University of Baghdad

Al Kindy College of Medicine

FIRST INTERNATIONAL SCIENTIFIC CONFERENCE 30-31 OCTOBER 2013

RESRACH DIGEST

Under the Patronage of His Excellency the Minister of Higher Education and Scientific Research

Mr. Ali Mohammed AlHussein Al-Adeeb

And under the supervision of the president of the University of Baghdad

Professor Dr. Alaa AbdulHussein AbdulRasool

Under the slogan

Al-kindy College of Medicine... Confident Steps Toward Global Accreditation

Al-kindy College of Medicine / University of Baghdad holds its international scien c conference on Monday and Tuesday (28 – 29 / October/ 2013)

The Aims of the Conference:

The conference aims to gather the scientists and distinguished minds from the colleges of medicine regarding all specialties both inside and outside Iraq to be aware about the update in the medical education and medical science fields. Besides, to exchange the experiences and encourage scientific research.

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CONFERENCE PROGRAM & Speakers

Day One: Wednesday 30 Oct.2013
Ceremony/Venue: Main Conference Hall

Time	Activity
9.00 am-9.05 am	Opening ceremonies
9.05 am-9.10 am	Qur'an and National Anthem
9.10 am	Alfatihah and a minute of Mourning
9.10 am-9.20 am	Welcoming speech by the Dean
9.20 am-9.30 am	Minister of Higher Education and Scientific Research, speech
9.30am-9.40 am	Chancellor of Baghdad University speech

9.40am-10.00am	A film show of Al-Kindy College of Medicine
10.00am-10.15am	Survey and voting activity
10.15am-10.45 am	Prof. Salman Al-Rawaf: Royal College – UK (Honor Guest) Medical education & researches in the 21st century: International perspective
10.45 am-11.00 am	Prizes
11.00 am-11.30 am	Coffee Break

Session Two: Medical Education and Accreditation/Venue: Main Conference Hall
Chairman: Prof. Thamer Al-Hilfy - Prof. Salman Al- Rawaf

Time	Topic	Speaker
11.30am- 11.50am	Steps towards accreditation	Prof. Dr. Alam Sher Malik Dean faculty of Medicine /Quest International University-Perak /Malaysia
11.50am-12.10pm	Quality Improvement of clinical care	Dr. Yousif Tawfiq/ Consultant / USAID – PHCPI
12.10pm-12.30pm	Quality of learning and education	Prof. Dr Mohamed Saed Dean of Kufa Medical College
12.30pm-12.50pm	Documentation for accreditation process	A/P Dr .Rukhasana Hussain Medical Education Dept /Faculty of Medicine /Quest University/Malaysia
12.50pm-1.10pm	WHO role in supporting medical college accreditation process	Dr. Ali Zu Al Fiqar / WHO
1.10pm-1.30pm	Towards curricular reform and accreditation	A/P Dr Ali Alshaham Alkindy college of medicine
1.30pm-2.00pm	Discussion and Feedback session	
2.00pm	Lunch break	

Day Two: Thursday 31st Oct.2013

Session one (Surgery)/Venue: Main Conference Hall

Chairman: Prof. Dr Mohamed Kamel

Co chairman: A/P Dr Hameed Al-Aarajy

Time	Topic	Speaker
9.00am-9.15 am	New aspects of neurosurgery.	Prof Dr Aron Saroha India
9.15am-9.30 am	New aspects of cardiothoracic surgery.	Prof Dr Magender Sing India
9.30am-9.45 am	Randomized trial of endoscopy with testing for H Pylori compared with non-invasive testing alone in the management of dyspepsia.	Prof Dr Ibtisam Al-shadidi KMC
9.45am-10.00am	Can biliary tract anatomical variation and congenital anomalies be a cause of acute cholecystitis?	Dr Reda Jawad Al-kindy Teaching hospital
10.00am-10.15 am	Bowel cleansing quality in morning versus evening preparation regimens; a prospective study.	A/P Dr Riyad Mohamed KMC
10.15am-10.30 am	Outcome of surgical resection of subcortical metastatic tumors in the central and paracentral region of the brain.	A/P Dr Basam Mohamed KMC
10.30am-11.00am	Discussion and feedback session	
11.00am-11.30 am	Coffee break	

Session two(Basic Science)/Venue : Main Conference Hall

Chairman : Prof. Dr Manal Adnan
Co chairman : A/P Dr Hamodi Al-Sinbili

11.30am-11.45am	Frequency of HLA DRB1 in Iraqi patients with knee osteoarthritis.	Prof Dr Batol Moter
11.45 am-12.00 am	Gallic acid inhibits E coli adhesion to bladder cells in induced urinary tract infection in diabetic rat.	Dr Nidal Mohamed Ali Hawler MC
12.00am-12.15pm	Distribution of HBV genotype among Iraqi patients with HBV infection	Dr Eman Al-obaidi Baghdad Teaching Hospital
12.15pm-12.30pm	Magnesium and calcium content of tap and bottled drinking water in Baghdad city.	Dr Thaar Al-bichari Lecturer /KMC
12.30pm-12.45pm	Tissue microarray based immunohistochemical assessment of apoptotic regulating protein (BCL2 and p53) in fibroadenoma.	Dr Haider Fadel Lecturer/Nahrain MC
12.45 pm-1.00pm	Interleukin -6, tumor necrosis factor-α and high sensitivity C reactive protein in Iraqi patient with fibromyalgia syndrome.	Dr Raghad Naji Lecturer /KMC
1.00pm-1.15 pm	Reno-protective effect of zinc sulfate and silymarin against thallium induced poisoning in rats.	Dr Saad Badi Lecturer/KMC
11.15 pm-12.00pm	Discussion and Feedback session	
12.00pm	Lunch break	

Session one (Medicine &Pediatric) /Venue: Lecture Hall –B

Chairman:Dr Jawad Ibrahem Rashed
Co chairman: Prof Dr Mohmod Daher

Time	Topic	Speaker
9.00 am-9.15 am	Exercise ECG and coronary computed tomography angiogram in Baghdad-Iraq: a preliminary report.	A/P Dr Eman Al-qaser Baghdad MC
9.15 am-9.30 am	Effects of erythropoietin therapy on the biochemical profile in patient with chronic kidney diseases.	A/P Dr Ali Al-Saedi KMC
9.30am-9.45 am	Frequency of Bacteria isolated from blood culture of children at a children hospital in Baghdad and study of their antimicrobial susceptibilities.	Dr Nadhema hmood College of science Mustansirya
9.45 am-10.00 am	Prevalence of microscopic colitis in patients with chronic watery diarrhea.	Dr Nafeh Al-esawi Anbar MC
10.00am-10.15 am	Evalution of serum level of adiponectin, leptin, erythropoietin and some indicators of anemia in diabetic patients.	Dr.Tahreer Itihad KMC
10.15 am-10.45 am	Update in hypertension and ischemic heart disease drugs/MEPHA drug company	

10.45 am-11.00am	Discussion and feedback session
11.00am-11.30 am	Coffee break

Session Two (Gynecology and Community Medicine)/Venue: lecture hall –B

Chairman: Prof Dr Sarmed Khonda

Co chairman: A/P Dr Mohamed Asaad

11.30 am-11.45 am	Relationship between in vivo concentration of anti Mullerian overian hormone-Acivin-A and Follistatin hormones with pregnancy rate following intrauterine insemination.	Prof Dr Saad Al-dijali Nahrain MC
11.45 am-12.00am	Clinical communication skills (CCS) Curriculum development using an outcome approach.	Dr Abdul Salam Sultan MOH
12.00am-12.15 pm	Mental health consequences of war and terrorisms in Iraq: a preliminary report.	A/P Dr Jawad Al-diwan Baghdad Mc
12.15 pm-12.30 pm	Tikrit medical teachers' opinions regarding current school curriculum.	A/P Dr Wafaa Mohmod Kirkuk
12.30pm-12.45 pm	The effect of leptin level in pregnancy complicated by intrauterine growth restriction on the neonatal outcome.	A/P Dr Taghreed Al-haidari KMC
12.45pm-1.00pm	Cutaneous leishmaniasis outbreak at Al Shirkat district January 2011.	Dr Yaser Younis Neinawa MC
1.00pm-1.15pm	In vitro determination of antibacterial properties of garlic extract against multi drug resistant bacteria isolated from women with asymptomatic bacteriuria.	Dr Arej Ateia Diyala MC
1.15pm-2.00pm	Discussion and feedback session	
2.00pm	Lunch Break and Closing Ceremony	

List of Content

Can Biliary Tract Anatomical Variations and Congenital Anomalies Be a Cause of Acute Cholecystitis?

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Abstract

Background: anomalous biliary anatomy is frequently encountered by surgeons during chlecystectomy. importance of its recognition lies in avoiding serious biliary injuries. they are not described as a risk factor for developing acute cholecystitis (ac) in patients with cholelithiasis. risk factors described for the development of acute cholecystitis (ac) include comorbid factors, i.e. diabetes mellitus, history of cardiovascular disease and respiratory disease and history of cerebrovasculer accident (cva); other two demographic factors are male gender and age >60 years. in the present study we try to see if "biliary tract anatomical variations & congenital anomalies" are risk factors for acute cholecystitis.

Objective : To highlight the different Biliary Tract Anatomical Variations & Congenital Anomalies described in the literature with study of 40 cases with anomalous biliary tract associated with Acute Cholecystitis (AC); and to conclude if these anomalies are the cause of AC, so we can add it to other causes of AC.

Methods: This is a prospective study of 40 cases of anomalous biliary tract associated with Acute Cholecystitis (AC); these cases were treated electively by laparoscopic chlecystectomy (LC) after a period of six weeks of conservative treatment for the acute phase. in Al-Kindi Teaching Hospital in the period from March 2011- August 2013.

Results: Thirty four (34) cases were females (85%) while 6 cases were males (15%). Average age was 35 years (range was 28-72 years). Clinical presentation includes mainly pain in right hypochondrium in 30 cases (75%), pain in right hypochondrium and epigastrium in 7 cases (17.5%) and pain in epigastrium alone 3 cases (7.5%) as main symptoms. Acute cholecystitis (AC) was present in 35/40 (87.5%). Anatomical variations were in cystic artery in 22 patients (55%), cystic duct in 10 patients (25%), right hepatic artery in 5 patients (12.5%) and gall bladder in 3 patients (7.5%). Laparoscopic chlecystectomy (LC) was successful in 34 cases (85%), while 4 cases (10%) were by open chlecystectomy (OP) and two cases were conversions (5%).

Conclusion : In this study we found that biliary tract anatomical variations & congenital anomalies can be considered as risk factor for acute cholecystitis in patients with cholelithiasis, in addition to other factors mentioned above.

Keywords :acute cholecystitis (AC), cholelithiasis,biliary tract anatomical variations, biliary tract congenital anomalies, risk factors, laparoscopic chlecystectomy (LC)

Introduction:

Congenital anomalies of the biliary tract may be clinically significant. These congenital anomalies involving the biliary tract include aberrant or accessory biliary ducts, aberrant cystic duct insertion; bile duct cysts, alterations of the biliary tract associated with situs anomalies, and anomalous junction of the common bile duct with the pancreatic duct. Recognition of these entities as anomalies may avoid diagnostic errors, aid in surgical planning, and prevent inadvertent ductal injury [1]. For instance, aberrant or accessory biliary ducts may predispose patients to inadvertent ductal ligation at laparoscopic cholecystectomy and may complicate surgeries [1][2]

The necessity of cholecystectomy for gallbladder stones depends on whether it is symptomatic or symptomless. When patient is complaining of recurrent biliary colic, then operative treatment should be considered [3]. Some of these patients may complain of mild symptoms such as indigestion or intermittent abdominal discomfort to severe form of gallbladder disease such as acute cholecystitis (AC) [4, 5], which required urgent intervention [6-9]. Acute cholecystitis (AC) symptoms are usually more severe than chronic cholecystitis, with increased morbidity, especially in patients with comorbidities [10]. Acute cholecystitis is defined when the patient had 2 or more of the following clinical and operative findings. The clinical factors (4 factors) consist of fever with a body temperature higher than 37.5°C, leukocytosis, right upper abdominal pain with tenderness and continuous symptoms for more than 48-hour duration despite medical treatment. The operative findings (4 factors) included a gallbladder wall thickness of greater than 4 mm, severe adhesion to an adjacent organ, distortion of the biliary anatomy, and gross inflammation of the gallbladder serosa. Complicated cholecystitis was defined if hydrops, empyema, pericholecystic abscess, or gangrene developed [11-13].

Laparoscopic cholecystectomy (LC) has become the standard procedure for gallbladder disease. Nevertheless it may be associated with risk of ductal injury, especially if anatomical variations or congenital anomalous duct system is present [14-15]. Therefore it is recommended that patients who are going to develop AC should be recognized and operated upon before they progress to AC [16].

In the present time the well known risk factors for developing AC are old age(>60 years), male gender, cardiovascular disease (CVD), respiratory disease (RD), diabetes mellitus (DM) and cerebrovascular accident (CVA) [17-19].

Anatomical variations and congenital anomalies of the biliary ductal or vascular system were not mentioned in the medical literature as apredictor for AC.

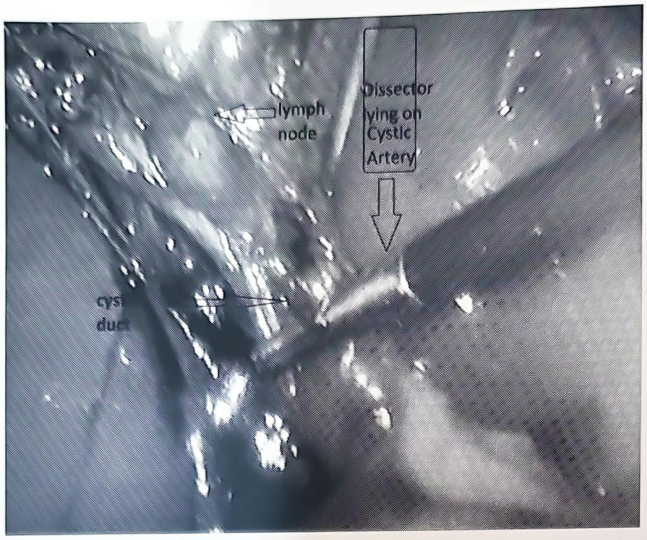

Figure-2 shows cystic artery anomaly.

Figure-3 reveal an anomalous cystic duct, were 2 short branches comes out of gallbladder & unite to form single cystic duct that descend behind lower portion of cystic artery.

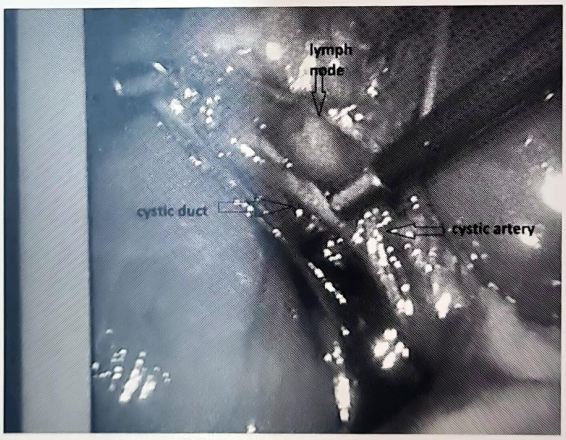

Figure-3: cystic dut anomaly.

Figure-4 shows cystic artery that was anterior to cystic duct in its whole length.

Figure-5 shows a large accessory bile duct that communicates to cystic duct in a patient with AC.

Figure-6 shows a cystic artery that coarse lateral to cystic duct and turn at its upper portion behind & medial to cystic duct.

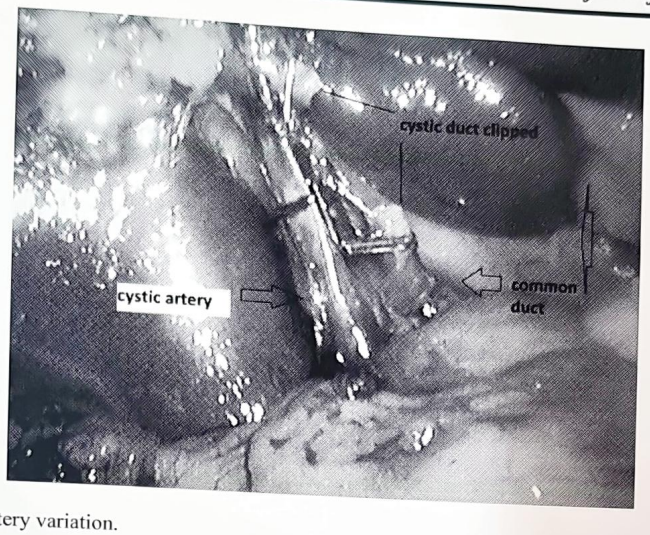

Figure-4: cystic artery variation.

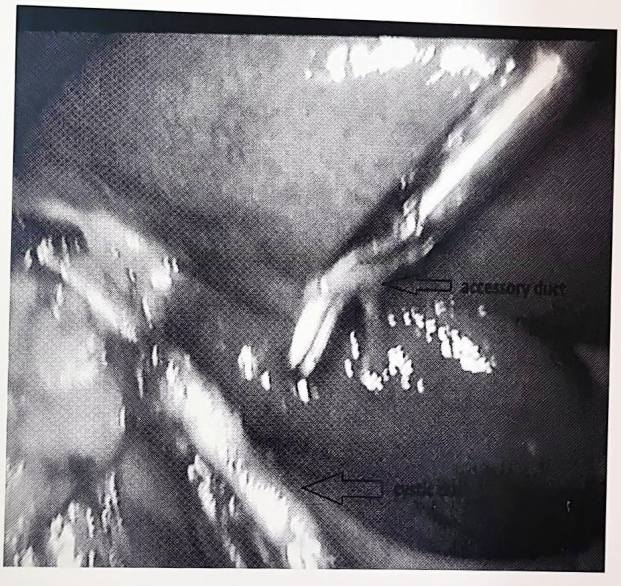

Figure-5 shows accessory bile duct.

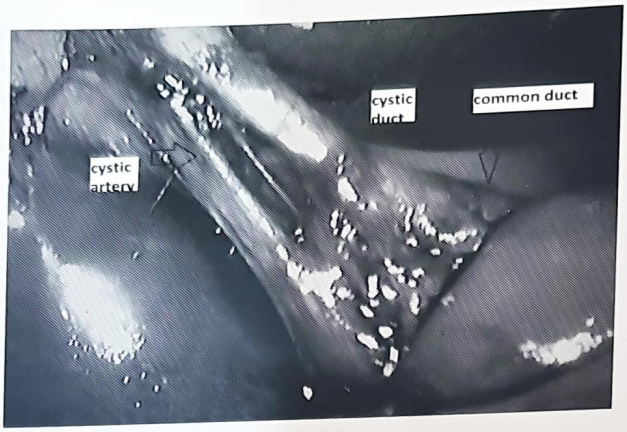

Figure-6 shows anomaly of cystic artery.

Discussion

Acute cholecystitis is a clinical syndrome when 2 or more of the following are present: (1) Fever with a body temperature higher than 37.5°C (2) Leukocytosis (3) Right upper abdominal pain with tenderness (4) continuous symptoms for more than 48-hour duration despite medical treatment (5) Gallbladder wall thickness of greater than 4 mm (6) Severe adhesion to an adjacent organ (7)distortion of the biliary anatomy (8) gross inflammation of the gallbladder serosa (Cho KS 2004)[20]. Complicated cholecystitis is defined if hydrops, empyema, pericholecystic abscess, or gangrene developed(Lee HK *et al* 2009)[11-13].

Many risk factors have been described for developing AC (Giger UF *et al* 2006)[13]. These risk factors include Old Age (Fagan SP *et al* 2003) [21] , Male Gender (Lee HK *et al* 2005)[18] and Comorbidities (such as Cardio-Vascular Disease (CVD), Respiratory Disease (RD), Diabetes Mellitus (DM) and Cerebro-Vascular Accident (CVA)[Hickman MS *et al* 1988] [22].

On the other side of the problemare thecongenital anomalies of the biliary tract which are clinically significant. These congenital anomalies involving the biliary tract include aberrant or accessory biliary ducts, aberrant cystic duct insertion; bile duct cysts, alterations of the biliary tract associated with situs anomalies, and anomalous junction of the common bile duct with the pancreatic duct.

Cholecystectomy should be considered in cases of AC. Options for the timing of cholecystectomy differs. Some prefer to operate after a period of conservative treatment for 4-6 weeks, to allow the acute phase to subside, thus reducing the rate of complications, morbidity and mortality. Others prefer to operate within the first 72 hours before development of adhesions to adjacent organs and distortion of anatomy, the so called "the Golden Period"; claiming that cholecystectomy is easier during this "Golden Period" (Shiptz B *et al* 1995) [23]. We search the internet (Cochran Library, Pubmed, Mediline) using the keywords "Acute Cholecystitis, Laparoscopic Cholecystectomy, Biliary Tract Congenital Anomalies & Anatomical Variations". Congenital anomalies were not mentioned as a risk factor in AC in the literature. We can say that this is the first time that these anomalies shown to be associated with AC. Although the cause is obscured, but it can be due to some sort of ischemic changes caused by vascular variations leading to dysfunctional disturbance. On the other hand the different anomalies of biliary tract lead to obstruction to the flow of the bile and this lead to alteration in nature of the bile and precipitation of its solute and formation of gallstones with consequent complications and development of AC.

Conclusion

Biliovascular Anatomical Variations & Congenital Anomalies should be considered as a risk factor for development of AC in cholelithiasis. So when anticipate which patient will develop AC, we have to take in account this risk factor. Since this factor is not mentioned in the literature we have searched and, to our knowledge, this is the first time to correlate it to development of AC.

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Distribution of Hepatitis B Virus (HBV) Genotypes Among Iraqi Patients With HBV Infection

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Abstract

Background: Hepatitis B virus (HBV), the prototype member of the family hepadnaviridae, is a human pathogen, causing the serious liver disease. The virus is the major cause of chronic hepatitis cirrhosis, and hepatocellular carcinoma worldwide. HBV has been classified into eight genotypes, which vary in geographic distribution.
Objectives: This study was established to assess the prevalence of HBV genotype among Iraqi patients.
Methods: genotyping was attempted on 240 HBV-infected Iraqi patients from different hospitals in the country. These included 155 males and 85 females with mean age 47.0 years . Testing for HBsAg, antibody to HBsAg (HBsAb), HBeAg) and anti-HBe were done using the available commercial kits of the third generation enzyme immunoassay (Foresight-USA). According to the manufacture instructions, a sample was considered positive if the optical density value was equal to or greater than that of a strong positive reaction control multiply by 0.2 (cut-off). The positive samples were re-tested in duplicate
HBV DNA was determined by a sensitive PCR based assay (COBAS Amplicor; Roche Diagnostics) with a lower limit of detection of approximately 200 copies/mL.
Results:. The majority of the patients were genotype D (200 of 240 patients; 89.5%). Two patient (0.8%) had genotype A, and three (1.2%) had genotype C. Seven patients (2.9%) were genotype E, and 13 patients (5.4%) were mixed genotypes (7 patients AD, 3 patients DE, 2 patients DC, 1 patient ACD).
Conclusions: This study highlights that the vast majority of Iraqi patients with chronic hepatitis B have genotype D. No correlation could be observed between the different genotypes and epidemiological or clinical factors. The relationship between genotype D and HBeAg status in terms of disease severity needs to be further elucidated in larger longitudinal studies.
Key words: HBV, Genotype, ELISA,PCR, Iraq.

Introduction

The hepatitis B virus (HBV) is one of the most common chronic pathogens in the world, and high percent of the world's population have been exposed to this virus [1].and part of them have been died due to the consequences of this infection such as cirrhosis and hepatocellular carcinoma(HCC) [2]. HBV is the prototype member of hepadnaviridae family [3] and the *Orthohepadnavirus* genus(3,4). The HBV genome is a partial, double-stranded DNA with four overlapping open reading frames [5,6].

In 1988, HBV was classified into four genotypes, and designated by capital letters of the alphabet from A to D [6]. In 1994 [6,7], an additional two HBV genotypes were found and named E and F, respectively. Genotype G was reported recently in 2000 (8), and genotype H, was proposed in 2002 [6]. Therefore HBV, the hepadnavirus infecting humans, is classified into eight genotypes, A-H, based on an intragroup nucleotide divergence of up to 4.2% of the S-gene sequences or in some cases up to 8.0% of complete genomes [9,10] until now. These genotypes arise during replication as a result of nucleotide misincorporations, in the absence of any proofreading capacity by the viral polymerase [11].

Different HBV genotypes have distinct geographical distributions. Genotype A is found mainly in Northwest Europe, the United States, India, and Sub-Saharan Africa. Genotypes B and C prevail in East Asia, while genotype D is common in the Mediterranean countries. Genotype E is only found in Africa and genotype F is found mainly in Central and South America[8].

To date, the isolation of genotype G has been limited to HBV carriers in France [8], Germany [12], United Kingdom, Italy [13] and the United States of America (USA) [8,13] while genotype H has been reported in patients from Central America [10,11].

Recently, a number of publications have examined the impact of HBV genotype on disease pathogenesis and the clinical outcomes in patients with chronic hepatitis B. Most of these natural history studies, in view of the bimodal distribution of HBV genotypes in Asia and Western countries, have compared either genotypes B and C[11,12], or genotypes A and D[14]. The clinical impact of HBV genotypes when studied in Indian patients with a mixed population of both genotypes A and D, has shown that genotype D has a higher likelihood of developing advanced cirrhosis compared to genotype A[15].

Chronic hepatitis B is an important medical problem in Iraq although, with the implementation of HBV vaccination of children. However, no data are available on prevalence and distribution of HBV genotypes in Iraq. Furthermore, the association between the distinct

genotypes and the severity of liver disease in the country remains unreported, therefore this study were designed to shed light on the frequency and clinical significance of HBV genotypes in Iraqi patients with chronic HBV infection.

Methods

Serum samples:

Patients were recruited prospectively from three hepatology centers, two at Medical City Hospital and the thired one at Al-Khadimya Teaching Hospital in Baghdad in period between May 2008 to July 2011

These medical centers serve as referral centers for population groups resident in different geographical regions of the country. Two hundred fourty consecutive adult HBsAg positive patients were recruited at the three centers. Patients were interviewed in person by participating investigators at recruitment by using a structured questionnaire.

Information on socio-demographic characteristics, alcohol consumption, personal medical and surgical history, time of disease diagnosis, area of birth and upbringing, and family history of liver disease or cancers was collected.

Patients who were HBsAg positive for a period exceeding 6 month and who had not received any antiviral therapy for HBV in the preceding 6 month were included in the study.

General exclusion criteria included: (1) anti-HCV antibody positive;(2) identifiable other causes of chronic liver disease (high serum iron and ferritin, abnormal serum ceruloplasmin, history of significant alcohol consumption, antinuclear antibody > 1:320, antismooth muscle antibody

> 1:320, antimitochondrial antibody > 1:40); (3) history of hepatotoxic medications in the preceding three months of presentation; (4) history of antiviral therapy in the last 6 month.

Blood sample were screened for hepatitis B surface antigen (HBsAg) by ELISA method and retested again. Two hundred and forty HBsAg-positive serum samples, were selected to determine the HBV genotypes. These serum samples were male/female = 88% /12% and mean age =34.6 years. These serum samples were stored at -70°C until using forDNA extraction.

Serological Evaluation

HBV markers [HBsAg and antibody to HBsAg (HBsAb)] ,hepatitis B envelope antigen (HBeAg) and anti-HBe antibody was performed using the commercial kits of the third generation enzyme immunoassay (Foresight-USA) . HBV DNA was determined by a sensitive PCR based assay (COBAS Amplicor; Roche Diagnostics) with a lower limit of detection of approximately 200 copies/mL.

Dna extraction:

The QIAamp DNA extraction kit (QIAGEN GmbH) was used for DNA extraction from serum samples according to the manual. The extracted DNA was used for amplification in the LiPA procedures. LiPA analysis was performed within approximately 5 days following DNA extraction. If DNA extracts were not used immediately, they were stored at -20°C.

Virologic Testing

Genotypic testing was performed in only those with a detectable HBV DNA (qualitative) in serum. HBV genotyping was determined from serum samples by performing nested PCR-mediated amplification of the target sequence and hybridization with sequence-specific oligonucleotides at Bioscientia Laboratory in Germany.

Statistical Analysis

Analysis of data was carried out with the aid of SPSS package version 10.0 software (Chicago, Illions, USA) Parameters were compared using the Chi-square test. *P* values less than 0.05 were considered statistically significant.

Results:

Mean age of the patients was 47 years, and 155 patients (64.5%) were male while 85 (36.5%) were female.

ble 1 Biochemical, hematological and

Table 1: Baseline characteristics of study population.

Parameter	Value
Total no. of patients	240
Age (years)	47.0 (8–67)
Gender (Male: Female)	155 : 85
Serum ALT (U/L)	38 (28-265)
Serum AST (U/L)	41 (20-233)
HBeAg Positive	41
Negative	199
Serum HBV DNA levels (copies/mL)	$2x10_6$ ($1.5x10_5$ to $>1.8x10_8$)
Histological activity index (excluding fibrosis)	6 (3–13)

The majority (185 of 240 patients, 75%) had acquired HBV through unknown risk factors, while 25 patients (10.4%) reported blood transfusion, 20 (8.3%) reported aprior history of surgery or dental procedures and 10 (4.1%) reported a family history of HBV infection.

Amongst the patients who were HBeAg positive, only ten patients (24.3%) had cirrhosis. The vast majority of HBeAg positive patients were either carriers with normal ALT (18 of 31 patients, 58.0%), or raised ALT (13 of 31 patients, 41.9%). five patient expressed HBeAg as well as anti-HBe.

Figure-1 shows the genotyping results. The majority of the patients were genotype D (200 of 240 patients; 89.5%). Two patient (0.8%) had genotype A, and three (1.2%) had genotype C. Seven patients (2.9%) were genotype E, and 13 patients (5.4%) were mixed genotypes (7 patients AD, 3 patients DE, 2 patients DC, 1 patient ACD).

Figure -1: The distribution of genotypes among HBV patient

Table-2 depicts the patients with genotype D and E according to disease severity. Patients with genotype E were older (mean age 61.5 years), tended to have higher levels of ALT, AST, and These differences were significant between the groups.

Table 2: Comparison of patients with genotype E and genotype D hepatitis B virus infection.

Parameter	Genotype E (n=7)	Genotype D (n=200)	P value
Age (years)	30.2 (10-67)	41.5(8-67)	0.05
Male: female	5:2	142:58	0.05
Abnormal serum ALT	5/7	35/200	0.003
Abnormal serum AST	6/7	35/200	0.007
HBeAg positive	6	39	0.001
HBV DNA levels	$5.8x10_7$	$1.1x10_6$	0.001
Histological activity index*	7 (4-13)	4 (3-12)	0.054

There is no geographical difference in hepatitis B genotype among different governorate, table-3.

Table-3: Distribu on of HBV genotypes among di erent governorate of Iraq

Governorate	Type of Genotype					Total no. of patients in each governorate
	A	C	D	E	Mixed	
Baghdad	1	1	90	2	4	98
Basra	0	0	38	0	1	39
The-Qar	0	1	17	1	1	20
Diala	1	0	16	0	1	18
Al-Anbar	0	1	22	1	1	25
Salah Al-Din	0	0	21	0	1	22
Kurdistan	0	0	6	2	3	11
Karkuk	0	0	5	1	1	7
Total	2	3	215	7	13	240

Discussion

Hepatitis B virus (HBV) infection is a global health problem with a continuously increasing burden in developing countries like Iraq. Even with the introduction of universal vaccination of all Iraqi children, the burden of decompensated liver disease secondary to hepatitis B is expected to increase significantly.

