# Libyan Journal of Medical Research

## General Director of the
## National Medical Research Center
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## Editorial

### The Promising of Molecular Biology in Treatment and Safety of our Patients

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It is estimated that the human body consists of 75 trillion cells, classified to 200 types. Each cell has recognition molecules called receptors. These receptors are found intracellular like steroid hormones, retinoic acid and thyroid hormone receptors or they are attached to the cell membrane like insulin and glucose receptors. These receptors recognize their ligands which are distributed in the extracellular fluid. In example: insulin released from *Beta* cells of the pancreas circulate in the blood will bind to its receptors which are localized in the plasma membrane of the liver or fatty cells or muscle cells. This binding will lead to further intracellular actions needed for glucose receptor synthesis and these glucose receptors will migrate to their location in the cell membrane, facilitating the glucose entry.

In the recent decades, these molecular structures have been extensively studied and led to manufacture of pharmacological substances (drugs) to imitate the ligand interactions with their receptors like *Beta* receptor stimulating and inhibiting agents.

It is well known that, 150 of the top best market leading drugs act on G protein coupled receptors.

Aljarari and Alexander in their paper entitled 'identification of adenosine receptor subtypes expressed in human neuroblastoma cell line SH-5Y5Y' which is published in this issue, have identified a group of sub receptors which belong to the G-protein coupled receptors. They have demonstrated that; these receptors which recognize Adenosine as a ligand are widely distributed in different organs of the human body including neuroblastoma cells, which contain the G-protein coupled sub-receptors A2B, A2a. Because of the wide distribution of Adenosine in the body and its recognition by the sub receptors mentioned by the authors, this gives rise to the possibility of using Adenosine in the treatment and control of the neuroblastoma which is a malignant disease. It is well known that this ligand (Adenosine) is used to control some types of arrhythmias like supraventricular tachycardia (SVT).

To continue with the great advances of molecular biology, Abdul M. Gbaj and co-authors in their paper: 'Titration of 12 mer CYP2C9 probe with 24 mer target in solution', published in this issue, describe a simple titration method using 5-pyrenyl probe to determine the concentration of CYP2C9 DNA. This simple process makes it possible to identify the genes responsible for the different sub types of cytochrome enzymes P450 which are the key enzymes in the metabolism of different drugs. This means we can identify how a patient will respond to a given drug. According to the enzyme sub-types which are the editions of his or her polymorphic genetic code. This is a very attempting to compare this procedure with the blood group cross matching. The identification of gene polymorphism, as the authors of this paper attempt to reach using the above mentioned titration method, will be of a great step forward to make drugs more safe and useful.

# Simulation of SAR and Temperature Distributions in 3D Model of the Human Head Exposed to Mobile Phone Radiation at 900 MHz

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## Abstract:
In this study, using the finite element method via ComsolMultiphysicssoftware package, specific absorption rate (SAR) distributions andtemperature increase are simulated in 3D human head model exposed to the field radiated from cellular phonewhich consists of square patch antenna. Both Maxwell and bioheat equations with suitable boundary conditions are solved to find SAR and temperature distributions. The maximum log scale of SAR calculated was of the 0.6 while the maximum temperature increase was 0.3 $^{\circ}$Cfor 900 MHz from the antenna

## 1. Introduction

As a result of the significant increase in portable phones use in recent years it has become the subject of research and studies.These studies indicated apotential health hazards owing to the absorption of radio frequency (RF) radiation emitted by portable telephones. RF waves emitted by these mobiles have been linked to brain cancer, salivary gland tumors, behavioral problems, and migraines. These risks have been shown to be higher in people who have used cell phones for at least ten years [1]. However, studies on brain cancer cast doubt on these results since it is difficult to accurately assess risk factors in humans [2].

It is broadly accepted that mobile phones cause heating of the human organ exposed to their radiation and specifically the human head. The current exposure limits are based on Specific Absorption Rate (SAR) of the exposure heat. The SAR parameter has been widely used to determine the possibility of health hazards in the human head because of radio

Most previous studies of human exposure to electromagnetic field were limited with respect to the electric field and SAR distribution. Nevertheless, they have to an incomplete analysis. Therefore, to approach reality, modeling of this work, it prefers a real link between the heat transfer and electromagnetic radiation.

frequency (RF) radiation [3, 4]. A SAR limit of 2W/kg averaged over any contiguous 10 g head tissue was recommended by the Council of European Union [5] for the general public.Some significant thermal damage can occur in sensitive organs under conditions of partial body exposure to RF electromagnetic waves. Mobile phones are electromagnetic radiation devices, which may be harmful to human health from their radiation. Thus, it is interesting to analyze the heat transfer in the human head due to electromagnetic wave exposures.In accordance with the development of the computer and numerical analysis techniques, an anatomical human head model can be incorporated into simulated studies. Recently, the modeling of heat transport in human tissue has been investigated. Pennesbioheat equation, introduced by Pennes [6] based on the heat diffusion equation, is frequently used for analysis of heat transfer in human tissues.

not been considering heat transfer in their model during exposure to electromagnetic fields. That leads                    …

Therefore, in order to provide information on levels of exposure and health effects from mobile phone radiation, it is important to simulate both electromagnetic

field and heat transfer within an anatomically based humanhead model to represent actual processes of heat transfer within the human head. In this study, a three-dimensional human head model was used to simulate the SAR distribution and the temperature distribution over the human head. The 900 MHz frequency was chosen for the simulations in this study, as it is used frequently in the areas of cell phone usage.

## 2. Method and model

The human head geometry is the same geometry (SAM Phantom) provided by IEEE, IEC, and CENELEC from their standard specification of SAR value measurements. Geometrical data file was created from a magnetic-resonance image (MRI) of a human head; these images contain 109 slices,each with 256-by-256 voxels (7). The model comprises four types of tissue including skin, fat, skull, and brain. These tissues have different dielectric and thermal properties (8,9).

Finite element method (FEM) via COMSOL[TM] MULTIPHYSICS version 5.1 carries out this study.In this study, a square patch antenna is considered as a source of electromagnetic radiations and is placed at the left side of the head model at a distance of 1cm. Figure (1) shows athree-dimensional finite element mesh of the human head model exposed to radiations from a mobile phone which consists of square patch antenna.
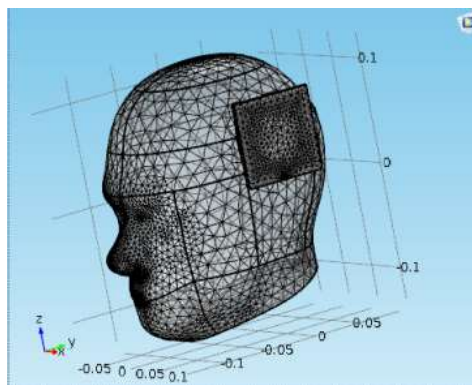


Fig. 1A three-dimensional finite element mesh of human head model with square patch antenna

## 3. Mathematical modeling

### 3.1. **Governing equation of Electromagnetic Wave Propagation**

Mathematical models are developed to predict the electric field and SAR with relation to temperature gradients within the human head. The electromagnetic wave propagation in a human head is calculated using Maxwell's equations [10,11]. The general form of Maxwell's equations is simplified to illustrate the electromagnetic field penetrated in human head as the followingequation:

$$\nabla \times \frac{1}{\mu_r} \nabla \times E - k_o^2 \varepsilon_r E = 0 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

where E is electric field intensity (V/m), $\mu_r$ is relative magnetic permeability, $\varepsilon_r$ is relative dielectric constant, and $k_0$ is the free space wave number (m[-1]).

### 3.2. Boundary Condition of Wave Propagation

As the electromagnetic energy is emitted from the patch antenna and interact with the human head with a particular radiated power. The lumped port is used to define a voltage drop in microstrip patch antenna. Therefore, the boundary condition for solving electromagnetic wave propagation, is described as shown in Fig. 2.

$$n \times (\nabla \times E) - jkn \times (E \times n) = -n \times (E_o \times jk(n - k)\exp(-jk.r)$$

$$n.(k\nabla T) = 0$$

$$n.(k_u \nabla T_u - k_d \nabla T_d) = 0$$

$$n \times (E_1 - E_2)$$

$$n \times E = 0$$

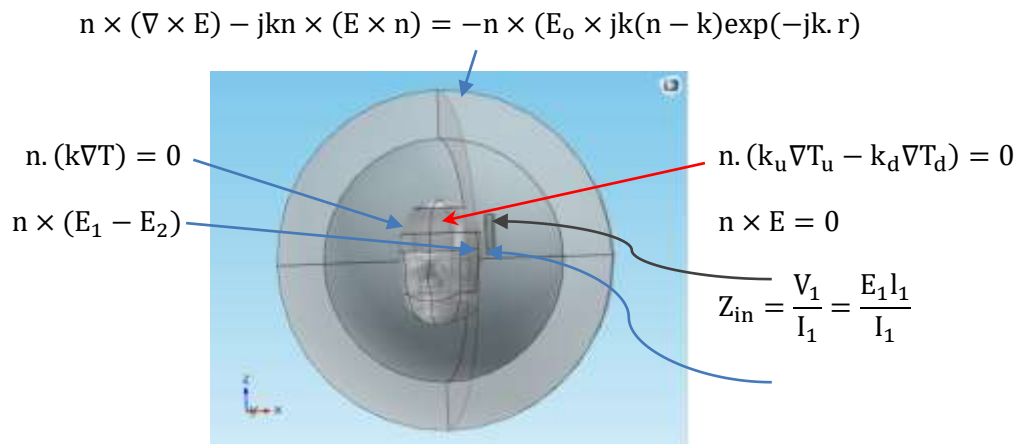$$Z_{in} = \frac{V_1}{I_1} = \frac{E_1 l_1}{I_1}$$

Fig. 2 Boundary condition for both electromagnetic wave propagation and heat transfer

At the bottom of the patch antenna, an electromagnetic simulator employs lumped port boundary condition with specified radiated power

$$Z_{in} = \frac{V_1}{I_1} = \frac{E_1 l_1}{I_1}$$ …………………………………………….……………….(2)

where $Z_{in}$ is the input impedance ($\Omega$), $V_1$ is the voltage along the edges (V), $I_1$ is the electric current magnitude (A), $E_1$ is the electric field along the source edge (V/m), and $l_1$ is the edge length (m).

the perfect-electric-conductor boundary condition along the patches on the antenna is considered

$$n \times E = 0$$ …………………………….............……………….……….. (3)

Boundary conditions along the interfaces between different mediums, for example, between air and tissue or tissue and tissue, are considered as continuity boundary conditions

$$n \times (E_1 - E_2)$$ ………………………………………….…………… (4)

The outer sides of the calculated domain, i.e., free space, are considered as scattering boundary conditions [10]

$$n \times (\nabla \times E) - jkn \times (E \times n) = -n \times (E_o \times jk(n - k)\exp(-jk.r)$$ ….. (5)

where k is the wave number (m$^{-1}$), $\sigma$ is the electric conductivity (S/m), n is the normal vector, $j = \sqrt{-1}$ and $E_o$ is the incident plane wave (V/m).

### 3.3. Equation of Heat Transfer

The temperature distribution within the human head is obtained by solving Pennes' bioheat equation [10,12], the equation can be written as

$$\rho C \frac{\partial T}{\partial t} = \nabla.(k\nabla T) + \rho_b C_b \omega_b (T_b - T) + Q_{met} + Q_{ext}$$ ……………………… (6)

where $\rho$ is the tissue density (kg/m$^3$), C is the heat capacity of tissue (J/kg K), k is the thermal conductivity of tissue (W/m K), T is the tissue temperature ($^o$C), Tb is the temperature of blood ($^o$ C), $\rho_b$ is the density of blood (kg/m ), $C_b$ is the heat capacity of blood(3960 J/kg K), $\omega_b$ is the blood perfusion rate (1/s), $Q_{met}$ is the metabolism heat source (W/m$^3$), and $Q_{ext}$ is the external heat source(electromagnetic heat-source density) (W/m ).

The heat conduction between tissue and blood flow is approximated by the blood perfusion term, $\rho_b C_b \omega_b (T_b - T)$. The external heat source term is equal to the resistive heat generated by the electromagnetic field (electromagnetic power absorbed), which is defined as [10]

$$Q_{ext} = \frac{1}{2}\sigma_{tissue}|E|^2 = \frac{\rho}{2}.SAR \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (7)$$

Where $\sigma tissue = 2\pi f \varepsilon_r' \varepsilon o$

Where SAR is the energy of electromagnetic wave propagation absorbed by the tissue.The specific absorption rate is defined as power dissipation rate normalized by material density [10,13]. The specific absorption

rate is given by

$$SAR = \frac{\sigma}{\rho}|E|^2 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (8)$$

### 3.4.**Boundary Condition of Heat Transfer**

Heat transfer is considered only in the human head, which does not include parts of the surrounding space. As shown in Fig. 2, the outer surface of the human head corresponding to assumption (3) is considered to be a thermally insulated boundary condition

$$n.(k\nabla T) = 0 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (9)$$

It is assumed that no contact resistant occurs between the internal organs of the human head. Therefore, the internal boundaries are assumed to be a continuous

$$n.(k_u\nabla T_u - k_d\nabla T_d) = 0 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.. (10)$$

### 4. **RESULTS AND DISCUSSION**

In this study, the mathematical model of bio heat transfer and electromagnetic wave propagation performed for a mobile phone consisting of a patch antenna radiating maximum 1W power at 900 MHz.. For the simulation, the dielectric and thermal properties are directly taken from [8,9], respectively.The exposed radiated power used in this study refers to ICNIRPstandard for safety level at the maximum SAR value of 2 W/kg[20]

### 4.1.**SAR Distribution**

The results of the simulations performed with COMSOL™ MULTIPHYSICS are shown in Figs. (3-5). It has been shown in the Figure 3 the maximum amount of SAR locates on the ear region and also it has the value of (0.603-0.654) W/kg. It is obvious that the regions near the antenna have the largest SAR values and by keeping away from these regions the SAR values diminish.Figure (4) show the distributions of the local SAR, at the y=0 plane; in xz plane in (W/kg), on the human head. It is evident from these results that the dielectric properties, [8,9], become significant to SAR distributions in human tissue when electromagnetic energy is exposed in these tissues. The magnitude of dielectric properties in each tissue will directly affect the amount of SAR within the human head. Comparing these results to the ICNIRP limit of SAR value (2W/kg), one sees that the resulting SAR from this study does not exceed the limit value
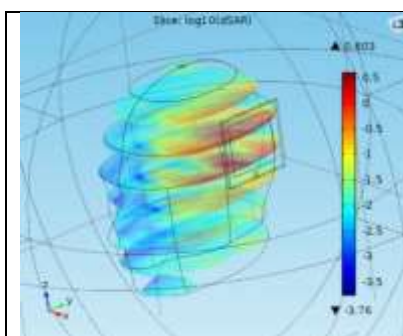


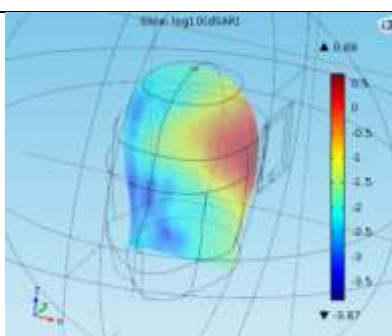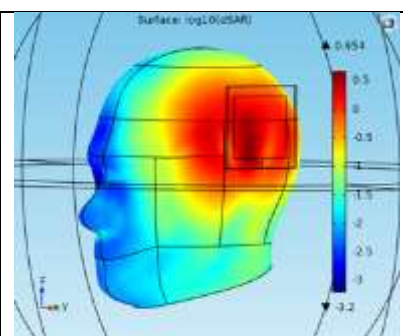| | | |
|---|---|---|
| Fig.3 Log scale of the SAR distribution in brain region of human head model at 900 MHz. | Fig.4 Log scale of the SAR distribution in brain region of human head model at xz plane for 900 MHz | Fig.5 Log scale of the SAR distribution over surface of human head model at xz plane for 900 MHz. |

4.2.Temperature Distribution.

Electromagnetic wave propagation and unsteady bioheat transfer are coupled together to study the heat transfer within the human head. Due to these coupled effects, the electric field distribution in the headisconverted into heat by absorption of the tissues. Simulations to obtain the temperature distributions using 900 MHz aredepicted in Fig. (6-8).
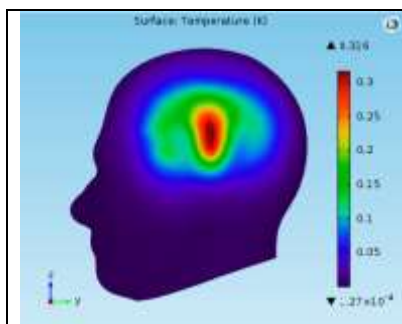


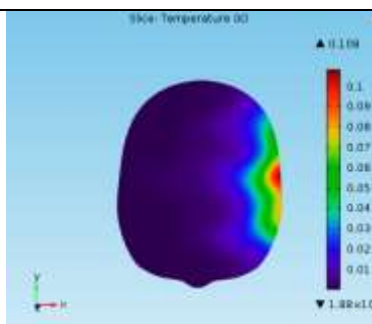Fig. 6 The local temperature distribution in brain region of human head model at 900MHz.



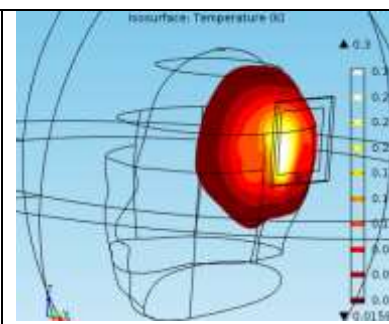Fig.7Top view of the local temperature distribution in brain region of human head model at 900 MHz.



Fig.8 Isothermal contours of the temperature profile of the whole head at 900 MHz.

The temperature is highest closest to the antenna. The maximum temperature increase (from 37 °C) is approximately 0.3 °C, and drops rapidly inside the head. The obtained results confirm the importance of performing a thermal analysis together with the dosimetric one. SAR levels in the tissues are less than the safety limit recommendations [3,5,15]. However, it is found that the induced temperature elevation in the brain, in all the examined conditions, never exceeds 0.4ºC.the obtained results were very close to those presented in the literature using more sophisticated models (10,12)

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# Estimated Risk of RadiationfromCardiac Nuclear MedicineImaging

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## ABSTRACT

In light of the rapidly increasing frequency of cardiac nuclear medicine examinations and as a part of a nationwide survey to estimate patient exposure to radiation from diagnostic nuclear medicine at Tripoli Medical Center. The purpose of our study was to assess the effective dose and cancer risks attributable to radiation from heart scanning.

Organ effective doses and cancer risk factor for males and females as a function of different doses for three hundred cases for different cardiac protocols (rest and stress ) using $^{99m}$Tc-SistaMIBI wereestimated by using RADAR and Olinda software package.

The results indicate that , The collective effective dose for the female and male cases were 15.35 human-Sv, 12.23 human-Sv respectively, while the effective dose and cancer risk factor were:20.2mSv ,10.1 and 19.23 mSv , 9.23 respectively.

**Key words**: nuclear medicine, cancer, cardiac,sistaMIBI,RADAR,effective dose .

## INTRODUCTION

Several different radiopharmaceuticals have been used in recent years for cardiac imaging in nuclear medicine. The dosimetry (i.e., published doses) of these agents may be quite different, and dosimetry issues for several of these agents have generated some confusion and concern. Here the technical basisfor the dose estimates for various agents used in nuclear cardiology is described and differences in the dosimetry of the agents are discussed. Product package inserts, limited dose compendia, and other sources sometimes have presented conflicting and confusing information about the dosimetry of these agents.

Practitioners sometimes have expressed concern about how differences in radiationdosimetry may affect the choice of a radiopharmaceutical. The dosimetry (organ absorbed doses and effective doses) of radiopharmaceuticals currently used in nuclear cardiology is reviewed, and uncertainties in the doseestimates are discussed. Relative radiation risks for these radiopharmaceuticals also are discussed. The principal motivation for this effort was to address questions from nuclear cardiologists about whether a particular radiopharmaceutical is preferred over another on the basis of their respective radiation risks. In addition, I describe an analysis demonstrating that the differences in most radiation dose estimates for the agents in question are small compared with the absolute uncertainties of these estimates. The use of risk models in evaluating the radiation risks of diagnostic studies also is briefly discussed. In the absence of universally accepted standard son cardiac radiopharmac euticalad ministered doses, practicevarieswidely across Nuclear medicine center in Tripoli. To obtain objective data regarding the effectivedoseandradiationriskfactoronsuch variations,wesurveyedmainlyTripolimedica lcenterwheremostofnuclearmedicinediagno sis are performed.

## Materials and Methods

Tc-Mo generator with total activity  500 m Ci  was  imported  regularly  from  France from

Biosiscompany,pharmaceuticalkits(Metho xyisobutylisonitrileMIBI)from  the  same source. The imaging tool was dual head gamma camera from Philips company ,the full    description    and    pacifications mentioned below.

The dual head skylight gamma camera was installed at T.M.C on(26/9/2006).

The Skylight Dual Head gamma camera features    two    detector    heads,    each consisting of remove able Low Energy High      Resolution      (LEHR)collimator, aNaI(Tl)(sodium      iodide      doped      with thallium) scintillation crystal, alight guide and an array of(55) photo multiplier tubes PMTs).The LEHR collimate or features parallel

holes(2.45cmheight,0.16mmseptalthicknes s)withhexagonalcellsof1.11mmdiameteran disused  with  low  energy  sources  such as[99m]Tc.The    NaI(Tl)scintillating    crystal was  a  9.5mm  thickness  single  planar crystal

withalightyieldofabout40000photonsperMe V    of    deposited    energy    and anemissionspectrumpeakedat415nm.

### PATIENT DATAANDPREPARATIONS

This    study    included    data    from 379cases(187male,192female;          age range,( 3 5  -7 2 y,mean;4 2 y) that were referred    from    different    cardiology departments in Libya to Tripoli Medical Center for nuclear medicine scan.

### [99m] TC-SESTAMIBI

Myocardial Perfusion Imaging with ^{99m}Tc-Labeled Radiopharmaceuticals (Sestamibi ) Two-Day Protocol. Two-day protocols are best in terms of taking advantage of the physical properties, pharmacokinetics, and acquisition,   processing,   and   display parameters  of  99mTc-labeled  agents. Typical doses for 1- and 2-d protocols are shown in Table 3. The advantages of 2-d

Theskylightgammacamerahasresolutionofa pproximate5mm                  (intrinsic resolution),thestandards of ware  provides filtered      back      projection(FBP) (OSEM)(Internal).This    dissertation    was conducted specifically on this camera.

Sky light camera canals have ability to image wide variety of patient son many types of bed sin variety of positions, there will  also  behigh  through  put  capability with fully automate acquisition work flow for all procedure types, and dual planar simulate eosin mating of two patient son one system.

Sky  light  cameras  unique  architecture allows gamma detectors to be mounted directly  into  room's   structure;  creating gantry- free or" open floor" design  by removing  the  limitations  associated  with conventional  floor-mounted  systems, Sky light can image awed orange of patient son many types of  bed sin variety of positions. Sky light camera allows clinicians to image two different patients simulate oily, off erring  unparalleled  efficiency  gains  for busy image Nuclear Medicine departments.

Different    can    protocols    have performed(rest and stress) for the above mentioned cases, the full procedures for each scan will be   mentioned bellow in details.

protocols are as follows: flexibility of scheduling  stress–rest  imaging;  better patient flow;

ability to image obese patients; higher dose; better gated rest–stress imaging; no cross talk or cross contamination; optimal defect contrast with minimal background activity;          elimination          of

day 2 study if stress study is normal; andhigh accuracy for detecting coronary artery disease in patients with a low likelihood of coronary artery disease. The disadvantages of 2-d protocols are as follows: the need for 2 d; inconvenience for patients; delay in diagnosis;

and camera time relative to that used for dual-isotope simultaneous acquisition, which is not recommended because of energy window cross talk.

Imaging Protocol for [99mTc]-Labeled Agents. For stress imaging, 555 MBq–1.11 GBq (15–30 mCi) is injected at peak exercise. Gated SPECT is performed from 15 min to2 h after injection, preferably within 15–30 min, to maximize stress myocardial defect contrast and minimize hepatobiliary and gastrointestinal interference. For rest imaging, 555 MBq–1.11 GBq (15–30 mCi) is injected at rest. Gated SPECT is performed within 45–60 min after injection. A 60-min delay is optimal for adequate hepatobiliary clearance of the radiotracer.

Imaging too soon after injection will result in increased residual liver activity and increased counts in the adjacent inferior wall because of scatter and scaling. Waiting too long will decrease total myocardial count density and increase gastrointestinal interference. The optimal imaging window is when radiotracer activity has cleared from the liver and not concentrated in the gastrointestinal tract, that is, loops of bowel and the stomach (retrograde flow). In the event that there is a significant splanchnic or bowel overlap with the inferior wall, various maneuvers, such as drinking water or milk or eating fatty food, can be tried to

alleviate the problem before repeating delayed imaging. One-Day Rest–Stress or Stress–Rest Protocol Performed with Sestamibi . The rest–stress sequence issignificantly better than the stress–rest sequence in terms of detecting the reversibility of stress-induced perfusion deficits. With the stress–rest sequence, the rest activity obscures some of the stress defect, resulting in the degradationof image contrast and a reduction in the detection of true reversibility.

With the rest–stress sequence, there is a greater difference in counts between normal and abnormal areas of the myocardium on stress images. This difference results in better normalization of the abnormality on rest images. A 3- to 4-h delay between rest imaging and stress imaging allows radioactivity to decay by 29%–37%, thereby providing better image contrast (7,8,9). However, the American Society of Nuclear Cardiology guidelines (10) offer the option to proceed immediately from rest imaging to stressimaging during a 1-d protocol as long as the higher dose is 3.5–4.0 times the lower dose. Images from a 2-d protocol and images from a 1-d (rest– stress) protocol are shown in Figure 1. Both studies are normal. The studies were obtained with the same camera.

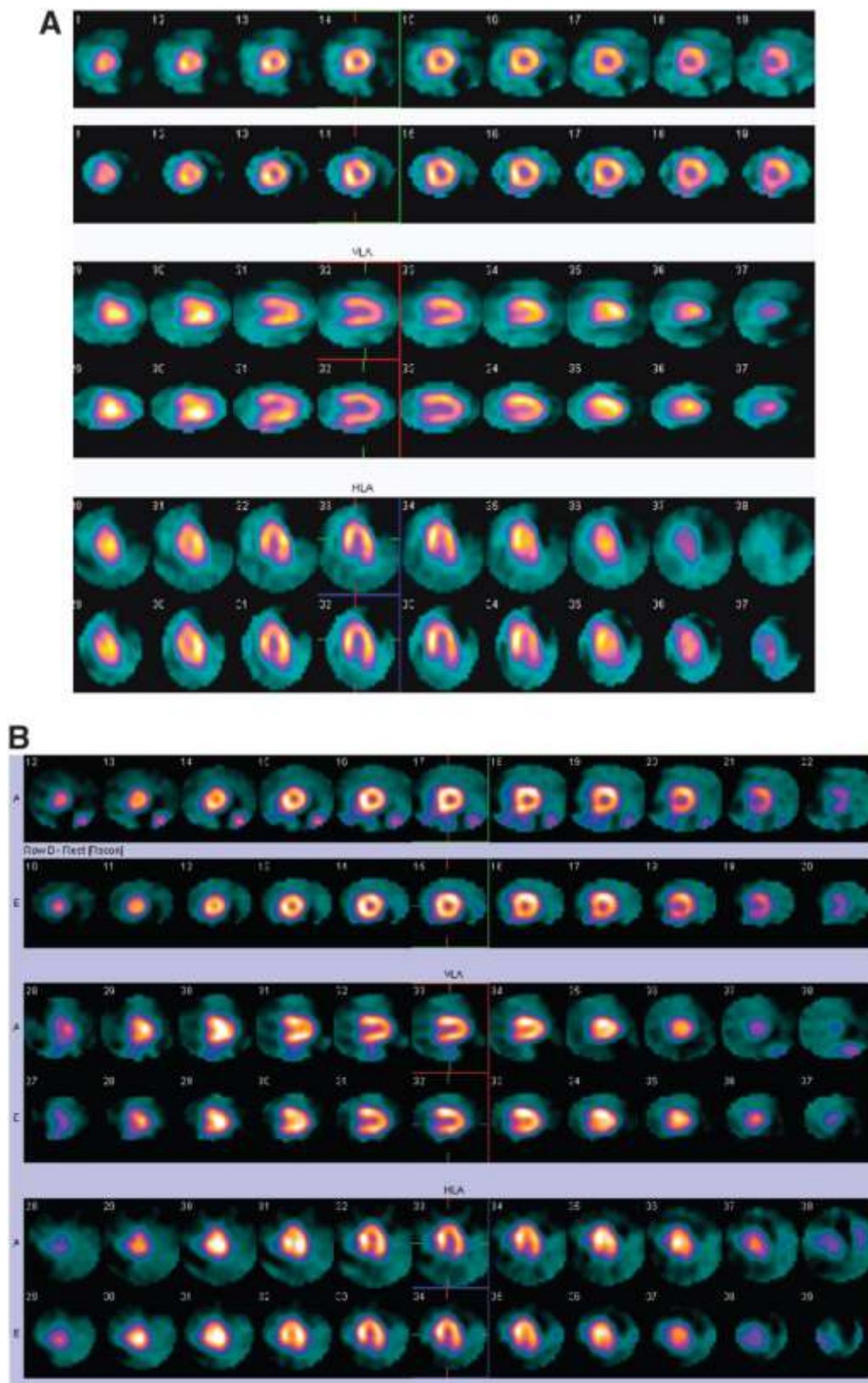The images from the studies are very comparable in features and quality.

Figure 1. (a) Images obtained with 2-d $^{99m}$Tc-sestamibi protocol. Findings are normal.
(b) Images obtained with 1-d $^{99m}$Tc-sestamibi protocol. Findings are normal.

Table1Injecteddoses for some female and male patients for cardiac scan with$^{99m}$Tc-SESTAMIBI

| sex | age | Weight(kg) | Dose in stress (mCi) | Dose in rest (mCi) |
|---|---|---|---|---|
| F | 73 | 109 | 35 | 30 |
| F | 57 | 77 | 15 | 30 |
| F | 56 | 82 | 25 | 30 |
| F | 72 | 75 | 15 | 38 |
| F | 60 | 49 | 13 | 38 |
| F | 65 | 97 | 13 | 30 |
| F | 44 | 85 | 16 | 25 |
| F | 66 | 76 | 10 | 30 |
| F | 50 | 55 | 10 | 30 |
| F | 80 | 75 | 10 | 30 |
| F | 47 | 76 | 15 | 39 |
| F | 68 | 79 | 13 | 30 |
| F | 79 | 79 | 15 | 30 |
| F | 35 | 76 | 15 | 30 |
| M | 58 | 103 | 30 | 15 |
| M | 57 | 73 | 30 | 16 |
| M | 42 | 86 | 35 | 12 |
| M | 44 | 77 | 15 | 30 |
| M | 49 | 75 | 10 | 30 |
| M | 49 | 76 | 10 | 30 |
| M | 49 | 75 | 10 | 30 |
| M | 55 | 105 | 33 | 30 |
| M | 51 | 49.5 | 16 | 35 |

## SOFTWARE USED

OLINDA(copyrightVanderbiltUniversity,2003) perform internal dose calculations, principally for radiopharmaceuticals, using the RADAR method of dose calculations and RADAR dose factors. RADA Ris the Radiation Dose Assessment Resource, which his a working group that maintains resources for internal and external dose calculations, mostly giv e non a web site[73], but also in a number of open literature publications. The Organ Level Internal Dose Assess men code, OLINDA, implements the dose factors from the RADAR web site in a code that permits users to enter kinetic at a for radiopharmaceuticals (or fit them from time-activity data).A number of models are provided, established by various authors in the literature.

## RESULTS ANDDISCUSSION

Fig 1.1 shows the relationship between the injected dose (mCi) and effective do seinfemale cardiac scanning using Sistamibi labeled with$^{99m}$Tc fordifferentagegroups.Fig1 .2showstherelationbetweentheinjected dose and risk factor for the same group of female patients. The trend shows linear or relation between effective do send risk factor for different age groups. The results also indicate the same trend between injected dose and risk factor .Its of ten the case that an increase in injected dose will improve the

image quality at the expense of higher effective dose and consequently higher risk factor swell. Conversely, when the injected dose is reduced as shown inFig1.1&1.2the effective dose and risk factor will decrease dramatically.
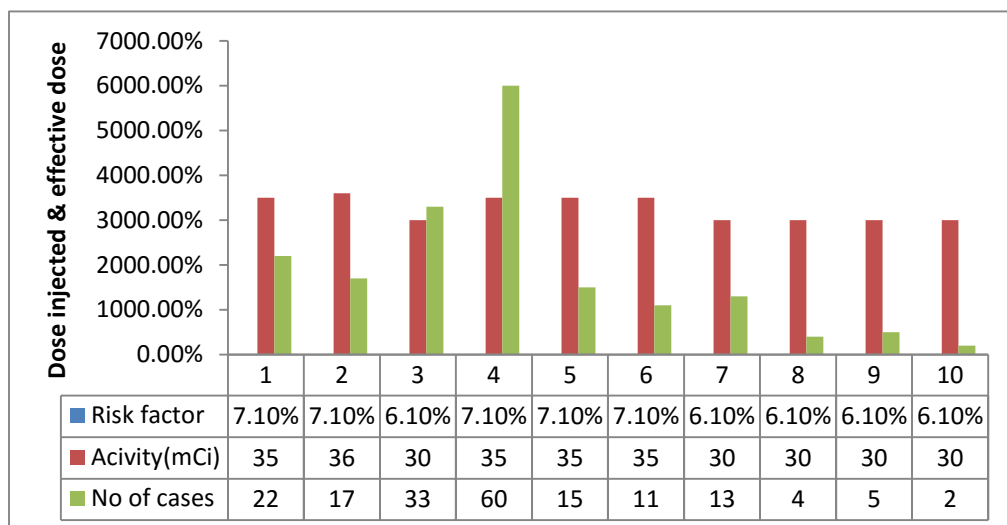


| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Risk factor | 7.10% | 7.10% | 6.10% | 7.10% | 7.10% | 7.10% | 6.10% | 6.10% | 6.10% | 6.10% |
| Acivity(mCi) | 35 | 36 | 30 | 35 | 35 | 35 | 30 | 30 | 30 | 30 |
| No of cases | 22 | 17 | 33 | 60 | 15 | 11 | 13 | 4 | 5 | 2 |

Fig1 .1RelationshipbetweenInjecteddoseandEffectivedoseinfemale cardiac  patients scanned with $^{99M}$Tc-Sistamibi.



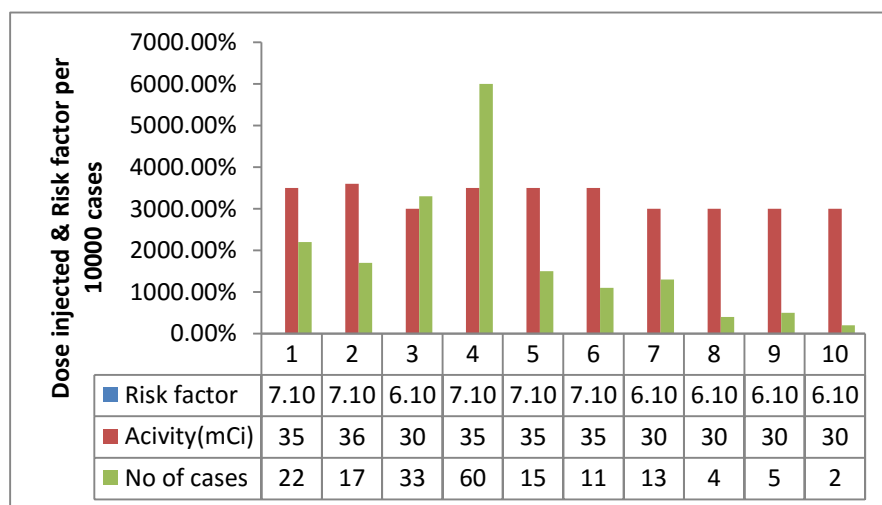| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Risk factor | 7.10 | 7.10 | 6.10 | 7.10 | 7.10 | 7.10 | 6.10 | 6.10 | 6.10 | 6.10 |
| Acivity(mCi) | 35 | 36 | 30 | 35 | 35 | 35 | 30 | 30 | 30 | 30 |
| No of cases | 22 | 17 | 33 | 60 | 15 | 11 | 13 | 4 | 5 | 2 |

Fig1 .2 RelationshipbetweenInjecteddoseandRisk factor percentage  per 10000  cases in female cardiac patients scanned    with $^{99m}$Tc-Sistamibi.

These potential risks of low-dose radiation must be weighed against the benefits of cardiac imaging. For this discussion, we will focus on single-photon emission computed tomography (SPECT) because it has the most substantial evidence base among the high-dose, rapid-growth cardiac imaging modalities .Conceivable benefits of cardiac imaging include correct diagnosis, accurate prognostication, and improvement of patient outcomes. Such outcomes could include appropriate refocus on lt ergative on cardiac diagnoses (e.g., in patients with chest pain and normal SPECT studies), improved quality of life (e.g.,

attributable to relief of chest pain), and improved survival. Patients with high risk of disability and death from cardiovascular disease have the greatest potential absolute gain from appropriate diagnosis and management. This risk varies with age, cardiovascular risk factors, symptoms, and previous evidence of coronary artery disease (CAD) (i.e., myocardial infarction or revascularization) (11,12).In symptomatic patients, the ability of stress (either exercise or pharmacologic) SPECT myocardial perfusion imaging (MPI) to diagnose potentially treatable coronary artery disease is well established. For example, in patients who have uninterruptable baseline electrocardiograms as the result of pre-excitation or left bundle branch block, there is a class I recommendation for SPECT MPI (evidence and/or general agreement that the procedure is be neficial, useful and effective) in the American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) Guidelines forthe management of chronic stable angina. The use of SPECT MPI also can help establish the prognosis of patients with CAD. For example, the risk of cardiac death or myocardial infarction increases as the overall size of myocardial perfusion defects on SPECT MPI increases and left ventricular ejection fraction (which can be determined from

gated images)decreases (13). The ACCF/AHA guidelines include several class I indications for the use of SPECT MP Ian risk stratification (14).However, the dentition of diagnosis or prognosis does not necessarily imply improved outcomes inters of patient survival, except in certain subgroups of patients. A potential survival be net conveyed by cardiac imaging is very relevant to our discussion because it must be balanced against the projected risk of reduced longevity from cancer. Although some radiation-related cancers (leukemia, thyroid cancer, bone cancer) can have short latency

periods of 2 to 5 years, most solid cancers have latency periods of 10 to 40 years.

