 0.38 mg/dl); however, this difference was statistically insignificant (P<0.06).

The present study revealed a drastic elevation (P<0.00) of serum levels of FGF-23 and iPTH in dialyzed patients (9.84, 23.6 times, at P< 0.001), compared to control subject.

Univariate correlation analysis of FGF-23 with the other parameters revealed that serum FGF-23 was positively correlated with phosphorus (r= + 0.62, *P*= 0.00); iPTH (r= 0.73, *P*= 0.00) and ALP (r= 0.77,*P*=0.00) as shown in table (2) and figures (1) and (2).

Table 1: Clinical and biochemical level in studied group.

Variable	Patient (n =20)	Control (n = 20)	t- test	*P* value
Age (years)	13.15 ± 2.91	13.42 ± 2.78	0.26	0.8
Weight (Kg)	21.79±4.16	32.17±3.07	7.48	0.00*
Height (cm)	120.85±9.29	135.83±6.04	4.97	0.00*
Hemoglobin (g/dl)	9.09 ± 0.91	11.60 ± 0.67	8.25	0.00*
Urea (mg/dl)	113.15±42.01	22.67±8.89	7.32	0.00*
Creatinine (mg/dl)	8.84±2.51	0.85±0.23	10.92	0.00*
Calcium (mg/dl)	8.47±0.8	8.99±0.38	2.3	0.02*
Phosphorus (mg/dl)	6.15±1.68	3.46±0.63	5.29	0.00*
ALP (U/L)	513.40±116.5	57.75±10.54	-17.3	0.00*
PTH (Pg/ml)	357.00±92.9	26.83±4.6	- 15.8	0.00*
FGF 23 (ng/L)	510.25±65.8	53.33±9.5	-30.5	0.00*

Values shown are means (± SD), n = 20 individuals per group. (*)Values shown are significantly different from the normal control group at P < 0.05 (t-test).

Table 2: Correlation between FGF-23 and other parameters

	Pearson correlation "r"	*P* value
Urea	**0.91**	**0.00***
Creatinine	**0.95**	**0.00***
Calcium	**- 0.49**	**0.004***
Phosphorus	**0.62**	**0.00***
ALP	**0.77**	**0.00***
PTH	**0.73**	**0.00***

*Value shown resulted in a significant Pearson Correlation (r) at P < 0.05.

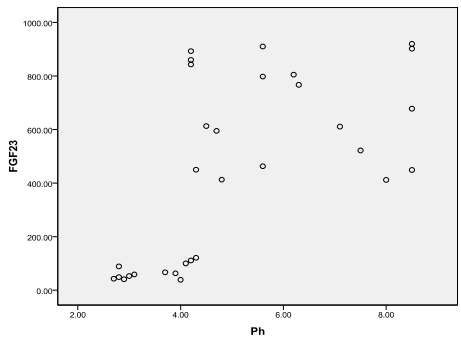

Fig. 1: Correlation between FGF-23 and Phosphorus levels. An r = 0. 62 was determined for the plot shown

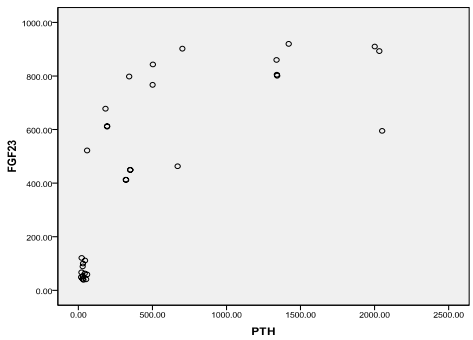

Fig. 2: Correlation between FGF-23 and PTH levels. An r = 0. 73 was determined for the plot shown.

4. Discussion

In patients with CKD, circulating FGF-23 levels are progressively elevated to compensate for persistent phosphate retention ***(Komaba and Fukagawa, 2009).*** In late CKD, FGF-23 cannot reduce serum phosphate levels, and abnormally high FGF-23 concentration appears to exert unwarranted effects, including left ventricular hypertrophy, faster CKD progression and premature mortality ***(Jüppner, 2011).***

Although many studies have been performed in CKD adults, few data are available on FGF-23 metabolism in CKD children ***(Magnusson et al., 2010).***

In the present study, we studied 20 dialysis patients compared to their matched age and sex healthy controls. There was a significant decrease in weight and height of dialysis patients when compared to healthy controls. These findings were consistent with ***Stefandis and Klaus (2007)***who reported that growth failure is a common and significant clinical problem for children on dialysis and often remains a major impediment to their rehabilitation.

In our study, levels of FGF-23 in patients on dialysis were significantly higher in comparison with those in the control group. These increases in the FGF-23 levels are in agreement with ***van Husen et al. (2010)*** who proved that the highest levels of FGF-23 were found in stage 5 compared to stages 1 and 2 CKD.

The increased level of FGF-23 was positively correlated to the elevation of serum creatinine and blood urea, as illustrated in the present study. These findings are in agreement with ***Nakanishi et al.(2005)*** who suggested that serum FGF-23 levels are progressively increased as kidney function declines and are markedly elevated once on dialysis.

Phosphorus is an important mineral for cell structure and energy ***(Giachelli, 2009).*** It is filtered freely in the glomerulus and then reabsorbed in the proximal tubules under the effect of various hormones. The amount of reabsorbed phosphorus is the main regulator of the serum phosphorus levels in subject with normal renal function, or moderately reduced glomerular filtration rate ***(Prie et al., 2009).***

In the current study, the phosphorus levels were significantly elevated in the studied patients and positively correlated with FGF-23 levels. These findings were consistent with ***Fourtounas (2011)]*** who found that, in CKD, the kidneys fail to excrete the phosphorus, resulting in positive phosphorus balance. The skeleton through the disorders of the bone that accompany CKD, contributes to this hyperphospatemia, as it fails to handle the exceeding phosphorus. Furthermore, our findings were in agreement with ***Komaba and Fukagawa (2009)*** who stated that reduced renal function directly affects phosphorus reabsorption. The kidney becomes incapable of filtering enough phosphorus and its high level in blood directly stimulates the parathyroid gland which in turn stimulates FGF-23 synthesis and secretion by the osteocytes.

Sliem et al.(2011) explained this positive correlation providing two explanations; the first is the kidney, which is the principal target of FGF-23, becomes no longer responsive to FGF-23 in CKD. Moreover, Klotho production by the kidney is reduced in end stage renal disease. The second is that, in early stage CKD, serum FGF-23 is elevated to maintain normal serum phosphate levels, by promoting urinary phosphate excretion,. However, in patients at the advanced stage, overt phosphate loading may overcome such compensation for decrease glomerular filtration rate (GFR) despite markedly elevated FGF-23 levels.

Parathyroid gland is the main organ responsible for PTH production and Ca^{2+} homeostasis in the organism. It senses serum Ca^{2+} concentration via the Ca^{2+} receptor (Ca R) and vitamin D receptors (VDR) ***(Duran et al.,2010).*** Extracellular ionic Ca^{2+} is the main parathyroid regulator; low levels stimulate PTH secretion and high levels inhibit hormone release and furthermore, favor its degradation within the parathyroid cells ***(Silver and Levin, 2005).***

The present study revealed marked increase in the parathyroid hormone levels, which was positively correlated with FGF-23 levels. These data were supported by ***Rodrinuez et al. (2006)*** who stated that, in CKD the incorrect control of PTH secretion was attributed to the reduced VDR and CaR expression which occur in parallel to the parathyroid gland growth. Parathyroid gland hyperplasia and the consequent increase in PTH secretion are responsible for hyperparathyroidism observed in CKD.

Komaba and Fukagawa (2010) explained t the failure of increased FGF-23 levels to suppress PTH, by the parathyroid resistance that might be due to the decreased expression of the Klotho-FGF R1 complex in the hyperplastic parathyroid gland. ***Nakanishi et al.(2005)*** pointed out that elevated level of serum FGF-23 is suggested to be an important predictor of secondary hyperparathyroidism in patients who are undergoing dialysis treatment.

The findings of this study were also in agreement with ***van Husen et al. (2010)*** who recorded a positive correlation of the levels of FGF-23 with parathyroid hormone and phosphate concentration. ***Gutierrez et al. (2008)***documented that in CKD, serum FGF-23 levels are increased together with secondary hyperparathyroidism, indicating resistance of the parathyroid to FGF-23. Measurement of FGF-23 seemed to have prognostic significance in the treatment of secondary hyperparathyroidism.

Hemoglobin levels were significantly lowered in the studied patient group. The development of anemia

in patients with CKD was explained by ***Rao et al.(1993)***who reported that high PTH levels directly inhibit the production of RBCs and increase their fragility. Hyperparathyroidism can also cause marrow fibrosis decreasing thereby the production of red blood cells.

Alkaline phosphatase (ALP) is an enzyme measurable in most body fluids and usually originates from the liver or bone. In CKD patients without liver disease, ALP can be elevated in high-turnover bone disease ***(Regidor et al., 2008).*** However, measuring this readily available and inexpensive biomarker has not been singled out as an individual therapeutic target of CKD ***(Eknoyan et al., 2003).***

The present study showed significant elevation of serum ALP in the studied patient group in comparison to the control group; these data are supported by the study of ***Fahrleitner-Pammer et al.(2008)and Kovesdy et al. (2010).***

Concerning calcium levels, a significant decrease was observed in comparison to the control group. This hypocalcemia was supported by the study of ***Levin et al.(2007)*** who reported that, in advanced cases of renal failure, serum calcium levels drop in response to decreased intestinal calcium absorption, resulting from low circulating levels of 1,25(OH)2 D3. On the other hand, ***Wesseling et al.(2008)*** state that, hypocalcemia is quite uncommon in CKD stage 3 and early stage 4, but more often observed in stage 5.

In conclusion FGF-23 could represent a promising therapeutic target that might improve the fatal prognosis of dialysis children with chronic renal failure in regard to management of disordered phosphorus metabolism. Further research is needed to show whether lowering FGF-23 levels improve outcomes in children on maintenance hemodialysis

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The Importance of Antioxidants with the Marine Origin in Inhibit Free Radicals

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Abstract: Free radicals are substances that can exist independently and At least one unpaired electron, which is why it is very active. The types of free radicals, superoxide radicals can be Hydroxyl radical, Radical Nitric Radical and nitrogen dioxide cited. In addition, oxygen free radicals produced during metabolism is also very dangerous. Most damage that free radicals in the human body include: Chemical abuse, such as carbon tetrachloride poisoning, inflammation, kill germs, the destruction of nuclear acids (DNA , RNA), Serious damage to the lungs and impaired breathing process, aging (lipid per oxidation of cell membranes) and many diseases including cancer, diabetes, arthritis, Alzheimer's and cataracts. Or antioxidant compounds are produced in the body or through Power supplies are. In fact, antioxidants, this combines with free radicals into harmless molecules in the reaction and the production and protects the body against radical damage. Antioxidants are capable of even very low concentrations, the oxidation of the material considerably oxidation can be inhibited or delayed throw. Blossoming of new technologies in recent decades due to the biological importance of marine origin has increased antioxidants. Results of research on aquatic life in many countries shows that such a potentially rich source of vital compounds, salts and minerals vitamins, essential fatty acids, anti-tumor, anti-virus and antioxidants are the most unique of its kind, or are unique.
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1. Introduction

About 361 million square kilometers (70.8%) of the total land area are covered by seas and oceans. Followed by an increase in world population and increasing food needs of the uncontrolled exploitation of marine life has been.

Plus shipping marine, oil pipelines, buried deep in the hazardous waste, drainage, water balance, and finally the giant ships discharge of wastewater, industrial and agricultural by rivers to the sea all caused extensive environmental catastrophes in the Earth's vital ecosystems has been. Including many endangered species and subsequent loss of aquatic biodiversity seas. In recent years, extensive research specialist life sciences and marine sciences, the need for greater attention to the sea and protection of marine resources has been emphasized.

However, parallel development of sciences and biological technology, once again focused the attention of researchers to the fish in most countries where consumption of marine species is not confined only to the power sector, but has pharmaceutical applications, marine of Health and extensively in is increasing. In this respect the unique characteristics of some species of marine biotechnology specialist attention Technology and Bio Marine technology was used.

Blossoming of new technologies in recent decades due to the biological importance of marine origin has increased antioxidants.

Results of research on aquatic life in many countries shows that such a potentially rich source of vital compounds, salts and minerals vitamins, essential fatty acids, anti-tumor, anti-virus and antioxidants are the most unique of its kind, or are unique.

1.1. The role and importance of antioxidant compounds:

Antioxidants, compounds that are vital in maintaining health and preventing disease are involved in some of the most important cases and the use of antioxidants, the following is noteworthy:

A. Protect cells
B. Protect cell membranes and other lipids
C. Prevention of heart disease, cardiovascular and cancer
D. Prevent eye and vision disorders (cataracts and retinal disorders)
E. Prevention of nervous system disorders (Parkinson)
F. Delaying the aging process of cell and tissue
G. The role of antihistamines in controlling and improving a Lor Zhyk diseases, asthma and ...
H. Protect the body's extracellular fluid (ECF body including blood, lymph, carpeted fluids in the lungs, seminal fluid, cerebrospinal fluid and synovial fluid)

2. Free radicals

Free radicals are substances that can exist independently least one unpaired electron, which is why very active free radicals are the most important features:

Free radicals with oxygen or carbon centers are of special biological significance.

The free radicals are very unstable and can be inorganic chemicals and organic acids and nuclear membranes, especially with key molecules to react.

A free radical, chain reactions are initiated, free radical reacts with molecules that have means to become free radicals and the chain to create a progressive damage.

The combination of free radicals to neutralize them but to deal with a free radical or free radical, a radical new arise.

Types of free radicals, superoxide radicals can be , Hydroxyl radical , Radical Nitric Radical and nitrogen dioxide Cited. In addition, oxygen free radicals that are produced during metabolism are also very dangerous.

2.1. Production of free radicals

Generally, two categories of factors can cause the production of free radicals in the human body are:

I. *External factors (physical or chemical factors), such as ion beam instrument and even the sun, air pollution, especially carbon dioxide and nitrogen oxide, chemicals, pesticides, anesthetics, and industrial solutions and ozone, particularly drugs chemotherapy.*

II. *Intrinsic factors (factors Biological), including free radicals and oxidizing reagent that arise due to factors such as oxidase, including mitochondria, and peroxisomal phagocyte, local inflammation and anemia, Bi S K E T noted both internal and external factors With the creation of free radicals, causing lipid oxidation and lipoproteins are Lypyfvshyn cell mutagenicity and harassment.*

The most significant damage caused by free radicals in the human body to make include:

Chemical abuse, such as carbon tetrachloride poisoning, inflammation, kill germs, the destruction of nuclear acids (DNA , RNA), Serious damage to the lungs and impaired breathing process, aging (lipid per oxidation of cell membranes) and many diseases including cancer, diabetes, arthritis, Alzheimer's and cataracts.

3. How to neutralize free radical damage

The human body has two types of immune system against the harmful effects of free radicals, particularly oxygen free radicals are.

I. *Enzymatic defense systems, including glutathione peroxides, superoxide dismutase and catalase must be noted that several minerals including selenium, copper, manganese and zinc are essential for the production and activity of these enzymes, which must be supplied through diet.*

II. *Antioxidant defense system: Antioxidant compounds are produced in the body or through the supply chain are actually a combination of antioxidants, which reacts with free radicals and molecules to produce safe and protects the body against radical damage in antioxidants able, even with very low concentrations, significantly inhibited the oxidation or oxidation of materials to the pitch lag.*

So what was said to be the body's antioxidant defense system of enzymatic and non enzymatic breakdown into two parts, the enzymatic antioxidant defense system includes enzymes such as superoxide dismutase (SOD), Glutathione peroxides (GPX) And catalase (CAT) And non-enzymatic antioxidant defense system that can be group including the most important antioxidants, carotenoids, ascorbic acid, uric acid and bilirubin cited.

However, the remarkable thing is that the human body in perfect health during the first 20 to 30 years (youth) can well protect itself from oxidative stress that is always a balance between production of free radicals and the defense system of anti through nutrition, food supplements and medicines are also inevitable and unavoidable due to the toxic effects of kinetic antioxidants, the researchers explored the use of natural antioxidants has been paid.

4. The role of antioxidants in the treatment of diseases (cancer)

In the case of cancer, especially in advanced stages, the methods used to remove the tumor and chemotherapy The majority of essential drugs used during chemotherapy side effects are several steps Treatment and recovery can undermine patients' symptoms such as hair loss, digestive disorders, skin disorders, weight loss, anemia and blood disorders including neutropenia , decomposition of red blood cells and ...

Medical experts recommend that oncology patients with chemotherapy of diets rich in antioxidants and antioxidant supplements to control mitigate and prevent use of the above complications.

The beneficial effects of antioxidant compounds can be significant in protecting cell membranes against lipid per oxidation mechanisms, which are caused by free radicals caused by chemical agents, was observed.

5. Sources of natural antioxidants

Some foods that are rich in natural antioxidants are consumed in the diet over a healthy, it can be to get the antioxidants your body needs to strengthen the antioxidant defense system to meet most of them can be fruits, vegetables, grains, legumes, red and white meat, fish and many aquatic cited. New research has shown that some fish are rich sources of antioxidants, which in some cases several times higher antioxidant capacity of plant antioxidants are the mines of the fish eggs, crustaceans, especially shrimps and marine crabs, jellyfish and some sea urchins.

Of Many aquatic organisms, various compounds with antioxidant properties have been extracted from some of these antioxidants and how to identify building performance have been used in pharmaceutical processes.

6. Research on free radicals and antioxidants

Researchers believe that the increased absorption of dietary antioxidants by increasing consumption of dietary supplements rich in natural antioxidants, the tissue damage caused by free radicals and reduces the incidence of complications, including disease prevention will prove very Free radicals are highly reactive and difficult because they have very short half-life of free radicals and thus cannot be directly measured and evaluated, but the damage caused by them as an indicator, search, and review other problems, however, For example, free radicals are combined with a protein can produce over 100 different products, or when combined with lipid free radicals, highly unstable material is not only complex but also break a lot of complexes, in addition to measuring each The compounds in the blood cannot be permanent damage to the body because the body is able to largely prevent the disposal of these used successfully to prevent the next injury.

Researchers to overcome these problems and advanced are examples of systems of biochemical reactions of free radicals is able to show any identification and discovery of new signs of damage from free radicals, for being informed of what is really inside the body gives a necessary and unavoidable.

Table 3-1 antioxidants extracted from marine

Reference	Extractive compounds	species
Sun & etal (2004)	Exopolysaccharide	Keissleriella
Abdol – ó lateff (2006)	Isobenzofuranone	Epicoccum . sp
Zhang & etal (2007)	Fatty acid fraction	Agrocybe aegerita
Miyake & etal (2005)	Bio chnological formation from flarone	Aspergillus saitoi
Strobed & etal (2003)	Isopestacin	Pestalotiopsis microspora

7. Evaluation methods of free radicals (oxidative stress):

Since free radicals have a short half-life methods of measuring them **ESR** Advanced devices has been very expensive for the assessment of oxidative stress directly by simply not possible, but can be examined to determine the amount of antioxidant activity. However, the results based on more accurate measure of antioxidant activity and are considered.

8. Antioxidants evaluation methods

Different methods to evaluate the activity and capacity of antioxidants Biological fluids (blood, lymph, etc.) exist, but all these methods are based on three main:

I. Techniques ORAC
II. FRAP
III. Randox

The technique **ORAC** Ability of biological fluids from absorption of oxygen radicals that are produced in trace amounts during the metabolism, in order to measure the amount of enzyme super Aksyda Dsymvtaz (**SOD**) Is measured as the most important enzymes in the body's antioxidant defense system against oxygen free radicals Is.

The technique **FRAP** Ability of biological fluids in the recovery of ferric ions (Fe^{+3}) and down (Fe^{+2}) to be measured by spectrophotometer methods.

Technique **Randox** The only way that the amount of antioxidants to M (TAS) was measure the biological fluids of this method is based on spectroscopy.

Oxidative stress indices and their application in the evaluation of antioxidants :

Although from 1996 onwards the **FRAP** was used for measuring antioxidant capacity in this way but the fact that some antioxidants such as iTunes Glvta peroxidase are ignored, then use it to

limit non-specific and led to extensive research since 2005, other indicators to evaluate the anti- The most important indicator of oxidant may be selected include:

I. *The amount of thiol groups (- SH) Plasma*
II. *Liz rate Rbc (Lyses of red blood cells)*
III. *Assessment of lipid per oxidation*

Very sensitive to oxidative damage of plasma thiols levels in conditions of oxidative stress can be greatly reduced, usually by spectrophotometer methods of plasma thiol groups in the presence of a suitable reagent (reagent **Ellman**) was measured.

Mainly red blood cells (**Rbc**) An appropriate model for assessing and measuring the damage caused by free radicals because the free radicals attack the cell membrane lipid per oxidation and membrane proteins **Rbc** Ultimately caused the destruction of the membrane and hemolysis hemolysis provide **Rbc** , in the cells (hemoglobin) is released to the review and measurement methods, spectroscopy is obviously much stronger antioxidant defense system and antioxidant capacity of blood is higher than the percent lyses **Rbc** (Under the effects of free radicals) is reduced.

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Effect of yoga training on attention and anxiety preschool children

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ABSTRACT: The purpose of this study is investigated the effects of yoga as a non-competitive sports in anxiety of children 6 years preschool. Experts believe that due to non- competitiveness, this exercise in children, does not create anxiety. Because many sports are competitive and competition lies in their natural, they can be a source of anxiety. Also anxiety have cognitive affects, cause reducing the concentration, Confusion and reduced accuracy and is impaired Children's educational performance. Method is Semi-empirical and field, 307 preschool boys and girls from Tehran city choose randomly and then screening with CAT. 164 persons were diagnosed without anxiety and removed from the study. In yoga, 135 children remained were participated in the twelve-week training, based on three sessions of 30 minutes per week. Their anxiety levels before and after the exercises were compared to test their search hypotheses to reduce Interference of confounding variables and integration training, and implementation yoga techniques, One coach from each nursery was selected and trained under yoga training an hour for 10 sessions. CAT test were selected for the study of anxiety, for grading test results, were used revised child anxiety scale RCMAS, and to study accuracy, set of motor developing Orzetski Lincoln passing the Maze sub tests were selected. Research data were analyzed with statistical tests, Pearson correlation test, T-dependent test, three- factor mixed analysis of variance that include on factor between subjects(gender) and tow within subjects factors (anxiety and accuracy).(0/05>P). Results showed significant differences in anxiety in children before and after yoga has occurred. Their accuracy also has significantly different in tow stage at the before and after exercise. Wilkes Lambda test results showed a significant interaction effect of anxiety and accuracy and size of Eta = 0/85, respectively. But according to Wilkes Lambda test, the interactive effects of gender, anxiety, and accuracy was not significant. Overall results showed that yoga is non-competitive sports and was very effective to overcome anxiety in children and increase their accuracy. In addition, it has positive physiological and psychological benefits for children. The ultimate goal is that, when children are faced with a stressful situation can quickly be quiet and to achieve this it is essential that children daily do breathing exercises.

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Key words: Yoga, anxiety, accuracy, pre-school children.

Introduction:

Century is called the age of anxiety, in recent years alot of attention has been given to the issues of child psychology. Because, they are exposed to various stressors with great negative results. These results include a range of inappropriate physiological, psychological and cognitive effects.(1,9)
Exercise therapy has been suggest as a low cost and non-drug method for prevention, treatment and reduce mental health problems in children.(8)
Including methods of exercise therapy, Yoga breathing exercises in comparison with drug have been no side effects. While applicable to individual and group, due to the non-competitive does not produce anxiety. Movements, breathing exercises, effective steps to mindfulness, meditation, and ethical behavior, The main idea in Yoga thinking is control think fluctuations (18), eight stages of yoga support strengthen focus and calm the mind (17), yoga exercise for children agespre-school have been established on rearing practices of imagination and fantasy,(20) Yoga instructor can also give them little data in their understanding, about yoga and its effect on the reduction of anxiety, fear and daily agitated. The exercises depend on the children's creativity, physical fitness and strength of Innovation.(4) Peti Tell (2005) said Yoga breathing techniques are very important for relaxation. These techniques are effective for all children as well as for children with special problems, because this technique can be helpful to avoid the fear, aggression and anger. Although it seems, children are unfailing reserves of energy and restless, but often as adults are faced with a lot of mental and physical challenges, including stress, anxiety, depression, feelings of tire, etc. But yoga can help children to be quiet and focus. Yoga breathing

technique training is an important and effective tool that helps children manage anxiety, stress, depression, and increase lung capacity. (16) Matnz et al. (1990) were distinguished between the two aspects of anxiety.
Somatic anxiety, anxiety related to the physiological changes such as increased heart rate, increased respiration and peristalsis of the stomach. Cognitive anxiety usually is associated with somatic anxiety and related to anxiety thinking, including concern, self-doubt, failure imagining and humiliation. (16) Kalkes (1998) points out that cognitive anxiety has a negative relation with performance and as soon as cognitive anxiety increases, performance drops. (15) Hall and Zaglin (2002) and Kalks et al (2004), reported that long-term high-intensity aerobic exercise is a factor in the increased anxiety. (24)
Kertis (2000) reported that exercises effective in reducing anxiety (14), Brown et el. Showed that yoga is effective in reducing stress and anxiety in children. (20) karden Moudi et el. (2008) in their study have introduced yoga as a confounding factor that is suitable for children with blood diseases and tumors. (21) Christine Mkvist (2010) noted that children with cancer are experiencing symptoms of stress such as anxiety. He had participated children in individual yoga sessions and reported significantly reduce in their anxiety after performing yoga. (22) Linda J Harris ec el. (2004) stated that the most common treatment to treat agitation of children's hyperactivity and attention in North America and Australia is meditation techniques mental stimulus. (19) TifaniFild (2010) has reviewed the effects of yoga on psychological conditions including anxiety, depression, pain symptoms, cardiovascular conditions, basic safety and tolerance. (17)
Faith Attain and Safari (1381) have investigated the effect of light, mediate and heavy exercise to reduce anxiety of 90 people with highest anxiety score. They showed, there are significant differences, in anxiety of people in heavy exercise group compared with two another groups. (12) In view of the adverse effects of anxiety on the physiological, psychological, movement and educational children's functions, it is necessary to search and offer an appropriate solution for that. Several researches refer anxiety reduction as a result of Yoga breathing exercises. Exercise therapy for anxiety is low cost, safe and useful. The present study is going to investigate impact of regular physical activity such as Yoga exercises on pre-school children's anxiety and accuracy.

Research method
Semi-empirical method is selected and the data it has collected to form field. Different tests were used to measure variables, to study the children's accuracy, one of motor development subtests Lincoln - Avzrtsky as passing the labyrinth, to study the anxiety was used CAT and test result was scored by Revised Children's Manifest Anxiety Scale(RCMAS).

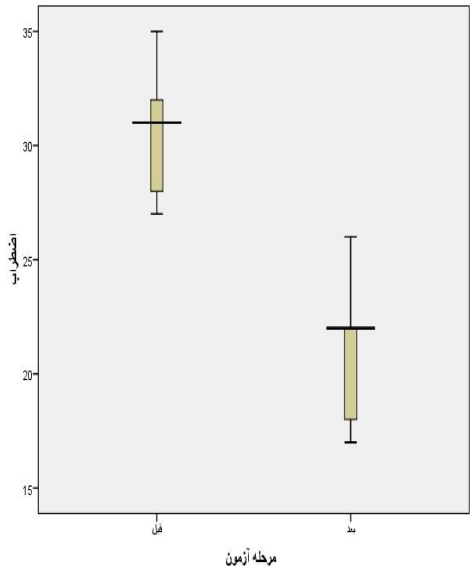
Community and Sample
Community included all preschool children who were six years from kindergarten in Tehran, both boys and girls in school years 1390-91. The sample of 135 persons, including 68 males and 67 females, was selected.

Sampling:
With preparation the sampling frame, a list of names and addresses of kindergartens in Tehran were prepared based on municipal area. Using cluster sampling, were selected by random draw three regions (17, 2, and 14)from Tehran and five kindergartens from each area.
In the starting stage all children were examined with anxiety test. 139 children with highest anxiety score were divided into sample. Yoga exercise was presented to them. During the study 4 people were reduced from sample size.

Method:
After screening test, students without anxiety were removed from the study and to reduce interference confounding variables, integrate training, run Yoga technique, an instructor from each kindergarten was trained Yoga training under the expert for ten one-hour session.
After this stage, children with anxiety were participated in twelve-week training-including three thirty-minute sessions per week- before starting the training, sample were test by the accuracy test. The results of the accuracy and anxiety test for each child were recorded based on norm tables. At the end of the last training session, anxiety and accuracy test for children again held. The results were recorded as before. To test the hypothesis, the results of both phases of the study were compared with each other.

Method of data analysis:
To analyze the data collected, were used both descriptive statistics index and inferential statistics index such as dependent T-test, Pearson correlation, analysis of variance.

Results and findings
In table1, children's anxiety was compared in both pre- and post-test and descriptive results indicate after Yoga training children's anxiety decreased.



To provide more descriptive information, measuring anxiety scores of participants in the two phases were classified in three categories in Table 1. Comparing the percentage of each class in the post-test showed a decrease in children's anxiety.

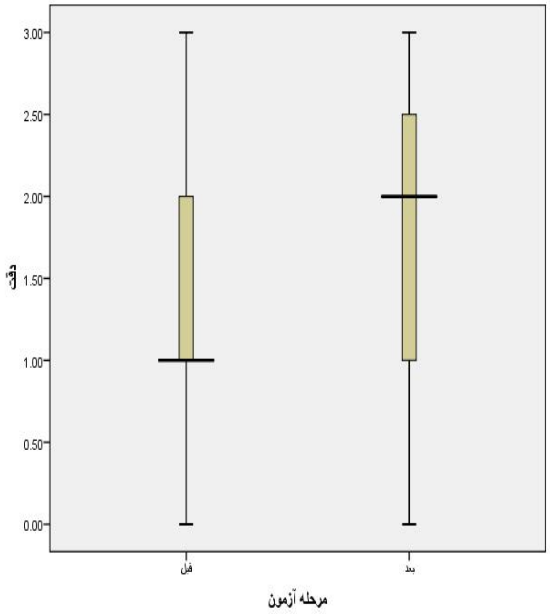
Table 1: Descriptive statistics anxiety before and after yoga

Dependent			SD	M	Variable	
T	d.f	P				
28	134	0/0001	2/67	30/7	Pre	Anxiety
			2/77	20/8	Post	
3/8	134	0/0001	0/853	1/47	Pre	Accuracy
			0/957	1/79	Post	

In table 2:The accuracy of children in pre-test and post-test were compared and the results showed that the accuracy of children was increased after receiving Yoga training.

Anxiety		Category
Post test	Pretest	
48/1	32/6	low
30/4	43	Average
21/5	24/4	high
100	100	total

Figure 2: accuracy box plot



To provide descriptive information, the result scores ofmeasurmentaccuracy of subjects in two stages were classified in four categorysin Table 2.Compare the percentage of children in each class, indicating increased accuracy in the post-test.

Accuracy		Category
Post test	Pretest	
25/2	10/4	high
40	39/3	Average
23	37	low
11/9	13/7	Careless
100	100	total

For data analysis, and determination how Yoga trainings effect on children's anxiety and accuracy, anxiety and accuracy scores in two stages were compare with each other with T dependent test.

Significant difference can be seen in pre-test and post-test anxiety scores (0001/0 = P, 28 = t). This means, Yoga has been able to reduce the average anxiety score preschoolers. Also there is significant difference in pre-test and post-test accuracy scores. (t=8/3, P=0/0001). This means that Yoga have increased mean accuracy score preschoolers.(Table3)

Table 3: Comparison of anxiety and accuracy before and after yoga

F	Effect size	sig	df	Mean square	Source
4/42	0/997	0/000	1	101577	**intercept**
5/1	0/037	0/024	1	11/890	**Sex**
			133	2/29	**error**

For further analysis of the data, determine the effect of yoga on anxiety and accurately that means to determine how many percent of anxiety and accuracy variance was determine separately by yoga training and how many percent of that was determine by yoga training interaction with children's gender, was used mixed analysis of variance test, that is a three- factor plan. In this plan, a factor between subjects was gender with two levels and 2 factors within subjects were anxiety and accuracy.

Result of multivariate test according to Wilkes Lambda test showed that the main anxiety affect, regardless of gender and accuracy, was significant, this means in terms of anxiety that there is significant difference between subjects before and after Yoga and yoga training effect on this difference was 99%. (table4)

The Wilkes Lambda test results revealed no significant interaction effect of gender and anxiety. With this test was determined also a significant interaction effect of anxiety and accuracy, that means Yoga could simultaneously affect the accuracy and anxiety of preschoolers. The size of this effect was 85%. (table4)

Wilkes Lambda test results are estimated the main effect of accuracy regardless of gender and anxiety was significant, effect size was calculated as 86%. That means the yoga is affected 86 percent of the accuracy variance accuracy. (table4).

To continue the analysis of multivariate data, Wilkes Lambda test results showed a significant interaction effect of gender and precision, Yoga and gender are simultaneously affect the accuracy but the estimated effect size (4%) is very small. (table4)

At the end, according to Wilkes Lambda test results, the interactive effect of gender and accuracy and anxiety was not significant. (table4)

Also the between subject factor test results indicated that the main effect of gender is significant and effect size was estimated to be 3%. (table5)

Table 4: Results of multivariate data and the size of the main and interactive effects of variables

Table 5: Test results between subjects factor

F-Test	The significance level	Effect size	Wilkes Lambda	EFFECT OF VARIABLES
1/41	0/0001	0/991	0/009	Anxiety
1/56	0/214	0/012	0/998	Anxiety and Sex
8/4	0/0001	0/864	0/136	Accuracy
6/05	0/015	0/044	0/956	Accuracy and Sex
7/6	0/0001	0/851	0/149	Accuracy and Anxiety
2/47	0/118	0/018	0/982	Accuracy, Anxiety and Sex

Discussion:

The review and analysis of hypothesis test results (about anxiety and accuracy), it can be concluded that the twelve-week workout yoga has positive impact on preschool children and has been cover much change in these two variables and has been reduce anxiety and increase their accuracy.

Reduction anxiety observed in this study is consistent with the results of the Moody and et. (2008), Faith Attain and Safari (1388), Christian McBride West (2010), Faye Martin (2011), Keila Richard (1997), that all, in the different ways, examined the effects of physical exercise on anxiety, especially yoga.

Linda J. Harrison (2004) has introduced Yoga meditation as a method of treatment suitable for ADHD children and knows that as a common procedure to treat turmoil of attention deficit hyperactivity in North America and Australia. (19)

In confirmation of her theory, this study reports a significant increase in children's accuracy level after doing yoga.

In previous research in not mentioned to the differences between boys and girls but in this study, we mentioned to it, but there isn't significant difference in anxiety after yoga between them. It means, Yoga affects equally boys and girls anxiety. Also there was no significant difference in accuracy rates of boys and girls after yoga. Kilarchard said: By engaging in yoga relaxation techniques, stress hormones (cortisol) decreases, resulting of that, consequences of stress in the body is reduced, blood pressure and heart rate decreases, anxiety, Depression, fatigue, asthma and insomnia passes. Also writes, Yoga helps person to more attention to surrounding natural environment. (6) The results of the present study are consistent with his views

because significant negative relationship between anxiety and accuracy was observed.
Jeremy West (2004) who studied the effects of Hatha yoga and African dance on stress, using saliva to measure cortisol of students, reported yoga and dance are reduced stress and its negative effects. (24)

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Determining the extent of populist discourse in Iranian Press

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Abstract : The general purpose of this study is to determine the extent of dealing the different topics with which different media (in terms of political tendencies) have to do, and, hence, to compare them. Our principal question, therefore, is that what the populist discourse is in Iranian press in 2008?The most important concern, thus, of this article is identifying the indexes of populist discourse to offer an explanation of populist discourse in Iranian press. To do so, the available texts and papers were studied, first of all, and, then, the enumeration was drawn from the indexes considered. In the next stage, enumerated indexes, all together, were presented to the professors and theorists of the field so, while reading it, they would do the modifications. Finally, the proper indexes were selected, taken into consideration, and analyzed in details, respectively.To answer the principal question the hypotheses were set, in the next step. Being determined, thereby, indexes of populist discourse were explored on the basis of questions, hypotheses, and purposes of the study. Using content analysis method, we considered two cases of Keihan newspaper (right wing) and Etemad Meli (left wing) to explore, comparatively, the populist discourse in Iranian press in 2008. In order to examine the proposed hypotheses, therefore, two national newspapers were selected which had different political and socio-cultural attitude, and, also, of same public written structure (what is printed and published), namely, Keihan and Etemad Meli.

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Keywords: populism, populist discourse, press, political tendencies.

Introduction

Populism, as a political concept, according to Herbert Schiller, is based on the primary hypothesis that ‘the public is unaware and passive, without being able of discrimination’. Characteristic of someone being populist, on the other hand, is her/his enthusiasm ‘to be seen’, that is, those who are more enchanted with "visual attractions based on feelings" rather than "reasonable analyses". Populism, in other words, means presenting fine images to those who ‘don’t see well’. Populism, in other reliable sources, has been defined as having ‘belief in people’, ‘being popular’ and ‘people-ism school’, etc. In political sciences, principally, a populist one would like his/her image to be displayed by dramatically inciting emotions before public more than thinking to introduce his/her thoughts or offering fundamental strategies in politics or other fields. Such an attitude is well sufficient that media – once in hands of people with political power, specifically those believing in populism – would convert to powerful means of, provisionally, expansion of that belief. Beside the political incentives, however, commercial and profitable aspects, maybe not that much political, are to be considered too in perspective. It is here that social conception of populism, that is, immediate impressing or inciting the people, is taken into account more than political one.

As the name of populism shows, thus, it is most often based on having dominance over the pubic. The term "People" is a word, although, but it has abundant semantic and concrete consequences. In all socio-political systems, almost, in the recent centuries and even before that, people have had played fundamental and, occasionally, unique role. People themselves, sometimes, would have caused a riot or revolution; people's revolt has been set out, caused sometimes by stimulation of a number of profiteers and opportunists, sometimes by freedom - loving and liberal ones, who've been on top of people's stream of demand and volition, or familiar with their language and pains. People, in any way, were a necessary and inseparable part of the most of socio–political events and changes and, also, revolts and revolutions which have had occurred during the whole history and scope of the world. But "all the revolutions were which set out in the name of people and all the revolts and revolutions were which directed by the same people have been specified and classified by different names and titles. It has been said, although, in these revolts and developments, that every thing and everybody is people, that there is nothing outside their volition considered, and that whatever is done is to satisfy the same volition; but once the result and process of actions along with decisions is considered, differences would have clearly been revealed; and here is taken as the origin of such "isms", whose emblem, purpose and basis is the people. One which would reminds more, among all these "isms", of people, by name and title, is nothing but populism. Its history has been traced back to mid 19th century, and, to different movements which have occurred in such lands as former Soviet Union."

In populism, therefore, we thoroughly speak of people and their demand and wish. People are everything and there is nothing outside them or their willing; whoever and whatever resists this is the enemy of people, of nation, of their demand and volition, of a slogan and a target.

Whatever volition of people aimed, it should be realized; not only no one should oppose it, but also there is no one who does so, because everybody in society is one of the people, since people are all integrated and sincere, their slogan is the same and any imagination of "other voice" in such society would abolish initially.

Nor multiplicity of creeds, thus, nor multiple voices and thoughts one would find in such systems. The Populist system calls for, and sees only, univocality and sincerity. Differences should be either ignored or omitted, since these differences, these opposing voices, are all against society's willing. All of them just want to sow discord and worry about, beside their unity, rising of people's wish. They are not concerned about people at all. All they worry about, therefore, besides seeking their needs and desires, is just fulfillment of demands of their own: all the oppositions are proceed from this raven. Populism is the system which not only is not glad by opposed votes and different thoughts, but also is afraid of such diversity. Differences of thoughts, to this tenet, has no meaning; everybody should say the same as what people say, so even though people is a plural noun itself but actually is singular, the singular which speaks in the form of the leader of movement, who speaks only of people's demands. In such a system, differences of colures, of races, of classes, and of wealth should not be mentioned and all the people should be alike.

"Mass media" or "media populism" emerged, clearly and obviously during the World War I, in the world. Mass mobilization for future war and fighting with other nations, and even with internal political opposition, was their most important goal. The most important symbol of such media in the world was Josef Goebbels in Germany, propaganda minister of Hitler administration. Leader thinkers of this school refer to, as their major influences, thinkers such as Gustave Le Bon and Jean Gabriel de Tarde. Populist media define their prophecy, at least, to conduct heterogeneous society toward uniformity and homogeneity.

What is under consideration, as a result, in this article is comparative study of populist discourse in Iranian press in ۲۰۰۸. The most important issue in this research is: in which society and circumstances populist media are formed in and what is their origin? With what trait and context endurance and stability of mass media massages would increase or decrease? In other words, how and under which conditions, on one hand, can written media make society uniform and homogenous, in order to conduct them toward their favorite goals, and, on the other hand, to what extent effects and endurance of such political, social and economic messages is stable. Finally, within what environment and social, economic and political grounds are they reduced and undermined?

Indeed, populism grows, in the modern world, through media and develops into democracy and the society. It reproduces itself too, basically, in this way and breakthroughs into different social levels. Accordingly, populist discourse can be distinguished by reading media and, with different methodological instruments, the way in which they deal with different subjects.

Research methodology

Any study or research seeks to reach certain aims. In this regard, the primary principle and the first step for reaching the aims is the method.

After being specified, research aims should be applied, by researcher, to practical issues; because, basically, principal aim of every research is to explore, to explain, to interpret and to expand knowledge about special phenomena. So too, this research tries, using general principles and rules of such study, to select proper measuring tools for research.