Because HBV is an etiologic agent of acute and chronic disease throughout the world [30], and also its genotypes might influence mutation patterns in precore and core promoter regions, severity and activity of liver disease,

patterns of serological reactivity, replication of the virus, prognosis and response to antiviral treatment (16), detection of HBV genotypes is very important to clarify the pathogenesis, route of infection and virulence of the virus.

genotype D was reported as the predominant HBV genotype in the study subjects (17).This study also concurs with previous studies in other countries , indicating that HBV genotype D prevails in the Mediterranean area, near and Middle East (18,19,20). A similar study performed in Syria showed that 97% of the studied patients were of genotype D, and 72% were HBeAg negative (21). Moreover, study in Turkey revealed that all 44 patients studied had genotype D (22). Another study in Yemen demonstrated that genotype D was the dominant genotype in a settled population, while genotype A was found only in communities with continuing African links (23). In addition, two studies in Iran revealed genotype D was the most prevalent HBV genotype (24,25).

There is no geographical difference in hepatitis B genotype among different governorate when compared with the number of patients from each governorate.

The clinical impact of HBV genotype D has been studied less extensively. However, initial

studies have found that it may be associated with lower rates of sustained remission and HBsAg

clearance and more severe liver disease compared with genotype A (26). Emerging evidence suggests

that patients with genotype D infection may develop fulminant hepatitis with high frequency (27).

A study from Syria and India indicated that genotype D is more often associated with HBeAg negative chronic hepatitis B (CHB), more severe diseases and may predict the occurrence of HCC in young patients (22, 28, 29). Several studies have reported lower response rates to interferon and peglated interferon-á therapy in patients with genotypes C and D than in those infected with genotypes B and A (30). Evidence suggests that the emergence of lamivudine resistance develop later and less frequent in patients with genotype D infection than in those with genotype A infection (30,31). However, our study reveled that patients with group E were older (mean age=30.2), tended to have higher levels of ALT, AST, higher HBV DNA levels and a tendency towards higher grades of histopathological liver injury.

Several studies reported a correlation between HBV genotype and HBeAg clearance(32,33,34,35), Similarly in our study, we found a lower prevalence of HBeAg among our patients with genotype D (18.1%), suggesting that HBeAg clearance occurred at higher rates among patients with genotype D.

the association between HBeAg status and genotype D, in terms of severity of liver disease needs to be studied further before any additional conclusions can be derived.

Mixed infection with two different HBV genotypes has been known since typing was done

serologically (36,37). Mixed infection was accompanied by acute exacerbation of the chronic

disease (31), and may be provoked by population migration (15,39). However in this study, In view of their small number, we could not study the clinical implications of such coinfection. Such cases could arise either from superinfection with a second HBV genotype on top of one genotype or from simultaneous infection with both genotypes.

We therefore suggest that HBV genotyping become a routine exercise in clinical medicine and

molecular epidemiology. As genotypes have different biological and epidemiological behavior, their

detection and monitoring is more than just academic but also medically significant. Continued efforts for understanding HBV genotypes through international co-operation will reveal further virological differences of the genotypes and their clinical relevance Furthermore, efforts to prevent mixed infections (super-infection or co-infections) in patients with chronic hepatitis B should not be overlooked, especially in areas endemic for HBV infection.

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Effects of Erythropoietin Therapy on the Biochemical Profile in Patients with Chronic Kidney Disease – A Single Center Study

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Abstract

Introduction: Chronic kidney disease (CKD) is a chronic and progressive loss in renal function over a period of months or years. Many biochemical parameters need to be monitored on an ongoing basis to assess clinical and biochemical status of CKD patients.

Methods: Two hundred Iraqi patients who were diagnosed as having CKD were studied from June 2011 to January 2013 in Al-Kindy teaching hospital. They were given erythropoietin (EPO) for seven months. One hundred sixty patients (80%) showed response to treatment with an improvement in haemoglobin (Hb) g/dl, haematocrit (Hct) % [recombinant erythropoietin (rhEPO) responders] and biochemical profile including blood urea, creatinine, serum sodium and potassium, total serum protein and albumin along with total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C), while the other 40 patients (20%) showed no significant response to treatment.

Results: In the rhEPO responders results showed increase in Hb (g/dl), haematocrit (Hct) (%), HDL-C (mg/dl), total protein (g/dl) and albumin (g/dl) with decrease in TC (mg/dl), TG (mg/dl), creatinine (mg/dl), urea (mg/dl), potassium (meq/l) and sodium (meq/l) levels during the period of EPO therapy. While in the rhEPO non-responders results showed decrease in Hb (g/dl),Hct (%) levels, total protein (g/dl) and albumin (g/dl)with increase in creatinine (mg/dl), urea (mg/dl), potassium (meq/l) and sodium (meq/l) levels. There was no change in TC (mg/dl), TG (mg/dl) and HDL-C (mg/dl)levels during the period of EPO therapy.

Conclusion: treatment with erythropoietin seems to positively influence the biochemical profile in patients with CKD.

Introduction:

Chronic kidney disease (CKD) is a chronic and progressive loss in renal function over a period of months or years. The symptoms of worsening kidney function are unspecific and might include feeling of generally unwell and experiencing a reduced appetite [1].Many biochemical parameters need to be monitored on an ongoing basis to assess clinical and biochemical status of CKD patients [2]. The informed use of biochemical tests plays an important part in the investigation of renal integrity and function, although many other types of investigations are also used in the investigation and management of patients with renal disease [3]. Studies attempting to investigate whether EPO treatment has an effect on patients biochemical profiles including blood urea, creatinine, serum sodium and potassium , total serum protein and albumin along with total cholesterol(TC), triglyceride(TG) and high density lipoprotein cholesterol (HDL-C)[4-7].

Methods:

From June 2011 to January 2013, a total of 200 patients (100 males &100 females) were studied. Those patients were seen in the department of nephrology in Al-Kindy Teaching Hospital, Baghdad / Iraq. The age ranged from 45-75 years, all patients were diagnosed as having renal anaemia from history, clinical examination and laboratory investigations including biochemical profile TC, TG, HDL-C, creatinine, urea, total protein, albumin, sodium and potassium . Patients were given epoetin alfa(4000 IU), subcutaneously(SC) once, twice or three times a week, depending on the degree of anaemia. All patients were not on dialysis. Fifteen ml of blood sample has been collected from the big veins in the ante-cubital fossa of each patient.

Results:

In this study200 CKD patients with renal anaemia were given EPO treatment(4000 IU) SC. Patients depending on the response to EPO therapy were classified into two groups, rhEPO responders 160 patients(80%) and rhEPO non-responders 40 patients(20%). EPO dose(4000 IU syringe SC).

In the rhEPO responders results showed increase in Hb (g/dl), haematocrit (Hct) (%), HDL-C (mg/dl), total protein (g/dl) and albumin (g/dl) with decrease in TC (mg/dl), TG (mg/dl), creatinine (mg/dl), urea (mg/dl), potassium (meq/l) and sodium (meq/l)levels during the period of EPO therapy.

In the rhEPO non-responders results showed decrease in Hb (g/dl),Hct (%) levels, total protein (g/dl) and albumin (g/dl)with increase in creatinine (mg/dl), urea (mg/dl), potassium (meq/l) and sodium (meq/l) levels. There was no change in TC (mg/dl), TG (mg/dl) and HDL-C (mg/dl)levels during the period of EPO therapy table (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11), respectively

Table (1): Comparison of haemoglobin between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	14.00 ± 0.88 BC	8.38 ± 1.43 A	8.09 ± 1.68 A

M2	14.00 ± 0.88 BC	9.30 ± 1.11 A	9.04 ± 1.35 A
M3	14.00 ± 0.88 BC	9.85 ± 1.06 AC	9.05 ± 1.36 AB
M4	14.00 ± 0.88 BC	10.45 ± 1.04 AC	9.43 ± 1.67 AB
M5	14.00 ± 0.88 BC	11.05 ± 0.99 AC	8.63 ± 1.65 AB
M6	14.00 ± 0.88 BC	11.66 ± 0.97 AC	7.89 1.43 AB
M7	14.00 ± 0.88 BC	12.31 ± 1.02 AC	6.76 ± 1.27 AB

A: Significant at level P < 0.05 / B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (2): Comparison of haematocrit between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	42.94 ± 2.26 BC	27.17 ± 4.31 A	26.07 ± 4.36 A
M2	42.94 ± 2.26 BC	29.96 ± 3.36 AC	28.68 ± 3.91 AB
M3	42.94 ± 2.26 BC	31.61 ± 3.16 AC	29.25 ± 3.86 AB
M4	42.94 ± 2.26 BC	33.46 ± 3.12 AC	30.38 ± 4.76 AB
M5	42.94 ± 2.26 BC	35.21 ± 2.97 AC	27.95 ± 4.98 AB
M6	42.94 ± 2.26 BC	37.07 ± 2.93 AC	25.52 ± 4.12 AB
M7	42.94 ± 2.26 BC	38.94 ± 3.07 AC	22.83 ± 3.84 AB

A: Significant at level P < 0.05 / B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (3): Comparison of cholesterol between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	174.46 ± 9.36 BC	212.06 ± 34.68 A	219.18 ± 37.89 A
M2	174.46 ± 9.36 BC	200.24 ± 32.51 A	218.15 ± 37.83 A
M3	174.46 ± 9.36 BC	194.48 ± 31.24 AC	218.78 ± 38.55 AB
M4	174.46 ± 9.36 BC	189.83 ± 29.91 AC	217.5 ± 41.90 AB
M5	174.46 ± 9.36 C	185.42 ± 27.90 C	216.38 ± 46.29 AB
M6	174.46 ± 9.36 C	180.57 ± 26.18 C	213.65 ± 51.08 AB
M7	174.46 ± 9.36 C	176.79 ± 24.28 C	210.53 ± 60.64 AB

A: Significant at level P < 0.05 /B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (4): Comparison of triglyceride between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	111.85 ± 12.55 BC	166.09 ± 12.06 AC	159.72 ± 12.73 AB
M2	111.85 ± 12.55 BC	163.12 ± 11.53 AC	159.04 ± 12.69 AB
M3	111.85 ± 12.55 BC	161.32 ± 11.17 A	159.04 ± 12.64 A
M4	111.85 ± 12.55 BC	159.09 ± 11.13 A	158.81 ± 13.93 A
M5	111.85 ± 12.55 BC	156.59 ± 11.37 AC	158.07 ± 15.04 AB
M6	111.85 ± 12.55 BC	153.86 ± 11.37 AC	158.16 ± 15.71 AB
M7	111.85 ± 12.55 BC	150.61 ± 11.30 AC	157.31 ± 17.70 AB

A: Significant at level P < 0.05 /B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (5): Comparison of HDL between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	52.42 ± 4.67 BC	34.83 ± 5.25 A	35.96 ± 1.12 A
M2	52.42 ± 4.67 BC	36.74 ± 2.65 A	36.01 ± 1.29 A
M3	52.42 ± 4.67 BC	37.99 ± 3.02 A	36.15 ± 1.68 A
M4	52.42 ± 4.67 BC	39.53 ± 3.34 AC	36.32 ± 2.20 AB
M5	52.42 ± 4.67 BC	40.58 ± 4.23 AC	36.49 ± 1.98 AB
M6	52.42 ± 4.67 BC	41.77 ± 5.36 AC	36.2 ± 1.87 AB
M7	52.42 ± 4.67 BC	43.20 ± 6.17 AC	35.81 ± 2.96 AB

A: Significant at level P < 0.05 /B:Signficant at level P < 0.05 / C: Significant at level P < 0.05.

Table (6): Comparison of creatinine between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	0.90 ± 0.09 BC	3.84 ± 1.89 AC	4.41 ± 2.11 AB
M2	0.90 ± 0.09 BC	3.62 ± 1.79 AC	4.30 ± 2.04 AB
M3	0.90 ± 0.09 BC	3.43 ± 1.78 AC	4.48 ± 1.94 AB
M4	0.90 ± 0.09 BC	3.22 ± 1.74 AC	4.69 ± 1.89 AB
M5	0.90 ± 0.09	3.00 ± 1.71	4.97 ± 1.99

	BC	AC	AB
M3	4.36 ± 0.18 BC	3.61 ± 0.27 AC	3.35 ± 0.18 AB
M4	4.36 ± 0.18 BC	3.70 ± 0.23 AC	3.43 ± 0.21 AB
M5	4.36 ± 0.18 BC	3.80 ± 0.22 AC	3.36 ± 0.21 AB
M6	4.36 ± 0.18 BC	3.89 ± 0.22 AC	3.12 ± 0.19 AB
M7	4.36 ± 0.18 BC	4.02 ± 0.27 AC	2.86 ± 0.21 AB

A: Significant at level P < 0.05 / B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (10): Comparison of K between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	4.15 ± 0.25 BC	4.82 ± 0.43 A	4.74 ± 0.33 A
M2	4.15 ± 0.25 BC	4.76 ± 0.44 A	4.65 ± 0.35 A
M3	4.15 ± 0.25 BC	4.62 ± 0.43 A	4.57 ± 0.39 A
M4	4.15 ± 0.25 BC	4.51 ± 0.43 A	4.59 ± 0.40 A
M5	4.15 ± 0.25 BC	4.43 ± 0.40 AC	4.64 ± 0.41 AB
M6	4.15 ± 0.25 BC	4.34 ± 0.37 AC	4.68 ± 0.39 AB
M7	4.15 ± 0.25 C	4.31 ± 0.54 C	4.79 ± 0.40 AB

A: Significant at level P < 0.05 / B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Table (11): Comparison of Na between controls and renal anaemia patients

Groups / Months	Control (No:26) Mean ± S.D.	rhEPO responders (No:160) Mean ± S.D.	rhEPO non-responders (No:40) Mean ± S.D.
M1	140.0 ± 1.67 BC	141.24 ± 1.56 AC	142.08 ± 2.69 AB
M2	140.0 ± 1.67 BC	141.04 ± 1.33 AC	141.99 ± 2.25 AB
M3	140.0 ± 1.67 BC	141.13 ± 1.37 AC	142.52 ± 2.62 AB
M4	140.0 ± 1.67 BC	140.71 ± 1.39 AC	142.8 ± 2.79 AB
M5	140.0 ± 1.67 BC	140.39 ± 1.56 AC	143.54 ± 2.76 AB
M6	140.0 ± 1.67 BC	139.83 ± 1.75 AC	144.3 ± 2.87 AB
M7	140.0 ± 1.67 BC	139.73 ± 2.10 AC	144.64 ± 2.70 AB

A: Significant at level P < 0.05 / B:Significant at level P < 0.05 / C: Significant at level P < 0.05.

Discussion

In our study, lipid profile was organized in rhEPO responders than rhEPO non – responders patients, also lipid level is positively associated with improvement of anaemia in CKD patients. This is compatible with kes *et al.* [8], Ponnudhali & Nagarajan[9], Nissenson & Fine [10], Naini *et al.* [11], Papavasiliou *et al.*[12], Siamopoulos *et al.*[13] and Tselepis and Siamopoulos[14] who showed that EPO therapy is associated with an improvement in the blood lipid profile especially long – term treatment with EPO. However, this effect is influenced significantly by food intake. In CKD, epoetin alfa improves quality of life, physical activity and increased tissue oxygenation that lead to an increase in activity of several enzymes and transferring proteins involved in lipid biogenesis as well as in HDL maturation and lead to the increase in HDL-C levels. [14,15].

In the present study, creatinine and urea were lower in rhEpo responders patients than rhEpo non-responders patients also creatinine and urea are positively associated with improvement of anaemia in CKD patients. This finding is in agreement with results of other studies. Kuriyama *et al.* [16], Gouva *et al.* [17], klahr [18] and Fougue and Lavill[19] who suggested that regulation of diet and reduced urea level may prevent the natural progression of CKD towards end stage renal disease thus delaying the start of maintenance dialysis treatment. Cao *et al.*[20] Show that, rhEpo therapy increased significantly Hb and decreased apparently creatinine, urea and inflammatory factors.

In the present study, total protein and albumin were higher in rhEpo responder than rhEpo non-responder patients and it are positively correlated with improvement of anaemia in CKD patients as had been demonstrated in other studies Hosokawa and Yoshide[21], Aki and Atasever [22], Chen *et al.*[23] and Lopez-Gomez *et al.*[24] which concluded that rhEpo therapy is very effective for the increase of total protein, albumin and other elements levels in patients undergoing chronic haemodialysis.

The results showed a decrease in the level of total protein and albumin in rhEpo non-responder patients. This is due to the severity of renal impairment, low protein diet and disorders to metabolize proteins in CKD[25-29].

In this study, potassium was slightly lower in rhEpo non – responder patients. However we showed that patients had higher average K levels when their renal function became worse. This is in agreement with Hsieh *et al.*[30]and Owiredu *et al.*[31] who concluded that, serum K level increases in correlation with the decline in the glomerular filtration rate (GFR) in the late stages of CKD. Also male gender, diabetes mellitus and anaemia might be risk factors for higher K level in CKD patients. The variation in the serum K level became wider as renal failure progressed.

In this study, Na was lower in rhEpo responders than rhEpo non-responder patients. Sodium level remains within normal limits throughout improvement and non-improvement of anaemia in CKD patients until landing renal function to about 5-10% of normal function , this is in agreement with other researches Bunke *et al.*[32], Kavukcu *et al.*[33], Owiredu *et al.*[31] and Mahaldar[34] concluded that EPO decreases urine sodium excretion after a sodium load in normal human subjects without altering GFR, blood pressure or plasma renin activity. While, Alcazar[35] showed that in CKD, fractional excretion of sodium increases so that absolute sodium excretion is not modified until GFR below 15 ml/min.

In the kidney, more than 75% of oxygen consumption occurs in a direct relationship with sodium reabsorption. Thus sodium reabsorption itself depends on renal blood flow and GFR that is, the greater the renal blood flow, the greater the GFR and Na reabsorption[36].

Our results, showed increase in electrolyte level which is associated with rhEpo non-responder patients, this may be attributed to the high level of electrolyte in food and worsening of kidney function as has been shown in other studies Morimote *et al.*[37], Sanders *et al.*[38], Reynolds *et al.*[39] and Fink[40] which showed that a diet high in electrolyte increases the risk of hypertension, kidney and cardiovascular disease in people.

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Evaluation of Serum Levels of Adiponectin, Leptin, Erythropoietin and Some Indicators of Anemia in diabetic patients

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Abstract

Background: Diabetic patients with anemia may be at increased risk of adverse outcomes from diabetic retinopathy, nephropathy, neuropathy, and cardiovascular disease. The etiology of anemia in diabetes is multifactorial and includes inflammation, nutritional deficiencies, concomitant autoimmune diseases, drugs, and hormonal changes in addition to kidney disease. Anemia that is associated with erythropoietin (EPO) deficiency may have prognostic significance for persons with nephropathy or heart failure. Adiponectin (ADPN) and leptin are considered to be primary adipocytokines that were found to have a vital role in diabetes.

Objective :Evaluation of serum levels of EPO and some adipocytokines like adiponectin, leptin, and some indicators of anemia. And to elucidate any correlation between these parameters and anemia in diabetic patients.

Methods: The studied group was consisted of (30) diabetic patients without overt complications precisely kidney disease as group2 (G2). This group is compared with (30) healthy controls as group1 (G1). Blood tests included Fasting serum glucose (FSG), glycosylated hemoglobin (A_{1C}), insulin, hemoglobin (Hb), Hematocrit (Hct) ,iron , total iron binding capacity (TIBC) ,Transferrin Saturation percentage (TSAT %), ferritin, EPO , ADPN, and leptin.

Results: Anemia was diagnosed in diabetic patients (G2) by Hb levels that were lower than healthy controls ($P< 0.05$). Iron levels were elevated in both types of diabetes in G2 compared to G1 ($P< 0.05$). ADPN and EPO levels were elevated in diabetic patients of G2 as compared to G1 ($P< 0.05$). While leptin levels were reduced in type1diabetic patients of G2 compared to healthy controls G1 ($P< 0.001$) and type 2 diabetes of G2 ($P<0.05$). There were significant negative correlations between (FSG and leptin), (Hct and leptin), and (ferritin and leptin).

Conclusion: Diabetic patients in this study were found to have a degree of anemia even in the absence of overt complications and kidney disease. The presence of anemia in diabetic patients in the absence of nephropathy, may explain the increase in EPO production and increased ADPN levels in response to low levels of Hb. There was a negative correlation between leptin and Hct in diabetic patients this correlation may add evidence that leptin can be reduced in case of adequate response of EPO. Subtle disturbance of iron metabolism were found in diabetic patients demonstrated that elevated iron indices are found in patients with diabetes.

Keywords: Diabetes, Anemia, Adiponectin, Leptin, Erythropoietin, Iron, Ferritin, Hemoglobin.

Introduction:

Diabetic patients with anemia may be at increased risk of adverse outcomes from diabetic retinopathy, nephropathy, neuropathy, and cardiovascular disease. The etiology of anemia in diabetes is multifactorial and includes inflammation, nutritional deficiencies, concomitant autoimmune diseases, drugs, and hormonal changes in addition to kidney disease. Anemia that is associated with erythropoietin deficiency may have prognostic significance for persons with nephropathy or heart failure [1].Whether or not anemia is a marker or mediator of adverse outcome still remains to be completely resolved. Treatment of anemia in diabetes has quality of life benefits and reduces transfusion requirements [2].

Many factors have been suggested as the reason for the earlier onset of anemia in patients with diabetes, including severe symptomatic autonomic neuropathy, causing efferent sympathetic denervation of the kidney and loss of appropriate erythropoietin (EPO) production; damage to the renal interstitium; systemic inflammation; and inhibition of EPO release. It has also been shown that a normochromic, normocytic anemia can occur before evidence of renal impairment is present [3].

During hematopoiesis, there are many growth factors, which stimulate the proliferation and maturation of erythroid progenitors, the main one is EPO which act in concert with other growth factors [4]. The obesity (*ob*) gene protein known as leptin (from Greek word leptos "thin") is one of the most important adipose derived hormones that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism [5]. It was suggested that leptincould be involved in the early stages of erythropoiesis and stimulate hematopoietic stem cells *in vitro*.The proliferative effects of leptin on hematopoietic stem cells suggesting a role for leptin in erythropoiesis [6].

There is growing interest in adipose tissue as an endocrine organ. In addition to leptin adipocytes secretes a variety of biologically active adipocytokines like adiponectin (ADPN). It exerts anti-diabetic, Anti-atherogenic and anti-inflammatory roles and involved in the regulation of insulin action and lipid metabolism [7]. Adiponectin has a potential protective role for the cardiovascular system. Plasma ADPN levels were decreased and were negatively related to the severity of cardiovascular injury in patients with coronary artery diseases and with type2 diabetes [8]. Previous Studies suggest that certain cytokines might have negative effects on normal hematopoietic cells; for example, the elevations of pro-inflammatory cytokine tumor necrosis factor a (TNFa) and ADPN in certain leukemias and depression of normal bone marrow hematopoiesis [9].

In the past, complications of diabetes such as hypertension, heart disease, polyneuropathy and foot problems were so devastating that anemia paled in comparison. Furthermore since late complications of diabetes are ischemic in nature; anemia may play an important part in the onset of these complications [10].

The purpose of the present study is to evaluate serum levels of EPO and some adipocytokines like ADPN and Leptin and some indicators of anemia in a group of healthy individuals and diabetic patients in the absence of overt complications.Evaluate Iron status in these groups. And to elucidate any correlation between these parameters and anemia in diabetic patients.

Methods:

This study was conducted at the Department of Physiological Chemistry, Baghdad College of Medicine and the Department of Biochemistry, Al-kindy College of Medicine, Baghdad University, in corporation with the Teaching Laboratories in the Medical City, and the National Diabetes Center, Al-Mustansiriya University.

A total of (60) subjects were enrolled in this study, (30) subjects (asgroup1 or G1) comprised the healthy controls were selected from Al-kindy College of Medicine. Group2 (G2) represent (30) diabetic patients were selected from the National diabetes Center, Al- Mustansiriya University.Group2 were also segregated according to the type of diabetes and divided into (9) type1 and (21) type2 diabetes, (G2T1 and G2T2). Patients with overt complications or kidney diseases or smokers were all excluded. Male to female ratio was (15/15) in both group1 and group2.

Glycosylated hemoglobin (A_{1C}) was measured by variant hemoglobin program utilize the principle of ion exchange high performance liquid chromatography (HPLC). Fasting serum glucose was measured by enzymatic methods using kits supplied by (Biomagreb, Tunisia). Iron and Total iron binding capacity (TIBC) were performed using kits supplied by (Human, Germany). Serum transferrin saturation (TSAT %) can becalculated using the following equation [11] :

$$[\text{TSAT } \% = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100] .$$

Hemoglobin (Hb) was determined by conversion of Hb into cyannohemoglobin using drabkin reagent kit (Crescent Diagnostics, Saudi Arabia). Hb was recorded for estimating the risk of anemia. Anemia was defined as (Hb≤ 11g/dl, irrespective of sex) according to the guidelines of the World Health Organization (WHO) [12]. Estimation of hematocrit (Hct) was done according to centrifugation method [11].

Serum Ferritin, EPO, leptin and insulin concentrations were measured using a commercially available ELIZA kits (DRG, International, Inc. USA). Each kit utilizes a principle based on polyclonal antibody to recombinant human Ferritin, EPO, ADPN, leptin and insulin respectively.

Statistical methods:

Statistical analysis was performed using SPSS for Windows version (12.0). Data were expressed as Mean ± Standard Deviation (X±SD). The least significant difference (LSD) method was used to compare individual groups and for comparison among type1, type2 diabetic patients and control subjects. Correlation coefficients were measured using Pearson's correlation coefficient. The criteria for statistical significance were determined at P-value less than (0.05).

Results:

Table (1) represents the clinical characteristics of the healthy controls and diabetic patients. There were no significant differences in age and BMI means of diabetic patients (G2) as compared to healthy subjects(G1), (P> 0.05). Mean duration of diabetes was (12.03±7.0, years). Table (2) shows the glycemic profile of the study groups. Anemia was diagnosed in diabetic patients by hemoglobin and hematocrit levels. Hb levels were lower than healthy controls (six patients of G2 were diagnosed to have anemia, Hb ≤ 11g/dl, table (3)). There were no statistical differences in the levels of Hct in the in G2 as compared to G1. Although G2T2 were insignificantly higher in hematocrit level as compared to G2T1 and G1 respectively, (P> 0.05), table (3).

Table (4) representsiron Status in the study groups. Iron levels were elevated in both types of diabetes in G2 compared to G1 (P< 0.05), while mean levels of TIBC in both type1 and type2 diabetic patients of G2 were reduced as compared to G1 (P<0.05).Type2 diabetic patients of G2 had elevated levels of TSAT% compared to control G1 (P> 0.05). On the other hand type1 diabetic patients of G2 had reduced levels of TSAT% as compared to healthy controls (P< 0.01), and type2 diabetics of G2, (P< 0.05) respectively. Ferritin levels were elevated in type2 diabetic patients of G2 as compared to type1 diabetes of the same group, (P< 0.05) and G1, (P< 0.001).

ADPN and EPO levels were elevated in diabetic patients of G2 as compared to G1 (P< 0.05). While leptin levels were reduced in type1diabetic patients of G2 compared to healthy controls G1 (P< 0.001) and type 2 diabetes of G2 (P<0.05) as demonstrated in table (5).

Many correlations were observed in the study groups. In group1; FSG was associated positively to age (r=0.386, P=0.039), BMI (r=0.380, P=0.042), A_{1C} (r=0.506, P=0.032), iron (r=0.406, P=0.029),ferritin (r=0.577, P=0.001)and negatively associated to EPO(r= -0.557, P=0.002). Also ADPN was negatively correlated to BMI (r= -0.572, P=0.001). On the other hand A_{1C} correlated positively with insulin (r=0.590, P=0.01) and negatively with leptin (r= -0.450, P=0.016). Hb had a significant positive correlation with Hct (r=0.896, P=0.000). Iron had apositive correlation with insulin (r=0.483, P=0.009) and ferritin (r=0.76, P=0.000).

In group2BMI was positively related to leptin but this correlation did not reached to the statistical significance (r=0.200, P=0.092) while negatively related to ADPN (r= -0.372, P=0.015). Duration of diabetes was positively associated with FSG (r=0.434, P=0.030) and negatively with Hb (r= -0.469, P=0.018).FSG was positively associated with A_{1C} (r=0.515, P=0.004) and negatively with leptin (r= -0.433, P=0.021). Hb had a significant positive correlation with Hct (r =0.54, P=0.002). Leptin was negatively associated to ferritin (r= -0.361, P=0.048) and Hct (r= -0.455, P=0.015). Iron was negatively associated to TIBC (r= -0.61, P=0.001). ADPN did not establish any association with the other parameters of the study in group2.

Table 1: The clinical characteristics of the healthy controls and diabetic patients.

	Control Subjects G1	Diabetic Patients G2	P
Number of Subjects	30	30	—
*Age (years)	47.1±12.12	47.9±14.28	NS
Sex (male/female)	15/15	15/15	—
*BMI (Kg/m^2)	27.0±2.85	27.02±4.66	NS
*Duration of Diabetes (years)	—	12.03±7.0	NS

*Data are (Mean ±SD), G1: group1, G2: group2, NS: not significant.

Table 2: The glycemic profile in the study groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
FSG (mg/dl)	99.25±17.19	273.0±165.4	189.54±15.45	G1 vs. G2 (T1) P=0.001 G1 vs. G2 (T2) P=0.0001 G2 (T1) vs. G2 (T2) P=0.01
A_{1C} %	5.29±0.88	9.85±5.95	8.69±2.7	G1 vs. G2 (T1) P=0.001 G1 vs. G2 (T2) P=0.0001 G2 (T1) vs. G2 (T2) NS
Insulin (µIU/ml)	11.24±3.21	19.52±15.13	12.23±10.6	ANOVA test was not statistically significant among the three groups.

*Data are (Mean ±SD), N: no. of subjects in group, G1: group1, G2: group2, G2T1: type1 diabetes of group2, G2T2: type2 diabetes of group2, LSD: least significant differences test, NS: not significant.

Table 3: Hemoglobin and Hematocrit levels in Study Groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
Hb (g/dl)	13.17±0.87	11.5±0.71	12.09±0.97	G1 vs. G2 (T1) P=0.016 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) NS
Hematocrit (%)	39.77±4.66	40.0±3.33	41.5±3.54	G1 vs. G2 (T1) NS G1 vs. G2 (T2) NS G2 (T1) vs. G2 (T2) NS

*Data are (Mean ±SD)

Table 4:Iron Status in the Study Groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
Iron (µg/dl)	107.67±42.33	276.8±181.14	159.16±123.46	G1 vs. G2 (T1) P= 0.021 G1 vs. G2 (T2) P= 0.047 G2 (T1) vs. G2 (T2) P=0.001
TIBC (µg/dl)	359.5±58.23	237.0±101.9	303.3±100.8	G1 vs. G2 (T1) P=0.036 G1 vs. G2 (T2) P=0.014 G2 (T1) vs. G2 (T2) P=0.001
TSAT (%)	30.70±13.11	24.3±8.0	35.29±7.56	G1 vs. G2 (T1) P=0.001 G1 vs. G2 (T2) NS G2 (T1) vs. G2 (T2) P=0.01
Ferritin (ng/ml)	77.95±39.68	68.25±31.97	129.6±24.16	G1 vs. G2 (T1) NS G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) P=0.01

***Data are (Mean ±SD)**

Table 5: EPO, ADPN and Leptin levels in the study groups.