In comparison, approximately one-half of patients with 3-vessel CAD and abnormal left ventricular function will die within 5 years with medical therapy. Because these patients would not otherwise survive the latency period of a radiation-induced cancer, cardiac imaging with ionizing radiation cane used to identify these patients and thereby

improve their management and longevity. The probabilities of either adverse outcome will vary greatly for septic clinical scenarios, but in the short-term (e.g., 5 years) the risk from CAD is generally far greater than the risk from radiation induced cancer.

**CONCLUSION**

Radiation dose estimates for radiopharmaceuticals used in nuclear cardiology may vary, depending on the source of data used in their generation. Uncertainties in applying dose estimates to individual subjects or populations are considerable because of the use of standardized biogenetic and anatomic models. Considerations such as diagnostic accuracy, ease of use, image quality, and patient comfort and convenience should generally dictate the choice of

radiopharmaceutical, with radiation dose being only a secondary or even a tertiary consideration. Counseling of nuclear medicine patients who may be concerned about exposure should include a reasonable estimate of the median dose for the type of examination and administered activity of the radiopharmaceutical; in addition, it should be explained that the theoretic risks of the procedure are orders of magnitude lower than the actual benefits of the examination.

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# Humoral Responses Toward Cercarial Secretions Of *Schistosoma Mansoni*: Relationships With Age, Sex and Prevalence of Infection

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## Abstract

Exposure to mercurial secretions induces specific antibody responses, which can be useful to evaluate exposure to *S. ma`nsoni* infection. This paper describes work designed to measure the anti-CTF response (IgG1, IgG4 and IgE) in individuals from a schistosomiasis endemic area of Piida village, Uganda. The predominant anti-CTF antibodies in sera were IgG1 and IgG4. IgG4 specifically recognized antigens at approximately 30 kDa, 46 kDa and 58 kDa molecules. In addition, IgE antibody weakly recognized some molecules of CTF at approximately 22 kDa, 58 kDa and 80 kDa. The effects and the interactions of age, sex, prevalence and intensity of infection on specific antibody levels were also assessed. This study demonstrated that there were different responses in sex dependent age groups. In addition, the anti-CTF IgG1, and IgG4 responses were significantly higher in the age groups of 10-14 and 20-24 years. There was however, no remarkable effect of age on IgE anti-CTF. In addition, there were significant positive correlations between egg-count and the anti-CTF antibody isotypes responses. This study also investigated the relationship between anti-CTF and antibody responses to other *S. mansoni* antigens, including adult (AWA), egg (SEA) and whole cercarial homogenate. Most of these antibodies were strongly correlated with each other. In conclusion, our results suggest that the anti-CTF antibody response appears a reliable indicator of exposure to *S. mansoni* in endemic areas, and might also be exploited for schistosomiasis epidemiological studies.

**Key words:**  *Schistosoma Mansoni*, Schistosomiasis, immune responses,  ELISA, SDS-PAGE.

## Introduction

Schistosomiasis infection with any of the five species that infect man causes a range of immune-related events at the site of infection in general, and in particular, stimulate antibody production (Standley *et al.*, 2012). Animal models were often used to study immune responses, but they had many strict limitations. Therefore, recent studies had focused on *in vitro* investigations using sera from people living in endemic areas (Silveira *et al.*, 2002; Naus *et al.*, 2003 a; Walter *et al.*, 2006; Pinot de Moira *et al.*, 2010). In the endemic areas, the population was exposed to the Schistosoma infection from a very early age (Vereecken *et al.*, 2007). Researchers pointed out the importance to investigate factors affecting the immuno-epidemiology in these areas such as age, sex and the intensity of infection (Webster *et al.*, 1997; Naus *et al.*, 1999; Naus *et al.*, 2003a; Pinot de Moira *et al.*, 2010). There was evidence of earlier changes in the equilibrium of antibodies in more intensely infected                          populations

(Mutapi *et al.*, 1997; Mitchell *et al.*, 2011). The protective immunity appeared to increase slowly and the susceptibility to decrease in older children or adults, in spite of evidence that some people were repeatedly infected from a young age (Mitchell *et al.*, 2011). It was pointed out that some people were more susceptible to re-infection, while others appeared resistant after treatment for schistosomiasis but the reasons behind these observations were not known (Wang *et al.*, 2009). The heterogeneous nature of the human exposure to contaminated water was perhaps one reason; therefore to be able to discriminate between lack of cercarial exposure and acquired resistance would be helpful (Pinot de Moira *et al.*, 2010). Several studies reported that the IgG1and IgG4 were the predominant anti-*S. mansoni* isotypes induced in the sera of infected humans (Naus *et al.*, 2003 b; Caldas *et al.*, 2008). Seroepidemiologic studies in Kenya (Dunne *et al.*, 1992) and in Brazil (Jiz *et al.*, 2009) indicated that the early and high levels of production of IgG4 against adult and egg antigens of *S. mansoni* may block the activity of IgE (Aalberse *et al.*, 2009). Aaccumulating evidence also indicated that the levels of IgE against worm and egg antigen tended to increase with age (Naus *et al.*, 2003 a; Fitzsimmons *et al.*, 2007; Pinot de Moira *et al.*, 2010). Both IgG4 and IgE antibodies had been characterized as markers for developing protection to infection, as well as a risk for immuno-pathology (Imai *et al.*, 2011). People's contact with water containing cercariae had been extensively studied and had been shown to correlate negatively with age (Dalton and Pole, 1978; Mitchell *et al.*, 2011).

In *S. mansoni* endemic areas, the intensity of infection peaked between 6-20 years of age and declined rapidly after this age suggesting that the adaptive immune response increased (Pinot de Moira *et al.,* 2010; Mitchell *et al.,* 2011). The difference in the intensity of *S. mansoni* infection between the genders from the same community was highlighted, females being with lower intensity of infection than males (de Lima e Costa *et al.*, 1993; Naus *et al.*, 2003 a). This was related to different sex behavioural and/or different social culture factors (Kabatereine *et al.*, 2004; Matthys *et al.*, 2007; Imai *et al.*, 2011). However, a study in mice illustrated that the gender difference might be due to the difference in susceptibility to infection, and due to decreased immunity to infection amongst males (Eloi-Santos *et al.*, 1992). A study by Webster *et al.* (1997 b) suggested that the difference in the infection rate between the sexes could be dependent on hormone changes around puberty. The skin penetration process was facilitated by the Cercarial transformation fluid secretions (CTF) containing molecules that induce cellular and humoral immune responses (Knudsen *et al.*, 2005; Curwen *et al.*, 2006). Although, antibody responses had been studied intensively to *S. mansoni* adult and egg antigens (Walter *et al.*, 2006; Pinot de Moira *et al.*, 2010), schistosomula tegument extract (Sepulveda *et al.*, 2010), and cercarial homogenate antigens (Fitzsimmons *et al.*, 2007), the antibody response to CTF was not investigated in detail. Few studies reported that anti-CTF antibody was considerably more specific than anti-SEA antibody for antibody detection diagnostic test in endemic area (Chand *et al.*, 2010; El Aswad Bel *et al.*, 2011; Smith *et al.*, 2012). The main objective of the present study was to measure the antibody response to CTF antigens in humans residing in high endemic area of *S. mansoni* in order to answer the main question: Does anti-CTF antibody responses predict exposure to *S. mansoni* infection?

## Materials and Methods

## Preparation of cercarial transformation fluid (CTF) from S. mansoni

The CTF that was used for the present experiments was provided by Prof. Mike Doenhoff, University of Nottingham. The material was prepared as follows: *B. glabrata* snails with patient *S. mansoni* infections were placed in distilled water in glass beakers and incubated under a 60 watt tungsten light to induce the snails to shed cercariae into the distilled water. The cercariae were concentrated over a glass fiber filter into a smaller volume of water (approximately 10 ml) and placed in ice to cause sedimentation by gravity. The

supernatant was discarded, and the cercarial pellet was resuspended in an appropriate volume of PBS, approximately 5 ml PBS per ml of gravity-packed cercariae and the larvae were mechanically stimulated to release the components of their acetabular glands and break off their tails by drawing the suspension through a 20 G needle approximately 15 times. Larval bodies and tails were incubated at 37 ºC in a 10 cm-diameter plastic Petri dish for 2 hours after which the suspension was centrifuged at 2000 g for 10 minutes. CTF was collected and stored at –80ºC.

## Human sera and population

The human sera were kindly donated by Prof Dunne form Cambridge University. Two hundred ninety nine sera, of persons aged 5 to 60 years old and described by Kabatereine *et al.* (2003), were kindly provided. The sera were randomly collected from people infected with *S. mansoni* in North-western Uganda. No

schistosomiasis treatment had been offered to the population before the sera collection. Parasitological status was assessed by analyzing three stool samples collected on three successive days. For each individual, eggs were counted using the microscope, while personal data was also recorded.

## Immunoassay ELISA

Nine of the 384 well flat-bottom microlon 600 high binding plates were coated with CTF diluted in a coating buffer at a final concentration of 5.0 µg/ml. Following the blocking of nonspecific binding sites of the plates with a blocking buffer for 1 hour at room temperature, human infected and uninfected sera were diluted 1:20 for detecting parasite specific IgE and 1:200 for detecting IgG1 and IgG4 with dilution buffer and incubated overnight at 4ºC. The following day, the plates were probed with monoclonal biotinylated mouse anti-human IgG1, mouse anti-human IgG4 and mouse anti-human IgE at 0.5 µg/ml and were diluted with an incubation buffer and

incubated for 2 hours at room temperature with gentle shaking. Poly-HRP strepatavidin complex was added to each plate at a dilution of 1:3000 and incubated for 2 hours at room temperature. After each step described above, the plates were washed with washing buffer 5 times for 15 minutes. The reaction was then visualized by adding substrate solution for 10-30 minutes. The reaction was stopped by addition of a stopping buffer; the absorbance was read at a test wavelength 450 nm, and reference wavelength 630 nm. A total of 299 individuals' sera samples were used for measuring antibody responses towards CTF.

## Western immunoblotting

Cercarial transformation fluid at final concentration of 2.0 mg/ml was analyzed by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-transferred onto a nitrocellulose membrane (NCM). Immunoblotting followed the

PAGE), following the method proposed by Laemmli (1970). The CTF molecules of the four sections of the gel were

method developed by Towbin *et al.,* (1979). Sera from healthy European

volunteers were used as negative controls. Seventeen human sera from the *S. mansoni*

## Statistical analysis

The statistical analyses were performed using SPSS version 19. The data included sex, age categories and egg-count as independent variables, and immunological parameters (antibody response) as dependent variables. The intensity of infection was analyzed using the Hierarchical Loglinear function. Quantitative analysis of egg-count was done by age and sex based on general linear models (GLIM), and the residuals were indicated if the egg-count was not normally distributed (Behnke *et al.*, 1994; Wilson and Grenfell, 1997; Behnke *et al.*, 1999 b). The statistical analysis of the effect of sex and age in the prevalence of infection were performed by a full factorial model. The population was divided into 7 age classes (1 = 5-9 years, 2 = 10-14 years, 3 = 15-19 years, 4 = 20-24 years, 5= 25-29 years, 6=30-39 years, and 7= 40-60 years). The intensity of infection was analyzed in the seven age groups and in two age groups, the age group (20-24) years old and all other age groups as the second age group. A non-parametric model (Kruskal-Wallis test) was used to analyze two or more groups. The Mann-Whitney U test was used to determine variation within two groups. It was further analyzed by categorizing the intensity of infection into 7 groups according to the egg load in the faecal samples. These were: group 1 = 0 egg-count, group 2 = 1-200 egg-count,

## Results

The study group is described in Table **1**, including the parasitological characteristics as measured by the antibody responses to CTF of *S. mansoni*. The

endemic area in Uganda were randomly selected for the test.

group 3 = 201-400, group 4 = 401-800, group 5 = 801-1200, group 6 = 1201-2000, and group 7 = 2001-8000.

In order to characterize the relationship between age and IgG1, IgG4 and IgE antibodies specific to CTF from *S. mansoni* infected sera, the individuals were divided into five age groups. The effect of sex, age categories and egg-count on antibody responses were analyzed using GLIM, multivariate and univariate approaches. The distribution normality of 12 immunological parameters was tested. These parameters were reduced by the Principal Component Analysis into four groups (Sokal and Rohlf, 1981). The antibody response to CTF was component 1, response to AWA was component 2 and response to SEA was component 3. The intensity of parasite infections with these principal components was examined for potential effects of age, sex and egg-count, using a non-parametric model (Kruskal-Wallis test). The four principal components were employed as dependent variables and the age (7 levels) and the sex (2 levels) were used as main factors. Pearson's correlation coefficients were used to evaluate the relationships between IgG1, IgG4 and IgE antibody responses to CTF, and between the four antigens of *S. mansoni*. Significance was indicated at the 5% level.

prevalence of *S. mansoni* infection, in the study population, was very high, 89%.

## Age and gender-profiles of intensity and prevalence of infection

Although age was significantly related to the intensity of infection, the parameters of infection of *S. mansoni*: the intensity and the prevalence, did not show any significant differences between the genders ($P > 0.05$). However, females had a slightly higher intensity of infection than males in the (5-9) and (20-24) age groups, and it was higher in the males in the (10-14), (15-19), (20-24) and (25-29) age groups (Figure 1). In both genders, the intensity of infection was high around the age group of 20-24. The analysis of the intensity of infection in the two age groups (20-24 and all other age groups) confirmed that the parasite egg yield dramatically increased in the 20-24 years old and that during all other age groups the egg-count decreased or stable (Kruskal-Wallis test, $X^2_1 = 6.194$, $P = 0.013$). The prevalence of infection by age and sex (Figure 2) showed a similar trend to that of intensity of infection.

## IgG4 and IgE antibodies specifically recognize antigens of *S. mansoni* CTF by sera of the residents in an endemic area

Figure 3 A characterizes the molecules of CTF recognized specifically by anti-IgG4 and anti-IgE antibodies in the sera from 17 residents infected with *S. mansoni* by the Western blotting analysis. The figure shows that IgG4 antibody specifically recognized antigens with approximately 30 kDa, 46 kDa and 58 kDa molecules. On the other hand, the IgE antibody weakly recognized some molecules of CTF at approximately 22 kDa, 58 kDa and 80 kDa

## Specific antibody levels from human infected and uninfected sera in response to CTF

The mean of different isotypes, anti-CTF IgG1, IgG4 and IgE levels, in the infected and un-infected human sera are shown in Figure 4. The comparison of antibody responses between the age groups demonstrated that for both IgG1 and IgG4 very similar levels were observed in infected and un-infected sera. This corresponded to the prevalence of infection. There was a noticeable increase of antibody responses, particularly in the age groups (10-14) and (20-24), Figure 4. A and B but no such increase was observed with the level of IgE antibody (Figure 4 C).

## Relationship of CTF specific IgG1, IgG4 and IgE antibodies with age

Comparing the antibody responses between the five age groups, the IgG1 and the IgG4 showed very similar levels (Figure 5 A and B). The specific antibody responses to CTF demonstrated an age related increase over all the age groups with the exception of the 10-14 and 20-24 age groups, which showed remarkable increase in the level of response (Figure 5 A and B).

The age profiles of anti-CTF IgG1 and anti-CTF IgG4 reflected the levels of prevalence and intensity of infection. However, anti-CTF IgE responses were low in all the age classes (Figure 5). The effects of gender on CTF-specific IgG, IgG4 and IgE antibodies were examined but there was no significant difference in response with sex. Females presented a slightly higher response than males in the (30-39) and (40-60) age groups for IgG1,

and in the age group (5-9) IgG4 response was high. In the age groups (5-9) and (15-19) the IgE responses were high. Males were somewhat higher than females in all

the other remaining age groups. The IgG4 and IgE responses in males showed increase with age, especially from 20 years old (Figure 5 B and C).

## Comparison of serum antibody levels towards *S. mansoni* antigens

Pearson's correlations test indicated a significant positive correlation between the different antibody isotypes (IgG1, IgG4 and IgE) towards CTF (Table 2). Individual responses to CTF, AWA, SEA

and cercariae antigens showed positive and highly significant correlations for IgG1, IgG4 and IgE levels (Table 3). (r (299) = 0.402, *P* = 0.000).

## *S. mansoni* antigens specific IgG1, IgG4 and IgE antibodies and egg-count

There were significant positive correlations between the egg-count and the antibody response specific to CTF. The increase in the egg-count related to the increase in the antibody response to CTF (Error! Reference source not found.). The comparison of the antibodies in the 7 egg-count categories and their responses to CTF indicated that all antibody isotypes' responses were higher in individuals with egg-count from 1201-2000 epg (Figure 6). The IgG1 levels were significantly higher in the egg-count category 2001-8000 when compared to group 1 (epg = 0) and group 2 (1-200 epg) (Mann-whitney *U*-test, $z = -2.587$, *P*=0.01 and *P*= 0.03 respectively), Figure 6 A. A significant positive correlation was found between IgG4 antibody responses to CTF and approximately all egg-count categories in individuals with 2001-8000 epg when compared with groups 1 and 2 (r (299, 0.66) = 0.521, *P* = 0.000), with group 3 (*P*

= 0.005) and with group 4 (*P* = 0.015). Group 1 significantly differed with group 4 (*P* = 0.012), with group 5 (*P* = 0.006) and with group 6 (*P* = 0.000), Figure 6 B. Additionally, anti-CTF IgE response was significantly different between group 1 and the highest egg-count group (Mann-whitney *U*-test, $z = -2.485$, *P* = 0.012), Figure 6 C). Consequently, principal components (PC1-3) were examined in relation to sex, age groups and egg-count (infected and uninfected). For this, the non-parametric test (Kruskal-Wallis test) was employed; egg-count did indeed significantly change between all the principal components (Table 5). However, in relation to gender, no significant differences were found with all principal components (reflective of antibody responses). Similarly, there was no significant influence in terms of age classes (7 groups and 2 groups) in terms of all principal components.

## Discussion

The present study aimed to assess the importance of specific antibody responses to CTF in an *S. mansoni* infected community. In the endemic areas, individuals are frequently exposed to cercariae and their secretions continuously since an early stage in life. This leads to the production of antibodies against cercarial molecules. It is suggested that IgG4 is a marker to susceptibility to re-infection, whereas, IgE is a marker to

resistance to re-infection after treatment (Hagan *et al*., 1991; Dunne *et al*., 1992; Demeure *et al*., 1993). It is of interest to determine whether such observations are true for untreated and/or uninfected individual residing in an *S. mansoni* endemic area.

In Schistosomiasis endemic areas, the egg distribution among the infected population is not randomly distributed. However, the intensity of infection is significantly higher

in children, with a peak in those between 6-20 years old and then rapidly declining in the adults. The prevalence of *S. mansoni* infection was very high among the followed by a decline in males only. The finding of the current study is consistent with the study carried out in Kenya by Fulford *et al*. (1992). The stated that the intensity of infection, in heavily endemic areas, tends to occur at an earlier age (5-8 years old), and in areas with low level of infection, the peak of intensity of infection tends to occur at an older age (9-20) years old (Woolhouse, 1998; Duerr *et al.*, 2003; Mitchell *et al.*, 2011). The peak intensity of infection in the females, in this study, was in the age groups (5-9) and (20-24) years old, and the intensity of infection increased significantly from 10 to 30 years as in males. These results were not consistent with the study by Naus *et al.* (2003 a). They reported that males were more heavily infected than females in all age groups. This discrepancy may be due to occupational differences. The mentioned study was performed in an endemic region, where most male individuals were fishermen, who are heavily and repeatedly exposed to infection.

The results show that the molecules of CTF induce a number of antibody isotypes in an infected individual's sera, including IgG1, IgG4 and IgE. The percentage of individuals sero-positive within the study group indicates that IgG1 (94%) and IgG4 (83%) are the predominant subclasses in the CTF. These findings are consistent with other studies (Naus *et al.*, 2003 b; Caldas *et al.*, 2008). However, the level of IgE is low (62%). This observation is common in such communities as a result of diverse susceptibility and resistance to re-infection (Dunne *et al.*, 1992; Satti *et al.*, 1996; Naus *et al.*, 1998; Jiz *et al.*, 2009). The comparison of antibody responses between these age groups demonstrated similar IgG1 and IgG4 levels in sera of the infected and the un-infected. These results obviously demonstrate that antibody levels are result and related to the prevalence as well as to the intensity of infection.

population under study. The peak intensity of infection was around the age of 20 years, but there was other obvious peaks

peak intensity of *S. mansoni* infection was in the 12-25 years old. Other studies reported that the peak of the

This study has characterized and identified the molecules of CTF that are recognized by IgG4 and IgE antibodies. The dominant CTF molecule recognized by IgG4 antibody was at 30 kDa. A 30 kDa molecule has also been identified previously as a cercarial elastase (Landsperger *et al.*, 1982; Knudsen *et al.*, 2005; Hansell *et al.*, 2008), and has been suggested as a vaccine candidate (Cardoso *et al.*, 2006; Ingram *et al.*, 2012). Whether the 30kDa antigen recognized here is a elastase is not definite but previous studies did not find any antibody reactivity to any molecule at 30 kDa by Western blot technique(Bahgat *et al.*, 2001; Cardoso *et al.*, 2006). However, Pino-Heiss *et al.* (1986) reported that a 30 kDa protein reacted with sera, both from infected mice and humans. Further research is needed to investigate and identify the nature of the 30kDa molecule that reacts with human IgG4.

Another 22 kDa antigen was also preferentially recognized by IgE. This is most probable to be an allergen-like molecules released from dying *S. mansoni* adult worms and schistosomula (Dunne *et al.*, 1992; Dunne *et al.*, 1997; Fitzsimmons *et al.*, 2007). The released 22 kDa molecule by the dying schistosomula binds to IgE. The binding results in inflammatory reaction and creates the hostile environment for other invading cercariae (Fitzsimmons *et al.*, 2007). An earlier study showed that rat monoclonal IgE strongly reacted with a 22 kDa specific molecule present in schistosomula (Verwaerde *et al.*, 1987). Future studies are recommended before suggesting that this protein is a promising new biological marker of resistant individuals.

The results indicate that the high level of specific IgG1 and IgG4 antibodies responses towards CTF correspond particularly with the peaks of the studies reported that antibody response against AWA and SEA were associated strongly with age and with the intensity of infection (Satti *et al.*, 1996; Naus *et al.*, 1999). This peak of antibodies in these age groups (10-14 and 20-24) is most probably associated with the variation in the individual personal and behavioral period of exposure to infection.

The findings suggested a significant positive correlation between egg-count and antibody responses. This supports the reports by previous researchers (Webster *et al.*, 1997; Naus *et al.*, 1999; Naus *et al.*, 2003a). The analysis by two age groups, the group of 20-24 years old (group 1) and all other age groups (group 2) showed that group 1 is characterized by a significantly higher prevalence than group 2 and that the antibody levels of the individuals of this group are also very high, especially IgG1and IgG4.

There is a strong correlation between IgG4 and the different *S. mansoni* antigens (CTF, AWA, SEA and cercariae homogenate) as well as a positive correlation between IgG4 and IgF responses to CTF. The strong correlation between the IgG4 and the different S. mansoni anti-genes is most probably due to that these antigens expressed identical common epitopes, which directly bind to IgG4. Hussain *et al.* (1986) suggested that IgE and IgG4 antibodies might bind to the same epitopes. Thus, the effector function of IgE is blocked by IgG4 as they are both directed to the same epitopes (Demeure *et al.*, 1993). However, Li *et al.* (1999) demonstrated that the two antibodies are independently regulated by different mechanisms.

The IgG4 response is significantly different in the different age groups, depending upon the prevalence and the intensity of the infection, whereas no such observation is demonstrated with IgE response to CTF. Also there are no gender

prevalence of infection with age groups with the highest percentage of infection (10-14 years and 20-24 years). Previous

differences between IgG1, IgG4 and IgE responses. However, the levels of IgG4 and IgE increase from 20 years old in the males, while an increase in the levels of IgG1 level is observed from 30 years in females. The IgG4 level is considerably higher in females than males in age 5-9 year old, an observation reported by several workers (Webster *et al.*, 1997; Naus *et al.*, 1999; Naus *et al.*, 2003a). Two possible explanations were suggested. The first is that males are exposed to cercarial antigens more than females, because of behavioural differences (Kabatereine *et al.*, 2004). The second is that the difference could be due to different hormonal factors between sexes (Webster *et al.*, 1997). It will be useful to conduct further studies with a large number of adults over 20 years to assess the cumulative exposure to cercarial secretions, combined with a water contact survey.

It is interesting to observe that only egg-count is significantly related to all *S. mansoni* antigen, CTF, AWA, SEA, the principal components. This suggests that the antibody response is a reliable indicator of infection with *S. mansoni* in endemic areas and it might be also exploited for schistosomiasis epidemiological studies. The antibody responses are significantly correlated with the prevalence of infection. The results indicate that IgG4 and IgE responses are associated with sex, age as well as with the prevalence of infection and that anti-IgG4 and anti-IgG1 against CTF increase significantly with the egg abundance.

Sera from uninfected individuals, according to negative egg-count results (zero), had sero-positive results to CTF antigen with detectable levels of IgG1, IgG4 and IgE. This conclusion is consistent with those of other studies and suggests that such a reaction reflects the limited of microscopic sensitivity of egg combined with a very high sensitivity and

specificity of ELISA to detect antibody (de Vlas and Gryseels, 1992; Utzinger *et al.*, 2001). The sensitivity of antibody n conclusion, the results suggest that anti-CTF antibody responses predict exposure

detection is a more effective method than parasitology (Doenhoff *et al.*, 2004). I

to *S. mansoni* but further in depth studies are needed.

**Table 1 : Description of the study group.**

[a] Numbers and ranges of study group are detailed in table 7.1, [b] percentage of infected people (different genders) and prevalence of infection. [c] mean of egg-count (eggs per gram of faeces) of individuals.

| Number of Individuals | Age | Sex | Prevalence | Intensity |
|---|---|---|---|---|
| 299[a] | 5/60[a] | 134/165[a] 47% /55%[b] | 266[a] 89%[b] | 0-8226[a] (1060.24)[c] |

**Table 2 : Characterisation of the relationship between different antibody isotypes to CTF , plus the correlation between levels of IgG1, IgG4 and IgE antibodies to CTF.**

| Correlation | Pearson's R | $P$- Value |
|---|---|---|
| CTF-IgG1XCTF-IgG4 | 0.319** | 0.000 |
| CTF-IgG1 X CTF-IgE | 0.229** | 0.000 |
| CTF-IgG4 X CTF-IgE | 0.372** | 0.000 |

**Table 3 : The relationship between different antibody isotypes to some *S. mansoni* antigens.**

| Correlation | Anti-body isotype | Pearson's R | $P$- Value |
|---|---|---|---|
| CTF X AWA | IgG1 | 0.441 | 0.000** |
| | IgG4 | 0.239 | 0.000** |
| | IgE | 0.173 | .003** |
| CTF X SEA | IgG1 | 0.542 | .000** |
| | IgG4 | 0.114 | .050 |
| | IgE | 0.227 | .000** |
| CTF X Cercariae | IgG1 | 0.390 | .000** |
| | IgG4 | 0.144 | .013 |

Antibody levels specific for CTF, AWA, SEA and cercariae were measured by ELISA and correlated with one another using Pearson's rank correlation model.

**Table 4 : Correlation between egg-count with IgG1, IgG4 and IgE antibody response to CTF.**

| Correlation | Pearson's R | P value |
|---|---|---|
| IgG1 | 0.216** | 0.000 |
| IgG4 | 0.354** | 0.000 |
| IgE | 0.159** | 0.03 |

Positive relationship of egg-count reflective of antibody responses to CTF (Pearson's R), *P* value revealed that the correlation was also significant.=+[3]

**Table 5 : The effect of sex, age and egg-count in principal components (PC 1-4) of study.**

Principal component 1 is (antibody response to CTF), Principal component 2 is (antibody response to AWA), and Principal component 3 is (antibody response to SEA) a Represents egg-count divided into two groups infected and un-infected individuals. b Age classes characterised into 7 groups, whereas, c represented age into 2 groups. For more information the eigenanlysis and component matrix are presented.

**Eige nanalysis**

| Comp onent | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 1.616 | 53.881 | 53.881 | 1.616 | 53.881 | 53.881 |
| 2 | .776 | 25.867 | 79.748 | | | |
| 3 | .608 | 20.252 | 100.000 | | | |

Extraction Method: Principal Component

| **Component Matrix**[a] | |
|---|---|
| | Component |
| | 1 |
| Mean OD CFT IgG1 | .679 |
| Mean OD CFT IgG4 | .790 |
| Mean OD CFT IgE | .728 |
| Extraction Method: Principal Component Analysis. | |

| Variable | Egg-count[a] $X2, P$ | Sex $X2, P$ | Age1[b] $X2, P$ | Age2[c] $X2, P$ |
|---|---|---|---|---|
| PC1 | 10.226, 0.001 | 0.085, 0.77 | 7.755, 0.257 | 0.753, 0.386 |
| PC2 | 7.689, 0.006 | 0.159, 0.688 | 10.163, 0.118 | 0.933, 0.334 |
| PC3 | 24.942, 0.000 | 0.100, 0.752 | 9.925, 0.135 | 0.858, 0.354 |
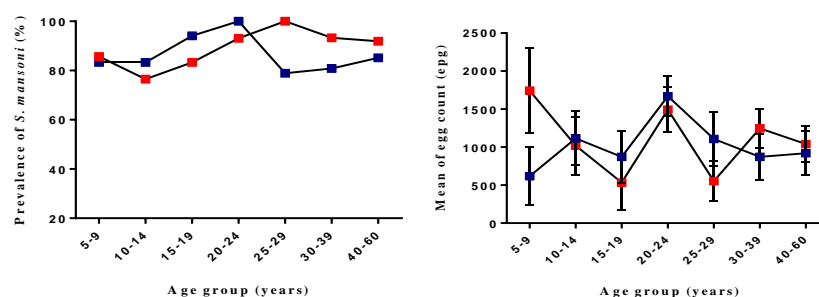


**Figure 1 : Intensity of infection by age group and sex.**

Red symbols represent the means ± SD of females, while the blue symbols represent means ± SD of males in each age group (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60. The standard error bars show the range of egg-count data, excluding extreme values.

**Figure 2 : Relationships between age-prevalence of *S. mansoni* and sex in North-western Uganda.**

Blue symbols represent males, while the red symbols represent females in 7 age groups (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60).
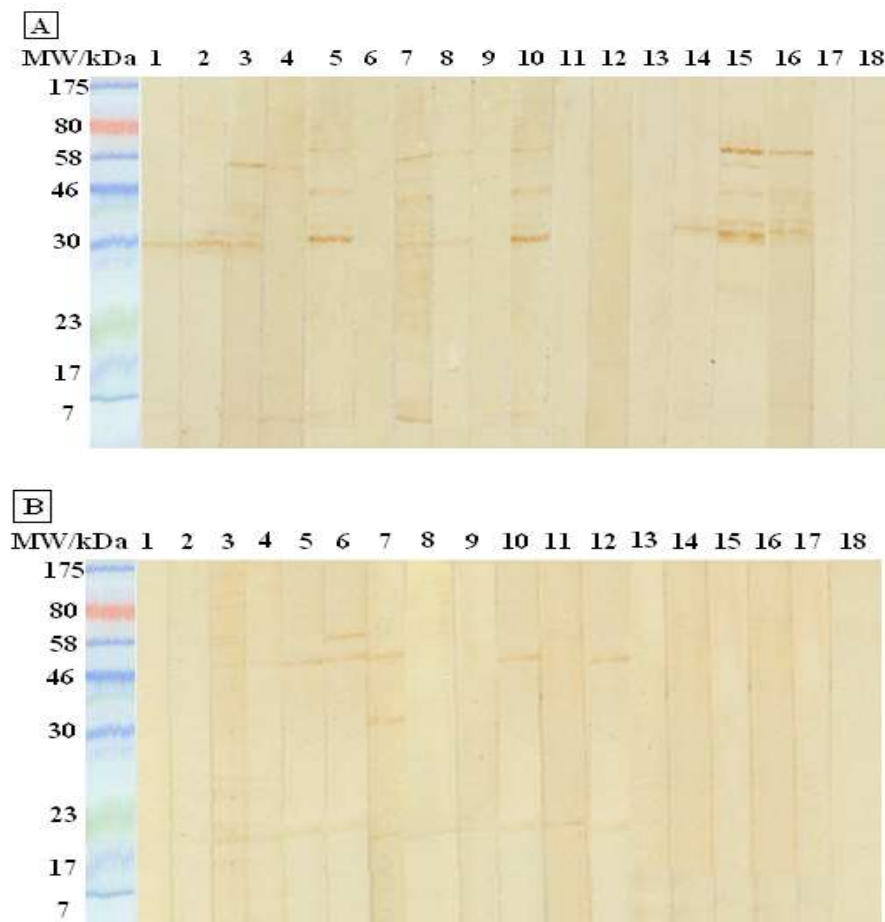


**Figure Error! No text of specified style in document.3 : Characterisation of specific antigens of CTF recognised by IgG4 antibody (A) and by IgE antibody (B) from human infected sera.**

Lanes 1 to 17 represent sera from infected individuals living in a parasite endemic area. Lane 18 represents negative control (healthy European volunteers). Lane MW/kDa presents molecular weights of protein standards in kDa.
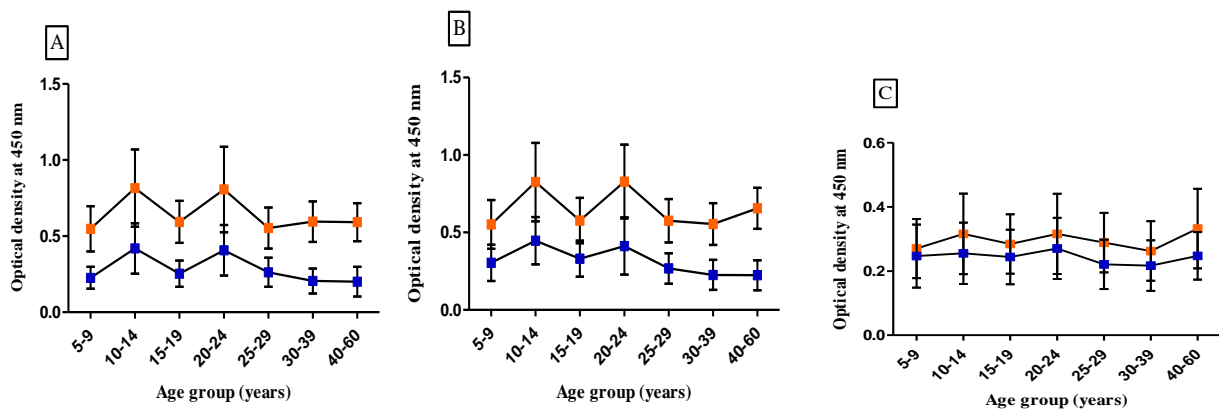
**Figure 4 : Distribution of sero-positive of infected and uninfected people sera.**

Infected sera are represented as orange, squares, and uninfected human sera (Blue, squares) according to antibody levels in each age group (5-9, 10-14, 15-19, 20-24, 25-29, 30-39 and 40-60). The horizontal lines indicate the arithmetic means with SD. A. IgG1, B. IgG4 and C. IgE antibodies response to CTF.
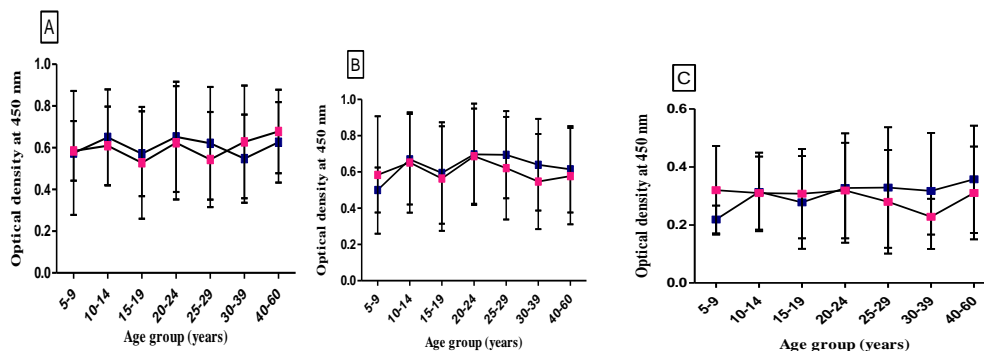


**Figure 5 : Relationship between CTF specific IgG1, IgG4 and IgE antibodies with age and sex.**

**A.** IgG1, **B.** IgG4 and **C.** IgE antibodies' response to CTF over age profile for endemic *S. mansoni* area; data points are optical density (OD) values at 450 nm. The horizontal lines indicate the arithmetic means with SD. Pink symbols represent females, while the blue symbols represent males.
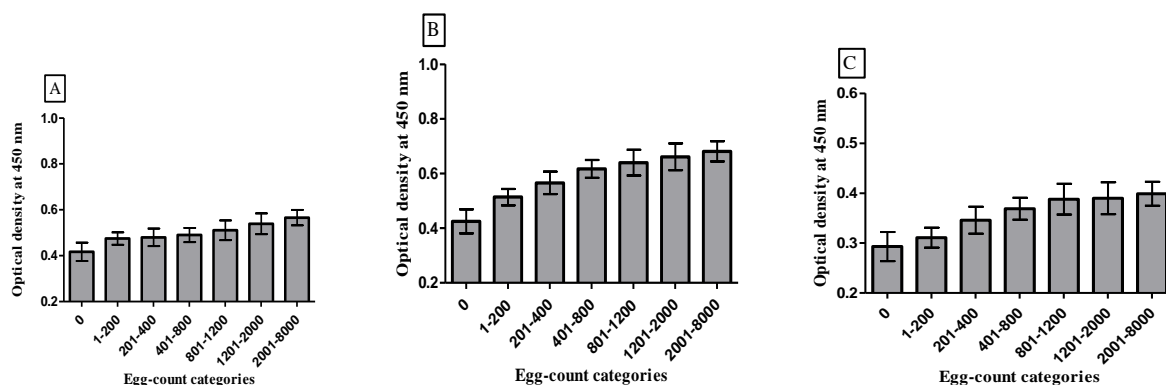


**Figure 6 : The relationship between antibody responses towards CTF and egg-count.**

IgG1, B. IgG4 and C. IgE of individual subjects (n = 299), 7 egg-count category; 0, 1-200, 201-400, 401-800, 801-1200, 1201-2000 and 2000-8000 were designed to test this relationship. Data points are means of optical density values at 450 nm.