Accordingly, this research tries, methodologically, by content analysis of two newspapers, namely, Keihan (right wing) and Etemad Melli (left wing) to study populist discourse in Iranian press comparatively. To achieve this goal, therefore, information gathering tools are suggested as following:

1- In the first stage, that is, identification of dimensions and components of populist discourse, the method was information gathering in libraries;

2- In the second stage, that is, comparative study of populist discourse in Iranian press, the method was information gathering using content analysis;

3- In the third stage, evidently, the professors and authorities were interviewed in order to determine the indexes of populist discourse.

Research hypotheses

1- It seems that populist discourse has been used in all media considered;

2- It seems that populist discourse is the characteristic of political excitation, through newspapers, in Iran;

3- It seems that, no matter which political tendency, populist discourse is the dominant way for political figures to promote their plans;

4- It seems that different newspapers, which are affected, highly, by diverse political events (such as election), tend to use populist writing style;

5- It seems that there is no difference between political newspapers in respect to ways in which they apply and pay attention to populist discourse.

Statistical population and sample

Statistical population includes all the elements which have one or more common group traits and may be selected for study. It is this element to which measuring tool is applied, and the statistical population includes all such elements or intelligible observations relating to the research subject. In other words, statistical population includes all the elements by which research matter subject manifests and from which conclusion can be drawn.

Our statistical population: Etemad Melli and Keihan newspapers in 2008.

Our samples: about 52 numbers have been selected from each newspaper.

Studied newspapers; (1) Keihan (2) Etemad Melli.
The reasons for such selection: both newspapers have obvious political tendencies; populist discourse, therefore, can be more clearly assessed in these texts.

Data analysis method

Two newspapers, as mentioned above, were selected among national newspapers in order to examine validation of the hypotheses. These two newspapers, as we said earlier, are different in terms of political attitude, socio-cultural approaches, and in terms of written structure of news (what is printed and published). These two newspapers are Keihan and Etemad Melli.

Differences of these two newspapers were as following:
1- Administrative and organizational structure;
2- Financing sources;
3- Form and size of newspaper;
4- Internal structure of newspaper (Etemad newspaper used to have one appendix and Keihan didn't. They were different in terms of form and composition as well);
5- Political tendency;
6- Social and cultural tendency.

To examine theses newspapers equally, in spite of their differences, the same sampling was done.

Within 52 weeks of the year, one number was sampled every week. Monday, on the basis of random sampling, was selected sampling day for both papers out of six days a week. No newspaper, nevertheless, was published in the first two weeks of the year; this defect was compensated by increase of sampling, in the next two weeks, with two additional numbers. In each month, hence, Mondays were selected as sampling days. However, next Tuesday was selected, in the case of Monday being vacation, as the first substitute, and Wednesday was selected as the second substitute for such vacations. From the first newspaper (Etemad) and the second newspaper (Keihan) 52 numbers were selected. The mentioned newspapers were selected because they represented two extremes of political and social attitudes and, therefore, could properly cover the hypotheses of research.

Indexes under consideration in these papers were:
- Main pictures of the front page or first title;
- Texts and articles.

Pictures:

The most important picture of news or main story, published in the front page. Significance of the picture is determined from paper's own point of view, and importance which the newspaper gives to it.

Indexes considered in pictures were:
- Paying attention to non-organizational political elites (the elites who are not identified as member of parties, that is, non-party characters even if they were a member.);
- Paying attention to the organizational political elites (the elites who are identified as a member of parties or other political organizations);
- Paying attention to the public in general, that is, as a totality not divided into social system units;
- Paying attention to the public in terms of the social groups;
- Formal picture of persons or subjects;
- Informal picture of persons or subjects;
- Paying attention to the subject in picture;
- Paying attention to the person as the one and only subject in picture;
- Paying attention to the person in terms of a more general subject or in a broader scope;
- Paying attention to the appearance of persons (the person's clothing, face or look being highlighted);
- Showing the person being inexpensive (to accentuate simple appearance of the person as regard to dress and behavior);
- Showing individual pictures with mighty appearance (despite the fact that grasping the picture is based on personal feeling of the addressee, or interpreter, some authoritative pictures are, indeed, discernible on the basis of general common principles. For example, to put emphasis on personal prestige, medals, bottom up picture, etc, indicates regarding the subject by Insinuation of his power or authority);
- Showing public pictures with authoritative appearance (displaying people or social groups in a position so as to transfer authority or power to the reader);

The texts studied in the newspapers include:
- Editorial article or the main note of newspaper which is substitute for editorial one or associating it;
- First title of the newspaper;
- Main story of the newspaper (in case that newspaper, rarely, had more than one main story, so as they could not be distinguished, one of them has been selected randomly). In this section, two main axes were considered: (1) Title of the main story (2) Text of the story;
- Main article and important notes (in case of several articles or notes to be not possible to discern – which occurred in the most numbers of newspapers - random sampling was done.)

Indexes studied in the texts :
- Paying attention to organization;
- Paying attention to political and social groups;
- Paying attention to different aspects of the Constitution;
- Freedom as the right of all individuals and a fundamental right – no matter the limiting aspects;
- Freedom under special conditions (freedom as a right but under conditions and limitations where display confiding aspects of enjoying freedom);
- Legality as an important affair, besides to determine its instance and use, mentioned in the text;
- Political parties as main actors of political affairs (no matter the instances, nor other attitudes, nor positive or negative assessment of role of political actors under special conditions);
- Election as a situation of competition between political organizations and parties (election as demonstration of organized competition);
- Election as a situation for competition of people;

- Paying attention to democracy;
- Using democratic ways in political competition and to seize the power (presence of dominant democratic discourse);
- Political disclosure (in terms of political groups or manners or actions);
- Individual disclosure;
- Comparing administrations in economic issues based on documents, statistics and figures and using instances;
- Comparing administrations in economic issues in general;
- Comparing administrations in social issues, based on documents and by use of instances;
- Comparing administrations in social issues in general;
- Comparing administrations in cultural issues in general;
- Comparing administrations by their foreign policy;
- Comparing administrations by their local policy;
- Positive approach to private life of the individuals (for elevating the person in public opinion);
- Negative approach to private life of the individuals (to make the person look bad in public opinion);
- Politics as an important and positive issue in personal and social life;
- Politics as a forbidden and negative issue in personal and social life;
- Social organization as a positive phenomenon (necessary and proper for social life);
- Social organization as a negative phenomenon;
- NGOs as positive instruments;
- NGOs as negative instruments;
- Approaching to NGOs with no positive or negative attitude;

Also following indexes have been considered in the sample newspapers:
- Percentage of political news (out of total news);
- Percentage of political stories (out of total reports);
- Percentage of political articles or notes (out of total articles or notes);
- Percentage of social news (out of total news);
- Percentage of social stories (out of total stories);
- Percentage of social articles or notes (out of total articles or notes);
- Percentage of political subjects by elitist approach (written for elites);
- Percentage of social subjects by elitist approach (written for elites);

Media populism

To intensify populist behavior of media depends, partly, on their relation to political groups of the country. In other words, newspapers take populist route in dominant political air, and use such manner instead of extending reasonable criticism. The same action, in fact, is found in political groups and their elite's behavior. Press, in this way, deviates from normal direction, and tend to intensify extreme behavior in society. In sphere where emotions and inflammations are dominant, and these are means by which movement fans are attracted, press is following, consequently, this direction as well. The press which promotes populist thought is the one which avoids, strongly, from thought, rational discussion and moral codes. They prefer factional benefits over public benefits and even, for such benefits, maybe victimize democracy. These are specifications of populist press.

Many analysts recognize the term "populism" synonymous to the idiom "to be seen", a synonymy that is such a criteria in which, if not according to Persian grammar, one can cover, bestow meaning or associate the other.

Populists, in this regard, don't lose any opportunity to be seen. They like to be seen because they are noticed by others, and this can accelerate applying their populist policies. Populists don't like to sit behind the desks for expert discussions, and listen to their boring speech, because, in this way, they are not to be seen and, therefore, don't show any tendency to work behind closed doors. For this reason, populists copiously tend to be among people, since they can attend vehement public gatherings and persist on their policies, so as to receive approval of their plans on different subjects, instead of using the expert views, by people's applause. This tendency of populists to receive credit from people to execute their plans, usually, leads to seek confirmation of policies not in experts gathering but in the large squares of cities and communication with the public face to face.

In a populist's belief, confirmation of executed, or future, policies should be applauded by the public to be legitimately actualized or realized, since this is shouting of the public that is the best evidence to justify policies and plans; populists persist on authenticity of this confirmation, even if one asks whether is the people's belief or opinion the best criteria for many complex and expert subjects which require specific education or knowledge. For this reason, a populist needs to be seen continually in order to introduce, and execute, his popular policies ceaselessly after being confirmed by people; and this is the way in which such false accreditation would be the best propaganda to dominate populism in all dimensions of a society.

It should be said, indeed, that according to populists it is not necessary to restrict it, in enlivening their beliefs, only to the country borders, since border, for populism, is a meaningless concept. Advocates of this thought find each other everywhere in any rank, and embrace each other brotherly before TV cameras to prove, to the public, that a common thought based on adoring populace can unite peers and which, notwithstanding religion, culture and tradition, lead to expansion of geographical scope.

Survival of populism is rest on extravert demonstration whether emerging such extraversion is shown by appearance and attending among the people or as article or picture printed on front pages of papers or, as well, by exciting motion pictures in magic frame of TV.

Populism, by any possible means, needs to bring itself to the people, which prove its opposition to individuality and its support for totality. For this reason, populism highly tries to execute policies in which essential trait is supporting poor and unknowing majority.

Generally, dividing society into majority and minority parts and everyday emphasis on existence of poor and rich classes in those parts, frequently accentuated and promoted by populists, not only is not by accident but results from such support for poor majority.

In this school, the value of majority support for populist policies is more than attention of minority to those policies; so, populists always try to accompany the majority and satisfy them.

Populists get excited by majority attention, since the excitement result from this attention to accelerate the execution of their populist plans. This is the direction in which what is called, by them, wealthy minority class is ignored, because such minority doesn't care to "being seen" and nor to the manner in which it is performed; therefore populists can't grant any share of benefits to such class, since their role helps destroy populism rather than support it. A populist, by any price, should be seen and what is important to them is not the form of display, but its doing by essence. Therefore, to choose doing it or not is not the case or, if one prefers, is not an option for the populists, but it is replaced by another important concern which can have many limitations per se, that is, the way of doing.

A populist, in order to be seen, requires media and specially those which necessarily accentuate and promote him, because the less speak of the rivals the more his success and victory; populism hates rival and rivalry and prefers to remain as a single hero.

Populists, accordingly, don't deny tend to play in an arena as the only actor, since it is evident that while winner of this play can be a single hero, why bothering to share such sweet victory with a rival. On the other hand, media can play an important role to organize the minds of individuals into a special direction; populism is well aware of this mechanism, so that some media populism is born in a close interplay of these two apparently incompatible components and, also, in an arena without rival.

Facts

On the basis of findings of this research, the following results are obtained in pictures analysis:

- In the second newspaper (Keihan) paying attention to non-organizational political elites (the elites who are not identified as a member of parties and are non-party characters even if they were member of a party) is more than the first newspaper (Etemad Melli). By contrast, there was more paying attention to organizational political elites (the elites who are identified as a member of parties or other political organizations) in the first newspaper (Etemad Melli) more than other.
- In the first newspaper (Etemad) paying attention to people as a totality not divided into social system units was a bit less than second newspaper (Keihan). In this case differences are not significant.
- In the first newspaper (Etemad) paying attention to people as social groups is considerably more than second newspaper (Keihan).
- Official picture of individuals or subject: it was observed in the first newspaper more than the second newspaper; in the case of non-official pictures this proportion is inverted for two newspapers, but these differences are not significant.
- Paying attention to the person as the one and only subject in picture in Keihan newspaper is more than Etemad newspaper, but these differences are not statistically significant.
- Paying attention to the person in terms of a general subject or in a broader scope in Keihan is more than Etemad newspaper.
- Paying attention to look and appearance of individuals (clothing or face or look of the person being accentuated) and showing the person's inexpensiveness (simple appearance of the persons in terms of dress and behavior being accentuated) was found not in Etemad but in Keihan newspaper. These differences are significant at level of 95%.

- Showing individual pictures with mighty appearance is found in Etemad more than Keihan newspaper, although public pictures with mighty appearance (displaying social groups or populace in a situation to transfer hegemony or authority to the reader) were found in Etemad just once, but was not found in Keihan Newspaper .

Studies and results above show that Keihan newspaper has potential more than Etemad to use pictures which can serve to populist attitude of the newspaper.

On the base of this research and results obtained from content analysis of sample numbers of papers, the following results obtained:

- Research results show that Keihan newspaper has experienced less variation in numbers of political news than Etemad newspaper during the study. In other words, covering political news by Keihan in considered samples was less dispersed than Etemad newspaper. Significance of this subject has been statistically shown by using Chi-Square test at level of 95%.
- Survey of different numbers shows that studied newspapers would contain more political subjects in volumes while acceding to the end of year (except for the last 2 weeks of the year). As regard to the fact that year 2008 was a busy year to campaign and candidateship for presidential election of June ۲۰۰۹, this was reasonable. Dividing volumes by seasons, moreover, shows that winter 2008 included more political issues than other three seasons for both newspapers. The question that whether upcoming election affected such matter, or whether the press accentuated the subject, should be object of another study, but it can be mentioned that there is a significant relationship between increase of political news of studied papers and upcoming of election.

- This study shows that Etemad newspaper assigned 25% of its stories in 52 numbers to political ones, while this rate was 14% for Keihan newspaper. Variation rate of this in Kehan newspaper was less than Etemad newspaper. To put it better, Keihan newspaper had clearer thread and less changed trend. While Keihan newspaper had assigned at most 20% of the stories to political ones, but Etemad newspaper had 40% of its stories as political. These differences are statistically significant. In other words, it seemed that Etemad was more into politics, as to contents of stories, than Keihan, while at the same time Keihan had clearer and less changed policy, as regard to stories percentages.
- In the case of political articles and notes, in two newspapers, it was observed that 60% of total articles and notes of one of them was assigned to political subjects. Also, by upcoming the election and during the weeks in which political debates were hot (and announced that Khatami or Mir Hossein Moosavi run for president), political articles and notes were significant on the basis of Chi-Square test at level of 95%. The first newspaper (Etemad) was, significantly, more sensitive to ongoing political issues than the second newspaper and contained more political stories.
- Although a newspaper being political doesn't mean it has populist tendency, there is a clear relationship between air of public sphere and studied newspapers.
- While results of study show that there are some differences in two newspapers as regard to inclusion of social news, but following table which is the result of applying Chi-square test, on the basis of difference of two newspapers, indicates that these differences are not significant.
    - The second newspaper (Keihan) tried more to reports social stories than the first, but these differences are not statistically significant.
    - In the political articles and notes, there are no significant differences between two newspapers.
    - The first newspaper (Etemad) has, significantly by statistics, more elitist material, whether in social or political issues, than the second newspaper (Keihan). In fact, it can be said that elitist approach of Etemad is stronger than Keihan.
    - To consider contents of Etemad and Keihan newspapers, by stories, show that Etemad newspaper has less people-oriented approach (in terms of addressee) rather than elitist approach as compare to Keihan.

    On the basis of results obtained above from quantitative content analysis of the subjects, it seems that we can conclude Keihan newspaper, due to tendency to public subjects, and also lack of attention or little attention to social and political issues from elitist point of view, has more potential for populist approach than Etemad.

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The Impact of Medical Education on Saudi Medical Students' Awareness of Cell Phone Use and its Health Hazards

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Abstract: The data surrounding the effect of cell phones' electromagnetic radiation on human health, particularly on the auditory and vestibular systems, are controversial. This issue is of particular relevance since it may affect billions of people worldwide. The study aimed to highlight the impact of medical education on the awareness of cell phone use and its health hazards in students at King Abdulaziz University's Faculty of Medicine in Jeddah, Saudi Arabia. In this study, a mixed research design was adopted as a complementary approach. Closed ended questionnaires were distributed to 400 medical students to determine their knowledge and practices regarding the use of cell phones and their possible health risks. The questionnaires were followed by discussions with four focus groups to further analyze the studied area. Both the questionnaires and focus groups covered many points, including the most common health hazards associated with the use of cell phones. The results showed that most of the students were aware of the potential risks arising from the use of cell phones, and at least half of them reported experiencing some of the negative effects.
[Saad Al-Muhayawi, Bassam Eldeek, Hussain Abubakr, Rawan BenKuddah, Adel Zahid and Hashem Abukhashabah. **The Impact of Medical Education on Saudi Medical Students' Awareness of Cell Phone Use and its Health Hazards.** *Life Sci J* 2012;9(2):1143-1148] (ISSN:1097-8135). http://www.lifesciencesite.com. 170

Keywords: Cell phones, awareness, and medical students

1. Introduction

While the use of cell phones has become widespread, many users are unaware of the potential health risks associated with cell phone use. Concerns regarding the potential physiological effects of exposure to radio frequency (RF) radiation have been increased recently. These waves may have a negative impact on the tissues that are near the handset, such as the auditory nerve. These risks include tumors, acoustic neuromas, and other potential concerns [Lönn, 2004]. Audiologic disturbances may be increased with increases in cell phone usage. Other factors may be related to the duration of exposure to radiation or the condition of a person's central nervous system and immune system [Galeev, 2000]. Some medical studies have shown that the use of cell phones might result in health problems, including warmth behind or around the tissues of the ear, brain tumors, headaches, sleep disturbances, impairment of short-term memory, lack of concentration, and high blood pressure [Knave, 2001].

This research aims to improve the understanding of medical students regarding cell phone use risks, and to gain better knowledge on medical education and a better understanding of the possible health hazards related to cell phone use. We chose King Abdulaziz University because it has two medical education curriculums: traditional and a new hybrid.

2. Subjects and Methods:

Type of study:

This is a mixed methodology study, using both quantitative (cross-sectional descriptive study) and qualitative (focus group discussion) methods. The aim of this design is to triangulate and complement the evidence from both the qualitative and quantitative data. Four focus group discussions (two being with sixth-year students and two with interns) were conducted at the hospital auditorium during break time. Each focus group discussion lasted about thirty minutes. For each group discussion, the research team met with 10 to 12 students.

Target group:

Interns from the old curriculum and sixth-year medical students from the new hybrid curriculum.

Time period of the study:

From October 2011 to January 2012, during the free time of the interns and sixth-year students.

Pilot study:

A pilot study was done with 42 students, including sixth-year students (n=22) and interns (n=20), to enhance the validity of the questionnaire. Modification of the questionnaire was done to avoid the pitfalls of the study and to increase the internal validity of the research. Members from the Faculty of Engineering revised the questionnaire to ensure both the content and discriminant validity.

Tool of the study:

The questionnaire was designed to measure the knowledge, attitudes, and practices of medical students regarding the negative effects of using cell phones. The

questionnaire was divided into four variable parts: personal data, knowledge, attitudes, and practices. Each question had a unique code to ease entering and **processing the data.**

In the quantitative study, the tool was in English. Arabic translation of the questionnaire was carried out for data collection purposes. Those questionnaires were distributed to all medical students in their sixth year (n = 325) and to all medical interns (n = 310). The return rates of the questionnaires were 61% (198/325) and 63% (195/310) from sixth-year students and interns, respectively. The Cronbach's alpha of the questionnaire was 0.78 for the sixth-year students, 0.85 for the interns, and 0.82 for the whole sample. Open-ended questions were used in the focus groups discussions to help the students express their knowledge and practices regarding cell phone use.

Monitoring for the quality of data:

There were four groups responsible for quality control. Group 1 was responsible for checking if the data was complete or not; they reviewed the questions to ensure that nothing was missing. Group 2 was responsible for coding; they made sure that every code was relevant to the right question. Group 3 was responsible for data entry; they entered the data into the computers to be processed. Group 4 took random samples and reviewed them completely to ensure that there were no pitfalls.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS), version 18, was used for statistical analysis. The qualitative data was presented in the form of numbers and percentages. A chi-square test was used as a test of significance for qualitative data, and Yates correction was used when the expected cell was less than 5. The quantitative data was expressed as mean and standard deviation. Significance was considered at a *P*-value less than 0.05.

3. Results:

Table 1: This study included 393 participants: 50.4% were sixth year medical students and 49.6% were interns. Regarding nationality, in the first group, 91.9% were Saudis and 8.1% were non-Saudis. The second group consisted of 93.3% Saudis and 6.7% non-Saudis. The age of the participants was ranging from 22 to 26 years with a mean ± SD of 23.25 ± .85 in the first group and 24.02 ± .83 in the second group. Participants were equally chosen between females and males. Regarding marital status, in the first group, 93.9% of these participants were single and 6.1% were married. However, in the second group, 67.2% of these participants were single and 32.8% were married. Most of the participants in the first group (86.9%) were born in the western region and in the second group, 86.2% were from the same region. Almost half of the participants in the first group (54.5%) make <1000 S.R monthly, 33.3% have a monthly income ranging from 1000–3000 S.R, and 12.1% make > 3000 S.R monthly. Regarding the second group, 93.8% make > 3000 S.R monthly and 6.2% have a monthly income ranging from1000–3000S.R.

Table (1) Demographic Characters of the Participants on the Research

	6th years		Intern		*P*-Value
Nationality Saudi	**93.3%**	182	91.9%	182	*P* = 0.366
Non Saudi	6.7%	13	8.1%	16	
Gender Male	**52.3%**	102	50.5%	100	*P* = 0.399
Female	47.7%	93	**49.5%**	98	
Status Single	67.2%	131	**93.9%**	186	***P* = 0.000**
Married	**32.8%**	64	6.1%	12	
Age	23.25 (0.85)		24.02 (0.83)		***P* < 0.001**
Place of Birth Western Re.	**86.2%**	168	**86.9%**	172	***P* < 0.001**
Central Re.	1.5%	3	4%	8	
Eastern Re.	8.2%	16	4%	8	
Northern Re.	0%	0	1%	2	
Southern Re.	4.1%	8	3%	6	
Outside Country	0%	0	1%	2	
Income < 1000	108	**54.5%**	0	0%	***P* < 0.001**
1-3000	66	33.3%	12	6.2%	
>3000	24	12.1%	183	**93.8%**	

Table 2: 87.9% of the participants in the first group and 88.2% in the second group have heard about the side effects of cell phones. More than half mentioned hotness, pain, headache, and tinnitus as side effects, with hotness being the most chosen (79.8% for first group and 90.3% for second group). Wax, brain tumors, and disturbances in brain functions were chosen by less than 50% of the participants as cell phone side effects, with significant differences as to whether wax is/should be regarded a side effect. Regarding the source of knowledge about these side effects, community discussions (71.7% for the first group and 73.3% for the second group), Internet, TV, and magazines were the most chosen sources, though there was no significance regarding the source of knowledge. When asked about the best way to minimize these side effects, 39.4% from the first group and 47.2% from the second group chose turning the phone on speaker mode; 34.3% from the first group and 27.7% from the second group chose headphones; 5.6% from the first group and 8.2% from the second group chose using the phone directly; and 3.5% from the first group and 2.1% from the second group chose using a Bluetooth earpiece, while 17.2% from the first group and 14.9% from the second group answered "I don't know." Only 1.5% total reported that the side effects of cell phone use are included in their curriculum.

Table (2) Knowledge of the participants towards the Health Hazards of Mobile Phone Magnetism

	6th years n=198		Intern n=195		Total	%	*P* - Value
Did you hear about Mobile Side effects "SE"	174	87.9%	172	**88.2%**	346	88%	*P* = 0.522
Source of Knowledge Magazine	103	52 %	87	44.6%	190	48.3%	*P* = 0.086
Radio	72	36.4%	72	36.9%	144	36.8%	*P* = 0.496
TV	81	40.9%	73	37.4%	154	39.2%	*P* = 0.274
Community	142	71.7%	143	**73.3%**	285	72.5%	*P* = 0.403
Internet	139	**70.2 %**	120	61.5%	259	65.9%	***P* = 0.044**
Advertisements	29	14.6%	34	17.4%	63	16%	*P* = 0.269
The Side Effect "SE" Hotness around the ears	158	79.8%	176	**90.3%**	334	85%	***P* = 0.003**
Pain	141	71.2%	154	**79%**	295	75%	***P* = 0.048**
Headache	109	55.1%	110	56.4%	219	55.7%	*P* = 0.433
Tinnitus	107	54%	104	53.3%	211	53.7%	*P* = 0.484
Disturbance in brain function	83	41.9%	82	42.1%	165	42%	*P* = 0.530
Brain Tumor	58	29.3%	69	35.4%	127	32.3%	*P* = 0.118
Wax	62	**31.3 %**	40	20.5%	102	26%	***P* = 0.010**
SE of mobile use & Curriculum are include	6	**3%**	0	0%	6	1.5%	***P* = 0.016**
Best way to minimize SE Through Phone	11	5.6%	16	8.2%	27	6.9%	***P* < 0.001**
Head Phone	68	34.3%	54	27.7%	122	31%	
Bluetooth	7	3.5%	4	2.1%	11	2.8%	
Speaker	78	39.4%	92	47.2%	170	43.3%	
I don't Know	34	17.2%	29	14.9%	63	16%	

Table 3: About 66.2% in the first group and 65.1% in the second group reported using their cell phones from 30 minutes to one hour daily. 70.7% in the first group reported having less than 10 calls per day and 65.1% in the second group reported having 10 to 20 calls per day, with the average call being from 15 to 30 minutes in about half of the participants (54% from the first group and 49.2% from the second group). More than half of our participants (66.2% from the first group and 50.3% from the second group) have been using cell phones for 5–10 years. The commonest reason for using cell phones among the participants was communication (54% from first group and 51.3% from second group). Other reasons included study purposes, multipurpose, and work purposes, in descending order. More than two thirds of the

participants (89.9% from the first group and 89.7% from the second group) take their calls through the phone directly; 12.6% from the first group and 22.1% from the second group take calls through headphones; 16.2% from the first group and 13.3% from the second group take calls through the speaker; and 12.6% from the first group and 5.6% from the second group take calls through a Bluetooth earpiece.

Almost half of the participants (52.5% from first the group and 67.7% from the second group) reported having hotness as a result of using cell phones. Other side effects such as tinnitus, wax, dizziness, headache, and sleep disturbance were reported as follows: 19.2%, 22.2%, 8.1%, 32.3% and 21.2% for the first group and 34.4%, 24.1%, 21.5%, 18.5% and 17.4% for the second group. From those who reported having side effects (144 and 151 participants in the first and second groups, respectively), 59.7% and 51.7% reported having these side effects after using a cell phone. 30.6% and 41.1% reported having them while using it, and 9.7% and 7.3% had them all the time.

Result of the focus groups discussion:

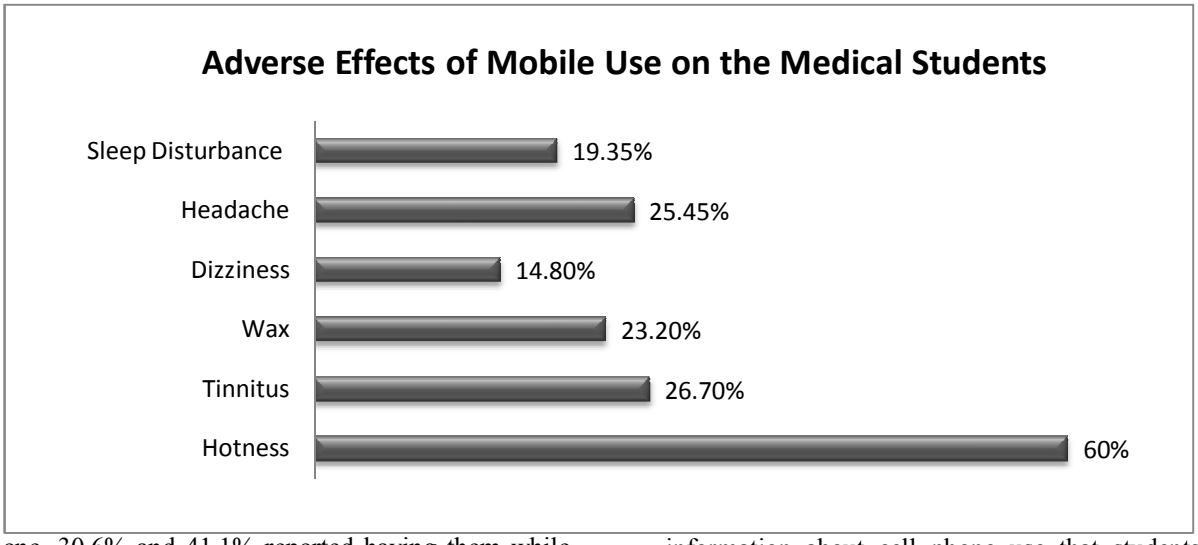
The research team conducted four focus groups discussions: two for the sixth-year students of the new curriculum and two for the interns from the old curriculum. Both students of old and new curricula stated that they were not taught specifically about cell phone hazards. They might have obtained some scattered knowledge from their teachers during lectures, tutorials, and clinical sessions in community and family medicine clerkships. They used different brands of cell phones (iPhone, Nokia and Blackberry). About half had more than one brand. About a third from each group used Blackberry messenger, which is considered another source of exposure to RF. The interns stated that they are exposed to another source of RF, which is a pager that is used mostly during their on-call shifts. In conclusion, their knowledge about cell phone use risks is not considered a limiting factor.

Table (3) Practices of the participants towards the Health Hazards of Mobile Phone Magnetism

	6th years n=198		Intern n=195		Total	%	*P* -Value
Daily Usage time <30 min	28	14.1%	14	7.2%	42	10.7%	*P* < 0.001
30 min - 1 hr	131	66.2%	127	65.1%	258	65.6%	
> 1 hr	39	19.7%	54	27.7%	93	23.7%	
Min\Call <15min	67	33.8%	66	33.8%	133	33.8%	*P* < 0.001
15 min - 30 min	93	47%	96	49.2%	189	48%	
> 30 min	38	19.2%	33	16.9%	71	18%	
Reason for Mobile using Work purpose	8	4%	22	11.3%	30	7.6%	*P* < 0.001
Study purpose	59	29.8%	16	8.2%	75	19%	
Communication	107	54%	100	51.3%	207	52.7%	
Multipurpose	24	12.1%	57	29.2%	81	20.6%	
Duration of used mobile < 5 years	7	3.5%	17	8.7%	24	6.1%	*P* < 0.001
5 to 10 years	131	66.2%	98	50.3%	229	58.3%	
> 10 years	60	30.3%	80	41%	140	35.6%	
Call\ day < 10 Calls	140	70.7%	52	26.7%	192	48.9%	*P* < 0.001
10 to 20 Calls	48	24.2%	127	65.1%	175	44.5%	
> 20 Calls	10	5.1%	16	8.2%	26	6.6%	
Way of receive calls Through Phone	178	89.9%	175	89.7%	353	89.8%	*P* < 0.001
Head Phone	25	12.6%	43	22.1%	68	17.3%	
Bluetooth	25	12.6%	11	5.6%	30	7.6%	
Speaker	32	16.2%	26	13.3%	58	14.8%	

4. Discussion:

RF, an invisible electromagnetic radiation, is emitted by cell phones and is absorbed by the skin, inner ear, cochlear nerve, and the temporal lobe surface of cell phone users [Pereira, 2008]. A cross-sectional study was performed at King Abdul-Aziz University to estimate the potential health risks generated by RF electromagnetic waves of cell phones on the auditory function of medical students. Despite a lack of scientific proof regarding the association of the use of cell phones and thermal damage [Pereira, 2008], half of the participants from the first group and more than two thirds of the second group reported that they felt warmth in the tissue surrounding their ear. The thermal effect is caused by water molecule polarization in which electromagnetic waves produce heat. It has been reported that the temperature measured in the head is increased by not more than 0.11°C during the use of a cell phone [Wilen, 2003]. The other effects are non-thermal, and their impact requires further study to explain the conflicts and the controversial issues associated with them, since many studies have different results [Pereira, 2008]. The most controversial issues are related to the effects of cell phones and their risk for causing tumor development. Many studies have reported a link between analog handsets used for ten years or more and the risk of developing tumors [Lonn, 2004] [Hardell, 2005]. Other negative sensations and subjective symptoms, such as tinnitus, wax formation, dizziness, headache, and sleep disturbance, were reported in the range of 19.2–32.3% in the first group and 17.4–34.4% in the second group. It was found that the warmth of tissues, tinnitus, headaches, nervousness or distress, fatigue, concentration difficulties, and sleep disorders were the most common complaints. From those who participated in the study, 59.7% and 51.7% reported having these side effects after using their cell phone. 30.6% and 41.1% reported having them while using it, and 9.7% and 7.3% experience them all the time. These results indicate that cell phone use may have a temporary effect, which may increase in the future. The students' awareness has increased mostly via electronic sources, printed media sources, and through social communication in the community. Cell phone use is mainly for social communication, followed by study purposes and other reasons. It can be reported that most students take their calls through mobile phones directly, and only a small number of students use speakers and headphones. Education and raising awareness are the key factors for improving the behavior of people regarding the extensive use cell phones. The results indicated that the interns were more aware of the risks associated with the usage of cell phones than the sixth-year students; however, they were found to use their cell phones more frequently than the sixth-year students, and hence experienced more side effects. Our results are in agreement with a study Martha and Griffet (2007) in which the level of schooling was shown to have a constructive impact on social risk awareness; this aspect should be supported since the level of education has vital importance on the duration and the way cell phones are being used. However, other factors may have contributed to interns' need to use their cell phones, such as the need to answer more calls from the hospital when they are on duty. Moreover, they are paid more, and so they can afford to pay higher bills. In addition, the use of pagers may increase RF exposure. Furthermore, all anticipated high health risks connected with the exposure and use of cell phones should be available to the public so that they can take precautions [Hutter et al., 2004]. Some of these would include either using a headset or putting the caller on speaker while conversing and avoiding wearing a wireless or Bluetooth headset continuously in order to cut down the dose of radiation. Finally, the university's new medical curriculum should include information about cell phone use that students may need, and should provide more tutorials and lectures



concerning the matter. We should also use the media to raise public awareness regarding the harmful side effects of cell phones.

Limitations:

The research team didn't measure the specific absorption rate, which is used to assess the amount of energy, in which the person can be exposed to RF, differs according to many aspects. These aspects include the distance, the spatial orientation of the place relative to the person, the cell phone handset model, the distance between a cell phone and its station, and the space between the user's head and the handset antenna. All these factors could affect the results of this study. For example, the Canadian government has restricted many models from entering the country because of the negative impact of some features on user health. In this study, it was difficult to classify the models of cell phones used as well as all the other parameters. These differences could be due to reasons such as cell phones' location, specific model, and exposure methodology. Inclusion of student from non-medical field might be considered in the future research.

Conclusion and Recommendation:

Some neurological symptoms and sensations caused by the daily use of cell phones were reported in this study. The use of cellular phones may affect the auditory system in the short term. We plan to continue monitoring these students in order to find out the possible negative effects and the appearance of any long-term hearing changes that may affect their auditory system. Therefore, it is advised that excessive use of cell phones should be avoided and health awareness must be increased through the new medical curriculum at King Abdul-Aziz University.

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Environmental Education in Malaysia, Progresses and Challenges Ahead (Review)

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Abstract: This paper reviews public awareness, knowledge and attitude on environmental aspects in Malaysian educational system and highlights gaps and challenges. The review found that however environmental education components were existed in various sources within the existing educational system; there are still huge gaps on public awareness in Malaysia. The educational system needs to invest more on teachers who are involved in related topics. Teachers would play an important role to increase the public awareness throughout students in primary and secondary schools. This is concluded that more effectiveness of environmental educational system relies on integrity of topics in one independent subject rather than appearances in various subjects. Students would perform an acceptable level of understanding if topics are being actively taught practically in which trainees experience tangible issues than theoretical. Budget however is always concerned in various countries; Malaysia demonstrated a good economic growth that facilitates enough financial allocation if government is convinced to approach towards sustainable development.

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1. Introduction

In the end of the 20th century, the environmental concerns grew much greater, not only among the developed countries, but also in some developing and underdevelopment nations. The common reason was that the consequences of environmental damages to some vital resources became so apparent and horrifying that governments became worried and mass media found great value for their headlines due to the public concern (Karimi, 2003). Besides destructive trend on environment during and after the industrial revolution, sluggish grew on the environmental awareness and initiatives revealed bitter consequences of the environment. The most helpful initiative, activities and movements resulted in international, regional and local agreements addressing specific issues are the increase of environmental awareness. International Environmental Events and Multilateral Environmental Agreements are few of the many improvements that emphasize the importance of the role of environmental education in safeguarding different components of the environment. Also there are different ways such as environmental education and introducing environmental issues to public for protection of the environment (Khorshid Dost, 2003).

In 1978, UNESCO (United Nations Educational, Scientific and Cultural Organization) at the Tbilisi Declaration stressed the need of common use of environmental education (EE) in educational system. During the Tbilisi Convention, environmental education was defined as: 'a process of developing a world population that is aware of and concerned about the total environment and its associated problems, and which has the knowledge, skills, attitudes, motivation and commitment to work individually and collectively towards solutions of current problems and the prevention of new ones'. The Agenda 21 is stressed on sustainable development in the 21st century for all level which leads to people in businesses and governments in economically, socially and environmentally for have a sustainable planet at worldwide, national and local levels. Ibarahim, (2006) in her interview about environmental education stated that, "Malaysia, a signatory party to the Rio Earth Summit, has an obligation to adopt and implement Agenda 21 at the national and local levels in which states clearly as: "One of the fundamental pre-requisites for the achievement of sustainable development is broad public participation in decision making". Individuals, groups and organizations should have free access to information relevant to environment and development held by national authorities". In Malaysia, environmental education subject is still as critical issue which is important for creating of an action to have environmental society. Malaysian

Ministry of Environment (1993) defines environmental education as following:

'It is through knowledge and awareness that positive values and attitudes emerge; values and attitudes that will prompt action to make the adjustments to lifestyles and consumption habits that will reduce the burden we place on the environment'.

Despite, growth of interest in environmental education, several of the studies and agreements recognized absence of environmental awareness and proper environmental education as the main cause of major environmental problems and issues. Therefore, emphasizes providing of public environmental awareness and environmental education for all age levels of human societies. Most of agreement was recommended of public participation in decision making, planning, implementation and monitoring environmental plans.

2. The Environmental Education Elements in Malaysian Education System

There has been a strong movement in Malaysian educational system in the past years towards an outstanding quality. This educational system follows primary to tertiary levels which is under categories including six years of primary, five years of secondary, and two years of pre-university before the student apply to get enrolled into public or private universities inside the country or oversea. There are three type of certificates in Malaysian education system namely LCE, MCE and HSE that refers lower certificate of education, the Malaysian certificate of education which is equivalent to "O" level in the G.C.E. system, and the higher school certificate which is equivalent to "A" level", respectively (MOE, 2010).

Malaysian educational system emphasizes the building of a multiracial and multicultural society where good and useful citizens are being built. Suhaimi (1982) mentioned that under these conditions the practice of liberal education would find limited expression for the development of free, school based, innovations on Environmental Education in schools. He emphasized that in elite system, the focus point for success is learning and tends to provide academic curriculum. In this condition negative trend of environmental education was concerned. This has been shown that volunteer contribution of pupils may provide a better stamina towards the meeting of environmental education's objectives.

Science curriculum in schools has been changed in primary and secondary in past decades and initiated a progressive replacement of traditional courses with modern approaches. Science curriculum was started in the late sixties had no specific objective on environmental education. Environmental elements were included into the curriculum gradually and enhanced by interested subject teachers. In the late seventies, there has been some official move to encourage adding topics on environmental education somehow to do not weaken the current academic contents. This gradual change has been developed environmental education on multidisciplinary infusion method. This integrated environmental elements into single subject lessons, such as geography, integrated sciences, biology, chemistry and the humanities.

According to Daniel (2006), there are two types of education in Malaysia which is including formal and informal education. Formal education consists of primary and secondary schools. These schools are under law, order and monitoring of the ministry of education (MOE). In 1991, Education Planning Committee (of MOE) has decided to integrated environmental education in national educational system. Followed by this, the environmental education was integrated to the new primary and secondary school curriculums. After this, the outcome from the committee, the MOE was committed to conduct the projects, activities, programs and all materials which were approved by the committee. In 1994, there was three main objectives and elements introduced for primary levels. These elements were containing Islamic education, Moral education, Mankind and environment. Later on, in 1994, the subject of environments was divided into science and moral studies. The subjects of environmental education were detailed into curriculums. For better understanding about the environmental subjects, the curriculum was divided into 3 syllabuses such as textbook, work book and activity book. The Secondary School Integrated Curriculum had similar elements to primary school. The subjects for these groups contained Islamic, Moral, Science and Social Education. The target and objective for the Secondary School Integrated Curriculum are to enhance the student's ability and to unify their skills and noble values, integrating in a national language (Bahasa Malaysia), having a real knowledge and finally cooperation and integrate of curriculum with co-curriculum (Mok, 2003). As mentioned earlier, the ministry of education has to lead and implement to the development of environmental education among schools. Also the MOE is granted to increase the student's awareness about the environment using appropriate programs and relevant requirement. Malaysian education system is moving towards to develop according to vision 2020. In this line, the MOE is trying to update the subject of environment in their strategies. Some of the policies, strategies

and action plans are shown in Table 1 (Nadson and Rasid, 2005).

Table 1, Policies, strategies and action plans in Malaysia (Nadson and Abd Rasid, 2005)

Policies, strategies and action plans in Malaysia No.	National Policy/Plan	Strategies/Action Plan/Statement
1.	National Policy on Biodiversity (1998)	Incorporate the study of biological diversity and related fields into the curricula of schools and institutions of higher learning
2.	National Policy on the Environment (2002)	To achieve a deeper and better understanding of the concepts of environmentally sound and sustainable development, and a caring attitude towards nature, EE and awareness will be promoted across the board, incorporating information dissemination and training, in line with Agenda 21
3.	National Integrity Plan (2004)	Community Institution – emphasizing on increasing the awareness of environmental conservation
4.	9th Malaysia Plan (2006-2010)	Appropriate interventions and changes will be made through the school curriculum to create a deeper and longer lasting awareness of the need to care for the environment. In addition, the energy, ideas, enthusiasm of the environmental NGOs will be harnessed to complement and supplement efforts by the government in promoting the environment.