Analyte	G1 N=30	G2 N=30		Multiple Comparison LSD
		Type 1 N=9	Type 2 N=21	
EPO (mU/ml)	34.35±7.48	91.23±12.65	122.91±20.71	G1 vs. G2 (T1) P=0.026 G1 vs. G2 (T2) P=0.000 G2 (T1) vs. G2 (T2) P=0.001
ADPN (µg/ml)	9.14±1.93	31.15±11.11	13.79±4.89	G1 vs. G2 (T1) P=0.001 G1 vs. G2 (T2) NS G2 (T1) vs. G2 (T2) P=0.008
Leptin (ng/ml)	10.19±5.97	4.11±3.70	19.16±9.93	G1 vs. G2 (T1) P=0.000 G1 vs. G2 (T2) P=0.001 G2 (T1) vs. G2 (T2) P=0.038

***Data are (Mean ±SD).**

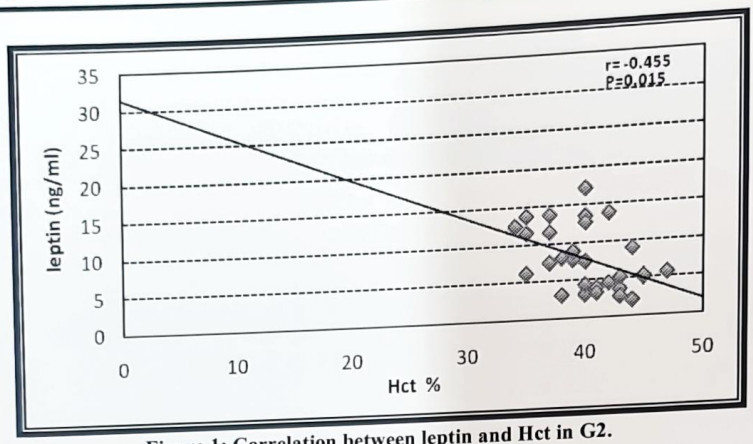

Figure 1: Correlation between leptin and Hct in G2.

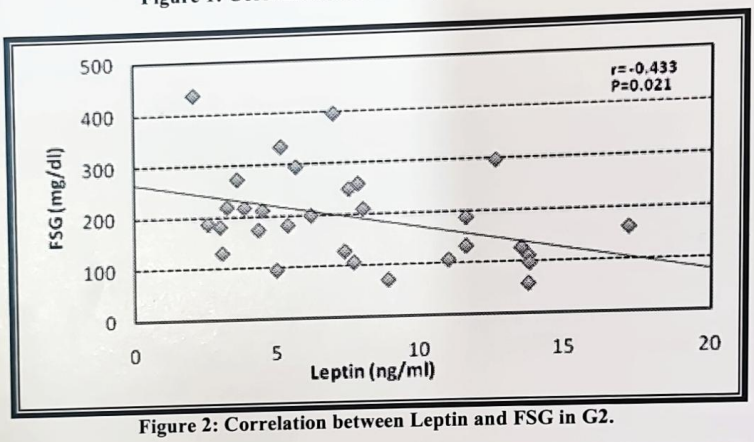

Figure 2: Correlation between Leptin and FSG in G2.

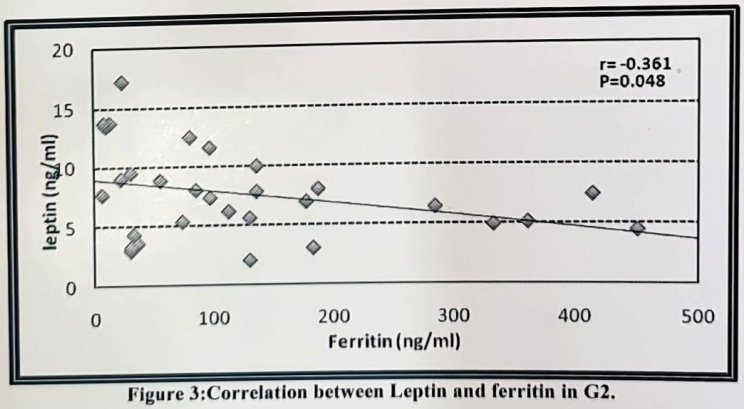

Figure 3:Correlation between Leptin and ferritin in G2.

Discussion:

Anemia is a common complication of chronic kidney disease. It is often more severe and occurs at an earlier stage in patients with diabetic nephropathy than in patients with chronic kidney disease of other causes. Numerous studies have addressed the interaction between diabetes and renal failure in its pathogenesis. The anemia associated with nephropathy results from EPO deficiency, which seems to develop in patients with type1 diabetes who have even relatively normal levels of serum creatinine. Early EPO-deficiency anemia occurs in both type1 and type2 diabetes [10].

The present study has shown that diabetic patients (G2) were found to have a degree of anemia even in the absence of overt kidney diseases.Although only (20%) of subjects in the diabetic patients group were overtlyanemic, all subjects had an ongoing, small but significant decrease in Hb. Thisstudy of diabetic patients without nephropathy shows an expected increase in EPO production inresponse to lowering levels of Hb.The results of the present study were coincided with the results Craig*et al.* [3],Thomas *et al.*[12], **Stevens** [13], Dimković [14].

In most studies to date, the predominant risk factor for the development of anemia in a diabetic population has been found to be the presence of renal disease, manifested as impaired renal function or albuminuria [10]. A cohort of type1 diabetic patients in the published study by Thomas *et al.* [15], where 14% of patients were found to be anemic. It is of note, however, that the study cohort of Thomas *et al.* [15] contained patients with nephropathy of varying degrees, whereas the groups studied within this study attempted to add to our understanding of the mechanisms behind the early onset of anemia in diabetic patients by separating the impact of diabetes from that of nephropathy.

In contrast to studies performed in patients with nephropathy, this study of diabetic patients without nephropathy showed a different picture in terms of EPO response. The present study demonstrated the expected normal increase in EPO production in response to low levels of Hb in G2 of diabetic patients in the absence of nephropathy. This is in contrast to the characteristics of anemia associated with diabetic nephropathy, in which impaired function of EPO producing fibroblasts associated with interstitial fibrosis and a defect of "anemia-sensing" mechanisms associated with autonomic neuropathy may both contribute to EPO deficiency [16].

In the present study, the levels of EPO were dramatically increased in type2 diabetes as compared to type1 diabetes and healthy subjectsrespectively (table (5)). The most important stimulus for an enhanced production of EPO is a lowered oxygen (O_2) supply to the tissue as a result of anemia or hypoxia. When anemia occurs, circulating Hb determines renal EPO response, which in turn stimulates erythropoietic activity. The disruption of the expected feedback mechanism of EPO production leads to an inadequately low serum EPO or inadequate response to anemia [17].

Previous study estimated EPO levels in normal subjects, patients with renaldysfunctions, hypothyroidism, diabetes mellitus, and in hematological disorders. There was a negative correlation between EPO and Hct values in anemic patients with normal renal function, whereas serum EPO levels is within the normal range in anemic patients with renal disorders, suggesting that serum EPO levels were relatively low in patients with chronic renal failure (CRF). On the other hand serum EPO levels were rather increased in patients with diabetes mellitus. Although the study did not report renal dysfunction in diabetic patients [18].

In the current study, there was a negative association between EPO and FSG (in G1). Although diabetic G2 lack this correlation that may require large sample size, as there were fluctuations of EPO concentrations in diabetic patients compared to controls. This correlation can be explained by a study which found that EPO mediated decrease in blood glucose levels in mice models injected with EPO as well as transgenic mice constitutively overexpressing EPO, and exposed to constantly high EPO serum levels thus suggesting interplay between EPO/glucose metabolism [19].

Some causal factors of anemia in diabetes are not related to EPO deficiency. It is important to recognize that erythrocyte half-life is abnormal in diabetic patients. This is due to several pathologies that have an impact on erythrocyte viability, such as increased osmotic stress which is a consequence of accumulation of sorbitol (as a result of activation of polyol pathway) and decreased Na^+/K^+- ATPase activity [20].

According to table (5), ADPN levels in diabetic patients (G2) were increased in both types of diabetes as compared to (G1).It could be speculated that insulin deficiency may contribute to elevated level of ADPN in type1 diabetes. However, as it has been reported that insulin therapy did not change ADPN levels [21]. In the current study also indicated that type1 diabetes of G2 had elevated levels of ADPN as compared to type2 diabetes and healthy controls. These results were in line with the studies of El-Maksoud *et al.* [21]; and Mather *et al.* [22].

The previous study [21] reported elevated plasma ADPN levels in groups of type1 diabetic patients, but without renal failure as compared to healthy subjects. On the other hand type2 diabetes was reported to have reduced levels of ADPN that associated with insulin resistance. ADPN is considered a powerful marker in subjects at higher risk for the development of type2 diabetes [22].

Although the results of the present study showed that diabetic patients (G2) had increased levels of ADPN as compared to healthy subjects (G1). Twenty percent

ofthese patients were diagnosed to have anemia (Hb ≤ 11g/dl). In addition Hb levels in G2 were lower as compared to healthy subjects even in the absence of overt kidney disease. These results were in line with the previous study [23].

The study of Aso *et al.* [23] demonstrated a higher incidence of anemia in diabetic patients with chronic kidney diseases (CKD) compared with diabetic patients without CKD and among many factors; Hb had the strongest independent influence on the serum ADPN level in patients with type2 diabetes. One possible explanation is the influence of tissue hypoxia caused by anemia on the expression of ADPN in adipose tissue.

Diabetic patients with marked elevation of the ADPN levels may have advanced diabetic nephropathy and or anemia. Anemia may explain the link between a high serum ADPN level and the severity of a patient's condition in diabetic patients with advanced CKD. There seems to be an "ADPN paradox" that clinicians need to consider when interpreting the serum ADPN level. Thus, because the "ADPN paradox" seems to mirror a complex progressive process. The clinical utility of measurement of ADPN for current risk prediction in individual patients is likely to be complex and is still vague [23].

Although adipose tissue is the only source of ADPN and the relationship of this adipocytokine to fat and body mass is a negative correlation. This was found in the present study. BMI in both healthy subjects of G1 and diabetic patients of G2 were associated negatively to ADPN. These results were in line with the study [23].

Leptin was found to be positively related to BMI [24], but this correlation was not statistically significant in the present study which may require larger sample size. Results from previous studies suggested a link between leptin and insulin homeostasis. Zoccali *et al.* [24] postulated that leptin levels were related to insulin. Although a correlation between leptin and insulin is not found in the presentstudy, but a negative correlation between leptin with A_{1C} (in G1), and FSG (in G2).

Moriya *et al.*[25] demonstrated that A_{1C} may be a factor to influence serum leptin levels and that hyperglycemia for a long period or poorly controlled diabetes may reduce leptin levels and directly increase insulin resistance and thereby worsening the condition. Taking in account the positive correlation between FSG and A_{1C} , this also was found in this current study. On the other hand leptin levels were elevated in type2 diabetes (G2) as compared to type1 diabetes and healthy controls respectively in this present study. These results were in line with the previous study [26].

Leptin also plays a major role in hemopoiesis and blood cell formation. Nasri [27] found a positive association between leptin and Hb in hemodialysis patients.Interestingly, there is a link between EPO and leptin, because EPO treatment induces a significant decline of leptinemia among hemodialysis patients. These cytokines seem to work in harmony and when EPO fails to be increased in response to lower levels of Hb, leptin will act instead and vice versa [27].

In addition, there was a negative associationbetween leptin and Hct in diabetic patients (in G2, figure (1)) in this current study. This correlation may add evidence that leptin can be reduced in case of adequate response of EPO which was found in group2 and this is similar to the findings of Togo *et al.*[28].

Togo *et al.*[28] study the relationship between levels of leptin and Hb in healthy male workers. A negative correlation was observed between the levels of leptin and those of Hb. The bone marrow contains adipocytes in which the *ob* gene is expressed. It has been speculated that the fat cells content of human bone marrow reflects the requirement for leptin in active hemopoiesis.This means that in clinical states with anemia and insufficient production of EPO such as in chronic renal failure other hemopoietic factors like leptin will be increasingly important in stimulating erythropoiesis [29].

The structural similarity between leptin and its receptor and cytokine-receptor system that control hemopoiesis has also promoted investigation for the potential for this hormone to influence blood cells formation .The studies have shown that the leptin receptor is expressed on a diverse range of hemopoietic cells. Leptin itself appears to enhance the proliferation of hemopoietic cells *in vitro*, and may augment some mature hemopoietic cells functions. In previous studies, minor hemopoietic deficiencies have been reported in mice lacking leptin or its receptors .Combined with leptin and ADPN data, these adipocyte derived proteins were related to hemopoiesis; therefore it has the possible existence of (adipose tissue/bone marrow) function linkage more clearly[29].

El-Maksoud *et al.* [21] study looked at type1 diabetics in relation to leptin and adiponectin levels. They found that type1 diabetics had higher ADPN and lower leptin. Other studies reported higher leptin and lower ADPN levels in type2 diabetics, which were found in this current study. Type1 diabetes has a different metabolic profile than type2 diabetes. Whereas type2 diabetes is disorder of insulin resistance, type1 diabetes is a deficiency of pancreatic production of insulin. ADPN levels in type2 diabetes decreases, which worsen insulin sensitivity. Furthermore, prior to a decrease of ADPN levels, leptin increases and the cause for this increase is leptin resistance. The study for El-Maksoud *et al.* [21], explained high ADPN levels in type1 diabetes is due to attempt of the fatty tissue to compensate for a lack of intracellular energy .With the lack of insulin , less glucose is translocated into the cellular compartments.

In the current study a negative correlation between leptin and ferritin was found in diabetic patients (in G2, figure (3)). This correlation may be explained as leptin

considered as negative acute phase protein while ferritin is positive acute phase protein [30].

In this current study the levels of Hb were lower in diabetic patients of G2 as compared to healthy subjects. The presence of anemia was confirmed in diabetic patients (As Hb ≤ 11g/dl, irrespective of sex in whole group according to the WHO criteria) [12]. In diabetic patients the presence of anemia was found in (20%) of the patients (six patients of total thirty in G2). The Hct levels were insignificantly higher in diabetic patient as compared to healthy subjects. Possible interpretation of the increased Hct levels in patients with diabetes as one of a range of hemorheologic abnormalities that enhance the risk of vascular disease indiabetes.

Linking type2 diabetes and raised Hct levels were associated with major compounds of obesity, glycemia, hyperinsulinemia, hypertension, and hypertriglyceridemia [31].Another study reported that Hb is linked to renal function in type1 and type2 diabetic patients. The study declared positive correlation between Hb and eGFR may be therefore Hb a powerful predictor of declining renal function [32].

Table (4) demonstrated the iron status in the study groups. Iron levels were significantly elevated in both types of diabetes (G2) as compared to healthy controls. These results were in line with the previous studies [33, 34].There were few reports suggesting subtle disturbance of iron metabolism are found in diabetic patients [33]. A previous study demonstrated that elevated iron indices are common in patients with diabetes. Excess iron may have a role in the development of diabetes and subsequently affect glycemic control. Also Excess iron has been implicated in the pathogenesis of diabetes and its complications.Although the exact mechanism of iron-induced diabetes is uncertain, it is likely to be mediated by three key mechanisms: (1) insulin deficiency, (2) insulin resistance, and (3) hepatic dysfunction. An understanding of the pathogenicpathways of iron-induced diabetes is derived mainly from studies on animal models of hemochromatosis [34]. Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species. In addition, iron indices are correlated with obesity and insulin sensitivity. It is possible that the excess of diabetic patients with elevated iron reflects the implicated role of iron in the development of insulin resistance and diabetes [34].

There is considerable interest in the relationship between insulin and iron pool in the body. Insulin influences the iron uptake and storage by increasing the cell surface transferrin receptors, reciprocally iron influences the insulin activity by interfering with glucose uptake and utilization. In addition, iron causes hyperinsulinemia by decreasing the insulin uptake and metabolism by hepatocytes.

This may explain and support the positive correlation between insulin and iron that was found in this current study (found in G1 and statically insignificant in G2) which was confirmed by the study of iron stores and insulin [33].

The non-transferrin bound iron or free iron has been found in various disease conditions like hemochromatosis, thalassemias and in patients on supplemental iron therapy, diabetes, and in hemodialysis patients. Previous studies have demonstrated the release of such free iron from ferritin, transferrin, and heme under certain conditions. Most of the free iron estimated in different disease conditions was found to be in its ferric state [35].

Although in this current study, the bond form of iron was measured (not the free iron), but a positive correlation between FSG and iron was found which may indicates the possibility that hyperglycemia may cause iron load (found in G1, and statically insignificant in G2).While in the previous study, a positive correlation was found between iron and A_{1C} that was correlated to FSG which may speculate that hyperglycemia, and poor glycemic control causes increase in glycation of Hb is contributing to the increase in free iron pool which is known to increase oxidant generation [35].

Proinflammatory cytokines and inflammatory mediators suppress native EPO production and blunt response to EPO at the receptor level. They also inhibit erythroid progenitor proliferation and differentiation and accelerate destruction of erythrocytes in the circulation. Inflammation has profound effects on iron traffic by diverting iron from erythropoiesis to storage sites within the reticuloendothelialsystem. Pro-inflammatory cytokines such as interferon-γ(IFN-gamma) and tumour necrosis factor-α (TNF-alpha) have marked inhibitory effects on erythroid progenitor cells. It has been suggested that the suppressive effect is related to the ability of these cytokines to generate nitric oxide (NO). Both pro-inflammatory and anti-inflammatory cytokines play major roles in the regulation of intracellular iron homeostasis. Anti-inflammatory cytokines are able to increase iron sequestration in activated macrophages and may, under certain conditions, contribute to the development of anemia [36].

The TIBC blood test measures the ability of transferrin to carry iron in the blood including the maximum amount of iron that can be attached to transferrin [37]. The findings of the present study in table (4) demonstrate that as iron levels increases TIBC levels decreased in (G2) diabetic patients, these results were in agreement with previous studies of iron status in diabetes [35]. Data in previous studies indicated significant increase in concentrations of serum iron, ferritin and lower TIBC levels in diabetic patients [35, 36].

As better index of iron saturation is transferrin saturation (TSAT). It was found from previous study that GFR,

A_{1C}, iron stores, microalbumin are predicators of Hb and the most powerful predictors are TSAT, and GFR. Previous study [33], demonstrated that type2 diabetes had iron overload with higher TSAT index as compared to healthy subjects which was in line with the data in type2 diabetes of group2 in this current study. The previous study interoperated these results by the positive correlation between hyperinsulinemia or insulin resistance and TSAT and negative correlation with TIBC in type2 diabetes; this may explain the reason for increased levels of TSAT in type2 diabetes in this present study.

Serum ferritin is the main storage molecule for iron; it is also an acute phase reactant. Its concentration tends to increase in the presence of inflammation [38].

Inflammatory cytokines may block iron from going to bone marrow for utilization or to increase the release of serum ferritin in the blood and subsequently elevate serum ferritin as low TSAT could indicate a negative acute phase reactant [36].In this current study positive correlations link ferritin with iron [in G1] which were similar to the findings of the previous study [38]. Although ferritin was not associated with iron in the diabetic groups which is may be due to the disturbance in iron mechanism [38] or the correlation required larger sample size for statistical significance.

On the other hand previous study revealed that serum ferritin concentrations in individuals with type2 diabetes or glucose intolerance are significantly higher than serum ferritin concentrations in control subjects which was in line with the findings in this current study. Furthermore the study demonstrated that diabetic patients in subgroup analysis to men and women had higher levels of ferritin and lower TSAT as compared to nondiabetic men and women, without analysis of diabetic patients according to the type of diabetes [39].Another study implicated inflammatory mechanisms rather than iron overload as a cause of increased ferritin levels in type2 diabetes in absence of nephropathy [33].

In this current study, mean levels of serum insulin were insignificantly elevated in diabetic patients of group2 as compared to healthy controls, but this elevation did not reach the statistical significance .This may be due to the small size of samples that was divided into two types (type1 and type2 diabetes). It should be taking in account that type1 diabetes has the higher mean level of insulin, which may be linked to the synthetic insulin (the long acting treatment) that used by some of patients. Although the samples were taken in fasting state but the effects of treatment is persistent in some patients.

Conclusions:

Diabetic patients in this study were found to have a degree of anemia even in the absence of overt complications and kidney disease. In this study the leve of Hb were lower in diabetic patients as compared healthy controls (20% of diabetic patients we diagnosed to have anemia, Hb ≤ 11g/dl.). The presen of anemia in diabetic patients in the absence nephropathy, may explain the increase in EP production in response to low levels of Hb. Diabet patients in this study showed increased levels in ADF and this may be linked to the reduced levels of Hb these patients. There was a negative correlation betwe leptin and Hct in diabetic patients this correlation m add evidence that leptin can be reduced in case adequate response of EPO. Subtle disturbance of ir metabolism were found in diabetic patients demonstrat that elevated iron indices are found in patients w diabetes. Excess iron may have a role in the developm of diabetes and subsequently affects glycemic control.

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Exercise ECG and Coronary Computed Tomography Angiogram in Baghdad, Iraq: a preliminary report

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Abstract

Background: Recently, business mentality in medicine enhances a wide application of new technology, and clinical practice run a head of guidelines. In Iraq, literature on accuracy of exercise ECG and coronary CT angiogram is scarce.
Objective: to throw a light on accuracy of exercise ECG and coronary CT angiography.
Methods: Fifty three patients scheduled to undergo percutaneous coronary angiography were included in this study. The patients underwent exercise and coronary CT angiography. The type of the study was intention- to- diagnose design. Questionnaire was filled for each patient. The requested information was demographic data, history, physical examination and findings of investigations. Exercise ECG and coronary CT angiography were evaluated against the findings of percutaneous coronary angiography using 2x2 table.
Results: Age of the patients was 51.2 ± 3.9 years, 50.9% were males and 81.1% of them with intermediate to high pretest probability. Sensitivity of exercise ECG and coronary CT angiogram was 64.1% and 71.8%, respectively, and specificity was 57.1% and 0%, respectively.
Conclusion: Low accuracy figures for exercise ECG and coronary CT angiogram were reported. Guidlines according to Iraqi situation may enhance accuracy.
Keywords: exercise ECG, coronary CT angiography, percutaneous angiography, Iraq, accuracy of test

Introduction

Over the last 4 decades, in developed world, the diagnostic ests are highly dynamic, new tests are developed and echnology of existing tests in being modified and nproved [1]. Biased and exaggerated results from poorly esigned studies may influence their premature ssemination and also incorrect treatment decisions will taken by physicians [1,2].

was noticed that in USA, the business mentality of edicine often embraces a application of new technology ore establishing their correct role adequately [3,4] which urn mounting health care costs and threaten Americans npetitiveness [3]. The cost of cardiovascular disease is n in the United States [4] which is attributed to the ensive modern equipments, intensive use of diagnostic . Cardiovascular imaging represent 29% of all imaging t load [5].

is undergoing an epidemiological transition with an asing burden of chronic non- communicable diseases) [6]. Cardiovascular disease represent the main causes spital admission and account for 40% of all causes of [6,7]. A recent survey for NCD risk factors in Iraq [8] d that the prevalence of risk factors of coronary disease (CAD) i.e. hypertension, overweight and lycemia were 40.4%, 66.9% and 10.4%, ively.

0, Iraq spend about 8.4% of its estimated gross ic product (GDP) of $ 82.2 billion on health [9], and of health expenditure was estimated to be out of [10]. Observers commented that noninvasive s of CAD is run without a head of guidelines. The Iraq faces reform of health care system and methods of funding this system [11]. Almost all health care reform proposals advocate improved efficiency and value of health care spending and seek to better align medical care with evidence based guidelines.

Literature, in Iraq, on accuracy of noninvasive diagnosis of CAD is scarce. This work, therefore, was carried out to through a light on accuracy of noninvasive diagnosis of CAD in Iraq.

Methods

Fifty three patients were scheduled to undergo percutaneous coronary angiogram (PCA) were included in this study. Exercise ECG (exECG) and coronary computed tomography angiography (CCTA) were done before PCA, which serves as the reference standard in this study. All patients scheduled for PCA had exECG and CCTA.

The study population were patients referred for diagnosis. The study was carried out according to the intention-to-diagnose design [12-14].

Both non-invasive tests (ex ECG and CCTA) were done within 2 weeks before PCA. PCA was performed without regards to the results of both index tests to avoid partial verification bias [14]. Pretest probability of CAD in the study population was calculated according to Gibbons et al [15].

Patients with unstable angina, cardiac arrhythmia, pregnancy and known CAD were not eligible for the study.

All patients were examined in the Iraqi centre for cardiac disease (medical city complex) and Ibn el Betar hospital of cardiology.

The study project was approved by the Baghdad College of Medicine review board.

A questionnaire was developed and filled through a personal interview. This questionnaire includes: demographic data (age, gender, residence, occupation, body mass index), detailed history of smoking, alcohol consumption, hypertension, diabetes and present complaints, review of prior tests (electrocardiography, echocardiography, ex ECG test,...etc), referral indication as stated by the physician and outcome data. The case record of every patient was reviewed.

Ex ECG was performed on motorized treadmill using Bruce Protocol up to a predicted maximal heart rate for age. [16] CCTA in both centers was performed using Brilliance 64, Philips. The patient was informed few days before the test to practice 15 second breath holding exercise, and to make appointment to his/her physician in order to control heart rate around 60 beats/minute. Patients found to have heart rate more than 65 beats/minute will receive additional dose of oral or intravenous metoprolol [17]. Results of ex ECG and CCTA were dichotomized into positive and negative.

PCA done at a specialized catheterization lab. using femoral or radial access as indicated, and the result classified as negative or positive test, also.

The results from ex ECG and CCTA (index tests)were compared with those of PCA (reference standard). The discrepancies in the results were used to measure diagnostic accuracy of ex ECG and CCTA. Sensitivity and specificity, which represent the proportion of correctly diagnosed diseased and non diseased persons, respectively, [12] using 2x2 table for comparison with reference test.

Results

The mean age of patients ± SD was 51.2 ± 3.9 years, 50.9% of them were males. Out of the total, 16 (30.2%) were smokers. Twenty seven (50.9%) and 24 (43.3%) were hypertensive and diabetics, respectively. Twenty three (43.3%) patients had dyslipidemia. ECG ischemic changes was noticed in 20 (37.7%) patients and chest pain in 52 (98.1%). These findings are shown in **Table 1.**

Low pretest probability of CAD was observed in (17.0%) patients and intermediate to high probability among 43 (81.1%).

Table 2 shows the accuracy parameters of exECG and CCTA. Sensitivity of exECG and CCTA were 64.1% and 71.8%, respectively, and specificity were 57.1% and 0%, respectively. Positive and negative predictive values of exECG and CCTA were 80.6%, 66.6%, 36.4% and 0%, respectively.

iscussion

e major purpose of exECG and CCTA as noninvasive ts, is to identify patients with intermediate to high probability of CAD who may get early PCA and consideration of revascularization [18,19]; they are used as a gate keeper for PCA. The finding that 81.1% of patients examined by exECG and CCTA were with intermediate to high pretest probability means that examined patients got no benefit from the tests. A positive exECG and CCTA may increase a low pretest probability to intermediate or high posttest probability which may influence a therapeutic decision making toward revascularization. Patients with an already high pretest probability of CAD may not benefit from CCTA as most of them will be referred to PCA. The objective of exECG and CCTA is to demonstrate ischemia which is recognized as critical in the clinical management. This was reflected in the PCA intervention guidelines in both United States and Europe. [20]

Test performance was used to evaluate exECG and CCTA. Diagnostic test performance usually measured by sensitivity, specificity and predictive values (positive and negative) which are influenced by prevalence of the disease i.e. pretest likelihood of CAD affect post-test accuracy. The revealed sensitivity and specificity of exECG (64.1% and 57.1%, respectively) and of CCTA (71.8% and 0%, respectively) were lower than that reported in literature [18,19,21,22]. This finding might be attributed to high percentage of patients with intermediate to high pretest probability (81.1%). Furthermore, the design of the study might be contributed to lower reported accuracy figures.

The demonstrated specificity of CCTA in this study was 0% which indicated that negative CCTA finding could not ruled out a CAD and the revealed specificity was much lower than that reported in literature [18,19,21,22]. The high accuracy figure in literature might be attributed to the referral bias (design of studies). CCTA was use for identification of coronary artery stenosis to support decision indicating in performing PCA because of the result of CCTA. The difference might be attributed the recent application of CCTA in Iraq. The accuracy of CCTA is a function of the reader experience [23] which in turn appears to be a determinant of proficiency in CCTA interpretation. The ACC/AHA guideline for competence in cardiac CT angiography mandate minimum experience of > 150 cases. [24]

The tests (ex ECG, CCTA and PCA) were carried out to all patients enrolled in this study regardless of the finding of any test. Most patients were with intermediate to high risk which in turn affect the accuracy of CCTA [25,26]. Gibbons et al [15] formulated a stable angina guideline that indicated that high risk patients do not need noninvasive tests. The ex ECG and CCTA b are used for detection or exclusion of CAD in patients with low probability of CAD which is useful clinical [27] and economical. [28]

Conclusion:

Low accuracy was demonstrated for ex ECG and CCTA in Baghdad centers. The tests were used to detect or exclude CAD in patients with intermediate to high pretest

probability. Guidelines according to Iraqi situation might enhance the accuracy of studied tests (exECG and CCTA) and participate in reforming health system.

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(Table -1) Characteristics of the Studied Sample

Variable		Mean ± SD	
Age		51.2 ± 10.3	
BMI		29.8 ± 3.9	
Variable		No.	%
Sex	Male	27	50.9
Residence	Baghdad	43	81.1
Smoking	Smoker	16	30.2
	Ex-smoker	11	20.8
	Nonsmoker	25	47.2
Hypertension		27	50.9
DM		24	45.3
Dyslipidemia		23	43.4
ECG Ischemia		20	37.7
Chest pain		52	98.1

(Table- 2) Accuracy of exECG and CCTA

Accuracy	Index test		P value
	Ex ECG	CCTA	
Sensitivity	25/39 (64.1%)	28/39 (71.8%)	0.4
Specificity	8/14 (57.1%)	0/14 (0.0%)	
Positive predictive value	25/31 (80.6%)	28/42 (66.6)	0.2
Negative predictive value	8/22 (36.4)	0/11 (0.0%)	

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Frequency of Bacteria Isolated from Blood Cultures of Children at a Children Hospital in Baghdad and Study of their Antimicrobial Susceptibilities

Nadheema Hammood Hussein

Abstract

A eight months study (1 January till 1 September 2013) of bacteremia in febrile patients was carried out in one children hospital at Baghdad, to estimate the rate of positive blood culture from children patients and to determine the frequency of bacterial isolates from blood cultures and their antimicrobial susceptibility patterns. A total of 846 blood specimens were collected from children suspected with blood stream infections. Blood cultures and isolation pathogens were done according to standard bacteriological tests and identified by using VITIC 2 system. The rate of bacteremia in the studied patients was 36.6%. Coagulase negative *Staphylococcus* (CoNS) was the most frequently isolated bacteria in blood cultures, 164(52.9%) isolates (*P-value*< 0.05).The isolation rate of Gram positive bacteria more than Gram negative bacteria in patients. The highest rate of isolation was within the age group less than three months. It was shown that *Escherichia coli* was the most frequently isolated Gram negative bacilli in blood specimens 35(11.3%). The most effective antibiotic on the blood culture Gram-positive isolates was Vancomycin. In Gram negative bacilli the most effective antibiotics on these isolates was Imipenem followed by Amikacin.