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# Growth inhibitory Effect of Chlorhexidine and Hexetidine Containing-mouthwashes Against Oral Bacteria

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**الملخص**

الاستعمال المنتظم لغسولات الفم التي تحتوي في تركيباتها علي مضادات جرثومية فعالة ، يعتبر من أنجح الطرق فاعلية للوقاية من الالتهابات الجرثومية وتسوس الأسنان . من المواد الكيميائية التي وجد لها فاعلية قصوى ضد البكتيريا ، مركبي الكلور هيكسيدين و الهكستدين.

استهدفت هذه الدراسة تقييم كفاءة التضاد البكتيري لعدد 4 من محاليل غسول الفم المتوفرة بالسوق الليبي .. في هذه الدراسة تم اختيار 60 متطوع من البالغين وتم تقسيمهم إلي 4 مجموعات ، كل مجموعة تحتوي علي 15 فرد ، كل فرد تم تزويده بغسول للفم لاستعماله كمضمضة بمقدار 15 مل ولمدة 30 ثانية بمعدل مرتين يومياً ولمدة أسبوعين مع مراعاة المحافظة على التنظيف الاعتيادي بالفرشاة ومعجون الأسنان. ولغرض المقارنة والتعرف علي فاعلية غسولات الفم تم جمع اللعاب من كل مجموعة قبل البدء في الاستعمال .. وبعد انقضاء مدة الاستعمال تم تجميع اللعاب وإخضاعه لتجارب معملية لعد البكتيريا الحية المتبقية قبل و بعد تعرضها لغسول الفـــم.

النتائج الأساسية لهذه الدراسة أظهرت تنوعا في التضاد البكتيري حيث أن غسولات الفم التالية زوردايل و اوراكسين ، التي تحتوي في تركيبها على مادة الكلور هيكسيدين كان لها التأثير الأكبر في التقليل من عدد البكتيريا في لعاب المتطوعين بنسب 90% و 60% على التوالي ، بينما كانت النسب المئوية لغسولات الفم هيكستريل و جفالكس التي تحتوي في تركيبتها على مادة الهكستدين فكانت على التوالي 34% و25% .

كما تم في هذه الدراسة تحديد الزمن اللازم لقتل البكتيريا لهذه المحاليل الأربعة وعد البكتيريا لها بعد تعريضها لها في أزمنة مختلفة ابتداء من زمن دقيقتين حتى 60 دقيقة ، وكان زمن القتل لغسولات الفم التي تحتوي على مادة الكلور هيكسيدين هو الأقل حيث تراوح من 2 إلى 4 دقائق ، بينما غسولات الفم التي تحتوي على مادة الهكستدين استغرقت وقتا أطول ما بين 15 الى 30 دقيقة لكي تظهر تأثيرا قاتلا على البكتيريا . كما تم في هذه الدراسة تقييم فاعلية التضاد البكتيري لغسولات الفم بقياس قطر دائرة تثبيط النمو البكتيري لها على كافة أنواع البكتيريا التي تم عزلها من لعاب المتطوعين وأثبتت النتائج أيضا أن غسولات الفم التي تحتوي علي مادة الكلورهيكسيدين هي الأكثر فاعلية في الحد من النمو البكتيري بإعطائها اكبر قطر لدائرة تثبيط النمو البكتيري.

## ABSTRACT

Frequent use of mouthwash (MW) is one of the most effective methods used to prevent oral bacterial infections and to assist individuals in their efforts to achieve and maintain better oral health. Using a MW containing antibacterial agents would be a simple way to prevent growth and multiplication of pathogenic organisms in oral cavity causing dental caries and other mouth diseases.[1,2,3] **Chlorhexidine (CHX)** and **Hexetidine** (**HX**) have been proposed as a potent chemotherapeutic agents against oral bacteria.

The present study was performed to investigate the inhibition of growth of oral bacteria by four mouthwashes commercially available in the Libyan market containing either **CHX** (*Zordyl®* and *Oraxin®*) or **HX** (*Hextril®* and *Givalex®*).
Sixty adult volunteers have been chosen and divided into four groups and their saliva samples were assessed for microbial count at the beginning and the end of two weeks of treatment, during which they rinsed with 15 ml of mouthwashes for 30 seconds twice daily (morning and evening) in addition to their usual oral hygiene procedures.
The results showed wide variations in their effectiveness: those containing **CHX** mouthwashes were more effective (**P ≤0.05**) than formulations based on **HX** as active ingredient on oral microbial count. The main findings of the present study were that *Zordyl®*, *Oraxin®* and *Hextril®* exerted high effects on the salivary microbiota, causing 90%, 60% and 34% reduction in salivary bacterial counts respectively. 25% reduction was observed for *Givalex®*. On the other hand, the zone of inhibition test was showed

that *Zordyl®* and *Oraxin®* had large zone inhibitory effects, while *Hextril®* and *Givalex®*, were less effective on some species of bacteria. This indicates that chlorhexidine-containing mouthwashes proved to be the best solutions to treat oral infection.

This concludes that twice daily use of CHX or HX-containing mouthwashes reduce oral bacterial load counts in healthy subjects when used as an adjunct to their normal oral hygiene procedures. This also suggests that inhibitory power of CHX is greater on oral bacteria than HX containing mouthwashes.

## *ACKNOWLEDGMENT TO:*

INTRODUCTION:

The accumulation of microorganisms in dental plaque is related to the etiology of oral diseases, with a high prevalence worldwide. Cariogenic micro-organisms enter the dental biofilms early in life and can subsequently emerge under favorable environmental conditions, to cause dental caries and periodontal diseases.

Frequent use of mouthwash (MW) is one of the most effective methods used to prevent oral bacterial infections and to assist individuals in their efforts to achieve and maintain better oral health. Using a mouthwash containing antibacterial agents would be a simple way to prevent growth and multiplication of pathogenic organisms in oral cavity causing dental caries and other mouth diseases.[1,2,3]

Today, a variety of chemical substances which are usually added to MW formulations to inhibit microbial growth have also been subjected to numerous investigations against different types of bacteria. Out of all these chemical biocides, CHX and HX.[4] are the most potent antimicrobial agents included in wide range of MW solutions; therefore, they are subjected to extensive research studies The interaction and uptake by bacteria was shown to be extremely rapid, with a maximum effect occurring within 20 seconds.[5] CHX molecules are positively charged and bacterial surface as well as the most surface structures in the oral cavity, including the surfaces of teeth and mucous membranes, are negatively charged. In accordance with the principle that opposite charges attract, CHX binds strongly to all these surface structures (figure 1).
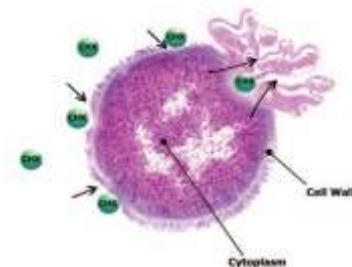


*figure 1.* *The chlorhexidine molecules adhere to and damage the surface of bacteria osmotic imbalance and the precipitation of cytoplasm  resulting  in cell  death*[6]

## MATERIALS AND METHODS

### Microorganisms

The following strains were used in this study: Streptococcus mutans, Staphylococcus aureus, Staphylococcus albus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Serratia marcescens.

## Maintenance & Growth of Organisms

One ml of bacterial culture was taken and inoculated into 10 ml of sterile Mueller-Hinton broth and incubated for 18 to 24 h at 37$^o$C. From this overnight culture, loopfuls were streaked onto 15 ml MH agar slopes and incubated at 37$^o$C for 18 h. The slopes were kept at 4$^o$C before being used. At the beginning of each week a fresh agar slopes was taken and sub-cultured daily in 5 ml MH broth after incubated at 37$^o$C for further 18 h. A Regular check for

organism's identity was maintained throughout this work and each time a new bacterial culture was used. The identification of the organisms was usually carried out either by biochemical tests or microbiological examination. The latter involved the use of Gram stain technique whereas; the biochemical characteristics of organism were determined by using (API system S.A., Montalieu-Vercieu, France).

### Determination of antibacterial activity

The antibacterial activity of the different mouthwashes was determined

### In-vivo test

The in-vivo study was conducted using volunteer's saliva which then used to evaluate the effect of different mouthwashes on bacteria most frequently found in oral cavity and commonly

associated with mouth infections. Sixty adult (female 45 and male 15)

### Mouthwash rinses procedure

All volunteers were advised to rinse their mouths with 15 ml of provided mouthwash for 30 seconds twice a day, in the morning (after breakfast) and in the evening (after dinner). In addition, rinsing with water, eating, and drinking for 30 minutes were avoided following

by an evaluation of the in-vivo and in-vitro tests.[7]

volunteers aged between 18-55 years were studied, in 14 days-self controlled study, mostly have no history of periodontal diseases, had not taken antibiotics for the last three months prior study.
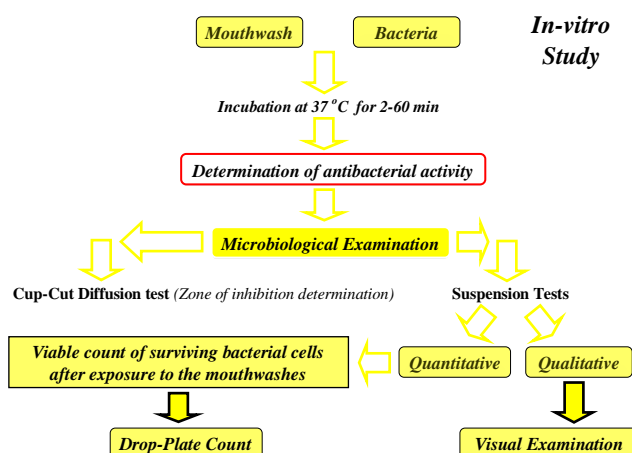
the use of the mouthwash.

Each volunteer was provided with a 15 ml volume dispenser to be used in each rinse otherwise rising procedure and volume will not be identical in all groups.
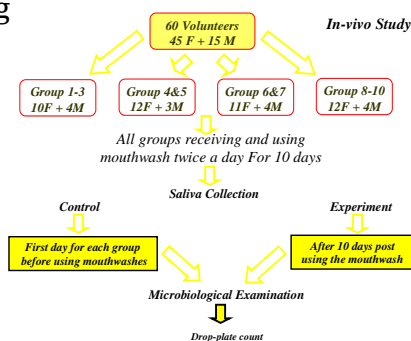
### In-vitro test

The antibacterial activity of the different mouthwashes was evaluated using agar viable count and cup-cut diffusion measurements.



### Viable cell count

The viable counting method of Miles and Misra (1938) was used. Triple 20 µl aliquots of 10-fold dilutions ($10^{-4}$, $10^{-5}$, $10^{-6}$ & $10^{-7}$) of the bacterial suspension were dispensed onto supper-dried agar plates and colonies were counted as colony forming unit per ml (cfu/ml) after overnight incubation at the appropriate temperature.
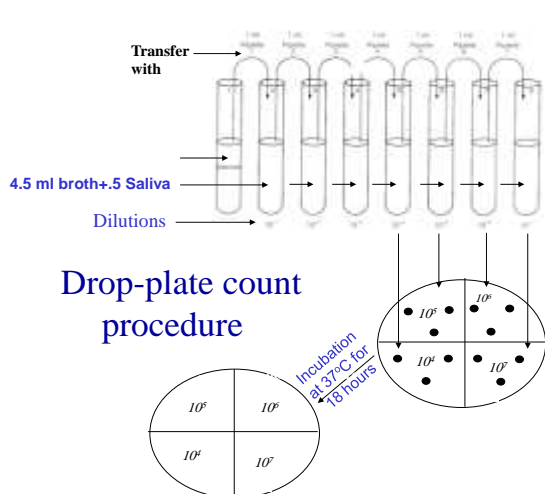


Number of cells .per/ ml
= number of colonies x dilution factor
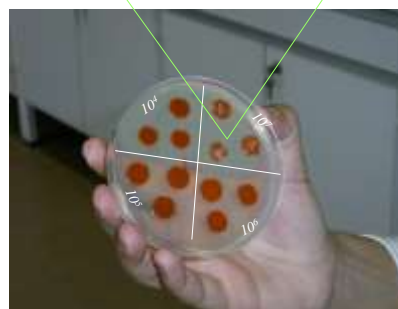
## The cup-cut diffusion assay

Tests of bacterial sensitivity to studied mouthwashes were done using cup-cut diffusion method.

A culture was developed on the surface of nutrient agar plate inoculated with the test strains for 18 hours. Using this culture a bacterial suspension of 0.5 McFarland was prepared. The McFarland turbidity standard is used for standardization of number of bacteria when required. The basic 0.5 McFarland standard contains approximately $10^8$ to $10^9$ cfu/ml. Then 200µl of bacterial suspension was then swabbed onto the surface of nutrient agar plate at 40 °C and left to dry. Cups were then bored in the agar plate, and each cup was filled with specific amount of mouthwash. The plate was incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition.

- *The Cup-Cut Diffusion Method*
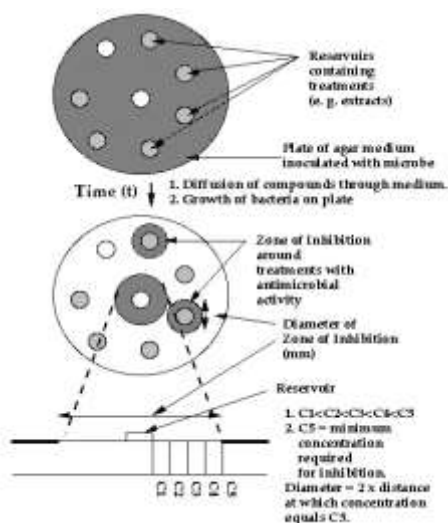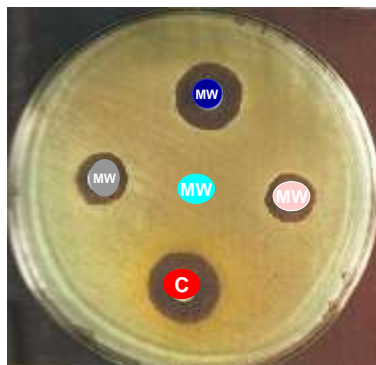


## Statistical analysis

Statistical analysis was carried out by using SPSS for windows version 14 program. The significance level ( 0,05 parametric) was used to indicate statistical significance. The one way ANOVA was used to define the effect of mouthwash on all groups.

## RSULTS:

Saliva samples which serve as a control were collected only once, first day for each subject before using mouthwashes and after two weeks post treatment. Specimens were then subjected to serial dilution and plating on a variety of selective agar tops Chlorhexidine containing mouthwash rinses have a substantial effect on the count of bacteria before and after use. The levels of total oral bacterial count in the saliva samples at the end of the study were significantly lower in groups used chlorhexidine containing mouthwash products. Zordyl® and Oraxin® were the most effective products achieving high to isolate the naturally occurring bacteria which later on exposed to in-use concentration of mouthwashes containing Chlorhexidine or Hexetidine to evaluate both viable counting and zone of inhibition developed.

reduction in bacterial viability. Chlorhexidine containing mouthwash rinses (Figures $2_{a\&b}$ respectively) exhibit appreciable antibacterial activity (90%, and 60% respectively). While Hexetidine containing mouthwash products, Hextril® and Givalex®, which were less effective, they exerts 34% and 25% reduction in the bacterial count (Figures $2_{c\&d}$ respectively).
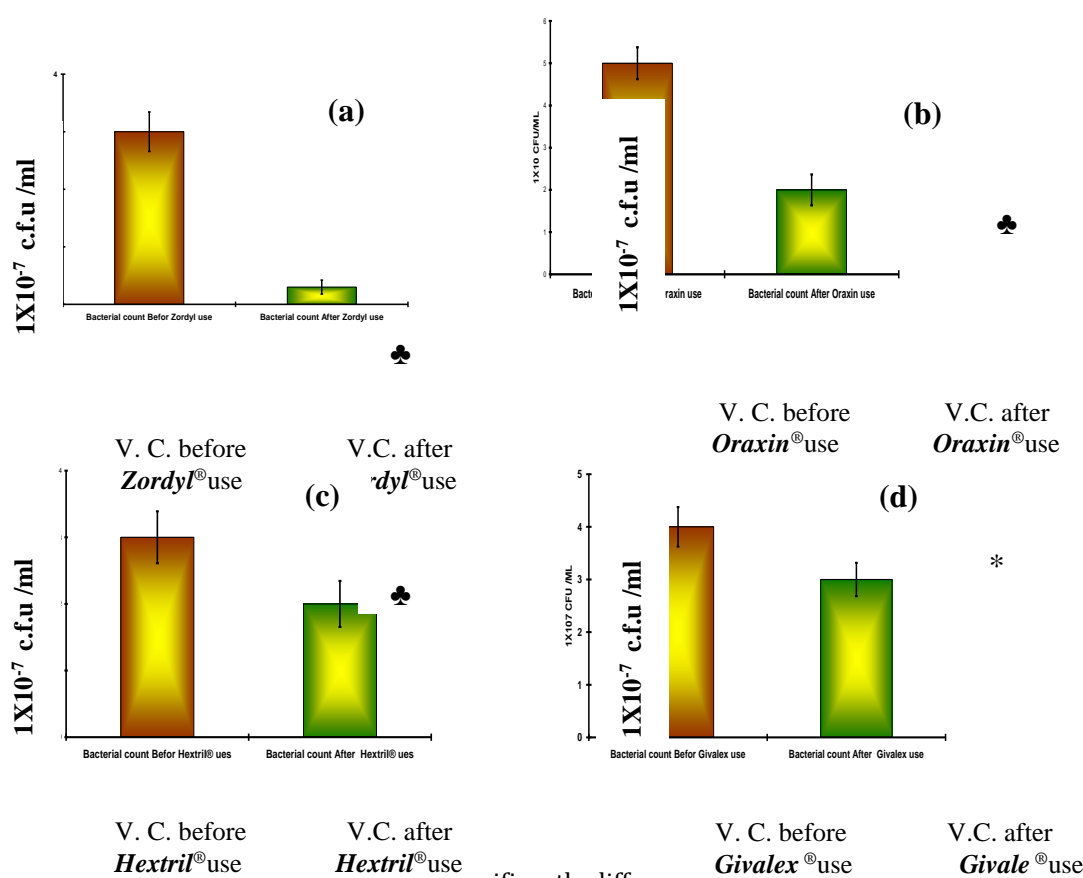
(*) No significantly different from the control

**Figure 2.** *Salivary bacterial count of volunteers before and after rinsing with different mouthwashes*
(a), ***Zordyl***®; (b), ***Oraxin***®; (c), ***Hextril***®; (a), ***Givalex***®

In order to determine the growth inhibitory activity of the four mouthwashes, a suspensions of isolated bacteria at final cell density of ($10^7$ cfu.ml$^{-1}$) were challenged with the use concentrations of mouthwashes solutions and viability was determined by colony counting after 2, 4, 8, 15, 30 and 60 minutes incubation time (Figures 3$_{a-g}$).

**Table 1.** *Killing times for different mouthwashes against isolated bacteria from volunteers saliva.*

| Bacteria | Killing times (min) at which mouthwashes(*) exhibit bactericidal effect | | | | | |
|---|---|---|---|---|---|---|
| | **(2)** | **(4)** | **(8)** | **(15)** | **(30)** | **(60)** |
| *Staph. aureus* | - | - | 1&2 | 3 | 4 | - |
| *Staph. albus* | - | 1&2 | - | 3&4 | - | - |
| *Strept. mutans* | - | 1 | 2 | 3&4 | - | - |
| *E. coli* | - | 1&2 | - | 3&4 | - | - |
| *Ps. aeruginosa* | - | - | 1 | 2&3 | 4 | - |
| *Kl. pneumoniae* | - | 1 | 2 | - | 3&4 | - |
| *S. marcescens* | - | - | 1 | 2&3 | 4 | - |

*(*) 1, Zordyl®; 2, Oraxin®  3, Hextril® & 4,Givalex®*

Figure 3 shows the inhibitory activities of Chlorhexidine containing mouthwash rinses (*Zordyl®* and *Oraxin®* ) and Hexetidine containing mouthwashes  (*Hextril®* and *Givalex®)* against isolated organisms. This was determined by colony counting over a period of 60 minutes incubation time.
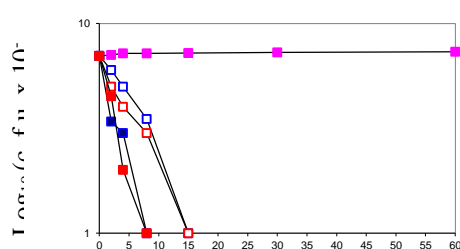


**(a)**

Time (min.)
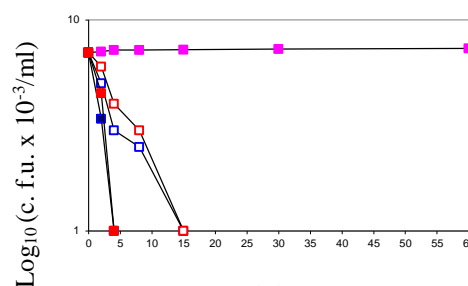
**(b)**

Time (min.)

**(c)**

Time (min.)

**(d)**

Time (min.)

**(e)**

Time (min.)

**(f)**

Time (min.)
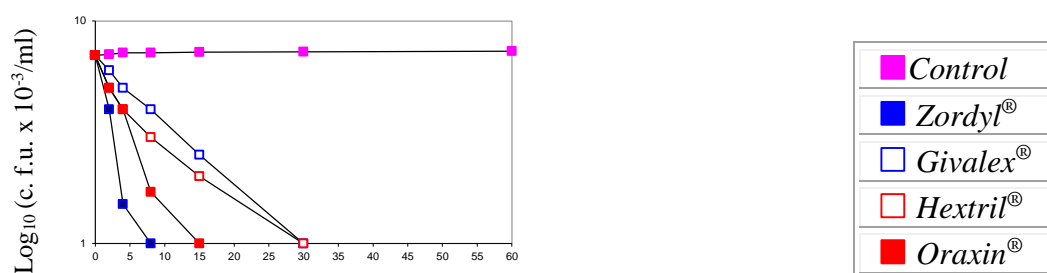
**(g)**

**Figure 3.** Growth inhibitory effect of different mouthwashes against isolated bacteria from volunteer's saliva (a), *Staph. aureus;* (b), *Staph. albus*; (c), *Strept. mutans*; (d), *E. coli*; (e), *Ps. aeruginosa*; (f), *Kl. Pneumoniae* and (g), *S. marcescens*

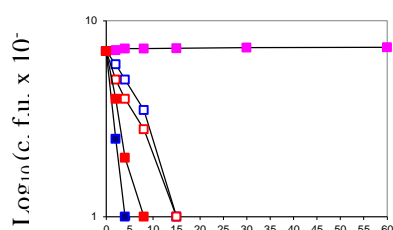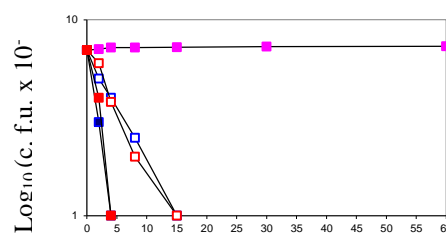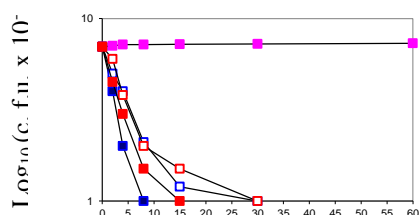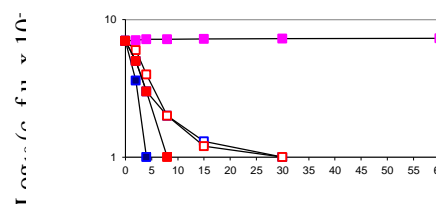On other hand **zone of inhibition** technique was used to assess the antibacterial effects of four mouthwash products on the growth of different types of bacteria isolated from volunteer's saliva. As shown in table 2 and figure 4 (plates 1-7) the antimicrobial effect of these mouthwashes was compared with effective causing inhibition on the growth of all isolated bacteria. The zone of inhibition was ranging from 17 to 40 mm, while Hexetidine containing mouthwash products, *Hextril*® which were considerably less effective. The phenol solution (0.5%) as a control which has an effect on the bacterial growth with a zone of inhibition ranging from 10-20 mm. Individual assessment of antibacterial activity of these agents revealed that chlorhexidine-containing mouthwash rinses, *Zordyl*® and *Oraxin*®, were the most

zone of inhibition was ranging between 9 to 20 mm. and *Givalex*® had low activity against gram positive bacteria and no effect against gram negative bacteria.

**Table 2.** *The effect of different mouthwashes* produced *on different types of bacteria.*

| Type of MW | Zone of inhibition mm | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Ps. aeruginosa* | *K. pneumoniae* | *Strept. mutans* | *Staph. aureus* | *Staph. albus* | *E. coli* | *S. marcescens* |
| **Control** | 18 | 16 | 14 | 10 | 18 | 20 | 16 |
| *Zordyl*® | 20 | 20 | 17 | 30 | 40 | 25 | 20 |
| *Oraxin*® | 17 | 20 | 20 | 30 | 30 | 20 | 20 |
| *Hextril*® | 00 | 10 | 9 | 20 | 13 | 06 | 16 |
| *Givalex*® | 00 | 00 | 10 | 20 | 25 | 00 | 00 |
| | | | | | | | |
| | | | | | | | |

**(1)**          **(2)**          **(3)**

**(4)**          **(5)**          **(6)**

**(7)**
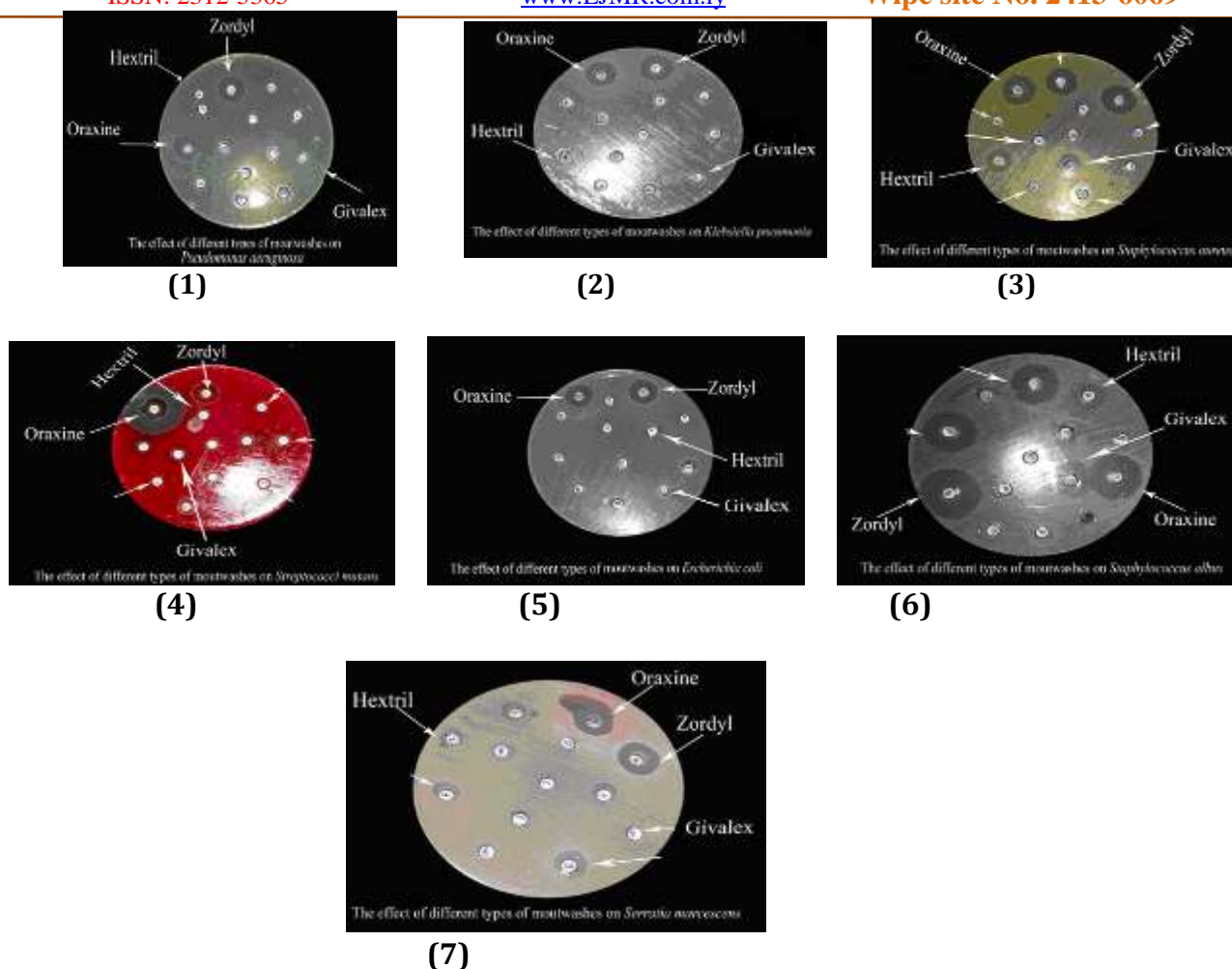
**Figure 4.** Zone of inhibition of different mouthwashes against different bacteria; (1), *Pseudomonas aeruginosa*; (2), *K. pneumonia*; (3), *Staph. aureus*; (4), *Strept. mutans*; (5), *E. coli*; (6 ), *Staph. albus* and (7), *S. marcescens*

DISCUSSION

A total of 60 volunteers (45 male and 15 female) were involved in this study. Subjects were divided into 4 groups and their saliva samples were assessed for microbial counts at the beginning and the end of two weeks of mouthwash use. Saliva specimens were subjected to serial dilution and plating on a variety of selective and non-selective agar tops to isolate the naturally occurring bacteria which were then exposed to in-use concentration of Chlorhexidine and Hexetidine-containing mouthwash to evaluate both viable counting and zone of inhibition developed.

Chlorhexidine-containing mouthwashes, showed better effect on oral microbial count tested by different experimental techniques than Hexetidine-containing mouthwashes, based on this active ingredient.
This inhibitory effect can be attributed to the presence of Chlorhexidine digluconate in essential antibacterial agent in their formulation. It was also observed that in subjects who rinsed with Hexetidine-containing mouthwash in their formulation, that total viable count of salivary bacteria was reduced but in much less degree than Chlorhexidine-containing mouthwash.

In addition, we evaluated the antibacterial activities of these mouthwashes using agar cup-cut diffusion method to determine effects against all bacterial strains, including pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus mutans, Staphylococcus aureus, Staphylococcus albus, Escherichia coli and Serrate, with zones of inhibition ranging from 17 to 40 mm in diameter for Zordyl® and 17 to 30 mm in diameter for Oraxin® (table 1). In contrast, Hexetidine-containing Mouthwashes were unable to produce zone of inhibition against all the tested bacterial species. Therefore, Zordyl® and Oraxin® might be promising lead mouthwash products for development of antibacterial agents against human oral pathogens. Furthermore, Chlorhexidine as the main active constituent in the formulations of both Zordyl® and Oraxin® mouthwashes, was proved to be an their zone of inhibition against some of salivary isolated bacteria which showed that Zordyl® and Oraxin® had inhibitory excellent biocide applicable for use in mouthwash preparations. because of the short treatment time (4 min.) required to kill all salivary isolated bacteria as shown in (table 2).

By contrast the killing times for Hextril® and Givalex® ranging from 15 to 30 minutes (in which the time profile is very important in the use of mouthwash, because of their way of application) the shorter the better, because there is no long contact time between the saliva and the product during use

## CONCLUSION

From this study, it can be concluded that, CHX & HX-containing mouthwashes proved to be an adequate method to maintain a good oral hygiene by reducing the bacterial load of oral cavity

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# Awareness, perception and factors affecting utilization of cervical cancer screening services among female nurses medical staff in Zawia teaching hospital , Libya

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**Abstract**

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix. One of the most common symptoms of cervical cancer is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer has progressed to an advanced (Kumar V , 2007). Among all malignant tumours, cervical cancer is the one which can be most effectively controlled by organized screening programmers(Arabian M,2009). The aim of the present study is to examine women's awareness of cervical cancer, to investigate women's perception of screening programmers , Finally To determine factors influencing utilization of services. This is a descriptive and cross sectional study was conducted in Zawia teaching hospital, from August 2015 to September 2015with total of 200 respondents. With self-administered questionnaire was used to obtained information on the socio-demographic characteristics of the respondents, Knowledge, perception about cervical cancer screening, as well as barriers against screening services. Fifty-three point five percent(107) of the women were aged 30–39 years and 63 (31.5%) were aged 40–49 years. Ten percent (20) of the respondents were aged 50 years and above. This means that the majority of the  respondents were still within the reproductive age group 90% of the respondents were heard of cervical cancer and 51% heard of cervical cancer screening programmed . 91(45.5%) and 88(44%) of staff heard about cervical cancer from a physician and TV/Radio respectively. While 89(44.5%) and 56(28%) of staff heard about cervical cancer screening from TV/Radio and physician respectively.

*Key words*:  Knowledge, attitude, barriers, cervical cancer, screening , Libya

INTRODUCTION

Cervical cancer is well recognized as the third most leading diagnosed in overall women's  cancer disease in the world (Jemal et al., 2011). Most cases were detected in the developing  countries in comparison to the developed countries with an estimated 529,409 new cases and 274,883 deaths in 2008. About 86% of the cases occur in developing countries, representing 13% of female cancers(WHO/ICO  2010). A literature search identified studies that examine factors influencing women's  participation in screening program, their psychological reaction to the receipt of an abnormal cervical smear result, and  experiences of colposcopy. Reasons given for nonparticipation included administrative failures, inconvenient clinic times, unavailability of a female screener, lack of awareness of the test's indications and

benefits, considering one self not to be at risk  of developing cervical cancer, and fear of  embarrassment, pain, or the detection of  cancer. The receipt of an abnormal result and referral for colposcopy cause high levels  of distress owing to limited understanding of the meaning of the smear test, many women  believe the test aims to detect existing cervical cancer( Oboma YI and Online FM , 2012). Inadequate knowledge (Al Sairafi and Mohamed, 2009) and lack of awareness can become a barrier to cervical cancer prevention (Al-Naggar, 2012). Many participants in previous screening studies revealed that they have little knowledge of cervical cancer (Oon et al 2011) and early screening using the Pap test can save their lives . Respondents also reported that they perceive that cervical cancer ultimately leads to death and can never be cured

(Sankaranarayanan et al., 2001; Austin et al., 2002; Wong et al., 2009; Institute, 2012 Respondents in a Malaysian study stated that cervical cancer arises from contracting sexually transmitted diseases (Wong et al., 2009). Some respondents also feel that insufficient information is made available about the centres providing the screening facilities (Abotchie and Shokar, 2009; Al-Naggar et al., 2010;

## Materials and Methods    Study design

about cervical cancer screening and risk factors for cervical

cancer; reasons for non-participation in the national cervical cancer screening programme ; A face to face interview technique according to a form translated into simple Arabic languish to ensure its comprehensibility. Respondents were given a free hand in response to questions and were only guided in their responses when they voluntarily called for assistance. They were also assured that the information provided would be kept confidential.

**Study setting :** The data will be collected from Service departments in researcher area are grouped into clinical and non-clinical departments. The clinical department consisted of 8 departments (Anaesthesia, Obstetrics & Gynaecology, Dialysis , Radiology, General Surgery, Medicine, Theatre, Paediatrics) and the non-clinical comprised 3 departments (Pharmacy, Nursing Records, and Administrative/Finance Departments).

**Sampling Procedure :** Stratified, proportionate and simple random sampling techniques will adopted for the selection of

**Data Collection Process**

Each interview started with an introduction and overview of the research including the objectives of the study. The respondents will told not to write any name on the self-administered questionnaire. Respondents were encouraged to ask questions on what they do not understand in the

**Statistical Analysis**

Data entry and analysis were performed with using statistical package for social sciences (SPSS) version 14. demographic data were summarized using descriptive

Aniebue and Aniebue, 2010 . Other respondents expressed concern that they would lose their virginity if they undertook the cervical screening test. This may relate in part to lack of knowledge regarding Pap smear screening process and test. the socio-background of the family (Abotchie and Shokar, 2009; Al-Naggar et al., 2010).

This is a descriptive and cross sectional study was conducted in Zawia teaching hospital, the study view point was conducted over a 9 month period from November 2014 to August 2015with total of 200  respondents. aimed at assessing and documenting the perception and utilization of cervical cancer screening services among female medical staff. It sought to understand the perception of this population about cervical cancer, its risk factors, severity and prevention. With self-administered questionnaire will be designed to assess the view, knowledge, level of perception and the attitude of female medical staff towards cervical cancer screening based upon similar studies conducted elsewhere and literature review. The questionnaire was used to obtained information on the socio-demographic characteristics of the respondents, Knowledge, perception about cervical cancer screening, as well as barriers against screening services. The questionnaire included 19 questions and was divided into three sections: awareness

questionnaire. Explanations were given to respondents as required to aid their understanding of unfamiliar terms. The questionnaires were retrieved back from each respondent immediately after completion and they were reviewed for completeness.

statistic Data were collected from mid-June to early August, 2015. Descriptive and inferential statistics such as percentages, Chi-square test , and factor  analysis were

used to determine the nature of the problem. The test of significance was

considered when $p < 0.05$.

## RESULTS

reproductive age group. In terms of their marital status, 53% of the respondents were single, 44% married, 1% widowed, and 2% divorced. Most of the respondents resided at rural area within 49% and 64% o f respondents were Libyan nationality and 36% were non Libyan , most of them were Filipino (30%) , 2% from Bangladesh ,1.5% from Indian and Sudan and about 1% were from Egyptian. Fifty one percent 0f population study had 3 children.