3. Environmental Awareness, Knowledge and Attitude in Malaysia

Malaysia as a developing country is serious to provide outstanding quality of education for citizens. The country is being changed in the education consideration of national needs. The major reform in Malaysian Educational System has happened since the late 1980's and early 1990's (Nadson et.al. 2008). Several studies are found on environmental awareness, knowledge and attitude among schools teachers in Malaysia. Lee (1987) in his study reported that, in Malaysian education system the science teachers in secondary school did not have enough information regarding to integrate of the environmental education subject in school curriculum. For prevent of destruction of environment have a high level of awareness is required (Sham, 1993). Ali (1999) and Osman (2003) reported that, the knowledge of environmental issues among teacher still in a moderate level. Daniel et al (2006), reported in his study, in 1997 the public awareness regarding to environmental issues especially haze problem in Sarawak State were renewed. After this, the local government targeted to measure the air pollution which was produced by many of old vehicles and considered fine for that owner of cars which had more pollution and collected these cars until improve and repair their vehicles. Vehicles spewing excessive smoke were fined and, some vehicles were taken off the road until the problem was rectified. However, he believed that, environmental awareness is in early stage in Malaysia (based on UNESCO, 2003) and the education system was not fully successful to create a responsible citizen regarding to environment. This means, there is some problems and disagreements between citizens related to environment which do not allow them to achieve a proper situation regarding to environment. Another study on Malaysian student's understanding on environment showed that, there was lake of correlation between student's understanding with their willingness on protect of environment (Shamsudin, 2003).

A descriptive study by Fong (2005) in two Selangor schools is in agreement with results reported by Sharifah et al.(2005) the students understanding on environmental issues was in surface level in Malaysia. In general the students' knowledge about environment was high while awareness and sensitivity was low. Also she reported the important resource for students to obtain environmental knowledge was television, radio and newspapers. Overall Lim's study revealed that the students' environmental awareness level was low and the teachers' environmental awareness level was only moderate. In the same study, it was found that environmental management of noise levels and waste management in the schools were not satisfactory. Some of study showed that psychology elements affect the knowledge of students. Aroff and Kasa (1987) believed that, the students are thinking based on the concept of psychology such as the "emotion, observation, learning, as well as the aspect of integrate". The knowledge based on experience and sense will develop the student's opinion about their surroundings. Hence, the role of educators is very important to encourage the behavior of the students that be responsible to do the right and good attitude,

along with the well and healthy environment (Alsagoff, 1992).

4. Environmental Awareness, Knowledge and Attitude in other countries

Palmer (1998) believe that for achieve to suitable level of knowledge, skill and values which are elements to figure of environmental awareness and ethics must be attention to the character of people and student's experiences. Also he stated that, when students achieve a suitable knowledge, understanding, attitude and concept toward environment they can do appropriate action than to environmental issues. Hence, student's awareness, knowledge and attitude will developed due to the achieved skill.

Environmental awareness, knowledge and attitude are major and important component to use as a tool for increasing of opinion of students towards environment (Worcester, 1996). These components can effect on students life whether inside or outside of the school (NAAEE, 1996). However, Ballard and Pandya (1990) have supported the above statements they believed that, the subject of environmental education possibly present as an independent subject in school curriculum. Orr (1992) stated that, the knowledge is not only element in environmental literacy. He referred that knowledge is important to connected to other element and attitude is needed to caring. Thus, attitude and knowledge is necessary for environmental literacy. He believed that, "All education is environmental education". Some researchers were not agreeing with this statement. They believed that, there is a biased in the Orr's statement, they said, "education is a favorable time to act and a chance to receive a sustainable society". Awareness, knowledge and attitude are important components in environmental education. Awareness is a force which motivate the knowledge (Madsen, 1996). It will be happen when the students see the fact of problems regarding to environment. So, the personal commitment to solving of environmental problems will be involve. He stated that for achieved to environmental awareness level, the people should be recognize and confidence regarding to problems in around them, also has a knowledge in scientifically point about environmental problems is compulsory and finally they should be responsible to answer and solve the problems. He believed that, for solving of environmental problems the decision makers and leaders should be have awareness and knowledge about environment. Also he referred, if the leaders have enough information regarding to environment, they can performed a suitable action when they faced with these problems. Another study by Blum (1987) was reviewed five such nation-wide studies on the knowledge and beliefs of ninth- and tenth-grade students on environmental issues. These studies were conducted in the United States (Bohl, 1976; Perkes, 1973), Australia (Eyers, 1975), England (Richmond, 1976) and Israel (Blum, 1984). The questionnaire was used as a means of collecting information about students' knowledge and attitudes about environmental issues. Since then researchers in several other countries have conducted similar types of environmental inquiry (Aminrad et al, 2010). In the Dominican Republic, Roth and Perez (1989) surveyed the environmental knowledge and attitudes of twelfth-grade students; in the Philippines, Cortes (1991) surveyed the environmental knowledge, comprehension, responsibility, and interest of secondary level students and teachers; and in Singapore, Lau (1992) conducted a pilot survey to find out the extent of environmental knowledge and commitment of school children. These three studies were conducted in less technologically developed countries than the United States, where Manning (1992) conducted a study similar to his predecessors. Lau's (1992) study on environmental knowledge and commitment of school children showed that students' performance in the knowledge section of the survey was very poor. Students fared better in their commitment to environmental protection. Aminrad et.al (2010), in her study state that overall environmental awareness and attitude about environmental issues is moderate and high, respectively. In addition, the majority of the students had adequate awareness and attitude about environmental issues. In conclusion, with increasing student's environmental awareness and attitude, they increase their environmental views.

Finally against this background of growing interest in environmental education, this review paper will show to the importance of environmental education and it's components in Malaysia and it's situation against the world.

5. Challenges of EE in Malaysia

The implementation of EE in Malaysia is an exciting duty. The challenges faced by environmental education researcher or implementers are:

<u>To strong support for EE</u>

-Attention of other organization to environmental education and put a priority in their program regarding to environmental education subject due to the longer period of impacts of environmental education program in society. However, should be exist some principle of precautionary to destroy of environment and principle regarding to protect of environment between organization.

Create a change in people's attitude

-One of major challenge in EE is changing people's attitude to be more environmentally friendly. Environmental education should be start from earlier of age on younger generation due to they are as a future leader are so important.

Reaction of new generation regarding environment

-Next challenge is creating awareness on the importance of the environment which achieved with technology (such as the internet and multimedia presentation) and also through electronic and printed media.

Lack of budget to implement of EE program

-One of big problem for implementer of environmental education program is lack of funding.

Less skill in implement of EE program

-Another challenge is limited skills among implementers, who wants solve the environmental problems. They do not want to share of lessons learned and their knowledge's to overcome problems.

Lack of integration

- The lack of collaboration among implementers is still a challenge but the situation now is much better due to improved communications amongst implementers.

6. Conclusion

Malaysia, in a long term plan of the year 2020 vision, has highlighted the importance of environment education in which appears in school books. The current research has shown a huge gap to an environmentally utopia in implementation of the policy. Throughout the study, this is found that family economy and education is highly effective in environmental understanding of public. Teachers are an area of severe concern where lack of their environmental knowledge's are predominantly influences children as a future generation. The study has suggested further researches that focus on stimulating factors may boost environmental awareness, attitude and knowledge of school teachers as a key factor in environmental education. This may happens by providing face to face and/or remote in-service education programs for teachers to improve their environmental understanding.

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Life Skills Education for Secondary Education

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Abstract: Life skills study is intended to strengthen a pupil's overall development. This involves, for instance, having pupils make an effort to develop spiritual values, physical health and psychological strength. They strengthen their social skills, moral competence and respect for others and them- selves. In addition, an effort is made to strengthen their courage, initiative, natural creativity and adaptability to meet the demands and challenges of everyday life. The emphases in life skills underline the fact that the school is the pupils' workplace, where valuable upbringing takes place. Basic responsibility for children's upbringing must, however, always be in the hands of their parents/guardians. As the school assists parents in their role as child-raisers, pupils' education and welfare is thus a joint project of schools and households. This co-operation must be based on mutual respect, mutual trust and joint responsibility. One of the emphases of life skills is to have the school create a positive and secure study environment, characterized by the support and co-operation of everyone in the school, both pupils and staff. A positive school spirit, together with realistic demands and expectations of pupils, facilitates them in achieving the study objectives set. Adolescence is a period of experimenting, experiencing and expanding. Adolescents need help and guidance indecision-making, problem solving, critical thinking, developing interpersonal skills, self-awareness, empathy, coping with stress and managing emotions. The rebelliousness and dislike for parental intrusion usually keeps parents at bay because teenagers do not relish the idea of help and guidance from parents. However, this may not always be so. Beneath frequent violent outbursts, sudden mood swings and related interpersonal problems of an adolescent, there may be a person crying out for professional help. All adolescents need support and guidance. When parents find it difficult to handle signs of trouble, professional help should be sought at the earliest. Extra care is needed while offering help to adolescents problems because it is not easy for teenagers to accept the fact that they need help. Attempts should be made to understand the adolescent, and to safeguard, protect and guide him/her. The Family Life & Life Skills Education Programmed is a good support system for adolescents at the community level.
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Key word: Life Skills, Education, Secondary Education, community level, Education Programmed, violent, self-awareness, spiritual values

1. Introduction

Life skills study is intended to strengthen a pupil's overall development. This involves, for instance, having pupils make an effort to develop spiritual values, physical health and psychological strength. They strengthen their social skills, moral competence and respect for others and them- selves. In addition, an effort is made to strengthen their courage, initiative, natural creativity and adaptability to meet the demands and challenges of everyday life. The emphases in life skills underline the fact that the school is the pupils' workplace, where valuable upbringing takes place. Basic responsibility for children's upbringing must, however, always be in the hands of their parents/guardians. As the school assists parents in their role as child-raisers, pupils' education and welfare is thus a joint project of schools and households. This co-operation must be based on mutual respect, mutual trust and joint responsibility. One of the emphases of life skills is to have the school create a positive and secure study environment, characterized by the support and co-operation of everyone in the school, both pupils and staff. A positive school spirit, together with realistic demands and expectations of pupils, facilitates them in achieving the study objectives set. Making life skills a special subject is a response to contemporary demands that pupils be better prepared to face the challenges of life. To do so, they need to work on them- selves, respect themselves but know their strong and weak sides. Life skills provide valuable opportunities to strengthen pupils' social development. The aspects dealt with are connected with participating in a democratic society, belonging to a family, having friends and comrades, working with others and placing oneself in another's position. The subject looks at the pupil as a whole, his/her ability to communicate, express opinions and give reasons for them, set objectives, show initiative, find his/her way around in the local environment, avoid dangers and be independent. In addition, creative ability and practical skills need to be developed. Life skills also gives schools the opportunity to take a closer look at issues which may come up and concern pupils' well-being

and feelings.

Adolescence is a period of experimenting, experiencing and expanding. Adolescents need help and guidance indecision-making, problem solving, critical thinking, developing interpersonal skills, self-awareness, empathy, coping with stress and managing emotions. The rebelliousness and dislike for parental intrusion usually keeps parents at bay because teenagers do not relish the idea of help and guidance from parents. However, this may not always be so. Beneath frequent violent outbursts, sudden mood swings and related interpersonal problems of an adolescent, there may be a person crying out for professional help. All adolescents need support and guidance. When parents find it difficult to handle signs of trouble, professional help should be sought at the earliest. Extra care is needed while offering help to adolescents problems because it is not easy for teenagers to accept the fact that they need help. Attempts should be made to understand the adolescent, and to safeguard, protect and guide him/her. The Family Life & Life Skills Education Programmed is a good support system for adolescents at the community level.

2. Defining Life Skills

Life skills are abilities for adaptive and positive behavior that enable individuals to deal effectively with the demands and challenges of everyday life. Described in this way, skills that can be said to be life skills are innumerable, and the nature and definition of life skills are likely to differ across cultures and settings. However, analysis of the life skills field suggests that there is a core set of skills that are at the heart of skills-based initiatives for the promotion of the health and well-being of children and adolescents. These are listed below: 1.Decision making 2.Problem solving 3.Creative thinking 4.Critical thinking 5. Effective communication 6. Interpersonal relationship skills 7. Self-awareness 8. Empathy 9. Coping with emotions 10. Coping with stress

Decision making helps us to deal constructively with decisions about our lives. This can have consequences for health if young people actively make decisions about their actions in relation to health by assessing the different options, and what effects different decisions may have similar

Problem solving enables us to deal constructively problems in our lives. Significant problems that are left unresolved cab cause mental stress and give rise to accompanying physical strain.

Creative thinking: Contributes to both decision making and problem solving by enabling us to explore the available alternatives and various consequences of our actions or non-action. It helps us to look beyond our direct experience, and even if no problem is identified, or no decision is to be made, creative thinking can help us to respond adaptively and with flexibility to the situations of our daily lives.

Critical thinking: is an ability to analyze information and experiences in an objective manner. Critical thinking can contribute to health by helping us to recognize and assess the factors that influence attitudes and behavior, such as values, peer pressure, and the media.

Effective communication: means that we are able to express ourselves, both verbally and non-verbally, in ways that are appropriate to our cultures and situations. This means being able to express opinions and desires, but also needs and fears. And it may mean being able to ask for advice and help in a time of need.

Interpersonal relationship skills: help us to relate in positive ways with the people we interact with. This may mean being able to make and keep friendly relationships, which can be of great importance to our mental and social well-being. It may mean keeping good relations with family members, which are an important source of social support. It may also mean being able to end relationships constructively.

Self-awareness includes our recognition of ourselves, of our character, of our strengths and weakness, desires and dislikes. Developing self-awareness can help us to recognize when we are stressed or feel under pressure. It is also often a prerequisite for effective. Communication and interpersonal relations, as well as for developing empathy for others

Empathy is the ability to imagine while is like for another person, even in a situation that we may not be familiar with. Empathy can help us to understand and accept others who may be very different from ourselves, which can improve social interactions, for example, in situations of ethnic or cultural diversity. Empathy can also help to encourage nurturing behavior towards people in need of care and assistance, or tolerance, as is the case with AIDS sufferers, or people with mental disorders, who may be stigmatized and ostracized by the very people they depend upon for support.

3. Life Skills Education

For health promotion, life skills education is based on the teaching of generic life skills and includes the practice of skills in relation to major health and social problems. Life skills lessons should be combined with health information, and may also be combined with other approaches, such as programmers designed to effect changes in environmental and social factors which influence the health and development of young people. The methods used in the teaching of life skills builds upon what is known of how young people learn from their own experiences and from the people around them, from observing how others behave and what consequences arise from behavior. This is described in

the Social Learning Theory developed by Bandura (1977). In Social Learning Theory, learning is considered to be an actively involved in a dynamic teaching and learning process. The methods used to facilitate this active involvement include working in small groups and pairs, brain storming, role play, games and debates. A life skills lesson may start with a teacher exploring with the students what their ideas or knowledge are about a particular situation in which a life skill can be used. The children may be asked to discuss the issues raised in more detail in small groups or with a partner. They may then engage in short role play scenarios, or take part in activities that allow them to practice the skills in different situations – actual practice of skills is a vital component of life skills education. Finally, the teacher will assign homework to encourage the children to further discuss and practice the skills with their families and friends. Life skills have already been taught in many schools around the world. Some initiatives are in use in just a few schools, whilst in other countries, life skills programmers have been introduced in a large proportion of schools, and for different age groups. In some countries, there are several important life skills initiatives, originating in different groups in countries, there are several important life skills initiatives, originating in different groups in countries, there are several important life skills initiatives, originating in different groups in countries, there are several important life skills initiatives, originating in different groups in countries, there are several important life skills initiatives, originating in different groups in the country, e.g. Non-governmental organizations, education authorities, and religious groups

Identifying an optimal strategy for life skills education

The wide range of motives for teaching life skills to children and adolescents include the prevention of drug abuse and teenage pregnancy, the promotion of mental well-being and cooperative learning. For adults, life skills appear in programmers such as communication and empathy skills for medical students and counselors, problem solving and critical thinking for business managers, and coping with emotions and stressors for people with mental health problems.

Given the wide ranging relevance of life skills, an optimal strategy for the introduction of life skills teaching would be to make it available to all children and adolescents in schools. Life skills teaching promote the learning of abilities that contribute to positive health behavior, positive interpersonal relationships, and mental well-being. Ideally, this learning should occur at a young age, before negative patterns of behavior and interaction have become established.

The school is an appropriate place for the introduction of life skills education because of:

- The role of schools in the socialization of young people.
- Access to children and adolescents on a large scale
- Economic efficiencies (uses existing infrastructure);
- Experienced teachers already in place;
- High credibility with parents and community members;
- Possibilities for short and long term evaluation

Even in countries where a significant proportion of children do not complete schooling, the introduction of life skills education in schools should be a priority. Life skills education is highly relevant to the daily needs of young people. When it is part of the school curriculum, the indications are that it helps to prevent school drop-out. Furthermore, once experience has been gained in the development and implementation of a life skills program me for schools, this may help with the creation and implementation of programmers for other settings.

Developing life skills programmers

Designing and implementing a life skills program is only a part of the life skills programmers development process. It is equally important to secure long term support and resources for life skills education, and to engage, from the very beginning, all of the potential agencies that would have a role to play in the process of life skills programmers' development. Implementing life skills programmers will require the introduction of teaching methods that may be new to teachers, and the success of the programmers will depend very much on the availability of in-service training, as well as efforts to include training in participatory learning methods in teacher training colleges.

The introduction of life skills education will require input from the school and education authorities, for teacher training and the development of teaching manuals, as well as for the ongoing support of teaching programmers once they are in place. This investment is worthwhile considering that the potential gains of life skills education are so far reaching. Apart from the impact on child health, there may be other benefits for the school as an institution. For example, evaluative studies of life skills programmers suggest that the methods used can help to improve teacher and pupil relationships (parsons et al., 1988), and there are indications that life skills lessons are associated with fewer reports of classroom behavior problems. There are also research indications of improved academic performance as a result of

teaching life skills (Weissberg et al., 1989). Other positive effects include improved school attendance (Zabin et al., 1986), less bullying, fewer referrals to specialist support services and better relationships between children and their parents. A life skills programmers will have to be proven worthy of the resources allocated to it. Process and outcome evaluation studies should be carried out, and results shared with all the relevant decision makers that could affect the future of the life skills programmers. A programmers that has a component of ongoing assessment of its use and impact offers scope for keeping in touch with changing priorities, and is more likely to be modified and maintained over time.

Well designed, tested and delivered life skills programmers can achieve much in helping children and adolescents become more responsible, healthy and resilient both during childhood and as adolescence.

ii) What is the target group of the life skills program?

If a life skills program is to be developed for the promotion of health and well being, it should ideally be targeted at all children and adolescents, as a positive response to health needs, rather than as an intervention aimed only at those already a risk or who already have hath problems.

If the plans are that the life skills program should eventually be implemented wide scale in a country, then the original program is likely to be developed first for the most dominant, majority language and culture in the country. This may mean that minority groups will not be reached, especially if there are no representatives from such groups in the Life Skills Advisory Panel or Development Group. Plans should be made for program adaptation, or life skills program development, for such groups once a program has been implemented and is being maintained. Life skills program can be developed for all ages of children and adolescents in school. Experience gained in countries where life skills program have been developed groups to be targeted are likely to be determined by education policy and the resources available, as well as by the age at which children are most groups, to help select who it is for and for how many year groups. Given the role of life skills in the promotion of positive health behavior, it is worthwhile ensuring that life skills program are available in the pre-adolescent or early adolescent years, since young people of this age group seem to be most vulnerable to behavior-related health problems.

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Behavior of R.C Columns with Poor Concrete Strength at Upper Part

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Abstract: Reinforced concrete columns are the main load bearing elements of any structure. It support the beams and slabs and transfer the loads to the foundations. Hence they have to be designed and detailed adequately to resist both gravity and lateral loads. In some buildings, especially when quality control is poor; the upper part of column contains few amount of mortar cement and large amount of aggregates, this mean that compressive strength of concrete is weak in this part. Low compressive strength for upper part of the column will lead to a reduction in bearing capacity of column. Moreover; upper part does not behave in the same manner as is the rest of the column body. This study is carried out to investigate the behavior of 12 types of reinforced concrete columns subjected to a concentric compressive load. The experimental specimens were made of concretes with compressive strengths ranging from 12.5MPa to 40MPa. Grade 400 was used for steel ties reinforcement in order to investigate the ability of higher yield strength steel to confine the concrete core of column. The behavior of the tested specimens is compared to predict the effect of studied parameters on the ultimate load, vertical and horizontal strains of the columns. This research presents a proposed equation for calculating the ultimate load of column taking into considerations the effect of poor concrete strength at upper part.
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Key Words: R.C columns, poor concrete, upper part, concentric.

1. Introduction

. Structural column failure is one of major significant concern in terms of economic as well as human loss. Thus, extreme care needs to be taken in column design, with higher conservative precautions than in the case of beams and other horizontal structural elements, since compression failure provides little visual warning.

This study is carried out to investigate the general deformational behavior of R.C Columns with Poor Concrete Strength at Upper Part of column body. These columns are subjected to axial compression loads acting at their top level. The cross section of columns and their reinforcing steel are kept constant, while the spacing between stirrups at the upper part is changed in some tested specimens. Also, the height of concerned upper part of column is changed in some tested specimens.

A lot of previous researches focused on the behavior of column with constant strength along its full height, even if there is a local defect in the column. In the present study the construction deficiencies such as bad quality concrete at upper part of column are precisely considered as shown in figure (1).

Soliman[1] Carried out a study to investigate the behavior of concrete columns strengthened by using circular concrete jackets. This study indicated that the use of spiral stirrups as a transversal reinforcement of the jacket improves the strength and the ductility of the strengthened or repaired column than using of rectangular separate stirrups.

Usama[2] carried out an experimental investigation on strengthening of the defected part in the column. The construction deficiencies such as bad quality of concrete and bad arrangement of horizontal reinforcement (stirrups) are only investigated. This study concluded that ultimate load of defected part of a column is increased by wrapping its cross section by a concrete jack, the presence of a defected part along the column height significantly changes its behavior (deformations of upper zones are more than the deformation of middle zone), and the compressive strength of the column has a great effect on the load capacity of the column compared with the bad arrangement of the stirrups.

Shamim *et al.*,[3] Fifteen 12-in. (305 mm) square cross section and 9-ft (2.74 m) long reinforced concrete columns were tested under flexure to large inelastic deformations while simultaneously subjected to constant axial load to investigate the behavior of column sections confined by rectangular ties. Major variables considered in this program included distribution of longitudinal and lateral steel. It was found that a larger number of laterally supported longitudinal bars results in higher flexural strength and ductility.

Unsupported longitudinal bars tend to buckle and push the ties outward at large deformations, resulting in a brittle behavior caused by a loss of confinement.

Němeček *et al.*, [4] The behavior of six series of reinforced concrete columns with a square cross section was investigated experimentally and numerically. Two different grades of concrete (normal and high strength) and three different densities of stirrups were chosen. The columns were loaded in

eccentric compression with small eccentricity. The major experimental and numerical results are as follows:

- Compression failure (crushing) accompanied by concrete softening and steel buckling developed in the columns.
- Failure of columns localized into the middle part, where a wedge-shape failure pattern developed in the concrete, together with buckling of the reinforcement between the stirrups. The damage zone had approximately the same dimensions for all tested series.
- The influence of the density of the stirrups on the column strength was negligible in the investigated cases (i.e. square cross section, stirrup density 50 mm–150 mm).

Kim *et al.*, [5] The effects of concrete strength and longitudinal steel ratio on the ultimate load and axial force, bending moment relation of a column were investigated.

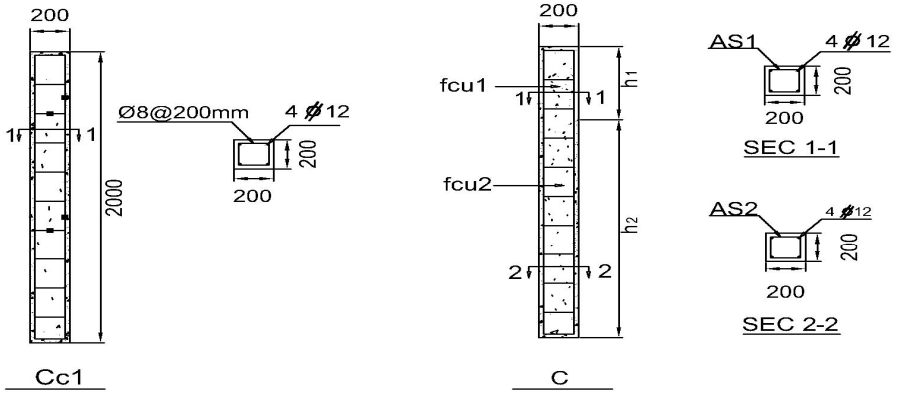
A series of tests was carried out for 30 tied reinforced concrete columns with 80 mm square cross section and three slenderness ratio of 10.60 and 100 three different concrete strengths of 25.5 , 63.5 and 86.2 Mpa, and two different longitudinal steel ratios of 1.98 and 3.95% were used. Experimental results revealed that the ultimate load for a short high-strength concrete column was significantly increased .The possibility of stability failure for a slender column was increased with the increase of concrete strength. The increment of ultimate load due to increase in longitudinal reinforcement for the short column was less than for the slender one, and the heavier reinforcement for the slender column led to a more stable column

Fig. (1) Bad quality concrete at upper part of column

2. Experimental Work

Test Specimens

Twelve 200 × 200 × 2000 mm rectangular columns of concrete strength (f_{cu}) 40 MPa were cast and tested. The main variables of the studied parameters were the upper poor concrete strength (12.5 – 20) MPa, the spacing of stirrups (200 -100 mm), and the stirrups diameter (8-10 mm). (36/52) steel grade was used for longitudinal reinforcement (diameter of 12 mm). The details of the specimens are as given in Figure (2).The columns were cast vertically to simulate the columns in actual practice of construction and compacted with a needle vibrator., Instrumentation, casting, and curing are shown in figures (3 , 4 , 5).Also the tested specimens properties are given in table (1).



h_1 = height of poor concrete part of column

h_2 = height of column part with normal constant strength

Fig (2) test specimens details

Fig. (3) Fixing electrical strain gages

Fig. (4) Specimens casting

Fig. (5) Specimens curing

Table (1) test specimens

Test specimen	h1(mm)	h2(mm)	Ast1	Ast2	fcu1	fcu2
Cc1	0	2000	Ø8@200 mm	Ø8@200 mm	40	40
Cc2	500	1500	Ø8@150 mm	Ø8@200 mm	40	40
Cc3	500	1500	Ø8@100 mm	Ø8@200 mm	40	40
Cc4	500	1500	Ø10@200 mm	Ø8@200 mm	40	40
C1	500	1500	Ø8@200 mm	Ø8@200 mm	14	40
C2	500	1500	Ø8@200 mm	Ø8@200 mm	18	40
C3	500	1500	Ø8@200 mm	Ø8@200 mm	20	40
C4	500	1500	Ø8@150 mm	Ø8@200 mm	14	40
C5	500	1500	Ø8@100 mm	Ø8@200 mm	14	40
C6	500	1500	Ø10@200 mm	Ø8@200 mm	14	40
C7	350	1650	Ø8@200 mm	Ø8@200 mm	14	40
C8	650	1350	Ø8@200 mm	Ø8@200 mm	14	40

f cu1 = concrete characteristic strength of upper part of column.
f cu2 = concrete characteristic strength of column.

Equipment and Instrumentation:

All columns were loaded with 500 ton hydraulic machine in Cairo university concrete research lab. The applied load was read out on the load cell scale. The set up of loading of the tested columns is shown in figure (6). To ensure concentric loading; displacement dial gauges of 0.01 mm accuracy and LVDT were placed at upper and middle position along column two perpendicular sides to monitor its deflected shape at different increments of loading as shown in fig. (7).

Concrete strains were measured by mechanical strain gauges of 100 mm gauge length, using demic points mounted on the upper and lower columns' sides at the same positions in all columns. Also, electric srain gauges are fixed on longitudinal steel bars and transversal stirrups.

Fig. (6) Set up of loading of the tested columns

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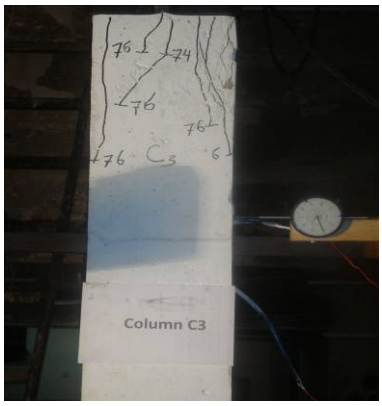

Fig. (7) Location of dial gauges and LVDT at upper and middle position along column

3. Analysis and Discussion of the Experimental Results

Mode of failure and failure load

The applied load was increased gradually from zero to the ultimate load with a constant increment of 10 tons. The load remained constant at each increment of load to record the concrete strain, the longitudinal reinforcement strains, the transversal reinforcement strain and crack patterns till collapse

For the twelve tested columns, the first crack appeared at a load level about 70% to 80 % of the ultimate (the failure) load for upper poor concrete strength specimens. While it was appeared at a load level about 80% to 90 % of the ultimate (the failure) load for control specimen of normal concrete strength, table (2) shows the value of loads at which the first crack appeared at upper part for each column. In addition; figures (8) to (10) show the crack pattern and the shape of failure of the tested columns.

Fig. (8) crack pattern and the shape of failure of cc1, c1,c2,c3

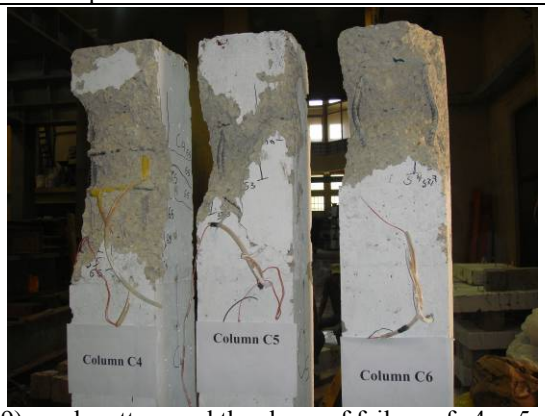

Fig. (9) crack pattern and the shape of failure of c4 , c5 , c6

Fig. (10) crack pattern and the shape of failure of c7 , c8

Table (2) Cracking and Failure Loads

Column No.	Cracking load (ton)	Failure load (ton)	Cracking load % of Failure load	Mode of failure
Cc1	100	113	88	Crushing
Cc2	130	143	91	Crushing
Cc3	121	134	90	Crushing
Cc4	127	143	89	Crushing
C1	45	63	71	Crushing
C2	55	78	70	Crushing
C3	57	79	72	Crushing
C4	51	65	78	Crushing
C5	50	66	76	Crushing
C6	47	67	70	Crushing
C7	52	72	72	Crushing
C8	43	61	70	Crushing

Figures (11, 12) illustrate that the ultimate load decreased by about 45,31 and 30% in specimens C1, C2, and C3 as the concrete strengths in the upper part of the column were 14, 18, and 20 MPa respectively compared with the control specimen of constant concrete strength of 40 MPa. While the column longitudinal strain at the same ultimate load decreased in lower portion of column with normal strength concrete compared with upper poor strength concrete part.

Figures (13 , 14 , 15) showed that the effect of decreasing the spacing between stirrups or increasing its diameter in the upper poor concrete strength part of the specimens C4 , C5 ,and C6 had little effect on measured ultimate load. While, this effect was increased in control specimens Cc2, Cc3, and Cc4.

Figure (16) showed that the height decreasing of upper poor concrete strength (h1) of specimens C7 by about 30% increased the ultimate load by about 14% compared with tested specimen C1. While the height increasing of upper poor concrete strength (h1) of specimens C8 by about 30% decreased the ultimate load by about 4% compared with tested specimen C1.

Figures (17, 18) illustrate that longitudinal steel – strains in upper poor concrete part of specimens C1 and C2 was more than that of lower normal concrete part. Also longitudinal steel – strain increased in upper part compared with control specimen Cc1.

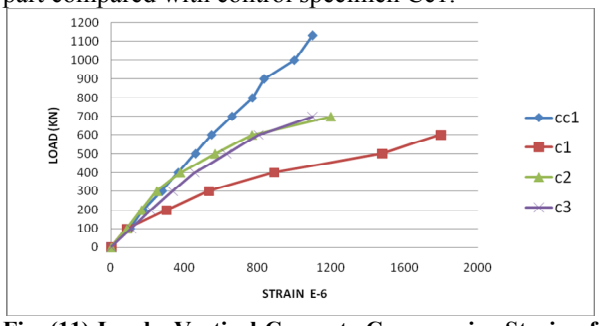

Fig. (11) Load - Vertical Concrete Compressive Strain of Upper Part

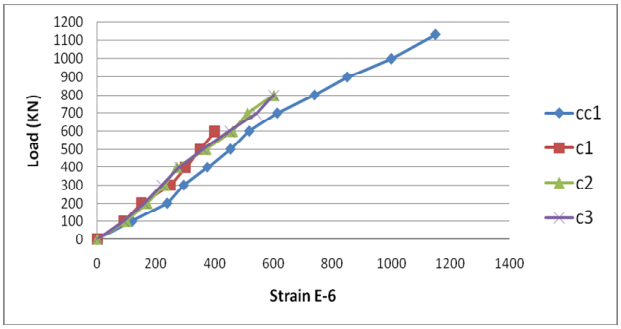

Fig. (12) Load - Vertical Concrete Compressive Strain of Lower Part

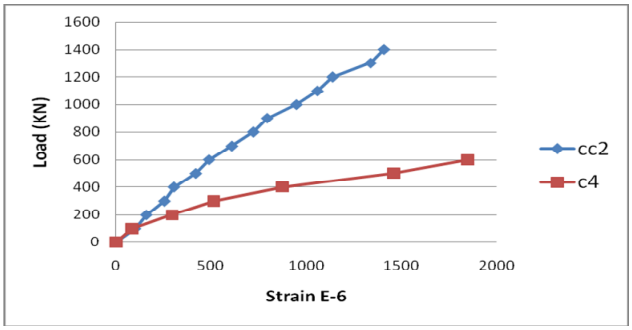

Fig. (13) Load - Vertical Concrete Compressive Strain of Upper Part

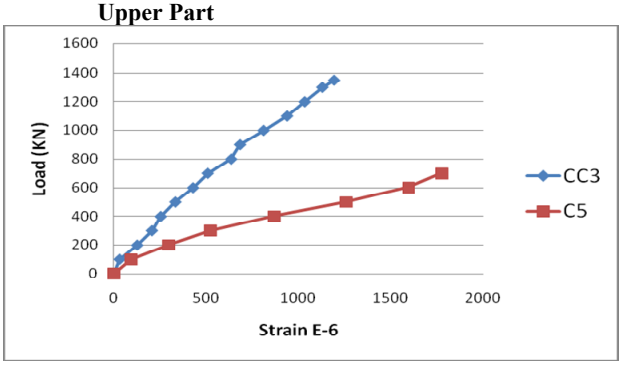

Fig. (14) Load - Vertical Concrete Compressive Strain of Upper Part

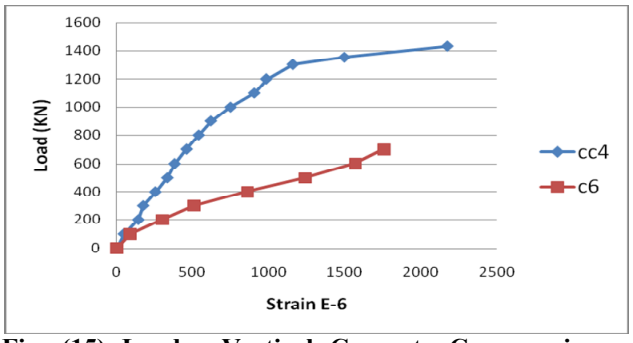

Fig. (15) Load - Vertical Concrete Compressive Strain of Upper Part

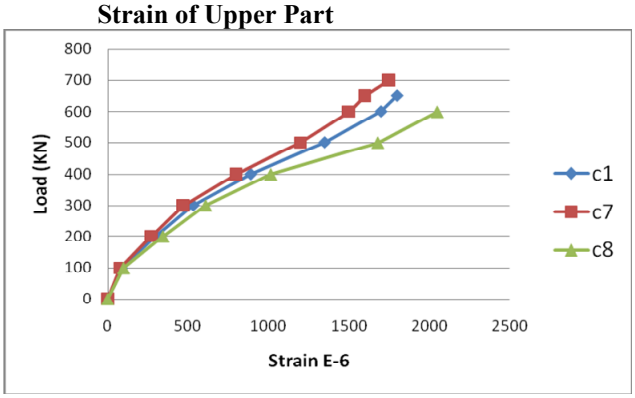

Fig. (16) Load - Vertical Concrete Compressive Strain of Upper Part

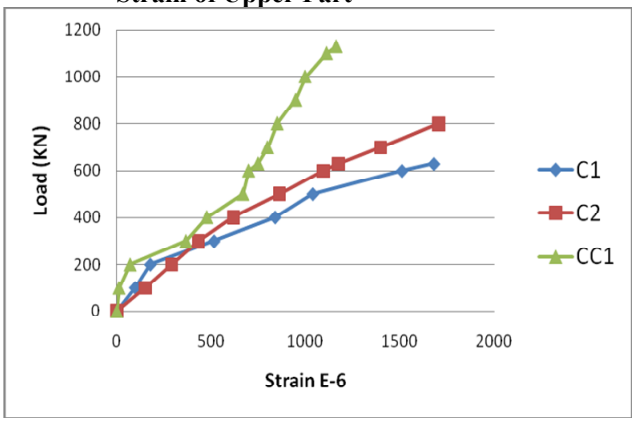

Fig. (17) Load - Vertical Steel Compressive Strain of Upper Part

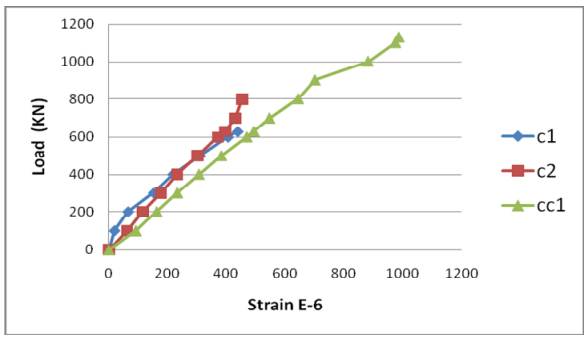

Fig. (18) Load - Vertical Steel Compressive Strain of Bottom Part

Calculating the load carrying capacity of columns using code equation with suggested modified factors.

A relation between the load carrying capacity and the concrete strength at upper and lower parts of tested specimens can be obtained by regression analysis which used best fit of the test results. Accordingly; a usable formula was obtained as follows.

Assuming that columns considered in the present study is part of a braced structure; the ultimate load carrying capacity of the column according to ECP 203 code [6] can be calculated as follows:

$Pu = 0.35 * f_{cur} * AC + 0.67 * f_y * A_{SC}$

Where:

Pu = Design load of tied columns, (ton).

$$f_{cur} = 0.92 \sqrt{f_{cu1} * f_{cu2}} * \sqrt{h_2 / h_1}$$

f_{cu1} = Concrete characteristic strength of upper part of column.

f_{cu2} = Concrete characteristic strength of column.

Ac = Area of column's cross section.

Fy = Yield strength of steel.

Asc = Area of longitudinal reinforcement.

h_1 = height of poor concrete part of column.

h_1 = height of column part with normal constant strength.

Table (3) and figure (19) showed good agreement between experimental measured ultimate load and the calculated one using the proposed equation.