Introduction

Bloodstream infections (BSI) are potentially life-threatening and require rapid identification and also antibiotic susceptibility testing of the causative agent in order to facilitate specific antimicrobial therapy [1] It is a serious problem that needs immediate attention and treatment. It is a cause of high mortality especially if caused by multidrug resistant bacteria [2,3]. Bacteriological culture to isolate the offending pathogen and knowledge about sensitivity pattern of the isolates remain the main stay of definitive diagnosis and managementof bloodstream infections [4,5].

Blood culture is one of the most important bacteriological examinations with important clinical and therapeutic consequences. Blood cultures should be ordered in all patients with signs suggesting septicemia, endocarditis or severe infection [6,7] In developing countries, more than 14 million deaths of children under five years of age occur during the childhood(8), with infections accounting for up to 70% of total mortality for this age group [9]

This study was aimedTo determine the frequency of bacterial isolates from blood cultures of children and their antimicrobial susceptibility patterns.

Methods

Period of Study

This study was conducted during the period from 1 January 2013 until 1 September 2013.

Collection of blood specimens

Blood samples were collected from inpatients suspected with blood stream infections prior to initiation of antimicrobial therapy. For each blood culture, 5 mL from infants and children (≥0.5 mL for infants <1 month of age, ≥1 mL for children between 1 month and 36 months of age, and ≥4 mL for children ≥36 months of age) (10).

Isolation and Identification of bacterial isolates

Blood specimens were cultured and microorganisms isolated from all specimens according to standard microbiology methods (11). then microorganisms were identified at species level by using VITEK 2 system (Bio-Merieux).

Antibiotic susceptibility test

The antimicrobial susceptibility test was performed according to Kirby-Bauer (disk diffusion) technique (12),using Muller-Hinton agar and different single antimicrobial discs supplied commercially (table-1).Inhibition zones developed around the discs were measured by millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute (13).

Table-1:Antibiotic discs used in this work.

Antibiotic discs	Code	Disc potency (µg/disc)	Manufacturing Company/ Origin
Amikacin	AK	30	Bioanalyse/ Turkey
Amoxicillin-Clavulanic acid	AMC	20/10	Bioanalyse/ Turkey
Aztreonam	ATM	30	Bioanalyse/ Turkey
Cefepime	FEP	30	Bioanalyse/ Turkey
Ceftazidime	CAZ	30	Al-Razi/ Iraq
Ceftriaxone	CRO	30	Bioanalyse/ Turkey
Erythromycin	E	15	Bioanalyse/ Turkey
Gentamicin	CN	10	Bioanalyse/ Turkey
Imipenem	IPM	10	BD/ Ireland
Oxacillin	OX	1	Bioanalyse/ Turkey
Penicillin	P	10 (unit)	Bioanalyse/ Turkey
Tobramycin	TB	10	Al-Razi/ Iraq
Trimethoprime-Sulphamethoxazole	TMP	1.25/ 23.75	Bioanalyse/ Turkey
Vancomycin	VA	30	Bioanalyse/ Turkey

Statistical Analysis

The Chi-square (χ^2) test was employed for comparison among groups. P value ≤ 0.05 was considered statistically significant (14).

Results

Study patients

Through a period of eight months, blood specimens were collected from 846 patients suspected with blood stream infections. During this study we noticed that the majority of patients were within the age grouping less than three months and male patients were higher than female patients, 502 (59.3%) vs. 344 (40.7%) out of 846 children patients.

Blood culture

Out of 846 blood specimens, the frequency of positive blood cultures that indicates bacteremia in the studied children patients were 310 (36.6%) cases (figure-1). According to age and gender, frequency of positive blood culture was higher in male than female patients, 186(60%) vs. 124 (40%), as shown in table - 2. From the same table, the majority of patients with positive blood culture were within the age group less than three months, 179 (57.7%) patients (*P-value*< 0.05).

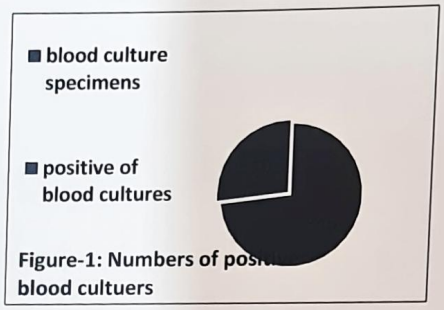

Figure-1: Numbers of posi... blood cultuers

Table-2: Frequency of positive blood culture according to age and gender

Age groups	Infected patients with bacteria					
	Male		female		Total	
	No.	(%)*	No.	(%)*	No.	(%)*
<3 M	113	36.5	66	21.3	179	57.8
3 M-2 Y	45	14.5	23	10.3	77	24.8
3-4 Y	15	4.8	15	4.8	30	9.6
5-6 Y	7	2.2	4	1.3	11	3.5
7-8 Y	3	1	4	1.3	7	2.3
9-10 Y	3	1	3	1	6	2
Total	186	60	124	40	310	100

M= month Y= year

Table -3 summarized the frequency of isolated bacteria detected in blood specimens from children patients, out of the total positive blood cultures (310), the isolation rate of Gram positive and Gram negative isolates was 191(61.6%) and 119(38.4%), respectively (figure-2). Coagulase negative *Staphylococcus* (CoNS) was the most frequently isolated bacteria in blood cultures, 164(52.9%) isolates (*P-value*< 0.05). It was shown that *Escherichia*

coli was the most frequently isolated Gram negative bacilli in blood specimens from patients followed by *Klebsiella pneumoniae*.

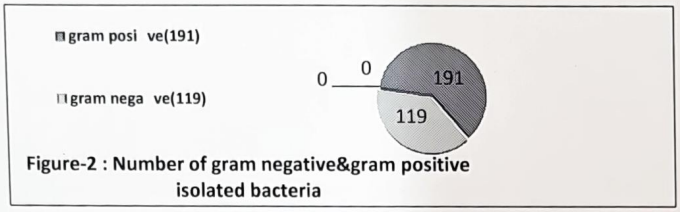

Figure-2 : Number of gram negative&gram positive isolated bacteria

Table-3: Frequency of blood culture isolates from patients specimens.

Gram positive bacteria	Number of strains (%)
Coagulase negative *Staphylococcus*	164 (52.9)
Staphylococcus aureus	21 (6.8)
Streptococcus spp.	6 (1.9)
Gram negative bacteria	**Number of strains (%)**
Escherichia coli	35 (11.3)
Klebsiella pneumoniae	26 (8.4)
Acinetobacter spp.	24 (7.8)
Pseudomonas spp.	13 (4.2)
Enterobacter spp.	12 (3.9)
Citrobacter spp.	5(1.6)
Salmonella spp.	2 (0.6)
Serratia spp.	2 (0.6)
Total	310 (100)

Antimicrobial sensitivity test results of Gram positive isolates from blood specimens to various antimicrobial drugs were shown on table -4. It was shown that the most effective antibiotics on these isolates was Vancomycin. Gram positive isolates in this study were mostly resistant to Penicillin and Ampicillin.

Table-4: Antimicrobial resistance pattern of Gram-positive bacteria isolated from blood cultures of children (patients).

Antimicrobial drugs	Coagulase negative *Staphylococcus* N=164		*S. aureus* N=21		*Streptococcus spp.* N= 6	
	S%	R%	S%	R%	S%	R%
Ampicillin	-	100	-	100	-	100
Erythromycin	12	88	60	40	100	-
Gentamicin	56	44	40	60	40	60
Oxacillin	-	100	-	100	70	30
Penicillin	-	100	-	100	-	100
Trimethoprime-Sulphamethoxazole	50	50	25	75	80	20
Vancomycin	88	12	75	25	80	20

S= Sensitive R= Resistant

Antimicrobial sensitivity test results of Gram negative bacilli from blood specimens to various antimicrobial drugs are shown on **Table -5.**

It was shown that the most effective antibiotic on these isolates was Imipenem followed by Amikacin. Gram negative bacilli in this study were mostly resistant to Ampicillin, Aztreonam, andTobramycin

Table-5: Antimicrobial resistance pattern of Gram-negative bacteria isolated from blood cultures of children (patients).

Antimicrobial drug	*Escherichia coli* n=35		*Klebsiella penumoniae* n=26		*Acinetobacter spp.* n=24		*Pseudomonas spp.* n=13		*Enterobacter spp.* n=12		*Citrobacter spp.* n=5		*Salmonella spp.* n=2		*Serratia spp.* n=2	
	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Amikacin	100	-	94	6	80	20	80	20	80	20	80	20	90	10	80	20
Amoxicillin-Clavulanic acid	29	71	40	60	17	83	-	100	-	100	-	100	30	70	10	90
Ampicillin	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100
Aztreonam	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100
Cefepime	14	86	30	70	30	70	20	80	70	30	20	80	75	25	30	70
Ceftazidime	8	92	25	75	20	80	-	100	80	20	80	20	80	20	80	20
Ceftriaxone	14	86	25	75	6	94	-	100	50	50	40	60	50	50	50	50
Gentamicin	50	50	44	56	40	60	60	40	70	30	65	35	45	55	80	20
Imipenem	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-
Tobramycin	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100
Trimethoprime-Sulphamethoxazole	42	58	30	70	6	94	-	100	10	90	15	85	40	60	20	80

S= Sensitive R= Resistant

Discussion

Prompt diagnosis and effective treatment are necessary to prevent complications and to reduce mortality from BSI (5). In this present study, out of 782 blood specimens, the frequency of positive blood culture that indicated true bacteremia in the studied patients was 310 (36.6%) cases. In comparison with other studies, In an Indian study the positivity of blood culture was 42% (770/1828). In a study conducted at the University College Hospital Ibadan, Nigeria, the prevalence of septicaemia in the infants studied was 38.2% (15).

In the current study, the isolation rate of Gram positive and Gram negative isolates was 191 (61.6%) and 119 (38.4%), respectively. Coagulase negative *Staphylococcus* was the most frequently isolated bacteria in blood cultures, 164 (52.9%) isolates. One study showed that the most common bacterial pathogens isolated from blood cultures were CoNS (1 250; 67.4%), *S. aureus* (245; 13.2%), *E. coli* (131; 7%) and *Klebsiella* spp. (130; 7.0%). Other bacterial pathogens detected were *Proteus* spp. (34; 1.8%), *Pseudomonas* spp. (22; 1.2%), *Streptococcus* spp. (28; 1.5%), *Salmonella* spp. (11; 0.6%), *Enterobacter* spp. (3; 0.2%) and *Acinetobacter* spp. 1 (0.1%) (16).

Gram positive isolates in this study were mostly resistant to Penicillin and Ampicillin, whereas Gram negative bacilli were mostly resistant to Ampicillin, Aztreonam, andTobramycin. The Tunisian study showed that the methicillin-resistance concerns 14% of the whole strains and 5.2 of the *S. aureus*. No resistance was found as regard the Vancomycin and the Pristinamycin; Ofloxacine was inactive on 14.8% of strains and the gentamicin on 11.3% (17).

Conclusions

- Coagulase negative *Staphylococcus* was the most frequently isolated bacteria in blood cultures, 164 (52.9%) isolates.
- It was shown that *Escherichia coli.* was the most frequently isolated Gram negative bacilli in blood specimens from inpatients 35(11.3%).

- The most effective antibiotic on the blood culture Gram-positive isolates was Vancomycin. In Gram negative bacilli the most effective antibiotics on these isolates was Imipenem followed by Amikacin.

Recommendations

- Automated blood culture systems must be used in all our hospitals to avoid the need for invasive methods and the risk of contamination in addition to saving time.
- Cohort studies are needed to evaluate the prevalence and incidence of blood stream infections in our children and mixed hospitals.

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Frequency of HLA –DRB1 in Iraqi Patients with Knee Osteoarthritis

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Abstract

Background: Osteoarthritis (OA) is the most common form of degenerative joint disease and the leading cause of musculoskeletal disability among the elderly individuals with severely impaired quality of life.
Aim of the study: to investigate whether we could confirm the role of HLA-DRB1 alleles and knee OA in Iraqi Arab Muslims patients.
Methods:A cross –sectional case control comparative study included twenty –six Iraqi Arab Muslims patients who had knee OA and admitted in the Orthopedic Department at Al-Kindy teaching Hospital between September – 2012 to June – 2013 compared to control ethnically matched group. HLA –DRB1 genotyping was done by PCR-SSOP method using Autolipa-48 and results were analysed by software version 5.0.
Results: OA of the knee is more common in females than males .There was an increased frequencies of HLA-DRB1*03:01:01:01 in patients with OA compared with healthy controls (p=0.01, odd ratio=4.766 , 95% CI: 1.287-17.6485).
Conclusions: Genetic factor (HLA) system - HLA DQB1*03:01:01 play an important role in the etiopathogenesis of OA.

Key words : Osteoarthritis, HLA, PCR.

Introduction:

Osteoarthritis is the most common form of degenerative joint disease leading to impaired quality of life [1] Efforts to recognize early stages of knee OA, it is characterized by loss of articular cartilage resulting from imbalance between cartilage breakdown and regeneration [2]. Genetic factor is one of leading causes of this disease [3,4]. The genetic marker is HLA class I and II located on chromosome No 6 which is highly polymorphic and may play a role in pathogenesis of this diseases [5]. A number of studies had been done like Nakajima etal 2010 [6] has identified two single neuclotide polymorphisim and strongly associated with knee OA. One of the markers is rs 7775228 mapped to the HLA class II gene DQB1 and the other one is intron I of the butyrophilin like 2 gene (BTNL2) gene which is implicated with T cell activation. Other study by Shi and coworkers tested the same two SNPs in Chinese case control study that failed to find such association [7]. Other researchers found increased frequency of HLA –A1B8 haplotype in OA patients [8]. This HLA encodes a protein that play a role in self non-self discrimination [9] This leads to T cell activation and cytokines secretion that contribute in chronic inflammation [10]. T cells and chondrocytes are interacted through cell surface molecules like HLA, CD4, CD8 in OA patients [11] These explanation supports the role of HLA variation in genetic susceptibility to OA.
We aimed in this study to investigate whether we could confirm the role of HLA-DRB1 alleles and knee OA in Iraqi Arab Muslims.

Methods:

A cross –sectional case control comparative study included twenty –six Iraqi Arab Muslims patients who had knee OA and admitted in the Orthopedic Department at Al-Kindy teaching Hospital between September – 2012 to June – 2013 . Age of the patients group was ranged from 35-70 years . Females were 24 and the rest were males. The patients were selected by the orthopedician according to the clinical presentation and X-ray changes. Any patients with secondary OA were excluded from the study .
The second control group consisted from thirty healthy volunteers among the staff of Al-Kindi College of Medicine that did not have any OA. The control group was ethnically similar to patients group, their ages were ranged from 30-60 years. Males were 18 in number and the rest were females.
The Scientific and Ethical Committee of Al-kindy medical college and Al-Kindy Teaching Hospital had approved the study. Informed consent was obtained from all patients and control group and have been approved by an appropriate ethics committee.
HLA genotyping: Peripheral venous blood samples from patients and control groups were collected in ethylenediaminetetraacetic acid-containing tubes and then stored at -20°C until testing for class II- HLA-DRB1. Genomic DNA was extracted using Promega DNA extraction Kit- USA. DNA product was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and was visualized under UV light. Locus- and allele-specific amplification of genomic DNA were

performed for DRB1. Amplification and Hybridization was performed using a panel of sequence-specific oligonucleotide probes (SSOP) using HLA-DRB1 amplification and hybridization kits (SSO HLA type DRB1 plus and Mastermix for HLA type DRB1 Amp plus kits -Innogenetics-Belgium) using automated method by AutoLipa – 48 Innogenetics-Belgum. The results were interpreted using LiRas version-5.0 software-Innogenetics-Belgium.

Statistical analysis was done using using MiniTab version . 3.0 software The distribution of HLA alleles in patients and control groups were compared using Chi-square for continuous variable . Fisher's exact test was used when necessary. In each comparison, the odds ratio (OR) along with the 95% confidence interval (95% CI) was used. P-value less than 0.05 was considered statistically significant.

Results:

Control and OA patients groups were typed for identifying the DRB1* alleles using DNA-based methodology (PCR-SSOP). Alleles frequencies of HLA-DRB1 for OA patients and control group is shown in table-1-. There was an increased frequencies of HLA-DRB1*03:01:01 in patients with OA compared with healthy controls (p=0.01, odd ratio=4.7667 , 95% CI: 1.2874-17.6485) ;also There is an increase in the HLA-DRB1* 11:01:01 in patients with OA compared with the control group, but the differences were not statistically significant

Table-1- Human leukocytes antigens (HLA-DRB1) alleles frequencies in patients with OA and healthy control groups.

HLA-DRB1* alleles	Knee OA patients group No.=26		Healthy control group No.=30		Odd ratio (95% con dence interval)	P- value
	No.	%	No.	%		
02:03	0	0	2	6.66	na	na
03:01	11	42.30	4	13.33	4.7667 (1.2874-17.6485)	0.01
03:13	4	15.38	0	0	na	na
03:17	0	0	4	13.33	na	na
04:02	6	23.07	0	0	na	na
07:01	5	19.23	7	23.33	1.4935	0.709
08:01	0	0	2	6.66	na	na
09:01	7	26.92	0	0	na	na
11:01	11	42.30	7	23.33	2.40 (0.7634-7.6053)	0.129
11:02	0	0	2	6.66	na	na
11:03	0	0	4	13.33	na	na
11:33	3	11.53	0	0	na	na
11:67	0	0	4	13.33	na	na
12:09	0	0	2	6.66	na	na
13:01	5	19.23	0	0	na	na
13:05	0	0	2	6.66	na	na
13:18	0	0	4	13:33	na	na
13:116	0	0	2	6.66	na	na
13:119	0	0	2	6.66	na	na

14:01	0	0	2	6.66	na	na
14:02	0	0	2	6.66	na	na
14:16	0	0	2	6.66	na	na
14:57	0	0	4	13:33	na	na
15:01	0	0	2	6.66	na	na

na=not applicable

Discussion:

In this study we found an association between HLA-DRB1*03:01:01 in patients with knee OA compared with healthy controls (P=0.01, Odd ratio= $.7667, 95% CI= 1.2874 to 17.6485 in Iraqi Arab Muslims in spite of a sample size small enough to have sufficient statistical power to confirm the effect of HLA typing. Other studies showed conflicting results, one demonstrated an association between DR2 and DR5 with OA in Caucasian ethenic group (2). Riyazi etal 2003 (12) showed that HLA-DR2 is more associated with distal interphalangeal joints in Dutch population. Other study showed an association between HLA-A*02 and B*38 with nodal generalized OA in Turkish population and concluded that HLA-B*44 positivity may be associated with familial nodal generalized OA and HLA-A*29 may be a preventative factor against the disease (13). Other work in Italy demonstrated no statistically significant difference of A1 and B8 antigens between patients and control group. By contrast, HLA-B35, B40, DQ1 and Cw4 antigens were over represented in the OA patients. Haplotype analysis showed an association of B35-DQ1, B40-DQ1 and DR2-DQ1 with increased OA risk (14).The genes that predispose to OA remained to be clarified. Many studies have pointed to different HLA class I and II association, perhaps indicating the heterogeneity of the condition. Several studies on generalized OA have revealed an association with HLA-B8. Linkage of HLA-B8 with A1 and DR 17 made it difficult to be certain the association is actually with HLA-B8 and not with some other genes on the haplotype. A Japanese study showed an association with HLA-Cw4. Other study has indicated association HLA-B35 with B40, Dr2 and DQ1 (15). Other study demonstrated an association with B27 in Brucellosis Iranian patients (16). These discrepancies between our study and other studies may be due to age of selected patients, gender, ethnicity, racial background, diagnostic and clinical criteria, sample size of patients and control groups which is too low in our study. Other cause is different in methodology, all the above causes served as a source of bias. Therefore, we recommended further study with larger sample and family study to clarify the association.

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In Vitro Determination of Antibacterial Properties of Garlic Extract against Multidrug-Resistant bacteria Isolated from Women with asymptomatic bacteriuria

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Abstract

Background: Urinary tract infections are most commonly prevalent infections among pregnant women, some bacteria have resistance toward many antimicrobial agents for treatment has led to therapeutic difficulties worldwide, in order that many investigators try to test plant extract to reduce bacterial infection.

Objectives: To evaluate the antibacterial effect of garlic extract on different types of bacteria which was isolated from pregnant women with asymptomatic bacteriuria.

Methods: One hundred five urine samples were collected from pregnant women with asymptomatic bacteriuria attending to Al-Batool teaching hospital for maternity and children in Diyala Governorate. Iraq, during the period from 1st /April /2012 till 1st/ July/2012.

All specimens were streak on blood agar and MacConke ager, then identified according to standard bacteriology and biochemical criteria. The susceptibility patterns toward 7 antimicrobial agents were done by disc diffusion methods. The resistant cultures of different types of bacteria, which were isolated from pregnant women with asymptomatic bacteriuria, were tested by 200mg/ml of garlic extract.

Results: Fifty three out of 105 samples (50.47%) were demonstrated negative bacteria growth while 52 out of 105 samples (49.52%) demonstrated positive bacteria culture. The isolated revealed that 15 *Escherichia coli*, 11 *Proteusmirabilis*, 10 *Klebsiella*, 8 *Staphylococcus saprophyticus*, 6 *Staphylococcus aureus*, and 2 *Streptococcus pyogenes*. The majority of bacterial isolates were sensitive to 200 mg/ml garlic extract.

Conclusion: Garlic extract was effective as antibacterial against antibiotic resistant strain.

Keywords: Garlic extract, antibiotic, urinary tract infections.

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Introduction

Urinary tract infection (UTI) is the second most common type of infection in the body. There are an estimated 150 million urinary tract infections per year worldwide [1]. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of these families include *E. coli, Klebsiell , Enterobacter* and *Proteus*. Also Gram positive *Stapylococcus sp*. plays a role in the infection [2]. The incidence of urinary tract infections is greater in women as compared to men who may be either due to anatomical predisposition or urothelial mucosal adherence to mucopolysaccharide lining or other host factors [3].

Urinary tract infections are a common complication of pregnancy. Symptomatic UTI occurs in 1% to 2% of pregnancies, while asymptomatic bacteriuria has been reported in 2% to 13% of pregnant women [4]. Several anatomical and hormonal changes in pregnant women lead to ureteral dilatation and urinary stasis [5]. Which contribute to the increased risk of developing urinary tract infections. Untreated UTIs can lead to complications, such as pyelonephritis, low-birth-weight infants, premature delivery, and, occasionally stillbirth [6].

Many drugs have been introduced for UTI such as norfloxacin, ciprofloxacin, gentamycin, etc., the problem of drug resistance and toxic manifestations of long term use of drugs are common. Unfortunately, decades of antibiotic use has given rise to antibiotic resistance. The increasing drug resistance among these bacteria had made therapy of UTI difficult and has led to greater use of expensive broad spectrum drugs. This resistance problem needs a renewed effort resulting in searching effective antibacterial agents against pathogenic microorganisms resistant to current antibiotics [7].

Garlic, a member of the Allium family (Liliaceae), has been used traditionally for ages to treat a wide array of diseases, namely, respiratory infections, ulcers, diarrhea, and skin infections, to mention just a few [8]. Reuter *et al.* [9] reported garlic as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and cardiovascular-protecting effects. This broad spectrum of activity has been attributed to the over 100 phytotherapeutic sulfur compounds

present in varying concentrations in garlic. They include allicin and thiosulfinates, which are formed by crushing-induced metabolic action of the enzyme allicinase (acysteine sulfoxide lyase) on the odorless amino acid allicin [10].

The present study was undertaken to investigate the antibacterial activity of garlic extract against bacteria isolated from pregnant women with asymptomatic bacteriuria.

Methods

Study Design: This study was conducted in Al-Batool teaching hospital for maternity and children in Diyala Governorate, Iraq during the period from 1st /April /2012 till 1st/ July/2012. A total of 105 pregnant women's were screened for significant asymptomatic bacteriuria. All the subjects were clinically identified to have no signs and symptoms of UTI.

Culture of Bacteria: The work of present study was done at department of microbiology - College of Medicine - Diyala University. For isolation, agars were prepared according to the manufacturer company; clinical specimens were collected using sterile swabs vaginal, urine samples were examined microscopically and cultured by placing on blood agar and MacConkey agar, then incubated at 37°C for 24 hours [11]. In next day many bacteriological and biochemical tests performed to recovered types of bacteria.

Antibiotic susceptibility Testing: Kirby and Bauer test were done to determine the susceptibility patterns toward 7 antibiotics most commonly used for the treatment of UTI were employed such as cefalexin, cefotaxime, erythromycin, nalidixic acid, amoxicillin, penicillin G and ciprofloxacin, were done by disc diffusion method [12]. The cultures which were resistant to the preceding antibiotics, which were selected according to the diameter of inhibition zone for testing the garlic extract (200 mg/ml stock solution).

According to this method, bacterial suspension of 0.1 X 10^{-6} CFU concentration was distributed on the surface of Muller-Hinton ager media for all bacterial species, then the antibiotic disc were put on the surface of culture media by sterile forceps. The plates were incubated under aerobic condition at 37°C for 24 hours. The results were read by measuring the inhibition zone in mm.

Garlic Extracts Preparation: Fresh bulbs of garlic (*Allium sativum*) were purchased from local markets in Diyala, Iraq. The cloves were separated and peeled to obtain the edible portion. Fifty grams of the edible portion was chopped and homogenized in 250 ml of autoclaved distal water and leave for 24 hours, then filtered by passage through a 25-mm pore-size filter to give a crude aqueous extract of 200 mg of garlic/ml. This was collected in a sterile vial and finally keeps in refrigerator at 4^{O}C until used.

Antibacterial Activity of Garlic Extract: The MIC and MBC of the garlic extract (200 mg/ml stock solution) against the bacteria which were resistant to the preceding antibiotics, which were selected for testing by agar dilution method [13]. As describe briefly, garlic extract stock solution at concentration of 200 mg/ml were used to prepared graduated concentrations at 10, 20, 40, 60, 80, 100 and 200mg/ml in normal slain, bacterial cell suspension which equilibrated their concentration to a 0.5 McFarland standard was distributed on Mueller-Hinton agar plate; plate were then incubated at 37°C for 24 hours. Minimum inhibitory concentration (MIC) of the garlic extractsolution that produced three or fewer colonies after incubation [14]. Minimum bactericidal concentration (MBC) also investigated for the bacterial strains which were determined as sensitive to the compounds in disc diffusion assay.

Data analysis: Fisher's exact test was used to obtain statistically significant differences between study groups with $P<0.05$ being considered statistically significant and $P>0.05$ was non-significant.

Results

The result of current study demonstrated that 52 out of 105 urine samples (49.52%) were positive for bacterial culture, while 53 out of 105 urine samples (50.47%) were negative for bacterial culture, these results occur after culturing.

According to standard bacteriological and biochemical tests 15 contaminated with *E. coli*, 11 of *Proteusmirabilis*, 10 of *Klebisella*, 8 of *Staphylococcus saprophytic*, 6 of *Staphylococcus aureus* and 2 of *Streptococcus pyogenes*. So the highest percentage of distribution is found in *E. coli* (28.84%) followed by *Proteusmirabilis* (21.15%), *Klebsiella* (19.23%), *Staphylococcus saprophytic* (15.38%), *Staphylococcus aureus* (11.53%) and finally *Streptococcus pyogenes* (3.84%). As showed in (Figure 1).

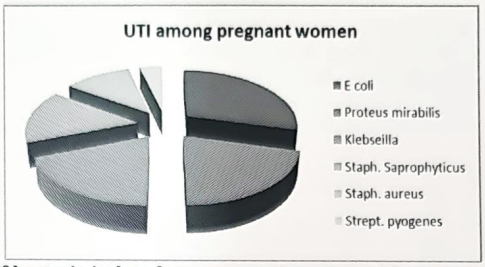

Figure 1: Type of bacteria isolate from pregnant women withasymptomatic bacteriuria

Age of pregnant women with asymptomatic bacteriuria ranged from 17 to 37 years. Minimum age was 17 years and maximum was 37 years (mean range = 25.5 years). As showed in (Table 1) highly significant differences noticed between three age groups, 21-30 years was the higher percent (67.3%), while 31-40 years was the lower percent (13.5%).

In the present study it was observed that Age of pregnant women with asymptomatic bacteriuriawas increased in the middle age group.

Table (1): Distribution of pregnant women with asymptomatic bacteriuria according to their age strata.

Age stratum	Number	Percentage	Comparison of Significance Chi²-value	Sig.
<= 20	10	19.2%	Highly Sig. P<0.0005	
21-30	35	67.3%		
31-40	7	13.5%		
Total	52	100%		

According to weeks of gestation the present study, it found that 50 pregnant women (96.2%) within third trimester, while second trimester, included 2 patients (3.8%) on the other hand not record any infected women during first trimester. However statistical significant differences were found among them (p<0.0005) as shows in **Table (2)**

Table (2): Distribution of pregnant women with asymptomatic bacteriuria according to their weeks of gestation

Weeks of gestation	Number	Percentage	Comparison of Significance Chi²-value	Sig.
First trimester (0-12 weeks)	0	0	138.9 P<0.0005	Highly Sig.
Second trimester (13-28 weeks)	2	3.8%		
Third trimester (29-41 weeks)	50	96.2%		
Total	52	100%		

The number of parity for the total number of pregnant women with asymptomatic bacteriuria is illustrated in Table (3), which revealed that 16 patients out of 52 (30.8%) had p0 and 12 out of 52 (23.1%) had p2 followed by p3, p4, p5. However there is no statistical significant differences noticed between each one of them (P=0.005) based on chi-square test analysis.

Table (3): Distribution of pregnant women with asymptomatic bacteriuria according to the parity.

Parity	Number	Percentage	Comparison of Significance	
			Chi²-value	Sig.
P0	16	30.8%	16.6	=0.005
P1	12	23.1%		
P2	9	17.3%		
P3	7	13.5%		
P4	6	11.5%		
P5	2	3.8%		
Total	52	100%		

The results of sensitivity test on bacterial growth which demonstrated that, the majority of bacterial isolate show resistant to more than one antibiotic. *E. coli*, *Proteus mirabilis* and *Klebsella* show resistant to pencilline G, amoxyicillin and nalidixic acid. While other type of bacterial isolate shows resistant to different antibiotic as showed in table 4.

Table 4: Number and percentage of the bacterial isolates resistant to different antibiotics.

Antibiotic	*E.coli*	*P.mirabilis*	*Klebsella*	*S. saprophytic* exact test	*S. aureus*	*S. pyogenes*	Fisher's
Cefotaxime	1(6.7%)	4(36.4%)	1(10%)	0	0	0	
Cefalexin	6(40%)	7(63.6%)	5(50%)	<0.005			
Erythromycin	6(40%)	3(27.3%)	4(40%)	4(50%)	2(33.3%)	2(100%)	
Amoxicillin	9(60%)	8(72.7%)	9(90%)	<0.005			
Nalidixic acid	3(53.3%)	5(45.5%)	9(90%)	0	0	0	
				<0.005			
				3(37.5%)	3(50%)	0	
				<0.005			
				5(62.5%)	2(33.3%)	1(50%)	
				<0.005			
Nitrofurantion	1(6.7%)	0	1(10%)	6(75%)	3(50%)	1(50%)	
Pencillin G	11(73.3%)	9(81.8%)	9(90%)	<0.005			
				1(12.5%)	4(66.7%)	0	
				<0.005			

Figure (2) described the sensitivity of bacterial isolate from pregnant women with asymptomatic bacteriuria. Nitrofurantion, cefotaxime, erythromycin and cefalexin show good activity toward most bacterial isolate comparing with other antibiotics.