The study was successfully conducted in the selected hospital. With a good response rate. Table 1 illustrates the background characteristics of the respondents.

**A** total of **200** female nurse were recruited for this study , Females aged 20–60 years participated in the study. Fifty-three point five percent(107) of the women were aged 30–39 years and 63 (31.5%) were aged 40–49 years. Ten percent (20) of the respondents were aged 50 years and above. This means that the majority of the respondents were still within the

**Table 1** Socio demographic data of respondents (n=200)

| Variable | Frequency | Percentage(%) |
|---|---|---|
| Age group | | |
| 20-29 | 5 | 2.5% |
| 30-39 | 107 | 53.5% |
| 40-49 | 63 | 31.5% |
| 50-59 | 20 | 10% |
| >=60 | 5 | 2.5% |
| Marital Status | | |
| Single | 106 | 53% |
| Married | 88 | 44% |
| Widowed | 2 | 1% |
| Divorced | 4 | 2% |
| Nationality | | |
| Libyan | 128 | 64% |
| Non Libyan | 72 | 36% |
| (Filipino | 60 | 30% |
| Indian | 3 | 1.5% |
| Bangladesh | 4 | 2% |
| Sudan | 3 | 1.5% |
| Egyptian) | 2 | 1% |
| Place of residence | | |
| Big town | 70 | 35% |
| Small town | 32 | 16% |
| Country side | 98 | 49% |
| Number of children | | |
| 0 | 69 | 34.5% |
| 1 | 10 | 5% |
| 2 | 8 | 4% |
| 3 | 102 | 51% |
| >=4 | 11 | 5.5% |

**Table 2** shows the awareness of cervical cancer and cervical cancer screening. 90% of the respondents were heard of cervical cancer and 51% heard of cervical cancer screening proramme . 91(45.5%) and 88(44%) of staff heard about cervical cancer from a physician and TV/Radio respectively. While 89(44.5%) and 56(28%) of staff heard about cervical cancer screening from TV/Radio and physician respectively.

**Table 2: Awareness of cervical cancer and cervical cancer screening**

| Variable | Frequency | Percentage(%) |
|---|---|---|
| **HEARD OF CERVICAL CANCER**     Yes<br>                                                            N | 180<br>20 | 90%<br>10% |
| **HEARD OF CERVICAL CANCER screening**    Yes<br>                                                                         No | 102<br>98 | 51%<br>49% |
| **Source of information for Cervical Cancer** (For those that demonstrated awareness) | | |
| Physician/Health worker | 91 | 45.5% |
| Family/ Friends | 10 | 5% |
| Newspaper | 6 | 3% |
| TV/Radio | 88 | 44% |
| Internet | 2 | 1% |
| No response | 3 | 1.5% |
| **Source of information for Cervical Cancer screening** (For those that demonstrated awareness) | | |
| Physician/Health worker | 56 | 28% |
| Family/ Friends | 8 | 4% |
| Newspaper | 0 | 0 |
| TV/Radio | 89 | 44.5% |
| Internet | 12 | 6% |
| No response | 35 | 17.5% |

In the adjusted model screening awareness depended on nationality - Non Libyan -speaking women were better aware of the programme than the others 95% CI: 6.72-7.78 and *P* value 0.03 those were explained in **table 3** below.

**Table 3: demographic characteristics of women awareness of cervical cancer screening programme**

| Socio-demographic characteristics | Categories | (CI 95%) | Test statistic P-value |
|---|---|---|---|
| Age group, yrs | 20-29<br>30-39<br>40-49<br>50-59<br>>=60 | 1.43-1.57<br>6.48-7.98<br>5.82-8.04<br>3.95-8.94<br>2.52-7.48 | <br><br><br><br>0.87 |
| Marital status | Single<br>Married<br>Divorced<br>Widowed | 6.90-8.75<br>6.11-7.29<br>6.61-8.28<br>5.48-8.52 | <br>0.11<br><br> |
| Nationality | Libyan  Non Libyan  (Filipino Indian Bangladesh  Sudan  Egyptian) | 4.81-6.99<br>6.72-7.78 | 0.06<br>0.03 |
| Place of residence | Big town<br>Small town<br>Country side | 6.12-7.78<br>6.61-8.28<br>5.08-8.04 | <br><br>0.55 |

shown in Table 4. These were lack of screening sites, screening sites being too far away, limited information on cervical cancer, and absence of health education programs. The personal factors were lack of knowledge about the Pap smear test and the facilities where it can be carried out. This is because these two barriers had accounted for 16.25% and 12.73% of the total variation in the data, respectively. This means that these are the most devastating barriers. Specifically, items such as "the screening sites are too far from where I live" and "there is limited information on cervical cancer in the community" were dominant in the institutional barriers to the Pap smear test among the respondents. With regard to the personal barriers, the respondents lacked adequate knowledge about the test and where it could be done. Similarly, negative beliefs, and negative misconception barriers collectively explained about less than 47% of the total variation. The negative beliefs identified by the majority of the respondents were that the Pap smear test was embarrassing and painful. the negative misconceptions identified were that women did not feel at risk and therefore felt no need for Pap screening.

In the questionnaire all the cervical cancer risk factors were given without stating this and women were asked whether they think that these are risk factors or not. About 93% of the respondents had no knowledge of the mode of transmission of this disease. In general Women did not have a good overview about cervical cancer risk factors ,for example, Respondents did not have any idea about the impact of smoking as a cause of cervical cancer and HPV was better known as a risk factor - 86.6% from the non Libyan women. 75.7% of the women responded that they were planning to participate in a cervical cancer screening programme. The overwhelming majority (97.7%) of the respondents had never heard about the Pap smear test. However, eight (2%) of the respondents had a correct understanding of Pap smears. Of the respondents( non Libyan) who had undergone the Pap smear test in the study, only

three (0.8%) had been screened. The three respondents who had had a Pap smear test reported that they were referred by their health care providers.

The main barriers identified by respondents for not seeking Pap smear tests were institutional and personal, as

**Table 4 Barriers for non-attendance in the cervical cancer screening programme**

| Scale | Loadings(MBS)* | | Barrier factors |
|---|---|---|---|
| There are no screening sites in the community | 0.849 | | Institutional barriers |
| There is limited information on cervical cancer in the community | | | Institutional barriers |
| The screening sites are too far from where I live | 0.873 | | Institutional barriers |
| There are no health education programs to promote screening | 0.939 | | Institutional barriers |
| I do not know what the test is all about | | | Institutional barriers |
| I do not know of any screening sites | | 0.376 | Personal barriers |
| Pap smear test is painful | | | Personal barriers |
| Recent visit to gynaecologist | 0.944 | | |
| Appointment times not suitable | 0.948 | | Negative belief barriers |
| It is not necessary for me | 0.885 | | Negative belief barriers |
| | | 0.188 | |
| | | 0.580 | Negative belief barriers |
| | | 0.77 | Negative misconceptions |

*(MBS)= Mean barrier score calculated by totalling the subject responses for each question in order to get the mean barrier score. Higher scores indicate a greater perceived barrier scale.

The study revealed that not having Pap smear tests had significant associations
with all seven barriers at the 5% significance level as shown in **Table 5**.
This confirmed the negative implications of these barriers on respondents' decision not to undergo a Pap smear test for cervical
cancer. Confirming the results from the factor analysis, the Chi-square test also revealed that institutional barriers were the main barriers to seeking a Pap smear test, followed by personal barriers, since they had the highest Chi-square values of 28.965 (*df* =4; *P*=0.000) and 26.055 (*df* =5; *P*=0.000), respectively.

**Table 5 : Association between Pap smear test and the barriers to Pap smear test**

| Barriers | Chi-square values | *Df* | *P*-value |
|---|---|---|---|
| Personal barriers | 26.055 | 5 | 0.000 |
| Institutional barriers | 28.965 | 4 | 0.000 |
| Negative belief barriers | 21.915 | 4 | 0.000 |
| Negative misconception barriers | 20.965 | 4 | 0.000 |

# Discussion

Libya has a population of 2.21 millions women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 241 women are diagnosed with cervical cancer and 95 die from the disease. Cervical cancer ranks as the 3rd most frequent cancer among women in Libya and the 7th most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Libya. However, in Northern Africa, the region Libya belongs to, about 3.0% of women in the general population are estimated to harbour cervical HPV-16/18 infection at a given time and 81.2% of invasive cervical cancers are attributed to HPVs 16 or 18.(ICO -HPV Cancer Libya 2014).

This study had three principal aims. First, to estimate which socio-economic characteristics are associated with female nursing awareness about cervical cancer. Secondly, to investigate women's perception of screening programmes and risk factors for cervical cancer. And finally, to study reasons why so many Libyan women do not participate in the cervical cancer screening programme. An important outcome of my study was that approximately a half of the respondents were not at all or were only partially aware of cervical cancer screening. According to Libya Human Papilloma virus and Related Cancers, Fact Sheet 2014 (Dec 15, 2014) , No data available on Cervical screening practices and recommendations.

The study results revealed that there is a strong need to improve women's knowledge about cervical cancer risk factors. Knowledge of women on cervical cancer and the Pap smear test are critical in cervical cancer prevention strategies. The results of this study shows that a great proportion (49%) of the sampled population had never heard of cervical cancer screening. This finding is consistent with other research, which reported a lack of knowledge about cervical cancer among women in neighbourhood countries (Getahun F, 2013). Education on cervical screening through the mass media and health talks in delivering health care are imperative to informing women about cervical cancer and the facilities available for them. Opportunistic screening in health facilities could be encouraged to improve screening uptake, especially in women in rural area . It is evident that information about cervical cancer needs to be made available to women through mass campaigns about the disease, especially specific preventive measures and the screening facilities available. According to the WHO, cervical cancer is caused by HPV, which is a sexually transmitted infection and mostly affects sexually active men and women. However, in the current study, only few respondent (non Libyan) knew that cancer of the cervix could be transmitted sexually. This implies that a greater proportion of sexually active women might acquire HPV through sex without them knowing the source of the infection. Awareness of the Pap smear test was low of the respondents had never heard about the Pap smear test. This is consistent with the findings of previous studies, in underdeveloped countries. In the present study, only 2% had correct understanding of Pap smears, ie, they could describe the test and identify facilities where one can obtain such services. This affirmed the findings of Paolino and Arrossi(2011), in which a significant proportion (49%) of those who had been screened had inadequate knowledge about Pap smears. In Libya and other developing countries, there is poor institutional framework to promote screening. This is different from industrialized nations, which have largely succeeded in implementing successful programs. A possible explanation is that women will engage in cervical screening if they are well-informed about it and the enabling factors are present to facilitate effective screening uptake e In the present study, institutional and personal factors

# Conclusion

cervical cancer screening into the health care of their staff. Adoption of alternative screening techniques, such as visual inspection with acetic acid (VIA) may be necessary to widen patients' coverage. Pap smears should be accorded priority like other Maternal and Child Health Programs. The state government needs to put in place a policy on screening for cervical cancer with appropriate screening guidelines.

Cervical cancer is a problem of global health concern. Cervical cancer screening services such as the Pap smear test might be effective in detecting early precancerous lesions.  A greater proportion of the staff respondents had little or no knowledge of cervical cancer screening. There is a need for the authorities of tertiary educational institutions and particularly those of Zawia teaching hosital to incorporate regular

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# The spiritual dimension and its impacton health

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Great advances to puzzle out the causes of illness and improve the quality of lifeand achieve higher levels of wellness within the physical, mental, social, and spiritual dimensions are rapidly taking place in the field of scientific and medical research. Furthermore, scholars in spirituality studies have contributed to the wealth of both qualitative and quantitative data that exist.[1]

All these have enriched therapeutic and preventive measures in combating illnesses. However, despite the advanced knowledge and technology which are disseminated through the mass media today, statistical data show that mankind still suffers from an endless series of physical, psychological and social diseases, particularly behaviour-related disorders.[2]

Examples are hypertension, smoking, sexually transmitteddiseases, violence and drug use. Traffic accidents associated with alcoholism, drug addiction, anxiety, depression, suicide, divorce, rape, illegitimacy, homosexuality, lesbianism, broken homes, murder, crime and the like. All these problems are related to disturbances and failure in behavioural aspects of events in social life.

In this respect a number of questions will be raised. For example, although people may be well acquainted with the preventive methods and treatments that are available today they may be unable to keep to the advice givenby concerned physicians. This indicates that certain ideas and attitudes may have already been formed which influence and determine people's behaviour in health and sickness. These ideas and attitudes will obviously be more positive, and will have a greater impact on human behaviour, if they are spiritually bound and religiously based; ideas and attitudes within a religious context will have a more dynamic and broader impact on the promotion of health and the prevention of behaviour related diseases. Yet most psychologists have little if any training onspiritual and religious issues. Perhaps psychologists and other health care professionalscould potentially use spiritual and religious principles to better serve their clients.[3]

This calls for a concept of health within its wider physical, social and psychological aspects, as well as within its spiritual dimensions. Therefore, Spiritual care is inseparable from physical, social and psychological care because together they form the whole. Promoting spiritual well-being supports clients in their journey to find meaning and hope in life and peace in death.[4]

## The role of religion

Recently many studies show increased interest in examining the role that religion might play in preventive holistic health care.

Since the dawn of history, religion has been well recognised for its preventive role. Its preventive determinants were tied to faith in the Almighty Creator.[2]

It was explained by the prophets that the preventive measures are orders from the Almighty who has created the human being and knows what benefits or harms him. This faith, which had a very powerful effect in the past, should be increasingly reinforced now that we have realised the great dangers to which humanity might have been exposed, had it not adhered to the religious orders with absolute faith. This absolute faith was very central in the role of prevention.

Alcoholism for example, was partly responsible for the deterioration which befell prehistoric civilisations.
 Islam faced that grave evil and succeeded step by step in overcoming its dangerous effects. It linked faith in God with the orders to abstain and succeeded in persuading the believers to give up the long standing habit and compelling dependence of alcohol. The true Islamic communities up till now are relatively free from the evils of alcoholic addiction. This is from the effect of the deep faith that true Muslims keep as regards the Quranic orders. This was also applied to other physical, psychological and social evils.

## Faith

The spiritual dimension is described and is interpreted as the need for: meaning, purpose and fulfillment in life; hope/will to live; belief and faith.[5]
Thus faith, once established in its proper spiritual dimension, worked as a strong preventive weapon and at the same time had sustained reinforcement through the relationship of the individual with the Almighty. This was the secret behind its success in the lives of people and in its influence on their behaviour, and consequently on their state of health.It is important to note that it is religious faith alone that can convert a man into a true believer and can suppress his selfishness and self-seeking under the impact of a doctrine and an ideology.
Though faith is of varying degrees, spiritual faith often creates in man satisfaction and pleasure depending only on the all-powerful God. A good example of this satisfaction and pleasure is manifested in the behaviour of the true believer who faces a very dangerous situation, but who is urged by his faith to go through it, in many cases succeeding.Without this faith, such an endeavour could not be fulfilled.
The early stages of faith are manifested in the child's love of his mother as long as she takes care of him. If this care disappears faith is lost. So love and faith are interrelated. Throughout life this relation matures and develops until it reaches its highest point in divine faith.
This faith is acquired step by step through love and satisfaction, meditation and contemplation of the universe and insight about life. The greatness and splendour of the creation all around us, of the earth and the universe, is the magnificent and enriching source which feeds this spiritual faith. Once attained this faith is surely a strong and vital force for leading a healthy and spiritually rich life.[2]

"إِنَ فِي خَلْقِ السَّمَاوَاتِ وَالْأَرْضِ وَاخْتِلَافِ اللَّيْلِ وَالنَّهَارِ لآيَاتٍ لِّأُوْلِي الْأَلْبَابِ"

"Indeed, in the creation of the heavens and the earth and the alternation of the night and the day are signs for those of understanding" (Al-e-Imran, 3:190).[6]

This meditation creates in man a treasure of insight that the Almighty God is not only a creator, but is also the absolute donator of health and all other endowments. It is He who created disease and it is He who created relief. Immunity is one of His blessings and medical resources throughout the ages are His gifts. This is the spiritual faith to which we refer, and by it we are urged on to conduct further and continual research in this endless field. So the believer is not just a passive receiver, but should participate in positive research for curative aids which the Almighty has created for him. Thus faith is not idle, but is charged with energy.[2]

## Morals and health

This meditation is also responsible for the creativity and development of many of our morals such as patience, courage, love, forbearance, generosity, pity, sacrifice and the like. These morals constitute the which arc a feature of the true believer.[7] spiritual elements which foster and support the physical, mental and social aspects of health. Thus a high standard of health is created by spiritual constituents

The essentials of the spiritual factors in health are unfortunately overlooked in our scientific and medical teaching, and are left to be taken care of by the religious teachers who are not generally conversant with this subject, nor are they in direct touch with scientific research. Scientific experiments with placebos based on faith have yielded some good results when controlled therapeutic measures were evaluated. These experiments as well as others clearly demonstrated the patient's faith in the placebo (capsules, etc.), in the modality of treatment and the provider of the drug, even when it was an inactive chemical. We need not stress here that faith in the treating physician is well known to cause many cases to improve spontaneously. This phenomenon should be given more attention to explore its essential basic elements and make more use of its potential powers. Thus it is to be realised that within the spiritual dimension there is a great potential of compassion and power of healing which needs more care and attention to be fully utilised. Consequently, when we speak about health, it is to be emphasised that faith is one of the foundations upon which health should be conceived and firmly built.[8]

The Holy Qur'an is the first Book which has described religious faith as a sort of concord between man and the entire creation:

" أَفَغَيْرَ دِينِ اللهِ يَبْغُونَ وَلَهُ أَسْلَمَ مَن فِي السَّمَاوَاتِ وَالأَرْضِ طَوْعاً وَكَرْهاً وَإِلَيْهِ يُرْجَعُونَ" **(آل عمران 83).**

"Do they seek anything other than the religion of Allah? But to Him submits whosoever is in the heavens and the earth." (Al-e-Imran, 3:83).

The Holy Qur'an has also described religious faith as a part of the innate nature of man:

فَأَقِمْ وَجْهَكَ لِلدِّينِ حَنِيفاً فِطْرَةَ اللهِ الَّتِي فَطَرَ النَّاسَ عَلَيْهَا لَا تَبْدِيلَ لِخَلْقِ اللهِ ذَلِكَ الدِّينُ الْقَيِّمُ وَلَكِنَّ أَكْثَرَ النَّاسِ لَا يَعْلَمُونَ **(الروم 30).**

**"Be devoted to the upright religion. That is the nature in which Allah has created man."** (Ar-Rum, 30:30).[9]

## A model of faith practice

A more searching look into the dimension of faith will enrich our scientific knowledge to the benefit of humankind. At this point we should assess, for example, the effects of the practices basically set by the Prophet Muhammad, which he gave as a model and asked all the believers to follow and maintainwith deep faith.Fundamentally these practices were crowned by absolute faith in the Almighty. This is stated verbally and devotedly in whatever the believer performs when he says "God is greater" (Allahuakbar).

The second constituent of that programme of faith and practice is ablution and praying five times daily. So the true believer should always keep cleanand meditative, thus combining a healthy state of physical and mental well-being.

The third is giving to others at large. It constitutes volunteering to give others money, treatment, help, care, a smile and even clearing any obstacles obstructing the pathway.

The fourth is fasting one month a year. In essence fasting implies avoiding indulgence in the daily necessities of life after satisfying the basic and essential ones, including food, drink, pleasures, sex, etc.

The fifth is pilgrimage and the visit to the holy city Makah, and it reinforces the feeling that the human being is always confronting the Almighty and should confess his ill behaviour and ask for forgiveness and relief. It also urges one to meditational recreation, deep insightful reflection for a better and healthier life, and it mobilises social feeling and the coming together with others for a noble cause.[10]

The combination of the physical and spiritual techniques in Islamic ideology brings the healthy and balanced believer to his full potential physically, psychologically and socially. This programme was responsible for creating the healthy Muslim needed in the dawn of Islam

to help to develop a healthy society in the entire of Islamic world. It is the essence of prevention and the backbone of a Every culture should make use of the dominant spiritual endowments to create a healthy community based on physical, psychological, social and above all, spiritual factors. This is why we ask for the addition of the spiritual factor to the other acknowledged factors. There are various ways and means for mobilising the compassion of the people with ennobling ideas within the spiritual dimensions of health, and for this purpose it is timely for the medical profession to set the relevant programme for undergraduates and for ongoing education.[12]

There are also a number of programme areas for the promotion of the health

**Practical Application**

Since the early days of Islam, the mosque has been used to practise worship as well as practising other everyday life activities. It was a centre for teaching, welfare, rehabilitation, guidance, medical care, planning for defence tactics, and so on. During the last decade, Muslim reformers, psycho-social workers and specialists in the medical professions called for a revival of the previous function of the mosque, to cope with the different problems facing the community. To realise this it was necessary to focus on the function of the mosque to adapt it to suit the newly arising demands.

Mosque is an institution. It is the source of spiritual and material guidance; it is the hall for worship, the school for knowledge and the centre for literacy pursuits.This was however put into practice by IbnTulūn who built a world famous mosque in Egypt with a well-equipped dispensary and library attached. The library was believed to have been stocked with a hundred thousand books on medicine and the dispensary used to witness many people who queue to receive treatment on Fridays.[14]

Modern mosques that will accommodate library, dispensary, multipurpose hall and

successful healthy life. It is also the catalyst for potentiating the other three foundations of health.[11]

system based on primary health care within the context of the spiritual dimension. The role of religious institutions, for example, in the promotion of health and the prevention of behaviour-related health problems has not been adequately explored, and should be optimally utilised. A model of utilising mosque facilities and religious teachers, for example, in the prevention and treatment of drug abuse has clearly demonstrated the useful potentialities of this institution, and this could be enhanced for wider application in the health field.[13]

other facilities should be given utmost attention by individual Muslim community. The design of the recently built mosques should take a new trend to suit the requirements of the mosque's new function. Thus, the new mosques should be made up of multi-storeys, each performing a different function. The ground floor is for praying, the second comprises different medical clinics. The third includes a library, social welfare centre, teaching classes, a rehabilitation office and a social centre to solve problems. This model satisfies the following needs:

• It is a centre for the healthy upbringing of children.

• It includes teaching classes, a club and a library for the benefit of the attending youth and thus gives the youth a breathing area in crowded cities caring for their general health.

• The different clinics charging nominal fees take care of a wide class of people who cannot afford the high expenses of treatment in special clinics or hospitals.

• The mosque is continuously propagating and spreading preventive means and so is very potent in this field.

• The spiritual approach vitalises the different activities adopted in the mosque, so the fourth dimension of health is well observed.

• Statistical data and continuous follow-up help to explore and define the benefits obtained for these activities.

During March 1984 the regional WHO conducted a seminar inviting responsible individuals in different ministries to activate that approach, and a project has been authorised for three years to conduct epidemiological studies about drug addiction in two of these mosque centres. This is the beginning of a movement in the Islamic countries to make use of the potentialities of the mosque for the welfare of health in general, and mental health in particular.

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# First experience with insulin analogues in type 1 diabetes mellitus in Tripoli diabetic hospital

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## ABSTRACT

The current study aims to determine the efficacy of insulin analogue on blood sugar control in patients with type 1 DM ,this study is a cross sectional study and was carried out in Tripoli diabetes hospital during the period (Nov/2009-April-2010). One hundred patients with Type 1 DM on basal –bolus insulin injections (after consent taken), were enrolled in this study, insulin glargin taken as basal single dose at night (mostly at 10 P M ) and Lispro injection before each meal (3 times/ day ), initially the total daily requirement calculated according to body weight or previous daily insulin doses and adjusted according to blood glucose profile done by patients (SMBG) ,the results were a significant regarding increase of total daily doses and body weight , decrease in number of hypoglycemic episodes /month ,and HBA1c .

The study concluded that insulin analogue (glargin &Lispro) administrations were associated with improve glycaemia control with reduction in hypoglycemia episodes in Type 1 DM.

**Key words**: Type1 diabetes, Insulin Analogue, self-monitoring Blood glucose(SMBG )

## INTRODUCTION:

The global rate of DMI is escalating,[1,2] Outcomes from the Diabetes Control and Complications Trial(DCCT)confirmed that intensified insulin treatment diminishes the risk of micro and macro-vascular events compared with conventional treatment.[3-6] The DCCT evidently demonstrated that intensive insulin therapy, definite as three or more injections per day of insulin or continuous subcutaneous insulin infusion (CSII) (or insulin pump therapy), was a solution of a better metabolic and improved results[7,8] . The study was carried out with short- and intermediate-acting human insulin, even with improved micro-vascular effects, intensive insulin therapy -term decline in macro-vascular disease. So a sensible A1C target for many non-pregnant adults with DMI is, 7%. Providers might reasonably suggest HBA1C goals of 6.5% for choose cases, if can be attained with no considerable hypoglycemia or other adverse effects of treatment. Suitable cases might consist of those with a short duration of diabetes, a

Was linked with a high rate of severe hypoglycemia (62 episodes per 100 patient-years of therapy). While the conclusion of the DCCT, a number of rapid-acting and long-acting insulin analogs have been appeared. These analogs are associated with less hypoglycemia than human insulin although present the identical reduction of A1C in cases with [9,10].

Reducing A1C to about 7% has been shown to diminish microvascular complications of diabetes, and, if realized almost immediately following the diagnosis, is related with long

long life expectation, hypoglycemia responsiveness, and no CVD. HB A1C goals of 8.5%) may be proper for cases with a history of severe hypoglycemia, hypoglycemia unawareness, limited life expectancy, advanced micro-vascular /macro-vascular complications, or extensive comorbid conditions.[11] Metabolic control for DMI at any age should be assessed based on regular

SMBG levels (and CGM data, if accessible), and HB A1C to facilitate modifications in treatment.

SMBG (Self-Monitoring Blood Glucose)
The DCCT established the advantages of intensive B sugar control on diabetes complications with SMBG as part of a multi-factorial role, signifying that SMBG is a vital part of efficient treatment. SMBG permits patients to estimate their individual reaction to treatment and evaluate if metabolic aims are being achieved. SMBG results are practical in avoiding hypoglycemia, regulating insulin dose (chiefly before meals), and appreciating the effect of suitable nutrition therapy and physical activity. More frequent SMBG is linked to lower A1C levels [12,13] SMBG rate and time should be ordered by the patient's particular requirements and aims. While advising SMBG, giver must guarantee that patients get continuing education and standard assessment of their SMBG skill and their facility to utilize SMBG records ,In cases with DMI should do SMBG before meals and snacks, at a least, and at further times, as after-meals to evaluate insulin-to-carbohydrate ratios; at bedtime; mid-sleep ( 3-4AM); prior to, during, The function of basal insulin (background insulin) is to maintain Blood sugar at steady degrees while abstain from food, is particularly used at mealtime to deal with B sugar levels after a meal[14].

The advantages of Multiple Daily Dose ,permit closely mimic normal insulin

and/or after exercise; if there is symptom of hypoglycemia ;after remedy of low/ high B sugar ; preceding to serious duties like as driving; and at more frequent intervals during sickness or strain.

The accessibility of insulin analogs has allowed insulin replacement that are planned to more intimately replicate natural insulin secretions. Particularly, pre-meal insulin analogs (lispro, aspart, glulisine) have action outlines nearer to normal, with resultant quicker initiation and neutralization of action to lower blood sugar in contrast with regular human insulin. Basal insulin analogs (glargine, detemir) have prolonged act, less variableness, more control, less hypoglycemia (especially nocturnal), and an encouraging result on weight[14].

Basal-bolus regime permits for accurate insulin dose regulations to attain glycaemic goals (HBA1c) and a glycaemic profile as close to physiological as possible with a low risk of hypoglycaemia.[15-27]

generally used 1-2 times a day .A bolus dose is insulin that

secretion ,flexibility in time of insulin injections ,amount of Carbohydrates intake each meal. Drawback of MDI, that more injections per day and weight gain .[28]

## MATERIALS & METHOD:

A cross sectional study which included a ( hundred) patients of DM I in Tripoli diabetic hospital from( Nov 2009 until April 2010).The data collected about Patient's demographics, Some important points in clinical history ,relevant investigations ,and then the patients were followed after 3month .

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS) version 16(compare means with paired samples test ) .

## RESULTS:

In National Diabetes Hospital in Tripoli – Libya where most of people with DM in west part of Libya receive their diabetes care, a total of 100 patients with DM I ,who attended outpatient clinic included

in the study , 72% of them were female, their age range was between (13-53years)with mean age(27.4±9.4years), the duration of diabetes ranged from newly diagnosed to(31years ) of DM .

while 46% of them were controlling their diet (assessed by registered dietitian ) ,(73%)were testing their blood glucose at home (SMBG) , with mean of total daily dose of human insulin (55 ±21.7 IU), most of them on Mixtard 30 Twice daily ,the remaining were on actrapid insulin +NPH in different combinations in four to two injections daily ,number of daily doses(3±1) ,history of hypoglycemic episodes (5.07±5.1),include minor ,major and nocturnal episodes, mean were weight (66.4±13.4Kg) ,BMI (24.7±4.4) ,and HBA1c pre study mean (10.6±2.2%)

## DISCUSSION :

Regular human insulin & intermediate-acting neutral protamine Hagedorn (NPH) insulin are both Conventional insulin . But, both do not mimic the outline of basal and post-meal physiological insulin release . Insulin analogues are adapted human insulin attend to overcome this restriction.[29]Insulin lispro in comparison with regular human insulin leads to a slightly lesser HB $A_{1c}$ concentration (weighted mean difference –0.09%, 95% CI –0.16% to –0.02%), a lower risk of severe hypoglycemia (relative risk 0.80, 95% CI 0.67 to 0.96) and a lower rate of nocturnal hypoglycemia (rate ratio 0.51, 95% CI 0.42 to 0.62). usually , Patients chosen rapid-acting insulin analogues over regular human insulin for the reason that flexibility of the dose in relation to meal.[30-34]Several reports shown that considerable enhancement in quality of life and patient pleasure with the utilize of rapid-acting insulin analogues, while further studies established no differentiation.[30-41]Insulin glargine (Lantus ) in contrast to, neutral

## CONCLUSION:

The study shows that insulin analogues (glargine, lispro) improved the glycaemic control in patients with DM I (i.e.HBA1c), with decrease in the number

After starting insulin analoug (insulin glargin as single basal dose at night +ultra rapid insulin analogue lispro/Aspart befor the 3 main meals ) regular follow up 3 month later **,** the total daily dose is significant increased (60.9±25 IU, P- value 0.05) ,the number of daily doses were 4 times , the number of hypoglycemic episodes/month were significantly diminished(1.1±2.1 P–value were 0.00),the mean weight increased (67±14.6Kg P-value 0.09),their mean HBA1c significantly reduced (9.5±2.3% P value were 0.00) .

protamine Hagedorn insulin (NPH), offered a small but statistically important reduction in HBA1c (weighted mean difference –0.11%, 95% CI –0.21% to –0.02%), as well ,the main hazard decline in night-time hypoglycemia support of insulin glargine (Lantus) use (relative risk 0.64, 95% CI 0.47 to 0.87). [42] For diabetic morbidity or mortality, still, incomplete statistics to compare insulin analogues and conventional insulin[42].

In this study we compare between the utilization of insulin analogue (insulin Glargin as basal and Lispro or Aspart as bolus doses) &human insulin (insulin Mixtard 30 twice daily, NPH and soluble insulin with different regimes) in the same patients with DM I, concerning with blood sugar control, incident of hypoglycemia, and weight gain. We concluded that insulin analogues provide a scientific benefit over human insulin for glycaemic control in DMI ,with less hypoglycemia especially nocturnal and may be considered a first choice for patients with recurrent hypoglycemia in spite of modification of conventional insulin treatment.

of hypoglycemic episodes/month; however, both total daily dose and mean body weight are increased.

## RECOMMENDATION:

In Type 1 DM the use of insulin analogue linked to significant improvement in blood glucose control ,less hypoglycemic episodes per month (especially nocturnal ) this is outweigh the high cost of insulin analogue than human insulin.

## ACKNOWLEDGEMENTS:-

Table (1) show mean &standard deviation for different variables on the study

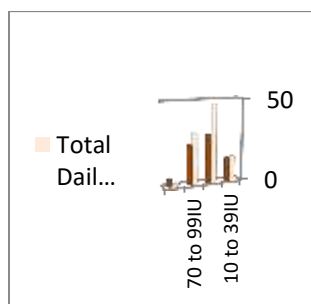| variable | Mean ±SD at start(twice daily human insulin) | Mean ±SD (after) basal-bollus insulin analogue | p-value |
|---|---|---|---|
| **Total daily dose** | 55±21.7 | 60.9±25 | 0.05 |
| **Hypoglycemia episods** | 5.07±5.1 | 1.1±2.1 | 0.00 |
| **Body Weight** | 66.4±13.4 | 67.7±14.6 | 0.09 |
| **HBA1c** | 10.6±2.2 | 9.5±2.3 | 0.00 |



Figure (1) show the total daily dose

difference at start &after 3 month of study

.
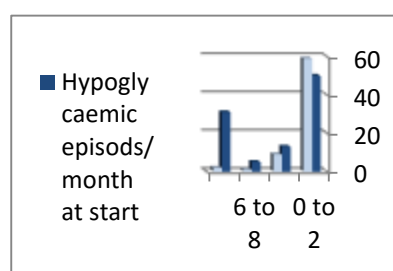


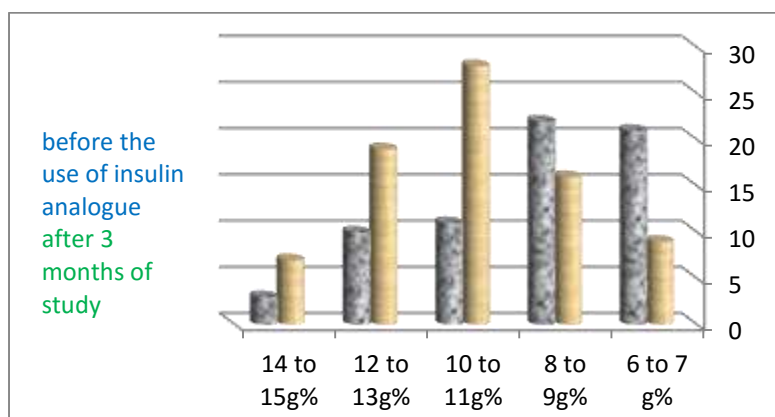Figure (2) show the total daily

episodes before &after 3month of study



Figure (3) : show difference in HBA1c levels before &after 3 months of study

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# Quantitative determination of lead and cadmium in samples of six brands of infant's milk powder formulae marked in Libya

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## ABSTRACT

Six commercial samples of Infant milk formulae (powdered form) which represent most kinds of milk formulae used for feeding infants from birth up to three years of age  were taken from reputed Pharmacies of  different cities of Libya for the estimation of heavy metals, lead (Pb) and cadmium (Cd). All samples were analyzed by Flame Atomic Absorption Spectrophotometer . The different kinds of infants milk formulae were found to have concentration of Pb and Cd ranging from 0.0000-0.0080 ppm and 0.0001 – 0.0015 ppm , respectively. Results obtained revealed that level of values of Pb and Cd were below the permissible limits as recommended by WHO and other standards .This study signifies its importance for consumers, manufactures and professionals in children's health care programs.

**KEYWORDS :**Infant's milk formulae, Analysis (FAAS),VARIN SPECTR AA 55B, Heavy metals, Cadmium,Lead.

## INTRODUCTION

Human milk contains optimal amount of carbohydrates, proteins and fats and is best source of nutrition for feeding infants (1).The milk is also an important source of major and trace elements necessary for normal development of infants. Milk powder is one of the important dairy products being used in the preparation of condensed milk, cheese, ice creams, infant milk formula , evaporated milk and as an ingredient in many bakery products. Infant milk formula is considered as nearly complete food and  an excellent source of protein, fat and major minerals for the normal growth of infants. During manufacturing of infant milk formula essential elements are added in appropriate quantity in order to meet nutritional requirement (2)). Excess from required quantity of the added elements may be a potential source of danger to health. Therefore, accurate measurement of the concentration of the trace elements in formula is very essential by using sensitive results gathered to an existing Egyptian standard as set for allowable amounts of and advanced methods of elemental analysis(3,4,5,6,7) . More than 20 different trace elements are reported in milk and milk products and most of them are essential and very important as a cofactors in many enzymes play important role in many physiological function and deficiency of these elements may produce pathological disorders (3,4). The low concentration of heavy metals such as Pb and Cd leads to metabolic disturbances and causing serious health problems including heart failure, cancer , kidney damage etc. (8,9,10).UNICEF, 1999, emphasize on control and assessment of babies food product's by purpose of their maintaining good health (11). The aim of the present study to test for the presence of toxic heavy metals, Lead and Cd in infant's milk formulae used in feeding to three years aged infants available in Standard pharmacy stores of Libyan markets through Atomic Absorption Spectrophotometry and to compare the toxic heavy metals for feeding to infants and manufacture of  food products .

## MATERIALS AND METHODS

### a. Collection of Samples :

A total of six random tin containers of milk powder representing most kinds of powdered milk formula for infants from birth up to third year of age were collected from local renowned pharmacy stores of Zliten and Alkhoms cities of Libya . The

**FormulaA1 :** for feeding new born infants.
**FormulaA2 :** for feeding new born infants.
**FormulaB1 :** for feeding infants at one year age.
**FormulaB2 :** for feeding infants at one year age.
**FormulaC1 :** for feeding infants at three year age.
**FormulaC2 :** for feeding infants at three year age.

samples were collected from its original packages in  clean polyethylene bags, labeled and taken to the laboratory and kept in refrigeration till analysis.

The  following Infant's  milk  powder formulae were taken for the analysis :

### b. Quantitative determination of heavy metals in  each sample :

All  chemicals were used of AR grades for each analysis. The samples were digested following the procedure described by Oddy, 2001(12). Briefly, 20ml $HNO_3$ was added to 10.0g of each sample and allowed to stand for 15min. The mixture was heated until the liquid reduced to 5ml. After cooling, 20ml $HNO_3$, 10ml $H_2SO_4$ and 8mL $H_2O_2$ were added and the contents were evaporated to 5ml. After cooling 10ml deionized $H_2O$ was added for the

removal of residual acid and the mixture was boiled for 10min (this was repeated twice). After cooling the digest was filtered into 25ml volumetric flask and made up to mark with deionized $H_2O$. The infant formula filtrates' were subsequently analyzed for the presence of heavy metals (Pb, Cd) by Flame Atomic AbsorptionSpectrophotometer (FAAS) Model VARIN SPECTR AA 55B,.