Table (3) Cracking and Failure Loads

specimens	b1 (mm)	b2 (mm)	F_{cu1} (Mpa)	F_{cu2} (Mpa)	h_1 (mm)	h_2 (mm)	P_u (exp) (kN)	$P_u(pe)^*$ (kN)	P_u (exp) / P_u(pe)
c1	200	200	14	40	500	1500	630	636	0.99
c2	200	200	18	40	500	1500	780	714	1.09
c3	200	200	20	40	500	1500	790	746	1.05
c4	200	200	14	40	500	1500	650	642	1.01
c5	200	200	14	40	500	1500	660	642	1.02
c6	200	200	14	40	500	1500	670	642	1.04
c7	200	200	14	40	350	1650	720	777	0.93
c8	200	200	14	40	350	1350	610	553	1.10

***$P_u(pe) = P_u$ using proposed equation**

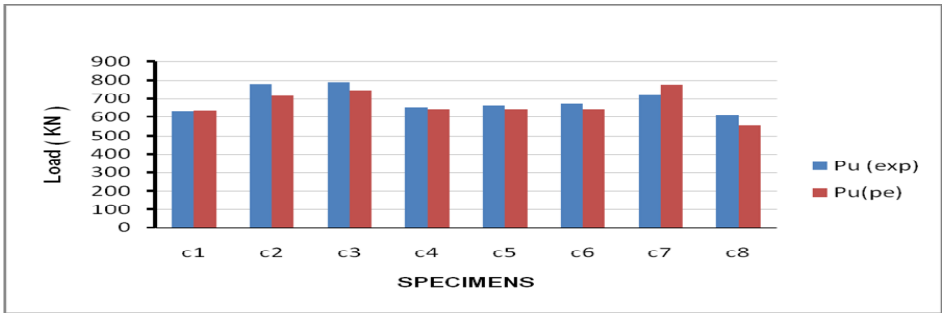

Fig. (19) comparison between P_u (exp) and P_u(pe)

Conclusions

1. Mode of failure of all poor concrete upper part tested specimens was the initiation of vertical crack at upper part followed by crushing of concrete at top of column. While horizontal and vertical cracks appear at middle of normal concrete strength control specimen.
2. Ultimate column load decreased as the concrete strength of upper part decreased.
3. Decreasing of stirrups spacing or increasing its diameter at upper poor concrete part had no significant effect on the ultimate column load. While, stirrups concentration in the upper part increased the ultimate column load by about 20% in case of normal concrete strength along the whole height of columns.
4. Ultimate column load increased as the height of poor concrete part decrease and visa versa.
5. Longitudinal steel – strains in upper poor concrete part of column was more than that of lower part of normal concrete strength.
6. A proposed equation for calculating the ultimate load for short braced columns which takes into consideration the effect of height and strength of upper poor concrete and lower normal concrete parts was presented as follows:

$P_u = 0.35 * f_{cur} * AC + 0.67 * f_y * A_{SC}$

Where:

$$f_{cur} = 0.92 \sqrt{f_{cu1} * f_{cu2}} * \sqrt{h_2 / h_1}$$

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5/2/2012

Growth and Mineral Status of Barley Plants As Affected By Drought and Foliar Fertilization

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Abstract: Two field experiments were conducted in the Experimental Farm of the National Research Centre, Shalakan, Kaloubia Governorate during 2005/2006 and 2006/2007 winter seasons to evaluate the foliar fertilization Foliar-X (commercial multi-nutrients) and water deficit at two growth stages and those irrigate regularly on growth and yield of barley c.v. Giza 125. Plant height, number of tiller and spikes / plant and spike length in the first and second seasons, did not show any significant effect by water deficit at heading or late at dough stage. In the first season, dry weight of shoots, spikes and whole plant values were lower when plants subjected to omitting of irrigation at heating than that at dough stage or control plants, however, the differences in whole plant were only significant. The differences in these parameters in plants exposed to water deficit at dough stage and that irrigated regularly were approximately equal. The highest negative effect by omitting of irrigation at heading was higher in the dry weight of the whole plant followed by that on shoots in the second season while in the first, season the degree of depression was similar. Phosphorus concentration in straw drastically decreased by subjection barley plants to drought at heading and at dough stages and at latter stage the effect was pronounced. However, the differences in N and K concentrations seemed to be equal with both drought treatments and the control treatment. Data showed that water deficit led to a depression in K, Fe, Mn and Zn uptake and the depressions continuous as the drought treatment delayed. The differences in K uptake were not great enough to reach the level of significant. Later deficit at heading gave the higher value of N uptake but at dough stage induced decrement but less than that resulted with that at heading. Nevertheless, the water deficit treatment at heading decrease the uptake of P, while, under deficit at dough stage this element pronouncedly increased compare to regular irrigation treatment.

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Keywords: Barley-Drought- Growth-Straw-Grains-Yield-Macro and micronutrients.

1. Introduction

Water scarcity is an increasingly important issue in many parts of the world. Climate changes predictions of increase in temperature and decrease in rainfall mean water will become even scarce. Since agriculture is the major water user, efficient use of water in agriculture is needed for the conservation of this limited resource (Farri and Faci, 2006).

In Egypt, the production of cereals in old lands still up tell now not enough to face the increasing demand of the population which increased rapidly in the last decades. The increase of areas and productivity of less water requirement crops in the new cultivated soils are considered one of the important ways for narrowing the cereal gap.

Barley (*Hordeum vulgare L.*) is grown under wide range of environmental conditions. Generally, it grows in areas where water supply is limited and where crop production depends mainly upon rainfall. In Egypt, barley grains and straw are mainly used for animal feed and sometimes grains are used for bread making by some bedowins (Ashour and Selim, 1994). Negative effect of drought on growth and mineral uptake of barley were studied before by many authors: among of them Angum, *et al*. (2002) and Hussein, *et al.* (2006).

Beneficial effects of fertilization through soil application or foliar spray and its interaction with water stress on growth and mineral status of barley plants were reported by Selim (1994); Angas, *et al.* (2006) and Li, *et al*. (2009).

This study was designed to investigate the positive effects of foliar fertilizer on growth and mineral status of barley plants grown under drought through some growth stages.

2. Material and Methods

Two field experiments were conducted in the Experimental Farm of the National Research Centre, Shalakan, Kaloubia Governorate during 2005/2006 and 2006/2007 winter seasons to evaluate the foliar fertilization (Foliar-X) and water deficit at two growth stages and those irrigate regularly on growth and yield of barley c.v. Giza 125. Some physical and chemical properties of soil in the experimental sites were noted in Table (1).

Table (1): Analytical data of the experimental site.

A . Soil mechanical analysis

Sand		Silt	Clay	Soil
Course >200 µ %	Fine 200-20µ %	20-2 µ %	< 2 µ %	Texture
7.20	14.25	30.22	48.33	clay

B. Soil chemical analysis

pH 1:2.5	EC dSm^{-1} 1:5	$CaCO_3$ %	CEC mole Kg^{-1}	OM %	Soluble cations and anions meq/100 g soil							
					Na^+	K^+	Ca^{2+}	Mg^{2+}	CO_3^-	HCO^{-3}	Cl^{-1}	SO^{-2}
7.15	1.3	2.53	33.5	1.3	1.82	0.23	2.38	1.27	0.0	0.91	1.9	1.89

Available macro-nutrients %			Available micro-nutrients (ppm)			
N	P	K	Zn	Fe	Mn	Cu
0.47	0.25	0.95	3.1	4.8	7.3	1.2

Soil physical and chemical analysis were done according to the methods described by Cottenie, *et al.* (1982) and Page, *et al.* (1982).

Every experiment included 9 treatments, three irrigation treatments in combination with three foliar fertilizer levels. The treatments were as follows **:**

I- Drought: 1- Regular irrigation. (D0). 2- Omitting of irrigation at heading stage (D1) 3- Omitting of irrigation at dough stage (D2)

II- II-Foliar fertilization: Foliar fertilizer: Foliar-X contains (10% N, 7% P_2O_5, 8% K_2O, 2500 ppm Zn ; 3000 ppm Mn ; 2500 Fe ppm; Cu traces , B traces, S traces, and Mg traces) was sprayed in the rate of (F_0) 0, (F_1)1 and (F_2)2 g/L. Control plants were sprayed with the same distilled water quantity.

The experimental design was split plot in six replicates which the drought treatments equipped the main plots and the foliar fertilization treatments were randomize distributed in the sub plots. Grains of barley (*Hardium vulgare. L*) c.v. Giza 125 were sown in the beginning of December in both seasons. Calcium super phosphate (15.5% P_2O_5) and potassium sulfate (48.5%K_2O) were broadcasted before sowing at the rate of 200 and 100 Kg, respectively. Ammonium sulfate (20.5%N) at the rate of 200 Kg/fed was applied in two equal portions. The 1st was applied after 21 days from sowing and the 2nd was added two weeks latter. The foliar fertilizer (Folia-X) was sprayed twice, 21 and 45 days after sowing. At the end of the growing seasons, data of some growth parameters i.e. stem length, fresh and dry weights of both tillers and spikes were collected. N, P, K Fe, Zn and Mn concentrations barley plants were determined according to Cottenie, *et al.* (1982).

Data collected were subjected to the proper statistical analysis using the methods described by Snedecor and Cochran (1980).

3. Results and Discussion

Growth

1- Drought

Plant height, number of tiller and spikes / plant and spike length, in the first and second seasons, did not show any significant effect by water deficit at heading or late at dough stage. Dry weight of shoots, spikes and whole plant values, in the first season, were lower when plants subjected to omitting of irrigation at heating than that subjected to drought at dough stage or control plants, however, the differences in whole plant were the only significant. The differences in these parameters in plants exposed to water deficit at dough stage and that irrigated regularly were approximately equal (Table 2 and 3). In the second season, drought by omitting of irrigation at heading or dough stages lowered the dry mass of shoots and whole plant, in spite of the depressive effect was less at heading than that at dough stage. Dry weight of spikes showed similar response but the differences not enough to reach the significant level. The highest negative effect by omitting of irrigation at heading was higher in the dry weight of the whole plant followed by that on shoots in the second season while in the first season the degree of depression was approximately similar. These data are in harmony with those obtained by: Qureshi, and Neibling (2009); Braune, *et al.* (2009) and Katerji, *et al.* (2009).

The adverse effect of water deficit in plant growth may be due to the less availability of water surrounding the plant roots which affected the root growth and efficiency of water extraction and the disturbance in water adjustment in the different plant organs (Premachandra, *et al.* 1992 and Kocheva, *et al*. 2004), mineral absorption (Ouda, *et al*. 2005) or through the effect on photosynthesis activity (Baker, 1991; Yadanov, *et al*. 2000 , Tambussi, *et al*. 2005 and Oukarroum, *et al.* 2007), protein formation, antioxidant

activity (He, *et al.* 1995 and Oukarroum, *et al*. 2007) and hormonal unbalance (Hare, *et al.* 1997 and Hoad, *et al*. 2001).

In addition, under water limited conditions, Farri and Faci (2006) mentioned that this phenomenon may be related to soil water extraction which considered the more important component to the seasonal Etc of some cereal crops.

2- Foliar fertilization

The application of Foliar-X in the first season, affected significantly the plant height and spike length. The differences in number of tillers and spikes /plant and the dry weight of shoots, spikes and whole plant were not significant (Table 2 and 3). In the second season, plant height, length of spike and dry weight of shoots, spikes and whole plant were significantly responded. Using commercial foliar compounds were raised up in the last decades in Egypt for enhancing growth and increased yield and its traits of different field crops (Deab, 1998; Sinebo, 2005 and Oukarroum, *et al.* 2007). For vegetable and fruit crops for prolonging the period of harvest, improved nutritional values (Reddy, *et al.* 2003) and improved marketing quality and in cereals for improving growth, yield and technological characters (Yassen, *et al*. 2010**).**

Table (2) Growth response of barley plants to Foliar-X spraying and drought First season

Treatment		Stem length	NO. of leaves	NO. of spikes	Length of spikes	Dry matter (g):		
Drought	Foliar-X					Spikes	shoots	Whole
Without (Regular Irrigation)	F0	92.5	5.5	6.44	12.0	8.35	9.10	17.45
	Fl	95.0	7.5	5.40	15.0	10.45	9.80	20.25
	F2	105.0	7.5	5.88	14.0	9.45	13.15	22.60
At heading stage	F0	95.0	6.5	5.13	11.5	13.50	11.20	24.70
	F1	97.5	6.5	5.25	13.5	7.65	9.75	17.40
	F2	95.0	7.0	6.72	12.5	9.40	12.55	21.95
At dough stage	F0	85.0	8.0	5.41	10.5	4.35	6.25	10.60
	F1	90.0	8.0	5.30	12.0	8.65	8.45	17.10
	F2	82.5	.0	5.50	14.0	4.75	4.75	10.50
L.S.D at 5 %		N.S	N.S	N.S	N.S	N.S	N.S	N.S

Table (3): Growth response of barley plants to Foliar-X spraying and drought Second season

Treatment		Stem length	N0. of leaves	N0 of spikes	Length of spikes	Dry matter (g):		
Drought	Foliar-X					Shoots	Spikes	Whole
Without (Regular .Irrigation)	F0	85.2	5.33	8.33	13.0	3.62	3.11	6.73
	Fl	86.3	5.33	8.33	12.2	4.04	6.47	10.51
	F2	92.7	6.33	9.33	13.3	7.87	4.45	12.32
At heading stage	F0	81.3	5.67	8.33	13.3	2.28	1.86	4.64
	Fl	87.8	6.33	8.33	13.4	3.96	4.28	8.24
	F2	90.7	5.67	8.33	13.2	4.25	3.73	7.38
At drough stage	F0	76.3	5.67	8.33	12.3	4.49	3.57	8.06
	Fl	81.4	5.67	8.67	13.6	4.10	3.64	7.74
	F2	82.6	5.67	8.67	13.9	5.27	5.05	10.32
L.S.D at 5 %		N.S	N.S	N.S	N.S	2.14	N.S	3.78

Selim, *et al.* (1992) reported that foliar spry with Metalosite (Commercial foliar fertilizer) increased most of growth and yield components criteria comparing with control. The increase in growth and yield and its components by foliar fertilization may be mainly due to the foliar application of nutrients is readily absorbed by leaves and enhancing the physiological processes (Robredo, *et al*. 2007), to face the great needs of nutrients during some growth stages especially at grain formation and filling(Oosterhuis, 1997) and not lost by evaporation, fixation (Tiemeyer, *et al*. 2007) or solved in the drainage water. El-Kholy and El-Bawab (1998) noticed that the foliar or soil application of fertilizers exerted a positive response on barley and wheat. They added that the superiority of Stimifol as a foliar fertilizer may be attributed to its greater content of N, P and K and vitamins and amino acids and also EDTA. Ahmed and Shalaby (1994) and Shalaby and Ahmid (1994) confirmed these results.

3- Drought x foliar fertilizer

The interaction effects of varietals differences and drought on yield of barley c.v. Giza 125 were illustrated in Table (2 and 3). These data indicated that in the 1st season, all growth measurements did not

significantly responded, but in the 2nd season, dry of stem, spikes, and whole plant significantly affected. In plants irrigated regularly stem, spikes and whole plant dry weight increased with Foliar-X (commercial multi-nutrients) spraying by: 70.35, 13.81 and 61.47%, however, in plants subjected to drought (omitting of irrigation) at heading stage these decline were: 86.41, 100.54 and 92.75 % . In the case of expose barley plants at dough the decrement were 17.37, 32.37 and 63.55% for stem, spikes and whole plant dry weight, compare to plants regularly irrigated, respectively. Asare-Boamah, et al. (1988) recorded that Triadimefon reduced transpiration and protected the plants from drought. It increased leaf diffusive resistance indicating partial closure of the stomates, and treated plants maintained their water potentials while those of the controls were declined. Osmotic potentials of both treated and control leaves fell, but values in the controls were significantly lower than those from the treated plants. They added that after three days after treatment with Triadimefon in both water stressed and non-stressed plants, the abscisic acid (ABA) levels in the leaves of the treated plants were more than twice the levels of the controls. It appears, that the protection conveyed by Triadimefon during water stress is mediated at least partially, via its effects on ABA levels in treated tissue.

These data could be concluded that foliar fertilizer act positively to ameliorate drought negative effects. This phenomenon was very clear when irrigation omitted at heading stage. Furthermore, the enhancement of foliar fertilizer lowered when plant subjected to drought at dough stage to be less than the control plants (Regular irrigation).

Mineral status

1) - Drought

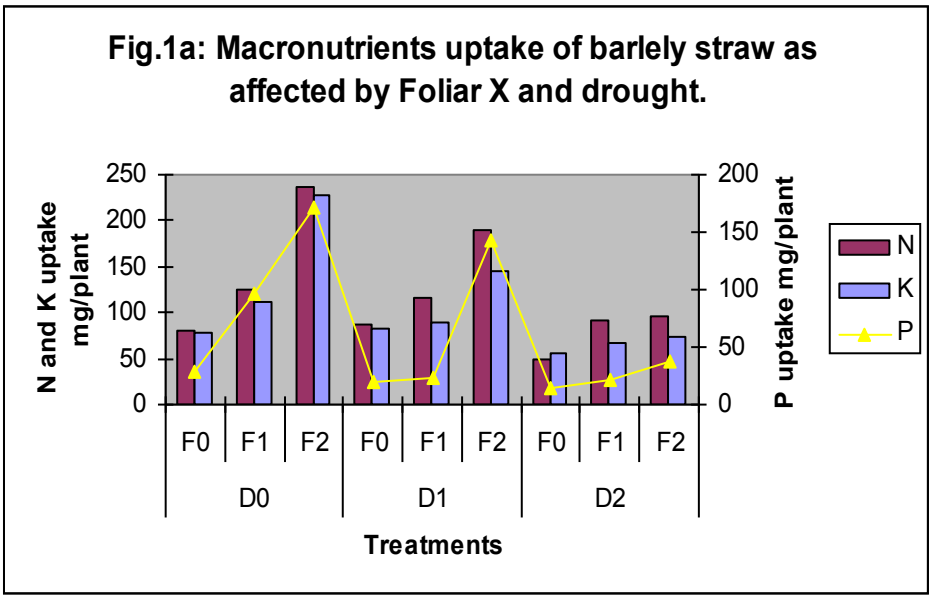
Data in Figs. 1(a and b) showed that phosphorus concentration in straw drastically decreased by subjection barley plants to drought at heading and at dough stages and at latter stage the effect was higher. However, the differences in N and K concentrations seemed to be equal with both drought treatments and the control treatment. Data presented in Fig. 1 (a and b) showed that water deficit led to a depression in K, Fe, Mn and Zn uptake and the depressions continuous as the drought treatment delayed. The differences in K uptake not great enough to reach the level of significant. Water deficit at heading gave the higher value of N uptake but at dough stage induced decrement but less than that resulted with drought at heading. Nevertheless, the water deficit treatment at heading markedly decreased the uptake of P, while, under deficit at dough stage this element pronouncedly increased compare to regular irrigation treatment. Mantagero, *et al* (2007) mentioned that adjustment of water in plant tissues necessary to the adjustment of P status in plants. Hussein, *et al*. (2006) on barley Giza 124 found that the concentration of N and K percentages increased slightly in shoots by omitting of irrigation at elongation stage but the increase in K by drought treatment at dough stage was more than that showed at elongation stage. However, the percentage of P was not affected by omitting of irrigation. Marketable decreases in Fe, Mn and Zn concentration in straw of barley plants were detected by missing of irrigation at heading and dough stages. Moreover, the effect was more by delaying the drought treatment from heading to dough stage. Hussein, *et al*. (2006) also revealed that the concentration of Fe and Cu in shoots of barley plants c.v. Giza 124 clearly decreased by omitting of irrigation and the rate of decreament raised by delaying the omitting of irrigation. Mn slightly increase by both irrigation treatments while Zn showed approximately the same response of P (Youssef, et al., 1999). In desert plants noticed that the majority of ions (K, Ca, Mg, Cl and Na) increased with decreases in soil moisture whereas, the concentration of P and K diminished with deficiency in soil moisture (Angum, *et al*., 2002). Tocker, *et al*. (1999) reported that chloride accumulation in the leaves was generally higher in drought and saline treatments where the control K concentration remained higher in both treatments. Nevertheless, El-Kholy and Hamed (2002) revealed that the drought caused reduction in sodium and potassium uptake of barley shoots. Negative relationship between drought and macronutrients concentration in grains of barley plants were observed. The depression in N,P, K, Fe Mn and Zn concentrations when plants exposed to drought at dough stage was more than that caused by drought at heading stage This means that this finding hold true for the all determinate elements. The uptake of Mn and Zn showed approximately the same response of its concentration in straw. P uptake responded reversely, however, Fe and N showed similar response. El-Zieny, *et al.* (1990) noticed that water deficit increased the concentration of both P and K in stem, leaves and roots but the total amount was decreased. Also, this treatment increased the total carbohydrates in leaves, stem and spikes of barley. El-Faham, *et al.* (1993) on wheat, revealed that K content in grains increased when irrigation skipped at jointing stage and P at jointing and milk ripe stages. They added also, that water stress had a depressive effect on Fe, Mn and Zn contents in grains. Kandil, *et al.* (2000) on maize, found that N and protein content in grains considerably depressed by widening irrigation intervals from 18 to 24 days. Similar responses were shown with macronutrients concentration in grains of barley plants as that of micronutrients by drought treatments.

2) Foliar fertilizer

As was expected that macro or micronutrient

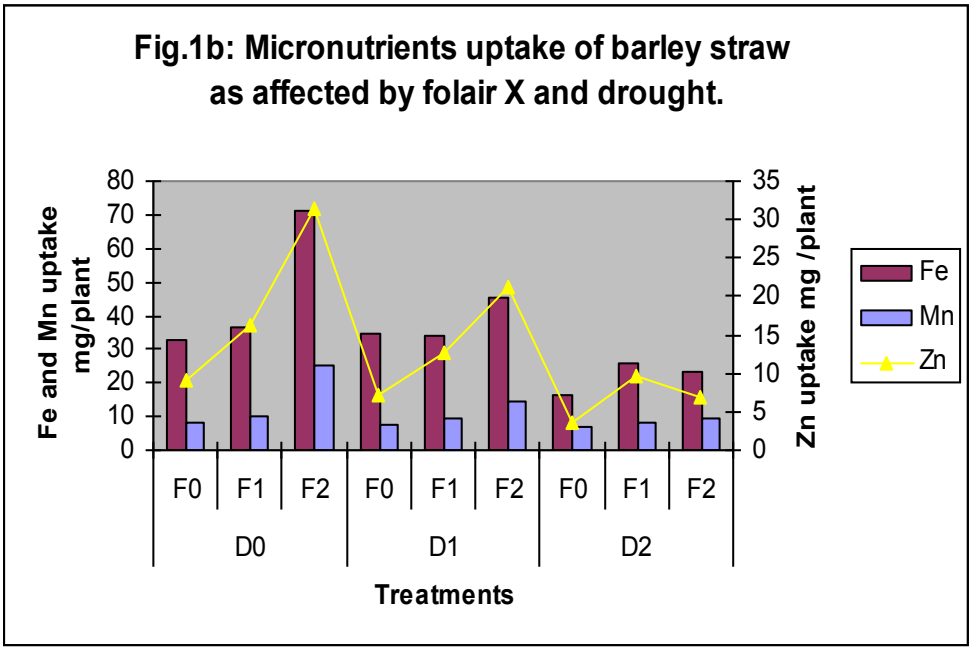
concentrations increased with the foliar fertilizer sprayed on vegetative parts of barley plants as shown in Figs (a and b).

Application of Foliar-X increased the concentration of different nutrients. Data illustrated that Foliar-X spraying increased N, K, and Mn uptake in straw and the increments parallel to the concentration increase in the spraying solution. However, both fertilizer levels gave the same effect on zinc uptake. Furthermore, Fe uptake increased by the used of fertilizers. In grains a positive relationship was detected between the increase of fertilizer concentration and the values of different elements. This was true for the uptake of N, and Zn Fig 2 (a and b). However, P uptake decreased with the first level of fertilizer and tended to increase by the 2nd level but still less than that of the control



D_0: Regular irrigation D_1: Drought by omitting of irrigation at heading stage D_2: Drought by omitting of irrigation at dough stage.

F_0: Sprayed by distilled water F_1: Spraying by 1 g/L Foliar-X F_2: Spraying by 2 g/L Foliar-X.



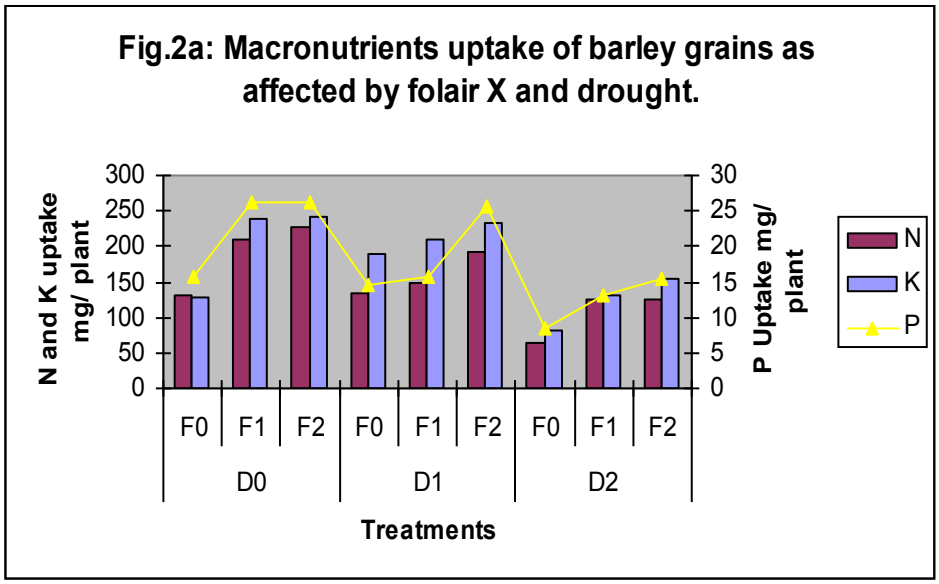
See notation In Fig. 1a

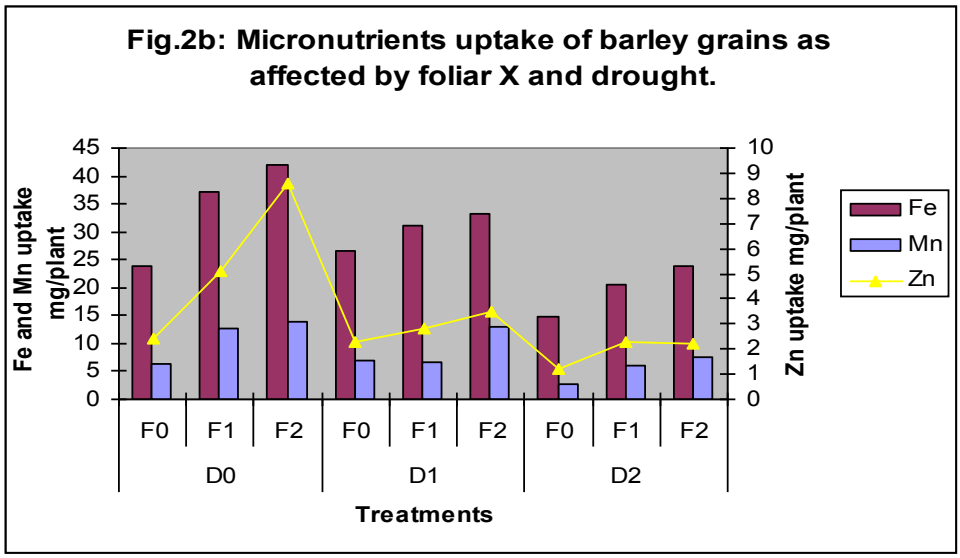
The uptake of K and Fe responded similarly to both fertilizer levels, while, the increment from the 1st fertilizer level more than that caused when spraying the 2 g/L fertilizer solution. Hu, *et al*. (2008) mentioned

that the application of foliar fertilization started on the same day as the drought stress began and lasted for 5 days. The optimal level of N, P and K nutrients was chosen. They noticed that nutrient uptake increased by foliar spray of fertilizers solution. Yassen, *et al* (2010) indicated that spraying wheat plants with micro-nutrients either as a single nutrient or as possible combinations increased grain nitrogen concentration and consequently, protein percentage as compared with that of control plants.

3)-Drought x Foliar fertilizer

Fig. 1 (a and b) and Fig 2 (a and b) showed the interactive effect of drought and foliar fertilizer on the concentration of some macro and micronutrients in shoots of barley plants. The concentration of N, P and Mn concentrations increased as the foliar fertilizer increased under two drought treatments and in plants irrigated regularly. As expected that data cleared that Foliar-x application increased the uptake of all estimated macronutrients except P which the reverse was true. The increments were more with the 2nd concentration than that from the 1st one except for Zn uptake which in the 1st level exceeded that with the 2nd level. Under the irrigation treatment in which barley plants subjected to water deficit at heading raised the uptake of different nutrients except for K and Fe with 1st level of Foliar-X. While, when plants exposed to water deficit at dough stage, the nutrients uptake were improved by the 1st as well as 2nd fertilizer dose except for Zn with the higher fertilizer concentration in the sprayed solution.





See notation In Fig. 1a

Macro as well as micronutrients concentrations in grains were affected positively by the two Foliar-x concentrations but the increases raised up to the highest level used . Generally, the increment in all elements measured, with few exceptions, by the 2nd level of fertilizer more than one fold of that caused by the 1st fertilizer level. Concerning the macronutrients, the highest increase was in Fe concentration (91.12 % compare to grains of the non-sprayed plants) by the use of the 2nd level of fertilizer. In the case of micronutrients, the highest increase was in Mn which reached 131.82 and 127.59 % compare to grains of non-sprayed plants by application the 2nd rate of fertilizer on plants subjected to drought at heading or at dough stages.

Continuous increases were shown either in macro or micronutrients uptake as a result of increasing the foliar fertilizer rates in plants irrigated regularly. However, when drought induced at heading, only N showed the same response, meanwhile, the 1st rate of fertilizer sprayed resulted in higher values of K and Mn. P, Fe and Zn uptake gave the similar trend to both fertilizer rates. Grains of plants received 1 % Foliar-X showed the great values of mineral uptake with one exception for P which this rate of fertilizer did not exert difference but increase by the second rate of this fertilizer. Ouda, *et al.* (2005) concluded that the application of Potassium-P fertilizer during plant growth provided the growing plants with potassium, which enhancing the ability of barley plants to tolerate water stress. Similarly, it provided the growing plants with phosphorus which enhanced the metabolic activities. Furthermore, Hussein, *et al*. (2006) demonstrated that application of some complete foliar fertilizers improved the uptake of nutrients in shoots of barley plants and this reflected in the water stress tolerant.

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Falls Epidemiology at King Abdulaziz University Hospital, Jeddah -Saudi Arabia-2009

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Abstract: Aim: Fall is considered usually as a sensitive quality indicator associated with patient safety, quality of care, and unfortunately risk of morbidities including head injuries and fractures. Hospital falls were found to be related mainly to the patient characteristics, plus some circumstances and activities which may facilitate these falls to occur. It affects approximately 2% to 17% of patients during their hospital stay and falls rate varies from 1.4 up to 17.9 falls per 1000 patient days depending on hospital type and patient population. Although there is some researches about falls in developing countries, however most of these lack investigating the underlying causes and SA is not an exception of this rule. **Objectives:** To determine the magnitude of falls among hospitalized patients at King Abdulaziz University Hospital (KING ABDULAZIZ UNIVERSITY HOSPITAL) in two wards; medical and surgical and to study the predisposing factors and co-morbidities. **Design and setting:** A cohort prospective study for a period of 3 months was applied targeting male and female patients in the two selected wards using an structured interview questionnaire **Main outcomes measure:** Number of cases sustaining falls and fall risk factors related to the patient health status, environment and nursing. **Results:** Total fallers were 2.4% of the total cases reviewed (1115 cases; mean age: 48.59 ± 19.931years) with 70.4% and 29.6% observed in medical and surgical wards, respectively with significant difference (P<0.05). Among the fallers, males represented 51.9% of the cases. Syncope, vertigo, degree of alertness before fall, a previous history of fall in the past three months, wet floor, lowered bed side rails, malfunctioning of emergency system were among the significant predisposing factors to falls among studied sample(P<0.05). **Conclusion:** Falls are not uncommon among hospitalized patients (2.4%) with various predisposing factors such as Syncope, vertigo, a previous history of fall in the past three months, degree of alertness before fall, wet floor, lowered bed side rails, malfunctioning of emergency system. Large scale studies should be conducted in the future to establish the various factors contributing to falls over a longer period of time.
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Keywords: Epidemiology, risk factors, falls, hospital.

1. Introduction

Falls are known to be one of the most common inpatient adverse events[1] It is considered usually as a sensitive quality indicator in the delivery of inpatient services, and also one of the monitors used in the hospital programs that aim at improving and reading excellence in hospital structure[2]. It is important to emphasize that literature reviews used different definitions for falls, leaving the interpretation to the study participants. The most common one used in most of these literatures is that it is a sudden, unexpected decent from standing, sitting or horizontal position (including slipping from a chair to the floor, patients found down on the floor, and assisted falls) .[1,3,4]

Falls affect approximately 2% to 17% of patients during their hospital stay and fall rate varies from 1.4 up to 17.9 falls per 1000 patient days depending on hospital type and patient population.[5]

Falls can result in serious physical and emotional injuries, poor quality of life, increased length of hospital stay and increased costs, so in return many hospitals succeed to routinely report inpatient falls. **Fisher *et al.,*** reported that inpatient falls may lead also to permanent disabilities and even death [3] . In **Diccini *et al.,*** study, the fall rate was 23% among hospitalized patients which resulted in a wide array of lesions; 83% of these lesions were bruises, sprains and lacerations, while fractures represented about 9% [2] . Prevention of falls in the hospital setting is therefore an important patient safety and public health issue.

Hospital falls were found to be related usually to the patients characteristics plus some circumstances and activities which may facilitate these falls to occur such as; being over 65 years old, the level of patient's awareness, use of some medications, syncope and postural hypotension, bladder or bowel incontinence, balance disturbances, motor impairment, sensory impairment, lack of confidence in the environment and history of previous occurrence of falls [2] .

Hitcho *et al.,* found that falls affected young as well as older patients. It was usually unassisted types and involved elimination related activities [1] .In 2005, another study was conducted retrospectively at the same hospital and reveled considerable variation in fall rates and percentages of fall related injury by services conducted [3] .In 2006, **Akihito Nakai** team at Tama-Nagayama Hospital in Japan demonstrated that there is a difference in the risk factors of inpatient falls among

the different clinical services such as internal medicine, surgery, pediatrics and others. The results suggested that fall prevention strategies should be linked to the patient characteristics and circumstances that cause a patient to fall [4].

Most research of falls has been conducted in elderly populations from the community and long-term-care facilities. Less is known about falls among hospital inpatients [6,7]. The majority of published inpatient fall studies are retrospective one which based mainly on data from medical records or incident reports while few are prospective. Information from risk management database studies is often incomplete and don't identify clearly potential causal factors for falls [1].

So, beside the sensitivity and the importance of the topic and due to lack of similar epidemiological studies in our community, this prospective study was conducted.

Aim of the study:

To describe the epidemiology of hospital inpatient falls in KING ABDULAZIZ UNIVERSITY HOSPITAL; including the magnitude of the problem, characteristics of patient who fall, circumstances of falls and fall-related injuries.

2. Methodology:

A prospective study was conducted at KING ABDULAZIZ UNIVERSITY HOSPITAL at Jeddah region, SA, for a period of 3 months started from Aug 1, 2009 through Oct 31, 2009. Patients included were those admitted to medical and surgical departments; both male & female wards with a total of 167 beds. Falls during physical therapy sessions or reported from psychiatry service were excluded.

Data collection started after approval of ethical research committee. The need for written informed consent from patients was waived because this study was part of hospital-based quality improvement project and posed no risk to patients. The study was performed by a detailed fall data collection questionnaire which was developed based on an extensive review of the literature and the fall protocol policy at KING ABDULAZIZ UNIVERSITY HOSPITAL to identify possible factors contributing to falls. The patients' medical records offered detailed information on the patient's medical history. A patient was considered confused or disoriented if the nurse documented the patient as not being alert to person, place, and time at the time of their fall.

The fall related variables in this reporting system include three categories of risk factors that are related to the patients characteristics, environment and nursing factors.

Risk factors related to the patient are age, gender, mental status prior to the fall (alert & oriented, or not), history of syncope, postural hypotension, dizziness and vertigo, visual disturbance, bladder & bowel incontinence, balance deficit, hearing disturbances, osteoporosis, diabetes mellitus, hypertension, musculoskeletal problems, neurological problems, history of physiotherapy and previous history of fall in the last 3 months.

Risk factors related to the environment include easy reach to the belongs and call bell, presence of watcher, dim light, use of full-length side rails, uneven, wet or slippery floors, uncomfortable bed height, malfunctioning emergency call systems and availability of transfer aids in need.

Risk factors related to nursing include education about fall, preventive procedures and patient assistance to the bathroom.

In case of fall incidence, the patient who fell was interviewed by a trained nurse to assess the circumstances of fall regarding to: hospital stay duration, location (patient room, bathroom, others), time (7:00 am – 6:59 pm, 7:00 pm - 6:59 am), relation to any surgical operation, request of nurse assistance and his/her response to call, type of assistance received after fall (assurance, clinical examination, X-ray, other investigations, assign a watcher or none).

We consider a fall to have occurred if the patient was seen falling, was found on the floor, or reported having fallen (The few patients who fell more than once were counted only once). The operational definition of fall used in this study is the one mentioned earlier in the introduction paragraph.

Training session was held to provide the nurse staff with a solid knowledge about the purpose of the study and the structural questioner designed to collect the data suggested in the methodology which will be used to interview the inpatient that fell, a family member or a health staff. The data was collected in nearly daily base as regard the new admissions, the incidence of a fall taking in regard the occurrence of fall and number of patients/day.

The data was analyzed using SPSS for widows, version 15. Descriptive analysis and appropriate analytical tests were performed in form of Chi square test for the qualitative data and student t-test for quantitative one. Tests were two-tailed and a *P* value of < 0.05 was considered significant.

3. Results:

A total of 27 inpatients fell during the study period (representing 2.4% of all patients admitted between August 2009 through October 2009 and met our inclusion criteria (total =1115).

Demographics of the inpatients who fell were displayed in table (1). Those fallers were analyzed against non fallers to determine whether the two groups differed significantly regarding the studied demographics. Fall percent differed significantly ($P<0.05$)regarding the service. Medicine had the higher

percentage of fall than surgery (70, 4% vs. 29, 6%). Fallers were slightly younger on average than non fallers with mean age equal two 41.81 ± 18.15 and 48.61 ± 19.981 years, respectively. They were also more thinner with average BMI=22.7 ± 4.9 instead of mean=27.32 ± 7.8 in the other group the difference was statistically significant (P<0.05). Men were more likely to experience fall (53%) during study period than women (47%) but the difference was statistically insignificant (P>0.05). Most of the medical conditions studied were more prevalent among the fallers vs. non fallers but the significant difference was only observed as regard history of vertigo, syncope (P<0.05) and degree of alertness before fall , history of fall in the past three months (P<0.000).

Description of Falls: The largest proportion of patients fell in the evening or at night (14/27:51.9%), in patient's room (11/27:40.7%) (Table 2). Twenty one of fallers requested nurse help; of them 15 (55.6%) reported immediate nurse response while 5 reported late response and one patient reported no response at all. One of the 8 fallers with history of operation, 5 inpatients (62.5%) had no relation to the operation. Three patients of faller group (11.1%) received only assurance, while 20 (74.1%) fallers required an intervention (Table 2).

Environmental and Additional Circumstances of fall

Table (3) shows that approximately 15% of faller reported history of wet floor during their period of stay at hospital against only 3.4% among non fallers and the difference was statistically significant (P<0.05). A significant difference was also observed as regard the history of malfunction emergency bell (14% among fallers vs. 3.4% among non fallers) and presence of full length side rails (18.5% among fallers vs. 26.4% among non fallers).

Only 18.5% among fallers and 19.1% among non fallers reported that they received an educational message from the nurse about the hospital fall policy and the same percent among fallers received nurse assistance while in the bathroom against 17.4% among the non fallers and the difference between the two groups was insignificant (P> 0.05).

Table (1): Demographics & distribution of risk factors related to patients:

Characteristics	Total N=1115	Fallers N=27 (2.4%)	non fallers N=1088 (97.6%)	*P*
Ward:				
Medicine	**560 (50.2%)**	**19 (70.4%)**	**541 (49.7%)**	**0.034**
surgery	**555 (49.8%)**	**8 (29.6%)**	**547 (50.3%)**	
Mean age± (SD)	**48.59 ±19.93**	**41.81± 18.151**	**48.61 ± 19.981**	**0.203**
Mean BMI ±(SD)	**28.37± 5.12**	**22.7± 4.9**	**27.32± 7.8**	**0.027**
Gender:				
Male	**591 (53%)**	**14 (51.9%)**	**577 (53%)**	**0.903**
Female	**524 (47%)**	**13 (48.1%)**	**511 (47%)**	
Previous history of falls in the last 3 months	**206 (18.5%)**	**14 (53.8%)**	**182 (17.6%)**	**0.000**
level of alertness prior to fall:				
Non Alertness	**35 (3.1)**	**6 (22.2)**	**29 (2.7)**	**0.000**
-Syncope	**129 (11.6%)**	**7(26.9%)**	**123 (12%)**	**0.023**
-Postural hypotension	**302 (27.1%)**	**9 (33.3%)**	**293 (29.5%)**	**0.485**
-Faintness	**190 (17%)**	**5 (18.5%)**	**185 (18.3%)**	**0.748**
-Vertigo	**299 (26.8%)**	**12 (44.4%)**	**287 (28.5%)**	**0.034**
- Visual disturbance	**433 (38.8%)**	**11 (40.7%)**	**422 (40.6%)**	**0.862**
-Balance disturbances	**387 (34.7%)**	**10 (37%)**	**377 (36.6%)**	**0.725**
- Hearing disturbances	**179 (16.1%)**	**5 (18.5%)**	**175 (17%)**	**0.828**
-Incontinence	**227 (20.4%)**	**5 (18.5%)**	**222 (21.7%)**	**0.923**
- Osteoporosis	**130 (11.7%)**	**7 (25.9%)**	**123 (13%)**	**0.121**
- D.M	**387 (34.7%)**	**11 (40.7%)**	**376 (35.5%)**	**0.472**
- HTN	**366 (32.8%)**	**10 (37%)**	**356 (34.1%)**	**0.537**
- Musculoskeletal disorders	**533 (47.8%)**	**14 (51.9%)**	**519 (48.1%)**	**0.565**
-Neurological disorders	**592 (53.1%)**	**16 (59.3%)**	**576 (53.4%)**	**0.295**

Table (2): Description of Falls:

Description of Fall	N (%)
Fall location	
Patient room	11 (40.7%)
Bathroom	10 (37.0%)
Ward	1 (3.7%)
Others	5 (18.6%)
Time of fall	
7:00 am-6:59 pm	13 (48.1%)
7:00 pm-6:59 am	14 (51.9%)
Relation to operation (8 patients)	
6-24 hours post operative	2 (25%)
More than 48 hours post operative	1 (12.5%)
No relation	5 (62.5%)
Requesting nurse help	
Yes	21 (77.8%)
No	5 (18.5%)
Unknown	1 (3.7%)
Nurse response to call	
Immediate response	15 (55.6%)
Late response	6 (18.5%)
Intervention ordered	
Reassurance only	3 (11.1%)
Clinical examination	9 (33.33%)
X-ray	1 (3.7%)
Providing a "special visiting card"	1 (3.7%)
Multiple intervention	7 (25.9%)
Others*	2 (7.5%)
None	3 (11.1%)
Unknown	1 (3.7%)

*Others: health education, ECG.