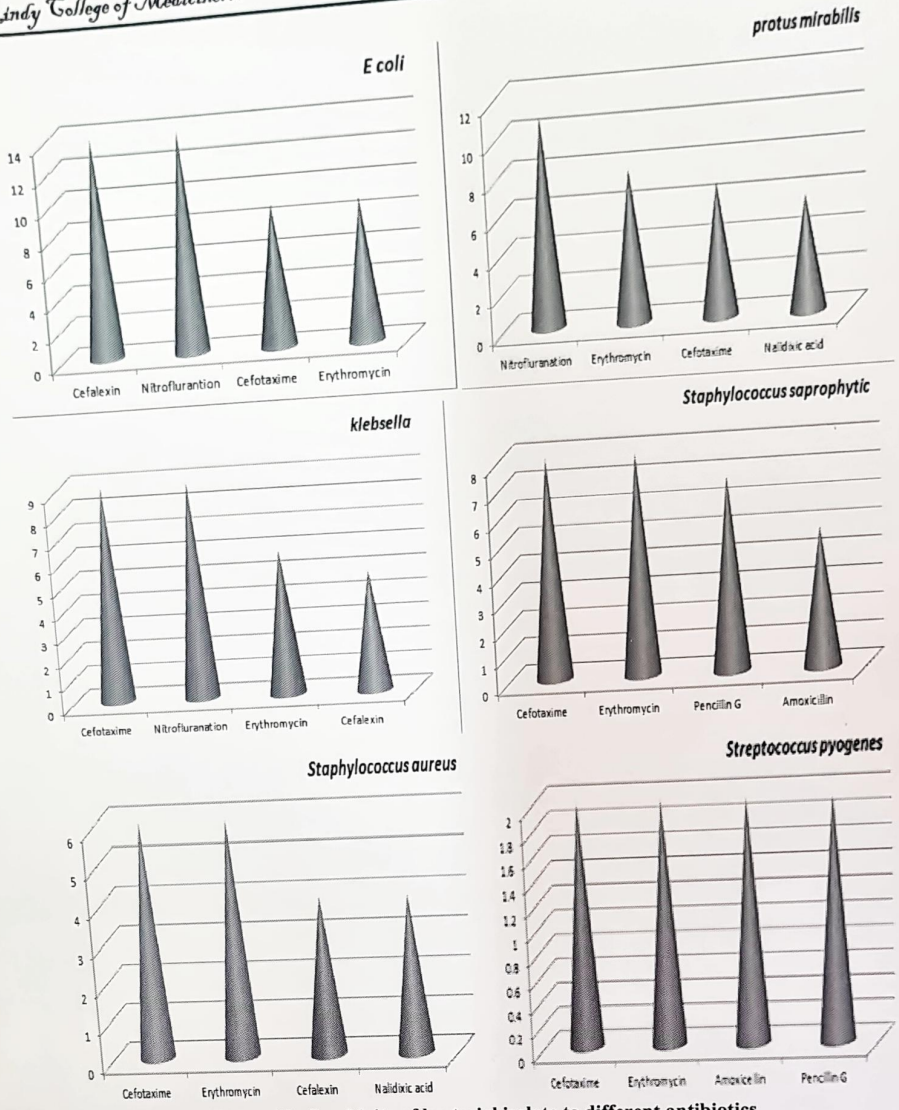

Figure (2): Sensitivity of bacterial isolate to different antibiotics

Bacterial isolates were taken from each bacterium which shows high resistant to antibiotics for testing their sensitivity to garlic extract. All concentration of the garlic extract solution show effect on bacteria than distal water in different percentage.

Regarding to the values of MIC and MBC as shown in (table 5), the results of present work which demonstrated that garlic extract were more effective as antibacterial agent against *E. coli, Klebsella* and *Staphylococcus saprophytic* comparing with *Proteusmirabilis*

Table 5: Values of MIC and MBC of garlic extract.

Type of bacteria	Garlic extract MIC mg/ml	MBC mg/ml

	20 mg/ml	40
E. coli	mg/ml	
Proteus	40 mg/ml	60
	mg/ml	
Klebsella	40 mg/ml	60
Stap.saprophytic	mg/ml	
	20 mg/ml	40
	mg/ml	

Discussion

Urinary tract infection is an old problem that continues to present new challenges due to change in theetiology of urinary tract infection and in the antimicrobial susceptibility of urinary pathogens over the years. Factors such as the changing in patient population and extensive use andabuse of antimicrobial agents could contribute to changesin the microbial profile of urinary tract isolates [15].

Urinary tract infections are more concerning in pregnancy due to the increased risk of pylonephritis. During pregnancy, high progesterone levels elevate the risk of decreased muscle tone of the ureters and bladder, which leads to a greater likelihood of reflux, where urine flows back up the ureters and towards the kidneys, if bacteriuria is present they do have a 25-40% risk of a pylonephritis [16].

In this study we found that the prevalence of urinary tract infection in pregnant women admitted to the Al-Batool teaching hospital for maternity and children was 49.52% most of these (69.23%) belong to gram negative organisms Enterobacteriaceae constitute the main group, while gram positive cocci was responsible for only (30.76%). Our results agreed with [17]. who found that most isolate of urinary tract infection belong to gram negative bacteria 77% during performing his study among patients in Egypt. Also my study agreed with study done in Kuwait by Mady and Helmi [18]. Who found that 83.1% of isolate belong to gram negative organism.

In this study, *E.coli* was the commonest organism causing urinary tract infection with ratio 28.84%, the incidence of *E.coli* is comparable to those of Okada *et al.*, (1994), Nunezsanchez *et al.*, (1999) and Al-Jiffri (2011) which reported most predominant pathogen causing urinary tract infection is *E coli* in 32.2%, 40% and 43.9% respectively. However, other study performed in France gave a higher value 75% [22]. Study done by Nicolle[23]. Who referred to, percentage of *E. coli* in urinary tract infections is 80-85%.

Escherichia coli bearing adhesions of the Dr/Afa family frequently causes urogenital infections during pregnancy in humans and has been associated with mortality in pregnant rats. Two components of the adhesin, Dra/AfaE and Dra/AfaD, considered virulence factors, are responsible for bacterial binding and internalization [24].

The change in immune responses may result from the increase in progesterone level during pregnancy, which modifies maternal immune responses in order to protect semiallogenic fetal antigens from rejection, the increased progesterone level might encourage infection with Dr/Afa$^+$ *E. coli*, up-regulating the expression of DAF, which acts as an epithelial receptor for this bacterium [25, 26].

Other bacterial cause's infection include: *Proteus mirabilis* and*Klebsiella*,. These are fellow up *E. coli*, these results agree with results of Ronald [27]. Who refer many other urinary tract infection causing genera are also isolated from patients with variable degree of infection such as *Klebsiella, Enterobacter, Proteus, Serratia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

The percentage of *Staphylococcus saprophytic* was (15.38%) agreed with [23]. Who found that the *Staphylococcus saprophytic* is cause of 5-10% of urinary tract infections cases in his study.

Based on microscopic examination and different biochemical tests, following bacterial isolates were identified from positive urine culture investigated in present study *Staphylococcus aureus* and *Streptococcus pyogenes* constituted the lower percent. These results agree with the observations reported by Shalini and Sharma (2010) who concluded that, Gram-negative bacteria *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus vulgaris* were the most common of uropathogens responsible for UTI with 90% in comparison to 10% of Gram-positive bacteria *Staphylococcus aureus*[28].

Regarding the urinary tract infection rate revealed that the prevalence of UTI was higher

in age group 21-30 (67.3%) than others as recorded. This may be related to the rising incidence with young age group according to type of bacteria. Also the Gram-positive *Staphylococcus saprophyticus* plays a role in the bacterial panorama, especially among young women. *E. coli* dominates as causative agent in all patient groups [29]. This result disagree with [21] who found that the incidence of bacteriuria was higher in patient's ages 41-52 years old (35.3%) and the percentage decrease by 15.5% with age 29-40 years old and less incidence among age of 77-87 years (1.7%). This may be related with collect the samples from male and female at different age groups.

According to type of weeks of gestation the present study demonstrated that most women with asymptomatic bacteriuria occurred within third trimester (96.2%). Pregnant women are at increased risk for UTI (starting in week 6 through week 24), because uterus sits directly on top of bladder and displaces it Shift in position of urinary tract and hormonal changes during pregnancy make it easier for bacteria it travels up urethras to the kidneys. For these reasons, many doctors recommended periodic testing of urine [30]. Also this study showed that no significant association between parity and urinary tract infection.

In this study nitrofurantionwas the mostcommon antibiotics to which the organismswere sensitive to pregnant women without urinary tract infection followed by cephalexin, and lastly erythromycin.The may be related with cephalexin or nitrofurantoin are typically used because they are generally considered safe in pregnancy [31].

The results of sensitivity test on bacterial growth which demonstrated that, the majority of bacterial isolate show resistant to more than one antibiotic this resistant related with the widely used of these antibiotic. In addition, the development of the bacterial resistant due to change in the site of antibiotic activity and bacterial membrane permeability or may be enzymatic resistant [32].

The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections [33].

So the present study was undertaken to investigate the antibacterial activity of garlic extract and due to garlic has been known for ages to have anti-infective properties against a wide range of microorganisms. Its application has been diversified on good number of diseases and infection such as diabetes, tuberculosis, hypertension, viral, fungal, bacterial, worm infestation and different types of cardiac problems. It has been tested against a good number of gram negative and gram-positive bacteria and was found to be effective against them [34].

In the present study the sensitivity of these isolates to garlic extract belongs to the antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis [36, 37].

This results is nearly compatible with the results of Iwalokun *et al.*,(2004) who reported that activity of garlic extract against Multidrug-Resistant Bacteria and Candida Species in Nigeria. And Harjai *et al.*, 2010. Who evaluated as a prophylactic agent in vivo in a mouse UTI model and in vitro data showed decreased elaboration of virulence factors and reduced production of quorum-sensing signals by *P. aeruginosa* in his study also suggest that decreased virulence of *P. aeruginosa* in garlic-fed mice can be attributed to the quorum-sensing inhibitory property of garlic. This might have contributed towards reduced production of virulence factors in vitro. Also my study in agreement with the study done by Ankri and Mirelman [40]. Demonstrated that Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities and exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; antifungal activity, particularly against *Candida albicans*; antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia* and antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism.

In conclusion, urinary tract infections in pregnant women are considered the most serious health problems facing the world, *E. coli* is the most frequent isolated species, garlic extracts was effective plants against selected isolated and can be used for treatment specially antibiotics

are sometimes associated with side effect, better patients tolerance, relatively less expensive due to long history of use and being renewable in nature.

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Interleukin-6, Tumor Necrosis Factor-α and high sensitivity –C Reactive Protein in Iraqi patients with Fibromyalgia Syndrome of Acute Cholecystitis?

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Abstract

Background : Fibromyalgia Syndrome (FMS) is a common chronic widespread pain syndrome , usually associated with other somatic and psychological symptoms including fatigue , sleep disturbance , and cognitive difficulties like difficult concentration . The prevalence of fibromyalgia is reported to be 2-4% with a female to male ratio of about 9:1, its prevalence increase with age from (30-50) , declining thereafter .

The etiopathology of fibromyalgia is not clear , though cytokines like interleukin-6 is a potent stimulator of hypothalamic-pituitary axis via activation of the hypothalamic corticotrophin-releasing hormone (CRH) .

Objective : To evaluate interleukin-6 (IL-6), tumor necrosis factor -alpha (TNF-α)and high sensitivity-C Reactive Protein (hs-CRP) and their relation with symptoms included in the underlying etiopathology of fibromyalgia patients since interleukin-6 is a potent stimulator of hypothalamic pituitary axis via activation of the hypothalamic CRH .

Setting : The study was performed at the Rheumatology and Rehabilitation Consultation unit , Baghdad Teaching hospital/medical City during the period from November 2010 to July 2011 . The laboratory tests were done in The Teaching Laboratory / Medical City .

Subjects : The study included 57 patients with FMS (49 females + 8 males) , their mean age (43 ± 10.9) years , and 34 healthy control individuals (28 females + 6 males) who their age and sex matching with the FMS patients . Interleukin-6, tumor necrosis factor-α , and high sensitivity – C Reactive Protein were estimated by using ELISA technique

Results : The results show that the mean (± S.D.) values of serum IL-6 , TNF-α , and hs-CRP concentrations were significantly higher in fibromyalgia patients than healthy control ($p<0.005$).

Conclusion : The elevated IL-6 and TNF-α in FMS patients considered a promotion to fatigue , hyperalgesia , pain and depression . TNF-α is also associated with stress , rapid eye movement

Key words : fibromyalgia syndrome, IL-6, TNF-α, hs-CRP, disturbed sleep, fatigue, and depression.

Introduction :

Fibromyalgia syndrome (FMS) is a chronic disorder of diffuse pain in the muscle or joints , accompanied by tenderness on examination at specific , predictable anatomic sites known as tender points *(Buskila and Cohen 2007, and Simms 2007).* It also can be defined as an idiopathic , non-articular pain defined by a widespread musculoskeletal pain and generalized tender points *(Chakrabarty and Zoorob 2007).*

Fibromyalgia has been estimated to affect up to 2-4% of the general population of industrialized countries *(Clauw and Corffford 2003)* . Although , it can affect both genders , females are almost 10 times more likely to be diagnosed with FMS, and 85% of the fibromyalgia population seeking treatment is females *(❖ Wolfe et al., 1990).*Thus FMS has a prevalence of approximately 2% in the general population and is the third most common diagnosis in rheumatology clinics , ❖ and approximately 30% of patients who have FMS also suffering from a clinical depression . It is known to affect children and adolescents as well as adults ; however , the average age at onset is about 45-48 years *(Pae et al., 2008)* . It was reported that , most patients present with FMS between the age of 30-50 years , and the prevalence increased with age *(Anthony and Longford 2010).*

FMS is associated with a wide range of symptoms . Most studies reported that these symptoms can be remarkably persistent and pervasive over the years . Most symptoms , however , do tend to improve over time . The symptoms include :-

Chronic , widespread pain , and heightened pain , tender Points, Depressive Disorder , Fatigue and sleep problems *(Wolfe et al., 1990)* .

The diagnosis was narrowed down to the most widely accepted set of classification criteria for research purposes which was elaborated in1990 by the Multicenter Criteria Committee of the American College of Rheumatology . These criteria , which are known informally as " the ACR 1990 " , define fibromyalgia according to the following criteria :- *(Wolfe et al., 1990,Chakrabarty and Zoorob 2007, and Simms 2007*)

A history of widespread pain lasting more than three months , affecting all four quadrants of the body , i.e., both sides , and above and below the waist.

Tender points ; there are 18 designated possible tender points (although with the disorder may feel pain in other areas as well) . During diagnosis , four kilograms of force is exerted at each of 18 points .

Although now there is a new ACR criteria for diagnosis of FMS depending on symptoms severity (SS) *(Wolfe et al.,2010)*

The cause of FMS is currently unknown , several hypotheses have been developed including :-

1-Brain disorder :- The frequent association of fibromyalgia with stress related disorders , such as chronic fatigue , posttraumatic stress disorder ,

irritable bowel syndrome , and depression , as well as similarity of many central nervous system (CNS) abnormalities , suggest at least a partial common substrate for these disorders *(Schweinhardt et al., 2008)* .

2-Stress :- Stress may be an important precipitation factor in the development of FMS . FMS is frequently co-morbid with stress-related disorders such as chronic fatigue , posttraumatic stress disorder , irritable bowel syndrome and depression *(Clauw and Corfford 2003)* . Researches have proposed that , because exposure to stressful conditions can alter the function of the hypothalamic-pituitary-adrenal (HPA) axis , the development of FMS may stem from stress-induced disruption of the HPA axis *(Mc Beth et al., 2005).*

3-Dopamine dysfunction (hypodopaminergia) :- The dopamine hypothesis of fibromyalgia proposes that the central abnormality responsible for symptoms associated with FMS is a disruption of normal dopamine – related neurotransmission . Dopamine is a catecholeamine neurotransmitter with roles in pain perception and natural analgesia *(Cervenka 2006)* .

4-Abnormal serotonin metabolism :- Researchers hypothysized that serotonin , a neurotransmitter that regulates sleep patterns , mood , concentration and pain , could be involved in the pathophysiology of fibromyalgia – associated symptoms *(Palmer et al., 2010)* .

5-Abnormal HPA axis :- An abnormality of hypothalamic-pituitary-adrenal axis showing hyperactive pituitary release of adrenocorticotropic hormone and relative hyporesponsiveness of the adrenal cortex was reported *(Griep et al., 2002)* .

6-Obesity :- Obesity is a well-known aggregating factor for certain rheumatologic conditions , emerging evidence are exploring the hidden link between obesity and rheumatic diseases , such as fibromyalgia *(Ursini et al., 2001).*

Epidemiological data show that fibromyalgia patients have higher prevalence of obesity (40%) and overweight (30%) . Multiple studies have been proposed to explain " the hidden link " , but at the same time is not possible to ascertain whether obesity is a cause or consequence of fibromyalgia *(Ursini et al., 2001).*

Cytokines ; are low-molecular-weight (15-25 KDa) regulatory proteins or glycoproteins secreted by white blood cells , and various other cells in the body in response to the release of endogenous " danger signals " that betray the presence of cell dying by necrosis . These proteins assist in regulation and development of immune effector cells , inflammation , immunity differentiation , migration , and repair . Some cytokines posses direct effector function of their own . *(Kindt et al., 2007)*

Cytokines produced at inflammatory sites signal the brain to produce sickness-related behaviors, including depression and other symptoms such as fever (*Watkins et al., 1995 and Dantzer et al., 1998).*

Cytokines signal the brain not only to activate the HPA axis but also to facilitate pain and induce a series of mood and behavioral responses generally termed sickness behavior *(Watkins and Maier 2000 and Dantzer 2001).*

Inflammatory processes may play a significant role in cycles of pain and sleep disturbance. The association between pain and sleep disturbance is bidirectional, *(Heffner 2011).* The relationship between cytokines and depression is complicated as a variety factors could directly or indirectly influence cytokine activity . While cytokine elevations are most pronounced in severe depression, their activity may also be related to chronicity of illness, neurovegetative features of depression (altered sleep patterns, food intake, weight changes, fatigue) or high stress perception characteristic of depression *(Anisman and Merali 2002).*

In a study of cytokines and their interaction with sleep , it was found that since IL-6 present in the peripheral and the central nervous system , this comprise a link between peripheral immune stimulation and CNS-mediated behaviors and experiences such as sleep , and fatigue . The debilitating fatigue experienced in chronic fatigue syndrome and related diseases may also be related to altered cytokine profiles *(Mullington et al., 2001).*

In a study it was found that IL-6 produces fatigue and pain in healthy people , decreases cognitive function , correlates with depression , influences the hyperalgesia of corticosteroid withdrawal and promotes B- and T-cell proliferation *(Wallace et al., 2001).*

In clinical studies higher levels of IL-6 have been associated with greater pain severity in individuals with rheumatoid arthritis and fibromyalgia, as well as with greater post-operative pain *(Starkwheather 2005).*

In other studies , there was other hypothesis , which indicate that there is some abnormality with the hypothalamic-pituitary-adrenal (HPA) axis , with elevated activity of corticotrophin-releasing-hormone (CRH) and substance P , that may not only affect the HPA axis , but other endocrine and immune response *(Neeck 2002 and Mease et al., 2005).*

Clinic patients with fibromyalgia have been reported to have hyperactive HPA axis function ,

and this hyperactivity is more marked when compared with other chronic pain patients with less widespread symptoms *(Mc Beth et al., 2005)*

.

Several studies have reported increased IL-6 in plasma and serum of FMS patients *(Wallace 2001, Gür 2002, Kashipaz 2003, Bazzichi 2007, Wang 2008, and Hernandez 2010)* . These studies were done using ELISA method . Other studies reported increased IL-6 level in PBMC *(Wallace 2001, Mollie 2003, Ortega 2010, and Geiss 2011)*

TNF-α promotes rapid eye movement sleep and allodynia , including pain producing excitatory amino-acids , and regulatory substance-P expression *(Wallace et al., 2001)* . While Dina et al.,(2011) found that the increase in the TNF-α in the muscles induce skeletal muscle hyperalgesia mediated by its cognate receptors on nociceptors .

In a study , it was found that the soluble receptors of TNF-α , and IL-6 present in the periphery and the CNS , comprise a link between peripheral immune stimulation and CNS-mediated behaviors and experiences such as sleep , sleepiness , and fatigue *(Mullington et al., 2001)* .

It was found that acute pain induction is associated with elevation in serum TNF-α levels that last at least one hour. These data are consistent with the notion that the experience of pain is associated with enhanced release of pro-inflammatory cytokines, which in turn sensitize the nervous system, promoting a further amplification of pain transmission *(Edwards et al., 2009).*

Increased levels of cytokines , key inflammatory mediators such as C-reactive protein have been associated with symptoms of pain , fatigue , and distressed mood in multiple conditions . These symptoms mimics the representative symptoms of FMS . However, up to date results examining the association of cytokine alterations with FMS and its symptoms have been mixed *(Menzies 2012).* Hepatic CRP production is generally stimulated by IL-6 , and to a lesser extent by IL-1 and TNF-α . This may explain the higher CRP (together with a higher monocyte production of IL-6 , IL-1β , and TNF-α) in FMS patients compared to the healthy volunteers *(Hernandez et al., 2010)* .

Studies found that elevated levels of pro-inflammatory cytokines or other inflammatory markers (e.g., C-reactive protein , fibrinogen) are associated with depression [because severe or overwhelming stress , and any resultant posttraumatic stress disorder (PTSD) , alters and dysregulates the key systems that are part of the stress response , and the immune system responds to stress by releasing pro-inflammatory cytokines . These cytokines increase inflammation and serve the adaptive purpose of helping the body heal wounds and fight infection] , inflammation , and heart disease . Several studies have found elevated C-reactive protein in depressed patients with heart disease *(Kendall 2009)* .

Methods :

Patients and controls:

The study was performed during the period from November 2010 till July 2011. The subjects were selected from the people attending the out-patient clinic in Medical city – Baghdad Teaching Hospital – Rheumatology and Rehabilitation Consultation Unit , where the anthropometric tests were performed .

The present study includes 57 patients with Fibromyalgia Syndrome (49 females and 8males) , their mean age 43 ± 10.9 years . The clinical diagnosis of these patients was confirmed by the consultant rheumatologists of the former hospital according to the ACR 1990 criteria for the diagnosis of FMS . Thirty four control individuals apparently healthy (28 females and 6 males) who were age and sex matching with FMS patients , were included in this study as control . The tests were done in the Baghdad Medical City Laboratories , Medical City Teaching Labs.IL-6 kit was provided by RayBio-USA, TNF-α and hs-CRP kits were provided by DRG international,Inc-USA.

Criteria of inclusion : -

- Cases of primary FMS approved by clinical , laboratory , radiological diagnosis .
- Medical treatments taken up by patients never effecting the laboratory tests .

Criteria of exclusion : -

- Diabetes Mellitus (DM) .
- Rheumatoid Arthritis (RA) .
- Osteoarthritis (OA) .
- Systemic Lupus Erthromatosus (SLE).
- Sjogren Syndrome (SS) .
- Previous breast surgery .

Principle of the assay:

The determination of (IL-6, TNF-α, & hs-CRP) by Enzyme-Linked Immunosorbant assay (ELISA) for the quantitative measurement of human (IL-6, TNF-α, & hs-CRP) in serum , plasma, cell cultures coated on a 96 well plate . Standard and samples were pipetted into the wells and

Cytokine present in a sample is bound to the wells by the immobilized antibody . The wells are washed and biotinylated anti-human (IL-6, TNF-α,& hs-CRP) antibody is added . After washing away unbound biotinylated antibody , Horse Radish Peroxidase (HRP

) – conjugated streptavidin is pipette to the wells . The wells are again washed , a tetramethyl benzidine (TMB) substrate solution is added to the wells and color develops in proportion to the amount of (IL-6, TNF-α,and hs-CRP) bound . The Stop Solution changes the color from blue to yellow , and the intensity of the color is measured at 450 nm.*(Delvis et al 2006).*

Results:

The present study shows the values of serum IL-6 in FMS patients was significantly higher than the control ($p<0.0005$) , and TNF-α results in serum of FMS patients were highly significant when compared with control as well the results of hs-CRP in serum of FMS patients when compared with healthy control, as shownthe table below;

Table : Mean ± S.D. of IL-6 in serum of FMS patients and control .

Parameters	Controls mean±SD	Patients mean±SD	P.value
IL-6 (pg/ml)	9.13±2.99	17.23±7.19	0.000**
TNF-α (pg/ml)	5.85±2.98	68.52±38.28	0.005**
hs-CRP (mg/L)	5.12±1.26	13.40±6.12	0.000**

**H.S.: Highly significant.

Discussion :

It is now known that FMS is not an inflammatory condition at least not in the typical sense . But a lot of research shows that inflammatory cytokines are elevated , sometimes dramatically in those with FMS . In fact IL-6 is often markedly elevated in those with FMS . It was suspected that IL-6 elevation is the cause of fibro fog . Of interest , IL-6 has also been shown to be elevated in other conditions , such as lupus , that are also associated with impaired memory and learning *(Sparkman et al., 2006).*

These results are in accordance with Wallace et al.,2001, Gür et al.,2002, Kashipaz et al., 2003, Bazzichi et al., 2007, Wang et al., 2008, and Hernandez et al., 2010 , who stated that IL-6 increase significantly in serum of FMS patients .

It was found that higher IL-6 levels were strongly associated with higher ratings of current pain . This finding suggests that individuals may attribute daytime fatigue to pain , rather than to its more likely source , sleep disturbance (*Heffner et al., 2011).*

In samples of pain , patients IL-6 and inflammatory markers correlate with higher pain severity . These findings support the conclusion , that pro-inflammatory cytokines are likely to play a facilitatory role in the development and maintenance of persistent pain syndrome , including neuropathic pains *(Edwards et al., 2008).*

Many mechanisms were concerned with the elevation of IL-6 , some suggest that IL-6 induces a profound ACTH secretory response that is thought to be mediated by the stimulation of hypothalamic CRH release (*Torpy et al., 2000*). 1-

This finding are of particular interest as mast cells have been proposed as the target of CRH outside the brain , leading to enhanced inflammatory processes that could contribute to pain *(Theoharides et al2-2004).*

TNF-α is widely considered the protolytic pro-inflammatory cytokine due to its principal role in initiating the cascade of activation of other cytokines and growth factor in the inflammatory response. There have been correlations between tissue levels of TNF-α and pain and hyperalgesia in a number of3- painful diseases . TNF-α has also been linked to the generation and maintenance of neuropathic pain *(Sommer and Kress 2004)* . Experimental sleep deprivation has been found to alter immune responses and is reported to increase circulating levels of inflammatory markers such as IL-6 , TNF-α , and CRP with significant elevations after only one night of sleep loss *(Irwin et al., 2006)* .

Sleep restrictions induced increases of TNF-α levels only immediately following awakening . Loss of sleep during part of the night is one of the most5- common complains of persons who experience environmental or psychological stress, or have a psychiatric disorder. The results show that a modest amount of sleep loss activates cellular and genomic markers of inflammation , and these responses are associated with up-regulation of molecular signaling pathways that mediate increases in the transcription of the IL-6 and TNF genes *(Irwin et al.,2006)* .

Thus , there seems to be a relationship between dynamic changes in sleep and variously cellular , hormonal , and immunological functions. Pain may influence the sleep processes and alters these essential parameters , thereby interacting with the course of the disease . On the other hand , sleep disturbances may decrease the pain threshold *(Editorials 1999).*

CRP in one study was found to be inversely associated with pain threshold , consistent with peripheral sensitization. Sleep problems were inversely associated with pain threshold at all sites , suggesting a defect in central pain processing . The association between CRP and pain threshold was only evident after accounting for the effects of non-inflammatory factors , such as sleep and psychiatric distress . The association between pain threshold , CRP and sleep problems did not differ based on the presence of fibromyalgia , consistent with other studies advocating a syndrome of widespread pain that spans a spectrum of symptoms and severity , rather than a discrete entity *(Lee et al., 2009).*

Conclusions:

The results suggested that FMS is not a homogeneous diagnosis but shows varying proportions of co-morbid anxiety and depression dependent on psychological characteristics of the patients .

The results support the contention that pain and stiffness in fibromyalgia may be accompanied by a suppression of some aspects of the inflammatory response system and that the presence of clinically significant depressive symptoms in fibromyalgia is associated with some signs of inflammatory response system activation .

The demographic study revealed that FMS is more predominant in middle-aged and older persons because of the stress of life, and women were more than men effected by the syndrome , and this due to biological , psychological , and sociocultured factors . As for the BMI , obese and overweight persons were more susceptible to FMS .

Cytokines are produced on demand and travel only over short distances. Due to this local action at low concentrations , their serum levels may not reliably reflect local activation .

The elevated serum IL-6 in FMS patients considered a promotion to fatigue , hyperalgesia , pain and

depression. One of the mechanisms suggest that , IL-6 induces a profound ACTH secretory response that is thought to be mediated by the stimulation of hypothalamic CRH release

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Prevalence of Microscopic Colitis in Patients with Chronic Watery Diarrhea

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Abstract:

Chronic watery diarrhea is a common cause for consulting a physician in general practice or in internal medicine and referral to gastroenterologist
Microscopic colitis now has emerged as a common cause of chronic watery diarrhea ,its prevalence is not clearly known. Some patients have mild symptoms that may be misinterepted as irritable bowel syndrome . A small percentage of patients with IBS are referred to outpatient gastroenterology clinics with symptoms include watery diarrhea or constipation or combination of bot Macroscopically , microscopic colitis characterized by normal or almost normal colonic mucosa and diagnosed by microscopic examination of a biopsy or sample of colon tissue obtained during colonoscopy . The exact cause of microscopic colitis is not known. There are several theories , one thought is that bacteria or a virus initiated the inflammation response. Another possibility is a malfunction in the immune system of an affected person. There are several reports of drug induced microscopic colitis and some have concomitant autoimmune disease .

Aim : The purpose of this study was to investigate the prevalence of microscopic colitis in patients with chronic watery diarrhea and compared with patients with diagnosis of diarrhea predominant irritable bowel syndrome.

Methods : Between January 2008 and January 2013, 138 patients (between 20 and 78 years) were recruited into the study prospectively who had undergoing colonoscopy for indication of chronic watery non bloody diarrhea with no apparent cause . Eighty four patients with chronic idiopathic watery diarrhea and Fifty four patients were diagnosed as IBS using the Rome III criteria and had watery diarrhea as predominant symptom with macroscopically normal colonoscopy findings .After looking into detailed clinical history of patients including number of daily bowel motion ,duration of diarrhea , history for autoimmune disease (rheumatoid arthritis , diabetes mellitus) , drug intake (Aspirin & NSAID)and laboratory evidence of chronic inflammation (ESR) , biopsy taken from each segment of colon and from any abnormal looking areas .The diagnostic criteria for microscopic colitis is applied according the universally accepted histological criteria . Statistical analysis were produced by the use of Chi square test and at the level of significant alpha < 0.05 to compare our results in those with IBS and those with chronic watery diarrhea with no definite cause .

Results : A total of 138 patients were included in the study .The prevalence of microscopic colitis (MC) in all patients with chronic watery diarrhea was 10.8 % (15/138) , 13 % (11/84) in those with no IBS ,while in the diarrhea predominant subgroup of IBS patients was 7.4 % (4/54) , i.e. 26.6 % (4/15) of patients with microscopic colitis full filled the Rome III criteria of IBS.Diabetes mellitus present in 8.3 % (7/84) and Rheumatoid arthritis present in 3.5 % (3/84) of patients with idiopathic chronic watery diarrhea Aspirin intake history present in 7.1 % (6/84) , NSAID intake history present in 4.75 % (3/84) of patients with idiopathic chronic watery diarrhea .