## RESULTS AND DISCUSSION

The results of the estimated concentration of  Pb and Cd in  six infant's milk powder formulae procured from  different Standard

Pharmacies of Al-khoms and Zliten cities of Libyaare shown in table 1.

**Table 1 :** Heavy metals concentration in different infant's milk powder formulae:

----------------------------------------------------------------------------------------------

| Infant Formula | Pb (ppm)* | Cd (ppm)* |
|---|---|---|
| Formula A1 | 0.0080 | 0.0015 |
| Formula A2 | 0.0060 | 0.0005 |
| Formula B1 | nil | 0.0011 |
| Formula B2 | 0.0032    0.0001 | |
| Formula C1 | 0.0007 | 0.0004 |
| Formula C2 | 0.0022 | 0.0005 |

-------------------------------------------------------------------------------------------

*All results are below permissible limit  (13-15).

Two formulae( A1 and A2) were for new born infants. They have shown Pb concentration ranging from 0.0060-0.0080 mg/kg and Cd concentration ranging from 0.0005-0.0015 mg/kg . Formulae B1 and B2 were for one year aged infants  have 0.0007 - 0.0022 mg/kg  and Cd concentration ranging from 0.0004-0.0005

shown Pb concentration ranging from 0 - 0.0032 mg/kg and Cd concentration ranging from  0.0001-0.0011 mg/kg . Formula C1 and C2 were for infants of three years age have shown Pb concentration ranging from mg/kg . The results indicate that nil /lowest concentrations of Pb and Cd were recorded

in Formula B1(nil ppm) and Formula B2 (0.0001ppm), respectively, however, highest concentration of Pb and Cd were recorded in Formula A1 (Pb : 0.0080 ppm ; Cd : 0.0015 ppm) . Thus it is apparent from the results that none of the infant's formulae exceeded the permissible limit for Pb and Cd levels as set by WHO and other Organization's Standards (13-15).However, the presence of Pb and Cd in infant food is of great concern since infants are particularly more sensitive to ingested toxicants even in very low concentration than adults  so infants health goes at risk(16).Exposure to Pb during infancy irreversibly affects development of the nervous system, causing reduction of IQ and learning disabilities(17). Chronic exposure to Cd and Pb is associated with kidney damage (18). It is reported that milk powder formulae containing soy flour were high in trace elements Pb and Cd (19,20). It is also reported that lead intake

was most strongly influenced by storage of infant formulae in lead-soldered cans. (21). It is apparent in the present study that all kinds of infant's formulae have shown very low levels of concentration  of Pb and Cd in acceptable limit .Thus this study demonstrates  that all kind of infant's formulae are devoid of any risk of untowards health hazards to infants.Numerous milk formulae and milk products are available in markets for all age groups of children and adults but it cannot be generalized from this study that all of the infant's milk formulae contains lead , cadmium or other toxic heavy metals below standard permissible limit. Many analyst  have reported higher concentration of toxic trace or heavy metals in milk powder formulae than recommended standard concentration of permissible limit which may have caused wide array of hazardous impacts on human health (22-26).

## CONCLUSION

All of six infant's formula samples tested were positive for lead and cadmium concentrations except Formula B1 which did not show the presence of  lead , however, the concentrations of both heavy metals were below the level of permissible limit as recommended by WHO and other Organization's Standards. However, this study can be used for general awareness as a reference for consumers, manufactures and health care professionals for the sake of maintaining good  health for children and adults using good quality milk formulae and milk products following their regular monitoring of heavy metals applying standard analytical techniques.

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# Otolaryngology Pediatric Emergencies update in Zawiya Teaching Hospital
## ENT Department, Zawia Teaching Hospital

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Despite advances in public health and medical care, pediatric Ear, Nose, and Throat emergencies are still prevalent around the world, and their incidence in Libya has actually increased over the past ten to twenty years.

The otolaryngologist is frequently called upon to deal with such cases when conservative measures have failed. Children are among the most commonly seen by the primary care physicians and the otolaryngologists.

The role of the otolaryngologist in evaluating the acutely ill child is to determine the etiology of the emergency through comprehensive evaluation and to assure appropriate treatment for the various underlying etiologies.

The object of the evaluation is to separate possible causes into the categories of life threatening or benign, and to distinguish easily treatable causes from more complex causes. Although the majority of these conditions are benign, there are several critical disorders that must be immediately recognized and treated.

Approximately one third of E.N.T attendance in our Department is comprised by the pediatric age group[1]. That, prompted us to analyze the E.N.T. emergencies of this age group in our setting, to provide a baseline data which may help a better planning in the future and thus improving medical services.

## Method

A retrospective analysis of cases presented as emergencies was done in the ENT Department of Zawia Teaching Hospital, from July, 2007 to December, 2014. All consecutive cases in the pediatric age group up to 14 years old were studied. These cases either came directly from casualty or were referred from Pediatric Emergency Services in the area.

## Analysis

Throughout 7 years (2007-2014)a total of 670 ENT emergency cases were attended. Out of these, 225 (33.7%) cases were from the pediatric age group and of which 130 cases were males (57%), and 95(43%) females noticing a slight preponderance of the male cases over females *(See Chart 1.).*

Chart 1.



Pediatric Age Group Cases by Gender

66.30%   43%   33.7   57%

- Pediatric Age Group
- Non Pediatric Age Group
- Males
- Females
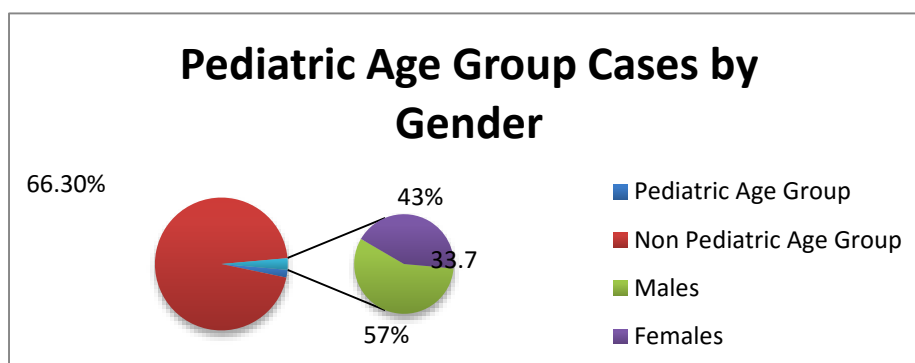
Only 43(18.8%) cases required admission either for close observation, systemic antibiotics or for surgical intervention.  Te maximum number of cases was seen in age group of 2-5 years old, distributed as shown in the following Chart.

Chart 2.
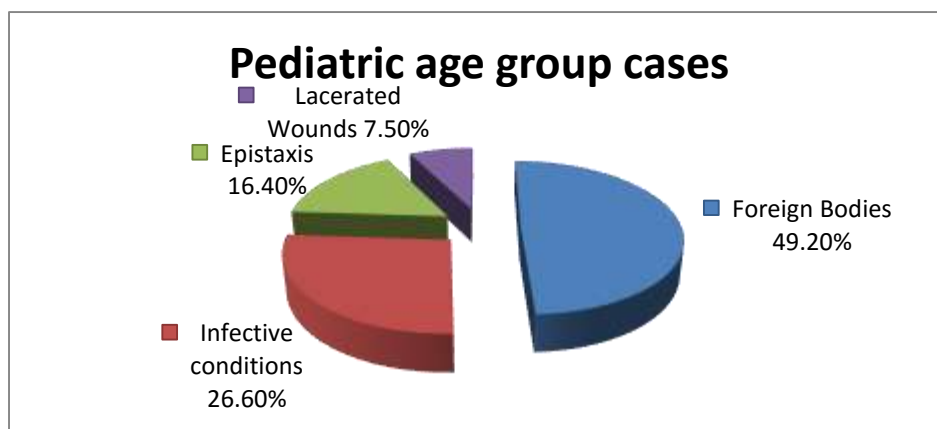


The foreign-bodies in nose, ear and aero digestive tract collectively, were 111 (49.2%) cases at the top of the list. Among the foreign-bodies *(Chart 3)*, nose 50 (45%) was the commonest site followed by ear 30 (27%), esophagus 25 (22%) and bronchus 7 (6%).

Chart3. Foreign Bodies Occurrences
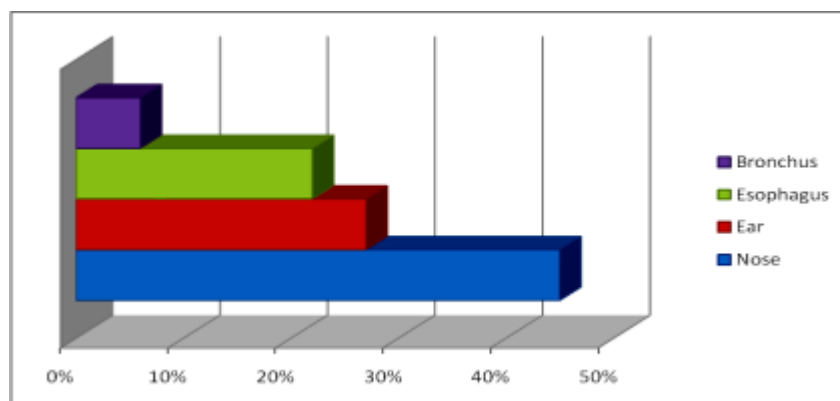


The next common problem was that of infective conditions 60 (26.6%) and the majority of these infections were related to ear as shown in *Table 1*.
And finally, Epitasis accounted for 37 (16.4%) cases, whereas 9 (7.5%) cases of lacerated wounds on lip, nose, palate and tongue.

Table I–Frequency Distribution of Infective Cases

| DISEASE | NUMBER |
|---|---|
| OTOLOGICAL: | |
| | |
| *ASOM | 15 |
| **CSOM | 06 |
| OTITIS EXTERNA | 04 |
| OTOMYCOSIS | 05 |
| UPPER AERODIGESTIVE: | 03 |
| ACUTE TONSILLITIS | 04 |
| LARYNGOTRACHEOBRONCHITIS | 01 |
| LUDWIG'S ANGINA | 02 |
| RETROPHARYNGEAL ABSCESS | 02 |
| ACUTE LARYNGITIS | 03 |
| ORAL THRUSH | 01 |
| PAPILLOMATOSIS | |
| RHINOLOGICAL: | |
| SEPTAL ABSCESS/NASAL INFECTION | 02 |
| OTHER: | |
| MUMPS | |
| SUBMANDIBULAR LYMPHADENITIS | 04 |
| PERITONSILITIS/PERIT.ABSCESS | 02 |
| CERVICAL LYMPHNODE ABSCESS | 02 |
| | 04 |

**\*ASOM – Acute Suppurative Otitis Media**
**\*\*CSOM – Chronic Suppurative Otitis Media**

## Discussion

Injuries form major bulk of emergencies in children followed by respiratory disorders and gastrointestinal problems. However, ENT problems were reported only in 4.2% of cases[2]. Only pediatric cases referred to our department were studied. In our study pediatric patients accounted for 33.7% (n = 225) out of total 670 ENT emergency cases.

In another study, children comprised 39% of subjects reporting to emergency otolaryngology services[3]. This figure is comparable to our study.

The majority of ENT emergencies in pediatric age group were not life threatening and could have been dealt with, on an OPD basis.

The commonest problem encountered was foreign body insertion and the nose was the most frequent site for this study. Different incidence has been reported for foreign bodies' insertion in other data sets [4, 5]. The next common problem encountered in our study was infections of different sites. The bulk of these were acute and chronic infections of the ear, accounting for 56% of all infective cases, where Acute Otitis media overruled all cases [5].

However, because of parental anxiety, these cases were consequently presented to emergency services. A lot of these cases could have also been managed in the Pediatric Emergency Department itself.

Therefore, our way forward should be:

A better training of pediatric residents in dealing with such cases might help to reduce the burden on ENT emergency services.

Parents, teachers and primary health care providers should be educated to identify these situations and bring them to medical notice immediately.

The help of mass media should be taken in consideration to propagate the message which should be simple and short and reinforced periodically [6].

An increased emphasis on the ENT training of medical students and general practitioners is required [5]. A short posting of pediatric residents in otolaryngology is also advisable to familiarize them with the common emergencies.

This study provides baseline data on pediatric otolaryngology emergencies for future planning of medical services and clinical training.

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# Validation of the Tunisian Version of the Oswestry Disability Index for Low Back Pain in the Zawia Region of Libya

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## Abstract

Lower back pain appears a major problem worldwide, accounting for many days of lost work, huge expense, and much suffering from the people affected by it. Incidence of it appears high in the West, but, to date, no studies have determined its incidence in many non-Western countries, including Libya.

To help remedy this problem, and to help people with LBP, one needs to measure it. However, this too is a problem. The major method of measuring LBP is use of the Oswestry Disability Index. This was first produced in English, but has now been translated into several other languages, including Tunisian Arabic and Saudi Arabian Arabic. The problem concerns using such Arabic translations for people living in Arab-speaking areas other than Tunisia and Saudi Arabia. Arabic has several dialects, and not all are mutually intelligible.

The present study seeks to validate the Tunisian Arabic version of the ODI for Libyans living in the Zawia region of the country. Sixty participants were recruited from patients receiving medical help for LBP in the region. They then had to complete the Tunisian Arabic version of the ODI, and report whether they found it intelligible. The participants were also given Arabic versions of the SF-36 and the PVAS. Scores on these tests were used to validate the Tunisian version for Zawia Libyans.

Results suggested that the Tunisian version of the ODI is a valid measure for this particular Arab sub-population.

**Keywords**: low back pain, oswestry disability index, pain visual analog scale, short form-36

## Introduction

The present research seeks to validate Tunisian Arabic version of the Oswestry Disability Index (ODI) for Libyans suffering from low back pain (LBP).

The ODI

The ODI is a measure of how much back or leg pain impairs normal living. People who score highly on the ODI cannot manage routine day-to-day activities. Originally produced in English, the questionnaire has been shown to be both reliable and valid for English speaking people (1, 2). It has also been translated into and validated in a number of languages other than English, including Greek (3), Turkish (4), and Tunisian Arabic (5)

LBP is pain originating from, or in or around, the lumbar region of the back. The pain may be acute (lasting less than 4 weeks), sub-acute (lasting 4–12 weeks), or chronic (lasting more than 12 weeks). The degree of pain varies according to patient (6). Pain may arise from two related areas: the coccyx, or base of the spine, and the sacroiliac joint, the joint that attaches the spinal column to the pelvis.

## Incidence

LBP is a common musculoskeletal condition.In the U.S.A., back pain is the second most common reason for visits to physicians (7), the fifth most common reason for hospital admission, and the third most common reason for surgery (8). In the U.K., during the period 1988–1989, LBP was the most important cause of days off work, accounting for 12.5% of the total of sick days off work (9). Swedish statistics are similar, with, in 1961, some 11–19%of

days off work attributable to back pain; in 1987, 8% of the insured Swedish population were reported as away from work, at some time during the year, because of back pain. Overall prevalence of LBP, at least in Western countries, appears to be at least 12% and may be over 35% (10).

As indicated, there appears to be no data on the incidence of LBP within Libya, and, in general, the incidence within Middle Eastern and North African countries appears uncertain. However, such evidence as exists suggests incidence within Arab countries is high (11), for example, in a study of health care providers in a Kuwait hospital, found a lifetime incidence of LBP of 70.9%. The point prevalence was 21.5%. The study also found that low levels of job satisfaction and self-reported health were associated with LBP. In addition, (11) reports LBP is a common problem in Arabian Gulf and North African countries

## *Methodology*

Three measures were used. The Pain Visual Analogue Scale (PVAS), the Short form SF-36, and the Tunisian Arabic oswestry disability index TAODI

## *Data collection*

Sixty Libyans from the Zawia region were recruited. All were suffering from LBP. Care was taken to ensure the LBP varied from mild to severe and from acute to chronic. Care was also taken that participants came from both urban and rural areas and that roughly equal numbers of males and females were represented. All were aged 18–65 Recruitment of the participants within the Zawia hospital was by informal approach to patients within the physiotherapy department. The researcher has worked in the department, and ethics and practical approval for the research had been verbally granted. In recruiting the patients, patients were asked to sign a consent form .On completion of the TAODI, they were then asked verbally to indicate how easy it was for them to understand and complete the TAODI

Some participants were illiterate or had poor general education (they had attended only primary schools). For these participants, the questions were asked verbally by the proxy researcher, who completed the questionnaire for them.

Participants had their diagnosis of LBP (acute or chronic) verified by a medical practitioner or a physiotherapist. In this regard, the medical practitioners helped determine the validity of the TAODI for Zawia Libyan Arabic speakers.

## Statistical analysis

All analyses were conducted using SPSS for Windows (Version 14). Alpha was set at p $\leq$ .05 for all comparisons.

## **Results**

Participants comprised 25 men and 35 women. The mean age was 42.17 (standard deviation: 14.45; range: 18–64). Their mean BMI (uncorrected for age or gender) was 26.94 (standard deviation: 4.55). Thus, on average, they were overweight. Eleven were obese (BMI $\geq$ 30), though 21 had BMIs in a healthy range (between 18 and 25). None was underweight (BMI < 18).

Table1.  Means and standard deviations of the 14 measures

| Measure | Mean | SD |
|---|---|---|
| GH1 | 3.233333 | 1.031153 |
| GH2 | 2.983333 | 1.185958 |
| PF | 2.12 | 0.452788 |
| RP | 2.7625 | 0.854233 |
| ER | 3.205556 | 0.788552 |
| SF1 | 2.883333 | 1.059128 |
| P1 | 4.033333 | 1.024557 |
| P2 | 3.133333 | 0.98233 |
| MH | 3.31 | 0.321912 |
| VIT | 3.3375 | 0.522431 |
| SF2 | 2.75 | 1.144256 |
| GH3 | 3 | 0.828517 |
| TAODI | 2.291667 | 1.157068 |
| PVAS | 5.25 | 2.191084 |

The importance of the table is only that it demonstrates that participants varied on all measures, and that many had severe problems.

Because the direction of each SF-36 item varies, Table 1 shows a code for each measure and the expected correlation (positive or negative).

Table2.  Meaning of low scores for each measure

| Code | Low score | Expected correlation with TAODI |
|---|---|---|
| PVAS | Good health | Positive |
| GH1 | Good health | Positive |
| GH2 | Good health | Positive |
| PF | Poor health | Negative |
| RP | Poor health | Negative |
| ER | Poor health | Negative |
| SF1 | Good health | Positive |
| P1 | Good health | Positive |
| P2 | Good health | Positive |
| VIT | Good health | Positive |
| MH | Poor health | Negative |
| SF2 | Poor health | Negative |
| GH3 | Poor health | Negative |

Because the direction of the measures varies, if the TAODI is valid, correlations between it and other measures should be positive or negative according to the individual measure

Table 3 shows the correlation coefficients for all measures with the TAODI

Table3 Correlations between TAODI and each of the measures.

| Measure | correlation | Significance | Direction of correlation |
|---|---|---|---|
| GH1 | 0.597 | <.0005 | Correct |
| GH2 | 0.523 | <.0005 | Correct |
| PF1 | −0.557 | <.0005 | Correct |
| RP | −0.409 | <.0005 | Correct |
| RE | −0.195 | 0.0132 | Correct |
| SF1 | 0.454 | <.0005 | Correct |
| P1 | 0.557 | <.0005 | Correct |
| P2 | 0.466 | <.0005 | Correct |
| MH | 0.085 | 0.517 | N/A |
| VIT | 0.497 | <.0005 | Correct |
| SF2 | −0.509 | <.0005 | Correct |
| GH3 | −0.264 | 0.039 | Correct |
| PVAS | 0.672 | <.0005 | Correct |

In the table, the direction of correlation (positive or negative) is deemed correct if the correlation is in the predicted direction. Clearly, all correlations are in the predicted direction save mental health, but this correlation is insignificant. Equally clearly, all significant correlations, save those for emotional responsiveness and the third measure of general health, are extremely significant.

Table 4 shows the adjusted correlations for each of the eight measures mentioned in the SF-36 Table 4. Adjusted correlations for each of the SF-36 measures

| Measure | Adjusted correlation |
|---|---|
| General health | 0.461 |
| Pain | 0.511 |
| Social functioning | 0.482 |
| Physical functioning | 0.557 |
| Physical role | 0.409 |
| Emotional role | 0.195 |
| Mental health | 0.085 |
| Vitality | 0.497 |

From the adjusted correlations, one can derive the measures of physical and mental health measured in the SF-36. The derived correlations are, respectively, .485 and .315

## *Discussion*

The SF-36 measures vitality in the other four questions within Section 9. Each is answered in the same manner as the mental health questions. The correlation between the TAODI and vitality, at r = .497, was highly significant (p < .0005(

If the TAODI is a valid measure, results of the present study suggest people with LBP have

The questions in the measure all concern feeling tired or lacking energy. It is to be expected that, the more serious the LBP, the more tired the participants would feel. So, again, the result corroborates the view than the TAODI is valid for Zawia Libyans

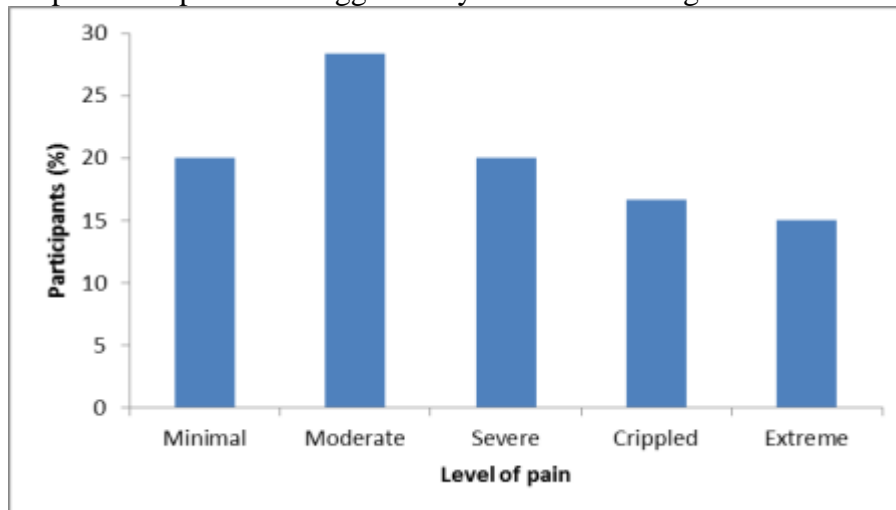the profile of problems suggested by                     Figure1



Figure2. Profile of problems of people suffering from LBP as suggested by results of the present study



The figure suggests that the major problems (left in the figure) are physical problems and pain. This, as indicated, is plausible. The most common symptoms of LBP are pain and tension of stiffness in the lower back (12); the latter two equate to physical disability, albeit mild. Other common symptoms include muscle spasms and a reduced range of motion that involves any use of the back (13)—that is, almost any gross physical movement. Again, these equate to physical disability. This, of course, is in addition to LBP caused by injury or disease. The finding that the next most common associates of LBP social problems and a lack of vivacity accord with common sense and with the literature. It is likewise unsurprising that the least important associate, but still significant, associate of LBP was emotional role (14). One would expect some impairment of emotional role caused by LBP, but perhaps not as much impairment as in physical activities. Thus the overall pattern of results suggests the TAODI is a valid measure.

Finally on this issue, as indicated, all participants indicated they had no difficulty using the TAODI. This not only in itself suggests that the questionnaire is valid, it also accords with results of the pilot study.

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# Carotid Body Tumor, a Case Report

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## Abstract

A carotid body tumor (**chemodectoma** *or* **carotid body paraganglioma***) is a highly vascular rare neck tumor arising from the para ganglion cells of the carotid body, located at the carotid bifurcation splaying ICA and ECA away from each other.*

## Report of case

My patient was 61 years old male with history of gradual increasing neck swelling, physical examination revealed pulsatile painless Lt Side neck mass

Neck CT scan was done in our radiology department and revealed there is a large soft tissue density mass of approximately 41x35x28 mm at the bifurcation of the Lt CCA, splaying ICA, ECA away from each other (figure D) and encasing the CCA (figure C)

The Lt internal jagular vein is compressed laterally by the mass effect

In post contrast films the mass exhibits rapid homogenous enhancement
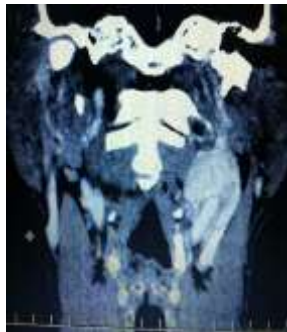
A
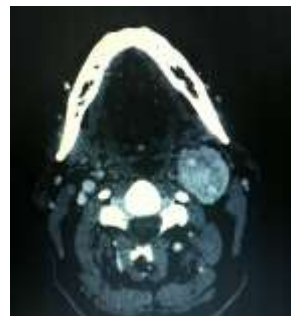
B

C

D

Coronal (A), sagital (B) and axial (C,D) CT neck images showing Lt sided carotid body tumor with splaying of ICA and ECA,

Diagnosis of carotid body tumor was made; the patient underwent a surgical resection

The histological result confirmed the diagnosis of carotid body tumor

### *Discussion*

Epidemiology:-

The carotid body tumor derived from para ganglion cells of the carotid body, sporadic form is the common one and about 5% are bilateral, the 2$^{nd}$ rare form with a pattern of autosomal dominant inheritance and about 32% of the cases are bilateral

Presentation:-
Painless pulsatile firm neck mass below the angle of the jaw, medially and laterally mobile but vertically fixed

Location:-
 Located within outside adventitial layer of CCA at level of carotid bifurcation, commonly along posteromedial wall.

Extension:-
 Extended inferiorly to lower cranial nerves, pharynx; superiorly to skull base and intracranial cavity

Radiological Features:-
Carotid body tumors are located at the carotid bifurcation with characteristic splaying of the ICA and ECA, described as the lyre sign. In all modalities the dense vascularity of these tumors is manifested as prominent contrast enhancement.

CT scan

Contrast enhanced CT scan is excellent at depicting these lesions. Typical appearances are:
- soft tissue density on non-contrast CT (similar to muscle)
- bright and rapid (faster than schwannoma) enhancement
- splaying of the ICA and ECA

MRI
- T1W images

Iso to hypointense compared to muscle

Salt and pepper appearance when larger, representing a combination of punctate regions of haemorrhage or slow flow (salt) and flow voids (pepper)

Intense enhancement following

gadolinium
- T2W images

Hyper intense compared to muscle

Salt and pepper appearance also seen on T2


Tretment:-

Surgical excision is the treatment of choice.

The larger tumor the higher risk of operative complications.

 In patients for whom the risks of complications preclude surgery, radiotherapy may be considered

Differential diagnosis:-

1. Vagal schwannoma: tends to displace both vessels together rather than splaying them

 2. Vagal neurofibroma: tends to displace both vessels together rather than splaying them

3. Lymph node mass: may look similar if hypervascular

4. Glomus vagale tumour: same pathology but located more rostrally

 5. Carotid bulb ectasia

## Conclusion

Carotid body tumor (chemodectoma or carotid body paraganglioma) is a highly vascular rare neck tumor arising from the para ganglion cells at the carotid bifurcation presented with painless pulsatile firm neck mass, the key imaging finding is splaying of the ICA and ECA, Surgical excision is the treatment of choice

## Consent
Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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# A study to investigate the effect on spinal angles of a self-selected and a standard position while sitting on the kneeling chair

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Abstract

Introduction: Back and neck pain are major problem amongst the growing number of seated workers, and enormous therapeutic and ergonomic design effort goes into reducing these problems. Educating the correct posture, choosing the right ergonomic chair, and readjusting the workstation have become very important element in any therapeutic plan. The objective of this study is to identify the difference in five spinal and pelvic angles between the self-selected and standardized position while sitting on the kneeling chair in healthy subjects. Methods: fifteen healthy subjected (≥18 years) participated in this pilot study. The spinal angles (neck angle, head tilt, cervico-thoracic, thoracic and lumbar angle) and pelvic tilt angle were measured while sitting on the kneeling chair in self-selected and standard position. Results: the study showed a significant difference in the ,lumbar spine and pelvic tilt angle when comparing the sitting posture with and without instructions. Conclusion: the study revealed that sitting on a specially designed chair does not position the body in neutral alignment, but it can be achieved by educating subjects on the correct sitting posture.

*Keywords: back pain, neck pain, sitting posture, kneeling chair.*

## Introduction

Working in an office typically involves spending a great deal of time sitting in an office chair in a position that adds stress to the structures of the spine. Therefore, to avoid developing or compounding back problems, it is important to have an office chair that is ergonomic and that supports the lower back and promotes good posture. There are many types of ergonomic chairs available for use in the office. No one type of office chair is necessarily the best, but there are some elements that are very important to look for in a good ergonomic office chair. In order to meet the user's needs by relaxing the muscles, reducing the physical load on the spine, avoiding fatigue, and helping users to do their work more efficiently. For instance, in the ordinary conventional office chair the adjustable seat height, seat width and depth, lumbar support, backrest, armrest, and the seat material are important to consider to ensure the user's comfort. Beside the conventional chair there are some more sophisticated ergonomic chairs that have been designed to give support, comfort and promote good posture (10). It has been thought that these newly designed chairs can be beneficial for office workers with discomfort or neck or back pain. Therefore, they can be used as an alternative to the ordinary chair such as kneeling chair. The kneeling chair is an office chair that has a forward tilted seat and two cushions for knee support but without backrest, and places the user in a kneeling position (figure 1). The design is thought to encourage good posture by sliding the hips forward and aligning the back, shoulder and neck. The seat pan gives the primary support, and additional support comes from the knee support cushions. This type of ergonomic chair distributes the weight between the pelvis and the knees, which reduces spinal compression, and therefore reduces the stress and tension in the lower back and leg muscles (10).

Fig1: kneeling chair

This chair could position the lumbar spine in a more natural alignment (lordosis) or very close to the neutral position (2). By searching the literature, three studies were found (Bennett et al, 1989; Fery and Tecklin, 1986; and Link et al, 1990) in which the authors compare the lumbar curvature when sitting on Balance Multi chair (kneeling chair) (BMC) or Standard Conventional chair (SCC) while performing a writing task at a desk, and standing posture. In addition, Link et al (1990) investigated the relationship between lumbar curvature and a) anthropometric factors and the length of hamstring and hip flexor muscles, b) prolonged sitting whereas Bennett et al, 1989 studied the electromyographic activity of the erector spinae (ES) muscles and measuring lumbar curvature during relaxed (comfortable) and erect sitting posture while sitting on three different chairs (a kneeling chair, an office chair, and a straight back chair) and during standing.

In the study by Fery and Tecklin (1986), forty four healthy university students (22 males and 22 females) participated in the study whereas Bennett et al (1989) used only 20 healthy young subjects, eight of which were men. In the study by Link et al (1990), sixty one 20-30 year old subjects were recruited for the study. Age and gender control were considered. This sample size in the study by Link et al (1990) was large enough to detect the differences; however, the postural alignment could vary between gender and age group (11), and the results cannot be generalized to females and the older male population. Therefore, another study is needed in which female subjects are used or which studies a sample from different age groups. The subjects in both of the above-mentioned studies had no previous experience in sitting on the BMC, which helped to eliminate the learning effect. Three measurements were taken for the lumbar spine in the three studies for each condition by a flexible ruler. In the Fery and Tecklin (1986) study, all measurement preparation and data collections were done by one researcher; this would have helped to standardise the procedures. Bennett et al (1989) managed to measure the lumbar curvature during standing and sitting on the kneeling chair and straight back chair; however, the authors were not able to measure the curvature during sitting on the office chair as the backrest support blocked the area. As a result, the straight back chair and kneeling chair were included in the lumbar curve measurements and analysis. Fery and Tecklin (1986) palpated the spinous processes (L1 and S2) before measuring the curve in each condition, which helped to reduce the effect of skin movement. The reliability and validity of

the testing procedures were not determined in these two studies. However, Hart and Rose (1986) established high reliability for these procedures (r= .97) and good validity (r= .87) between the lumbar curve standing position rather than the seated position. Further, the results revealed no significant difference between the two sitting (relaxed and erect) positions when standing and sitting on the kneeling chair. However, there was significant difference between the relaxed and erect posture during sitting on the straight back chair. Fery and Tecklin (1986) found a significant difference in the lumbar curvature among the three positions *(F = 120; df = 2,129; p <*.01). Also, they found a significant difference in the curve between the mean of all pairs; the mean of the lumbar curve in standing was (31.2± 14.8 degrees), for the SCC it was (-9.0 ± 10.4 degrees), and for sitting on the BMC it was (-2.0 ± 13.0 degrees. Link et al (1990) found that the young men in the study spent 7.8 hours per day sitting. The lumbar spine while sitting on SCC was flexed, whereas on BMC it was nearly $9^0$ more extended than on the SCC (< .05). A significant association between the lumbar curve and sitting order was found in the linear regression analysis (F = 4.35, P =

measurement obtained by the flexible ruler and radiograph. Bennett et al (1989) found that there was a significant difference in the lumbar curvature when in the

.04, $R^2$ = .08). These studies show that the kneeler chair could position the spine in neutral position; therefore, their findings can be accepted. However, more research is needed to establish and update these results and evaluate the long term use of this chair in a work setting.

The aim of this study is to investigate if there is any difference in six spinal angles (head tilt, neck, cervico- thoracic, thoracic, lumber, and pelvis angles) between the self-selected and standardized sitting position in the kneeler chair.

Material and methods

A three repeated measurements pilot study with sample size of healthy pain free subjects (5 females and 10 males with mean age 35.4± SD11.69 years) was used in the study. The subjects were excluded in case of having pain in the past six months prior to conducting the study. The Ethical approval was obtained from the Cardiff University School of Healthcare Studies (SOHCS) Research Ethics Committee, and informed consent was obtained from all subjects.

Eight Retro-reflective markers were placed over the right canthus, tragus, C7, T12, L4, PSIS, and ASIS (Fig 2).



Figure 7: Self-Selected Position (Comfort Position)

## Testing procedures:

The subjects sat comfortably (figure 2) on the chairs and carried out a typing task for 5 min during that time and about 4.5 min

from the start of the typing a flash photograph was taken, a two minute break was given. This procedure was then

repeated two more times. After that, the workstation was repositioned in a standard position in which the screen was placed at the edge of the desk with screen height at eye level. The subject was then asked to sit in a standard way (figure 3) on one of the chairs to continue the typing task for 5 minutes with a 2 minute break following (three trials), and then sit on the second chair and repeat the same

procedures and have his or her photograph taken. The sitting instructions included sitting upright and the thigh-trunk angle was measured by the goniometer as it should be ($90^0$-$120^0$) (6). The measurements were taken before each trial. Each photograph was analyzed using MAT-lab software which has shown very high to excellent reliability in previous



Figure 8: Standard Position          **Figure 9: measuring**          **Figure 10: measuring the Lumbar and thoraci**



**Figure 11: measuring the thoracic**          **Figure 12: measuring the head tilt and neck angles**

## Statistical analysis:

The mean and standard deviation (SD) of each angle were calculated using the Excel program, then imported to the statistical package SPSS version 18.0 (4). Histogram and Q-Q plot west were used to identify the normal distribution of the data. Parametric sample paired t-tests were used to serve the research question and p= 0.05 was considered as statistically significant. According to Portney and Watkins (2009), a paired t-test is used in the same or matched subject designs to compare between two conditions. As the t-test was repeated 6 times, Post Hoc Bonferroni's correction was carried out in order to avoid type I error due to the repeated t-test. Therefore, each angle was tested at the level of significance of 0.008 ($\beta$ = 0.05/6).

**Results**

Fifteen subjects, male (n=10) and female (n=5), participated in the study. As shown in table 1, the mean age of the subjects was 35.4 yrs. The mean height and the mean weight were 167.33 cms and 66.33 Kgs respectively (appendix 4).

Table 5: Participant's Demographic Data:

|  | minimum | maximum | mean | SD |
|---|---|---|---|---|
| **Age/yrs** | 22.00 | 64.00 | 35.4000 | 11.68516 |
| **Weight/kg** | 46.00 | 91.00 | 66.3333 | 12.02181 |
| **Height/cm** | 152.00 | 185.00 | 167.3333 | 8.59956 |

**Keys: SD= standard deviation, yrs= years, kg= Kilogram, cm= centimetre**

### *Descriptive data for each the studied spinal and pelvic tilt angles:*

Table 6: Descriptive data (mean and standard deviation) of the head tilt, neck angle, and cervico-thoracic angles.

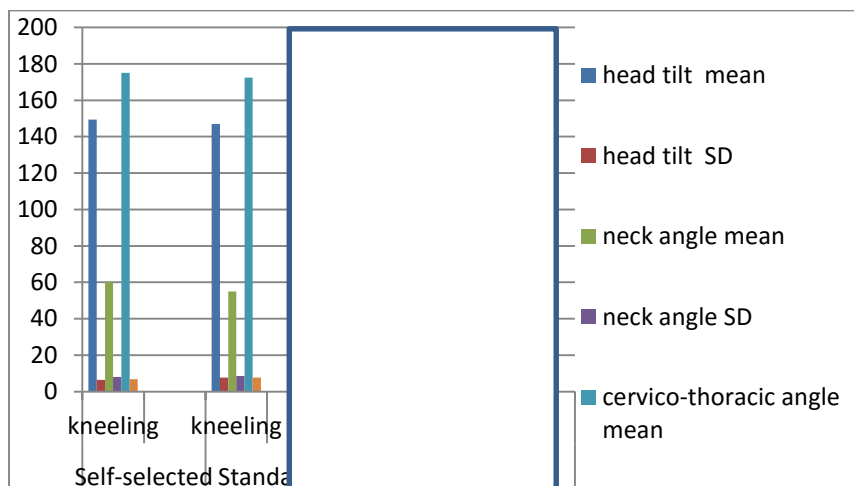| Position | Type of chair | head tilt (º) | | neck angle (º) | | cervico-thoracic angle (º) | |
|---|---|---|---|---|---|---|---|
|  |  | **mean** | **SD** | **mean** | **SD** | **mean** | **SD** |
| **Self-selected** | **kneeling** | 149.3333 | 6.53462 | 60.3222 | 8.02008 | 175.0622 | 6.76697 |
| **Standard** | **kneeling** | 146.8467 | 7.74905 | 55.0400 | 8.59239 | 172.3844 | 7.66161 |

**Keys: SD= standard deviation.**



Figure 13: mean and standard deviation of head tilt, neck angle, and cervico-thoracic angles.