Table (3): Distribution of risk factors related to environment & nursing:

Characteristics	Total N=1115	Fallers N=27(2.4%)	Non Fallers N=1088(97.6%)	*P*
-Absence of easy reach to belongs	254 (22.8%)	9 (33.33%)	245 (22.5%)	0.320
-Unreachable call bell	141 (12.6%)	5 (18.5%)	136 (12.5%)	0.648
-Absence of watcher	589 (52.8%)	13 (48.1%)	576 (52.9%)	0.780
-Dim light	120 (10.8%)	3(11.1%)	117 (11.4%)	0.070
-Lowered bed side rails	293 (26.3%)	5(18.5%)	288 (26.4%)	**0.037**
-Uneven floor	34 (3%)	1 (3.7%)	33 (3.2%)	0.830
-Wet floor	41 (3.7%)	4 (14.8%)	37 (3.4%)	0.002
-Uncomfortable bed height	52 (4.7%)	2 (7.4%)	50 (4.6%)	0.483
Malfunction of emergency bell	221 (19.8%)	7(25.9%)	214 (19.7%)	**0.021**
Presence of mobility aids in need	487 (43.7%)	8 (29.6%)	479 (44.1%)	0.136
Educational message about fall policy	212 (19%)	5 (18.5%)	207 (19.1%)	0.927
Assistance to bathroom	195 (17.5%)	5 (18.5%)	190 (17.4%)	0.885

4. Discussion:

This study documents the experience of inpatients fall and suggests that complex patient's characteristic, environmental and nursing factors may contribute to these falls. Fall frequency in our hospital was higher in the medical ward, which was consistent with Eileen study[1]. Patients in this service may have greater illness severity, longer stay periods, greater prevalence of balance and weakness problems or/and lower patients to nurse staffing ratios that could account for this significant association.

In agreement with **Fares *et al.*,**[7], the most important significantly associated risk factor for fallers was a previous history of fall; therefore, it might be beneficial to target this group during the prevention strategies. Among other risk factors were reported,

syncope and vertigo were significantly higher between fallers. This significance couldn't be compared with other studies [1, 2,4,7,8] due to either lack of data or the design of the study that couldn't elicit such as significant. Alertness has been documented as a risk factor for fall-related injures [1,3,7,8]. However, in our study, non alerted patients were more likely to fall as with **Diccini *et al*., 2006**[2]. This could be a result of patients with an altered mental status been less aware about the risk of falling and were less protected, and the fact that cognitive status was based on subjective assessment rather than assessment by standardized measures.

In the risk factors associated with hospital environment and nursing, both problems of structure and process were evaluated. Wet floor, lowered bed side rails, and malfunctioning of emergency system were all significant and indicated failure in the process of fall prevention, which it was also found in a recent study[2]. This can be explained by insufficient knowledge about fall prevention policy, a lack of commitment on the part of the physicians and nurses, or whether the high priority given to the acute care of treatment contributed to fall risk assessment protocol being neglected.

There were several limitations to this study. First, we couldn't determine what proportion of falls is not reported, nor which falls are more likely to go unreported. Second, patient-days were not available, which precluded the calculation of falls per 1000 patient-days (the more widely accepted metric for calculating fall rates). Third, the short duration of the study can only give us a hint about the risk factors and the process of prevention implementation. Forth, interpersonal variation in judgment could have substantial influence on the result. However, such differences in opinion are unavoidable in routine work, and occur also with more standardized questionnaire. Lastly, these findings are valid for patients in the medical and surgical wards only.

5. Conclusion:

Falls are not uncommon among hospitalized patients (2.4%) with various predisposing factors such as syncope, vertigo, degree of alertness before fall , previous history of falls in the past three months, wet floor, lowered bedside rails and malfunctioning of emergency system. Therefore, prevention effort can then be targeted to patients with previously mentioned risk factors.

Recommendation:

Large scale studies should be conducted in the future to establish the various factors contributing to falls over a longer period of time. Also, there is great need to understand the nature of inpatient falls and fall-related injuries. It is also important to ensure continuous education of nursing staff.

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Finite Elements Analysis Techniques of Vessel Collision with Cable-Stayed Bridge

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Abstract: Vessel collision design for bridges crossing navigable waterways is an important consideration since it significantly affects the total cost of bridges. Economical design requires appropriate determination of impact loads imparted to bridge piers. While the collision force is dynamic in nature, current provisions for bridge design are based on static approximations of structural behavior and limited experimental data, it prescribed by current bridge design specifications (Method II probabilistic approach as outlined in " the AASHTO Guide Specifications for Vessel Collision Design of Highway Bridges"). Collision force and structural deformations predicted by the static and the dynamic analysis techniques are compared for vessel collisions of varying mass (DWT) and Velocity.This research is concerned with the effect of vessel impact forces on long span cable-stayed bridge. The Contact-Stiffness Approach was applied to determine the maximum impact force of a vessel collision as a function of the vessel velocity, and the deadweight tonnage of the vessel. Impact force is applied to the tower of bridge at the point above water level. A comparative study was conducted to investigate the effect of vessel impact force on Tatara cable-stayed bridges, with a center span of 890 m, cases of loading with different values of the vessel velocity, and deadweight tonnage of the vessel were studied for Static and dynamic Analysis finite element bridge Structure using ANSYS program. Results from such comparisons indicate that, dynamic analysis technique are preferable. For more severe collision conditions, the use of equivalent static force for design purposes is acceptable.
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Keywords:Bridge design; Vessel Collision; Dynamic Analysis; Static Analysis; Ansys program

1. Introduction

Bridges crossing coastal or inland waterways are susceptible to collapse caused by vessels impacting bridge piers. The increase in vessel size and traffic density has put these bridges at higher risk of being hit *(Saul and Svensson,1983)* Direct inclusion of veseel -ship and barge- impact loads on bridge structures was neglected in bridge design until about twenty-five years ago. The possibility of such a catastrophic collision was considered very small and therefore disregarded. Additionally, designing bridges to resist such an extreme event could be overly conservative and uneconomical. Moreover, methods for determining impact forces were not well understood or established.

Consideration for the design of bridges against vessel impact is important in many countries around the world. Land-locked countries must be concerned with vessel traffic in rivers, channels and lakes, while countries by the ocean must account for vessel traffic entering and leaving its ports. Vessels have been known to collide with other vessels, with bridge piers, and with other obstacles. Countries like the United States, Japan, and Germany have, over the years, carried out numerous research studies dealing with vessel impact on bridges and other obstacles.

Vessel collisions on bridge structures may occur when vessels veer off-course, becoming aberrant. Factors that affect vessel aberrancy include adverse weather conditions, mechanical failures, and human error. It has been noted in the literature that, on average, at least one serious vessel collision occurs per year *(Larsen, 1993)*. During severe vessel collisions, significant lateral loads may be imparted to bridge structures. Engineers must therefore account for lateral vessel collision force when designing bridge structures over navigable waterways. If such bridges cannot adequately resist impact loading, vessel collisions may result in failure and collapse of the bridge; leading to expensive repairs, extensive traffic delays, and potentially, human casualties.

It was only after a marked increase in the frequency and severity of vessel collisions with bridges that studies of the vessel collision problem have been initiated in recent years. In the period from 1960 to 1998, there have been 30 major bridge collapses worldwide due to vessel collision, with a total loss of life of 321 people. The greatest loss of life occurred in 1983 when a passenger ship collided with a railroad bridge on the Volga River, Russia; 176 were killed when the aberrant vessel attempted to transit through a side span of the massive bridge. Most of the deaths occurred when a packed movie theater on the top deck of the passenger ship was sheared off by the low vertical clearance of the bridge superstructure.

Of the bridge catastrophes mentioned above, 15 have occurred in the United States, including the 1980 collapse of the Sunshine Skyway Bridge crossing Tampa Bay, Florida, in which 396 m of the main span collapsed and 35 lives were lost as a result of the collision by an empty 35,000 *DWT* bulk carrier, is shown in Fig. 1.

Fig. 1. The 1980 collapse of the Sunshine Skyway Bridge

2. Estimating Vessel collision force

In the design and evaluation of bridge structures that cross navigable waterways, the loads imparted to a bridge during potential vessel impact events must be carefully considered. The guidance on vessel impact forces can be classified according to the governing assumptions made to estimate the maximum forces. The three basic approaches are ***(Rober and Stevent, 2002)***[7]:

- **Contact stiffness**:The American Association of State Highway Transportation Officials (AASHTO, 1998)
- **Impulse-momentum**: The Federal Emergency Management Agency (FEMA, 1995)[11], U.S. Army Corps of Engineers, 1995)[10]
- **Work-energy**: The National Association of Australian State Road Authorities(NAASRA, 1990).

The aim of each approach is to estimate the maximum vessel impact force based on the velocity and total mass of vessel. Each requires an additional parameter: impulse-momentum requires the stopping time; work-energy, the stopping distance; and contact stiffness, the effective contact stiffness. In the following section we develop a one-degree-of-freedom model of the impact between vessel and a bridge structure. We review each of the above approaches and discuss the assumptions required by each for estimating the maximum vessel impact force. We then discuss other influences that can affect the vessel impact force: added mass and the vessel orientation on impact.

2.1 One-Degree-of-Freedom Model

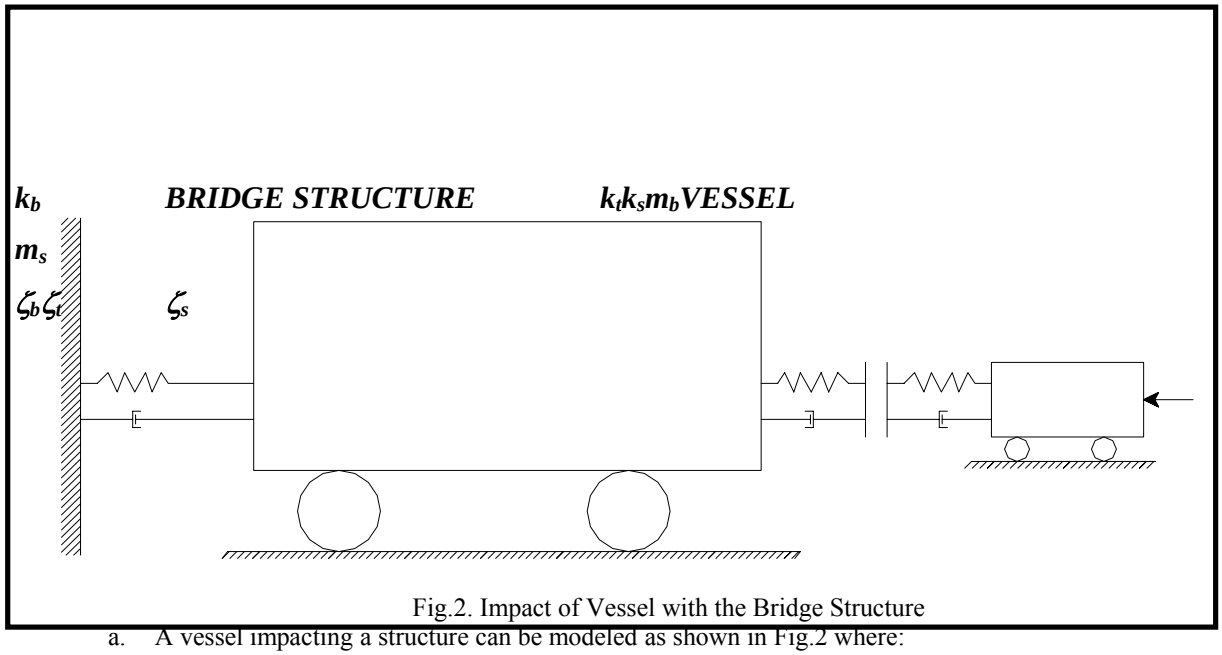

Fig.2. Impact of Vessel with the Bridge Structure

a. A vessel impacting a structure can be modeled as shown in Fig.2 where:

m_s is total mass of vessel,
K_s is stiffness (a linear constant of proportionality between penetration depth and force),
ζ is damping coefficient, and
u_s is velocity of vessel.

b. The bridge structure can be modeled as shown in Fig. 2 Where:

K_b is the stiffness of the bridge structure, (associated with the supporting foundation)
m_b is the mass of the bridge structure, and
k_t is The local deformation of the bridge structure at the impact zone.

The vessel has a total mass of m_s and an approach velocity of us. The elastic deformation of the vessel at impact is described by ks. It is reasonable to assume that *(Robert and steven, 2002)*[7]:

- The collision occurs over such a short duration that damping can be neglected.
- This system can be reduced to one degree of freedom if the bridge structure can be considered to be rigid. If $k_b >> k_s$, that is, the bridge structure support stiffness is much greater than the stiffness of the target zone or the vessel.
- The bridge structure will also act as if it is rigid if the mass of the bridge structure is so great that it doesn't move appreciably in response to the impact of the vessel.

The descriptive equation of such a one-degree-of-freedom model is

$$m_s \ddot{x} + \hat{k} x = 0 \qquad \text{Eq.1}$$

Where k is the effective contact stiffness of the collision

$$\hat{k}^{-1} = \frac{1}{k_s} + \frac{1}{k_b} \qquad \text{Eq.2}$$

The variable x is the summation of the compression of the target face and the vessel during impact and rebound (e.g., $x = x_s + x_t$), and the dot notation indicates the time derivative of x. At the moment of contact between the vessel and the bridge structure (i.e., t = 0), x = 0 and $x = u_s$, so the solution of equation (3.7) is:

$$x = u\sqrt{\frac{m_s}{\hat{k}}}\sin\left(t\sqrt{\frac{\hat{k}}{m_s}}\right) \qquad \text{Eq.3}$$

Given the linear relationship between the penetration depth and the normal force, $F=kx$, the maximum vessel impact force, $F_{i,max}$, predicted using Eq.3 is:

$$F_{i,\max} = u_s \sqrt{\hat{k} m_s} \qquad \text{Eq.4}$$

Thus, the maximum vessel impact force is a function of the impact velocity multiplied by the square root of the product of the effective contact stiffness and the total mass of vessel.

Note that the maximum vessel impact force is independent of the properties of the bridge structure if the bridge structure is considered to be rigid.

The analysis of a one-degree-of-freedom system given above is valid for vessel impact provided that both the inertia and the stiffness of the bridge structure are large enough that the bridge structure itself does not move in any appreciable amount in response to the impact. This assumption results in the maximum vessel impact forces for the relatively short impact durations we are investigating. However, a light bridge structure that has soft foundation stiffness will move in response to the impact, and the system can only be accurately described by the two-degree-of-freedom equations of motion; in this case we expect the equations based on the one-degree-of-freedom analysis to over-predict the actual vessel impact force.

2.1 Main approaches for estimating Vessel Impact Force

2.1.1 Contact-Stiffness Approach

Eq.5 has the same form as the expression adopted for calculating vessel impact forces on bridge piers *(AASHTO 1998)*, where the maximum collision force on the pier, F_v, is based on the dead-weight tonnage of the vessel, *DWT* (long tons), and the vessel velocity, *u* (ft/s). The adopted expression for the maximum impact force of a vessel collision, computed in English units and using an empirical coefficient for the stiffness, is:

$$F_{v(kips)} = 8.15u\sqrt{DWT} \qquad \text{Eq.5}$$

We refer to this approach for estimating the maximum impact force as the contact-stiffness approach because it requires only the effective contact stiffness of the collision to estimate the maximum impact force of an impactor with known mass and velocity *(Robert and steven, 2002)*[7].

2.1.2 Impulse-Momentum Approach

The impulse-momentum approach equates the impulse acting on the vessel in contact with the bridge structure with the change in momentum of the vessel. The governing equation for this approach is based on the definition of impulse, I:

$$I = \int F(t)dt = t_i \overline{F}_i = \int d(u_s m_s) \qquad \text{Eq.6}$$

where F is the force acting on the vessel and is a function of time, t, $\overline{F}_i$ is the time-averaged force, and I is equal to the total change in the momentum of the vessel over the course of the impact. Integration of E.6 requires the functional relationship between impact force and time. If we use F_i and assume that the momentum of the vessel goes to zero as a result of the impact, then Eq.6 becomes.

$$\overline{F}_i = \frac{u_s m_s}{t_i} = \frac{u_s w_s}{g t_i} \qquad \text{Eq.7}$$

Where w is the weight of the vessel, and g is the gravitational constant. The impact duration, t_i, is equal to the time between the initial contact of the vessel with the bridge structure and the maximum impact force. An independent estimate of t_i is required to estimate the impact force. The impulse-momentum approach has been adopted by FEMA (1995)[11] and the U.S. Army Corps of Engineers (1995)[10].*[For brevity we refer to these works collectively as FEMA guidance since the FEMA publication, Engineering Principles and Practices for Retrofitting Flood-prone Residential Buildings (FEMA, 1995)[11], provides the most comprehensive description of applying the design approach.]* Eq.7 is the expression used in the FEMA guidance. FEMA suggests that a value of 1 sec be used for t_i.

A limitation of Eq.7 is that it gives the average impact force, not the maximum force, an important point that is not explicitly stated in the FEMA (1995)[11] guidance. An expression for the maximum force, $F_{i,max}$, can be obtained if the function of the force with time, F(t), is assumed. A linear rise of force with time would be the simplest approach. However, based on Eq.3 we would expect that the functional dependence of force on time is sinusoidal, which results in *(Robert and steven, 2002)*[7]:

$$F_{i,\max} = \frac{\pi u_s m_s}{2 t_i} \qquad \text{Eq.8}$$

2.1.3 Work-Energy Approach

In this case the impact force is computed by equating the work done on the bridge structure with the kinetic energy of the vessel and assuming that the velocity of the

vessel goes to zero as a result of the collision *(Robert and steven, 2002)*[7]:

$$W = \int F(x)dx = \int d\left(\frac{1}{2}mu^2\right) \quad \text{Eq.9}$$

where W is the work done by the change in kinetic energy, *½mu²*. The force is a function of the distance, x, over which it acts *(F = kx)*. We define S, the stopping distance of the vessel, as the distance the vessel travels from the point of contact with the target until the vessel is fully stopped (u = 0). Then Eq.9 can be solved as follows:

$$\int_0^s kxdx = \frac{1}{2}mu_0^2 \quad \text{Eq.10}$$

Or

$$kS^2 = mu_0^2 \quad \text{Eq.11}$$

Since $F_{i,max} = kS$, equation (3.17) becomes:

$$F_{i,\max} = \frac{mu_0^2}{S} = \frac{wu_0^2}{gS} \text{ or } F_{i,\max} = \frac{2}{S}KE \quad \text{Eq.12}$$

This is the expression used by NAASRA (1990) to compute impact forces of vessel or woody debris on bridge piers.

Fenske,.1995) proposed a formulation nearly identical to Eq.12 except that a coefficient, C_f, is introduced to account for variations in the "stiffness of the bridge, relative angle of impact, fluid damping and [pier] mass:

$$F_{i,\max} = C_f \frac{mu^2}{S} \quad \text{Eq.13}$$

However, appropriate values of C_f and S were not presented in that work.

2.1.4 Equivalence of Approaches

Though the above analyses of the maximum impact force are presented as three separate approaches, the one-degree-of-freedom model can be used to demonstrate that they are ,equivalent. We can use Eq.3 to determine the values of t_i and S that coincide with $F_{i,max}$. These are the values required by Eqs.8 and 12 *(Robert and steve,2002).*

$$t_i = \frac{\pi}{2}\sqrt{\frac{m_s}{\hat{k}}} \quad \text{Eq.14}$$

And

$$S = u_s\sqrt{\frac{m_s}{\hat{k}}} \quad \text{Eq.15}$$

Substituting Eq.14 into Eq.8 or eq.15 into Eq.12 yields

$$F_{i,\max} = u_s\sqrt{\hat{k}\,m_s} \quad \text{Eq.16}$$

Which is identical to Eq. 4.

Eqs.14 and 15 show that impact duration and stopping distance are not constants that are independent of the properties of the vessel involved in the collisions. Indeed, the impact duration depends on the total mass of the vessel and the contact stiffness of the interaction, while the stopping distance depends on the approach velocity as well as the vessel mass and the contact stiffness. Treatment of ti and S as constants that are independent of debris mass and velocity has led to the disparate estimates of impact forces using these otherwise equivalent expressions.

In this study, the estimation of vessel impact force was according to the contact-stiffness approach.

2.2 Factors acting on the value of Vessel Impact Force

The analyses described above implicitly assume that the mass of the vessel is uniformly affected by the collision, and it does not account for added mass or vessel orientation. The mass of the vessel will not be uniformly affected in collisions that cause the vessel to rotate or merely redirect the trajectory of the vessel. Eccentric and oblique collisions tend to cause rotation of the vessel. Collisions perpendicular to the long axis of "long" vessel can cause the ends of the vessel to rotate as a result of flexure. We may expect the maximum impact force to be increased by added mass and decreased through oblique and eccentric collisions.

$$\frac{F_{i,\max}}{F_{i,\max}^{90}} = \frac{1}{\sqrt{1+\left(\frac{\varepsilon_0}{r_i}\right)\left(1+\mu\frac{r_0}{\varepsilon_0}\right)}} \quad \text{Eq.18}$$

2.3 AASHTO Guide Specification Design Method (II)

The AASHTO Guide Specification (1991)[1] defines the acceptance criteria for two bridge classifications: regular and critical bridges. For regular bridges, the acceptable annual frequency of collapse for the total bridge elements, AF_c, should be equal to, or less than, 0.1 in 100 years. For critical bridges, the acceptable annual frequency of collapse, AFc, should be equal to, or less than, 0.01 in 100 years.

According to the design Method II, the annual collapse frequency of the j th bridge component shall be computed by

$$AF_{c_j} = \sum_{i=1}^{n} N_i . PA_i . PG_{i,j} . PC_{i,j} \qquad \text{Eq.19}$$

Where:

AF_{cj} is annual frequency of the *ith* bridge component collapse due to vessel collision, $j = 1, \ldots, m$, and

m is the total number of bridge components susceptible to vessel collision;

N_i is annual number of the *ith* vessel category classified by type, size, and loading condition which can strike the bridge element, $i = 1, \ldots, n$, and

n is the total number of classified vessel categories;

PA_i is probability of vessel aberrancy of the *ith* vessel category;

PGi,j *is geometric probability of a collision by an aberrant vessel in the.Wang and Liu 2 ith category with the jth bridge component; and*

PCi,j is probability of the *jth* bridge component collapse due to a collision with an aberrant vessel in the *ith* category.

2.1 The Parameters Required for Estimating the Vessel Impact Force

The vessel impact force in direct head-on collision with a bridge pier can be approximated by the formula: $F_{v(kips)} = 8.15u\sqrt{DWT}$

Where: u is vessel speed (ft/sec),
DWT is deadweight tonnage of vessel (tons).

This formula requires some parameters, where:

- Vessel speed near bridge (3.08 m/sec up to maximum speed 18 m/sec. *(Bangash, 1993).*
- Loading conditions (fully-load vessel and unloaded vessel), (2000 ton up to 96386 ton).
- Vessel impact force was to be assumed to be a static load applied at the water level *(Vijay Chandra and Szecsei, 1980).*
- Draft and trim, (for dynamic analysis only and included in constant factor for static analysis).

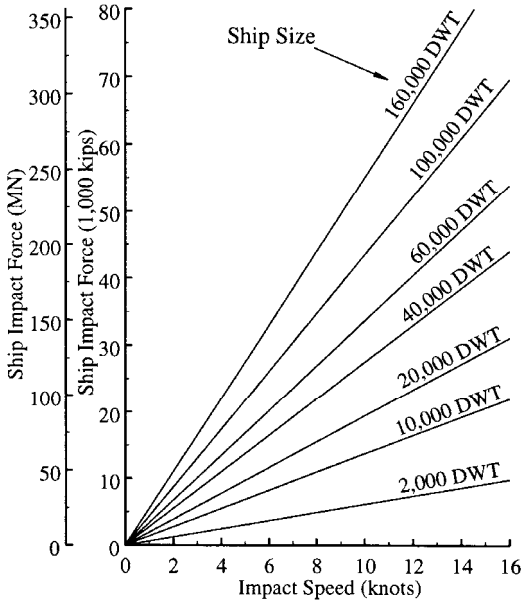

Fig.3. typical vessel impact forces

Finally, for bridges crossing over navigable waters, impact of vessels shall be taken into account in the design of piers and pier foundations, or adequate remote protection devices shall be provided. The number of piers to be designed against impact or requiring protection as well as the size of vessels to be considered in the calculation of collision forces shall be determined by the Concessionaire in consultation with the navigational authorities. Piers and pier foundations shall be designed to resist direct impact, head-on, oblique and sideways impacts by fully-laden vessels and unloaded vessels.

The bridge superstructure shall be designed to resist a local ultimate horizontal design force of 50 kN representing a collision between a vessel's antennae structure and the bridge superstructure unless a survey of vessels effectuated by the successful Proponent or the local Authorities indicate otherwise *(AASHTO, 1998).*

3. Dynamic Analysis

Many dynamic structural analysis problems require the engineer/analyst to prescribe time-varying parameters such as load, displacement or time histories of ground acceleration. However, in some cases such parameters cannot be determined ahead of time. For dynamic Bridge structure analysis under

vessel impact, the impact load is a function of the structure and soil characteristics and is therefore unknown prior to analysis *(Consolazio and Cowan, 2005).*

4. Case study

There are many cable-stayed bridges that could be chosen as a case studies, in order to investigate the previously mentioned iterative technique. However, it may be more convenient to choose a general and realistic case. For this reason, the case study presented here is as close as possible to the Tatara Cable-Stayed Bridge:

The "Tatara Bridge" is cable stayed bridge, whose 890 m center span is longer than that of the "Normandy Bridge" in France by 34 m. (Fig. 4, 5)show the general arrangement of the Tatara Bridge"،while the main tower and the main girder section are shown in (Fig. 6,7), respectively. *(Honshu-Shikoku, 1996)*

The main tower is 220 m high and designed as an inverted Y shape. It has a cross-shaped section with corners cut for higher wind stability and better landscaping. (Material properties, G= 8.10E+06 t/m2, E= 2.10E+07 t/m2, TC= 1.20E-05).

The main girder section consists of three spans, 270 m, 890 m, and 320 m, and measures 1480 m in total length. As either side span is shorter than the center span, PC girders are installed at each end of both side span sections as counterweight girders to resist negative reaction. This cable stayed bridge thus uses a steel and PC connection girder . The bridge has a total width of 30.6 m, including a road for motorized bicycles and pedestrians (hereafter called sidewalk) and a girder height of 2.7 m. It uses flat box girders attached with fairings to ensure wind stability. (prestressed concrete sections properties,G= 1.22E+06 t/m2, E= 2.80E+06 t/m2, TC= 1.00E-05 and steel sections properties, G= 8.10E+06 t/m2, E= 2.10E+07 t/m2, TC = 1.20E-05).

Cables installed in 21 levels were two-plane multi-fan cables (maximum cable length: about 460 m.(Cables of the bridge have indented surfaces in the polyethylene cable coating, similar to dimples on a golf ball, to resist vibration caused by both windy and rainy weather (rain vibration). (Material Properties of the Cables, E= 2.00E+07 t/m2, TC= 1.20E-05).

Different codes were adopted to cover all aspects. At first, the overall stability of the girder, considering different modes of instability was checked for each section of the girder by utilizing the results of an eignvalue analysis. Then, the ultimate capacity of the whole section was checked by, adopting an interaction equation of the Japanese code (JSCE). 1987 (Attia, 1997).

The ultimate strength of the flange has been evaluated based on British code (5400), 1983. Meanwhile, the ultimate strength of the web has been checked by equations of the American code (AISC), 1978. Furthermore, a large deformation analysis was performed to compare its results with the results of elastic analysis.

The complete three-dimensional finite element model for Tatara cable-stayed bridge was developed to similar to the Japanese model. Shells and Frames element were used to model the bridge elements in a fish-bone style (Fig.8).

Fig. 4. Tatara Cable-Stayed Bridge

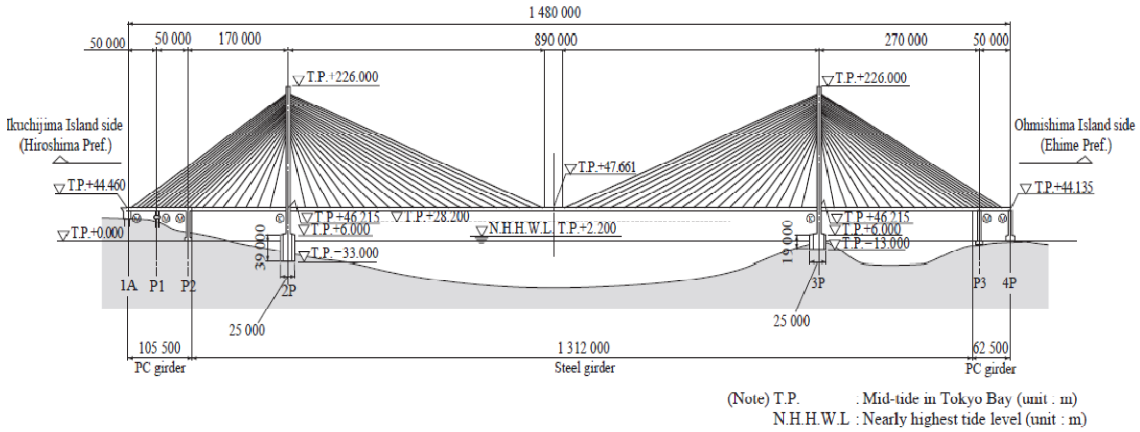

Fig. 5. General arrangement Tatara Cable-Stayed Bridge

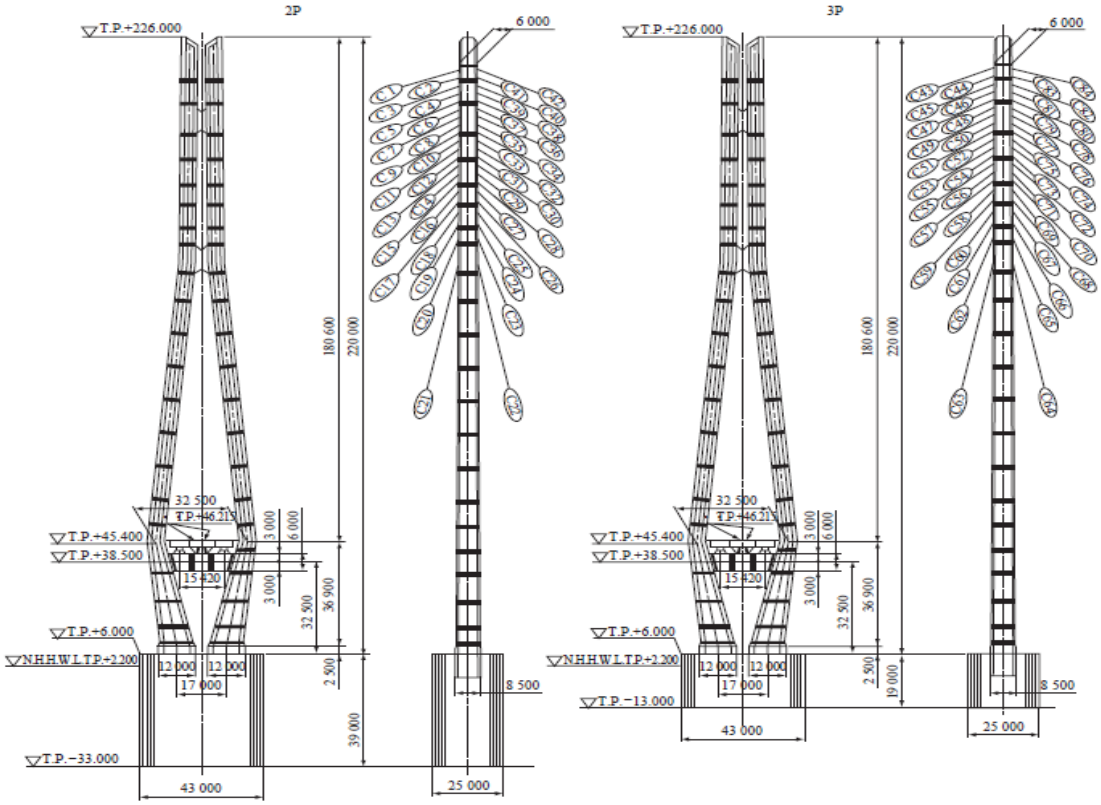

Fig.6. General arrangement (Main Tower)

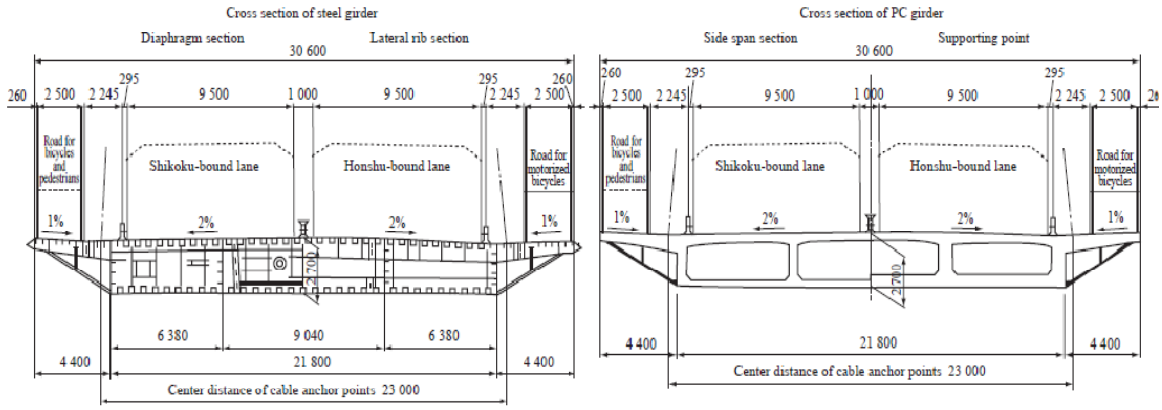

Fig.7. General arrangement (Main Girder)

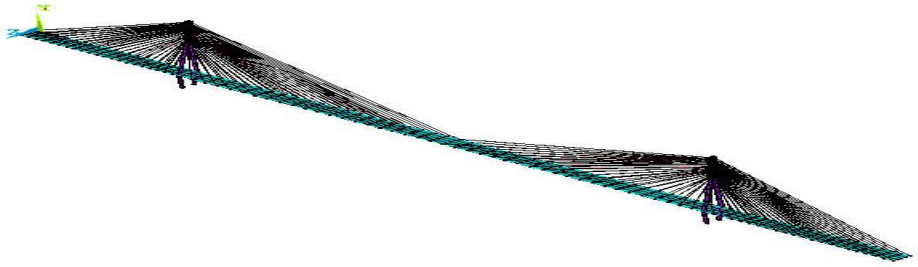

Fig.8. Three-Dimensional Finite Element Model of Tatara Bridge

5. Results and Discussion

A comparative study has been conducted between the results of the gravitational dead loads and the initial prestressing cable force for each model and the different cases of loading for the impact loads. The effects of the vessel velocity, deadweight tonnage on the displacements of the bridge girder and the tower was investigated for each studied bridge.

The gravitational dead loads and the initial prestressing cable force has the basic case of loading of the Tatara Cable-Stayed Bridge according to analyzing a three-dimensional model and the values of displacements of studied cases of loading for the impact loads are shown in the following figures

For evaluation of the effects of the vessel velocity and deadweight tonnage of the vessel on the displacement of the bridge girder and the tower with respect to the increase of the bridge span is considered in this thesis

The results of analysis showed that the effect of the factors in the impact load formula on the displacements of the main girder of the cable-stayed bridges increases with the increasing in the velocity and dead weight tonnage. In addition, the effect of the factors in the impact load formula on the longitudinal displacements of the top tower of the cable-stayed bridges increases with the increasing in the velocity and dead weight tonnage, but the effect of the factors in the impact load formula on the vertical but transversal displacements of the top tower of the cable-stayed bridges independent with the increasing in the velocity and dead weight tonnage.

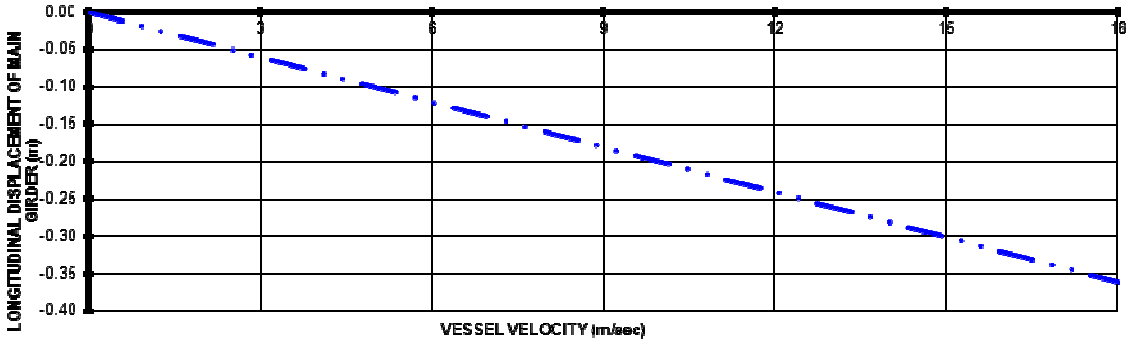

Fig.9. Effect of the Vessel Velocity on the Longitudinal Displacements at Midpoint of the Main Girder of the studied models

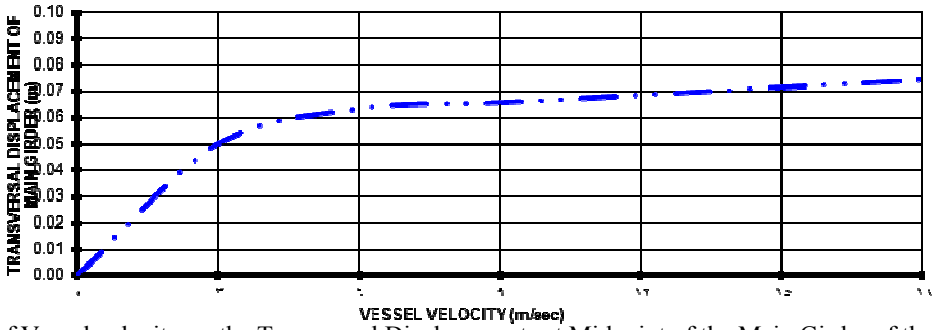

Fig.10. Effect of Vessel velocity on the Transversal Displacements at Midpoint of the Main Girder of the studied models

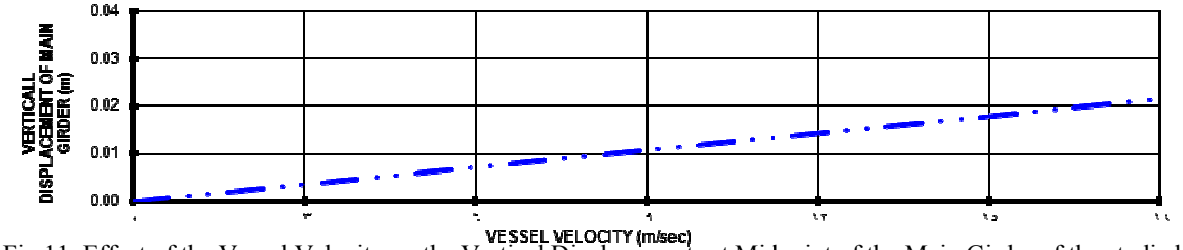

Fig.11. Effect of the Vessel Velocity on the Vertical Displacements at Midpoint of the Main Girder of the studied models

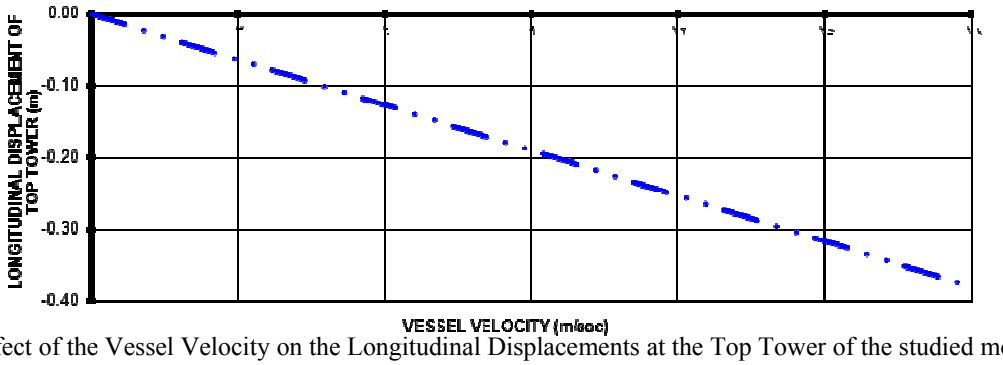

Fig.12. Effect of the Vessel Velocity on the Longitudinal Displacements at the Top Tower of the studied models

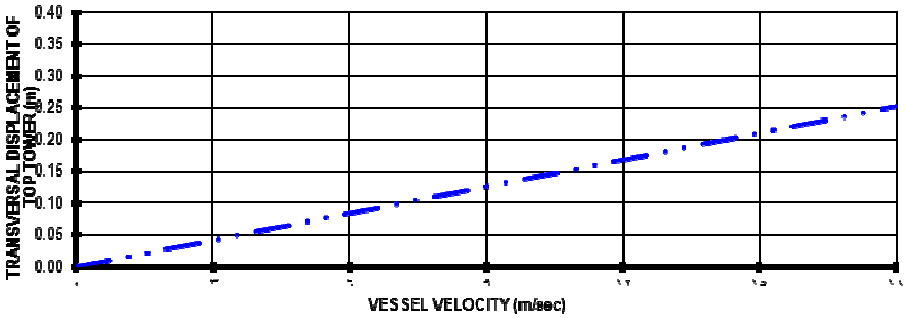

Fig.13. Effect of the Vessel Velocity on the Transversal Displacements at the Top Tower of the studied models

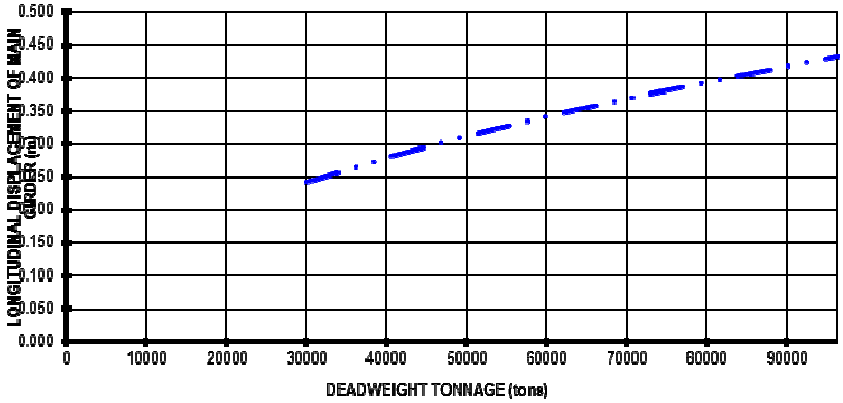

Fig.14. Effect of the Deadweight Tonnage on the Longitudinal Displacements at Midpoint of the Main Girder of the studied models

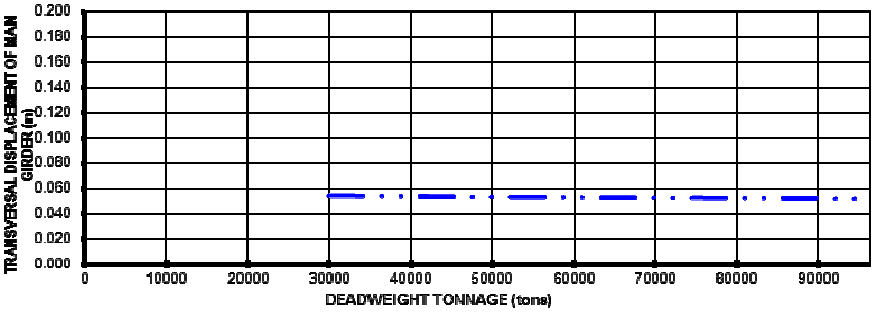

Fig.15. Effect of the Deadweight Tonnage on the Transversal Displacements at Midpoint of the Main Girder of the studied models

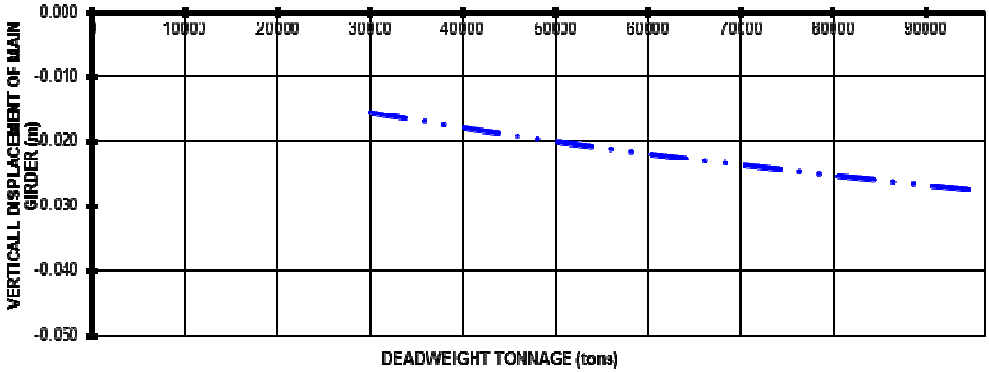

Fig.16. Effect of the Deadweight Tonnage on the Vertical Displacements at Midpoint of the Main Girder of the studied models

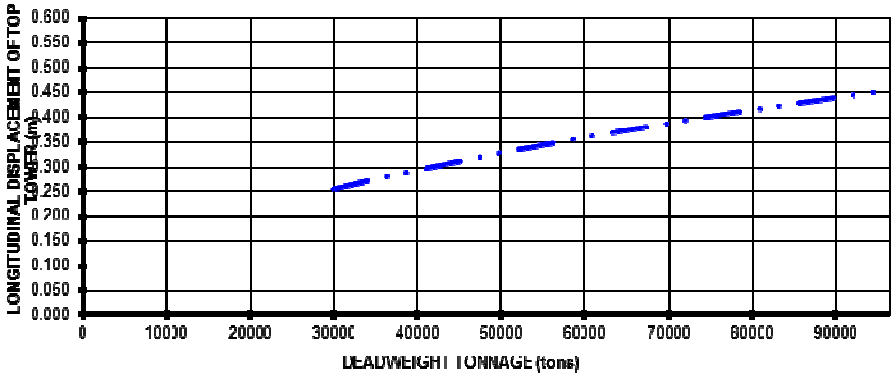

Fig.17. Effect of the Deadweight Tonnage on the Longitudinal Displacements at the Top Tower of the studied models

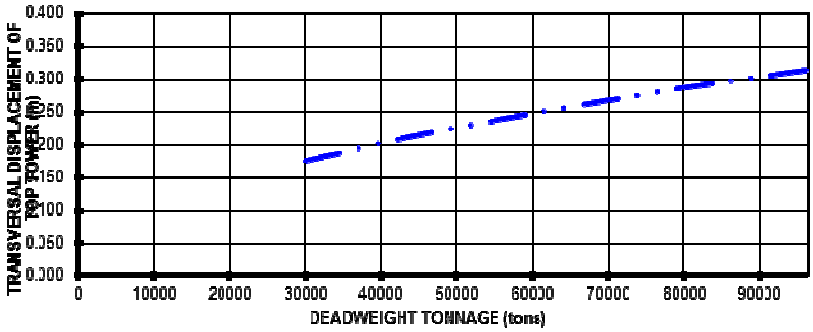

Fig.18. Effect of the Deadweight Tonnage on the Transversal Displacements at the Top Tower of the studied models

6. Conclusions

The current world trend is to adopt long-span cable-supported bridges for wide water crossing. Over the past 40 years, rapid developments have been made on long-span cable-supported bridges. Cable-stayed bridges are now entering a new era, reaching central span lengths up to 1,000 m and even longer. On the other hand, suspension bridges central span lengths reached up to 2,000 m and even longer. This research is concerned with the effect of vessel impact forces on Tatara cable-stayed bridge. A comparative study was conducted to investigate the effect of vessel impact force on the bridge deformations partical for the central span and top of the tower of the bridge structure. The Contact-Stiffness Approach - AASHTO, 1998 – Formula was applied to determine the maximum impact force of a vessel collision as a function of the vessel velocity and the deadweight tonnage of the vessel. Impact force is applied to the tower of bridge at the point above water level.