Conclusion

Microscopic colitis prevalence within the normal level similar to that in worlds in spite of high level of infectious causes of diarrhea in our society , this may support the non infectious cause of chronic watery diarrhea .MC can be identified in patients with chronic watery diarrhea and subset of diarrhea predominant IBS .

Keywords : Microscopic colitis ,Chronic watery diarrhea ,Irritable bowel syndrome .

Introduction:

Chronic watery diarrhea is a common cause for consulting a physician in general practice or in internal medicine and referral to gastroenterologist [1] . Some patients with chronic idiopathic watery diarrhea have an apparent non specific inflammation of colonic mucosa, this refer to microscopic colitis with unclear significance.

In 1980, microscopic colitis (MC) was defined as having symptoms of watery diarrhea and specific histological characteristic features when the colonic mucosa is macroscopically normal or near normal [2] .Currently, MC is divided into two subtypes- collagenous colitis (CC) involving chronic mucosal inflammation and a wide subepithelial collagen band and lymphocytic colitis (LC) involving chronic mucosal inflammation and no subepithelial band .

In 1976, Lindstrom showed for the first time that collagen deposits accumulated as a wide subepithelial band in the rectum and colon in patients with persistent watery diarrhea of unknown cause [3]Some experts claim that LC is an early phase of CC [4] .

Microscopic colitis , previously regarded as rare , and certainly overlooked , now has emerged as a common cause of chronic diarrhea and also may causes constipation ,which may be short-term or chronic [5] most commonly occurs in middle aged to elderly patients and is more common among women than men .

The prevalence of microscopic colitis is not clearly known. It is estimated that 10% to 20% of persons in the United states with chronic diarrhea may have microscopic colitis .

The primary symptom of microscopic colitis is chronic, watery diarrhea. , often associated with nocturnal diarrhea . Individuals with microscopic colitis can have diarrhea for months or years before the diagnosis is made. Typically, begin very gradually and are intermittent in nature with periods when the person feels well, followed by bouts of chronic diarrhea. Typically , a person with microscopic colitis can have 4 to 9 daily bowel motion and even 20 daily bowel

motion are possible .Some individuals may experience diffuse abdominal pain or mild abdominal cramps while blood in the stool is unusual . Less common symptom include fatigue , nausea , fecal incontinence and weight loss that may significantly impair quality of life of patient .[6] Some patients have mild symptoms that may be misinterepted as irritable bowel syndrome , as MC and IBS may have similar symptom [7] . Unlike irritable bowel syndrome , which is chronic functional bowel disorder of unknown etiology without a curative treatment , microscopic colitis is an inflammatory bowel disease. Both conditions may share similar symptoms ,where Chronic watery , not bloody diarrhea is a typical symptom in addition to abdominal discomfort and pain, relief from discomfort upon defecation and/or abdominal pain is associated with increase in bowel motion . A small percentage of patients with IBS are referred to outpatient gastroenterology clinics with symptoms include diarrhea or constipation or combination of both . The prevalence of IBS varies in different populations, where in USA and Europe are range between 6.2% and 25% [8]. Macroscopically , microscopic colitis characterized by normal or almost normal colonic mucosa [9]. Microscopic colitis is diagnosed by microscopic examination of a biopsy or sample of colon tissue obtained during colonoscopy .The inflammation is not visible during colonoscopy .The disease can affect the entire lining of the colon or present in patches on the colon lining even in constipated or asymptomatic patients [10] .

Collagenous colitis is identified by layers of collagen (> 10 µm) which is a connective protein, in the lining of the colon. It is more common in women than in men. Generally diagnosed in individuals age 50 and older, adults under 45 years old and children, ages 5 to 12 years old have been diagnosed with collagenous colitis. Lymphocytic colitis is identified by the presence of increased intraepithelial lymphocytes for more than 20 /100 surface colonic epithelial cells with surface epithelial damage and infiltration of lymphocytes and plasma cells in the lamina properia in the response of the immune system, among the cells lining the colon , but the collagen layer is normal . Both men and women are equally affected and typically diagnosed during the 5th decade of life .The exact cause of microscopic colitis is not known. There are several theories , one thought is that bacteria or a virus initiated the inflammation response. Another possibility is a malfunction in the immune system of an affected person.

An interchanges between inflammatory bowel disease and microscopic colitis has been reported occasionally [11] .Whether there is merely a chance association of two fairly common disorder occurring in the same individual or due to common genetic predisposition or shared immunologic pathways remain unknown thus far . There are several reports of drug induced microscopic colitis and a strong likelihood of association has been found with aspirin and non-steroidal anti-inflammatory drugs intake [12] . Many patients with microscopic colitis have concomitant autoimmune disease . [13] .

Aim :The purpose of this study was to investigate the prevalence of microscopic colitis in patients with chronic watery diarrhea and compared with patients with diagnosis of diarrhea predominant irritable bowel syndrome.

Materials and methods :

Between January 2008 and January 2013, 138 patients (between 20 and 78 years) were recruited into the study prospectively who had undergoing colonoscopy for indication of chronic watery non bloody diarrhea with no apparent cause ..

Eighty four patients with chronic watery diarrhea and other symptoms pertaining to lower gastrointestinal tract undergoing colonoscopy for reasons not related to IBS and who had macroscopically normal or near normal colonic mucosa.

Fifty four patients were diagnosed as IBS using the Rome III criteria and had watery diarrhea as predominant symptom with macroscopically normal colonoscopy findings.

After looking into detailed clinical history of patients including number of daily bowel motion ,duration of diarrhea , history for autoimmune disease (rheumatoid arthritis , diabetes mellitus) , drug intake (Aspirin & NSAID)and laboratory evidence of chronic inflammation (high erythrocyte sedimentation rate (ESR) , biopsy taken . Biopsy were taken from each segment of colon and from any abnormal looking areas .They were immediately placed in separate container with 10 % formalin , processed conventionally in paraffin blocks and cuts into 5 µm thick sections and stained with hematoxylin-eosin (H&E) stain .The diagnostic criteria for lymphocytic colitis is applied according the universally accepted histological criteria which include increase lymphocytic infiltration of lamina properia , increased intraepithelial lymphocytes over 20 intraepithelial lymphocytes / 100 epithelial cells , lymphocytic infiltration of lamina properia , surface epithelium degradation and increase crypt epithelial cells mitosis . Subepithelial collagen band thickness was evaluated for diagnosis of collagenous colitis . Statistical analysis were produced by the use of Chi square test and at the level of significant alpha < 0.05 to compare our results in those with IBS and those with chronic watery diarrhea with no definite cause .

Result :

A total of 138 patients were included in the study, 78 were female (52.7%), and the mean age of them was 53.5 ± 11.2 for all patients (range 20-78). Fifty four patients were in the IBS groups and their average age was 47.1 ± 9.6, 29 (53.7%) were female and 25 (46.3%) were male. In the remaining group of 84 patients, 35 were males (41.6%), 49 were females (58.3%) and the average age was 59.1 ± 9.7 years. Physical examination did not reveals any abnormality .The mean duration of diarrhea was 12 months (range: 3-28 months).114 patients (82.6%) had more than 8 stool/ day . Colonoscopic examination reveals that most of the patients with normal colonic mucosa, where 92.8 % (78/84) of patients with chronic idiopathic watery diarrhea had normally looking colonic mucosa and 7.2% (6/84) with mild hyperemia , while those with IBS ,96.3% (52/54) patients had normal looking colonic mucosa and only 3.7 % 92/54) patients had mild hyperemic colonic mucosa . The prevalence of microscopic colitis (MC) in all patients with chronic watery diarrhea was 10.8 % (15/138) , 13 % (11/84) in those with no IBS ,while in the diarrhea

predominant subgroup of IBS patients was 7.4 % (4/54) , i.e. 26.6 % (4/15) of patients with microscopic colitis full filled the Rome III criteria of IBS.(Table-1)

Focal active colitis was found in 1.4 % (2/138) of all patients with chronic watery diarrhea , 1.8 % (1/54) of the IBS patients and 1.1 % (1/84) of patients with idiopathic chronic watery diarrhea , these differences were statistically insignificant . χ^2 =1.589 , P = 0.662 .(Table-1).

Diabetes mellitus present in 8.3 % (7/84) of patients with idiopathic chronic watery diarrhea , all of them show microscopic colitis on microscopic examination and only one patients with IBS have diabetes mellitus ,1/54 (1.85 %). Rheumatoid arthritis present in 3.5 % (3/84) of patients with idiopathic chronic watery diarrhea and no patients with IBS . Although this results may show an association of microscopic colitis with autoimmune diseases , especially with diabetes mellitus , these differences were statistically insignificant . χ^2 =0.413 , P = 0.52 (Table-1).Aspirin intake history present in 7.1 % (6/84) of patients with idiopathic chronic watery diarrhea and only three patients with IBS have history of aspirin intake ,3/54 (5.5 %). NSAID intake history present in 4.75 % (3/84) of patients with idiopathic chronic watery diarrhea and one patients with IBS . Although this results may show an association of microscopic colitis with history of chronic drug intake , these differences were statistically insignificant .χ^2 =0.280 , P = 0.597 .(Table-1).

Table -1: The association of chronic watery diarrhea with various clinical , macroscopic and microscopic features.

DATA	CWD	IBS		P VALUE
DM	7 (8.3%)	1 (1.8%)	0.413	0.52
R A	3 (3.5%)	0 (0 %)		
Aspirin	6 (7.1%)	3 (5.5 %)	0.280	0.597
NSAID	4 (4.75%)	1 (1.8 %)		
ESR	45 (52.5 %)	4 (7.4 %)	0.425	0.515
M/E MC	11 (13 %)	4 (7.4 %)		
ACTIVE	1 (1.1 %)	1 (1.8 %)	1.589	0.662
NON SPECIFIC	3 (3.5%)	1 (1.8 %)		
NORMAL	69(82.1 %)	48 (88.8 %)		
Colonoscopy				
Normal	78 (92.8 %)	52 (96.3 %)	0.712	
Mild hyperemia	6 (7.2 %)	2 (3.7 %)		

Discussion

Microscopic colitis is a rare disease characterized by chronic watery , non bloody diarrhea with normal radiological and endoscopic appearance and diagnosed only by histopathological examination of colonic biopsies . The prevalence of microscopic colitis is differ in different communities as it may related to predisposing factors .Our study showed the overall prevalence of microscopic colitis was 10.8 % (15/138) , 13 % (11/84) in those with idiopathic chronic watery diarrhea ,while in the diarrhea predominant subgroup of IBS patients was 7.4 % (4/54) , i.e. 26.6 % (4/15) of patients with microscopic colitis full filled the Rome III criteria of IBS . This results is close to those reported according to a study by Olesen *et al*., MC was diagnosed in 10% of all Swedish patients (1018 patients) with idiopathic watery diarrhea referred for colonoscopy , and he document that in the subset of patients older than 70, the prevalence was 20% [14] Our results is lower than those reported by Tuncer *et al.* who reported that there is a 23.3% MC prevalence in IBS patients compared to a 5% prevalence in controls [15] .

In our study there was slight female predominance in patients with chronic watery diarrhea without significance colonoscopic abnormality ,correspond to earlier Faloda *et al* report from India [16]

Focal active colitis is characterized by focal crypt damage caused by neutrophils and may be associated with infections, ischemia , partially-treated ulcerative colitis and IBS . FAC was present in 2 of 138 patients (1.45%) who had biopsies but who were otherwise normal, as determined by endoscopic evaluation.In our study we found that 1.8 % of IBS patients had focal active colitis (1/54) and this ratio is correspond to that reported in previous studies carried by Che *et al* . [17].

Irritable bowel syndrome is a public health problem since it is widely seen and does not have a definite cure Fifty four (39.1 %) of our patients with chronic watery diarrhea were clinically diagnosed as IBS , had normal colonoscopy ,with 7.4 % of them show microscopic colitis on colonic biopsy , finding similar to earlier reports ,where microscopic colitis was the predominant abnormality observed in biopsies from patients with IBS [18] . But

according to recently published a study by Kao *et al.* in which a total of 547 cases of MC were examined, MC had a higher occurrence in IBS than in controls ($P < 0.001$)[19]. In view of the result of the present study ,mucosal pathology, including MC, could be identified in IBS patients using microscopic evaluation, given that macroscopically, the colonic mucosa was normal . In contrast ,Limsui *et al.* found that 56% of the 131 patients diagnosed with microscopic colitis fulfilled the Rome III criteria for IBS and that 33% had been diagnosed with IBS before receiving the diagnosis of microscopic colitis. [20]Chey *et al.* found that colonoscopy and colonic mucosal biopsies were able to identify an alternative diagnosis in 1.9% (9/466) of diarrhea predominant IBS patients [17] Of these nine patients, seven had microscopic colitis, one had Crohn's disease, and one had ulcerative colitis Therefore, patients with suspected diarrhea-predominant IBS should undergo biopsies of the colon to investigate possible microscopic colitis if symptoms are not well controlled by anti-diarrheal therapy , i.e. the possibility of microscopic colitis should be considered while examining colonoscopic biopsy in patients with chronic watery diarrhea & normal colonoscopy to avoid any misdiagnosis that may affect the treatment & prognosis of patients.

In our study all biopsy with microscopic colitis (2.8%) show lymphocytic colitis and non of patients in our study had collagenous colitis , similar to study results reported by Pradi *et al* , who showing that 7.1 % of patients with idiopathic chronic watery diarrhea had microscopic (lymphocytic) colitis and no one showing collagenous colitis .[21]

We acknowledge the limitation of our study is the difference between the groups in terms of age and gender. The percentage of women with IBS is 55.3%. This percentage is close to the female: male ratio of previous studies from Turkey.

The association of chronic idiopathic watery diarrhea , microscopic colitis with diabetes mellitus may support an autoimmune process .

History of aspirin and NSAID intake was more in microscopic colitis than IBS but no statistic difference was observed , this may suggest this drugs as implicated in pathogenesis of microscopic colitis .

Conclusion

Microscopic colitis prevalence within the normal level similar to that in worlds in spite of high level of infectious causes of diarrhea in our society , this may support the non infectious cause of chronic watery diarrhea .MC can be identified in patients with chronic watery diarrhea and subset of diarrhea predominant IBS .

It appears reasonable to test for microscopic colitis in those patients by performing a colonic biopsy and the clinical criteria for IBS are no sufficient enough to rule out the diagnosis of microscopic colitis ..

Autoimmunity may be a risk for microscopic colitis .

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Randomised trial of endoscopy with testing for helicobacter pylori compared with non-invasive H. pylori testing alone in the management of dyspepsia

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Abstract

Objective: To compare the results of treating dyspepsia and testing for Helicobacter Pylori (HP) by endoscopy or by non invasive testing.

Methods: Two hundred fifty- cases of dyspepsia enrolled in the study. Hundred cases with an alarm symptoms underwent endoscopy for H. pylori testing. One hundred fifty cases with no alarm symptoms using non invasive methods for H. pylori testing. The results of the two groups had been compared after treatment.

Result: Hundred cases with alarm symptoms (test and scope group), treated either by eradication or empirical therapy according to H. pylori results. Hundred fifty cases without alarm symptoms (Test and treat group), treated in the same way. Failure and success rates of the two groups were compared.

Conclusion: The H. pylori "treat" strategy in uninvestigated dyspepsia is an effective alternative to prompt endoscopy.

Keywords: Endoscopy, Helicobacter pylori, Dyspepsia.

Introduction:

Dyspepsia is a common gastrointestinal (GI) symptom and accounting for 3-4% of out patient consultations [1]. The term dyspepsia often refers to upper abdominal pain or discomfort attributable to the upper GI tract. This symptom may be episodic or persistent, it may be encompass symptoms often exacerbated by eating[2-4].

The **prevalence of dyspepsia** varies in different countries ranging from 26% in the United States to 41% in England [1,5].

The **pathophysiology of dyspepsia** remains obscure and thus has impeded development of precise and effective treatment strategies[6]

Dyspeptic symptoms may be caused by a variety of conditions such as peptic ulcer disease, gastroesophageal reflux, and malignancy [7].

History taking and empirical therapy, H. pylori testing and eradication therapy, endoscopy based diagnosis and treatment are the main strategies currently employed for management[8]. The major controversy has concerned optimal management strategy of patients with new onset dyspeptic who do not present with "alarm" symptoms[9].

The widespread use of acid suppression with treatment of uncomplicated dyspepsia prior to endoscopy leads to less likelihood of recognition of mucosal lesions caused by acid-peptic disease, but not to a high healing rate for these lesions, and it may mask malignancy [10].

The **prevalence of H. pylori infection** increases with age world wide, reaching 40-60% in asymptomatic elderly subjects and over 70% in elderly patients with gastro duodenal diseases[11]. A **"test and treat "strategy** for patients with dyspepsia who are positive for H. pylori is recommended by the European H. pylori study group and the American Gastroenterology Association[12].

Studies have shown 20-50% of dyspeptic patients with a positive H. pylori test will have evidence of underlying ulcer disease or duodenitis whereas less than 5% of those a negative test will have ulcer disease[13].

Management options of dyspepsia include empirical therapy, prompt endoscopy, **"test and scope"**, and " test and treat" patients [14].

patients older than 55 years and with **"alarm" symptoms or signs** (e.g. weight loss, dysphagia, vomiting, anaemia, or positive faecal occult blood), use of non steroidal anti – inflammatory drugs (NSAIDS) should undergo prompt endoscopy [7,15,16].

NSAID users infected with H. pylori have an almost doubled risk of bleeding peptic ulcer compared with uninfected NSAID users [17]. In young patients without "alarm" symptoms an initial empirical approach to treatment has been advocated[16].

Methods:

Three hundred fifty cases of dyspepsia lasting for a period of 2 months or more being evaluated between **September 2001 to December 2002** in Al-Kindy teaching hospital. It was further ensured that the patients symptoms were likely to be originating from the upper GI tract and not from abdominal wall muscles or elsewhere, patients with GI bleeding, acute or chronic liver disease, structural heart disease, chronic renal failure or any other apparent diagnosed organic disease were excluded.

The study **ages ranging** from 15-61 years, **mean age** 36.9±9.5 years. Those dyspeptic patients had been divided in two groups, according to the **"alarm symptoms** in the history which are (weight loss,

dysphagia, vomiting, anaemia, positive faecal occult blood, use of NSAISs).

The group with "alarm" symptoms **(200 cases)** submitted for endoscopy **(test and scope group)**. 100 cases, of this group showing organic causes of dyspepsia which were excluded from the study, the other **100-cases** which were regarded as **"non ulcer" or functional dyspepsia** (*), underwent a biopsy from the antrum and body of the stomach to test for H. pylori using **rapid urease test (Clo test)**. Those showing **positive H. pylori test** we start them on **eradication therapy** and those showing **negative H. pylori test** we start them on **empirical trial** (antisecretory).

(*)Functional dyspepsia is persistent or recurrent pain or discomfort centered in the upper abdomen with evidence of organic disease likely to explain the symptoms being absent, includuing at endoscopy[18].

The second group which show **no "alarm" symptoms (150-cases)** submitted for **non invasive testing** for H. pylori **(test and treat group)**. Employing serology testing of antibodies in the sera of the patients infected by H. pylori [13].

Those which show **positive H pylori test** we start them on **eradication therapy**, while those with **negative H. pylori test** , we start them on **empirical antisecretory trial.**

All treatments continue for 8 weeks and **failure and success** rates were evaluated. Failure of treatment or persistence of the symptoms of (test and treat group) should be submitted for endoscopy.

Results:

Three hundred fifty cases of dyspepsia had been evaluated. The dyspepsia lasting two months and even more.

The study group **ages ranging** from 15-61 years with a **mean age** 36.9± 9.5years, there were 245 males and 105 females, with a **ratio** of M/F. 2.3:1 (Table 1).

Table 1- Demographic characteristics of the study group.

Mean age standard deviation 36.9± 9.5 years		
Age group/ years	**No.**	**%**
15-30	92	26.3
31-40	**128**	**36.5**
41-50	90	25.7
51-60	32	9.1
> 61	8	2.3
Total	350	100%
Sex		
Male	**245**	**70%**
Female	105	30%

According to the "alarm" symptoms in the history , they were divided into two groups. Group 1 of 200-cases (57.1%) **"test scope group"** where there is an "alarm" symptoms, they were submitted for endoscopy, 100 cases (50%) had been excluded from the study because of organic of dyspepsia, the other 100- cases (50%)
were regarded as **functional dyspepsia** and biopsy taken from antrum and body of the stomach to test for H. pylori, 30 cases (30%) show **positive results** and start them on **eradication therapy** for 8- weeks.

70- cases (70%) show **negative results** and start them on **empirical antisecretory therapy ,** for 8 weeks also . Persistence of symptoms or recurrence after treatment is regarded as failures, which are 8 cases (26.6%), and 12 cases (17.1%) respectively. And total **failure and success rate** of this group was 20%, 80% respectively (Table 2) (Table 3,5).

Discussion

The development of both intestinal and diffuse gastric cancer is associated with H. pylori related gastritis, and the risk of gastric caner is cancer is extremely low H. pylori negative individuals [18]. One concern about **non ndoscopic management of dyspeptic patients** is the possibility of missing underlying malignancy, but in western countries this is rare in patients less than 55 years of age presenting with dyspepsia in the absence of sinister symptoms [13].

Analysis of symptoms does not, however, provide sufficient diagnostic yield to differentiate functional from organic dyspepsia, and appropriate investigation are needed in patients with a poor response to short-term therapy or frequent relapses [8].

H. pylori eradication is considered to be an appropriate option in infected patients with functional dyspepsia, as it leads to long- term symptom improvement in the upset of patients [19].

In our study about 50% of the cases of dyspepsia in the 1 st group with alarm symptoms are due to **organic causes** which is much less than in other studies [3,6] , possibly because most of the failure in the 2nd groupWho will be submitted for endoscopy will have an organic cause and will be additive to the above percentage .

The prevalence of H. pylori in the 1st group is 33.3% (Table 3,4). In reviewing of the articles the prevalence of H. pylori increased by age reaching up to 60% in elderly patients [11], the differences, possibly because the major sample of our study was 31-40 years (table 1).

Eradication therapy is recommended for both groups with H. pylori positive, and should never be given without first obtaining a proof of infection [20]. Economic constraints and long waiting lists have inhibited policies which include endoscopy. Eradication therapy for H. pylori resolves symptoms and reduces the endoscopic work load.

Failure of therapy in our study after eradication therapy was 26.6%, 30 % of the 1 st and group respectively (table 3, table 4). In reviewing of the articles only 27% of patients in the eradication group had no improvement in dyspepsia and underwent endoscopy[21] which is the same as in our result.

H. pylori negative dyspepsiain the 1 st group was 70%, and in the 2nd group was 66.7% (table 3, table 4). Those had been submitted for **empirical antisecretory therapy**. H. pylori negative dyspeptic patients aged less than 45 years, without "alarm" symptoms can be managed without endoscopy[22]. **A test and treat stragegy** for H. pylori in uncomplicated dyspepsia saves endoscopy without missing significant underlying pathology [12]. Employing serological testing **(non invasive)** , appears to be substantially less expensive than initial endoscopy had lead patients without sinister symptoms reducing the waiting time for endoscopy in older patients [24]).

Comparison between the two groups reading **failure rate** (20%. 23.3% respectively) and **success rate** (80%, 76.6% respectively) (Table 5), after therapy revels no statistical difference (P value > 0.05).

Conclusion:

A "test and treat" strategy with eradication of proven H. pylori infection may be as effective as endoscopy-based treatment of dyspepsia, and reduces costs, by reducing the need endoscopy.

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Tikrit Medical Teachers Opinions Regarding Current School Curriculum

Wafa Mahmood Jasim
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Abstract

Curricula usually define the learning that is expected to take place during a course or programme of study in terms of knowledge, skills and attitudes,

A descriptive study was conducted on Tikrit medical teachers during the period from 15th May /2012 till 20th February / 2013 to determine the opinions of Tikrit medical teachers regarding the current medical curriculum .

133 medical teachers were included in the study after distributing a special questionnaire form and face to face interview was conducted after receiving the written consent from them.

Majority of teachers are male (83- 62.4 %) , and they have a PhD certificate(59- 44.4 %) and they are from clinical specialty (82- 61.7 %) having a period of teaching between 5- 10 years (84- 63.1 %), and they are assistant professor (67- 50.3 %) .

49.3 % of study male teachers considered that continuous update planning of the curriculum while 54% of female teachers considered the requirement of specialized quality team for future curriculum improvement .

Tacit medical teachers suggested a small scale modification in both basic and clinical branches(53.6%) .

The study recommended that further researches should be done on different medical Colleges to evaluate the medical curriculum and detected the main defects in order to obtain a better learning results .

Key words : Medical teachers , Curriculum , Opinions

Introduction

Curriculum can be defined as an educational plan that spells out which goals and objectives should be achieved, which topics should be covered and which methods are to be used for learning, teaching and evaluation [1]

The curriculum represents the expression of educational ideas in practice. The word curriculum has its roots in the Latin word for track or race course. From there it came to mean course of study or syllabus. Today the definition is much wider and includes all the planned learning experiences of a school or educational institution [2, 3]

The curriculum must be in a form that can be communicated to those associated with the learning institution, should be open to critique, and should be able to be readily transformed into practice. The curriculum exists at three levels: what is planned for the students, what is delivered to the students, and what the students experience [4, 5]

In contemporary medical education it is argued that the curriculum should achieve a "symbiosis" with the health services and communities in which the students will serve. The values that underlie the curriculum should enhance health service provision. The curriculum must be responsive to changing values and expectations in education if it is to remain useful.

..[6 ,7]

There are two main types: prescriptive models, which indicate what curriculum designers should do; and descriptive models, which purport to describe what curriculum designers actually do. A consideration of these models assists in understanding two additional key elements in curriculum design: statements of intent and context [8]

The recent Curriculum for Tikrit medical college details what vocational general practitioners need to learn throughout their general practice learning life. The curriculum details the knowledge, skills and attitudes that general practitioners require for competent, unsupervised general practice , meeting their community's healthcare needs, and supporting current national health priorities and the future goals of the healthcare system. [9,10]

The aim of the study is to determine the opinions of Tikrit medical teachers regarding the current medical teaching curriculum

Methodology :-

1- A administrative agreement

Official permission was taken from the Tikrit university / Tikrit medical college before establishing the study

A descriptive study design was conducted on medical teachers in Tikrit medical college.

2- Setting of study :-

The study was carried out in Tikrit medical college on different basic and clinical departments .

Tikrit medical college was established since 1989 and conducted an innovative method of teaching which is based on problem- solving .

3- Study sample and sampling method :-

A descriptive study conducted among medical teachers in Tikrit medical college and 133 teachers were included in the study (51 teachers from basic departments and the remaining 82 teachers from clinical departments) .

A special questionnaire form was distributed to them after receiving the written consent from them and the data was collected by interviewing with the study teachers after complete explanation of the main objectives of the study .

4- Study period :

The study was conducted during the period from 15th May / 2012 till 20 th February / 2013.

5- Data collection tool :

A special questionnaire has been prepared by the investigator utilizing available related literature to the questionnaire item included three main parts :-

Part-1- Demographic characteristics including (age, gender, specialty , scientific degree, certificate , and period in teaching).

Part-2- Medical teachers opinions about the current curriculum characteristics in their college .

Part -3- Medical teachers suggestions for future curriculum changes and improvement

6- Reliability of the questionnaire form :

The data was collected through the use of special questionnaire which was presented to (6) experts in medical education , they were (4) Community physicians and (2) statistical experts .

The reliability of the questionnaire was 75 %

7- Statistical analysis of data :-

All the statements with yes and no answer , number and percent will be calculated .

Chi- square test was used to detect the relation between the studied variables and the level of significance is taken at level 5% ($P < 0.005$) .

Results

The total number of medical teachers are 150 distributed among basic and clinical department

The number of teachers response to study was 133 and the response rate was (88.6 %) .

Table 1: Frequency distribution of study medical teachers according to their socio demographic characteristics

Socio demographic parameter		Tikrit medical teachers N =133	
		No.	%
Gender	Male	83	62.4
	female	50	37.6
Age group (in years)	30- 39	17	12.7
	40- 49	69	51.8
	50 -59	35	26.3

Certificate	Master of science	31	23.3
	Board (Iraqi and Arabian)	43	32.3
	PhD	59	44.4
Specialty	Basic sciences	51	38.3
	Clinical sciences	82	61.7
Period in teaching	< 5 years	12	9.1
	5-10 years	84	63.1
Scientific degree	Professor	5	3.7
	Assistant professor	67	50.3
	Lecturer	48	36.2

Table 1 shows that medical teachers from Tikrit college were male constitute a percent (62.4%) within the age group between 40- 49 years (51.7%) , have an PhD certificate (44.4%) , from clinical specialty (61.7) with a period in teaching between 5- 10 years (63.1%) having a scientific degree of Assist prof (50.3%)

Table 2: Frequency distribution of study medical teachers according to their opinion regarding current undergraduate curriculum teaching characteristics .

Current undergraduate teaching characteristics	**Teacher opinion**				
	Tikrit medical teachers N=133				
	Agree		**Disagree**		
	%	**No.**	**%**	**No.**	
1- Current curriculum is suitable for Iraqi community needs	**73**	**54.9**	**60**	**45.1**	
2-The class schedule is suitable	**63**	**47.4**	**70**	**52.6**	
3-The current method of medical education stimulate the active role of the student in learning process	**63**	**47.4**	**70**	**52.6**	**P**

4-Current curriculum has a good coverage for all essential skills	73	54.9	60	45.1	Value
5-Hospital based learning is sufficient	52	39.1	81	60.9	
6-A need for training the students in PHC centers	52	39.1	81	60.9	
7- Agreement with current method of student assessment	67	50.8	66	49.2	

χ^2 – test was used

Curriculum improvement parameter	Tikrit medical teachers N=133				Total	
	Male		Female			
	N0.	%	No.	%		
1- Emphasis the need for continuous life long learning	11	13.3%	5	10%	16 12%	
2- Achieve a wide range of medical knowledge, communication skills and teamwork activities	7	8.4%	6	12%	13 9.8%	P Value
3- The requirement of specialized team in quality improvement	23	27.7%	27	54%	50 37.5%	
4- Continuous up-date planning of the curriculum	41	49.3%	9	18%	50 37.5%	
5 All of the above	1	1.3%	3	6%	4 3.0%	
Total	83	62.4%	50	37.6%	133 100%	

Table 2 shows that 54.9 % of study medical teachers. agree that current curriculum is suitable for Iraqi community needs and it covers all essential skills while 60.9% of them disagree with sufficiency of students training in hospital.

Table 3- Frequency distribution of medical teachers according to their opinions regarding curriculum improvements according to gender

χ^2 – test was used

Table 3 shows that 49.3 % of study male teachers considered that continuous update planning of the curriculum while 54% of female teachers considered the requirement of specialized quality team for future curriculum improvement .

Table 4: Frequency distribution of medical teachers according to their suggestions regarding the main modifications of medical teaching curriculum

Modification parameter	Tikrit medical teachers N=133				P Value
	Small scale modification		Large scale modification		
	No.	%	No.	%	
1-Basic science only	34	35.1	3	8.4	
2-Clinical science only	11	11.3	12	33.3	
3-Basic and clinical branches	52	53.6	21	58.3	
Total number	97 72.9%		36 27.1%		

χ^2 – test was used

Table 4 shows that medical teachers suggested a small scale modification (97- 72.9%) and 53.6% of them agree with the modification in both basic and clinical branches. Table 5: Frequency distribution of medical teachers according to their opinions regarding better learning

Better learning	Tikrit medical teachers N=133	
	No.	%
1-Only increase in theoretical hours	17	12.8
2-Only increase in practical hours	55	41.4
3- Both of them should be increased	61	45.8
Total	133	100%

Table 5 shows 45.8% of study medical teachers agree with the need for increase in both of theoretical and practical ho

Discussion :

Concerning the socio demographic characteristic of study teachers , it was show that they are male having a period of teaching between 5- 10 years while Vernone / 1995 [11] during his study in University of Missouri-Columbia school of medicine that overall response rate was 69% were female (882 of 1,287group, the respondents were curriculum has at least four important elements which are : **content; teaching and learning strategies , assessment processes and evaluation** that must be present in order to cover all the essential needs .

experienced in both PBL and traditional curricula, with an average of 3.75 years of teaching) .