**Key: SD= Standard deviation.**

It can be observed from table 2 that there was no large variation in the three spinal (head tilt, neck, and cervico-thoracic) angles in different positions as the mean value were large with relatively small standard deviation (Figure 8).

Table 7: Descriptive data (mean and standard deviation) of the thoracic spine, lumbar spine, and pelvic tilt angles.

| Position | Type of chair | Thoracic spine angle (º) | | Lumbar spine angle (º) | | Pelvic tilt angle (º) | |
|---|---|---|---|---|---|---|---|
|  |  | **Mean** | **SD** | **mean** | **SD** | **mean** | **SD** |
| **Self-selected** | **kneeling** | 46.7044 | 7.01384 | 0.1511 | 15.87447 | 1.9195 | 7.41048 |
| **Standard** | **kneeling** | 44.3022 | 7.47207 | 7.4356 | 13.25817 | 6.8849 | 7.01388 |

**Keys: SD= standard deviation**

In table 3 however, a large variation was observed in the lumbar and pelvic tilt angles, which can be understood from the small mean value of these two angles with relatively large standard deviation in each position. The values can be visually observed and understood in figures (9), it can be seen also in the table 3 that the hyper-lordosis and the posterior tilt of the pelvis are reported as negative values.
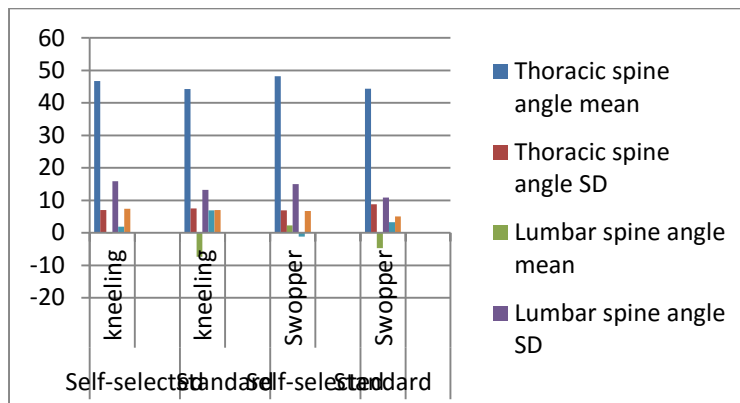


Figure 14: mean and standard deviation of the thoracic spine, lumbar spine, and pelvic tilt while sitting on the kneeling chair in the two sitting positions.

**Keys: SD= standard deviation**

Table 8: results of t-test to compare the spinal angles between the self-selected and standard position while sitting on the kneeling chair:

|        | **Spinal angle** | **t-value** | **Significance** |
|--------|------------------|-------------|------------------|
| **Pair 1** | Head tilt angle/SS-<br>Head tilt angle/S | 1.952 | .071 |
| **Pair 2** | Neck angle/SS-<br>Neck angle/S | 3.049 | .009 |
| **Pair 3** | Cervico-thoracic angle/SS-<br>Cervico-thoracic angle/S | 2.140 | .050 |
| **Pair 4** | Thoracic angle/SS-<br>Thoracic angle/S | 2.107 | .054 |
| **Pair 5** | Lumbar angle/SS-<br>Lumbar angle/ S | 5.039 | .000 |
| **Pair 6** | Pelvic tilt angle/SS-<br>Pelvic tilt angle/S | -3.698 | .002 |

**Keys: SS= self-selected position, S= standard position**

A comparison of the spinal angles between the self-selected and standard position while sitting on the kneeling chair (table 6). In the 6th table the t-value and level of significant are reported, and show some significant values. For instance, there is a significant difference between the self-selected and standard position while sitting on the kneeling chair in the lumbar spine as the P> 0.008 (P= .000) which was more lordotic in the standard position in comparison with the self-selected position. Also, a significant difference is observed in the pelvic tilt (P= .002) due to the posterior direction of the pelvic tilt in the self-selected position, whereas, no significant differences are observed in the other angles.

## Discussion

In order to understand and eliminate the problem of neck and back pain (NP, BP), sitting posture (postural analysis PA) has been regularly investigated in the field of physiotherapy and the healthcare profession. Ergonomic chair designs may influence the sitting posture and muscle activity; therefore, the type of chair has become an area of interest for many researchers. Despite this interest, there has been only limited research regarding posture while sitting on the kneeling chair (3,9). Therefore, there is a need for up to date research investigating the effect of using the kneeling chair in reducing NP and BP.

This study showed no large variation in the head tilt, neck angle, the cervico-thoracic angle, and thoracic angle while sitting on the kneeling chair and doing a typing task significant difference in the lumbar curvature between the two chairs, as it had 9 degrees more extension and was very kneeling chair than to the lumbar curvature in the standard conventional chair. This result is not supported by the present study, which could be due to the difference in the methodology as well as the chair design and the performed task. Regarding the neck angle, the results (table 6) showed that the level of significance of the neck angle was just over 0.008 (P= 0.009); this result could be significant with a larger sample size which should be applied in future. From the above mentioned results, it seems that sitting on the kneeling chair on its own does not position the body in the optimal position. However, educating workers and raising their awareness regarding the ideal sitting posture has a major impact on their posture. These results challenge the proposed aim and widespread idea of using the kneeling chair for good postural alignment without giving any instruction about sitting posture.

. On the other hand Bennett et al (1989) reported a significant difference in the lumbar curvature, as it was greater when sitting comfortably on the kneeling chair than on the straight back chair. Bennett et

(tables 2, 3). However, there were large variations in the lumbar angle and pelvic tilt (table 3). These findings could mean that any major changes happened in the lower spine (lumbar spine and pelvic tilt angles) due to sitting on an ergonomic chair not making any changes in the upper spine. On the other hand, it was found that significant changes happen in the lumbar and pelvic tilt angles only. Which could have been affected by two main factors: firstly, educating subjects about the correct sitting posture and secondly, the structural design of the kneeling chair and the presence of the forward tilt (10). , Fery and Tecklin (1986) and Link et al (1990) studied the difference in the lumbar curvature while sitting on the standard conventional chair and the kneeling chair in a comfortable sitting position. The authors revealed that there was a much closer to the lumbar curvature in the standing position in the

al (1989) studied the lumbar curvature as well as muscle activation while sitting on the kneeling and straight back chair in the relaxed (self-selected) and erect (upright) position using a flexible ruler. They revealed no significant difference in the lumbar curvature between the relaxed and erect position while subjects were seated on the kneeling chair. However in the current study there was a significant difference in the lumbar spine. This contradiction could be explained by the fact that Bennett et al (1989) studied young subjects whose ages ranged between 22 and 37 years old, whereas in the current study the age group was wider. Further, the measuring techniques of the spinal posture were different. In addition, two tasks were used in the study by Bennett et al (1989) (a typing and a writing task), whereas in this study the typing task was the only one performed. Bennett et al (1989) explained their findings by the fundamental function of the kneeling chair design and in this they were the same as Fery and Tecklin (1986).

## In conclusion

The ergonomically designed kneeling chairs is designed to maintain neutral postural alignment especially in the lumbar curvature, and sometimes are recommended to be used as part of a therapeutic plan for back pain patients. The current study revealed that sitting on a specially designed kneeling chair does not inherently position the spine in the correct posture. Also, the results significant difference between the two positions in the lumbar spine and pelvic tilt angles when sitting on the kneeling chair. These results raise an issue around the proposed aim of using kneeling chair to intentionally correct sitting posture.. Therefore, the results do not support the clinical claim that using the ergonomically designed chair as an alternative to the ordinary office chair will adjust the spine to a good postural alignment. Instead, more focus should be placed on educating sitters on how to sit correctly, which could help to reduce the prevalence of neck and back pain.

Word count: 3107

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# Iron Deficiency Anemia in women in Zawia Area

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## Abstract:

Anemia is a common health problem in women in developing countries, since anemia is more common in women than man due to physiological processes. There are many types of anemia and this study dealed with iron deficiency anemia which is quite common in women who are in childbearing age especially pregnant women. Iron deficiency anemia is characterized by a decrease of hemoglobin, serum iron, ferritin, and red blood cells (shape and size). Also it is characterized by an increase in total- iron binding capacity. Red blood cells become microctytic, and hypochromic due to a decrease in iron content. This study was conducted in Zawia area and included 210 women in childbearing age (18- 45 years) who were visiting women clinic Zawia teaching hospital. After filling the questionnaire, blood samples were taken and analyzed for hematological and biochemical profiles. Biochemical testes included measurement of serum iron, ferritin, and total-iron binding capacity. Among the total sample (210 women), there were 87 (41.42%) pregnant and 123 (58.57%) non-pregnant women (includes married and single). Pregnant women (87) were classified according to the gestational age into first, second, and third trimesters. Out of 87 pregnant women, there were 7 (8.04%) pregnant women in the first trimester, 34 (39.08%) in the second trimester, and 46 (52.87%) pregnant women in the third trimester.

The means of biochemical and hematological parameters in the studied samples were: Hb = $10.37\pm 2.02$ g /dl, RBC= $3.78\pm 1.037$ m/m3, serum iron $61.86\pm 40.28$ µg /dl, and TIBC = $386.01 \pm 94.91$ µg /dl. In our study, Weconsidered that any women have hemoglobin below 11.5 g /dl is anemic.

89.1%, 69.5%, and 47.8% of pregnant women who belong to third trimester had low (below normal value) Hb, serum iron, and ferritin. i.e. iron deficiency anemia was more common in third trimester among the first and the second trimesters. Third trimester pregnant women also had high TIBC more than first and second trimesters.

We have compared between pregnant and non- pregnant women in the terms of hematological and biochemical parameters. We found that 85%, 65.3%, and 36.7% of pregnant women have low Hb, serum iron, and ferritin. Our study showed that 45 (21.5%) out of 210 women (The whole samples) had iron deficiency anemia. i.e. 21.5% of women who included in this study in Zawia area had iron deficiency anemia.

Among 45 women who have iron deficiency anemia, there were 30 (66.6%) pregnant, and 15 (33.3%) non-pregnant. That means prevalence of iron deficiency anemia was more in pregnant than non- pregnant as we expected.

Our study showed the effect of tea on absorption of iron. In this matter, drinking tea women in this study showed 42.4% a decrease in serum iron level.

*Key wards:*Hemoglobin, Red blood cells,Total iron- binding capacity.

## *Introduction*

Anemia is present when there is a decrease in the level of hemoglobin in the blood below the reference level for the age and sex of the individual. (1) It means that anemia is indicated by a hemoglobin concentration in the blood of less than 13.5 g/dl in adult males and less than 11.5 g/dl in adult females or haematocrit of less than 41% in adult males and less than 36% in adult females.(2)

Iron absorption responds to daily need and is influenced by the amount and type of iron accessible from food, the functional state of the gastrointestinal mucosa and pancreas, current iron stores, and erythropoietic needs.(3) Iron absorption can thus be influenced at several different stages.(4)Much of the dietary iron is non-haem iron derived from cereals, with a lesser component of haem iron derived from haemoglobin or myoglobin in red or organ meats. Haem iron is more readily absorbed than non- haem iron, being less subject to influence by other dietary constituents (5). Even when animal foods form only a small part of diet, they have a disproportionate effect on the total iron absorbed, this is because an unidentified ligand present in meat promotes an enhanced availability of the non - haem iron in the rest of the diet. (6)

Iron absorption may be regulated both at the stage of mucosal uptake (possibly by varying the expression of metal transport proteins) and at the stage of transfer to the blood. Factors favouring increased iron absorption include iron deficiency, pregnancy, hypoxia and increased erythropoiesis. Iron absorption is usually decreased when the body is overloaded with iron, and in acute and chronic infections (7).

Very little iron is excreted by normal persons. Healthy adult males lose about 1mg each day, mostly as hemoglobin storage iron in desquamated cells and erythrocytes in feces (8). Nearly negligible amounts of iron are excreted in sweat and urine (9). With each menstrual cycle, women lose approximately 20- 40 mg of iron (10).

Iron deficiency is one of the most prevalent disorders known, with 30% of the world wide population affected (11). Those with a higher than average risk for iron deficiency anemia include pregnant women, both young children and adolescents, and women of reproductive age (12). Increased blood loss, decreased dietary iron intake, or decreased release from ferritin may result in iron deficiency. Reduction in iron stores usually procedes both a reduction in circulating iron and anemia, as demonstrated by a decreased red blood cell count, mean corpuscular hemoglobin concentration, and microcytic red blood cells (10).

Although a decrease in serum iron and an increase in transferrin / Total iron binding capacity are classic indices of iron deficiency, the serum ferritin concentration has evolved as a more sensitive and reliable test for confirming this condition (13).

## Materials and methods

This study was a cross-sectional survey including two hundred and ten blood samples were taken from non-pregnant and pregnant women in reproductive age in different stages of pregnancy and investigated for different biochemical and hematological parameters such CBC, blood film, serum iron, ferritin, and total iron binding capacity (TIBC). Its saturation with the purpose of assess the cases of IDA in pregnant and compare them with non-pregnant women. Questionnaires contain different data completed for each subject including personal data (name, address, age, nationality), and other data (age of gestation, data on variables of interest including, drinking tea status, education, diet, data were collected from each subject.

Iron kits (Fluitest iron- Bicon diagnostic Hecke 8- Germany)

TIBC kits (Fluitest TIBC- Bicon diagnostic Hecke 8- Germany)

Ferritin kits (Ortho- Clinical Diagnostics- Johnson- Johnson- Company)

Wrigts stain- UK.

- Sysmax (kk21- Japane)

- Microscope (hundwetzlar- Germany)

- Centrifuge (Eppendrof- Germany)

- Spectrophotometer (Biosynthesis BTs- 302- Spain)

- Vitros system (Ortho- Clinical Diagnostics- Johnson- Johnson- Company).

Data analysis was performed with computer software (SPSS, Version 14.0, SPSS Inc., Chicago, IL). Age was presented as mean ± SD, frequencies; percentages of different variables were computed. Chi Squire analysis for independence was used to examine the relationship significance between Hb, and different biochemical test and different gestation age and to examine the significance of differences in risk characteristics associated with Hb concentrations and iron status markers. Students T test was used to compares the means of Hb, Iron and RBC in both pregnant and non pregnant group of women.

210 Samples collected from Zawia central hospital (women clinic), and 2nd March polyclinic, analysis were performed Zawia hospital laboratory.Blood samples were taken, using needle (size 23Gχ1½). After taking 5ml of venous blood, the blood is transferred then in two tubes in one tube (type AFMA-Disg) and gently mixed for 3- 4 times. The tube contains an anti-coagulation substance called ethylene diamine tetra-acetic acid (EDTA) to prevent blood coagulation. Each tube labeled by a sticker contains number, name of the subject, time of collection, and the place of collection.Blood samples were transferred to the hospital laboratory for CBC (complete blood count) and blood film, the CBC analyzed by using sysmax kk21 machine.All hematological parameters for each subject were recorded in a strip paper from the sysmax machine. Each strip paper was numbered. The time consumed to get a one strip is ranged between one to a half minute. All obtained data of blood parameters for each woman were stored in the computer.

Blood films were examined to reveal any pathological changes in RBC in case of iron deficiency anemia. The slides were examined in hematology department in Zawia teaching hospital.In case of biochemistry samples, was separated from the clot or cells within 1 hour. Samples were centrifuged within at least 3000 rpm for 5 minutes. Biochemistry profile included measurement of serum iron and total iron binding capacity (TIBC) by using commercial biochemical kits (Biconfluitest B-Germany) according to standard spectrophotometeric methods which were in routine used in the biochemistry laboratory.

## *Results and discussion:*

This study included 210 women in Zawia area. During the study, 210 blood samples were taken from reproductive age (18 - 45 years). The samples analyzed to investigate the anemia and iron deficiency as assessed by biochemical and hematological parameters and determined the distribution of anemia among no pregnant and pregnant women. Moreover, to determine the frequency of anemia in the different stages of gestation. The age range was between 18 - 45 years old, with mean age of 30. 2 ± 7. 28 years in the whole sample. 122 married (58%), 88 single (41%). 87 were pregnant women (41.4%) and 123 non-pregnant women (that included single and married women).

The age structure among the sample was divided into four different age groups. Age concentrated between 18 years and 33 years 141(67%) figure (3).

The means of biochemical and hematological parameters in the study sample were as following: Hb was 10.37± 2.038 gm/dl, serum iron = 61.86 ±40. 288 µg / dl, TIBC = 386.01 ± 94.918 µg / dl, serum ferritin = 29.45 ± 30.592 ng / ml and RBC = 3.78 ± 1.037 m/m$^3$ (see table 1).

**Table 1:** Shows the mean and ± S.D of some biochemical parameters.

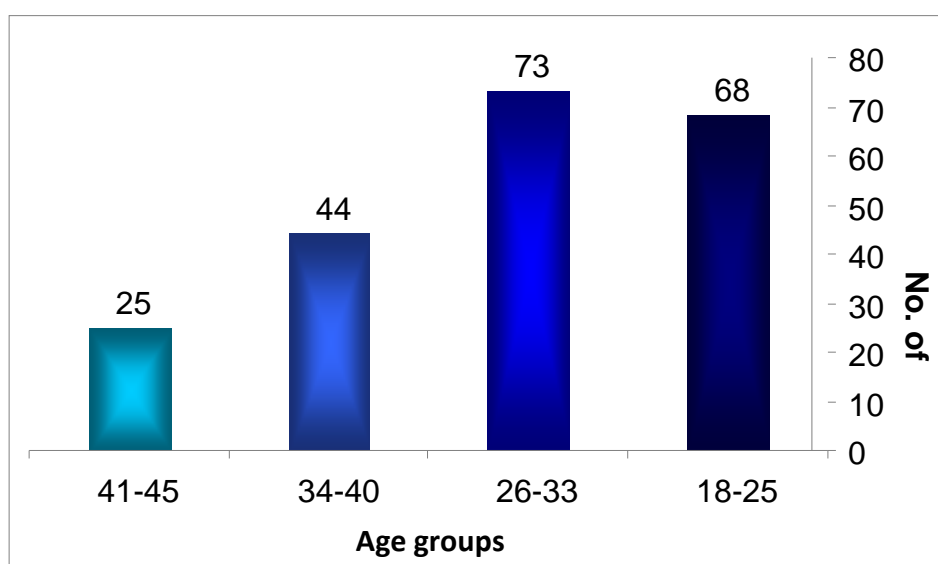| Total samples | Hb | Iron | TIBC | Ferritin | RBC |
|---|---|---|---|---|---|
| 210 | 210 | 210 | 153 | 77 | 210 |
| **Mean** | 10.37 | 61.86 | 386.01 | 29.45 | 3.78 |
| **Std. Deviation** | ±2.038 | ±40.28 | ±94.91 | ± 30.59 | ±1.03 |



**Figure 3:** Age grouping of sample population. Numbers above the bars

represent number of cases.

The pregnant women screened in this study were 87 and having different gestational stages. In first trimester, the number of pregnant women was 7 out of 210 women (Total studied sample) and that represents 8.04%, second trimesters were 34 pregnant women 39.08%, and third trimester were 46 pregnant women 52.8%. The highest percentage was third trimester of gestational stage in the study sample as shown in (Table2).

**Table 2:** Shows distribution of gestational age

| Pregnancy duration | Frequency | Percent |
|---|---|---|
| First trimester | 7 | 8.04 % |
| Second trimester | 34 | 39.08 % |
| Third trimester | 46 | 52.87 % |
| Non-pregnant women (single-married) | 123 | 58.09 % |
| Total | 210 | 100 % |

In the whole sample, the results show that 66 women have normal Hb (31.42%) whereas 144 women have low Hb (68. 57%). The mean value of Hb was 10. 37 ± 2.03 g/dl and RBC 3.78 ± 1.03 m/m$^3$. (Table 3).

**Table 3:** Shows frequency and percentage of hemoglobin

| Hb | Frequency | Percent |
|---|---|---|
| Normal* | 66 | 31.42 % |
| Low** | 144 | 68.57 % |
| Total | 210 | 100 % |

\* Samples equal to or greater than 11.5 g/dl considered normal
\*\* Samples less than 11.5 g/dl considered anemic (11).

We considered any women in childbearing age who has hemoglobin level below 11.5 g / dl is anemia according to WHO and many other studies (14, 15, 16, 17). The mean value of hemoglobin level in the whole studied sample (210 women) was 10.37± 2.03 g/dl which is below the normal value (11.5g /dl). My explanation for this low value of the mean hemoglobin is due to big variation between hemoglobin values in the non- pregnant and pregnant women. Since single females have a higher hemoglobin level than pregnant women. In my study, there were 87 (41.42%) pregnant women out of the total sample (210). That certainly decreases the mean value of hemoglobin of the whole studied sample. Serum iron concentration; the frequency of normal serum iron in the study sample were 128 women (60.95%) and low serum iron were 82 women (39.0%). The frequency of normal serum ferritin were 162 (77.14%), and low serum ferritin were 48 samples (22.9%). (table 4).

**Table 4:** Shows frequency and percentage of normal,and low serum iron

| Iron level | Frequency | Percent |
|---|---|---|
| Normal | 128 | 60.95 % |
| Low | 82 | 39.0 % |
| Total | 210 | 100 % |

Table 4 shows that 82 (39%) women out of the total sample (210) have low serum iron. Table5 shows that 48 (22.9%) women have low serum ferritin. Table 6 shows that 70 (33.3%) women have high TIBC level. So the percentage of low serum iron, ferritin, and high TIBC is ranging between 22.9% to 39%. These results are very close to results of some workers who have done studies on Lebanese women (14). It should be mentioned here that (14) have done their study only on non-pregnant women, but the similarity between my study and their study is the age of the studied sample (18- 45 years).

**Table 5:** Shows frequency and percentage of serum ferritin.

| Ferritin level | Frequency | Percent |
|---|---|---|
| Normal | 162 | 77.1 % |
| Low | 48 | 22.9 % |
| Total | 210 | 100 % |

Frequency of cases with normal TIBC were 132 women (62.9%), and 70 women with high TIBC (33.3%), and only 8 cases with low TIBC (3.8%).

**Table6:** Shows frequency and percentage of TIBC

| TIBC level | Frequency | Percent |
|---|---|---|
| Normal | 132 | 62.9 % |
| High | 70 | 33.3 % |
| Low | 8 | 3.8 % |
| Total | 210 | 100 % |

The mean hemoglobin concentration in 87 pregnant women was $9.38 \pm 1.86$ gm/dl, whereas the mean in 123 non- pregnant women (single-married) was $11.08 \pm 1.85$ gm/dl.

Figure 2 show that 74 pregnant women (85%) out of 87 pregnant women have low hemoglobin (less than 11.5 gm/dl). Non-pregnant women (single and married) have a lower percentage (56.9%) of low hemoglobin (70 out of 123 women).

The mean of hemoglobin concentration in married women was $10.56 \pm 1.68$ gm/dl and was $11.28 \pm 1.89$ gm/dl in single women.

In my study, there were 123 non- pregnant women (married or single). 56.9% of these non- pregnant women had low hemoglobin level (Hb< 11.5 gm/dl). In contrast, hemoglobin level was measured for 87 pregnant women, and 85% of them had low hemoglobin level. i.e pregnant women in Zawia area has lower hemoglobin level compared to the non- pregnant women

.
Some other studies were conducted in Kazakhstan (18) and Nepal (19). Both stuidies included pregnant and non- pregnant women and considered any women have hemoglobin level less than 12 gm/dl is anemic.
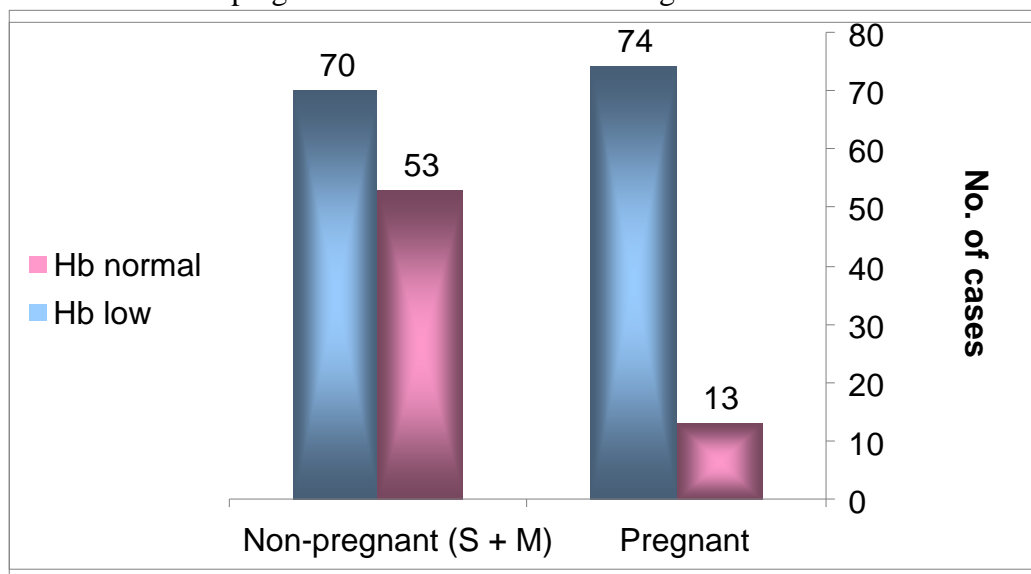


**Figure 4:** Shows the number of cases with low and normal hemoglobin level in the pregnant and non- pregnant women. S=single, M=married

The mean of plasma iron in 87 pregnant women was $54.44 \pm 39.58$ μg / dl, whereas the mean in 123 non- pregnant women was $66.43 \pm 40.20$ μg / dl.

Figure 4shows that 49 pregnant women out of 87 pregnant women have low serum iron (56.3%). Non - pregnant women (single and married) have a lower percentage (26.8%) of low iron (33 out of 123 women).

Tha mean of iron concentration in married women was $66.03 \pm 34.56$ μg / dl and was $66.33 \pm 42.03$ μg / dl in single women. It seems that ther is no significance differences in the mean of serum iron level in the married and in the single women.
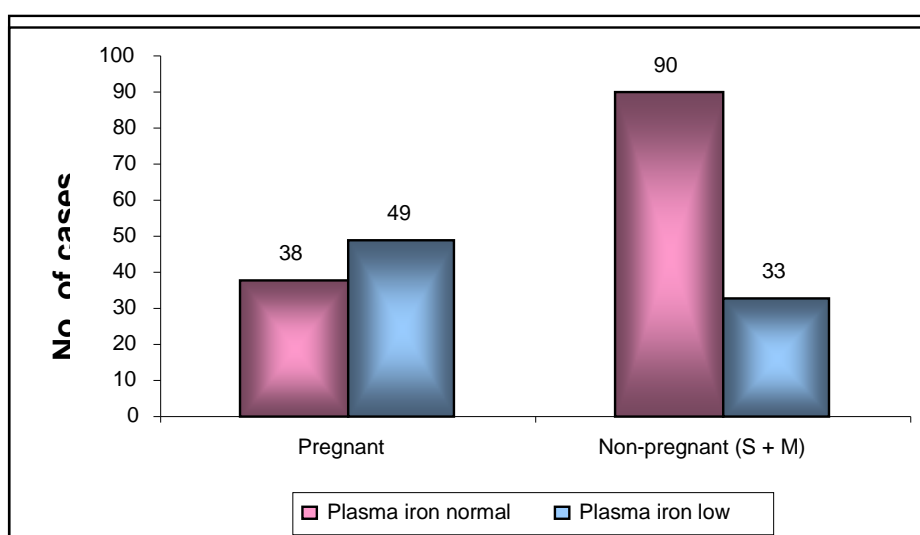


**Figure 5:** Shows plasma iron in pregnant and non-pregnantwomen. S=single, M=married

The mean of RBC in 87 pregnant women was $3.361 \pm 1.298$ m /m$^3$, whereas the mean in 123 non- pregnant women was $4.100 \pm .62315$ m /m$^3$.

Figure 5shows 30 pregnant women out of 87 pregnant women have low RBC (34.4%). Non pregnant women (single and married) have a lower percentage (14.6%) of low RBC (18 out of 123 women).

The mean of RBC in married women was $3.90 \pm 0.61$ m /m3 and was $4.14 \pm 0.619$ m /m³ in single women. Usually anemia is more common in pregnant women than in non- pregnant women due to fetus demand.
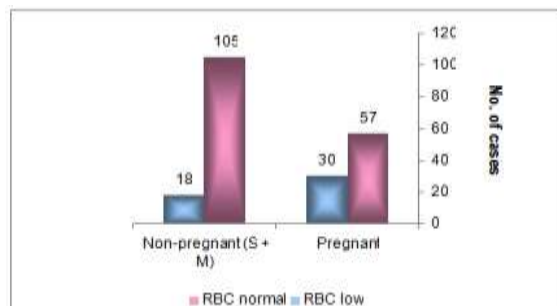


**Figure 6:**  Shows RBC in pregnant and non-pregnantwomen. S=single, M=married

The mean of ferritin level in pregnant women (87) was $20.105 \pm 24.312$
ng / ml. in non- pregnant (123) women the mean of serum Ferritin was $36.063 \pm 32.822$ ng / ml.

Figure 6show that 32 pregnant women out of 87 pregnant women have low serum ferritin (36.7%). Non- pregnant women (single and married) have a lower percentage (13.0%) of low ferritin (16 out of 123 women).

Tha mean of ferritin concentration in married women was $32.89 \pm 32.54$ng/L and was $37.20 \pm 32.92$ ng/L in single women.
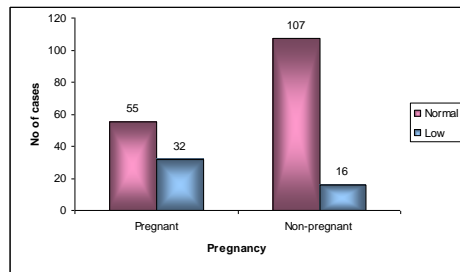


**Figure7:**  Shows ferritin level in pregnant and non-pregnantwomen. S=single, M=married

The mean of TIBC level in pregnant women (87) was $477 \pm 87.605$   µg / dl. in non- pregnant (123) women the mean of serum TIBC was $335 \pm 94.675$ µg / dl.

Figure 7 show that 45 pregnant women out of 87 pregnant women have a high TIBC level

(51.72%). Non- pregnant women (single and married) have a lower percentage (20.32%) of

high TIBC (25 out of 123 women).

Tha mean of TIBC level in married women was $369.97 \pm 77.02$ µg / dl and was $32.89 \pm 32.54$ µg / dl in single women.

Pregnant women have high TIBC level more than non- pregnant women and this in agreement with serum iron and hemoglobin levels.
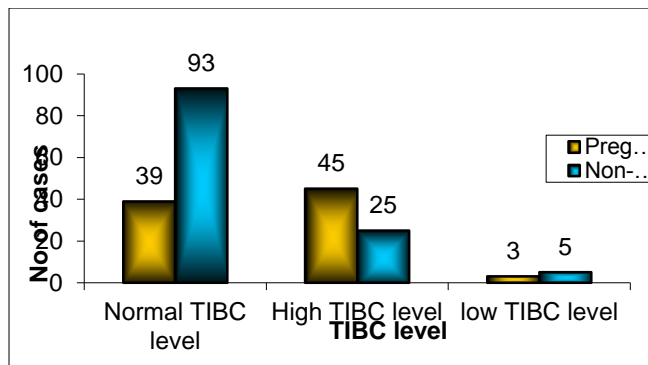
**Figure 8 :**Shows TIBC level in pregnant and non-pregnantwomen

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# Cyanotic congenital heart disease: a single center experience in Libya.

Zohra  S. Elmagrabi, Amnna  A. Rayani, Mabruka A. Zletni, Fathia A. Riani, and Elmukhta M.Habbas.

## ABSTRACT

**Background:** Congenital heart diseases are due to structural or functional heart defects. They can be detected during intrauterine life, at birth, or later on life. **Object**: To present a single center experience in cyanotic CHD. **Method and patients**: Eight hundred and twelve clinical records   of all pediatric cases referred to the cardiac department at Tripoli children hospital between January 2009 and December 2010. Those referred children were diagnosed as congenital heart disease patients. Clinical data was reviewed   including age, sex distribution type of cyanotic CHD and the age of patient at diagnosis of these lesions .The history, clinical examinations findings, chest X. ray, ECG, 2DECHO, and few CT angiography, mode of presentations, birth weight and type of cyanotic lesions were collected. **Results**: Out of the referred patients, 103 children were diagnosed as cyanotic congenital heart disease (13%). They were 68 males (66%) and 35 females (34%), aged between intrauterine life to 10 years a mean age of 186.5 days. Twenty five patients had transposition of great arteries ( 24.3%), 21patients with  Tetralogy of Fallot (20.4%),13 patients with tricuspid atresia (12.6%), 11patients had double outlet Right ventricle (10.6%),10 patients with single ventricle (9.7%), 9 patients with pulmonary atresia (8.7%). Nine more patients had different rare cyanotic lesions (Ebestien anomaly, Truncusarteriosus, total anomaly pulmonary venous return, persistent pulmonary hypertension and mitral atresia). Most of the patients presented at age of less than three months (78.6%).Cyanosis and murmur were common presentation (35%, 31% respectively). Nearly 50% of patient's weight was <3 kg at presentation. **Conclusion**: CHDs are common. TGA and TOF were the most common lesions this study. These two lesions are lethal if not detected early, diagnosis and early interference help to avoid inevitable complications, reducing disability, mortality and improving quality of life.

**Key words:** Cyanotic congenital heart disease, 2D echocardiography, TGA.TOF, Libya.

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## Introduction:

Congenital heart defects are the defects in the structure or function of the heart, detected at birth or later in life though they are present at birth (1). Congenital heart defects affect nearly 1% of live births and 25% of them are considered to be critical that require surgery or catheterization within the first year of life(2,3). Most of the critical congenital heart defects are of congenital cyanotic type of lesions. Cyanosis can occur when there is obstruction to right ventricle out flow leading to intra cardiac right to left shunting. Congenital heart disease (CHD) is an important cause of morbidity and mortality in infancy. CHD affects 6-8 children in every 1000 live birth (4). Out of them, 1-2 % had moderate to severe CHD (5). CHD is the most common congenital problem in children accounting for nearly 25% of all congenital malformations (6). Most of cyanotic heart defects are Life-threatening and presented in very serious condition which might lead to death in the first year of life especially in transposition of the great arteries, pulmonary atresia with intact septum, tricuspid atresia, hypoplastic left heart, and mitral atresia. Early diagnosis and proper management with appropriate intervention for these patients can give normal or near normal life expectancy.   patients born with sever forms of CHD have 12

folds increase risk of death during first year of life and the risk increase more if diagnosed after neonatal period (7).

**Patients and methods**

A retrospective cohort descriptive study has been done in Tripoli children hospital (cardiology clinic); from January 2009 till December 2010. Medical records of eight hundred and twelve of all pediatric cases referred to the cardiac department diagnosed with CHD were reviewed. 103 children were diagnosed as cyanotic congenital heart disease (13%). The age range was from intrauterine life –detected by fetal echocardiography to 10 years. Clinical data was reviewed including age, sex distribution, history, mode of presentations, birth weight, and clinical examination findings. Underlying cardiac lesion (s) of cyanosis was / were assessed by chest X ray, ECG, ECHO, and few patient had CT angiography. Data were arranged at excel sheets and analysis was done using Excel statistical package.

**Results:**
Eight hundred and twelve files of patients with congenital heart defect were retrospectively reviewed.The diagnosis of cyanotic heart lesions were confirmed in 103 patients (13%). Sixty eight were males (66 %) and35were females (34%), with male to female ratio of 1.9:1 (figure1).
Age distribution of patients was from intrauterine life to 10 years of age with a mean age of 186.5 days. More than half of the patients were diagnosed in the neonatal period (54%), and (24.6%) (78.6%) were diagnosed at age of three months. Fifty patients (49%) had a birth weight below three kilograms and 35% were between three and four kilogram at presentation. At presentation, Cyanosis was reported in (35%), murmur in (30%) while both cyanosis and murmur together were in (12.6%) figure (2). Heart failure was the presenting symptom in 3.8% of the patients and Down syndrome for screening in other 3.8% of the cases. 2.9% of the patients were diagnosed intrauterine by fetal echo.The commonest cyanotic lesion was TGA (24.3 %), followed by TOF (20.4%).

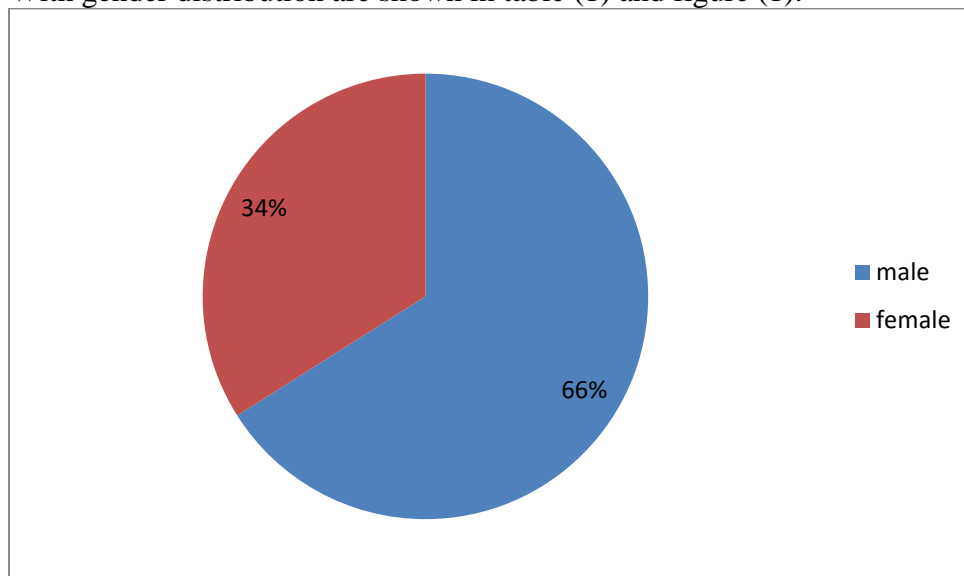With gender distribution are shown in table (1) and figure (1).