Excluding the original basic case of loading model of studied bridge, cases of loading with different values of the vessel velocity, and deadweight tonnage of the vessel were studied for bridge.The three-dimensional finite element models

of Tatara cable-stayed bridge under action of dead loads, which contribute the most to total bridge loads were analyzed. The finite element models were developed and generalized in a fish bone style mesh diagram. Shells and Frame elements were used to model the bridge elements. The geometrical nonlinear behavior due to cable sag and soil-structure in terms of were neglected. Piers were modeled as hinged supports. Towers were considered fixed to foundation. The three displacements of the midpoint of main girder and the top tower corresponding to different cases of loading were obtained and the results were compared for Tatara cable-stayed bridge. Based on the comparative study , the following conclusion can be drawn as follows:

1- Vessel velocity can influence linear on the value of the ship impact force where it has a maximum effect when it is the maximum value 18 m/sec.
2- Dead weight tonnage can influence linear on the value of the ship impact where it has a maximum effect when it is the maximum value.
3- Collision angle can influence nonlinear on the value of the ship impact force where it has a maximum effect when the ship is head on with the bridge axis (at $\alpha = 0$ o).
4- A linear relations is remarked between the vessel velocity and the longitudinal displacement of the main girder is increasing linear, and may be neglected in other direction.
5- A linear relations is remarked between the vessel velocity, the longitudinal and transversal displacements of the top tower is increasing linear, and may be neglected in the vertical displacement.
6- A linear relation is remarked between the deadweight tonnage of the vessel and the longitudinal displacement of the main girder is increasing linear, and may be neglected in other direction.
7- A linear relation is remarked between the deadweight tonnage of the vessel, the longitudinal and transversal displacements of the top tower is increasing linear, and may be neglected in the vertical displacement.

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Calculation of Hyperbolic Flow Past a Sphere using Indirect Boundary Element Method

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Abstract: In this paper, a steady and hyperbolic flow past a sphere has been calculated using indirect boundary element method (IDBEM), where as in our previous paper, we applied direct boundary element method for this purpose. The velocity distribution for the flow over the boundary of the sphere has been compared with the analytical results.

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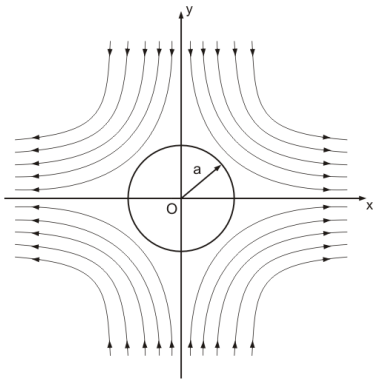
Keywords: Indirect boundary element method, hyperbolic flow, Sphere

Introduction

In recent past, the well-known computational methods such as finite difference method (FDM), finite element method (FEM), and boundary element method (BEM) etc have been applied for the flow field calculations around bodies. Out of these methods, BEM is a modern numerical technique in which only the boundary of the body under consideration is discretized in to different type of elements. BEM is well-suited to problems where domain is exterior to the boundary, as in the case of flow past bodies. The most important features of BEM are the much smaller system of equations and considerable reduction in data, which are essential to run a computer program efficiently. That is why; BEM is more accurate, efficient and economical than other domain methods. The BEM can be classified into two categories i.e. direct and indirect. (see Brebbia and Walker, 1980, Ramsey, 1942, & Milne-Thomson, 1968). Indirect method is a numerical method which depends upon the distribution of singularities, such as sources or doublets over the surface of the body and computes the unknown in the form of singularity strengths. This method is used in different areas like solid and fracture mechanics, fluid dynamics and potential theory etc. [1]. The initial work for potential flow calculations was done by Hess and Smith ([2], [3]). Indirect method is popular due to its simplicity because the discretisation only takes place on the surface of the body.

Calculation of Hyperbolic Flow Past a Sphere

Let a sphere of radius 'a' be taken as stationary and let U be the velocity of a uniform stream flowing in the positive direction of x – axis as shown in figure 1 (see [4] & [11]).



Hyperbolic flow past a sphere

Figure (1)

Now the stream function in this case is given by

$$\psi = \Omega x (y^2 + z^2) \left\{ 1 - \frac{5}{2}\left(\frac{a}{R_1}\right)^3 + \frac{3}{2}\left(\frac{a}{R_1}\right)^5 \right\}$$

Since $y^2 + z^2 = r^2$

$$\psi = \frac{U x}{\sqrt{4x^2 + y^2 + z^2}} (y^2 + z^2) \left\{ 1 - \frac{5}{2}\left(\frac{a}{R_1}\right)^3 + \frac{3}{2}\left(\frac{a}{R_1}\right)^5 \right\}$$

$$= \frac{U x r^2}{\sqrt{4x^2 + r^2}} \left\{ 1 - \frac{5}{2}\left(\frac{a}{R_1}\right)^3 + \frac{3}{2}\left(\frac{a}{R_1}\right)^5 \right\}$$

Thus $v_x = -\frac{1}{r}\frac{\partial \psi}{\partial r}$

= U

Error!

and $v_r = -\frac{1}{r}\frac{\partial \psi}{\partial x}$

= U

Error!

Equation of Indirect Boundary Element Method

The equation of indirect boundary element method for three – dimensional problems is given by ([7], [8], [9], [10]).

$$-\frac{1}{2}\Phi_i + \phi_\infty + \iint_{S-i} \Phi \frac{\partial}{\partial n}\left(\frac{1}{4\pi r}\right) dS = x_i$$

Discretization of Sphere:

The surface of the sphere is discretized into quadrilateral elements. The scheme of discretization is as shown in the figure (2).

The indirect boundary element method is applied to calculate the hyperbolic flow solution around the sphere for which the analytical solution is available

Consider the surface of the sphere in one octant to be divided into three quadrilateral elements by joining the centroid of the surface with the mid points of the curves in the coordinate planes as shown in figure (2) ([9], [10], [11]).

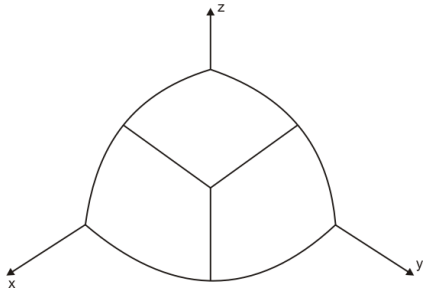


Figure (2)

Figure (3) shows the method for finding the coordinate (x_p, y_p, z_p) of any point P on the surface of the sphere.

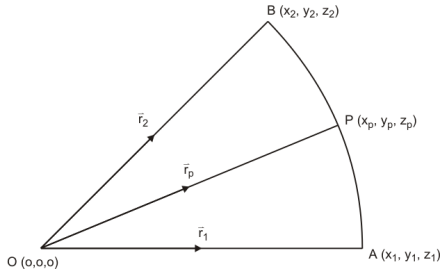


Figure (3)

Then each element is divided further into four elements by joining the centroid of that element with the mid–point of each side of the element. Thus one octant of the surface of the sphere is divided into 12 elements and the whole surface of the body is divided into 96 boundary elements. The above mentioned method is adopted in order to produce a uniform distribution of element over the surface of the body . From above figure, we have the following equation

$$|\vec{r}_p| = 1$$

$$\vec{r}_p \cdot \vec{r}_1 = \vec{r}_p \cdot \vec{r}_2$$

$$(\vec{r}_1 - \vec{r}_2) \cdot \vec{r}_p = 0$$

or in cartesian form

$$x_p^2 + y_p^2 + z_p^2 = 1$$

$$x_p (x_1 - x_2) + y_p (y_1 - y_2) + z_p (z_1 - z_2) = 0$$

$$x_p (y_1 z_2 - z_1 y_2) + y_p (x_2 z_1 - x_1 z_2) + z_p (x_1 y_2 - x_2 y_1) = 0$$

As the body possesses planes of symmetry, this fact may be used in the input to the program and only the non–redundant portion need be specified by input points. The other portions are automatically taken into account. The planes of symmetry are taken to be the coordinate planes of the reference coordinate system. The advantage of the use of symmetry is that it reduces the order of the resulting system of equations and consequently reduces the computing time in running a program. As a sphere is symmetric with respect to all three coordinate planes of the reference coordinate system, only one eighth of the body surface need be specified by the input points, while the other seven–eighth can be accounted for by symmetry.

The sphere is discretised into 96 and 384 boundary elements and the computed velocity distributions are compared with analytical solutions for the sphere using Fortran programming.

The following tables show the comparison of computed and analytical velocity distribution over the surface of sphere using 96 and 384 indirect boundary elements.

TABLE (1)

ELEMENTS	XM	YM	ZM	VELOCITY	EXACT VELOCITY
1	-.177E+00	-.934E+00	.177E+00	.14526E+01	.25097E+01
2	-.522E+00	-.798E+00	.157E+00	.12418E+01	.38214E+01
3	-.798E+00	-.522E+00	.157E+00	.82398E+00	.34106E+01
4	-.934E+00	-.177E+00	.177E+00	.34768E+00	.32259E+01
5	-.934E+00	.177E+00	.177E+00	.34768E+00	.32259E+01
6	-.798E+00	.522E+00	.157E+00	.82398E+00	.34106E+01
7	-.522E+00	.798E+00	.157E+00	.12418E+01	.38214E+01
8	-.177E+00	.934E+00	.177E+00	.14526E+01	.25097E+01
9	.177E+00	.934E+00	.177E+00	.14526E+01	.25097E+01
10	.522E+00	.798E+00	.157E+00	.12418E+01	.38214E+01
11	.798E+00	.522E+00	.157E+00	.82398E+00	.34106E+01
12	.934E+00	.177E+00	.177E+00	.34768E+00	.32259E+01
13	.934E+00	-.177E+00	.177E+00	.34768E+00	.32259E+01
14	.798E+00	-.522E+00	.157E+00	.82398E+00	.34106E+01
15	.522E+00	-.798E+00	.157E+00	.12418E+01	.38214E+01
16	.177E+00	-.934E+00	.177E+00	.14526E+01	.25097E+01
17	-.157E+00	-.798E+00	.522E+00	.14495E+01	.23718E+01
18	-.470E+00	-.703E+00	.470E+00	.13038E+01	.37699E+01
19	-.703E+00	-.470E+00	.470E+00	.96588E+00	.35883E+01
20	-.798E+00	-.157E+00	.522E+00	.82398E+00	.34106E+01
21	-.798E+00	.157E+00	.522E+00	.82398E+00	.34106E+01
22	-.703E+00	.470E+00	.470E+00	.96588E+00	.35883E+01
23	-.470E+00	.703E+00	.470E+00	.13038E+01	.37699E+01
24	-.157E+00	.798E+00	.522E+00	.14495E+01	.23718E+01
25	.157E+00	.798E+00	.522E+00	.14495E+01	.23718E+01
26	.470E+00	.703E+00	.470E+00	.13038E+01	.37699E+01
27	.703E+00	.470E+00	.470E+00	.96588E+00	.35883E+01
28	.798E+00	.157E+00	.522E+00	.82398E+00	.34106E+01
29	.798E+00	-.157E+00	.522E+00	.82398E+00	.34106E+01
30	.703E+00	-.470E+00	.470E+00	.96588E+00	.35883E+01
31	.470E+00	-.703E+00	.470E+00	.13038E+01	.37699E+01
32	.157E+00	-.798E+00	.522E+00	.14495E+01	.23718E+01
33	-.157E+00	-.522E+00	.798E+00	.14495E+01	.23718E+01
34	-.470E+00	-.470E+00	.703E+00	.13038E+01	.37699E+01
35	-.522E+00	-.157E+00	.798E+00	.12418E+01	.38214E+01
36	-.522E+00	.157E+00	.798E+00	.12418E+01	.38214E+01
37	-.470E+00	.470E+00	.703E+00	.13038E+01	.37699E+01
38	-.157E+00	.522E+00	.798E+00	.14495E+01	.23718E+01
39	.157E+00	.522E+00	.798E+00	.14495E+01	.23718E+01
40	.470E+00	.470E+00	.703E+00	.13038E+01	.37699E+01
41	.522E+00	.157E+00	.798E+00	.12418E+01	.38214E+01
42	.522E+00	-.157E+00	.798E+00	.12418E+01	.38214E+01
43	.470E+00	-.470E+00	.703E+00	.13038E+01	.37699E+01
44	.157E+00	-.522E+00	.798E+00	.14495E+01	.23718E+01
45	-.177E+00	-.177E+00	.934E+00	.14526E+01	.25097E+01
46	-.177E+00	.177E+00	.934E+00	.14526E+01	.25097E+01
47	.177E+00	.177E+00	.934E+00	.14526E+01	.25097E+01
48	.177E+00	-.177E+00	.934E+00	.14526E+01	.25097E+01

TABLE (2)

ELEMENTS	XM	YM	ZM	VELOCITY	EXACT VELOCITY
1	-.906E-01	-.983E+00	.906E-01	.14893E+01	.15503E+01
8	-.983E+00	-.906E-01	.906E-01	.17767E+00	.25889E+01
16	-.906E-01	.983E+00	.906E-01	.14893E+01	.15503E+01
24	.983E+00	.906E-01	.906E-01	.17767E+00	.25889E+01
32	.906E-01	-.983E+00	.906E-01	.14893E+01	.15503E+01
40	-.949E+00	-.830E-01	.276E+00	.43321E+00	.26147E+01
48	-.830E-01	.949E+00	.276E+00	.14881E+01	.15140E+01
56	.949E+00	.830E-01	.276E+00	.43322E+00	.26147E+01
64	.830E-01	-.949E+00	.276E+00	.14881E+01	.15140E+01
72	-.875E+00	-.813E-01	.459E+00	.70050E+00	.27134E+01
80	-.813E-01	.875E+00	.459E+00	.14883E+01	.15045E+01
88	.875E+00	.813E-01	.459E+00	.70051E+00	.27134E+01
96	.813E-01	-.875E+00	.459E+00	.14883E+01	.15045E+01
104	-.766E+00	-.784E-01	.625E+00	.94672E+00	.29172E+01
112	-.784E-01	.766E+00	.625E+00	.14886E+01	.14886E+01
120	.766E+00	.784E-01	.625E+00	.94672E+00	.29172E+01
128	.784E-01	-.766E+00	.625E+00	.14886E+01	.14886E+01
136	-.625E+00	.784E-01	.766E+00	.11607E+01	.31426E+01
144	.233E+00	.605E+00	.750E+00	.14499E+01	.23707E+01
152	.570E+00	-.382E+00	.716E+00	.12228E+01	.31934E+01
160	-.433E+00	-.242E+00	.859E+00	.13439E+01	.31063E+01
168	.242E+00	.433E+00	.859E+00	.14479E+01	.24167E+01
176	.813E-01	-.459E+00	.875E+00	.14883E+01	.15045E+01
184	.254E+00	.254E+00	.924E+00	.14465E+01	.24781E+01
192	.906E-01	-.906E-01	.983E+00	.14893E+01	.15503E+01

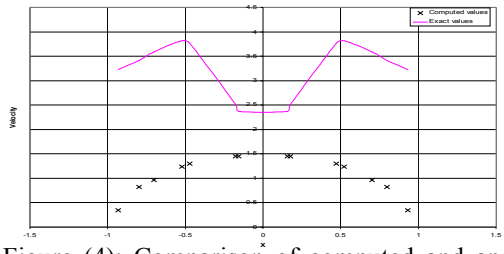


Figure (4): Comparison of computed and analytical velocity distributions over the surface of the sphere using 96 indirect boundary elements.

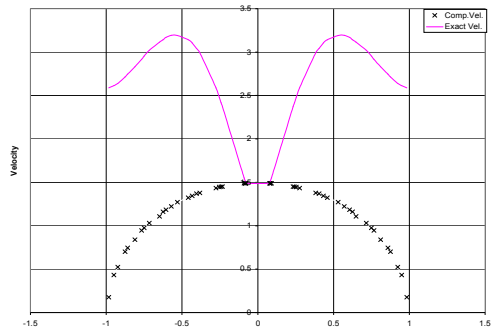


Figure (5): Comparison of computed and analytical velocity distributions over the surface of the sphere using 384 indirect boundary elements.

Conclusion

Indirect boundary element method has been applied to calculate the hyperbolic flow past a sphere. The improvement in results gained by taking 384 can be seen from the table (2) and figure (5) and such improvement increases with increase in number of boundary elements. Moreover, the computed results are in good agreement with exact results at the top of a body under consideration where viscous effects are minor.

Acknowledgement

We are thankful to the University of Engineering & Technology, Lahore – Pakistan for financial support.

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6/11/2012

Fast Writing of Surface Relief Gratings Based on Azo Dye-Poly(Methyl Methacrylate) Mixtures

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Abstract: We report the observation of surface relief gratings (SRGs) based on azo dye-poly(methyl methacylate) by fast writing of Nd: YAG 532 nm laser (s-polarized) of holographic grating method.The dependence of intensity of writing beams on the diffraction efficiency is studied. The first order diffraction of SRGs depends on the polarization of probe beam (s-polarized and p-polarized) were measured. The depths of SRGs were measured by atomic force microscopy (AFM), and the depths of SRGs can be obtained a relative high value for the high intensity of writing beams.[Ying-Chuan Wang. **Fast Writing of Surface Relief Gratings Based on Azo Dye-Poly(Methyl Methacrylate) Mixtures**. *Life Sci J*. 2012;9(2): 1196-1198] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: surface relief gratings, azo dye-poly(methyl methacylate), holographic grating method, the diffraction efficiency, atomic force microscopy

1. Introduction

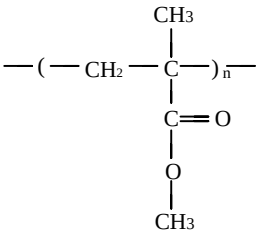
Thin films of polymers containing azo dye have been shown to offer interesting prospects for a variety of applications, such as optical data storage, optical switching devices, diffractive optical elements integrated optical devices, polarization splitters and electro-optical devices [1-6]. Many of these applications are due to photoisomerization and photoinduced anisotropy of the azo dye. The surface relief gractings can be inscribed by irradiation of interference fringes with two coherent laser beams [7, 8]. The irradiation of films with interference fringes can induce not only the alignment of the azo dye chromophores throughout the volume of polymer but also the controlled of surface modulations. SRGs, which are formed by the mass transport induced by the excitation and trans to cis isomerization of azo dye, and thermal diffusion enables rotation of dye molecules within the matrix.Thus, the reorientation of azo dye induces the large-scale molecular motion and the free volume requirements, and results in the expansion of irradiated azo dye doped polymer film [9-11].

In this study, the surface relief grating based on azo dye doped polymer film with a single pulse writing is reported. The first order diffraction efficiency dependes on the intensity of writing beams is studied. The diffraction efficiency depends on the polarizations of probe beam is also studied. The depths of inscribed surface relief gratings (SRGs) depends on writing energy is discussed.

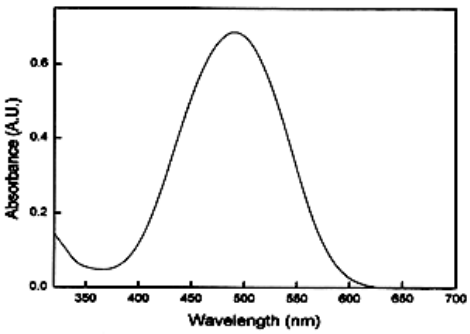
2. Experimental

Sample Fabrication

The azo dye and polymer are Disperse Red 1 (DR1) and poly(methyl-methacrylate) (PMMA) in the experiment, respectively. The weight concentration of DR1 and PMMA are 7wt% and 93wt%, respectively. The chemical structure of PMMA and the absorption spectrum of DR1 are shown in Figure 1(a) and 1(b). The absorption peak is around 500 nm. First, we dissolved DR1 and PMMA in toluene, and the azo dye in polymer solutions were filtered by filter with 0.2 μm. Samples were spin-coated films of the azo-dye (DR1) in poly(methyl-methacrylate).The films were dried in a vacuum and the temperature was about 80℃ under the glass temperature (Tg) of PMMA for more than 24 h . The thickness of the films were 3 μm.



(a).



(b)

Figure 1 (a) the chemical stucture of PMMA (b) the absorption spectrum of DR1

Setup

Figure 2 shows the experimental setup for the fast recording of surface relief grating in DR1 doped poly(methyl-methacrylate) (PMMA) thin film. A Q-switched Nd:YAG laser was employed for the single shot excitation beam at 532 nm. Two s-polarized writing beams were crossed in the sample. The He-Ne laser with s- or p- polarization was used as a probe beam and incident normally into the sample. The SRGs was inscribed by a single shot of a pair of s-polarized wrinting beams. The diffraction signals were probed by a He–Ne laser at 632.8 nm and detected by a photodiode and recorded with a oscilloscope.

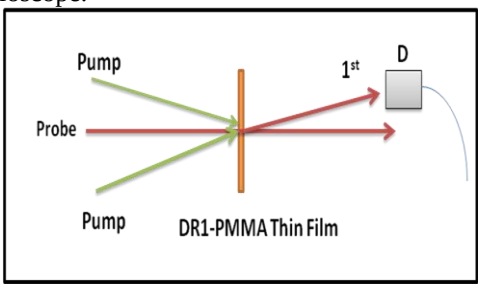


Figure 2 Experimental setup. Pump: Nd:YAG laser , Probe: He-Ne laser, D: Photodetector

3. Results and Discussions

Figure 3 plots the dependence of intensities of the pump beams on the diffricaton efficiency. The intensities of single pulse are 0.5, 1.0, 2.0 2.5 and 3.0 mJ/cm^2, respectively. The magnitude of diffraction efficiency and the relaxation mechanism depend on the intensity of the pump beams. Initially, the singal rises for all curves of single shot of pump laser. Subsequently, the signal relaxes down to almost zero and nonzero for 0.5, 1.0 and 2.0 mJ/cm^2, respectively. The signals maintain a constant value without relaxation for the pump energy is larger than 2.5 mJ/cm^2. The relaxation of diffraction efficiency is due to the thermal effect of cis to trans isomerization of azo dye.

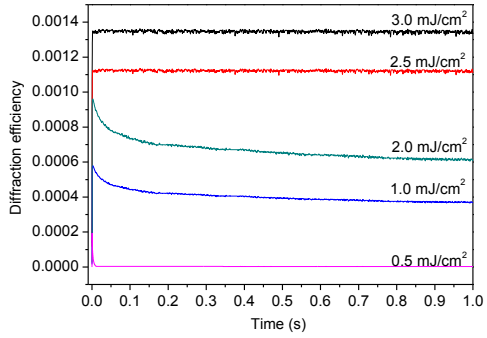


Figure 3 The dependence of intensities of pump beam on the diffraction efficiency

Figure 4 plots the first order diffraction of SRGs depends on the polarization of probe beam. The results show that the diffraction efficiency is independent of the ploarization of the probe beam. As SRGs is inscribed by a single shot of wrinting beams, the photoisomerization process of azo dye is completed. The independence of the diffraction efficiency on the polarization of probe beam implied that the diffraction efficiency was mainly attributed to the SRGs on the thin film. The diffraction efficiency is increasing with the increasing of pump energy.

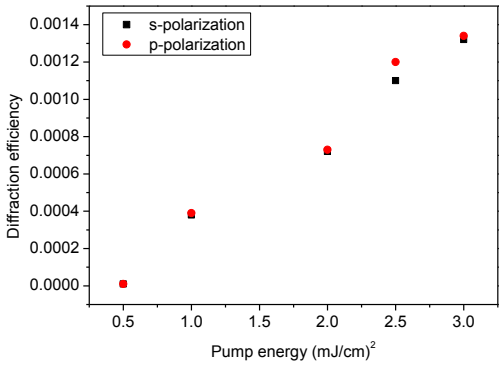
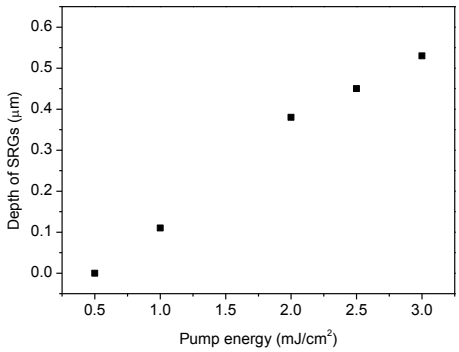


Figure 4 The dependence of the polarization of probe beam on the diffraction efficiency

Figure 5 plots the surface modulation of SRGs. The depths of SRGs are increasing with the increasing energy. The depth of SRGs can not be obtained in low energy of pump beam (0.5 mJ/cm^2). As the energy larger than 1.0 mJ/cm^2, the depth of SRGs can be measured, and the maximun value is 0.53μm in this experiment. The results also reflect the diffraction efficiency as shown in Figure 4.



4. Conclusion

In this study, we report the observation of surface relief gratings (SRGs) based on azo dye-poly(methyl methacylate) by fast writing of Nd:

YAG 532 nm laser (s-polarized) of holographic grating method. The diffraction efficiency is increasing with the increasing of pump energy. The diffraction efficiency is independent of the polarization of probe beam. The depth of SRGs is 0.53μm with pump energy of 3.0 mJ/cm^2 in this experiment.

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Evaluation of Carotid Intima- Media Thickness, Left Ventricular Mass and Left Atrium Diameter in Chronic Liver Diseases

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Abstract: Background: Cardiovascular disease is a common co-morbid disease process in individuals with chronic liver disease. Chronic liver disease is associated with abnormalities in cardiac geometry and function. The relationship between chronic liver disease and coronary atherosclerotic burden remains largely unknown. **Aim**: We aimed to evaluate the risk of atherosclerosis by measuring carotid intima-media thickness (CIMT) and to assess cardiac abnormalities in patients with chronic liver diseases (post hepatitis virus C infection liver cirrhosis and non alcoholic fatty liver disease NAFLD). **Methods:** Eighty patients with post hepatitis C liver cirrhosis (group 1), 20 patients with NAFLD (group 2) and 20 age and sex matched normal volunteers (controls) underwent echo-Doppler study for evaluation of left ventricular (LV) hypertrophy and mass, left atrium and left ventricular dimensions and EF%. Ultrasonographic measurement of CIMT, abdominal ultrasound and laboratory evaluation were done to all subjects. **Results:** There was a statistically significant increase in interventricular septum thickness (IVST), posterior wall thickness (PWT) and left ventricular mass (LVM) in both groups compared to the controls (P<0.01). IVST (1.01±0.17 in group1, 1.02±0.16 in group2 and 0.9±0.12 cm in the control group). PWT (1.03±0.12 in group 1, 1.03±0.15 in group 2 and 0.91±0.12 cm in the control group). LVM (186.89±52.18 in group 1, 195.57±65.46 in group 2 and 149.6±37.65 gm in the control group).There was also significant increase in left atrium diameter in group 1 compared to the controls (P <0.05) and significant increase in left ventricular end systolic diameter (ESD) in group 2 compared to the controls (P <0.05). Left atrium diameter (38.14±5.09 in group 1, 38.05±4.68 in group 2 and 35.8±3.79 mm in the control group) and ESD (3.10±0.58 in group 1, 3.36±0.75 in group 2, 2.89±0.43 cm in control group). CIMT was significantly increased in both groups compared to the control group (P <0.01) and in group 2 compared to group 1 (P <0.01). CIMT (1.03±0.11 in group 1, 1.17±0.1 in group 2 and 0.72±0.14 mm in the control group). **Conclusion:** Liver cirrhosis and NAFLD are associated with left ventricular hypertrophy (LVH), increased LVM and increased CIMT independently of classical cardiovascular risk factors. Also, patients with liver cirrhosis have increased left atrium size. Patients with NAFLD have increased left ventricular ESD diameter which may be a predictor of subclinical left ventricular dysfunction. Patients with liver cirrhosis or NAFLD having increased CIMT, which is indicator of atherosclerosis, should be evaluated for cardiovascular disease (CVD) risk and could be candidates not only for aggressive treatment of the liver disease, but also for aggressive treatment of underlying CVD risk factors; this would help to modify and potentially decrease the global CVD risk of these patients.

[Ashraf Abdel khalik, Amna Metwaly; Fatma Mohammad Nasr; Mervat Al damarawy and Ayman yousse. **Evaluation of Carotid Intima- Media Thickness, Left Ventricular Mass and Left Atrium Diameter in Chronic Liver Diseases.** *Life Sci J* 2012;9(2):1199-1206] (ISSN:1097-8135). http://www.lifesciencesite.com. 179

Key words: liver cirrhosis, nonalcoholic fatty liver disease, left ventricular hypertrophy, left ventricular mass, left atrium, left ventricular end systolic diameter, carotid intima-media thickness.

1. Introduction

Liver cirrhosis is one of the mysteries especially in developing countries where it affects a sizable portion of people mostly due to high prevalence of hepatitis C virus (HCV) infection. It is associated with a wide range of cardiovascular abnormalities including hyperdynamic circulation, cirrhotic cardiomyopathy, and pulmonary vascular abnormalities. The pathogenic mechanisms of these cardiovascular changes are multifactorial and include neurohumoral and vascular dysregulations [(1)]. *Matsumori et al.,* detected hepatitis C virus in the hearts of patients with myocarditis or dilated cardiomyopathy [(2)].

There is considerable evidence that HCV infection and the atherosclerotic process are related, but the role of infection in the pathogenesis of atherosclerosis remains controversial [(3)]. Some researches demonstrated that HCV seropositivity lead to carotid artery plaque and carotid intima-media thickening independent of other risk factors and these studies found that HCV seropositivity is an independent predictor for coronary artery disease (CAD) [(4,5)].

Carey et al., concluded that cirrhotic individuals particularly those with nonalcoholic liver disease (HCV and cholestatic liver disease), have a higher rate of CAD than that had been expected based upon the earlier autopsy and clinical observation made almost exclusively in the alcoholic population [(6)]. However, *Marchesini et al.,* reported that the incidence of atherosclerosis and subsequent coronary and cerebro-vascular diseases, which are major causes of morbidity

and mortality, is low in cirrhotics even in comparison with general population [7]. *Kalaitzakis et al.,* concluded that Liver cirrhosis, per se, does not seem to confer a protective effect against CAD [8].

Ultrasonographic measurement of carotid intima-media thickness (CIMT) is a noninvasive method for demonstrating subclinical atherosclerosis. Increased CIMT is associated with the presence and severity of coronary atherosclerosis and cardiovascular disease [9,10].

In most studies of patients with cirrhosis, the heart mass has been found to be within the normal range [11]. However, some have reported an increased left ventricular mass (LVM) [12]. Also, *Bernal et al.* reported increased LV mass index in the cirrhotic patients with higher values of left atrium diameter, interventricular septum thickness, and posterior wall thickness compared to controls [13]. In an experimental study of portal hypertensive rats, left eccentric hypertrophy was found to correlate directly with the degree of hyperdynamic circulation [14]. *De Marco et al.*, found that Patients with severe liver disease have LVM values exceeding the compensatory needs to sustain hemodynamic overload [15].

In echocardiographic studies, *Kelbæk et al.,* [16] and *Rector et al.,* [17] found the size of the left ventricle to be normal and that of the left atrium enlarged in liver cirrhosis [18].

These structural and functional changes in liver cirrhosis may be due to a hyperdynamic circulation status and a decreased diastolic compliance of the left ventricle, which may result in elevated left ventricular end diastolic diameter (LVEDD) and ventricular pressure, compensatory left ventricular enlargement and increased pressure in the left atrium [19].

Left atrium enlargement which reflects one aspect of increased cardiac output in patients with liver cirrhosis is an indirect marker of intra pulmonary right to left shunt [20].

Nonalcoholic fatty liver disease (NAFLD), a highly prevalent condition [21] is a feature of metabolic syndrome and characterized by excessive accumulation of fat in the liver cells [22, 23]. Patients with NAFLD have a higher mortality rate than the general population [24].

NAFLD is strongly associated with cardiovascular risk factors, such as obesity, dyslipidemia, type 2 diabetes mellitus, and insulin resistance [25]. From previous published data, it is obvious that coronary heart disease mortality rates in patients with NAFLD are close to those associated with cirrhosis [26].

In recent years, case-control studies have shown a relationship between NAFLD and the presence of early manifestations of atherosclerosis indicated by CIMT measurement [27]. Other studies have shown only a relationship between NAFLD and advanced atherosclerosis, such as carotid plaque [28, 29]. *Afshin et al.,* concluded that NAFLD may be an independent risk factor for developing atherosclerosis. Therefore, NAFLD without other cardiovascular risk factors can be associated with increased CIMT and increased risk of cardiovascular events [30].

Goland et al., found increased thickness of the interventricular septum, posterior wall and larger LV mass and LV mass/ height in NAFLD group compared to normal controls (31). Fallo et al., found that Patients with NAFLD had similar prevalence of left ventricular hypertrophy compared to patients without NAFLD (32).

In the present study, we aimed to evaluate the risk of atherosclerosis in two groups of patients with chronic liver diseases (post hepatitis virus C infection liver cirrhosis and NAFLD) and comparing them to normal controls. We used the ultrasonographically measured carotid intima-media thickness as a noninvasive method for demonstrating subclinical atherosclerosis.

We also evaluated left ventricular hypertrophy, left ventricular mass, left atrium and ventricular dimensions in the same groups of patients and comparing them to normal controls, to assess cardiac abnormalities in these groups.

2. Subjects and Methods:

The present study was conducted on 120 subjects from inpatients and outpatients services of Theodor Bilharz Research Institute Hospital, selected to represent 3 groups:

Group (1) included 80 patients with liver cirrhosis (all of them post hepatitis C virus infection)

Group (2) included 20 patients with NAFLD, matched for age and sex and

Group (3) included 20 apparently healthy volunteers as control group matched for age and sex and with normal liver ultrasonography , normal liver function tests and negative hepatitis markers.

Subjects with heart disease, diabetes mellitus, hypertension (blood pressure >130/85 mmHg), hyperlipidemia, acute or chronic kidney disease, any malignancy, alcohol consumption, pregnancy, liver masses, anemia with hemoglobin less 10 gm% or taking any medication with adverse effects on liver or cardiovascular system were excluded.

All patients were provided by informed consent, and the ethical committee of hospital approved this study.

All patients and normal volunteers were subjected to:

Thorough history taking and physical examination.

Blood sampling for blood picture including hemoglobin percent, liver function tests, renal function tests, serum electrolytes, cholesterol, triglyceride, HBs antigen and HCV antibody.

Twelve lead surface resting ECG.

Abdominal ultrasound scanning was performed in all participants by one trained radiologist who was blinded to all clinical and laboratory data, using a Toshiba Memo 30 scanner equipped with a 3.5 mHz linear transducer.

Liver cirrhosis was diagnosed based on the results of laboratory tests (low serum concentrations of albumin, high INR and low platelet count) and abdominal ultrasonographic findings (irregularity of the liver surface).

Hepatic steatosis was diagnosed by a characteristic liver echo pattern (diffuse hyperechogenicity of liver compared with right kidney) and negative hepatitis markers.

Echo-Doppler study:

All echocardiographic measurements were performed according to the recommendations of the American Society of Echocardiography ([33]) by a member of the study team in a blinded manner.

M-mode, Two dimensional echocardiography and Doppler ultrasound studies (pulsed, continuous wave and color flow imaging) were made using a high resolution (ALT 5000 HDI) Toshiba Memo 30 scanner equipped with a 2.5 mHz transducer.

With M-mode, measurements of interventricular septum (IVS) and left ventricle posterior wall (PWT) thicknesses separately at diastole and systole were done and left ventricle end-diastolic (LVED) and end systolic (LVES) diameters were determined.

Left ventricular mass was calculated according to Devereux and associates convention: LVM gm = 1.04 x $\{(LVED + IVST + PWT)^3 - LVED^3\} \times 0.8 + 0.6$ (34) where LVED was the left ventricular end diastolic diameter, IVST is the interventricular septum thickness, and PWT was the left ventricular posterior wall thickness.