Regarding the curriculum characteristics , medical teachers agreed with the suitability of it for Iraqi community and it covers all the essential skills while David Prideaux/ 2003 [12] during his study reported that the

He also considered that there is another prescriptive model of curriculum design which is called "Outcomes based education" is similar in many respects to the objectives model as shown in the figure 1.

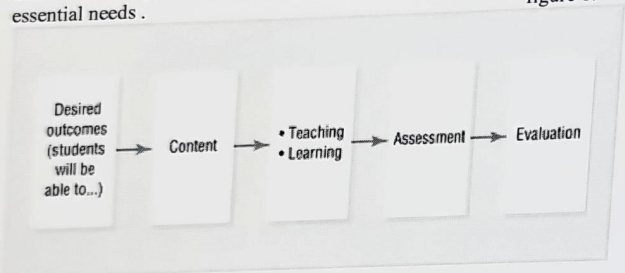

Fig -1 - Outcomes based curriculum

Ghanim .etal / 1997[13] published a research on the main difference between problem based curriculum in comparison with subject or lecture based in offering opportunities to discover early the importance of professional skills they need to acquire during study years, and they took a 3 medical colleges including (Tikrit, Mosul and Basrah) .

They found that more than 50% from Tikrit indicated the importance of 15 skills compared with 5% only from Mosul and Basrah, and this is attributed to the effect of the problem- based objective curriculum adopted by Tikrit only as compared with subject – based curriculum adopted by Mosul and Basrah colleges .

Concerning the curriculum improvement , Tikrit medical teachers agreed with the continuous update planning of the medical curriculum and the need for specialized quality team .

Levitt etal / 2012 [14] published a paper about the curriculum improvement for third year students and he reported that clinical-year students were able to conduct a self-directed quality improvement (QI) project. Lack of improvement in QI knowledge suggests that self-directed learning in this domain may be insufficient without targeted didactics. Higher order skills such as developing measurement plans would benefit from explicit instruction and mentorship. Lessons from this experience will allow educators to better target QI curricula to medical students in the clinical years.

WFME/ AMSC/ 2007 [15] conducted a global standards for quality improvement in medical education regarding the basic , post graduation and continuing professional medical education . They describe the main essential elements of each part of medical education with more concentration on pre-graduation period and determine a basic standards elements which must be follow systematically during the study years.

For the curriculum modification , medical teachers suggested that the clinical and basic branches should be modified while Ponce de/ 2011 [16] in University of Mexico suggested that an academic program with essential and necessary contents that integrates the essential core curriculum, which in turn leads to the achievement of the intermediate and final competences.

Dr. Syed / 2012 [17] said during the workshop on the accreditation of health professions education, with a special focus on medical education, that graduates

today are required to master core clinical competencies and acquire a range of cultural competencies to ensure they are in tune with the needs of populations and communities they serve. and necessary requirements should be included for accreditation systems including medical curriculum
Regarding the teachers opinion about better learning , they suggested an increase in both theoretical and practical hours.
This results is agreed with similar study was done in GCC countries **Gulf Cooperation Council** (Kingdom of Saudi Arabia, Oman, Kuwait, Qatar, Bahrain, United Arab Emirates and Yemen) / 2005 [18] to assess the current status of undergraduate curricula in these medical colleges in relation to SPICES (Student-centered, Problem-based, Integrated, Community-based, Elective and Systematic) model and they found that (40%) of them were following the traditional curriculum, while the remaining (60%) were following hybrid Problem-based learning (PBL) curricula and medical teachers were dissatisfied with the curriculum in traditional method because of in adequacy of practical hours in comparison to theoretical hours

Conclusions:

Medical teachers agreed that current curriculum is suitable for Iraqi community needs and it covers all essential skills .
They suggested that continuous update planning of the curriculum and the requirement of specialized quality team should be considered for future curriculum improvement with the need for small scale modification in both basic and clinical branches .
They agree with the need for more increase in both of theoretical and practical hours to achieve better learning .

Recommendation

Teaching and learning methods used in undergraduate medical education are continually changing in response to educational understanding, developing learning technologies and external healthcare agendas. As students become more dispersed and mobile and medical schools increasingly need to ensure they produce doctors who are safe, competent practitioners who can practice professionally in a range of contexts, teaching, learning and assessment methods need to adapt to reflect the demands of patients and healthcare systems.
Effective teachers therefore need to stay up to date, not only with their subject discipline knowledge but also with appropriate, contemporary educational theory, methods and techniques. Providing a variety of appropriate learning activities and assessments will help students and trainees get the most from their medical education .

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Mental health consequences of wars and terrorism in Iraq: a preliminary report

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Abstract

Background: Iraq has been at wars several times in its history, and at last the civil war and terrorism after 2003. There were few studies on the effect of war, civil war and terrorism on mental health in Iraq.
Objective: The work was carried out to report on mental health consequences of wars and conflicts on youth and children in Iraq.
Methods: A total of 210 university students resident in Baghdad (their age 18 - 24 years with male to female ratio of 0.8:1) selected randomly and 230 school children from Al-Sader city selected by multistage random sample (their age was 9 - 20 years with male to female ratio 1:1.2). A questionnaire was filled for each participant. Requested data were demographic information, data on school achievement and Harvard Trauma questionnaire (exposure to war trauma, posttraumatic stress disorder and substance use disorder). Variables were dichotomized (PTSD vs non PTSD; academic achievement (poor vs good). Chi square was used to examine the association between exposure and PTSD, substance use disorder and poor academic achievement. Regression was used to demonstrate dose effect association between exposed number of events and score of PTSD and depression.
Results: Out of the total, 209 (99.5%) and 151 (71.9%) of university children and school children, respectively, were exposed to trauma. Of the exposed there were 39 (18.7%) and 65 (43%), respectively, with PTSD; 22 (10.5%) and 19 (12.6%), respectively, with substance used disorder; and 53 (25.4%) and 49 (32.4%) had poor academic achievement. High rates of PTSD, substance use disorder and poor academic achievement were significantly associated with exposure to accumulated trauma events (> 5 events), among university students and school children ($p = 0.001, 0.001, 0.006, 0.003, 0.02$ and 0.002, respectively).
Conclusion: High exposure rates to trauma events and high prevalence of PTSD, SUD and poor academic achievement were observed. Accumulated exposure to trauma events was significantly associated with high rates of PTSD, SUD and poor academic achievement.
Key words: War, Terrorism, PTSD, SUD, Poor academic achievement, Iraq

Introduction

The implications of contemporary wars on the collective health status and well-being of affected populations go beyond the loss of life and destruction of infrastructure [1,2]. International studies indicate that who witness or are victims of traumatic events may experience a range of negative outcomes, including symptoms of depression, anxiety and posttraumatic stress disorder (PTSD) [3,4], poor academic achievement [5,6] and behavioral disorders that jeopardize their ability to function well later in life [7].

Iraq has been at wars at several times: a series of coups in the 1960s, Iraq - Iran war (1980 - 1988), Iraqi invasion of Kuwait resulting in the Gulf war (1991) and the conflicts starting in 2003 [8,9] (atrocities including civilian massacres, reprisal, bombing, shelling, mass displacement, disappearance and torture were norm in Baghdad, Iraq, with different intensities between districts). The economic sanction following the Gulf war 1991 had a profound effect on health of Iraqis [10,11]. Human right abuses had also been recorded [12].There are few studies on the effects of these conflicts on mental health [13-18]. Therefore, this work was carried out to report on health consequences of wars and conflicts e.g. post-traumatic stress disorder (PTSD), substance use disorder (SUD) and poor academic achievement, among youths and children in Baghdad, Iraq.

Materials and methods:

A total of 210 university students (US) from Baghdad University resident in Baghdad and 230 school children (SC) from Al-Sader city, Baghdad (area of many conflicts) [19,20] were included in the study. US were selected randomly from Baghdad University, College of Art. They were from first and second stages in the college. Their age was 18 to 24 years with male to female ration of 0.8:1. SC (230 students) were selected by multistage random sample from Al-Sader city (4 clusters from 80 clusters, 2 schools from each clusters, 2 classes from each school and 15 students from each class) (some students were refused to participate in the study) . Their age was 9-20 years with male to female ratio of 1: 1.2.

Data collection took place during the period 1st Feb. to 30th April 2010. The collected data was confidential and anonymous. Following standard university ethical review protocols, participants or their families were informed about the research and their right to refuse participation in the research.

A questionnaire was developed consisting of items on demographic characteristics of the

respondents (age, sex), poor academic achievement (skipping of school or college, absenteeism, difficulties in concentration and failure of academic year) and instrument to measure exposure to trauma events PTSD and self-reported alcohol and other substance abuse (substance use disorder, SUD) Harvard Trauma Questionnaire (HTQ) [21,22]. HTQ consisted of 28 items with a yes/ no response on exposure to traumatic event types throughout the respondent's lifetime and also within the previous 12 months. PTSD was measured using 45 questions on trauma symptoms with a 4 points severity scale (1, "not at all"; 2, "a little"; 3, "quite a bit"; 4, "extremely"). The first 16 items are based upon the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR). The remaining items were developed specifically for forcibly conflict affected population [23]. PTSD score > 2.5 was considered significant for meeting symptoms criteria of PTSD based on DSM-IV-TR. The HTQ has been used extensively in different countries and also in Iraq, and a good internal consistency, test-retestreliability and concurrent validity have been reported [13,23].

The number of exposed events was dichotomized (cut off 5 events) to measure the effect of multiple exposure on PTSD, SUD and poor academic achievements. The outcome of PTSD was dichotomized into respondent exhibiting or not exhibiting signs of PTSD (cut off > 2.5). Chi square was used to examine the effect of number of exposed events (independent variable) on PTSD, SUD and poor academic achievement (dependent variables). P value < 0.05 was considered as statistical significant.

Results:

Out of total US and SC, 209 (99.5%) and 151 (71.9%), respectively, reported an exposure to traumatic events.

Out of US and SC exposed to traumatic events, 39 (18.7%) and 65 (43.0%), respectively, met the criteria of PTSD. Exposure to > 5 trauma events was significantly associated with higher rates of PTSD in both US and SC, 30 (23.6%) and 41 (51.8%), respectively, than those exposed to ≤ 5 trauma events (9 (10.9%) and 24 (33.3%, respectively) (χ^2= 16.2, d.f.= 1, p= 0.001; χ^2= 18.9, d.f.= 1, p= 0.001, respectively).

In both US and SC, the prevalence of SUD among those exposed to trauma events were 22 (10.5%) and 19 (12.6%), respectively. Exposure to > 5 trauma was events significantly associated with higher rates of SUD in both US and SC, 17 (13.4%) and 15 (26.8%), respectively, than rates among those exposed to ≤ 5 trauma events, 5 (6.1%) and 4 (4.2%), respectively (χ^2= 38.3, d.f.= 1, p= 0.006; χ^2= 30.3, d.f.= 1, p= 0.003, respectively).

Poor academic achievement was noticed in 53 (25.4%) and 49 (32.4%) of US and SC, respectively. Significant differences were observed in poor academic achievement between those exposed to > 5 trauma events, 38 (29.9%) and 29 (52.7%) of US and SC, respectively, and those exposed to ≤ 5 trauma events, 15 (18.3%) and 20 (20.8%) of US and SC, respectively (χ^2= 5.2, d.f.= 1, p= 0.02; χ^2= 17.5, d.f.= 1, p= 0.002, respectively).

Score of PTSD was linearly correlated with number of traumatic events (Fig. 1).

Discussion:

Mental health is recognized as a key public health issue for conflict affected populations [24]. People experienced poor mental health suffer substantial distress and may be more vulnerable to violence, poor physical health and harmful practices such as substance abuse. Poor mental health can affect the ability of individuals, communities and societies to function both during and after conflicts. This study was a trial to explore the effects of exposure to wars and conflict traumas on mental health in Iraq.

The study revealed high exposure to war or conflict traumas (99.5% and 71.9% among US and SC, respectively). The high figure could be attributed to the civil war conflicts starting after 2003. It is consistent with that in Sri Lanka [25] which face a conflict between the majority, Sinhala, and minority, Tamil, populations in more or less similar to that in Iraq. Similar findings were reported in Afghanistan [26] after more than 2 decades of conflict, southern Sudan [27] and Balkans [28].

Although the same screening instrument (HTQ) was used for US and SC, a higher exposure rate was reported among US (99.5%) than that among SC in Al-Sader city (71.9%). Differences in educational might contribute to this finding as educational levels affect perception. There were more than 3 million population in Al-Sader city living in 31 square km which might suggest high exposure level of conflict traumas.

PTSD was noticed in 18.7% and 43.0% among US and SC, respectively). PTSD was reported in high rates among children in Kurdistan, Iraq, five years after military operation "Anfal" in Iraqi Kurdistan [29]. Low figure was reported by Iraqi Mental Health Survey [30] (IMHS) (1.6%), and this low figure was attributed to religious and spiritual coping measures in the Iraqi community. Limitation of IMHS (exclusion of internally displaced persons, those who migrated out of Iraq and residents of areas deemed too dangerous and completion of the survey during the period of ongoing violence 2006-2007) contributed to its low figure. Similarly high rates of PTSD were reported in Sri Lanka [25], Afghanistan [26], Balkans [28], Cambodia [31], Chechnya [32], Rwanda [33],

Somalia [34] and Uganda [35]. Differences in age between US and SC might be contributed to the differences in rates of PTSD between them (previous research found direct, inverse and no association between age and PTSD) [36]. Differences in education might be another factors for differences in rates of PTSD (studies reported an inverse association of PTSD with years of education) [37]. Among US and SC high rates of PTSD were significantly associated with exposure to > 5 trauma events. The finding is similar to that in Iraq, recently [13].It is consistent with that in Afghanistan [26], Cambodia [31] and Lebanon [38]. It was reported that high level of post- traumatic symptoms were related to the amount and type of exposure [39].

The prevalence of alcohol and other substance abuse were 10.5% and 12.6% among US and SC, respectively. Similar figures were reported in Baghdad [40], Iraq, recently. IMHS [30] reported low rate of alcohol and substance abuse (0.9% and 0.7%, respectively), and no relation with exposure to trauma events (war, civil war and widespread violence) was observed. Limitations of IMHS could be contributed to low figure reported by IMHS. In Africa, where many wars continues to engulf it, higher figure was reported (18.2%) [41]. Using alcohol, drugs or cigarettes might be regarded as modes of coping. However, this coping mean may vary between population. It was reporting that the active coping (substance use) was rare in Pakistan [42].Rate of SUD was significantly associated with high level of exposed conflict trauma (> 5 events). Literature [43,44] documented the association of SUD with exposure to trauma events, however, most of articles investigate SUD after single trauma event i.e. terrorist attacks or natural disaster, which limited in time. Researchers [21] in their trial to study SUD in war and post war situations e.g. Afghanistan, were faced that questions regarding alcohol and substance use were considered inappropriate in Muslim culture.

In the line of literature [42-44], a significant association of poor academic achievement with experienced of high levels of traumas was revealed (> 5 events). Children experienced high level of traumas are likely to show deficit in standardized test score in addition to the lower school grades [45]. correlation between I.Q. and PTSD in males combat veterans have also been reported [46].

The finding of linear relationship between number of war- related events and PTSD scores revealed a clear dose- effect relationship. It is in agreement with other epidemiological studies in war affected population [12,19-22]. This finding suggests that experience of cumulative trauma has harmful effect on mental health.

In third world, conflicts have increasing become internal within countries involving ethnic and civilian groups against each other. The use of terror is to exert social control by disrupting social, cultural and economic relations. The population and the psychological warfare are the targets. Studies from countries mentioned in the study (Afghanistan, Balkans, Cambodia, Chechnya, Lebanon, Palestine, Israel, Rwanda, Sri Lanka, Somalia and Uganda) show devastating consequences of civil war and terrorism. The findings of this study might be slightly optimistic. However, the small sample size, the restricted area, using a screening instrument with a cutoff point established in industrialized countries and neglecting the coping mechanisms in this study might affect the optimistic results of this study.

In conclusion this study revealed high exposure rate to conflict traumas, high rates of PTSD and SUD (mental health consequences of exposure to wars and conflicts). Poor academic achievement was significantly associated to accumulated events (war and conflict traumas).

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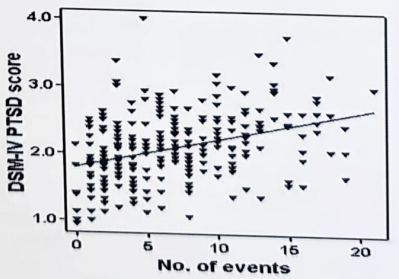
Table1 Distribution of PTSD, SUD and poor academic performance according to number of events in Baghdad university students and school children (highly exposed violence)

No. of events	Mental health consequences of exposure to conflict trauma											
	PTSD				SUD				Poor academic performance			
	University students		School children		University students		School children		University students		School children	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
>5	30	23.6	41	51.8	17	13.4	15	26.8	38	29.9	29	52.7
≤ 5	9	10.9	24	33.3	5	6.1	4	4.2	15	18.3	20	20.8
Total	39	18.7	65	43.0	22	10.5	19	12.6	53	25.4	49	32.4
	χ^2=16.2, d.f.=1, p=0.001		χ^2=18.9, d.f.=1, p=0.001		χ^2=38.3, d.f.=1, p=0.006		χ^2=30.3, d.f.=1, p=0.002		χ^2=5.2, d.f.=1, p=0.02		χ^2=17.5, d.f.=1, p=0.002	

Fig. 1Linear relationship of PTSD score and number of events



Integration of Communication Skills Teaching within Iraqi Medical College Curriculum - using an outcome Based Approach

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Abstract

Curriculum is defined as all planned and unplanned learning experiences in a medical education institute. It has three levels, planned, delivered and experienced [1]. For example, a planned curriculum should runs five interactive tutorials on respiratory medicine. Four case studies were delivered and the fifth was like cardio-respiratory tutorial. Meanwhile students experienced three well organized tutorials, half of the students came to the forth tutorial because of confusion in the timetable. And in the fifth tutorial there was limited interaction as the tutor had more of lecture style approach. Students learned only what they experienced and there was a gap between what the planned curriculum said and what was done actually. Poor curriculum might suffer from ossification or iatrogenic curriculitis. The worst scenario is what is called the hidden curriculum, it is believed when students learn through role modelling in which the values are never stated but everyone understands them [2,3].

Traditional teaching was subject-based and lecture style approach from experts which didn't influence graduates' practice. Accreditation Council for Graduate Medical Education demonstrated professional perspectives on educational outcome should be on patient & population care, medical knowledge, evidence-based practice, interpersonal and communication skills, ethics, professionalism, cultural sensitivities and values, health systems – based practice and social responsiveness [4].

Outcome based approach curriculum is defined by the outcomes to be obtained. Curriculum design proceeds by working "backwards" from outcomes to the other elements; content, teaching and learning experiences, assessment and evaluation [5]. It focuses on what student will do rather than what staff do. It considers clearly the purposes of what they do in terms of the effects and impact on students in broad outcome like; Graduates will attain knowledge and skills for treating common medical conditions.

The integration of **communication skills with undergraduate medical curriculum** will enable the graduates to communicate clearly, sensitively and effectively with patients and their relatives, and colleagues. Clear communication will help them carry out their various roles, including clinician, team member, team leader and teacher [6]. The Clinical communication skills "CCS" integrated curriculum should be based on a new evidence-based approach to teach the medical interview. The key components of CCS are Core medical interviewing skills, Specific communication issues and challenges, Communicating with others "relatives" and mastering professional communication skills; communicating with colleagues and other health professionals and presentation skills [7].

Clinical Communication skills at present is in its infancy in Iraqi medical schools, with the teaching medium being in English (neither being the first language of the doctor nor the patient), there are likely to be many instances in clinical practice where the message the medical student or qualified doctor wishes to convey, and the way the patient interprets this message may lead to misinterpretation and misunderstanding. This may even happen when the patient and the doctor are conversing in Arabic where it is the first language of both parties. Thus communication skills teaching must be fostered in local languages and developed new approaches and curricula to do so [8].

Principle of integration, CCS curriculum must be integrated vertically across the years of graduation and horizontally among subjects. It should have logical progression in which the material should be presented in a logical order and discernible by the students. The teaching methods should have planned repetition in spiral manner across the years. Experiential teaching and learner centred approach; CCS teaching should use limited traditional teacher centred lecture based, large group, sequential, didactic and passive sessions for increase awareness to the subjects. On other hand, the concentration should be on learner centred, active self directed contextual and small group learning on how to communicate? Evidenced showed that the most effective learning tools are systematic delineation and definition of the skills, observation of learners during interview, video or audio recording and review, well-intentioned feedback, rehearsal and active small group learning [9].

There are many experiential materials need to be adopted in Iraqi medical education; reviewing videos

of real Iraqi consultations, interviewing real patients, role play between the participants and the most important effective tool in CCS learning is simulated patients. This tool should grow up in Iraqi medical education from many variety of Iraqi population; Retired teachers, actors, real patients with chronic disease who are ready to work as simulated patients. They should be trained to demonstrate real patients' scenarios to enrich Iraqi practice. The advantages of using simulated patients during small group learning are substantial. The students can practice with ease without bothering real patient in Rehearsal, Improvisation, Standardization, Customisation. Simulated Patient can demonstrate specific issues and difficult situations like breaking bad news and dealing with angry patient. They also available on need and can give appropriate feedback, facilitation, and even evaluation.

Evaluation is the final step of integration, the faculty will see whether the integration is achieving the intended purpose. The evaluation need to be in different levels; quantitative and qualitative evaluation, and amongst all key stakeholders "students, Faculties, patients and society" to ensure that the integration is fit for purpose. Introduction of new subject within already loaded curriculum phase a lot of obstacles we need to overcome; trained expert in the field of CCS, adoption of Arabic in training "role play, recording interviews, inventing interviewing conversation skills with patients", Money, time and space "communication skills lab". During integration of curriculum and curricular roll out the faculty should deal with potential barriers to implementation and find ways to overcome such barriers.

It is proposed that in Iraqi medical schools, two communication skills textbooks originally published in English and now currently being translated into Arabic [7,9] should form the basis of the undergraduate and post-graduate teaching curricula in Iraq, and that the biopsychosocial method of interviewing should be used so that patients and their families should feel better understood, understand better what is happening to them and be more involved in decision-making.

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Magnesium and Calcium Contents of Tap and Bottled Drinking Water in Baghdad City

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ABSTRACT

BACKGROUND: Waterborne magnesium and calcium are known to be more bioavailable than those obtained from foods and consequently may be more important clinically. In some geographical areas, the magnesium and calcium contents of drinking waters, including tap and bottled waters, are extremely low and may provide little supplementation towards a person's daily requirement. The mineral contents of commercially available bottled waters throughout the world vary tremendously, and the shift in consumption from tap to bottled water, with different mineral constituents, may have important implications in health and disease.

AIM OF THE STUDY: To compare magnesium and calcium contents of commercially available, local and imported, bottled drinking waters, to household drinking water supplies in Baghdad, Iraq.

MEHTODS: Levels of magnesium, calcium and pH values were determined in tap water distributed through thepiped water delivery system in 10 municipalities of Baghdad City. Four local and seven imported brands of commercially available bottled drinking waters were also enrolled in the present study.

RESULTS: Measurements of pH, magnesium and calcium, indicates little differences in tap water samples utilized from the 10 municipalities, while, results of bottled drinking water show dramatic variations in both local and imported brands. Furthermore, dissimilarities between the estimated and labeled values were clearly observable, especially among local brands. Mean pH values of imported, but not local, bottle drinking waters were significantly lower than tap water ($P< 0.001$). For both brands, magnesium contents of bottled drinking waters were significantly lowers ($P< 0.05$ and $P< 0.001$) as compared to tap water levels, analogous results were observed for calcium concentrations ($P< 0.001$ for both brands). However, only local brands show a significant reduction in Ca: Mg ratio ($P< 0.01$), relative to that of tap water.

Key words: Magnesium , Calcium ,Tap ,Bottled ,Drinking Water.

Introduction:

Historically, "Drinking Water" was often regarded as water without pathogenic microbial or chemical contaminations, hence, in most countries, only few chemical elements that were assumed to define water quality were routinely analysed. Despite the fact that water calcium and magnesium may be useful in providing essential micronutrients, those are still not normally on the regulatory monitoring list, and so far there are no guidelines on minimum concentrations of these minerals in drinking water. Obviously, further efforts are needed to reorient and set new and better priorities for drinking-water practices, including potential benefits [1]Physiologically, waterborne minerals are in ionic form, which tend to be easily absorbed by the human gastrointestinal tract. Waterborne magnesium is known to be more bioavailable than magnesium obtained from foods and consequently may be more important clinically [2]. It has been estimated that drinking-water can contribute 40–100 mg magnesium/day. Thus, water may be a significant source of magnesium, accounting for 29–38% of the Estimated Average Requirement for adults [3]. Moreover, a review of published and unpublished data showed that the absorbability of calcium in the waters tested is comparable to the absorbability of calcium in milk when studied under similar conditions, suggesting that calcium in high-calcium mineral waters is highly absorbable and that mineral water consumption can potentially account for a substantial fraction of total daily calcium intake[4].

Calcium and magnesium are the main elements contributing to water hardness, and since the 1950s a causal relation between water hardness and cardiovascular diseases (CVD) has been hypothesized [5]. Many but not all geographic correlation studies showed an inverse association between water hardness and mortality from CVD. Most case-control and one cohort studies showed a statistically significant inverse relation between levels of water magnesium and mortality from CVD, while the relation with calcium was indecisive [6].

Martinez et al.[7], have provided statistical evidence sustaining the relationship between mortality from cardiovascular diseases and hardness of drinking water. The correlations were more apparent with magnesium than with calcium levels, suggesting a possibility of protectiveness but cannot be claimed as conclusive. In a more recent population-based ecologic study, each 1 mg/l increment in water magnesium level was shown to decrease the acute myocardial infarction (AMI) risk by 4.9%, whereas a one unit increment in the Ca:Mg ratio increased

the risk by 3.1%. Calcium did not show any statistically significant effect on the incidence and spatial variation of AMI. This seems to support the earlier findings of a protective role of magnesium and low Ca:Mg ratio against coronary heart disease but do not prop the earlier hypothesis of a protective role of calcium[8].

In some geographical areas, the magnesium and calcium contents of drinking waters, including tap and bottled waters, are extremely low and may provide little supplementation towards a person's daily requirement. The mineral contents of water from most Asian drinking-water supplies are generally in the range of 2–80 mg/l for calcium and below 20 mg/l for magnesium[1].

Very few studies have been conducted in Eastern Mediterranean countries on minerals in water supplies. A survey of trace elements, including magnesium, but not calcium, was conducted by al-Saleh and al-Doush in Riyadh, Saudi Arabia. This city is supplied mainly with desalinated seawater and water from deep wells. The study found that the household drinking-water contained magnesium at concentrations ranging from 0.78 to 0.88 mg/l, suggesting that the concentration of this trace element in drinking-water is minimal[9].

Bottled water consumption has been steadily growing in all parts of the world for the past 30 years, and it is now the most dynamic sector of the entire food and beverage industry[1]. Globally, consumption has increased by an average of 12% per year. The consumption of bottled water in Sweden, for example, increased from 92 million liters to 161 million liters from 1992 to 2001[10]. The annual per capita consumption of bottled water in the United States increased from less than 30 liters in 1991 to almost 42 liters in 1996. However, the fastest growing markets are in Asia and the Pacific, with an annual increase of 15% for the period 1999–2001. In newer markets such as India, bottled water consumption is increasing by as much as 50% annually[11].

The quality of bottled waters available throughout the world varies tremendously. Garzon and Eisenberg were the first to illustrate the large variations in mineral contents of commercially available bottled waters. The content of bottled water available in North America ranged from 1-120 mg magnesium/l, and from 1-240 mgcalcium /l, whereas bottled waters that are commercially available in Europe were in the range of 1-126 mg magnesium /l and 0-546 mg calcium /l[12]. A comprehensive follow-up study by Azoulay and colleagues have suggested that the bottled waters imported from Europe contained higher calcium and magnesium levels than local North American bottled waters[13]. More recently, Rosborg et al. studied the concentrations of about 50 metals and ions in 33 different brands of bottled waters on the Swedish market. Ten of the brands showed calcium concentrations around 10 mg/l and magnesium levels of less than 3 mg/l, implying that they could be soft water in origin[10]. In contrast, most of the local Asian bottled waters are low in all minerals, as they are usually obtained through membrane-treatment reverse osmosis or distillation[1]. The physical, chemical and microbial properties of 14 different bottled water brands in Baghdad city were recently evaluated by Rabeea and his colleagues. They found that calcium but not magnesium concentration was below the respective bottled drinking water standard[14].

Since the contribution of drinking-water, as a source of minerals, depends on the water mineral concentration and the amount of water consumed, the shift in consumption from tap water to bottled water, with different mineral constituents, may have important implications in health and disease. Hence, as a major concern, the present study was conducted in an attempt to compare magnesium and calcium contents of commercially available, local and imported, bottled drinking waters, to household drinking water supplies in Baghdad, Iraq.

METHODS:

Tap water distributed through thepiped water delivery system in 10 municipalities of Baghdad city was investigated in the present study. Within each municipality, four specimens were collected, in plain plastic screw-cap containers, from different tap water sources during a single day. Sources equipped with household water softener device orstorage tanks were disqualified, as it is known to be associated with diminution in magnesium and calcium water contents.

Four local and Seven imported brands of commercially available bottled drinking waters were enrolled. Four specimens from each brand were obtained, and their labeled pH, magnesium and calcium values were recorded.

Tap and bottled water levels of magnesium and calcium were determined spectrophotometerically, using the Blue Xilidil method (Giesse Diagnostics, Roma-Italy), and the O-cresolphtaleine method (Biomaghreb, Morocco), respectively. PH values were measured for all water specimens utilizing a pH meter (Great lakes, INC.). All samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >5%. The Mann-Whitney test was used to compare means of magnesium and calcium levels in different water sources, P values < 0.05 were considered to be statistically significant.

RESULTS:

Measurements of pH, magnesium and calcium, indicates little differences in tap water samples utilized from the 10 municipalities (Table 1). While, results of bottled drinking water show dramatic variations in both local (Table- 2) and imported (Table-3) brands. Furthermore, dissimilarities between the estimated and labeled values were clearly observable, especially among local brands.

Estimated mean levels (±SD) for tap water were 7.97±0.17, 22.42±0.54 mg/l, 107.2±11.48 mg/l and 4.78±0.46 for pH, magnesium, calcium and Ca:Mg ratio, respectively (Table 4). Mean pH values of imported (7.34±0.27), but not local (7.73±0.31), bottle drinking waters were significantly lower than tap water ($P< 0.001$). Alternatively, and for both brands, magnesium contents of bottled drinking waters were significantly lower (11.84±6.81 mg/l: $P< 0.05$ and 6.05±4.41 mg/l: $P< 0.001$ for local and imported brands, respectively) as compared to tap water levels, analogous results were observed for calcium concentrations (29.0±16.17 mg/l: $P< 0.001$ and 15.6±11.97 mg/l: $P< 0.001$ for local and imported brands, respectively). However, only local brands show a significant reduction in Ca:Mg ratio (2.68±0.67 : $P< 0.01$), relative to that of tap water.