Figure 15: Male: Female ratio.

| Type of CHD | % | No. ofpatients | Male | Female |
|---|---|---|---|---|
| TGA | 24.3 | 25 | 16 | 9 |
| TOF | 20.4 | 21 | 13 | 8 |
| TA | 12.6 | 13 | 9 | 4 |
| DORV | 10.6 | 11 | 6 | 5 |
| SV | 9.7 | 10 | 8 | 2 |
| PA | 8.7 | 9 | 6 | 3 |
| HLHS | 4.8 | 5 | 4 | 1 |
| Others | 8.7 | 9 | 6 | 3 |
| Total | 100% | 103 | 68 | 35 |

Figure2: frequency& distributions of CHD.
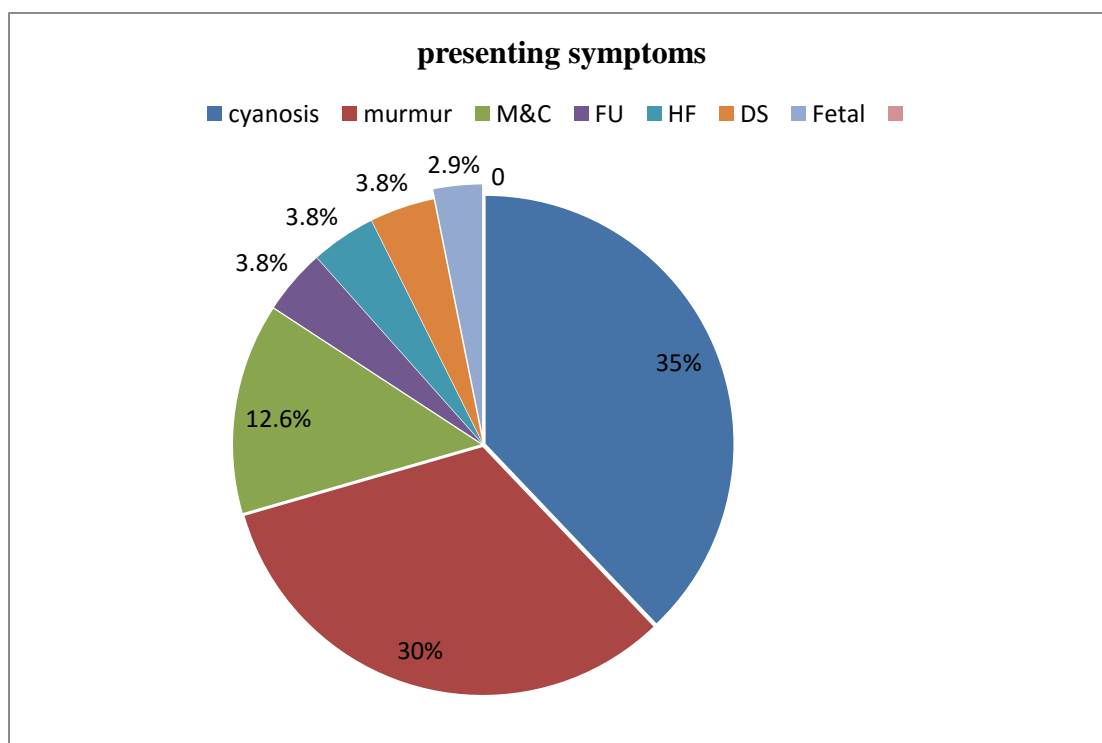


Figure 16: presenting symptoms
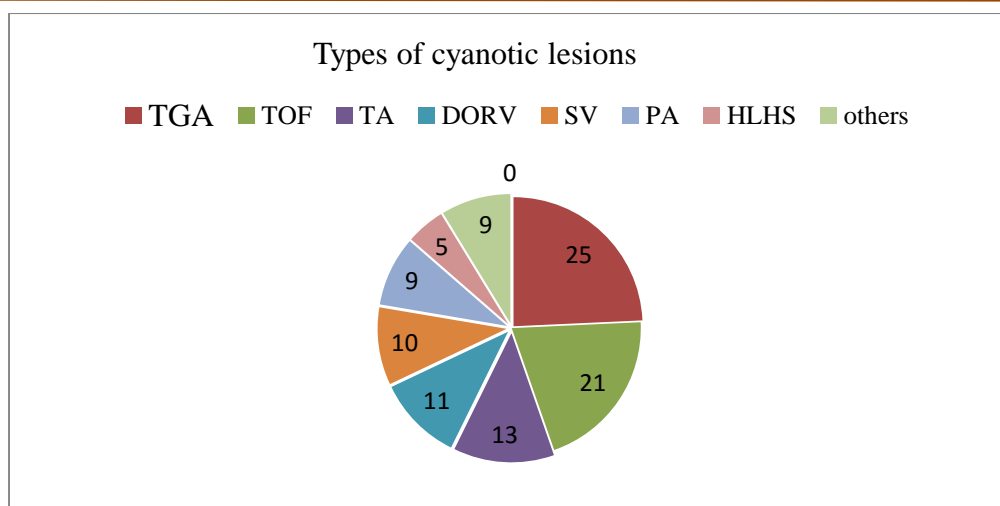
Figure 17: Types of cyanotic lesions

**Discussion:**

CHD is the most congenital anomalies in children. It affects 1% of live births, of which 25% presented with critical congenital heart disease that are cyanotic lesions requiring catheterization and / or surgical intervention in first few hours, days or months(3).Worldwide about 2-3 per 1,000 newborn infant with congenital heart diseaseare usually symptomatic during the first year of life. The diagnosis of CHD in 40–50% of patients is usually established during first week of life whereas 50–60% of patients are diagnosed during the 1st month of life (8).

In the present study, cyanotic CHD was reported in 13% of the total cases of CHD. Other studies in other Arabic countries in south Yemeni reported about the same percentage (15%) (9), while in Jordan (26%) of CHD has cyanosis (10). In Pakistan one study reported nearly half of CHD patients had cyanotic heart disease (11). These differences cited in the two studies (at Jordan and Pakistan might be due regional differences, ethnic or other unknown predisposing factors.

Our results showed that cyanotic CHD was diagnosed in 33% of the patients enrolled in the study during first week of age, while 21% of patients diagnosed at one month of age. At three month of age, a total of 24.6% of patients were diagnosed. Although the result in this study was not differ significantly from the international reported results. Our results showed less patients diagnosed during the first week of age and most of patients diagnosed latter. This delay mostly due to late presentation of patients to the cardiology services centers and / or negligence or incompetence of the doctor or midwife at nurseries.

Patent ducts arteriosus (PDA) plays an important role in timing cyanosis occurrence, especially in  ductal dependent lesions like TGA, PA, HLHS, sever coarctation of Aorta, interrupted aortic arch and critical aortic stenosis. This delayed closure leads to late diagnosis of these lesions or missed in early neonatal period. One study observed that 30% of patients with cyanotic CHD were diagnosed after baby discharge (11) while these babies are asymptomatic before closure of PDA. After the closure of the ductus in these babies, symptomatic features of cyanotic heart disease appear and may deteriorate leading to shock (12).

TOF is one of the most congenital cardiac lesions that cause cyanotic heart disease. TOF reported in Pakistan (51.7%) (13), and in India (44%)(14). TOF lesion reported in the present study in 20% of cyanotic patients. These differences in reported percentages between our results and the others might be due to late diagnosis in our clinic because of late referral or other reasons that we do not know.

TGA lesion was the most common lesion in our study (24.3%). In Pakistan TGA was (31.3%) and in India only (9%). These differences in the underlying lesions of CHD that lead to cyanotic heart disease in this study and others might be due to a number of factors such as socio-economic, cultural, ecological and genetic factors.

 **Conclusion:**

This study gives an overview of the CHD pattern in children. Earlier detection and correction of disease surgically or by catheterizations are of utmost importance to avoid late complications, reducing care cost, improving life quality. Many lesions are amenable to surgery; availability of local expertise -both pediatrician and pediatric cardiac surgeons, and awareness amongst parents and other professionals - will help to do correction of lesions at an optimal time.

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# Antibody Detection of Ornithodoros  Moubata Salivary Proteins as a Potential tool to detect exposure to ticks in small rodents

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## Abstract:

The detection of tick specific antibody has the potential to act as a marker for tick exposure. The ability to detect the exposure of rodents to ticks bites could aid our understanding of the epidemiology of tick-borne disease. Therefore, an ELISA was developed using tick salivary extracts from *O. moubata.* Sera from *A. sylvaticus, A. flavicollis* and *M. agrestis* that were naturally infected with ixodid ticks were also used. The study demonstrated that anti-tick salivary antibodies were predominantly detected in sera from infected animals using sera obtained during the period of heavy exposure to ticks (May and June). In contrast, rodents captured during the period of low tick exposure (December and February) showed no response. Furthermore, the levels of IgG1 and IgG2a were assessed as signatures of Th2 and Th1 responses respectively. The level of IgG1 was significantly higher in comparison to IgG2a in *A. sylvaticus* and *A. flavicollis* sera. In addition, the level of IgG1 was slightly higher than IgG2a antibodies when *M. agrestis* sera were tested. Immunoblot analysis indicated that sera from rodents infested by ixodid tick recognised several *O. moubata* salivary antigens ranging in size from 7 to 170 kDa. The predominant parasite specific antigen was determined to be approximately 46 kDa, which was recognised by all the IgG antibodies in both rodents' sera. This salivary protein may therefore represent a potential tool as a marker of exposure to ticks.
*key words*: marker, antibody detection, tick exposure, tick saliva

## Introduction

Tick salivary molecules have been shown to induce cellular and humoral immune responses (Heinze *et al.*, 2012). Antibodies to salivary antigens from ixodid ticks have been used to develop immunological assays for assessing the exposure of animals to the ticks' bites (Kashino *et al.*, 2005; Hall-Mendelin *et al.*, 2011). This approach was used in  tick transmitted disease surveillance programmers, including Lyme disease (Schwartz *et al.*, 1991). Previous studies showed that humans and animals exposed to ticks, or immunized with tick saliva, developed antibodies that recognise specific tick salivary molecules. Such responses have been observed in and reported from several laboratory experiments, using different experimental animals (Need *et al.*, 1991, Sanders *et al.*, 1996, Craig *et al.*, 1996, Szabo *et al.*, 2003, Kashino *et al.*, 2005 and Hall-Mendelin *et al.*, 2011). Investigations, using saliva from a variety of tick species, were carried out to evaluate human exposure to ticks' in different countries (Sanders *et al.*, 1998; Estrada-Pena and Jongejan, 1999; Waitayakul *et al.*, 2006). Recent studies, concentrated on small mammals because they are  capable of transmitting Lyme disease and they contributed to the natural circulation of some pathogens (Craine *et al.*, 1995; Gern *et al.*, 1998; Pawelczyk and Sinski, 2000; Michalik *et al.*, 2003; Stefancikova *et al.*, 2004; Tonteri *et al.*, 2011). The antibody production in the field studies was poorly investigated. The majority of the previous studies on tick-borne diseases were designed                       to

analyse immune responses to the pathogen itself, such as *Borrelia burgdorferi* (Brunet *et al.*, 1995).

Studies proved that the antibody response towards tick salivary proteins was a good biological marker for the exposure to the bites of ticks (Schwartz *et al.*, 1990; Schwartz *et al.*, 1993; Sanders *et al.*, 1999). It was shown that both hard and soft ticks induced diverse antibody response to their salivary antigens, with measurable levels of IgA, IgE, IgG and IgM being reported (Perez-Sanchez *et al.*, 1992; Baranda *et al.*, 1997; Das *et al.*, 2000; Vu Hai *et al.*, 2013). There was, however, significant variations in the cross reactivity of the immune sera between antigens derived from the saliva of different tick species (Need *et al*., 1991; Perez-Sanchez *et al.*, 1992; Janse van Vuuren *et al.*, 1992; Das *et al*., 2000; Brossard and Wikel, 2004; and Schuijt *et al.*, 2011).

Several proteins from *O. moubata* and *O. erraticus* were recognised by sera from rabbits naturally exposed to these ticks. The antigens identified were 19 kDa, 17 kDa and 20 kDa, (Canals *et al.*, 1990; Oleaga-Perez *et al.*, 1994; Astigarraga *et al.*, 1997; Baranda *et al.*, 1997). Among these proteins, the 20 kDa protein was a promising tool to be used in *O. moubata* surveillance and the control measures against tick-borne disease (Oleaga *et al.*, 2007; Diaz-Martin *et al.*, 2011). Oleaga-Perez *et al.* (1994); Astigarraga *et al.* (1997) and Baranda *et al.* (2000) used the 15 and the 17 kDa antigens from the saliva of *O. Moubata* obtained from the sera of animals immunised by anti-tick salivary extracts, but they could not detect these antigens in naturally infected mice. The salivary proteins of *O. moubata* and especially those detected by host specific antibody have not been fully identified. Therefore, studies of the antibody response to naturally infected wild rodents is required.

The present paper reports the assessment of the antibody response to the ticks' salivary components in order to develop immunological assays for assessing the natural exposure of animals to the bites of ticks.

## Materials and Methods

The mice sera was obtained from wood mice, *Apodemus sylvaticus,* and yellow-necked mice, *Apodemus flavicollis,* infected with ixodid ticks and were kindly provided by Gwenaël Vourćh, France; and from two wood mice, *Apodemus sylvaticus,* naturally infected captured in Tollerton, Nottingham, during the high and low transmission seasons respectively.

The voles sera was collected from 20 wild field voles, *Microtus agrestis,* captured in two different trapping sites within Kielder Forest in Northern England, UK. The animals' weight ranged from 30-49 g, 12 of them were adult females and the remaining were adult males.

The control sera was obtained from a specific pathogen free CD1 strain laboratory mice (positive Control). The human sera (negative Control) was kindly provided by a volunteer.

Animals were killed by formaldehyde and exsanguinated by cardiac puncture. Approximately 1.0 ml of blood was collected into a sterile tube. To encourage blood clotting, the blood was left 1.0 hour at room temperature. This was followed by a second incubation period for 1.0 hour at 4ºC. Subsequently, the clotted blood was centrifuged for 10 minutes at 9000 g and the plasma removed. Ten μl of plasma was aliquoted and stored at – 80ºC.

The salivary gland extract was obtained from fed ticks (females and males at an identical number) following the procedures described by Oleaga *et al.* (2007) and Garcia-Varas *et al.* (2010)

It was necessary to evaluate the commercial anti-mouse sera reactivity to the sera from *A. sylvaticus*, *A. flavicollis* and *M. Agrestis* exposed to the tcks' bites. ELISA technique was used for this purpose to test the sera reactivity from to Wood mice, voles, and laboratory mice (positive control), and normal human (negative control) to the Goat anti-mouse IgG antibody. The absorbance values were measured at 450 nm. Titration curves were constructed using GraphPad Prism software.

The optimum concentration of tick salivary gland extract (TSG) antigen was determined using wild rodent sera (*A. sylvaticus, A. flavicollis* and *M. agrestis*) by ELISA, with some modification. TSG antigens was coated on the microtiter plate with a 2-fold serial dilution (10.0 µg/ml, 5.0 µg/ml, 2.5 µg/ml and 1.25 µg/ml) of the coating buffer and incubated overnight at 4 ℃. After the incubation, the washing and the blocking, the plates were incubated with a 2-fold dilution (1:50 and 1:100) of sera from voles and wood mice. The bound antigen-antibody complexes were subsequently detected by the goat anti-mouse IgG antibody. The anti-goat IgG-peroxidase conjugate was added and incubated for 2.0 hours. This was followed by adding the substrate to develop the reaction. The enzymatic reaction was stopped and the optical density was read with a plate reader.

Twenty sera from infected *A. sylvaticus*, *A. flavicollis* and twenty infected *M. agrestis* sera were used as positive reference sera. Another 2 sera from *A. sylvaticus* captured from the field and 10 sera from *M. Agrestis*, also captured from the field, were used as negative reference sera. The microtiter plates were coated with 5.0 µg/ml of TSG antigen and incubated at 4ºC overnight. The following day, the plates were washed, blocked, and incubated with the sera in duplicate with 1:50 and 1:100 dilutions. The bound antigen-antibody complexes were subsequently detected by the goat anti-mouse IgG antibody. The anti-goat IgG-peroxidase conjugate was

added and incubated for 2.0 hours and followed by adding the substrate to develop the reaction. The enzymatic reaction was then stopped and the optical density (OD) was read with a plate reader.

The cut off value was calculated from the results of negative sera for each serum dilution using the formula described by Richardson *et al*. (1983) and Ruiz *et al*. (2001): mean of three optical density plus twice the standard deviations. Sera samples were considered positive when their OD value was greater than the cut off value. The sera that strongly reacted with TSG positive were then pooled to create a standard curve.

The IgG class and subclass antibodies in sera, at an optimal concentration of 5.0 µg/ml of TSG and sera dilution of 1:100 were determined using ELISA. Total anti-TSG specific antibody binding was measured using goat anti-mouse IgG, IgG1 and IgG2a antibodies at a dilution of 1:10000. This was followed by incubating with peroxidase conjugated anti-goat IgG. The assay was then developed with the SIGMA*FAST*™ OPD (*o*-Phenylenediamine dihydrochloride) peroxidase substrate and the optical densities were read at 450 nm.

The protein profiles of the *O. Moubata* TSG were demonstrated by the sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) technique described by Laemmli (1970).

The separated proteins, on the gels, were stained with Coomassie stain (PageBlue TM Protein staining solution) at a dilution of 1:4 (v/v) in distilled water to allow visualisation of protein bands. The gels were washed with 50 ml of distilled water and warmed in a microwave 3.0 times for 30 seconds. This was followed by covering the gels with approximately 50.0 ml of Coomassie blue and warming in a microwave for a further 20 seconds. To remove the excess stain, the gels were washed 3 times and incubated overnight in distilled water with gently shaking on an orbital shaker, at a gentle rotation speed.

The stained gels were subsequently photographed using a coloured scanner.

In order to identify which proteins elicit antibody responses in mammals, TSG was analysed by immunoblots (Towbin *et al.,* 1979) with natural infected rodent sera *A. sylvaticus*, *A. flavicollis* and *M. agrestis*. Three sera were selected from each group based on the levels of IgG and IgG subclasses antibodies from ELISA analysis and sera from *A. sylvaticus* (captured in December, low tick exposure); sera from laboratory mice were used as negative controls.

The binding affinity and specificity of IgG, IgG1 and IgG2a antibodies in sera from *A. sylvaticus* and *M. agrestis* reacted with TSG of *O. moubata* proteins were demonstrated by the Western immunoblotting technique described by Towbin *et al.,* (1979).

The data were statistically analysed by comparing the means. The differences in the OD values were analysed by one-way ANOVA test.

### Results

The reactivity of goat anti-mouse IgG antibody with the antibody in the sera from *A. sylvaticus* and *M. agrestis* showed a significant difference in optical density (OD) values. The mean absorbance of these sera with the normal human sera indicated high absorbance values for the laboratory mice (1.452), the *M. agrestis* (1.112) and the *A. sylvaticus* (1.066) compared with a very low OD value for normal human serum (0.012), $P = 0.0001$,). The reaction observed for wild rodents was significantly higher than human sera with goat anti-mouse IgG antibody, ($P = 0.0001$)..

Sera from heavily infected *A. sylvaticus* and *M. agrestis* showed a strong reaction in contrast with the normal sera of laboratory mice. The 5.0 µg/ml TSG antigen showed a significant difference between the sera tested ($P= 0.0001$),

Figure 3 shows that the dilution of 1:100 of *A. sylvaticus, A. flavicollis* and *M. agrestis* sera was appropriate for use in the detection assay. The cut off value was 0.155 (mean of three OD value of negative samples + twice the standard deviation).

illustrates the serum levels of IgG, IgG1 and IgG2a specific antibodies of the 20 infected sera of *A. sylvaticus* and *A. flavicollis* toward TSG of *O. moubata*. A negligible amount of antigen specific IgG2a was detected in either species to

TSG antigen. However, measurable levels of both IgG and IgG1 were found. Both IgG and IgG1 levels toward TSG proteins in *A. sylvaticus* and *A. flavicollis* sera heavily infected were significantly higher than the sera obtained from *A. sylvaticus* captured from the forest and from the laboratory mice ($P = 0.001$), and between IgG1 and IgG2a ($P = 0.008$).

indicates the levels of IgG, IgG1 and IgG2a specific antibodies of *M. agrestis* to TSG from *O. moubata.* The IgG was significantly higher than both IgG1 and IgG2a ($P= 0.001$). In contrast, no significant difference was observed between IgG1 and IgG2a antibodies for *M. agrestis* sera.

Although the TSG contained several molecules (Error! Reference source not found.), few molecules were reacted with individual serum from *A. sylvaticus* and *A. flavicollis.* The IgG antibody recognised antigenic bands of 7 kDa, 15 kDa, 20 kDa, 32 kDa, 30 kDa, 46 kDa, 64 kDa and 80 kDa, whereas, IgG1 antibody recognised only two bands at approximately 20 kDa and 46 kDa. IgG2a antibody reacted to only one molecule at 46 kDa  For *M. agrestis*, individual serum recognised only one antigenic molecule at 46 kDa, and there were some molecules that reacted weakly with these sera, presented at 7 kDa, 15 kDa, 20 kDa and 35 kDa).

## *Discussion*

The detection of antibodies against tick salivary proteins in sera from wild rodents naturally infected with ticks has the potential to be a marker of exposure to the ticks' bites. It is also a very useful tool for the epidemiological studies of tick-borne diseases (Schwartz *et al.*, 1991; Lane *et al.*, 1999).

The reactivity of the goat anti-mouse IgG antibody to detect antibody in other small rodents is good. A good reactivity to both *A. sylvaticus, A. flavicollis* and *M. agrestis* was observed, but not to the human antibody. The results are in consistent with the immunological facts that there are fundamental immunological differences between mice and humans (Capron *et al.*, 1999; Mestas and Hughes, 2004), and that the IgG isotypes are different between them (Mestas and Hughes, 2004).

The results of this study also show that there is significant cross-reactivity between argasid and ixodid ticks, indicated by the induced reactivity. The result is consistent with previous studies, which reported that there was cross-reactivity of salivary proteins present between soft and hard ticks (Need *et al.*, 1991; Janse van Vuuren *et al.*, 1992). This cross-reactivity is useful in the identification of those exposed to ticks' bites. The usage of *O. Moubata*, as a source of antigen for the detection of exposure to ticks, is advantageous, because of the ease of maintenance of this parasite in the laboratory compared to other ixodid ticks (Schwartz *et al.*, 1991).

The most antigenic molecules of *O. moubata* are 15 kDa, 17 kDa, 19 kDa, 20 kDa, 46 kDa and 70 kDa. The results are consistent with the findings from previous studies, which found that these molecules of saliva from *O. moubata* were recognised by sera from rabbits immunized with tick saliva (Canals *et al.*, 1990; Oleaga-Perez *et al.*, 1994). However, previous studies showed that the 15 and 17 kDa antigens present in the saliva of *O. moubata* were detected in sera from animals immunised with anti-tick salivary extracts, but there was no detection of those antigens in sera from naturally infected mice (Fivaz, 1989; Astigarraga *et al.*, 1997; Baranda *et al.*, 2000; Mejri *et al.*, 2002).

The present results proves that the specific antibodies to *O. moubata* TSG proteins are detected from *A. sylvaticus* and *A. flavicollis* infected sera. This suggests that these anti-salivary antibodies are not tick species-specific and that there are shared antigens in the salivary glands of the different tick species. The lack of a detectable specific antibody to TSG antigens by *M. agrestis* sera is due either to the fact that the hard tick species, which infect *M. agrestis*, have less cross-reactivity with those of *O. moubata*, or because the tick fails to induce measurable responses of antibody. Despite the fact that the antigen recognition pattern is different among the sera from the three ticks under study, *A. sylvaticus, A. flavicollis* and *M. agrestis*, there are TSG proteins that are consistently detected by IgG and IgG subclasses in all species, such as the 46 kDa protein. This molecule is a promising marker of exposure to the bites of the argasid and the ixodid ticks. The development of recombinant proteins from tick salivary component for other tick species can be used to determine those species-specific in tick infections.

IgG2a antibody is mainly produced by B-cells and controlled by Th1 cytokines, particularly IFN-γ (Collins and Dunnick, 1993; Else and Finkelman, 1998; Nagabhushanam and Cheers, 2001; Da'Dara *et al.*, 2003), while the production of IgG1 is controlled by the Th2 cytokine IL-4 (Paul *et al.*, 1987; Purkerson and Isakson, 1992). Thus these antibody isotypes reflect the balance between IFN-γ and IL-4 induced during infection. Despite IgG1 being less definitive as a marker for

Th2 than IgG2a as a marker of a Th1 to IgG2a presents a significant indicator of immune polarity (Da'Dara *et al.*, 2003; Li *et al.*, 2004).

The determination of IgG1 and IgG2a provides an indication of the polarisation of responses to tick saliva. The higher IgG1 levels than the IgG2a levels obtained from *A. sylvaticus,*and *A. flavicollis* ticks are suggesting that the salivary molecules are indicators of Th2 response. This conclusion was reported by several researchers (Wikel, 1996and 1999; Singh and Girschick, 2003). On the other hand, there is no difference in the levels of IgG1

responses, the ratio of the level of IgG1 and IgG2a from the sera of *M. Agrestis*. This observation indicates the Th1/Th2 response. It is possible that the short exposure of tick salivary proteins favours a purely Th2 response, whereas a long exposure induces both Th1 and Th2 responses.

Thus, it is concluded and recommended that the use of *O. moubata* salivary components to detect the antibody in exposed animals is a useful diagnostic indicator for the exposure to the bites of argasid ticks as well as the bites to ixodid ticks.
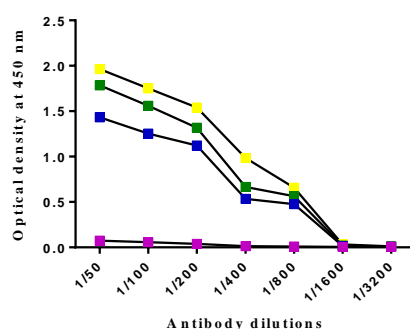


**Figure 1 : Reactivity of goat anti-mouse IgG with antibody in sera from wild rodents.**

The graph presents an ELISA result of ODs at 450 nm (Y-axis) versus the antibody dilution (X-axis). Normal laboratory mouse serum (Yellow, squares), *M. agrestis* (Green, squares), and *A. sylvaticus* (Blue, squares) normal human serum (Purple, squares). The results were presented as the mean, n = 3.
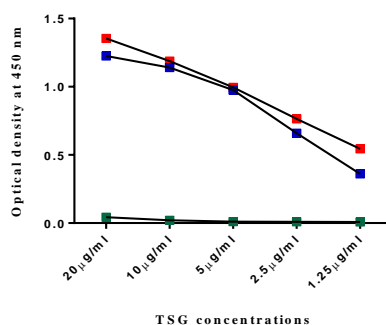


**Figure 2 : ELISA titration of TSG antigen reacted with antibody in infected *A. sylvaticus* and *M. agrestis* sera.**

Two-fold series dilutions of TSG concentration were examined (20 µg/ml to 1.25 µg/ml). The optimum concentration of TSG was 5µg/ml as indicated with two rodents sera. 1:100 dilution of *M. agrestis* (Red, squares), *A. sylvaticus* (Blue, squares) and sera from laboratory mice (Green, squares). The results were presented as mean, n=10.
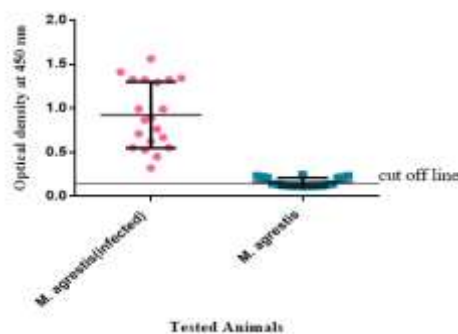
**Figure 3 : Distribution of sero-positive and sero-negative of *M. agrestis* sera.**

Sero-positive of *M. agrestis* infected (heavy tick infection period) (Pink, circles). Sero-negative of *M. agrestis* sera (Blue, squares). Samples of each type of sera (negative and positive) were used in triplicate at 1:100 dilution. The error bars represent the standard deviation of the arithmetic mean (n=40). The horizontal line indicates the cut off value as calculated to be 0.155.
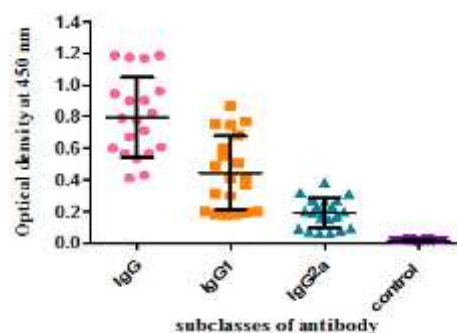


**Figure 18 : Detection of specific antibody of sera from *A. sylvaticus* to TSG of *O. moubata*.**

The mean ± SD of optical density of IgG, IgG1 and IgG2a antibody levels as measured by ELISA (n=20). Sera obtained from *A. sylvaticus* (captured in December, low tick exposure) used as a negative control. All the sera were examined at a 1:100 dilution. The TSG was used at a concentration of 5µg/ml. The error bars represent the standard deviation of the mean, n=20.
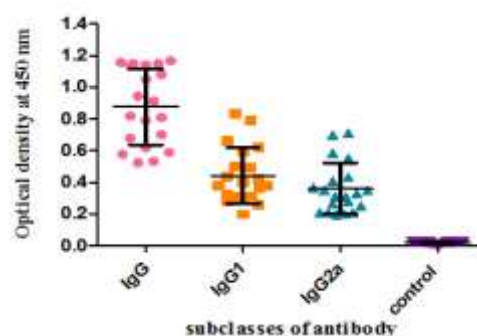


**Figure 19 : Detection of specific antibody of sera from *M. agrestis* to saliva of *O. moubata*.**

The mean ± SD of optical density of IgG, IgG1 and IgG2a antibody levels as measured by ELISA (n=20). Sera obtained from *M. agrestis* (captured in February, low tick exposure) used as a negative control. All the sera were examined at a 1:100 dilution. The TSG was used at a concentration of 5µg/ml. The error bars represent the standard deviation of the mean, n=20.
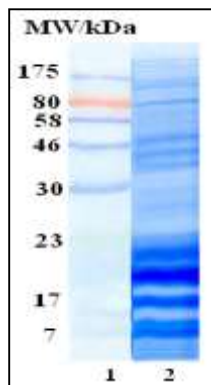
**Figure 20 : Protein components of *O. moubata* salivary glands.**

The salivary gland protein extracts were analysed by 12 % SDS-PAGE under reducing conditions (Lane 2) and stained with Coomassie blue. The protein molecular weight markers are in kDa (Lane 1).
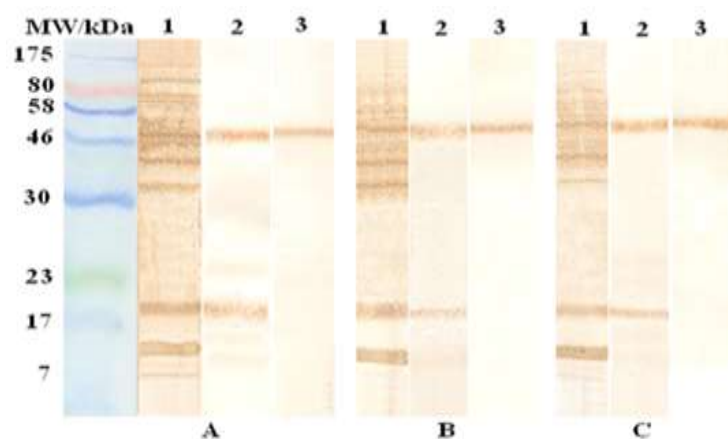


**Figure 21 : Immunoblot of TSG antigens of *O. moubata* recognised by antibody in infected *A. sylvaticus* sera.**

 Each set of three strips (A, B and C) was probed with infected *A. sylvaticus* sera to specific IgG antibody (Land 1), IgG1antibody (Land 2) and IgG2a antibody (Land 3). The protein molecular weight markers are in kDa.
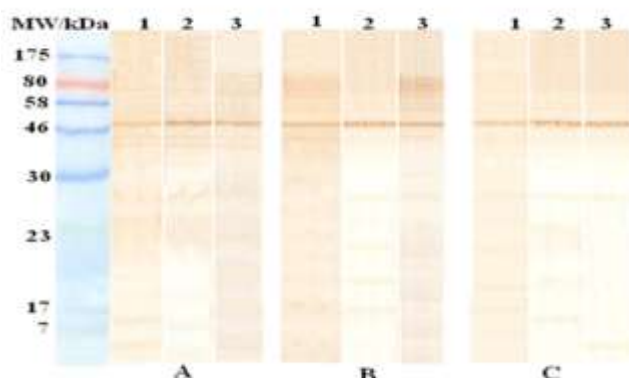


**Figure 22 : Immunoblot of TSG antigens of *O. moubata* recognised by specific antibody in infected *M. agrestis* sera.**

Each set of three strips (A, B and C) was probed with infected *M. agrestis* sera to specific IgG antibody (Land 1), IgG1 antibody (Land 2) and IgG2a antibody (Land 3). The protein molecular weight markers are in kDa.

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# Mobile phone Contamination by Microorganisms in Quinnipiac University: comparing health science students and non-health science students

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**ABSTRACT**:

This research investigates the microbial contamination associated with mobile phones of Quinnipiac University students and the roleof mobile phones play as a fomite. Investigates the presence of four bacterial species including *Staphylococcus ,Streptococcus*, *Proteus mirabilis* and *Escherichia coli* on mobile phones.

Mobile phones are easily contaminated with pathogenic bacteria and could be vehicles of transmission. The main objective of this study was to compare the contamination rate of mobile phones with pathogenic bacteria between health science and non-health science student's mobile phones.

A fomite is an object that can carry microbes, which infect people and increase the incidence and the prevalence of the diseases. Mobile phones come in close contact with the body and serve as a ready surface for colonization.

The goal of this study is to qualitatively and quantitatively investigate bacterial contamination of mobile phones. Cells phones from a variety of people were swabbed for bacterial culture. The level and type of bacterial contaminations were compared amongst health science students vs. non-health science students in an attempt to determine if the health science majors disinfect their phones more frequently because of their awareness of the role of fomites in the disease transmission.

To determine the most prevalent type of bacteria in the cell phones, the high-risk group of the contamination, and analysis any associations between the students major and the level of the cell phone contamination.

**INTRODUCTION**:

A mobile phone can act as a source that transmits microorganisms within the people who shared the mobile phones. They are considered fomites that are able to transfer a wide variety of pathogenic agents to others through indirect contact.

These public health concerns are important for the health care workers to be aware of the role of mobile phones in transmitting of these contaminants into the patients. It is widely known that fomites play an important role in spreading of infections in both community and hospital settings, causing outbreaks of nosocomial infections such as Methicillin resistance *Staphylococcus aureus* (MRSA) and other nosocomial diseases. Fomites transmit bacteria, which thrive and multiply on their surfaces and might cause infections. It is known that some diseases are more likely to be transmitted by fomites than others, people rarely clean their mobile phones due to the lack of knowledge about the role

including gastrointestinal and respiratory infections.

The majority of the people use their mobile phones in high-contaminated environments such as restrooms and kitchens. Consequentially, this behavior increases the potential of mobile phone contamination and disease transmission. This act puts them at a high risk of transferring potentially pathogenic microorganisms to their cell phones and to others.

The biggest concern is cross contamination between mobile phones and foods. This concern is more important in children environments such as day cares, schools and other public settings because young kids are vulnerable to disease. It's important to know and understand that most

mobile phones as a source of microbes' transmission.

In addition, mobile phones come in close contact with body surfaces such as the face, ears and mouth, which can act as a good area for colonization and potential source of microorganisms transmission, therefore the microorganisms can easily enter the mouth and ears where they can enrich and multiply and causing many diseases.

 Moreover, the majority of the people keep their mobile phones in their pockets and bags, which are warm environments that can act as an appropriate place for enriching rapid microorganisms growth and multiplication.The goal of this study was to investigate mobile phone contamination at Quinnipiac University to identify the colonization of four pathogenic microorganisms including *Staphylococcus aureus, Streptococcus*, *Escherichia coli* and *Proteus mirabilis*.

The second goal was to determine the level and type of bacterial contamination of the mobile phones of Quinnipiac students and to identify if there is a significant relationship between the knowledge of hand hygiene, and cleanliness and mobile phone contamination.

For this research, the hypotheses that have been investigated were the relationship between the major of the students and the level of mobile phone contamination. To identify if the hand hygiene and the awareness of the students about mobile phones as fomites could play significant role in the level and type of bacterial contamination.

**METHODS AND MATERIAL**:

 A questionnaire consisting of eight questions was conducted in this research experiment data not shown. A total of 151 samples were collected from both health science and non-health science students at Quinnipiac University.74 samples were collected from health sciences students including biomedical science students and biology majors, and 77 samples were collected from non-health sciences students including accounting majors, communication majors, and law students. All the samples were collected with the aseptic technique.  Mobile phones were swabbed with moist cotton emended in Trypticase soy broth(TSB).  Set of 6 tubes of 9 ml TSB were previously prepared labeled and placed on the tube rack. A serial dilution were performed by transferring 1ml of the original sample to the tube labeled $^{-1}$with vortex, and 1ml of the first TSB tube transferred into the second one, second to third, etc. Set of Trypticase soy agar (TSA) plates were labeled same as the serial dilution with three plates for each dilution. 0.1ml of each

dilatation was transferred into 3TSA plates, spread the diluents over the TSA surface and incubate all plates at 37 degrees Celsius for 48 hours. The original TSB samples were also inoculated at the same time into Mannitol salt agar and MacConkey agar  and incubated at 37 degrees Celsius for 24 hours. The characteristic bacteria isolates from each selective media plates wereGram stained to identify Gram-positive bacteria, ssp. The *Staphylococcus* ssp. is Gram-positive cocci, seen in clusters under the microscope, while *Streptococcus* ssp. is Gram-positive cocci, seen in chains under the microscope. However, the Gram-negative rods are seen red in color under the microscope.  The biochemical tests needed for further identification and differentiation of the isolated bacteria. These tests included catalase, coagulase for the Gram-positive bacteria, which grown on MSA, while the urease, idole, oxidase, citrate and methyl red and Voges-proskauer tests used for gram-negative bacteria, which grown on MacConkey.

Staphylococcus spp. can cause a variety of skin infections including boils and furuncles. The most important fact is that there is a strain of Staphylococcus that can be resistant to the first line antibiotics used in treatment to this kind of diseases including methicillin, and oxacillin.