The size of the left atrium is determined from the parasternal long axis view at end systole.

Left ventricular ejection fraction EF% was measured from M-mode dimentions using Teichholz formula [(35)].

With Doppler echocardiography accompanied by electrocardiogram, Flow characteristics and rates of mitral, tricuspid, aortic and pulmonary valves were evaluated .

High resolution B mode ultrasonography of both the common and internal carotid arteries was performed using an ultrasound machine (Toshiba Memo 30 scanner) equipped with a 7.5 mHz high resolution transducer. Patients were examined in the supine position with the head tilted backwards. After the carotid arteries were located by transverse scans, the probe was rotated to 90° to obtain and record a longitudinal image of the common carotid arteries.

The maximum CIMT was measured at the posterior wall of the common carotid artery, 2 cm before the bifurcation, as the distance between the first and second echogenic lines of the anterior and posterior arterial walls. The image was focused on the posterior wall of the common carotid artery, and gain settings were used to optimize image quality. Measurement was performed vertical to the arterial wall for accurate measurement of CIMT. Three CIMT measurements were taken at and the average measurement was used. All of the CIMT measurement sonograms were obtained by a member of the study team in a blinded manner to the results of abdominal sonography and the clinical and laboratory data for cases and control subjects.

Statistical Analysis:

Statistical analysis was performed using SPSS version 16. Data were expressed as the mean ± standard deviation (SD) for numerical variables. $P \leq 0.05$ was considered to be statistically significant.

3. Results:

The demographic data of group 1 and group 2 patients and controls revealed mean ages 43.5±9.95years, 41.55±10.53years and 43.9±8.8years respectively. In group 1 patients, 60 were males (75%) and 20 females (25%), in group 2 patients, 13 were males (65%) and 7 were females (35%) and in control group 14 were males (70%) and 6 were females (30%) Table (1).

Thirty two patients (40%) in group 1 had early cirrhosis and 48 (60%) has coarse shrunken pattern. The etiology of liver disease were HCV in all cases. The portal vein was dilated in 70 patients (87.5%), the spleen was enlarged in 57 patients (71.25%). Ascites was found in 61 patients (76.25 %); 21 (2.25%) with mild ascites ,33(41.25%) with moderate ascites and 7 patients (8.75) with marked ascites. The spleen was normal in both group 2 and the control group with no ascites. The liver span was decreased in group 1 compared to both groups, However the portal vein was increased in group 1 compared to both groups (P <0.01) Table (2).

The echocardiographic data showed a statistically significant increase in IVST, PWT and LVM in group 1 and group 2 compared to the controls (P<0.01, P<0.05 respectively) and significant increase in left atrium diameter in group 1 compared to the controls (P <0.05).There was also significant increase in left ventricular end systolic diameter (ESD) in group 2 compared to the control group (P <0.05).

Also, there was significant increase in CIMT in both groups compared to the controls (P<0.01) and significant increase in CIMT in group 2 compared to group 1 (P <0.01) Table (3).

Plasma levels of Na was significantly decreased and that of K was significantly increased in group 1 compared to group 2 and the controls (P <0.01), together with a statistically significant increase in ALT, AST, total bilirubin and direct bilirubin in both groups

compared to the controls and significant increase in AST, total bilirubin, direct bilirubin and INR in group 1 compared to group 2. However there were a statistically significant decrease in albumin, hemoglobin and platelets count in both groups compared to the controls and also a statistically significant decrease in group 1 compared to group 2 (Table4).

Table (1) Demographic data of the studied groups.

	Group 1(n=80)	Group 2 (n=20)	Group3(n=20)
Age	43.5±9.95	41.55±10.53	43.9±8.8
Gender Male Female	 60(75%) 20(25%)	 13(65%) 7(35%)	 14(70%) 6(30%)

Table (2) Comparison between liver span and portal vein diameter of the studied groups.

	Group 1(n=80)	Group 2 (n=20)	Group3(n=20)
Liver cm	12.5±1.8**##	16.25±0.9**	14.6±0.5
Portal vein mm	14.5±1.6**##	7.55±0.6**	6.44±0.5

* Group 1 or Group 2 vs Control # Group 1 vs group 2

P < 0.05= significant (*, #) ***P*** < 0.01= highly significant (**, # #)

Table (3)Echocardiographic &Duplex finding of the studied groups.

	Group 1(n=80)	Group 2 (n=20)	Group3(n=20)
IVST cm	1.01±0.17**	1.02±0.16**	0.9±0.12
PWT cm	1.03±0.12**	1.03±0.15**	0.91±0.12
LVM gm	186.89±52.18**	195.57±65.46**	149.6±37.65
EDD cm	4.95±0.65	5.06±0.6	4.77±0.47
ESD cm	3.10±0.58	3.36±0.75*	2.89±0.43
EF%	66.55±7.87	62.9±10.89	68.0±6.53
LA mm	38.14±5.09*	38.05±4.68	35.8±3.79
CIMT cm	1.03±0.11**# #	1.17±0.1**	0.72±0.14

* Group 1 or Group 2 vs Control # Group 1 vs group 2

P < 0.05= significant (*, #) *P* < 0.01= highly significant (** , # #)

IVST: interventricular septum thickness, PWT: posterior wall thickness, LVM: left ventricular mass, EDD: end diastolic dimension, ESD: end systolic dimension, EF: ejection fraction, LA: left atrium dimension, CIMT: carotid intima-media thickness.

Table (4) Laboratory data of the studied groups.

	Group 1(n=80)	Group 2 (n=20)	Group3(n=20)
Na mEq/L	131.96±4.74**# #	139.95±5.44	141.55±2.04
K mEq/L	4.78±0.56**# #	4.14±0.22	4.05±0.24
ALT U/L	31.78±33.85**	41.65±20.29**	13.85±2.06
AST U/L	61.96±77.76**#	43.8±16.89**	13.65±4.04
T bil. mg/dL	3.12±3.79**# #	1.46±1.56*	0.51±0.11
D bil. mg/dL	1.5±2.33**# #	0.56±0.92 *	0.12±0.02
Albumin g/dL	2.47±0.69**# #	3.71±0.43**	4.21±0.1
Hb g/dL	10.58±1.24**# #	12.39±1.14 *	13.09±0.62
Platelets count 10^9/L	62.6±17.3**# #	161.2±32.7**	224.7±58.7
INR %	1.69±0.5**# #	1.03±0.03	1.02±0.03
LDL mg/dL	96.4±58.5	117.6±29.5	109.6±30.4
HDL mg/dL	40.0±14.3	46.2±21.6	48.8±14.8
Total Choles. mg/dL	161.2±71.3	176.5±37.9	167.7±36.2
TG mg/dL	90.6±50.5	131.9±57.7	122.3±56.5

* Group 1 or Group 2 vs Control # Group 1 vs group 2

P < 0.05= significant (*, #) *P* < 0.01= highly significant (**, # #)

Na: serum sodium, K: serum potassium, ALT: alanine aminotransferase, AST : aspartate aminotransferase, Hb : hemoglobin, INR : international normalized ratio

T bil. : total bilirubin, D bil.: Direct bilirubin, LDL: low density lipoprotein, HDL: high density lipoprotein, Total Choles.: Total cholesterol, TG: triglycerides.

4. Discussion:

Chronic liver disease comprises a number of progressive disorders which culminate in liver cirrhosis. Liver cirrhosis is associated with a wide range of cardiovascular abnormalities including hyperdynamic circulation, cirrhotic cardiomyopathy, and pulmonary vascular abnormalities. The pathogenic mechanisms of these cardiovascular changes are multifactorial and include neurohumoral and vascular dysregulations [1]. Also,There is considerable evidence that HCV infection and the atherosclerotic process are related [2].

Plotkin et al. reported a higher rate of cardiac morbidity and mortality in cirrhotic patients with angiographic evidence of CAD than in those without such findings [36].

Nonalcoholic fatty liver disease which is excessive accumulation of fat in hepatocytes is associated with a range of pathologic lesions, ranging from simple steatosis to nonalcoholic steatohepatitis and cirrhosis. NAFLD is now considered to be a hepatic manifestation of the metabolic syndrome and may have a potential role in the development and progression of atherosclerosis [37]. Some investigators suggest that the outcome in patients with NAFLD is more dependent on cardiovascular events than on the progression of liver disease [38].

In this study, we evaluated atherosclerosis by measuring CIMT as an indicator of atherosclerosis and cardiovascular risk in two groups of patients having chronic liver disease (post hepatitis C liver cirrhosis and NAFLD). Our study revealed significant increase in CIMT in both groups compared to controls (P<0.01). There was also significant increase in CIMT in group 2 compared to group 1. The mean value of CIMT was 1.03±0.11 in group 1, 1.17±0.1 in group 2 and 0.72±0.14 in the controls.

Our results showed that fatty liver disease is a highly significant marker of increased CIMT. As regards a possible link between fatty liver disease and increased CIMT and cardiovascular risk, it has been suggested that fatty liver disease might contribute to accelerated atherosclerosis through increased oxidative stress, chronic subclinical inflammation, and decreased liver production of cytokines with antiatherogenic properties [39].

In consistent with our study *Ramilli et al.,* and *Afshin et al.,* studies showed that pure NAFLD without metabolic syndrome is strongly associated with increased CIMT [40,30].

In our study the increased CIMT in patients with post hepatitis C liver cirrhosis compared to the controls is in agreement of the studies of *Ishizaka et al.,* and *Vasalle et al.,* who demonstrated that HCV seropositivity lead to carotid artery plaque and carotid intima- media thickening independent of other risk factors and these studies found that HCV seropositivity is an independent predictor for coronary artery disease [4,5]. Also, *Kalaitzakis et al*., concluded that Liver cirrhosis, per se, does not seem to confer a protective effect against CAD [8].

Interestingly, O'Leary *et al.,* have previously reported that a carotid IMT value ≤0.86 mm carries a low risk of developing CVD, whereas an IMT value ≥1.10 carries a high risk of developing CVD [41]. Thus, our findings might have important clinical and public health implications. Our data further emphasize the importance of evaluating the CVD risk in patients diagnosed with NAFLD or liver cirrhosis. Patients with liver cirrhosis or NAFLD having increased carotid IMT could be candidates not only for aggressive treatment of the liver disease, but also for aggressive treatment of underlying CVD risk factors; this would help to modify and potentially decrease the global CVD risk of these patients.

Regarding the echocardiographic data , Our study revealed statistically significant increase in IVST,PWT and LVM in group 1 (post hepatitis C liver cirrhosis) compared to the control group (P<0.01). These findings are supported by the results of *Wong et al.* and *De Marco et al.* [12, 15]. Also, *Bernal et al*., demonstrated a high prevalence of LV hypertrophy in cirrhotic patients as compared to controls [13]. LV hypertrophy has been described in autopsies and living cirrhotic patients of any etiology, with or without ascites [42- 44]. It is thought to be secondary to volume expansion and activation of various neurohormonal systems (renin-angiotensin system, endothelin-1, sympathetic stimulation) commonly observed in these patients [45]. The prognostic value of echocardiographically detected LV hypertrophy has been unequivocally demonstrated by the Framingham Heart Study [46].

Also, our study revealed a statistically significant increase in IVST, PWT and LVM in group 2 (NAFLD) compared to the control group. This findings is similar to that of *Goland et al*., who found increased thickness of the interventricular septum, posterior wall and larger LVM and LV mass/height in NAFLD group compared to normal controls and they suggest that factors associated with insulin resistance perse have a pivotal role in the development of cardiac abnormalities in patients with NAFLD and the clinical implications of early changes in LV structure and function may have relevance for prevention and treatment of overt cardiac abnormalities and apparent heart failure in this population [31].

Mantovani et al., on their study on patients having NAFLD reported that NAFLD is associated with LVH independently of classical cardiovascular risk factors and other potential confounders [47]. *Fotbolcu et al.,* found mild abnormalities in the LV structure, including increased LVM, LVM index and LV wall thickness in normotensive, non-diabetic patients with NAFLD and they attributed this to higher

levels of BP recordings, higher BMIs (but they did not have morbid obesity), and higher levels of insulin resistance than the controls [48].

In the present study, left atrium diameter was significantly higher in group 1 compared to the controls ($P<0.05$). Our results agreed with that of *Kelbaek et al.*, [16], *Soyoral et al.,* [49] and *Bernal et al.*, [13] as they demonstrated increased left atrium diameter in patients with liver cirrhosis compared to the controls.

Zamirian et al., concluded that in cirrhotic patients, left atrium enlargement, which reflects one aspect of increased cardiac output, is an indirect marker of intrapulmonary shunt (IPS) and greater left atrium dimension is associated with the presence of intrapulmonary right-to-left shunt [20].

In our study, there was insignificant increase in the mean value of left ventricular end diastolic dimension (EDD) in patients with post hepatitis C liver cirrhosis compared to the controls and there was no significant difference in ejection fraction between the two groups. In accordance to our study, *Kelbæk et al.,* reported that significant left ventricular dilatation does not occur in cirrhotics presumably as a consequence of the reduced systemic vascular resistance in these patients but Left atrium dilatation has been reported [16].

In our study there was no significant difference in the mean values of left atrium diameter between patients with NAFLD and the controls, although left atrium diameter tends to be more in NAFLD group. Also, left ventricular EDD tends to more and Left ventricular ejection fraction (EF%) tend to be less in NAFLD patients than the control group but the difference is statistically insignificant. In consistent with our study, *Fotbolcu et al.,* found no significant difference in left ventricular EDD, left atrium size and ejection fraction between NAFLD group and the controls [48]. Also, *Bonapace et al.,* on their study on diabetic patients with NAFLD found the same results [50].

In our study, There was significant increase of end systolic diameter (ESD) in NAFLD group compared to the controls ($p<0.05$). According to the study of *Ramachandran et al.,* an increase in echocardiographic left ventricular internal dimensions (EDD or ESD) is a risk factor for the development of congestive heart failure and the knowledge of left ventricular dimensions improves predictions of the risk of congestive heart failure made on the basis of traditional risk factors, perhaps by aiding in the identification of people with subclinical left ventricular dysfunction [51]. Also, *Sandvik et al.* and *Lauer et al.* concluded that cardiac enlargement is associated with increased morbidity and mortality among healthy middle-aged and elderly people [52, 53].

So, in our study, the increased ESD in patients having NAFLD may be a predictor of subclinical left ventricular dysfunction and a risk factor for the development of congestive heart failure and increased morbidity and mortality. This is in consistent with the study of *Fotbolcu et al.,* who found that Patients with NAFLD have impaired LV systolic function even in absence of morbid obesity, hypertension, or diabetes [48].

Conclusion:

Liver cirrhosis and NAFLD are associated with LVH, increased LV mass and increased CIMT independently of classical cardiovascular risk factors. Also, patients with liver cirrhosis have increased left atrium size. Patients with NAFLD have increased left ventricular ESD which may be a predictor of subclinical left ventricular dysfunction. The clinical implications of early changes in LV structure and function may have relevance for prevention and treatment of overt cardiac abnormalities and apparent heart failure in this population.

Patients with liver cirrhosis or NAFLD having increased CIMT, which is an indicator of atherosclerosis, should be evaluated for CVD risk and could be candidates not only for aggressive treatment of the liver disease, but also for aggressive treatment of underlying CVD risk factors; this would help to modify and potentially decrease the global CVD risk of these patients.

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Ultrastructure of Vitellocytes in *Electrotaenia malopteruri* (Fritsch, 1886) (Cestoda: Proteocephalidae) A Parasite of *Malapterurus electricus* (Siluriformes: Malapteruridae) from Egypt

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Abstract: This study describes the Ultrastructure of mature Vitellocytes of the Proteocephalidae Cestode *Electrotaenia malopteruri* (Fritsch, 1886) a parasite of the common catfish *Malapterurus electricus* using transmission electron microscopy (TEM). The vitellocyte is characterized by the perinuclear cytoplasm that contains numerous parallel cisternae of granular endoplasmic reticulum (GER), several Golgi complex, its peripheral cytoplasm contains, lipid droplets, shell globule clusters, proposed glycogen like particles. The most characteristic feature of the mature vitellocyte of this Cestode species is the concentric arrangement of shell globule clusters.
[Salwa Z. A. Arafa **Ultrastructure of Vitellocytes in *Electrotaenia malopteruri* (Fritsch, 1886) (Cestoda: Proteocephalidae) A Parasite of *Malapterurus electricus* (Siluriformes: Malapteruridae) from Egypt.** *Life Sci J* 2012;9(2):1207-1211] (ISSN:1097-8135). http://www.lifesciencesite.com.180

Keywords: Proteocephalidae, *Malapterurus electricus*, vitellocytes, Ultra-structure, *Electrotaenia malopteruri*, TEM.

Introduction

Cestode class is known to be one of classes that has the highest reproductive capacities of all animal classes (Conn, 2000). Several TEM studies have been published on the ultra structure and differentiation of vitellocytes in cestodes, (Swiderski and Xylander, 2000). Vitellocytes in cestodes have two important functions, i.e. egg shell formation and the nourishment of the early embryo (Swiderski, *et al.,* 1970 a, b; Swiderski and Xylander1998 and 2000). So many authors studied the characteristic features of mature vitellocytes in different cestode groups

On Vitellogenesis in the cestode *Inermicapsifer madagascriensis,* (Swiderski, 1973); on Bothriocephalidea *Bothriocephalus clavibothrium, (*Swiderski and Mokhtar, 1974); on *Progillotia pastinance,* (Mokhtar-Maamour and Swiderski, 1976); on *Rhinebothriidea Echeneibothrium beaulchampi* (Swiderski and Mackiewicz, 1976); on *Proteocephalus longicollis* (Swiderski and Subilia, 1978); on *Grillotia erinaceus,*(McKerr, 1985); on Amphilinidea*, Gyrocotyle urna*, (Xylander, 1987) and on Amphilinidea, *Amphilina foliacea*,(Xylander, 1988); on Proteocephalidea *Proteocephalus exiguous* (Bruňanskà, 1997); on Cyclophyllidea *Catenotaenia pusilla (*Swiderski *et al.,* 2000)*;* on *Triaenophrous nodulosus, (*Koeneva, 2001); on *Moniezia expansa* and *Mosgovoyia ctenoides,* (Li *et al.,* 2003); on *Caryophyllaeus laticeps (*Swiderski *et al.,* 2004a); on *Khawia armeniaca., (*Swiderski *et al.,* 2004b); on Spathebothriidae *Cyathocephalus truncates, (*Bruňanskà *et al.,* 2005); on *Trypanorhynchea Dollfusiella spinulifera, (*Swiderski *et al.,* 2006a); on *Progillotia pastinance (*Swiderski *et al.,* 2006b); on *Didymobothrium rudolphii,* (Poddubnaya *et al.,* 2006); on *Paraechinophallus japonicus, (*Levron *et al.,* 2007); on *Paracharistianella trygonis,(*Swiderski *et al.,* 2007); on Caryophyllidea *Atractolytocestus huronensis, (*Brunanska *et al.,* 2009); on *Wenyonia virllis, (*Swiderski *et al.,* 2009); on Diphyllidea *Echeneibothrium euterpes and Clestobothrium crassiceps, (*Swiderski *et al.,* 2011).

In spite of all of these accumulated studies on different cestode groups, the work on Proteocephalidae still fragmented and less research has been done on the ultrastructure of reproductive system among proteocephalideans.

The aim of the work is to describe the aspect of Vitellocytes ultrastructure of *Electrotaenia malopteruri* a parasite of *Malapterurus electricus* to compare it with the results of previous reports of vitellocyte structure in other cestode species.

Materials and Methods:

Materials:

Mature specimens of *Electrotaenia malopteruri* were obtained from the intestine of the infested catfish, *Malapterurus electricus*, River Nile, Egypt. Living cestodes were dissected in a 0.6% NaCL solution and different portion of mature proglottids containing laterally the vitellaria and reproductive system were routinely processed for TEM examination.

Methods:

Specimen were fixed in cold (4°C) 3% glutraldehyde in a 0.1M sodium cacodylate buffer at PH 7.2, for 2 h., washed in a 0.1M sodium cacodylate

buffer at PH 7.2, post fixed in cold (4°C) 1% Osmium tetroxide in the same buffer for 1 h., dehydrated in an ethanol series, and finally embedded in Epon resin. Specimens were sectioned for histological study at 0.5-1µm using a Reichert-jung Ultracut E.ultramicrotom, mounted 0n glass slides, stained with 1% Toluidine Blue O.

Ultra thin sections were cut at thickness of 20-70 nm, placed on copper grids and double-stained with uranyl acetate and lead citrate. Sections were examined using a Joel 1010 Transmission electron microscopy (TEM) operated at 80 KV.

Results

The numerous vitelline follicles are arranged as a continuous series in the cortical parenchyma with some solitary follicles in the medullary parenchyma .In the centre of each follicle extend a small vitelline duct (plate 1,A,B,C)

Examination of ultrathin sections showed that the vitellaria of *Electrotaenia malopteruri* (Fritsch, 1886) is an extensive system of numerous oval or elongated vitelline follicles enclosed by the parenchyma (Plate 2,A).The characteristic arrangement of concentric distribution of shell globules clusters which is diagnostic to E. *malopteruri* (Plate2,A).

Vitellocytes generally have high Nuclei /cytoplasmic ratio (Plate2, A). The nucleus is round and contain large clumps of heterochromatin and the narrow cytoplasm contain large number of lipid droplets, proposed glycogen like droplets, (Plate. 2,B) and concentric rough endoplasmic reticulum (RER). Differentiation of vitellocytes was characterized by the increase of RER. Cisterna which is filled with an electron-dense material (Plate 2, F), Golgi vesicle gave rise to large membrane –bound inclusions (Plate 2, F). When completely mature they had a multigranular and were delimited by the smooth membrane. Oocytes are accompanied by the vitellocytes which have shell globules (Plate 2, D, E), few mitochondria scattered in the parenchyma (Plate 2, C).

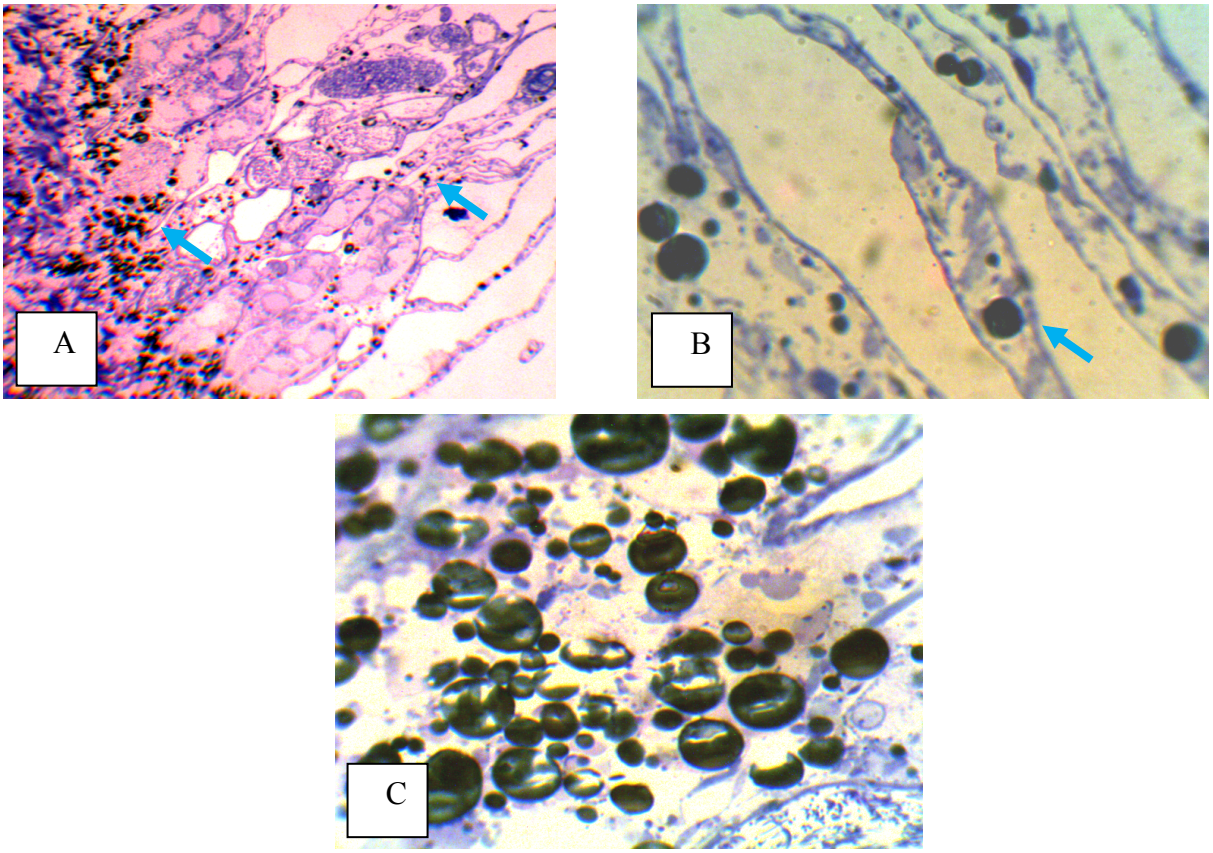

Plate 1: Semi thin section s of *Electrotaenia malopteruri* showing, A-Vitelline follicle arranged as a continues series in the cortical parenchyma (arrow) with some solitary follicles in the medullary parenchyma (arrow head), 400 x. B-Branches of vitelline duct containing vitellocytes (Arrow), 800 x. C-Higher magnification of A showing Vitellaria , 2000 x.

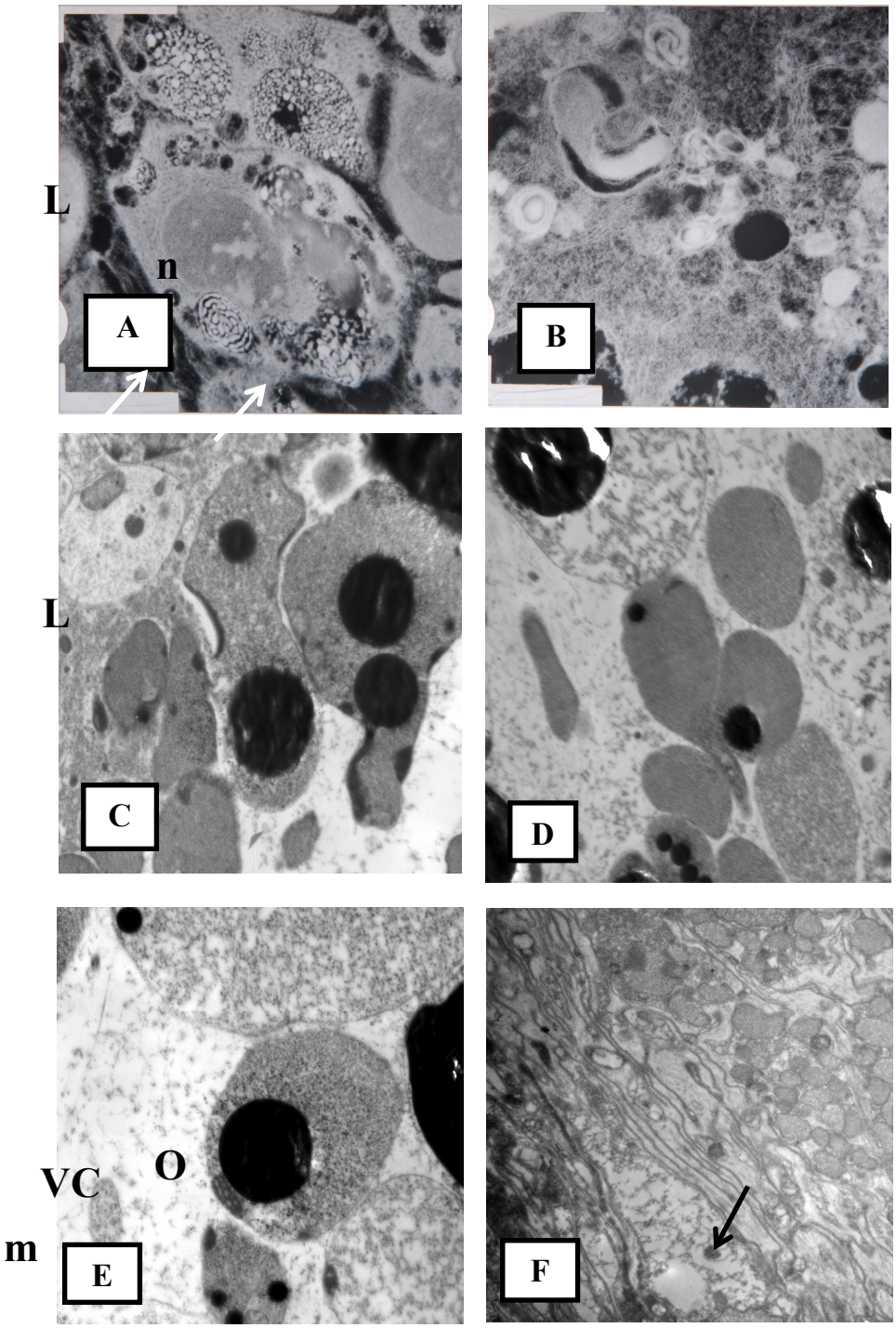

Plate (2): A-TEM micrograph illustrating the mature vitellocyte with the concentric shell globules **(arrows),** nucleus **(n),** Lipid droplets **(L),** 5000X. B- TEM micrograph illustrating the concentric arrangement of the endoplasmic reticulum (arrow, double arrow), proposed glycogen like-particles **(G)**, lipid droplets, 7500X. C- TEM micrograph illustrating the developing Oocytes **(Oo)** adjacent to the vitellocyte **(vc),** note the mitochondria **(m)**, 2500X. D-TEM micrographs illustrating the aggregation of shell globules (arrow), 2500X. E-TEM micrographs illustrating the scattered shell globules (arrows), 3000X. F-TEM micrograph illustrating the characteristic parallel cisternae of endoplasmic reticulum (white arrows), large membrane bound inclusions of Golgi complex (black arrow), 8000X**.**

Discussion

As summarized by Swiderski and Xylander (2000) in their extensive review, cestode vitellocytes are very important for egg formation and embryonic development. They play two significant functions including, the formation of hard egg shell or a delicate vitelline capsule and supplying nutritive reserves for the developing embryo.

Both roles are closely connected with the presence of two types of cytoplasmic inclusions in cestode vitelline cells such as:

Egg shell globules, vitelline vesicles or shell globules clusters that taking part in the egg shell or vitelline capsule formation.

Glycogen and/ or lipids (Sometimes mixtures of both in different proportion which represent recent various energy sources for the developing embryos).

In the present study lipid droplets were localized only in the vitellocyte cytoplasm this is in agreement with *Paracharistianella trygonis,* (Swiderski *et al.,* 2007). On the other side in Tetraphyllidean *Echeneibothrium beaulchampi* by the study of Mokhtar-Maamouri and Swiderski, (1976) and in *Didymobothrium rudolphii* studied by Poddubnaya *et al.,* (2006) it was reported inside the cell nuclei. Other studies on *Diplocotyle olrikii by* Bruňanskà *et al.,* (2005) and on Caryophtllidea *Atractolytocestus huronensis* by Bruňanskà *et al.,* (2009) their conclusion was that the lamellar heterogenous egg globules are represented in great amount, which was different from our studies that as we noticed that the egg shell globules have a characteristic concentric shape. Report of Swiderski *et al.,* (2004a) on *Caryophyllaeus laticeps* stated that lipid granules were absent in mature vitellocyte,while in our study lipid droplets were distributed through the cytoplasm of vitellocytes. Whereas, Swiderski and Mickiewicz (1976) work on *Glaediacris catostomi* found a great amount of cytoplasmic and nuclear glycogen. Study of Swiderski *et al.,* (2004b) on *Khawia armeniaca* reported the lamellar granules in the cytoplasm of this Caryophyllidean cestode. Swiderski *et al.*, (2009) work on *Wenyonia virllis* found moderate accumulations of cytoplasmic glycogen. In addition, Swiderski *et al.,* (2011) work on Diphyllidea *Echinobothrium euterpes* noticed a large amount of glycogen accumulations around the large, saturated lipid droplets of maturing and mature vitellocytes. Swiderski *et al.,* (2006a) work on *Trypanorhyncha Dollfusiella spinulifera* found very few glycogens in the cytoplasm. Many investigators; Swiderski and Mokhtar (1974); Mokhtar-Maamour and Swiderski (1976); Swiderski and Subilia (1978); McKerr (1985); Bruňanskà, (1997); Swiderski *et al.,* (2000), Koeneva (2001), Swiderski *et al.,* (2006 b), Swiderski *et al.,* (2007) and Levron *et al.,* (2007) discussed the ultrastructure of the vitellocytes in the following species: *Grillotia erinaceus Paracharistianella trygonis, Echeneibothrium beaulchampi, Progillotia pastinance, Bothriocephalus clavibothrium, Paraechinophallus japonicus, Proteocephalus longicollis, Inermicapisifer madagascarinesis, Triaenophrous nodulosus, Catenotaenia pusilla, Moniezia expansa, Proteocephalus exiguous* and *Mosgovoyia ctenoides* and compared their contents.

The discovery that lipids vary in the vitellocytes of different families raised important questions regarding the factors determining lipid types, functional significance and what role they might have in assessing evolutionary relationships at any level. As the nutrient reserves are related to the ecology and life cycle in some species and its accumulation in the vitellocytes may deflect the adaptation to the parasitic way of life in different groups of cestodes, Swiderski and Xylander (2000).

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Effect of Nutritional Educational Guideline among Pregnant Women with Iron Deficiency Anemia at Rural Areas in Kalyobia Governorate

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Abstract: All pregnant women are at risk for becoming anemic, that is because iron need for mother and fetus gradually increases during pregnancy and reaches its highest level at the end of the pregnancy. The aim of this study was to evaluate the effect of a nutritional educational guidline (NEG) on knowledge and knowledge related practice of pregnant women with iron deficiency anemia (IDA) at rural areas. The intervention design was conducted at three maternal and child health care centers in Kalyobia Governorate (Moshtoher, Kafr Shoukr, and Kaha). A total 200 rural anemic pregnant women were selected from these maternal and child health centers. One tool was utilized in this study, a structured interviewing questionnaire for assessment of the studied sample characteristics and their knowledge regarding iron deficiency anemia as well as their knowledge related practice. A significant increase in overall knowledge and knowledge related practice toward healthy nutritional habits after intervention was detected and subsequently the prevalence of anemia slightly decreased after intervention of the NEG (24%), than before**.** This study clearly showed that NEG can bring about an improvement in knowledge and knowledge related- practice of pregnant women toward IDA. The study recommended that nutrition should be mandated as a required component of comprehensive health care putting clear guidelines of the nutrition services, that nurse should provide to the pregnant women in primary health care facilitation in order to maintain proper health among pregnant women. Follow up HB level though pregnant women in rural area should be taken for early detection of anemia.
[Howyida S. Abd ElHameed, Aziza I. Mohammed and Lamiaa T. Abd El Hameed. **Effect of Nutritional Educational Guideline among Pregnant Women with Iron Deficiency Anemia at Rural Areas In Kalyobia Governorate**. *Life Sci J* 2012; 9(2):1212-1217]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 181

Key words: Nutritional educational guideline (NEG), iron deficiency anemia (IDA), pregnant women, rural area.

1. Introduction

Iron deficiency anemia is an important public health problem for pregnant women, living in developing countries,[(1)] affecting two thirds of pregnant women and contributing to maternal morbidity and mortality and to low birth weight[(2)]. Multiparty, poor socio economical and educational statuses are the principal reasons for high prevalence of anemia in development areas [(3)]. It is estimated that 20 – 50% of the world population is suffering from iron deficiency anemia. Iron deficiency is believed to be the most common cause of anemia in pregnancy[(4)]. Iron deficiency anemia does not only affect the mother but also has impact on cognitive and psychomotor functions and anemia in infant[(5)]. The WHO classifies the countries with prevalence of anemia higher than 40% as the countries, where anemia is a problem of public health significance [(6)]. It is defined by the WHO as HB less than 11gm in pregnancy, and is divided into three degrees; mild (9.0 –10.9%gm), moderate (7.0-8.9%gm) and severe degree (<7.0% gm) [(7)]. Anemia is more common in women especially if they are young, poor, pregnant or members of an ethnic minority[(8)]. It is the most common medical disorder in pregnancy related to increase maternal and prenatal morbidity, therefore antenatal care should be done for early detection and management[(9)]. The prevalence, etiology and degree of severity vary in different populations, it is 35% for non pregnant women, and 51% for pregnant women globally, and 3 – 4 times higher in developing countries [(10)]. In south Asia the prevalence of anemia among pregnant women is as high as 65%, in Indonesia, the prevalence of nutritional anemia based on scattered surveys, is between 50% and 70%[(11)]. Nursing responsibility for prenatal care includes physiological and psychological assessment of health, health education counseling for pregnant women identification of needs with appropriate community and specially referrals and provision of health promotion such as adequate nutrition, proper exercises and planned care[(12)].

Aim of study:

The present study aimed to evaluate the effect of **a** nutritional educational guideline on knowledge, and knowledge- related practice among pregnant women with iron deficiency anemia at rural areas.

This aim was achieved through the following:

1- Assessing the prevalence of anemia among pregnant women.

2- Assessing knowledge and knowledge- related practice of pregnant women regarding iron deficiency anemia.
3- Designing and implementing a nutritional educational guideline for pregnant women with IDA regarding knowledge related to IDA risk factors, iron rich foods and importance for intake of iron supplementation and their practice toward iron intake .
4- Evaluating the effect of nutritional educational guideline on prevalence of anemia among pregnant women, and their knowledge and knowledge related - practice regarding IDA.

Hypothesis:

1. Nutritional educational guideline improves knowledge and knowledge -related practice of pregnant women regarding IDA.
2. Prevalence of anemia is expected to be reduced among participant women.

2. Subjects and Methods

Research design:

An intervention study design was used.

Setting:

The study was conducted at three maternal and child health centers (**MCH)** in Kalyobia governorate, namely; Moshtoher, Kafr Shoukr & Kaha. These villages were selected as having the highest density of population, and each center is serving around 8.000-15.000 population of its catchment area.

Sample and sampling technique

Recruitment for the study sample included a total of 200 pregnant women, with IDA, at age group from 25–35 years, from different social levels, prime and multi gravid at the second trimester, can read and write, and resident at the selected rural areas. In addition, attending for follow up antenatal visit, mothers suffering from only anemia during pregnancy (HB less than 11gm), free from any medical disorders, and who attended at the selected MCH centers in a period of around 6 months from July 2011 to December 2011.

Tools of data collection: One tool was used:-

An Interviewing questionnaire:

It was designed by the researchers after reviewing of related literature. To collect the necessary data that cover the aim of the study, the interviewing questionnaire was used pre / post intervention. It involved both open/close ended questions. The time consumed to fill in the questionnaire was about 15 minutes. It consisted of four parts:

First part:

It covered the general characteristics, obstetric history, IDA prevalence among pregnant women.

Second part: It dealt with women's knowledge regarding IDA meaning, causes, symptoms, prevention, risk factors, source of iron rich foods, importance of iron supplementation, ect.) Knowledge scoring system:

Were scored as one score given for a correct answer while zero for an incorrect answer. Total knowledge scores were categorized as: >50% (0-10 score) poor knowledge; 50-75% (11-17) average knowledge; and ≤ 75% (18-22) good knowledge.

Third part:

It was used to assess women s' knowledge-related practice regarding eating iron rich food, don't drink tea with meals, regular intake of iron supplementation, and use of orange juice with iron supplementation. It was scored one score for an answer done, and zero score for an answer not done. Total knowledge-related practice scores were evaluated as >50% (0-7), unsatisfactory knowledge-related practice; and ≤ 50% (8-15) satisfactory knowledge- related practice.

Fourth part:

It is concerned with assessment of complete blood count through review of the studied women record to assess the prevalence and severity of anemia among them. It was repeated after treatment to evaluate their responses to treatment. Hemoglobin levels (Hb) were taken pre / post NEG intervention from studied women's records. The degree of anemia was estimated according to cut off point of the WHO (2001), it is divided into three degrees in relation Hb level as mild (9.0–10.9%gm), moderate (7.0-8.9%gm) and severe degree (<7.0% gm) [13].

Pilot Study:

A pilot study was carried out on 20 anemic pregnant women (10%) to test the tool content, applicability, clarity and time needed to fill in the sheet using the interviewing questionnaire. Some modifications and rephrasing of certain questions were done after validity of content was measured by four experts in the field of obstetric and community health nursing to test relevance and completeness of the tools. Those participants were excluded from the main study sample.

Study procedure

The study was conducted in three phases:

Phase one

An official approval was obtained to conduct this study according to a letter issued from the Dean of the Faculty of Nursing, Benha University to each MCH

centers director. Data were collected from the three MCH centers at Kalyobia Governorate over a period of 6 months from beginning of July 2011 to end of December 2011. Each of the MCH centers was visited two days/week. Each pregnant woman was interviewed for around 15 minutes to assess her knowledge and knowledge- related practice.

Phase two

A nutritional educational guideline regarding iron deficiency anemia was developed and intervened by the researchers for each pregnant woman. Two sessions were applied at the end of the day to clarify any questions asked by pregnant women; duration of each session was 2 hours, different teaching methods as lectures, group discussion, and demonstration were utilized. Each woman was informed about the time and place of each session.

Phase three

To evaluate the effect of nutritional guideline women`s knowledge and knowledge -related practice, the same format of interviewing questionnaire was utilized 3 months post intervention.

Ethical considerations

An oral consent was obtained from each mother who agreed to participate in the study in order to gain their cooperation. They were assured about confidentiality of information given and that it well be used only for the purpose of the study. They were also informed about their right to withdraw from the study at any time without giving any reason.

Statistical design:

The collected data were analyzed, simple statistic tests as frequency, percentage, arithmetic mean and standard deviation were used to present collected data. The level of significance was considered at $p<0.05$, and $P < 0.001$.