Streptococcus spp. is associated with many diseases such as rheumatic fever, rheumatic heart disease, and nephritic disease. Moreover, Escherichia coli can cause severe gastrointestinal illness, urinary tract infections and even renal failure. In addition, Proteus mirabilis can cause urinary tract infections, renal stones and renal failure.

**RESULTS**:

Based on the survey analysis, the numbers of health science students were74(49%) students equal to (49%), that are slightly less than number of non-health science student who were (51%) figure (1).

**Figure (1)Percentage of Health Science VS Non -Health Science students**
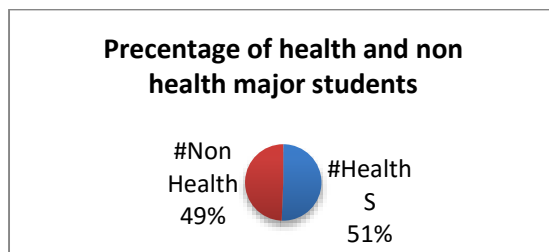


The most interesting outcome in the survey analysis was out of 151students that participant in the study only 30 (19%) students disinfected their phones. Among these students, 23(76%) students were health sciences students. This result showed that a significant number of students were never disinfected their phones in both groups.

**Figure (2)% of students who disinfected their phones vs. % of students who did not disinfected their phones**
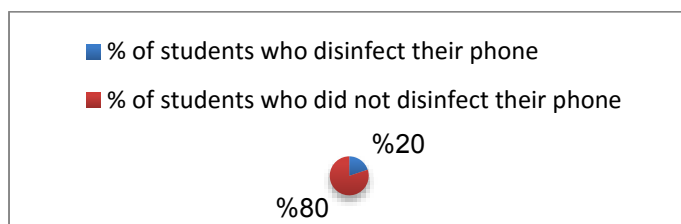


The survey revealed that among all Quinnipiac students who were participants in this study, only 7(4.6%) students cleaned their hands after use the mobile phone; all of those students were health science students figure (3).

**Figure (3) % of students who wash hands after Mobile phone use**
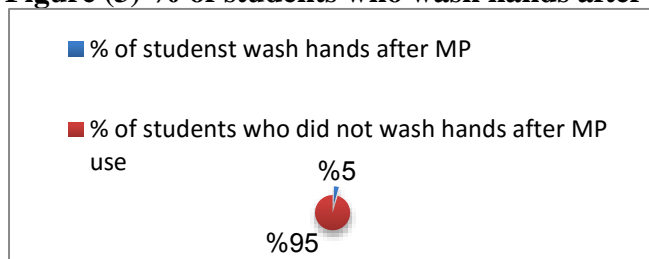


Based on the bacterial count(spread plate technique) analysis,the average colonies forming unit in the health science students mobile phones was $1.306 \times 10^4$while the average number of colonies forming unit in the mobile phones of non-health science students was $1.62 \times 10^4$.

**Table (1) the average # of CFU in health science vs. non health science students**

| The average #of colony forming unit (CFU) | Health science students | Non health science students |
|---|---|---|
| | $1.306 \times 10^4$ | $1.62 \times 10^4$. |

Data analysis "table 3"also showed that gram-positive bacteria were among the highest percentage of bacteria that have been found on Quinnipiac student's mobile phones, which found on the63.5% of mobile phones, while gram negative rod being discovered on 47% of mobile phones.

Based on gram staining, catalase and coagulase test, the result indicated that 48 (31%) samples out of 151 samples were harbored with *Staphylococcus aureus*, 40(26%) samples were grow *Staphylococcal epidermis*, and 6(3.9%) samples were contaminated with *streptococcal* ssp. "Table 3" data not shown.

The data show that14(9%) samples of gram-negative isolates were *E.coli* based on the biochemical testing, and 10 (6.6%) were *Proteus marbilis.*

In comparison of the study major in the table (2), the data revealed that 18 (37%)out of 48 *Staphylococcal aureus* isolates were present at mobile phones of health major, 14(35%) out of 40 of *Staphylococcal epidermis* were found on mobile phones of health major, 3(50%) out of 6 of *Streptococcal* isolate were found in the mobile phone of health major 7(50%) out of 14*E. coli* were health science students, and 3(30%) out of 10 of *Proteus* were present on health science students' mobile phones. This is clearly, pointed out to the fact that was less contamination level in the mobile phones of health science students in compared to the non-health students' mobile phones.

**Table (2)Bacteria isolates from mobile phones of Quinnipiac University students**

| Isolates | Health science mobile phones, n=74 | Non- health science mobile phones, n=77 |
|---|---|---|
| *Staphylococcal aureus* | 18(37%) | 30(63%) |
| *Staphylococcal epidermis* | 14(35%) | 26(65%) |
| *Streptococcal* spp. | 3(50%) | 3(50%) |
| *Escherichia coli* | 7(50%) | 7(50%) |
| *Proteus* | 3(30%) | 7(70%) |

The results support research hypothesis that was health science student's mobile phone would have less bacterial contamination due to their awareness of the mobile phone as a fomite.

The antibiotic sensitivity test was conducted in this research to all samples that had bacterial growth to detect if there were any resistant strains associated with mobile phones table (6) data not shown. The result as seen on table (10) data not gram-positive bacteria were susceptible to tetracycline, compared to 66% of gram negative

shown, shows the percentage of each bacteria strain and their susceptibility to the six antibiotics. It compares the sensitivity of both gram-positive strains and gram-negative strain for the six antibiotics have been used in the research. This data revealed that only 10.5 % of gram-positive bacteria were susceptible to erythromycin, and 8.3% of gram negative was susceptible to the same antibiotic. 80.9%                                          of

were susceptible to the tetracycline. The positive isolates were ciprofloxacin sensitive and 77% of gram-negative isolates were sensitive to ciprofloxacin. In contrast, in case of oxacillin there was only 2.2% of all bacteria isolates were sensitive to it and no strain of gram-negative was sensitive to it. Ceftriaxone was the most sensitive antibiotic for both gram negative and positive bacteria as the data revealed that 75% of gram-negative species were susceptible to it and 91.5% of gram positive were also sensitive to it. The data also indicate that clindamycin was one of the less sensitive antibiotic after the oxacillin with only 4.9%, and 8.3% sensitive of both gram positive and gram negative strain respectively. The results

data indicated that 85.2% of gram-recorded by comparing the zone of inhibition around each antibiotic to the diameter interpretative standards for the bacteria of interest. Overall, the result showed that gram-positive bacteria were more susceptible to all used antibiotics in comparison to the gram-negative bacteria except the clindamycin, in which the gram negative strain were more susceptible to it. Further analysis of data in table (10) data not shown, explain that the most susceptible antibiotics for all bacterial species that were investigated are ceftriaxone, ciprofloxacin, and tetracycline; in contrast the more resistant antibiotics areoxacillin, clindamycin, and erythromycin.

## CONCLUSION:

This research finding revealed that the most common bacteria isolates on mobile phones were *Staphylococcal aureus*, *Staphylococcal epidermi*s, *Streptococcal* spp. *E. coli* and *Proteus*. The overall contamination of mobile phone was 93%. The highest Total Viable Count was observed in non-health science student' mobile phones compared to the health science students' mobile phone. This is indicating poor personal hygiene.

The higher prevalence of microbiota in the mobile phones was found on the mobile phones of the non-health science students, this could be attributed to the poor hygienic and sanitary practices associated with their lack of awareness about mobile phone as a fomite. The research findings indicates that mobile phones can act as an important source of pathogenic organisms

for human and can serve as vehicle for cross-transmission.

The research has some limitations that couldinvestigate if the gender is associated with the level of contamination.The research may also investigate other bacterial species such as*Bacillus* spp. and *Pseudomonas aeroginosa* that shown to be associated with mobile phone contamination.

This study strongly recommend public to follow simple hygiene practice include washing hands after rest room use, and disinfect mobile phones with alcohol wipes can reduce the level of mobile phone contamination significantly. And emphasizes that mobile phones may act as a carriers in spread of pathogenic microorganisms in the community.

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# Role of fetal echocardiography in improving outcome of neonate with congenital heart diseases, Algala maternity hospital Tripoli/ Libya

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## Abstract:

Back ground: Congenital heart diseases (CHD) are the most common type of congenital anomalies and are a leading cause of mortality among children with congenital malformations. The estimated incidence of CHD is 8/1000 live birth.

**Objective and aims:** The aim of this study was to know the incidence of congenital heart disease among live born babies delivered at Aljala maternity hospital in Tripoli during the year 2009 ,and to compare the outcome of those diagnosed prenatally ( via fetal echocardiography ) with those discovered postnatally.

**Patients and methods:** The study involved 101 infants delivered in Aljala maternity hospital during one year interval from 1/1/2009 to 31/12/2009, who were diagnosed to have congenital heart defects.

**Results:**
The results revealed that the incidence of CHD is 7 per 1000 live births. There is no sex predilection as the M:F ratio is 1.06:0.94( almost 1:1 ), and positive family history of CHD increase the risk by only 2%. Septal lesion (VSD and ASD) found in 34% of patient, 57% have mixed type of lesion while 5% have complex heart diseases; by using Chi-square test the number of lesion affects the outcomes of patients significantly(Pvalue=0.002). Among 101 total patient, 14 patient (14%) need surgical treatment, 26 patient(26%) received medical treatment, while 61 patient (60%) just followed up in cardiac clinic. Prenatal diagnosis of CHD is of value in improving management of patients with congenital cardiac anomalies although the relation between antenatal diagnosis and outcome by using Chi-square test statistically not significant (Pvalue=0.310).

**Recommendations:**
Depending on the results of this study, a national screening program for congenital heart disease via the 5- views obstetrical ultrasound examination of pregnant ladies at 16 – 20 weeks of gestation is recommended (as first step in prenatal diagnosis), and also long term prospective studies are needed to further evaluate the role of prenatal diagnosis in improving prognosis of patients with congenital heart disease.

**Key words: CHD, Fetal Echocardiography,Libya.**

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## Introduction:

which is a non-invasive diagnostic ultrasound test that can detect congenital heart defects and rhythm disturbances in the fetus; the optimal timing is 18 to 22 weeks gestation. [4] The aim of our study to find out the incidence of congenital heart diseases among infants delivered in the Aljala maternity hospital, and to compare the outcome of infants with congenital heart diseases diagnosed antenatally (via fetal echocardiography) with those diagnosed postnatally. It is descriptive study conducted in Aljala maternity hospital and the cardiology outpatient clinic in Tripoli children hospital was reviewing the medical records of patients over one year period (1/1/2009 to 31/12/2009).

Congenital heart disease refers to a problem with heart structure and function due to abnormal heart development before birth .In the definition by Mitchell et al., congenital heart disease is a gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of functional significance [1]. It is the most common birth defects , occurring approximately in 8 / 1000 live births with higher incidence in premature infants ( about 2% ).[2] higher incidence shown in many recent studies ( reaching 12 per 1000 live births or higher ) [3]. This is largely attributed to the increasing use of echocardiography in diagnosing congenital cardiac defects including the expanding role of antenatal diagnosis via fetal echocardiography,

### Results and discussion:

The total live births in Aljala maternity hospital from 1/1/2009 to 31/12/2009 are 14617 newborn, out of which 101 new born babies diagnosed to have congenital heart disease with an incidence of 7 per 1000 live births, which is between the recorded international figure 8/1000 live births, and the figure recorded by the health region in England 6.5/1000 live births [5]. The highest incidence recorded in Taiwan between 2000-2006, where it was 13.1/1000 live births [6]. only forty (40%) of patients were diagnosed antenatal by doing fetal echocardiography, while 61 patients (60%) did not do fetal echocardiography and diagnosed during neonatal period, figure(1).

There is no sex predilection as the percentage of male patients was (51.49%) while female patients (48.51%) with Male to Female Ratio 1.o6: 0.94, almost 1:1, figure (2). We record 9% of the patients has family history of CHD, 2 of them their sibling are affected that will increase the risk to only 2%. This is lower than the usual risk factor when sibling is affected which is 4%, while 91% of patients have negative family history for the disease, figure (3). By analyzing the antenatal history we found 57% of patient's their mothers with no relevant antenatal history, 2% their mothers have TORCH infection. While 41% of patients are infants of diabetic mothers, the high percentage of diabetic mothers is largely because the diabetic clinic in this hospital is the main referral center in Tripoli and surrounding area. It is known that diabetes mellitus increase the risk of CHD by 3%, in our study the risk increased to 7% as 41 out of 567 infants of diabetic mothers delivered in the hospital during the year 2009 were diagnosed to have CHD, which is higher than the figure recorded in study done in Canada between 2002-2010, were the risk of CHD to infant of diabetic mothers increased to 4.38% [7]. Most of patients were full term babies (85%), while (15%) were preterm babies, it is similar to the result of study done in England from 1987 to 2001, they found that

preterm babies constitute 16% of patients with Congenital heart disease [8]. 60% of those preterm patients were infants of diabetic mothers, in our study 60% of preterm neonates having patent ductus arteriosus (PDA) which is the commonest congenital heart lesion found in preterm babies. Regarding the type of CHD, 34% of patients were found to have septal lesions; ventricular septal defect (VSD) account 3/1000 live birth, Atrial septal defect (ASD) 1.5/1000 live birth, while 2% of patients having valvular lesions ( mitral and tricuspid regurgitation , pulmonary stenosis ), another 2% with arterial lesions ( patent ductus arteriosus , coarctation of aorta ). Those with mixed type of lesions constitute 57% of patients and 5% have complex heart disease which account only 0.3% per 1000 live birth, table (1).This figures is similar to the result in a population based study from Atlanta, USA (done 1998-2005), the commonest lesions being VSD, ASD, Tetrology of fallot (3.8, 1. And 0.5 per 1000 live births) respectively [9].
 Among 101 total patients, 14 patients (14%) were treated by surgery, 26 patients (26%) received medical treatment (drugs), while 61 of patients (60%) were just followed up in the cardiac clinic, figure (4). The outcome  were 87 patients got improved ( 86% ) , 6 were not improved ( 6% ) , and 8 patients died ( 8% ) , figure ( 5 ).   We found that the gender does not affect the outcome as the number of deaths and non-improvements is equal in both males and females,p-value=0.993 by using $X^2$ test. The  high percentage of non-improvement among preterm babies (20%) compared to full term babies (3.5%) is largely attributed to the fragility of preterm babies making them vulnerable to complications and multiple health problems even with proper intervention (out of 3 preterm babies treated by surgery, 2 were not improved; compared to 11 full term babies treated by surgery only 1 patient not improved and 1 died postoperatively), the p-value is = 0.044, which is statistically relevant and means there is strong relation between gestational age and outcome of surgical

treatment, table (2). But when we study the relation between the number of lesions and the promising , going down from 100% improvement in case of single lesions to 50% improvement in patients with 5 lesions .the p-value is = 0.002 , which statistically significant, table(3).  By comparing the relation between the anti-natal diagnosis and the outcome we found that Out of 8 total deaths, 6 patients (75%) were not diagnosed antenatally by fetal echocardiography, while in 2 patients (25% of deaths) fetal echocardiography was done ( antenatally diagnosed ), and out of 6 patients were not improved; 5 ( 83% ) did not do fetal echocardiography compared to 1 patient ( 17%)  who did fetal echocardiography and diagnosed antenattaly. By applying the

outcome of patients  the results are less

statistical tests the p-value is = 0.310 which means there is no statistical significant relation between antenatal diagnosis and outcome, table (4). In our study, 61patients were did not receive any form of treatment (just follow-up ); among them 60 got improved ( 98% ) and one patient died ( 2% ). While 26 patients were treated medically ( drugs ); out of them 17(65%) got improved, 6 (23%) patients died, and 3(12%)  patients not improved. 14 patients were treated by surgery; 10(72%)  patients of them got improved, one patient died ( 7% ) and 3(21%) not improved. By applying $X^2$ test p-value is = 0.000 (highly significant) which means there is relation between type of treatment and outcome, table (5).

### Conclusions:

The incidence of congenital heart diseases among life born babies delivered in Aljala maternity hospital in the year 2009 was similar to the international incidence of the disease (7 versus 8 per 1000 live births). Septal lesion (VSD and ASD) is the most common CHD as it is found in 34% of patient.

### Recommendations:

There is a need for a national screening program for congenital heart disease to establish the overall national incidence of the disease. Post natal examination remains an essential part in the process of discovery of congenital heart disease. Long term

Prenatal diagnosis of CHD is of value in improving management of patients with congenital cardiac anomalies although the relation between antenatal diagnosis and outcome by using Chi-square test statistically not significant (P value=0.310).

study involving larger number of patients is required to prove the role of antenatal diagnosis via fetal echocardiography in improving the management and prognosis of neonate with congenital heart diseases.
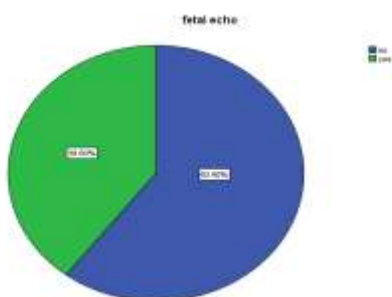
### Figures and tables



Figure (1): Distribution of patients
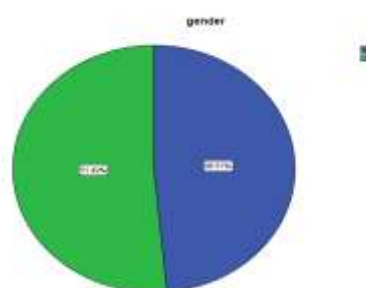
congenital heart disease.

according to time of diagnosis.



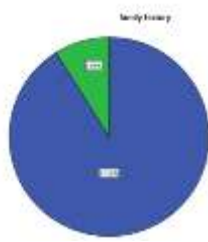Figure (2): Sex Distribution of patients with

Figure (3): Distribution of patients with CHD according

according to family history of the disease.
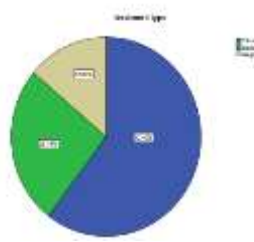


Figure (4): Distribution of patients

to type of management
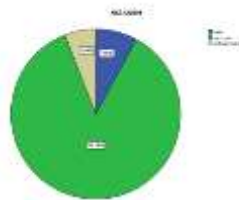


Figure (5): Distribution of patients with CHD according to overall outcome.

Table (1):Distribution of patients according to group of lesions ( type of CHD )

|  | Frequency | Percent |
|---|---|---|
| septal lesions | 34 | 33.7 |
| valvular lesions | 2 | 2.0 |
| Arterial lesions | 2 | 2.0 |
| Septal& valvular lesions | 8 | 7.9 |
| Septal & arterial lesions | 30 | 29.7 |
| valvular & arterial lesions | 3 | 3.0 |
| septal ,valvular& arterial lesions | 17 | 16.8 |
| complex heart disease | 5 | 5.0 |
| Total | 101 | 100.0 |

Table (2): Relation between Gestational Age &Outcome.

| | | | GA | | Total |
|---|---|---|---|---|---|
| | | | FT | PT | |
| out come | died | Count | 7 | 1 | 8 |
| | | % within GA | 8.1% | 6.7% | 7.9% |
| | improved | Count | 76 | 11 | 87 |
| | | % within GA | 88.4% | 73.3% | 86.1% |
| | not improved | Count | 3 | 3 | 6 |
| | | % within GA | 3.5% | 20.0% | 5.9% |
| Total | | Count | 86 | 15 | 101 |
| | | % within GA | 100.0% | 100.0% | 100.0% |

Table (3): Relation between Numbers of lesions & patients Outcome.

| | | outcome | | | Total |
|---|---|---|---|---|---|
| | | died | improved | not improved | |
| number of lesions | 1 lesion | 0 | 17 | 0 | 17 |
| | | .0% | 100.0% | .0% | 100.0% |
| | 2 lesions | 2 | 36 | 0 | 38 |
| | | 5.3% | 94.7% | .0% | 100.0% |
| | 3 lesions | 2 | 23 | 1 | 26 |
| | | 7.7% | 88.5% | 3.8% | 100.0% |
| | 4 lesions | 3 | 9 | 4 | 16 |
| | | 18.8% | 56.3% | 25.0% | 100.0% |
| | 5 lesions | 1 | 2 | 1 | 4 |
| | | 25.0% | 50.0% | 25.0% | 100.0% |
| Total | | 8 | 87 | 6 | 101 |
| | | 7.9% | 86.1% | 5.9% | 100.0% |

Table (4): Relation between Antenatal diagnosis and Outcome.

|  |  |  | fetal ECHO | | Total |
|  |  |  | not done | done |  |
|---|---|---|---|---|---|
| outcome | died | Count | 6 | 2 | 8 |
|  |  | % within outcome | 75.0% | 25.0% | 100.0% |
|  | improved | Count | 50 | 37 | 87 |
|  |  | % within outcome | 57.5% | 42.5% | 100.0% |
|  | not improved | Count | 5 | 1 | 6 |
|  |  | % within outcome | 83.3% | 16.7% | 100.0% |
|  | Total | Count | 61 | 40 | 101 |
|  |  | % within outcome | 60.4% | 39.6% | 100.0% |

Table (5): Relation between Treatment type & Outcome

|  |  |  | out come | | | Total |
|  |  |  | died | improved | not improved |  |
|---|---|---|---|---|---|---|
| treatment type | follow up | Count | 1 | 60 | 0 | 61 |
|  |  | % within treatment type | 1.6% | 98.4% | .0% | 100.0% |
|  | medical | Count | 6 | 17 | 3 | 26 |
|  |  | % within treatment type | 23.1% | 65.4% | 11.5% | 100.0% |
|  | surgical | Count | 1 | 10 | 3 | 14 |
|  |  | % within treatment type | 7.1% | 71.4% | 21.4% | 100.0% |
|  | Total | Count | 8 | 87 | 6 | 101 |
|  |  | % within treatment type | 7.9% | 86.1% | 5.9% | 100.0% |

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# Management of antenatal detected hydronephrosis

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## Abstract:

Abnormalities of urinary tract in fetuses are being recognized with increased frequency due to high resolution of fetal ultrasonography and greater staff expertise. The incidence of antenatal hydronephrosis ranged from 0.6-4% with up to 90% can be detected antenatly.  Most antenatal detected hydronephrosis are transient and resolve spontaneously however, severe urinary obstruction can lead to renal injury and end stage renal disease.  Very few cases need antenatal management while most of fetuses with antenatal hydronephrosis are investigated and managed postnataly. **The aim of this article** is to review the literature on the management of this condition and to identify infants requiring further investigation and management.

**Keywords:** antenatal hydronephrosis, urinary tract abnormalities, ultrasonography

## Introduction

Antenatal hydronephrosis (ANH) is defined as dilatation of the collecting system of the fetal kidney.  It is a common finding of antenatal ultrasound examination.  In nearly 1% of pregnancies, a significant fetal anomaly is detected by ultrasonography.  20-30% of these anomalies are genitourinary in origin. 50% of them manifest as hydronephrosis[1-3]. 41-88% of infants with ANH resolves by birth or during infancy [4], [5],[6] and Two main classifications exist [8]. The first is grading system developed by the appearance of renal parenchyma and pelvicalyceal system [9] [10] as shown in table 1:

most pelvic dilation is a transient finding [7].  However, urinary obstruction can lead to renal injury and end stage renal disease. If these anomalies are not detected by antenatal US and subsequently managed, many of these abnormalities manifest later in life as pyelnephritis, hypertension and end stage renal disease. Several systems are used to grade antenatal hydronephrosis by ultrasonography (US).
Society of Fetal Urology (SFU).  It is based on the long axis sonographic

**Table 1:** Society of Fetal Urology grading system of fetal hydronephrosis

| Grade | central renal complex | renal parenchymal thickness |
|---|---|---|
| 0 | Intact | Normal |
| 1 | Slight splitting of pelvis | Normal |
| 2 | Evident splitting of pelvis and calyces | Normal |
| 3 | Wide splitting of pelvis and calyces | Normal |
| 4 | Further splitting of pelvis and calyces | Reduced |

Measurements based on the long axis of the kidney

The second and more widely used classification for antenatal hydronephrosis is based on the measurement of the maximum antroposterior diameter of renal pelvis or the renal pelvis diameter (RPD) and the gestational age [7] as shown in table 2:

**Table 2:** grading system of fetal hydronephrosis by renal pelvis diameter (RPD) measurement.

| Gestation (weeks) | RPD (mm) | Grading of hydronephrosis |
|---|---|---|
| 15 to 20 | 4 to 7 | Mild |
|  | > 7 | Moderate |
| > 30 | 5 to 8 | Mild |
|  | 9 to 15 | Moderate |
|  | >15 | severe |

RPD measurement based on the maximum antroposterior diameter of the renal pelvis. However mild renal pelvic dilation shows no clinical impact on normal renal development [4, 11], while moderate and severe renal pelvis dilation associated with increasing risk of significant congenital abnormalities of kidney and urinary tract [12-14]. The controversy exists as to the threshold beyond which the fetal RPD is considered abnormal. Most recent studies suggest that antenatal hydronephrosis exist when RPD exceeds 5mm before 24weeks gestation or when over 7mm beyond 25 weeks of pregnancy[11, 15-17]. It has been suggested that using both methods in combination is superior to using each method alone [18]

## Epidemiology:

The reported incidence of antenatal hydronephrosis is ranged from 0.6-4.5% in different studies [4, 11, 20, 21]. In 20-40% are bilateral [20]. Antenatal hydronephrosis is more common in boys than girls (2:1) [19] . A 5 years cohort study [22] compare the incidence of renal abnormalities from 1999 to 2003 with those

reported previously from 1989 to 1993. They concluded that there was increased incidence of renal abnormalities detected antenatal. The

incidence was 7.6\1000 birth in recent cohort study versus 3\1000 live birth of previous one.

## Causes of antenatal hydronephrosis

Antenatal hydronephrosis may due to non-obstructive or obstructive causes. Non obstructive lesions such as primary vesicoureteric reflux (VUR) and multicystic dysplastic kidney (MCDK). Obstructive lesions particularly bilateral lesions are more harmful to developing kidneys. These include pelviureteric junction obstruction (PUJO), vesicoureteric junction obstruction (VUJO) and posterior urethral valve (PUV). Transient and physiological hydronephrosis is by far the most common form of antenatal hydronephrosis. It is accounted for 30-

50% of cases [12]. A study done at Tripoli Children's Hospital [36] which conducted 90 neonates (125 renal units) had presented with antenatal hydornephrosis from 1995 to 2007. Pelviureteric junction obstruction (PUJO) was found in 25.5% of cases, vesicoureteric reflux (VUR) was found in 14.4%, Posterior Urethral valve (PUV) was found in 17.7%, multicystic dysplastic kidney (MDK) was found in 20% of cases, uretero-vesical junction obstruction (UVJO) was found in 6.6% and transient hydronephrosis found in 15.5% of cases.

## Management of antenatally detected hydronephrosis:

Management of infants with hydronephrosis detected antenatally is a challenge to pediatric nephrologists and urologists. The aim of post natal management of these infants is to

identify those infants with severe congenital anomalies of kidneys and urinary tract while avoiding unnecessary testing in infants with transient dilation.

## Antenatal management

Detailed family history is important to exclude any genetic predisposition. If other anomalies are present amniocentesis for karyotyping should be strongly considered [23]. If RPD exceed 5mm in the second trimester, a repeat fetal US scan in the third trimester is required to assess its progression. If RPD exceed 7mm in the third trimester, a plane for postnatal management of newborn becomes mandatory [11].

Antenatal intervention either by direct and repeated bladder drainge or placement of vesico-amniotic shunt of infant with antenatal detected

hydronephrosis remain controversial and has failed to improve the natural course of congenital urinary tract obstruction. The main causes of failure of this type of management are renal dysplasia and pulmonary hypoplasia which are associated with urinary tract obstruction and are irreversible by the time the urinary dilation is first noticed by antenatal US. The main indication of invasive antenatal management is presence of markers of abnormal renal function. Which include presence of oligohydraminos and poor cortico-medullary differentiation in kidney with increased echogenicity.[8].

It is important to counsel the parent when fetal hydronephrosis is detected in sensitive way including reassurance that the majority will turn out to be transient and benign.

If fetal hydronephrosis persistent in the third trimester a multidisciplinary

approach is needed which include neonatologist, pediatric nephrologists, pediatric urologist and geneticist as necessitated by the underlying condition.

## Post natal management

Clinical examination will take place after birth to ensure that there are no other associated anomalies. If baby is well with no evidence of abdominal mass and passing urine with only unilateral lesion then discharge home should not be delayed. The role of prophylactic antibiotics is still contraversal. Infants with minor postnatal dilation do not need prophylactic antibiotics, as the urinary tract infection is uncommon in infants with two normal postnatal US examinations [24].

A prophylactic antibiotic is given for those neonates who had evidence of obstruction due to posterior urethral valve. Antibacterial prophylaxis is conventionally given to infants with VUR and for the first 6 months of life to infants demonstrating moderate to severe hydronephrosis [14].

Renal US should always be performed in neonates who had persistent hydronephrosis in the third trimester [17, 25]. It should be done after 48 hrs after birth to ensure that the infant is well hydrated and urine flow is established. However, renal US can be done early when there is severe bilateral hydronephrosis or a palpable abdominal mass at birth. The optimal timing of US at around 7 to 10 days of life, applying the same standard grading system to antenatal scan[26].

Most infants with postnatal hydronephrosis undergo voiding cystoureterogram (VCUG) to exclude VUR and bladder outlet obstruction. It should be performed, usually within 4 weeks in majority of cases[27]. However, it must be done within 48 hours of birth in any infant suspected to have posterior urethral valve. Several studies have recently demonstrated that gross degree of VUR can be associated with minimal or no dilation on post natal US [28, 29]. Some controversies still exist regarding the need of VCUG for cases of MCDK, PUJO and UVJO.

The radionuclide imaging is usually delayed until 3 months after birth unless clinically indicated (palpable mass at birth or severe pelvic dilation). A technetium-99m dimarcaptosuccinic acid scan (DMSA) is performed to confirm the non-function kidney and to define the differential function in infants with VUR and MCDK.

99mTc mercaptoacetyletriglycin (MAG3) is radionuclide scan of choice because of its high initial renal uptake to demonstrate the differential function and excretion in infants with hydronephrosis and usually associated with diuretic injection. Alternatively 99m technetium diethyl triamine pentaacetic acid (DTPA) may be used [11].

**Common specific etiology of antenatal hydronephrosis:**

1) **Transient hydronephrosis**: It is the commonest cause of antenatal hydronephrosis **[12].** The majority will resolve spontaneously either in third trimester or in early infancy **[30].** No need for prophylactic antibiotics and no further investigation apart from post natal US is needed.

2) **Uretero-pelvic junction obstruction (UPJO):** is the most common cause of non physiological obstruction. Its prevalence is approximately 1 in 2000 children with a male to female ratio in infancy of 3:1. 20-25% of cases had bilateral obstruction **[12].** Its management depends on the MAG3 renogram, if it is more than 40% a serial follow up by US is recommended while when the differential function is less than 40% with poor excretion surgical reconstruction is recommended **[31]**.

3) **Posterior urethral valve:** It is one of most cause of antenatal hydronephrosis in male infants. It was accounted for 17.7% of cases presented with antenatal hydronephrosis in **[36].** A study done at Tripoli children's hospital, 80 children with Chronic Kidney Disease (CKD) was conducted, from 2001 to 2010. Of the group 42(53%) of cases due to congenital nephropathy , PUV accounted for 52% of cases with late presentation to pediatric nephrologists **[35].** Antenatal intervention is required in severe cases with markers of impaired renal function and severe hydronephrosis by US, the bladder is generally decompressed using a percutaniously placed vesicoamniotic catheter or percutaneous endoscopic in utero ablation of the valve. These intrauterine procedures should be carried out in highly specialized centre. It carries many risks like fetal injury, intrauterine infection and premature labor. The risk of fetal mortality is 43% of cases [32]. After diagnosis is established with postnatal VCUG, a small polyethylene tube is inserted. Foley catheter should not be used because the balloon may cause severe bladder spasm and may produce severe bladder obstruction. Early referral to pediatric urologist is recommended. **Figure 1:-**
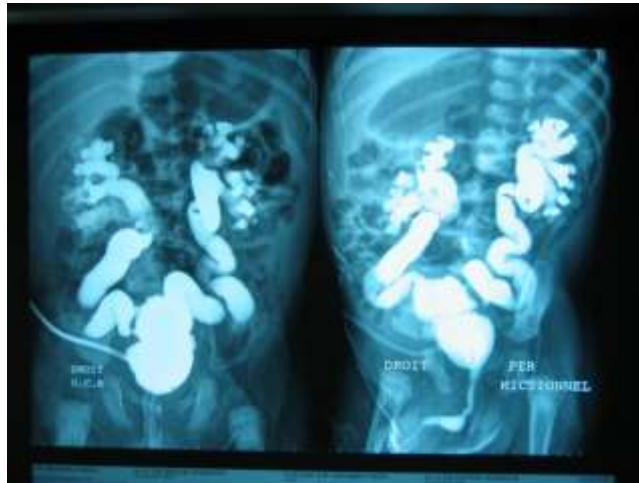
**Figure 1:-**VCUG showing posterior urethral valve with bilateral VUR

4) **Multi cystic dysplastic kidney (MCDK):** It is usually unilateral. Bilateral MCDK is incompatible with life. It is easily recognized by cystic appearance on pre and postnatal US with no function at all on the DMSA scan. Its management is usually conservative in **[33]** approach and they documented progressive involution with time 3% of cases disappear and 33% of cases reduced in size by 2years of age. **Figure 2**



**Figure 2:-Multicystic dysplastic kidney (postmortem and US)**

5) **Vesicoureteric reflux (VUR):** It constitutes between 10 to 38 % of cases of antenatal hydronephrosis **[8]**. When diagnosis is made by postnatal VCUG then the infants require a DMSA scan to define differential renal function and presence of renal scaring. It is predominates in males with high resolution rate 65% within 2 years**[34]**.

6) **Uretero-vesical junction obstruction (UVJO):** It is a rare condition and it is diagnosed when there is a dilated ureter as well as hydronephrosis without VUR on VCUG and it is confirmed by MAG3 renography.

**Postnatal scheme of management of antenatal hydronephrosis as adopted from [33] is shown below:**
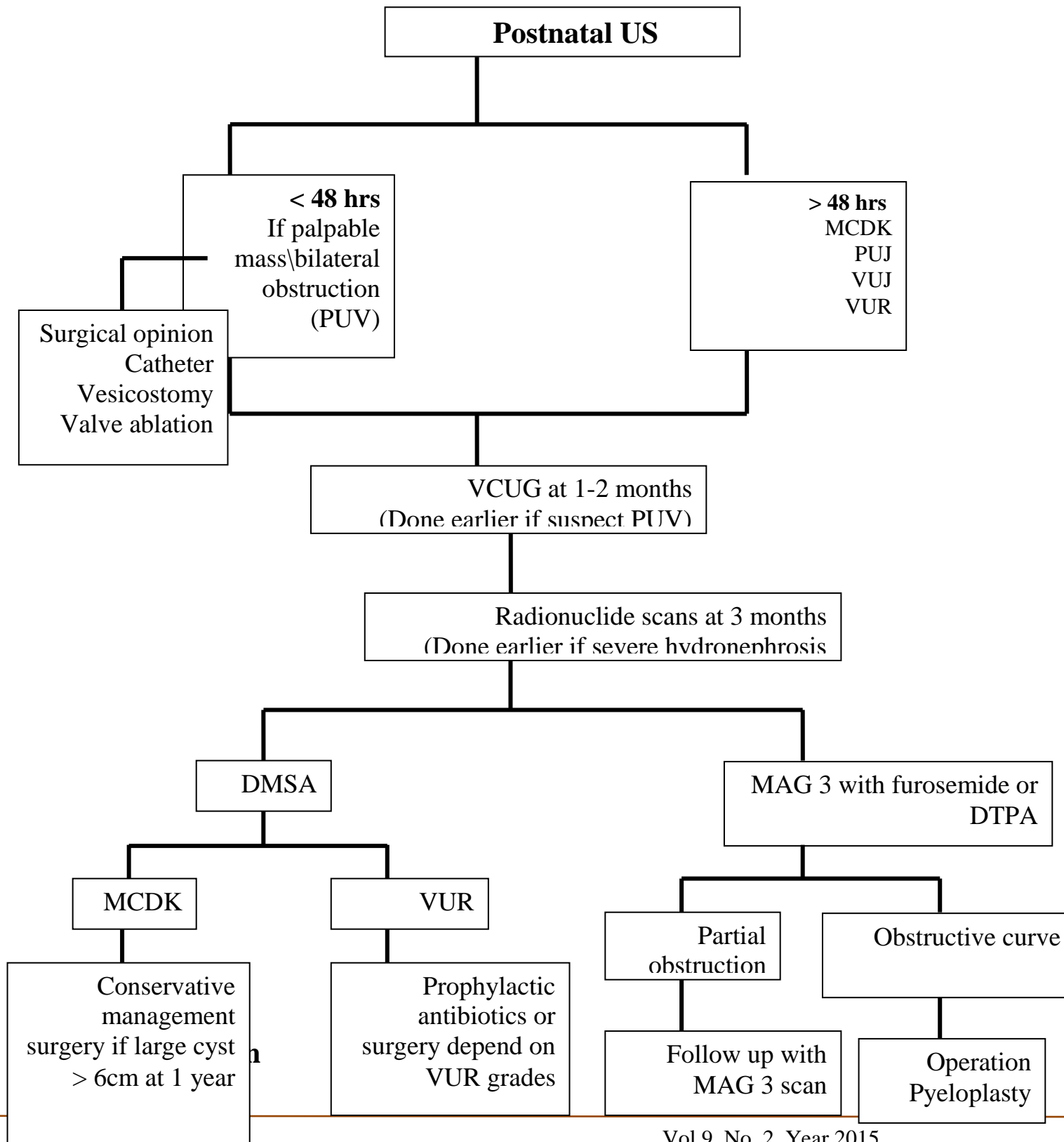
## Conclusion

Antenatal detected hydronephrosis is commonly associated with significant morbidity in early life. It can lead to parental anxiety extending well beyond the current pregnancy. The indication for choice to evaluate an infants with antenatal hydronephrosis should be based on evidence based protocols and guidelines. Multidisplinary approach remains the best way to offer a good care for these children.

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