3.Result:

Table (1) shows personal characteristics of the studied pregnant women. It reveals 50% of them had 18-32 years old, with a mean of 26.43±4.38.As regards educational level, 42.5% of them had university educational level, 58% and 46.5% of them were not working and have low income respectively.

Table (2) indicates obstetric history of the studied pregnant women, the mean gestational age among the studied group was14.80±1.30 weeks, and 49.5% of them were grand multipara. 43.7% of them have more than 3 years regarding birth spacing.

Table (3) displays the comparison between mean scores of studied sample knowledge before the nutritional educational guideline intervention and after three month. There were highly statistically significant differences between knowledge mean scores of studied pregnant women before and after intervention $p< 0.001$.

Figure (1): illustrates that more than three quarters of the studied sample (78%) had a good total knowledge score regarding iron deficiency anemia after intervention of nutritional educational guideline

Table (4): shows that there were highly statistically significant differences ($p<0.001$) between mean score of all knowledge related practice items of the studied women regarding iron deficiency anemia pre/post intervention, except for eat regular frequent meals where it was only significant ($P <0.005$).

Figure (2): Illustrates that less than three-quarter (71 %) of the studied women had a satisfactory knowledge- related practice after implementation of NEG, and 29% had unsatisfactory knowledge related practice as compared with 82% at pre-intervention.

Figure (3): Represents the prevalence of anemia among studied pregnant women before and after three months of NEG intervention. The figure shows that 24% of studied pregnant women had a normal level of hemoglobin and became free from anemia after 3 month post NIG intervention.

Table (1): Personal characteristics of the studied pregnant women(n=200).

Personal Characteristics	NO	%
Age(in years)		
18-	60	30
25-	100	50
≥32	40	20
Mean± SD	26.43±4.38	
Level of education:		
Preparatory	33	16.5
Secondary	82	41
University	85	42.5
Family income:		
High	35	17.5
High middle	16	8.0
Low middle	93	46.5
Low	56	28.0
Occupation :		
Working	84	42
Do not work	116	58

Table (2): Obstetric history of the studied pregnant women (n=200).

Obstetric history	Frequency	%
Gestational age in weeks:		
13-	68	34
18-	32	16
23-28	100	50
Mean± SD	14.80±1.30	
Parity:		
Primigravida	58	29
Multipara	43	21.5
Grand multipara	99	49.5
Birth spacing in years(n=142):		
< 1	25	17.6
1-3	55	38.7
>3	62	43.7

Table (3) Comparison of mean score of knowledge of the studied pregnant women regarding iron deficiency anemia (IDA) pre / post nutritional educational guideline intervention (N=200).

Women's Knowledge	Pre intervention NEG Mean ±SD	Post intervention NEG Mean ±SD	Paired t test	*P* value
Meaning of IDA	0.26 ± 0.59	0.93 ± 0.72	23.62	≤0.001**
Causes of IDA	1.11 ± 0.43	1.54 ± 0.34	15.39	≤0.001**
Signs and symptoms of IDA.	0.82 ± 0.66	1.56 ± 0.45	18.11	≤0.001**
Effect of IDA on pregnant women	0.56 ± 0.40	1.50 ± 0.52	15.24	≤0.001**
Effect of IDA on the neonate	0.46 ± 0.45	0.83 ± 0.80	12.27	≤0.001**
Measures of prevent IDA	0.42 ± 0.78	1.35 ± 0.45	18.80	≤0.001**
Risk factors of IDA	0.56 ± 0.61	1.32 ± 0.43	20.42	≤0.001**
Sources of iron rich foods	1.08 ± 0.71	1.57 ± 0.67	15.33	≤0.001**
Importance of iron supplementation	1.01 ± 0.87	1.56 ± 0.74	18.96	≤0.001**
Side effects of iron supplementation	1.43 ± 0.70	1.42 ± 0.70	18.58	≤0.001**
Measures to control iron supplementation side effects	0.50 ± 0.79	1.41 ± 0.75	16.88	≤0.001**

**** Highly statistically significant difference (p≤0.001)**

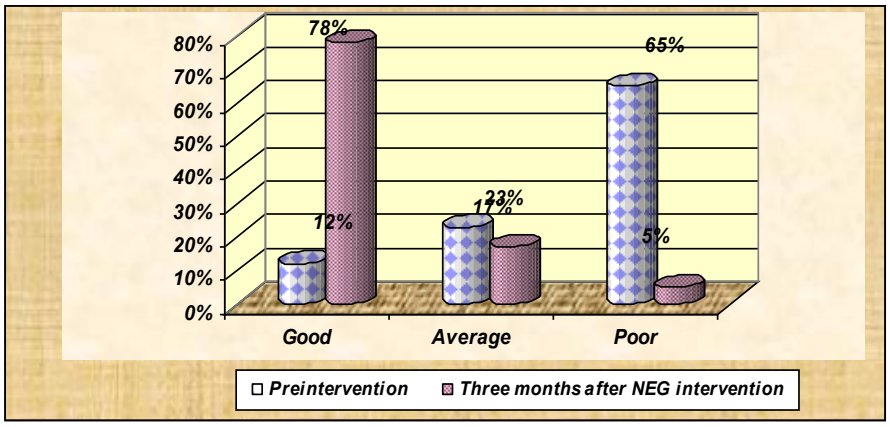

Figure (1): Percentage distribution of the studied pregnant women regarding their total knowledge score pre and three months after intervention of nutritional educational guideline.

Table (4): Comparison of mean score knowledge related practice of the studied pregnant women regarding IDA pre / post nutritional educational guideline intervention (n=200).

Women s' Practice		Pre-intervention		Post Three month after intervention		x^2	*P* value
		No	%	No	%		
Eating iron rich foods	***Done***	57	48.5	122	66	63.15	≤0.001**
	Not done	143	71.5	78	34		
Don't drink tea with meals	***Done***	153	76.5	136	68	81.93	≤ 0.001**
	Not done	47	33.5	64	32		
Regular use of iron supplementation	***Done***	35	17.5	152	76	57.53	≤ 0.001**
	Not done	165	82.5	48	24		
Administer iron supplementation	***Done***	72	34	168	84	56.74	≤ 0.001**
	Not done	128	64	32	16		
Eat regular frequent meals	***Done***	65	32.5	165	82.5	64.06	<0.05*
	Not done	135	67.5	35	17.5		
Use iron supplementation with milk	***Done***	28	14	110	55	81.68	≤ 0.001**
	Not done	172	86	90	45		
Use iron supplementation with fruit juice	***Done***	31	15.5	104	52	73.42	≤0.001**
	Not done	169	84.5	96	48		

*Statistically significant difference ($p \leq 0.05$).** Highly statistically significant difference ($p \leq 0.001$)

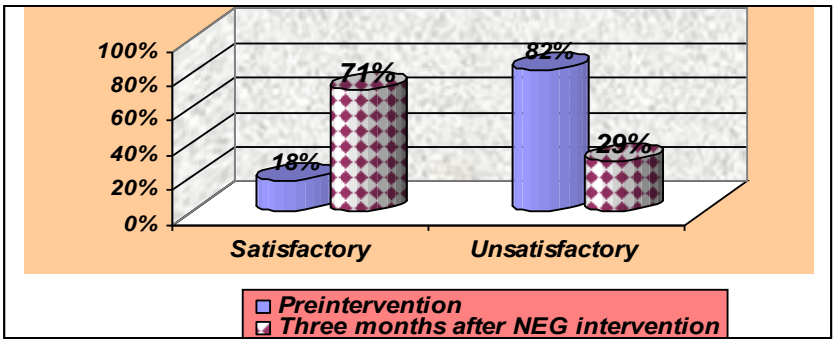

Figure (2): Percentage distribution of the studied pregnant women regarding their total practice score pre and three months after intervention of nutritional educational guideline.

4. Discussion

Anemia in pregnant women constitutes a real concern all over the world more so in developing countries. Various studies have reported variable prevalence rates of anemia during pregnancy and it varies from 33% to 75 %. [14,15]. The current study aimed to evaluate the effect of nutritional educational guideline on knowledge and knowledge- related practice of pregnant women with iron deficiency anemia at rural areas. According to the study findings, a total 200 pregnant women with iron deficiency anemia, residing rural areas with a mean age of 26.43±4.38 years, and almost three fifths of them were not working.

As regards obstetric history of studied pregnant women, the mean of gestational age was 14.80±1.30 weeks, about half of them were grand multipara, and more than two fifths of them have more than 3 years regarding birth spacing. These findings were supported by ***Schweitzer***[16] who pointed out that the prevalence of iron deficiency anemia increased with parity women with more than four children). In another study, done by ***Malhotra et. al.***[17] there results showed that repeated pregnancies is a factor for development of iron deficiency anemia in subsequent pregnancy.

The current study indicates that there were a highly statistically significant differences (p<0.001) in relation mean scores of both knowledge and knowledge- related practice of studied pregnant women regarding iron deficiency anemia per versus after intervention of NEG. This might be due to that the implementation of the NEG. helped pregnant women to identify different sources of iron rich foods, regulate their time to take iron supplementation, and overcome side effects of iron supplementation. These findings agreed with those of a similar study carried out by ***El Sayed*** [18]. Who mentioned that all rural pregnant women had knowledge about different sources of iron rich foods.

In addition, ***Pernilia et al.,***[19] highlighted the importance of health education to prevent iron deficiency anemia among pregnant women in developing countries to decrease maternal morbidity and mortality rate among women in reproductive age and, prevention of any corresponding complications regarding their future. Moreover, the **WHO**[13]recommended that the recent guidelines regarding prevention and control of iron deficiency anemia, are the most effective ways needed to decrease them. The current study estimated that, the prevalence of iron deficiency anemia among studied pregnant women was decreased after utilization of nutritional educational guideline, and approximately one quarter of the studied pregnant women had a normal hemoglobin level post intervention.

Conclusion

According to the result of the present study, it could be concluded that the prevalence of iron deficiency anemia among studied participants was still high after the NEG intervention except for only 24% of them who were treated and had normal hemoglobin level, and there was a highly statistically significant improvement regarding knowledge and practice of the studied pregnant women after intervention of NEG.

Recommendations:

- Follow up should be carried out to early detection of iron deficiency anemia among pregnant women at rural areas.
- Available booklet in certain knowledge about IDA in MCH centers should give to all pregnant women.
- Health education to all attending pregnant women in MCH centers are provide about balanced diet and source of iron rich food.
- Nurses should have regular, ongoing, specialized in-service training programs about prenatal nursing care. As well, nursing care standards should be applied in MCH centers to improve the care provided for pregnant women.
- Further study is needed to be conducted on a larger simple and different geographical settings to generalize the results of the study.

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Effects of Cardiovascular Endurance Training Periodization on Aerobic performance and Stress Modulation in Rugby Athletes

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Abstract: Background: Rugby is an intense sport that demands athletes to compete in frequent short bouts of high intensity exercise, consisted of both defensive and offensive play. Athletes must be periodized basic and specific fitness conditioning to improve their performance. **Aim:** To verify biological and psychological stress markers during strenuous cardiovascular endurance training (CET) periodization, using Profile of Mood States (POMS) questionnaires, heart rate variability (HRV) and blood urine nitrogen (BUN) as the criteria measurements, and determine the association of training intensity distribution in rugby training. **Methods:** Twenty-four male national level rugby athletes completed 8 weeks CET. Subjects were evaluated POMS, HRV and BUN five times during CET: at the beginning (week 0, date 0, T1), in the middle (week 1, date 5, T2; week 4, date 26, T3; week 6, date 40, T4) and at the end (week 8, date 52, T5) of the training programs. Peak oxygen uptake ($\dot{V}O_{2peak}$) and works of lactate threshold at 4 mmole/L (W_{LH}) were tested before and after CET. **Results:** There were significantly increasing in the absolute $\dot{V}O_{2peak}$ (20.7(16.5) %), relative $\dot{V}O_{2peak}$ (23.6(16.6) %) and W_{LH} (40.5(39.1) %) after the totally CET. Training increased BUN levels, LF/HF ratio and POMS total mood disturbance scores. The BUN and LF/HF were significantly correlated with POMS subscales before and during different CET stages. However, there was only significant association between HF distributions based on TMD (before: r= 0.795, P=.001; at the end of CET: r = 0.739, P=.001). **Conclusion:** 8 weeks periodized CET would increase aerobic performance and strengthen cardiovascular regulation in rugby athletes. A noninvasive monitoring method could be developed by POMS highly correlated HRV and BUN for physiological controlling in periodization.
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Key words: rugby, periodization, POMS, heart rate variability, blood urine nitrogen

1. Introduction

Rugby is a forceful sport in which the maximal performance relies on the various technical, tactical, physical and psychological developments. Rugby is a intense sport that demands athletes to compete in frequent short bouts of high intensity exercise, consisted of both running play and contact play. During these high-intensity bouts of activity, athletes are involved in defensive and offensive sprinting activities that power, strength, agility, and speed are needed. We believe that athletes must arrange basic and specific fitness conditioning to improve their performance.

The planned classification or structure of training loads (technical, tactical and physical) in periods or stages has been recommended to as periodization[1]. Periodization goals to strengthen the athletes' performance. Traditionally, periodization is separated into four phases: off-season (building of the athletic shape), pre-season (acquirement of the athletic shape), in-season competition (maintenance of the athletic shape) and transition (temporal loss of the athletic shape)[2].

Although, most elite athletes consecrate themselves to training programs that force them to the extreme of their exercise performance, thereby usually entering a condition typically recommended as "overreached" (OR). Optimal performance is only reachable if athletes can recover after optimally balance training stress and energetic recovery. Coaches should organize modifications in training load and compensation in regularly practice that include a transient short-term fatigue and effort followed by recovery leading to a long-term performance refinement. However, when the athlete is not well improved, mild hurts could develop into tissues damages. Athletes often train an extended period of severe training called "overtraining syndrome" (OTS) and are characterized by reducing performance in spite of an expanded rest period, associated by physiological, biochemical, and psychological stress symptoms.

The assessment and monitoring of stress markers during training programs would assign the remodeling and individualization of an athlete's training workload[3]. Therefore, the best management to evaluate the training adaptation and to avoid

overreaching and overtraining during training programs is the regular monitoring of selected biochemical, physiological and psychological markers.

Cardiovascular endurance training (CET) is a key component in periodization, because it is strongly combined with specific conditioning and beneficial metabolic, physiological and psychological effects. Intense or prolonged exercise during the training period may have caused skeletal muscle trauma, protein catabolism, and degradation of body tissue proteins. The byproduct serum blood urine nitrogen (BUN) concentrations should increase after training. Analyzing heart rate variability (HRV), which indirectly quantifies the influence of autonomic controlling on cardiac, could also be an important tool to analyze of cardiovascular stress in athletes [4]. Psychological adaptations have also been used to record the state during different training periods. These changes have been measured through the Profile of Mood States (POMS) questionnaire[5]. It has been shown that mood flutters increase similarly to training volume and/or intensity, and that individual POMS scores may get used to qualify training.

However, different metabolic stress indices might have various time courses in response to the periodization of intense training and following effective recovery. This suggests that training monitoring in athletes should be effected not only in a multi-level approach using estimations of performance but various biological and psychological indices as well. The present study analyzed psychological and physiological recovery in rugby athletes by comparing BUN to examine metabolic effect. HRV parameters and POMS scores were to verify the responses of adaptations during 8-week CET separately. In addition, any correlations between elements of physical and mental modifications were assessed with reference to the periodization. It was hypothesized that physiological and psychological markers during strenuous training section should be significantly modified sufficiently physical conditioning, maintained to provide adequate stimuli and altered the neural mechanisms of cardiovascular regulation.

2. Methods

2.1. Subjects

Twenty-four male national level rugby athletes were recruited in this study. The study protocol and purpose were explained and informed consent was obtained from all subjects before the study. Approval for the study was obtained from the Human Ethics Committee of the Cheng Shiu University. In a pre-study interview, information on routine use of vitamins and other nutritional supplements was obtained from each subject. Volunteers found to be taking regular medication that might interfere with cardiac autonomic function were used as exclusion criteria from the study. Two weeks prior to the tests, subjects were required to cease vitamin and supplement intake. Subjects were instructed to avoid exercise or strenuous physical activity for 3 days before and after CET aerobic tests. In the twenty-four hour period preceding the study, subjects recorded all food and drink intake and this dietary pattern was duplicated in other parts of monitoring. Peak oxygen uptake ($\dot{V}O_{2peak}$) and works of 4mM (4 mmole/L) lactate threshold (W_{LH}) of each subject were determined before and after the CET period.

2.2. Experimental design and procedures

This study was 8 weeks in duration including a total of 206 hours of rugby training. The first 4 weeks of endurance training (ET1, ET2) involved LT speed training (32 h). The 5th to 8th weeks of endurance training (SIT1, SIT2) involved interval speed training (28 h). The rugby training program included of rugby related basic physical, tactical and technical training, and recreational training (Table 1). CET included field exercises, interval training and circuit training. The SIT1 and SIT2 program consisted of mainly endurance type of sport activities such as high intensity interval training could improve endurance performance, resulting in enough time for the enhancement of other limiting skills such as rugby technique, starts and turns as well as sprint and strength endurance.

Subjects were evaluated five times in the study: at the beginning (week 0, date 0, T1), in the middle (week 1, date 5, T2; week 4, date 26, T3; week 6, date 40, T4) and at the end (week 8, date 52, T5) of the training programs. Diets or lifestyles were not controlled during the course of the season (table 1).

The tests were always performed in the same order in the testing session. On the day of the experimental test, measurements were executed at 6:20 am- 7:20 am before breakfast on Friday morning. Athletes did not perform strenuous physical activities in the 12 hours before recordings. No subject was taking drugs at the time of the recording sessions. The experiments were performed in a comfortable room at ambient temperature (22°C to 24°C) and relative humidity (55-60%). Subjects reported to the laboratory following a 10-h overnight fast. Subjects were instructed to consume 240 mL of water to increase hydration when they arrived at the laboratory. The athletes lay supine for 15 minutes before experiments to relax in the room made noiseless. Firstly, subjects were tested HRV and then asked to complete the POMS questionnaires. Blood samples were collected in a rest position from the earlobe in order to analyze hemoglobin (HB) and serum BUN (Table 1).

Table 1. The monitoring stages during the cardiovascular endurance training programs of the study

Training period	Before-training		CET				After-training
		Base line	$ET1_k$	ET2	SIT1	SIT2	
Test	before	T1	T2	T3	T4	T5	after
Testing Date	-7^{th}- -3^{rd}	0	5^{th}	26^{th}	40^{th}	52^{th}	56^{th} - 58^{th}
Testing parameters	$\dot{V}O_{2peak,}$ LH	HB, BUN, HRV, POMS					$\dot{V}O_{2peak}$, LH

2.3. Training program

The training program was designed by researcher and the team's coach. There were 37 sessions with a mean volume of 24.8 h per week between ET1-ET2. During SIT1-SIT2, the rugby athletes performed 37 sessions with a mean volume of 26.2 h per week extending entire rugby-training program (Table 2).

Table 2. Training characteristics between ET1-ET2 and SIT1-SIT2

Type of Training	Duration of a session (min)	Weekly frequency	
		ET1–ET2	SIT1–SIT2
Recovery	30	5	6
Endurance	60	6	7
Specific rugby	30	6	4
Specific speed	40	3	4
Tactical	30	3	4
Technical	40	6	6
Simulated match	60	5	5
Recreational	60	1	1

The training program consisted of recovery training (continuous running/swimming/dancing at 45–55% of maximal heart rate), endurance training (continuous running at 65–80% and 80–90% of maximal heart rate between ET1-ET2 and SIT1-SIT2, respectively), specific rugby training (rugby activities according to playing position), specific speed training (sprints between 10–30m), tactical training (rugby activities up to tactical objective), technical training (attack against defense in the fields), simulated match (a simulated match including of two halves, each of 30 min) and recreational training (like a simulated match with the athletes playing in different sports like badminton).

Table 3. Summarizes the training characteristics between ET1-ET2 and SIT1-SIT2.

Variable (n=24)	**ET 1**	**ET2**	**SIT 1**	**SIT2**
Protocol	40–60 min running	30 min running× 2–4 repeats, 20 min rest	30 s × 8–12 repeats, 2.5 min rest	30 s × 10–15 repeats, 1.5 min rest
Training intensity	(5 × per week)	(5 × per week)	(5 × per week)	(5 × per week)
Training intensity	45% $\dot{V}O_{2peak}$	~ 65-75%$\dot{V}O_{2peak}$ (~ 4mM LT)	''All out'' maximal effort	''All out'' supra-maximal effort
Weekly training	~8 h	~4-8 h	~7 h	~7 h
Training session	7days	21days	14day	14days

2.4. Psychological parameters assessment

A validate Chinese version of the POMS questionnaire [5] was used to evaluate exercise related mood using the subscales tension (Ten), depression (Dep), anger (Ang), vigor (Vig), fatigue (Fat), confusion (Con) and esteem (Est). POMS total mood disturbance (TMD) obtained by adding all other measures and subtracting vigor (TMD=Ten+Dep+Ang+Fat+Con+Est+100-Vig).

2.5. Blood Collection and Analysis

Earlobe blood samples were collected from each subject for determination of HB and blood urine nitrogen (BUN). The serum BUN levels were measured immediately with an Ektachem DT60 II chemistry analyzer (Johnson and Johnson, Rochester, NY, USA). The hemoglobin was measured with Analyzer (HemoCue® Hb 201+, Ängelholm, Sweden).

2.6. Aerobic Performance.

Peak oxygen uptake ($\dot{V}O_{2peak}$) was measured using a bicycle ergometer (cyclus2 record trainer, h/p/COSMOS, Nussdorf-Traunstein, Germany). The initial workload of the test was 100 W (70-80 rpm), and it was increased by 50 W every third minute until exhaustion. Oxygen uptake ($\dot{V}O_2$) was measured continuously using a gas analyzer (ML870B80 & MLS240 Metabolic Module, PowerLab, ADInstruments, Australia). The blood samples (20 μL) were taken from the earlobe every third minute to measure blood lactate concentrations by using the Biosen_C_line Clinic (analyzer for glucose and lactate, EKF Diagnostics, Barleben, Magdeburg, Germany) and defined works of 4mM (4 mmole/L) lactate threshold (W_{LH}) by para-analysis program (Para-analysis, h/p/COSMOS, Nussdorf- Traunstein, Germany). The heart rate was recorded continuously using heart rate monitor (Polar Electro, Kempele, Finland). Volitional exhaustion was the main criterion indicating that the $\dot{V}O_{2peak}$ was achieved, and the highest mean $\dot{V}O_2$ over 1 minute was set as $\dot{V}O_{2peak}$. The subject's exhaustion was ensured of at least two of the four following criteria: (a) no longer maintaining the required speed; (b) heart rate within 10 beats of age-predicted maximum; (c) a respiratory exchange ratio above 1.05 and (d) rating of perceived exertion (RPE) >18.

2.7. Power Spectral Analysis

The athletes were instructed to avoid physical activity and to maintain their usual food intake, but not to drink alcohol or caffeine for 48 h before the testing. Heart rate (HR) recordings for HRV spectral analysis were performed between 6:20 and 7:20. During the first 15min, the athletes were in a supine position. Electrocardiograms (SSIC, Enjoy Research Inc., Taipei, Taiwan) [6] were used to record the athletes' HR in supine position, and the respiratory rate was set at 12-15 cycles/min. Spectral analysis was performed using the autoregressive model [7]. This procedure allows for the automatic quantification of the central frequency and the influence of each relevant oscillatory component present in the interval series. Components in the frequency band from 0.03 to 0.15 Hz were considered low frequency (LF), and those in the range of 0.15 to 0.4 Hz, which is synchronous with respiration, were considered high frequency (HF). LF/HF components of RR interval are considered to be an expression of cardiac and vascular efferent sympathetic regulation, respectively, whereas the HF component of RR interval variability is considered to be an expression of cardiac vagal modulation [8].

2.8.Statistical analysis

Standard statistical methods were used to calculate the mean and standard deviations (SD). The haemocrit level was used to adjust the other parameters for different dehydration. The differences between the before and after training programs values were statistically examined by paired *t* test. ANOVA with repeated measures was used to determine the differences between tests. When a significant *F* value was achieved, appropriate LSD *post hoc* tests procedures were used to locate the difference between means. Pearson product-moment correlation coefficients (r) were used to determine association between POMS, HRV and BUN variables. The $P<0.05$ criterion was used to establish statistical significance.

3. Results

3.1. Anthropometric characteristics and metabolic values

Table 4 shows the variables concerning the anthropometric and metabolic characteristics of the athletes examined before and after the CET period. There were significantly increasing in the metabolic variables of $\dot{V}O_{2peak}$ and LH after CET.

Table 4. Anthropometric characteristics and metabolic values of the rugby athletes before and after CET

Characteristics	unit	Before training	After training	Change (%)
Age	y	22.7(2.3)	22.7(2.3)	
Height	cm	175.4(5.7)	175.4(5.7)	
Weight	kg	76.5(6.3)	77.2(6.8)	0.92(.67)
Absolute $\dot{V}O_{2peak}$	L/min	3.5(0.6)	4.2(0.7)*	20.7(16.5)
Relative $\dot{V}O_{2peak}$	mL/kg/min	47.8(7.2)	58.6(9.5) *	23.6(16.6)
Works of lactate threshold(4mM)	watts	119.9(49.7)	162.1(54.2)*	40.5(39.1)

Values are expressed as mean (SD). *: $P<0.05$, vs. before training programs.

3.2. Monitoring parameters responses

Table 5 shows the monitoring parameters during CET. We observed significant reduction in HB and elevation in BUN from T1 to T5, especially during SIT1 and SIT2 periods. Training increased BUN levels (T1, 15.0(2.6) mg/dL vs. T5, 27.9 (2.6) mg/dL, $p<.05$), LF/HF (T1, 1.44 (0.18) vs. T5, 1.62 (0.19), $p<.05$) and POMS total mood

disturbance (T1, 100.6 (4.5) scores vs. T5, 136.3(12.3) scores, p<.05). There were similarly increasing significantly in HF and LF/HF at T5 (SIT2). POMS levels at T5 were significant differences compared with T1-T3.

Table 5. Responses of physiological and psychological stress markers in the rugby athletes during CET

Parameters	unit	T1	T2	T3	T4	T5
Hb	g/dL	14.1(0.5)	13.9(0.9)	13.6(0.7)	13.1(2.3)^{AB}	12.3(1.8)^{AB}
Bun	mg/dL	15.0(2.6)	16.3(4.8)	17.6(4.6)	23.8 (5.2)^{AB}	27.9 (2.6)^{ABC}
HF	ln(ms^2)	5.01 (0.44)	4.81 (0.54)	4.94 (0.61)	5.67 (0.74)^{B}	6.48 (0.43)^{ABC}
LF	ln(ms^2)	6.62 (0.34)	6.27 (0.62)	6.43 (0.57)	6.63 (0.56)	6.81 (0.36)^{B}
LF/HF	ln(ratio)	1.44 (0.18)	1.38 (0.55)	1.46 (0.67)	1.52 (0.72)	1.62 (0.19)^{ABC}
Tension	scores	5.0(1.9)	4.7 (2.8)	4.9 (2.7)	6.7 (3.6)^{B}	8.7(2.2)^{ABC}
Anger	scores	3.0 (1.9)	3.3 (2.5)	3.8 (3.1)	4.3 (2.7)^{A}	5.3(2.9)^{ABC}
Fatigue	scores	6.0 (2.8)	5.7 (2.9)	6.1(3.5)	7.5 (5.5)^{B}	9.1 (4.8)^{ABC}
Depression	scores	4.0(1.9)	4.2 (1.5)	4.4 (2.3)	6. 9 (3.9)^{ABC}	7.8 (2.8)^{ABC}
Vigor	scores	12.5 (3.3)	14.4 (2.1)	14.9 (2.6)	12.7 (4.8)^{C}	9.0 (3.2)^{ABCD}
Confusion	scores	3.5 (1.5)	3.1 (1.3)	3.7 (1.9)	4.9 (1.9)^{B}	6.1 (1.8)^{ABC}
Esteem	scores	8.5 (2.3)	8.1 (1.8)	8.4 (2.5)	8.4 (3.1)	8.2 (2.5)
TMD	scores	100.6 (4.5)	98.5 (5.7)	99.5 (6.9)	109.2 (14.8)	136.3(12.3)^{ABCD}

Values are expressed as mean (SD). ^{A}: P<0.05, vs.T1; ^{B}: P<0.05, vs.T2; ^{C}: P<0.05, vs.T3; ^{D}: P<0.05, vs.T4.

3.3. Correlations analysis between physiological and psychological stress monitoring

The BUN and LF/HF were significantly correlated with POMS subscales before and during different CET stages (T1, Table6; T5, Table 7). However, there was only significant association between HF distributions based on TMD.

Table 6. Correlational analysis between physiological parameters and POMS scores before CET (T1).

	Tension	Anger	Fatigue	Depression	Vigor	Confusion	Esteem	TMD
BUN	R=.685	R=.570	R=.615	R=.632	R=.743	R=.657	R=.751	R=.785
	P=.001 *	P=.002 *	P=.008 *	P=.005 *	P=.001 *	P=.003 *	P=.001 *	P=.001 *
HF	R=.079	R=.276	R=.165	R=.176	R=.123	R=.126	R=.089	R=.795
	P=.698	P=.171	P=.420	P=.387	P=.546	P=.539	P=.663	P=.001 *
LF	R=.519	R=.276	R=.432	R=.433	R=.667	R=.469	R=.653	R=.991
	P=.065	P=.171	P=.027 *	P=.027 *	P=.002 *	P=.015 *	P=.003 *	P=.001 *
LF/HF	R=.736	R=.695	R=.660	R=.671	R=.822	R=.695	R=.824	R=.910
	P=.001 *	P=.001 *	P=.002 *	P=.002 *	P=.001 *	P=.001 *	P=.001 *	P=.001 *

*: P<0.05

Table 7 Correlational analysis between physiological parameters and POMS scores at the end of CET (T5).

	Tension	Anger	Fatigue	Depression	Vigor	Confusion	Esteem	TMD
BUN	R=.720	R=.442	R=.471	R=.553	R=.586	R=.627	R=.610	R=.943
	P=.001 *	P=.031 *	P=.015 *	P=.003 *	P=.001 *	P=.006 *	P=.009 *	P=.001 *
HF	R=.297	R=.066	R=.088	R=.132	R=.088	R=.237	R=.189	R=.739
	P=.140	P=.746	P=.667	P=.518	P=.666	P=.243	P=.355	P=.001 *
LF	R=.683	R=.376	R=.375	R=.487	R=.512	R=.582	R=.588	R=.965
	P=.001 *	P=.057	P=.058	P=.011 *	P=.007 *	P=.001 *	P=.001 *	P=.001 *
LF/HF	R=.769	R=.607	R=.541	R=.717	R=.697	R=.763	R=.770	R=.969
	P=.001 *	P=.001 *	P=.004 *	P=.001 *	P=.001 *	P=.001 *	P=.001 *	P=.001 *

*: P<0.05

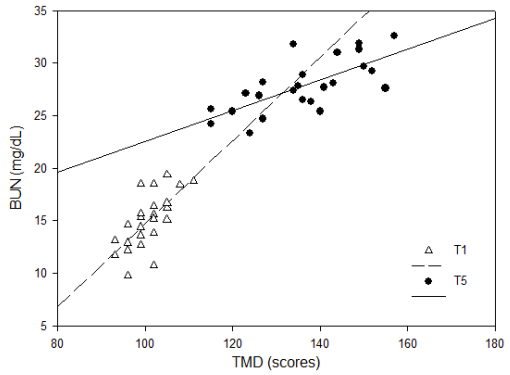

Figure 1. Relationship between the BUN and TMD changes in POMS before (T1) and during (T5) CET.

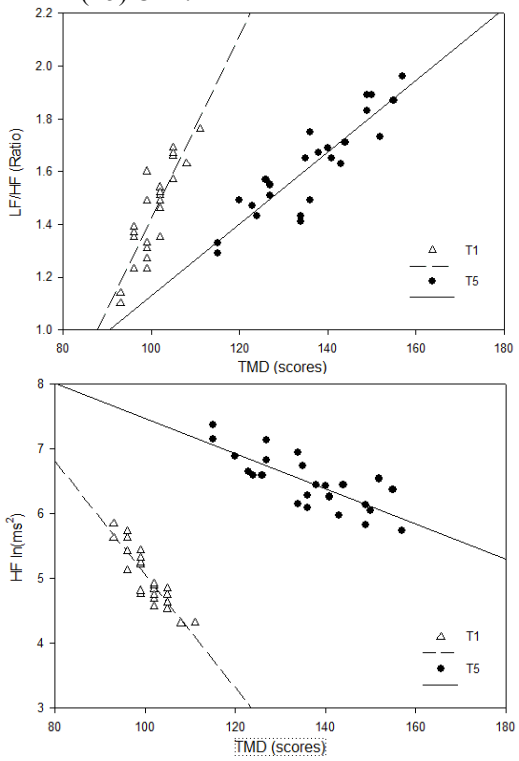

Figure 2. Relationship between the LF/HF ratio, HF and TMD changes in POMS before (T1) and during (T5) CET.

Discussion

The amount of red blood cells (erythrocytes) in the blood of an athlete can have a great impact on performance during both training and competition, particularly for an endurance athlete. Red blood cell content in the blood can be described as the balance between red cell production (erythropoiesis) and red cell destruction (hemolysis). The higher the red cell count in the blood, the better the oxygen-carrying capacity to the exercising muscles. Although endurance training is most often associated with improved oxygen-carrying capacity, intensive training can also induce a substantial hemolysis. This, along with iron stores that are often compromised because of excessive training or poor nutritional habits, may endanger the hematological status of endurance athletes. In this section, the potential benefits of a taper in terms of enhancing an athlete's hematological status and iron stores are discussed. Hematological changes strongly suggest that the training reductions usually applied during the taper facilitate a positive balance between erythropoiesis (i.e., red cell production) and hemolysis (i.e., red cell destruction), which contributes to taper-induced performance improvements. However, increased red cell production could compromise the iron status of athlete because erythropoiesis requires significant iron.

Serum BUN levels increase significantly after intermittent exercise. It means that intermittent exercise may induce renal dysfunction during vigorous exercise. In the present study, serum BUN elevated clearly during SIT1-SIT2, In spite of signals of muscle injury and degradation of somatic tissue proteins. It is possible that the reduced water intake caused concentrated BUN level in blood in the subjects due to increased urination.

When high-intensity exercise is performed, there may be an imbalance between the energy supply and the demand for ATP, eventually leading to the formation of BUN. BUN is also produced when muscle protein reserves are broken down and oxidized during exercise, especially when carbohydrate availability is limited. Blood BUN concentration could be used as a stress marker of the muscles' ability to produce ATP from ADP and also as evidence of increased protein degradation during times of increased training stress.

Many studies have reported that highly intensive exercise causes protein catabolism in blood or tissues. The current result showed that protein catabolism were greater in the SIT than in the ET2 (lactate threshold). One of the objectives of this study was to clarify the relationship between physiological stress (blood biochemical/physiological parameters) and mental fatigue (POMS) caused by physical activity during a series CET. Therefore, our results show that the ET2-CET built up athletes' experiences of physiological stress, mental fatigue and more effective protein catabolism than baseline. These latter results may due to the different styles of training modes of high intensity intermittent training.

However in the stages of SIT1-SIT2, mental fatigue is likely to be influenced by protein catabolism, energy metabolism, degeneration, and injury to muscle tissue. The athletes tolerated intense muscular impact at a high frequency. These results suggest that (a) playing in W_{LH} ET2-CET caused athletes both physical/mental fatigue and new balance adaptation,

(b) the training modes related difference activity were responsible for differences degrees in physical/mental fatigue during CET sessions and (c) health management during a rugby CET periodization is very important for the athletes and should be tailored to each athlete's performance. In the ET2-CET, where metabolism and energy consumption are associated with mental fatigue, ingestion of some quickly acting energy source is thought to be important to adjust recovery from fatigue. In SIT1-SIT2, protein catabolism, degeneration of muscle tissue, and injury are associated with long-term higher metabolic rate and mental fatigue as well as energy metabolism and consumption. We might supply proteins with the energy source and suggest as an effective modes to accelerate recovery from training-evented fatigue.

Psychological condition was modulated during CET. Psychological stress in rugby CET is not only the intensity of the exercise, but also to the athletes' satisfaction with their own performances probably. Therefore in this study the athletes' psychological states may have been affected by the training loads. Thus future studies should apply these results to the series training from pre-season to in-season. However, a decreased POMS tension scores were significant in only the subjects whose rest incorporated low intensity exercise. This result is in accordance with previous findings, that moderate exercise enhances relaxation an decreases psychological stress. Therefore incorporating low intensity exercise into the rest period after CET may achieve better psychological recovery in the athletes. With regard to the psychological responses, POMS questionnaire has been widely applied to investigate the relationship between mood state and athletic performance. Experimental studies with athletes have shown that physical activity can both improve and decrease the POMS scores. Morgan [9] defined the typical athlete profile of the POMS as the "iceberg profile". In the "iceberg profile", the athletes score below the 50th T score on tension, depression, anger, fatigue and confusion and above the 50th T score on vigor in comparison with other subjects. In our study, as noted by other authors, the athletes presented a typical "iceberg profile" over the training program. We also verified a reducing of the fatigue score from T1 to T2, which coincided with the best team performance.

Therefore, we believe that the athletes presented positive adaptations in response to the training program developed between T1, T2 and T3. The vigor scores decreased in T4 and T5 compared to T1 and T2. It response corresponded exactly to the worst physiological conditioning. The descent in the vigor scores may be explained either by the increase in intensity of training and protein catabolism. Higher BUN concentrations and correlation could prove. We find the decrement in the team performance affected the athletes' mood. In respect to the serum BUN response to the CET program, our athletes presented a rise in T4 compared to T1. Kumae et al. [10] did not observe alterations in the serum BUN concentrations in response to an increment in training volume during four weeks in experienced middle and long distance runners. Kumae et al. did not verify changes in the serum BUN of experienced sessions of distance runners (one with increase in the training volume and one with increase in the training intensity) during four weeks either. Besides the difference between the sports modalities used by us and by Kumae et al.'s studies, during training stages in which our subjects presented a rise in the serum BUN concentrations, there was an increment in both volume and intensity (i.e. SIT1-SIT2) of CET (Table 3). In our study, the serum BUN concentrations did not demonstrate any changes in response to the training program.

It is likely that the parasympathetic nervous system is altered in OTS, but specific biomarkers remain to be identified. Changes in heart rate at rest, reduced maximal heart rate during exercise, and changes in HRV, that is, the beat-to-beat changes of the heart, have all been noted as signs of OTS. An increase in resting heart rate has been reported in athletes' OTS, but these findings are not consistent and have not been confirmed in prospective studies. Likewise, we observed HF and LF/HF elevations reflecting higher sympathetic nervous system activity from CET. Studies examining the effects of CET on HRV in athletes are confusing because studies had shown no significant change in parasympathetic modulation until T5. Investigations of HRV in OR or in OTS has been limited[2], but if methodologies are standardized, HRV may be a potential, noninvasive tool for forecasting OR and the development/progress of OTS.

In the highly selected athletes of this study, we observed an effect associated with marked increases in the HF and LF/HF ratio; and decreases in the LF component of HRV. These findings of the studies would confirm that athletes enhances vagal and sympathetic cardiac modulation after CET. Sympathetic vasomotor control also did change from baseline, as indicated by significant variations in the LF component. However, when the training load approached the SIT2 period, neural cardiovascular regulation showed a clear increasing shift from vagal to sympathetic predominance, with concordant changes in hemodynamic variables and in cardiac and vascular indices of autonomic modulation.

The possibility that the increased markers of sympathetic activation would represent an trend of the daily intermittent exercise, because athletes did train vigorously from at least 15 hours before the recording

sessions and signs of enhanced sympathetic activation were detected in the recordings sessions performed with the same experimental schedule at supra-maximal training load. Finally, short-term overtraining in athletes did not alter frequency domain indices of HRV. Our results suggested that enhanced sympathetic activation and attenuated vagal inhibition could represent the neuro-vegetative adaptation for increasing athletic performance. Increasing the inhibitory influences of vagal mechanisms while coexistent enhancing sympathetic activity might serve to prepare the cardiovascular system to the rapid and wide, even anticipatory, variations in heart rate, cardiac output, blood flow redistribution, and muscle microvesscular perfusion of highly performing. Power spectral analysis of short-term HRV might represent a valuable tool to assess the time course of neurovegetative cardiovascular adaptations to CET.

Meeusen, *et al* [11] concluded three factors predominate the prevention of OT. There are (a) that the structure of the training programs must allow adequate regeneration and recovery; (b) the training programs must incorporate monitoring of OT symptoms; and (c) that the normal fatigue associated with training is not confused with that associated with OT. Periodization of training is important in order to allow for performance monitoring, training modification and to avoid training monotony[9]. Intensive training in short bursts with frequent intermittent modes, and sudden increases in training load should be avoided. But team sport training generally involves a diverse range of training activities, often under quite variable environmental conditions, as well as interindividual variability in the responses and adaptations to training. These issues complicate the integration of training variables into quantifiable units.

The main limitation of this study is the lack of a control group. However, this would be more a theoretical than an actual methodological limitation within the framework of our investigation. The ideal protocol of CET with a crossover design is virtually impossible in all levels athletes. A final potential limitation of this study includes the indirect testing autonomic function by HRV.

CET elevated biological stress increasing BUN, POMS scores and HRV modulation. According to training loads periodization, there are strong correlations among training load and physiological/psychological stress. CET should be significantly modified sufficiently physical conditioning, maintained to provide adequate stimuli and altered the neural mechanisms of cardiovascular regulation shifted the cardiovascular autonomic modulation from a parasympathetic toward a sympathetic predominance with enhanced sympathetic activation and reduced vagal inhibition.

In conclusion, the results of this study indicate that 8 weeks periodized CET would increase aerobic performance and strengthen cardiovascular regulation in rugby athletes. A noninvasive monitoring method could be developed by POMS highly correlated HRV and BUN for physiological controlling in periodization.

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