DISCUSSION:

Owing to the growing apprehension that certain constituents or contaminants of potable water may affect health, consumption of tap water in Baghdad, Iraq, during the past few years has decreased and consumption of bottled water has drastically increased in spite of its high unit price relative to tap water. Nevertheless, an accurate measure of the annual increase in bottled water consumption in this city is yet to be established.

According to the WHO, drinking water with an elevated pH above 11 can cause skin, eye and mucous membrane irritation. On pH value below 4 also irritation can occur due to the corrosion effect of low pH levels.

In this study, the range of pH values of bottled water for both local and imported brands were generally less than that of tap water (Table 4). This could be attributed to the corresponding magnesium and calcium concentration, which found in tap water, relatively higher than that of bottled water. Nevertheless, the observed values of pH for all studied samples, whether tap or bottled water, were within the recommended standard WHO limits of (6.5 – 8.5).

Calcium and magnesium are important minerals of drinking water and are of both direct and indirect health significances. A certain amount of these minerals in drinking water is desirable since their deficiency possess a health risk. Inadequate intakes of calcium have been associated with increased risks of osteoporosis, nephrolithiasis, colorectal cancer, hypertension and stroke, coronary artery disease, insulin resistance and opacity. Low level of magnesium statutes has been implicated in hypertension, coronary heart disease, type 2 diabetes mellitus and metabolic syndrome.

The daily intake requirements, which also known as Dietary Reference Intakes (DRI), for adult humans are estimated by 1000 mg calcium and 310-420 mg magnesium. Although water may be a useful source for providing calcium and magnesium minerals, there is no guideline on minimum concentration of these minerals in drinking water. The contribution of water to calcium and magnesium intake depends on the amount of the minerals in the water and the amount of water consumed.

With the respect to the gender and age, the dietary references intakes (DRI) of Calcium is nearly 3 times of Magnesium. For the studied tab water, and on the basis of mean values, person in Baghdad city, by drinking 2 liters per day, may take not more than 12% of magnesium DRI and 21% of calcium DRI. On the other hand, for both local and imported bottled water, the contribution of magnesium and calcium minerals to DRI values are approximately 6.5%, 5.8% and 3.2%, 3.1% respectively. These results show that tap water in Baghdad city can be classified as minerals-rich resource in comparison to local and imported bottled water. Calcium level in tap water represent about three times local bottled water and seven times of imported bottled water. Magnesium level, on other hand, that present in the tap water can go up to twice and four times of both local and imported bottled water content respectively. According to the classification of water hardness, which resemble the concentration of dissolved magnesium and calcium in mg/l (Table 5), Baghdad city tap water can be sorted as slightly hard water while local bottled water is soft water and the imported once even softer.

Since the magnesium in water is absorbed approximately 30% faster than magnesium from food[13], therefore the supplementation of magnesium may be best achieved using drinking water. Calcium level and thus Ca:Mg ratio of the water influence the absorption of magnesium from intestine. The magnesium is located inside the cell (intracellular) while calcium is predominantly located outside the cell (extracellular) . Consequently, the role of magnesium in

intracellular metabolic functions such as energy production, respiration and muscle contraction-relaxation is antagonistic to calcium . The physiological role of magnesium in the body is achieved through two important properties; the ability to form chelates with important intracellular anionic- ligands, especially ATP, and is ability to compete with calcium for binding sides on proteins and membrane. [15]

This study show that Ca:Mg ratio for tap water in Baghdad city has a higher values in comparison to both local and imported bottled drinking water. The increment in these values can arrange as 1:1.38:1.78 for local bottled water, imported bottled water and tap water respectively.

CONCLUSION:

The remarkable shift in consumption from tap to bottled water, with extremely low levels of magnesium content, for both local and imported brands, may suggest possible implications on community health. Further efforts are recommended to explore the scope of these implications, as well as the need to set new and better priorities for drinking-water practices

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Table 1: Magnesium and Calcium content (Mean ± SD) of Tap Water from different municipalities of Baghdad City.

Municipalities	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg
Mustansiriyah	7.9± 0.08	22.1± 0.06	108.0± 1.08	4.88± 0.03
Salihyia	7.9± 0.07	22.8± 0.08	111.0± 1.08	4.87± 0.03
Manssor	8.1± 0.09	23.2± 0.09	125.0± 1.08	5.38± 0.03
Durra	8.2± 0.07	22.8± 0.07	95.0± 1.08	4.17± 0.03
Sha'ab	8.1± 0.07	22.2± 0.08	106.0± 1.08	4.77± 0.03
Zyona	8.0± 0.08	22.7± 0.06	127.0± 1.08	5.60± 0.03
Saydia	8.1± 0.06	21.2± 0.08	100.8± 1.08	4.75± 0.03
Sa'adon	8.0± 0.08	22.3± 0.08	105.9± 1.08	4.74± 0.03
Sumar	7.7± 0.09	22.7± 0.07	101.0± 1.08	4.45± 0.03
Alwiyah	7.7± 0.08	22.1± 0.08	92.0± 1.08	4.16± 0.03

Table 2: Magnesium and Calcium content of commercially available Local Bottled Water in Baghdad City.

Brand name (Country)	Labeled Values				Measured Values			
	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg
Furat (Iraq)	7.5	11.0	19.0	1.7	8.0	13.2	27.0	2.1
Fayha (Iraq)	7.0	4.0	15.0	3.8	7.3	2.0	7.0	3.5
Al-Sabah (Iraq)	7.5	17.0	38.0	2.2	7.9	14.7	43.0	2.9
Mina (Iraq)	7.3	19.0	37.0	1.9	7.7	17.5	39.0	2.2

Table 3: Magnesium and Calcium content of commercially available Imported Bottled Water in Baghdad City.

Brand name (Country)	Labeled Values				Measured Values			
	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg
Juda (Kuwait)	7.2	10.0	15.0	1.5	7.3	9.5	16.4	1.7
Rawdatain (Kuwait)	7.8	5.2	39.0	7.5	7.8	4.8	39.0	8.2
Aquafina (Jordan)	7.0	13.0	5.0	0.4	7.0	11.8	5.3	0.4
Nestlé (Jordan)	NA	9.5	20.0	2.1	7.6	10.4	23.0	2.2
Hana (Saudi Arabia)	7.4	3.7*	12.0	3.2	7.2	1.7*	7.1*	4.3
Honey (Saudi Arabia)	7.3	2.4	8.8	3.7	7.3	1.8	9.1	5.1
Azbah (Saudi Arabia)	7.2	3.0	10.0	3.3	7.2	2.4	9.6	4.0

Table 4: Magnesium and Calcium content of different Drinking Waters in Baghdad City.

Water Source	pH	Mg (mg/l)	Ca (mg/l)	Ca:Mg
Tap Water				
Mean ± SD	7.97±0.17	22.42±0.54	107.2±11.48	4.78±0.46
Median	8.00	22.51	105.94	4.76
Range	7.7- 8.2	21.2- 23.2	92.0- 127.0	4.2- 5.6
Local Bottled Water				
Mean ± SD	7.73±0.31	11.84±6.81	29.0±16.17	2.68±0.67
P –Value*	NS	< 0.05	< 0.001	< 0.01
Median	7.80	13.93	33.00	2.58
Range	7.3- 8.0	2.0- 17.5	7.0- 43.0	2.1- 3.5
Imported Bottled Water				
Mean ± SD	7.34±0.27	6.05±4.41	15.6±11.97	3.70±2.56
P –Value*	< 0.001	< 0.001	< 0.001	NS
Median	7.30	4.76	9.60	4.00
Range	7.0- 7.8	1.7- 11.8	5.3- 39.0	0.4- 8.2

*versus Tap Water.

Table 5 Classification of water hardness

Type of Water	Conc. Of dissolved Mg and Ca (mg/l)
Soft	0 – 60
Moderate	61 – 120
Hard	121 – 180
Very hard	>180

Renoprotective effects of zinc sulphate and silymarin against thallium-induced poisoning in rats

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Abstract

Background: Thallium (Tl) is a heavy metal that its salts are highly toxic. This element is widely used in manufacture, and still being used as a rodenticide in some countries. Many occupational, accidental and criminal thallium toxicity cases present to clinics all over the world. Thallium toxicity affects all body compartments including renal tissue.

Objective: to study the renoprotective effects of zinc sulphate and silymarin therapy in rats poisoned with thallium.

Methods: 48 albino rats of both sex were classi ed into 4 groups, each group contains 12 rats, rst group animals were given only dis lled water orally for 5 successive days, second group were given a single oral dose of thallium acetate (16 mg/ kg) followed by a daily oral dose of distilled water. Third group given the same oral dose of thallium followed by a daily dose of zinc sulphate solu on (20 mg/kg) for 5 successive days. Fourth group rats were given the same oral dose of thallium acetate followed by a daily oral dose of silymarin solu on (25 mg/kg) for 5 successive days. Serum urea and crea nine were measured and renal tissue sections were taken for histopathological study.

Results: rats treated with zinc sulphate and silymarin had shown significant changes in serological and histopathological results in comparison to normal and thallium groups.

Conclusion: zinc sulphate and silymarin have renoprotective effects against induced thallium poisoning in rats.

Key words: thallium, zinc sulphate, silymarin, renoprotective.

Introduction :

Thallium is a chemical element referred to by the symbol (Tl) . It was discovered in 1861 by the English chemist William Crookes and metallic thallium was rst prepared by a French scien st Lamy in 1862[1]. The discovery of thallium occurred incidentally by spectroscope when the English chemist Crookes was examining a sludge left over from the production of sulfuric acid (H_2SO_4) in which he was looking for Selenium. After removing all of the selenium from the sludge, he inspected it with a device known as a spectroscope. He observed a bright green line that no one had ever seen before. He named the new element that was producing the green line thallium, after the Greek word for 'green twig', thallos [2] . Thallium is a natural constituent of earth crust, it presents in nearly all environmental media[3].

Thallium is present mainly in industrial wastewaters. Information on specific waste streams is not difficult to find; however, individual mines often do not want their thallium concentrations published. [4]

Absorption of thallium compounds is rapid after ingestion, inhalation, or skin contact and may be complete after ingestion.[5] Thallium is widely distributed in the body after its rapid absorption in animals. Acute and chronic studies agree that the highest concentration is found in the kidneys.[6] Thallium is mainly excreted in the faeces though this may be decreased significantly by paralysis of the small intestine, a characteristic feature of thallium

poisoning. [7] Thallium is also excreted in the urine, but about half the amount in the glomerular filtrate is reabsorbed in the tubules. The ratio of faecal to urinary elimina on is approximately 2:1.[8]

The exact mechanisms that mediate thallium toxicity are still poorly understood and not known since thallium interacts with cells at different levels. [9]There are similarities between the ions of thallium and those of potassium: they have similar ionic radii, and there is experimental evidence to suggest that the biological handling of thallium and potassium ions is interrelated[10]. Thallium can mimic potassium ion in most biological processes because of the same ionic radius and inability of the cell membrane to differentiate between these two cations. Moreover, thallium follows potassium distribution pathways and in this way alters many of potassium-dependent processes. For example, thallium may substitute potassium in the Na+/K+-ATPase. Interference with potassium transport has been demonstrated in rabbits, where thallium had a 10 mes higher affinity for Na+/K+-ATPase than potassium[11].

Thallium has a high affinity for the sulfhydryl groups present in many enzymes and other proteins. Due to the presence of empty d-orbitals in electronic configuration, thallium has a high affinity for sulphur ligands. It can form complexes with sulfhydryl groups of proteins which are usually involved in reactions catalysed by enzymes leading to their inactivation[12]

When bound to membrane phospholipids, especially to the anionic head groups, thallium changes membrane rheology, lipid packing, lipid arrangement in the lateral phase of the bi-layer, and the hydration of the polar head groups. [13]

Toxic injures to the kidney have been indicated by albuminuria and haematuria, however, the renal function is not grossly impaired. [14]. The toxic effects on the kidney, notably resistant to dehydration and decubitus, also strain the body's resources. In most of cases, the kidney present limited toxic injury. During the rst 2 weeks a er exposure, there is albuminuria with erythrocytes, leucocytes and cylindrical casts in the urinary sediment, and sometimes porphyria. In this serious phase of the illness, there is a fall in the concentrating ability of the kidneys, often with recovery afterwards. In some cases, there is also hemorrhagic damage to the kidneys. [15]

Methods :

The present study was conducted in the animal house / Baghdad college of medicine, between June 2012 and March 2013.

48 healthy albino rats of both sexes with body weight between 150 - 200 gm were divided into 4 groups, each group contains 12 rats. They were supplied by animal house of Baghdad college of medicine. They were kept in a well controlled hygienic environment, every rat was housed in a single cage . Rats had taken food and water *ad libitum*.

The groups were arranged as the following:

group A in which 12 rats were given only dis lled water orally for 5 successive days, group B in which 12 rats were given a single oral dose of thallium acetate (16 mg/ kg) dissolved in dis lled water followed after 2 days by a daily oral dose of 3 ml of dis lled water.

Group C in which the same oral dose of thallium was given followed a er 2 days by a daily dose of zinc sulphate solu on (20 mg/kg) for 5 successive days. Group D in which 12 rats were given the same oral dose of thallium acetate followed a er 2 days by a daily oral dose of silymarin solu on (25 mg/kg) for 5 successive days. Serum urea and creatinine were measured and renal tissue sections were taken for histopathological study.

The results are expressed as means ± standard deviation (SD). Data were analyzed by paired t- test to elucidate di erences between each 2 groups of the study. First, to compare between normal group and thallium group and to study the effects of thallium on normal parameters. Then, to compare the effects of each drug group with thallium group. Results considered signi cant when P value < 0.05.

Results :

Serum urea and s. creatinine:

In comparison with normal group, thallium caused an extremely statistically significant increase in both serum urea (P = 0.000) and crea nine (P = 0.002).

Table (1) : the serum urea and crea nine levels (M + SD) comparison between normal and thallium groups.

Biochemical Test	Thallium M + SD No.=12	Normal group M + SD No.=12	value
Urea (mg/dl)	58.25* + 13.91	28.17 + 5.13	.000*
Creatinine (mg/dl)	0.61*+ 0.14	0.45 + 0.07	.002*

Values represent M + SD

Results were considered statistically signi cant when ($P < 0.05$).

Serum creatinine values have shown a statistically significant change in zinc sulphate treatment ($P = 0.017$) group. While no sta s cally signi cant change

Table (2): comparison of renal function tests (s. urea and s. creatinine) values between thallium group and zinc sulphate treatment group.

Biochemical Test	thallium (M + SD) No.=12	Zinc sulphate post-treatment (M + SD) No.=12	P value
Urea (mg/dl)	58.25+ 13.91	45.92 + 13.31	0.114
Creatinine (mg/dl)	0.61 + 0.14	0.49* + 0.07	**0.017***

Values represent M + SD

Results were considered sta s cally signi cant when ($P < 0.05$)

serum crea nine also reduced signi cantly in silymarin treatment ($P = 0.004$) groups. And no sta s cally signi cant change occurred in serum urea levels occurred between thallium and silymarin group.(Table 3)

Table (3): comparison of renal function biochemical tests (s. urea and s. creatinine) values between thallium group and silymarin treatment group.

Biochemical Test	thallium (M ± SD) No.=12	Silymarin treatment (M ± SD) No.=12	P value
Urea (mg/dl)	58.25± 13.91	46.4 ± 11.6	0.085
Creatinine (mg/dl)	0.61 ± 0.14	0.45* ± 0.06	**0.004***

Values represent M ± SD

Results were considered sta s cally signi cant when ($P < 0.05$)

Histopathological results :

Micrographs ofrenal histopathology sections from thallium group rats had shown extensive vascular congestion and RBCs extravasation in both cortical and medullary areas. In many sections wide cortical destruction revealed by glomerular shrinkage and cellular necrosis. Tubular degeneration and even destruction and loss of normal architecture revealed in different sections and areas of the same section. (gure 2).

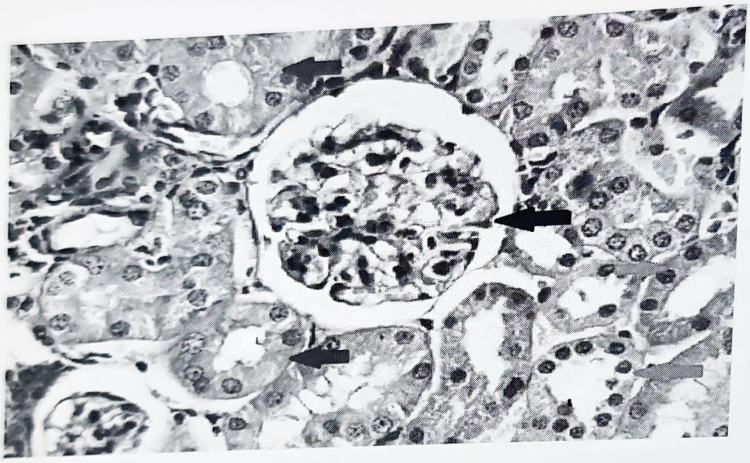

Figure (1) : **normal renal histology : A** photomicrograph of renal cortex of a normal rat showing the glomerulus (black arrow), proximal convoluted tubules (blue arrows) and distal convoluted tubules (green arrows). . **(H&E staining, 400x)**

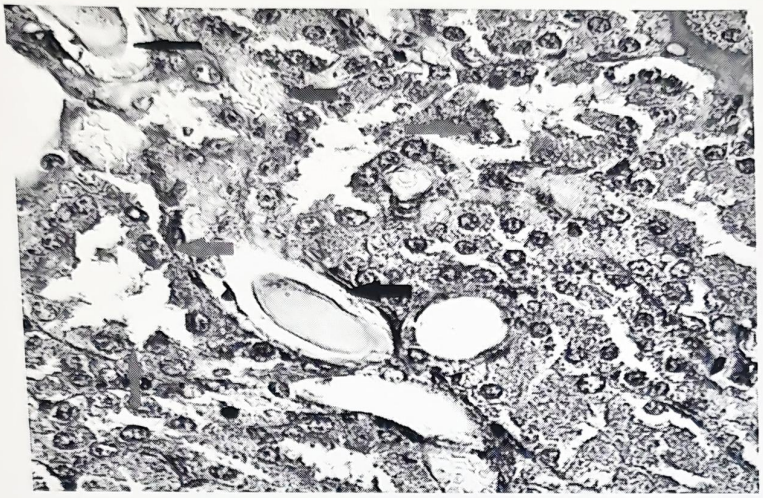

Figure (2) : thallium histopathological changes in renal ssue : A photomicrograph of renal cortex showing amorphous acidophilic tubular protein casts (black arrow), RBCs extravasation (blue arrow) and tubular necrosis (red arrow). there is also tubular degeneration (white arrow) .**(H&E staining, 200x)**

In most sections of zinc sulphate treatment group, the main histopathological feature was mild to moderate vascular congestion and RBCs extravasation, the architecture was preserved and degenerative changes were limited and mild in nearly all sec ons (gure 3)

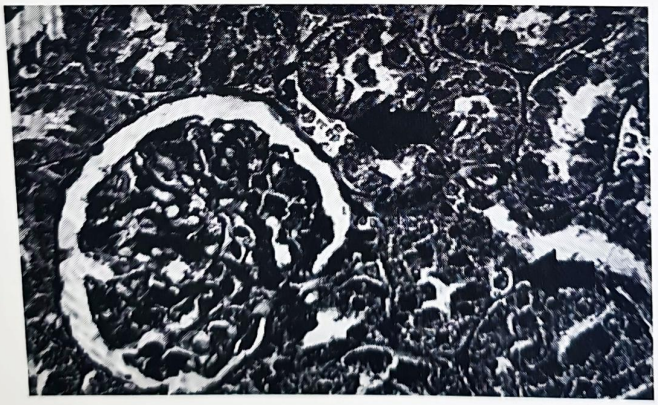

Figure (3) : : histopathological changes in renal ssue of thallium poisoned rats treated with zinc sulphate: this micrograph reveals a mild RBCs extravasation between tubules (black arrow), tubulo-glomerular architecture is preserved normal size of bowman space .**(H&E staining, 400x)**

Micrographs of silymarin treatment group revealed frequent vascular congestions (usually mild), RBCs extravasations in most sections and infrequent protein casts. Degenerative changes appeared in some sections but in general normal architecture was preserved. (gure 4)

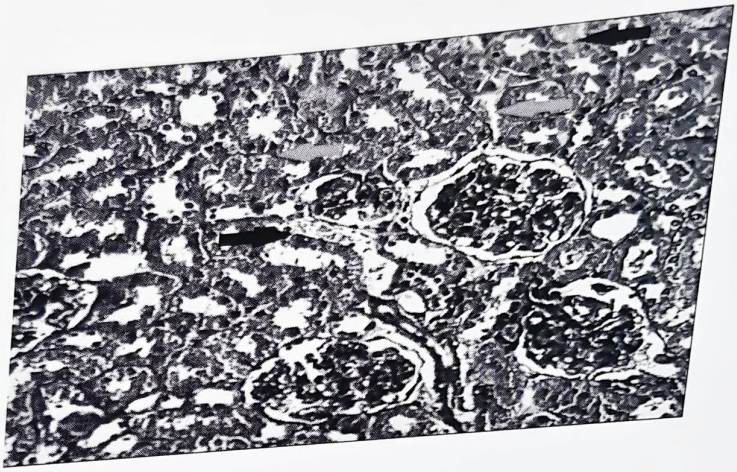

Figure (4) : histopathological changes in renal ssue of thallium poisoned rats treated with silymarin: In this micrograph there is mild vascular congestion (black arrows), and RBCs extravasation (green arrows) . **(H&E staining, 200x)**

Discussion :

The present study results of s. urea and s. creatinine had shown an extremely significant increase in both parameters. Many studies reported this elevation in rats. [xvi]This elevation indicates renal failure to excrete these metabolic waste products due to thallium toxicity-induced renal tissue damage.

Zinc sulphate caused a reduction in both parameters but significant reduction was confined to s. creatinine level . These results confirm the tissue protective effects of zinc outlined previously. Also, silymarin caused a significant reduction in creatinine . A rise in blood creatinine level is observed only with marked damage of functioning nephrons. Therefore, this test is unsuitable for detecting early-stage kidney disease[xvii] Thus, the significant reduction of s. creatinine is a clear effect reveals the reduction of renal damage severity by zinc sulphate and silymarin.

Thallium histopathological changes can occurwithin 24 hours.[xviii] Renal histopathological changes observed were extensive vascular congestion, RBCs extravasation in most of cortical and medullary parts, degenerative changes included cellular necrosis resulting in glomerular shrinkage and renal tubular damage. In addition, characteristic tubular eosinophilic casts were present in most sections.

zinc sulphate treatment caused a prominent improvement in histopathological features of poisoning which included preservation of general architecture, reducing the severity of congestion, extravasation and cellular degeneration and reduced distal tubular casts.

Zinc is a well-known antioxidant agent. The antioxidant properties of zinc were recognized for many years either through increase of oxidative stress and/or free radicals generation due to zinc deficiency or via reduction of oxidative stress and/or its consequences in the living organism by zinc supplementation. [xix] Zinc may exert many protective antioxidant effect by stabilizing lipid membranes and preventing lipid peroxidation by free radicals. [xx]. Zinc has a property of sulfhydryl groups protection. It maintains protein sulfhydryl stabilization thus preventing intramolecular disulfide formation.[xxi]

Silymarin treatment group micrographs had shown less frequent casts and well preserved architecture with mild degenerative changes. These results indicate that the antioxidant mechanism of silymarin had caused a renoprotective effect against thallium poisoning.

Conclusion:

Oral zinc sulphate and silymarin have renoprotective effects against renal damage caused by thallium poisoning.

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Gallic Acid Inhibits *E.coli* Adhesion to Bladder Cells in Induced Urinary Tract Infection in Diabetic Rats

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Abstract

Background: Diabetic people have a higher incidence of urinary tract infection than non-diabetics. *E. coli* bacteria are the most common pathogen associated with these infections, causing over 80% of urinary tract infections. Recurrent use of antibiotics can lead to pathogen resistance and result in side effects. Consequently there is a need for safe alternative medications as natural source that can be used to prevent urinary tract infections.

Gallic acid is found as part of tannins in some plants. Gallic acid is shown to possess antimicrobial properties, reduce albuminuria and hyperglycemia. This study was undertaken to investigate the role of gallic acid in inhibiting bacterial adhesion to bladder epithelial cells in induced diabetes and urinary tract infection in rats.

Methods: Diabetes was induced in rats by intraperitoneal injection of alloxan (135 mg/kg) to induce pancreatic islet cell death. Blood glucose levels were measured daily after the IP injection. Blood glucose levels of >250 mg/dl was considered as positive for diabetes initiation. Bladder infection was induced by transurethral catheterization of $1X10^8$ *Escherichia coli* (ATCC 252922).

After 18 hours of infection, gallic acid was administered orally at a dose of 2.5,5 and 10 µg/ml. the rats in each group were sacrificed after 72 h of infection and the bladder epithelial cells were collected and processed for the determination of adhesion of *E.coli* to the epithelium.

Conclusion: Gallic acid at concentrations of 5 and 10 µg/ml significantly inhibited the adhesion of *E.coli* to bladder epithelial cells compared to controls.

Key words: Gallic acid, *E.coli* adhesion

Cutaneous Leishmaniasis Outbreak at Al-Shurqat district, Iraq, Jan, 2011

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Background: Cutaneous leishmaniasis (CL) is parasitic disease caused by different protozoan species of the genus Leishmania and transmitted by bites of infected sandflies. It is endemic in over 80 countries, and emerged recently in new foci, posing a public health problem. In Iraq it is endemic in mid and southern provinces, recently it start affecting Northern provinces.

Objective: To determine epidemiological chracteristics of victims of CL outbreak in Shurqat district, north Iraq, 2011.

Methodology: A cross sectional study conducted on available victims; data was collected using a questionnaire including basic data, lesion chracteristics and potential risk factors especially travel history, sleeping outdoor, and presence of dogs.

Results: Among the 81 victims 75 were approached, male to female ratio was 1.6:1, Around 39% were <5years, and similar proportion aged 5-15 years. About 57% of lesions were on the face, 24% on upper limb, and only 4% were on the trunk. 71% of patients had solitary lesion. 90% of patients used to sleep outdoors, 38.66% of patients have direct contact with dogs, and 4 patients have travel history to an endemic district.

Conclusion: CL is spreading to new non endemic areas in north Iraq reflecting inefficient control measures. Collaboration of health, environment and agricultural agencies are needed to control this rapidly growing public health problem.

Key words: Cutaneous Leishmaniasis, Al-Shurqat district.

Outcome of surgical resection of subcortical metastatic tumors in the central and paracentral regions of the brain

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Abstract

Background. The operative treatment of subcortical metastatic tumors within the central and paracentral area is still under discussion. Against the background of possible new postoperative neurological deficits and of evolving new radio oncological techniques, the indication for surgery is limited only to a subgroup of patients.

Objectives. In this study we present the clinical results after operative treatment of metastases within the central and paracentral brain regions, with an emphasis on the short-term and mid-term functional outcome. With this study, we present the clinical results after surgery of patients suffering from symptomatic subcortical metastases located within the central and paracentral regions. Furthermore, we discuss the results against the background of the recent literatures.

Methods. This study was carried out in the period between January 2007 and January 2011 in the neurosciences hospital in Baghdad/Iraq.

The prerequisite inclusion criteria for the patients to be included in this study were:

1- A contrast enhanced C.T and/or MRI showing a secondary lesion in the central or paracentral regions of the brain.
2- The patient has a known primary tumor source with a confirmed histopathological diagnosis.
3- The postoperative histopathological result of the removed brain tumor correlates with the histopathology of the primary tumor.
4- A stable extra cerebral tumors dissemination controlled by a systemic oncological therapy and an estimated life expectancy of more than 6 months.

If the patient did not have, even one, of the above criteria he or she has not been included in this study.

The Karnofsky Performance Status (KPS) was used to measure the patients' functional ability. The Functional Assessment of Cancer Therapy-Brain (FACT-Br) determines various quality of life (QOL) aspects in brain tumors patients. On a 54-item scale patients respond from 0 (not at all) to 4 (very much). With the FACT-Br five major components of QOL are assessed: physical, social, emotional, functional well-being, patient-physician relationship and other activities of daily life (ADLs).Tumor localization and its extension were defined on preoperative gadolinium-enhanced T1-weighted MRI-scans. Additional information was gained from T1-, and T2- scans, which were done in all patients. After updated MRI those patients were evaluated for surgery. Early postoperative control was done by MRI within the first 10 days. Follow-up took place in the outpatient department, assessing clinical criteria 2 and 6 weeks postoperatively, followed by clinical control and MRI-scans every 3 months. In all patients, surgery was performed under general anesthesia.

Results. We report on 20 patients suffering from subcortical brain metastases within the primary sensori-motor area, with a median volume on MRI-scans of 8.18 cm . Patients were admitted to the hospital with a progressive hemiparesis ($n = 11$), focal seizures ($n = 6$) or other unspecific symptoms ($n = 3$) like headache, nausea, and neuropsychological disturbances, respectively. Surgery and the early postoperative course were uneventful in all cases. After a 6 months follow-up, two patients had died. The motor deficits improved in seven patients and remained unchanged in four cases. One patient suffered from a new persistent hemiparesis. A temporary paresis occurred in two cases. In five patients there was no motor deficit pre- and postoperatively. The KPS improved in ten patients 6 months after surgery. Quality of Life, measured by the FACT-Br score, improved in 12 patients and remained unchanged in one patient.

Conclusions. Even preexistent deficits can improve with positive influence on the quality of life for oncological patients, being disabled by the symptoms caused by the cerebral lesion.

Keywords : Brain metastases • Central region • paracentral region • Operative treatment • Neurological outcome

The effect of leptin Level in Pregnancy Complicated by Intrauterine Growth Restriction on the Neonatal Outcome

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Abstract

Background: Fifteen percent of small for gestational age are small as a result of fetal growth restriction, which could be due to maternal, placental or fetal factors. It is an important clinical problem associated with increase perinatal mortality and morbidity. Leptin is a protein that produced by many tissues including the placenta (syncytiotropholoast). Dysregulation of leptin metabolism may be implicated in preeclampsia and IUGR pathogenesis.
Aim of the study: To study the trend of leptin level alteration in maternal serum and cord blood in pregnancies complicated by fetal growth restriction and its relation with neonatal outcome.
Methods: An Analytic, cross- sectional study conducted in Al-Elwyia Maternity Teaching Hospital and Alkindy College of Medicine, from October 2009 to June 2010. Sixty seven pregnant women were included and they were divided into two groups: The first group (A) included 34 pregnant women with Intrauterine growth restricted fetuses with and without maternal diseases and the second group (B), included 33 pregnant women with normal pregnancies. Samples from maternal blood and umbilical cord blood were obtained at the time of delivery and leptin level was measured by Enzyme linked immunesorbant assey (ELISA) test.
Results: Umbilical cord leptin level was significantly lower in group A (medain1ng/ml) compared with group B (median10.2ng/ml); P<0.001, and maternal serum leptin was also significantly lower in group A (median19.8ng/ml) compared to control group B (median31.8ng/ml), P 0.042.
Newborn weight for age (Z score) and maternal body mass index were the most important and the only statistically significant determinants of cord blood leptin, while only maternal body mass index had a strong and statistically significant positive association with maternal serum leptin.
In group A, there was a linear correlation between cord blood leptin and placental weight, P<0.001, and a linear correlation between Apgar score at 5minutes and cord blood leptin level, P<0.001.
Conclusion: Women who had growth restricted fetuses had significantly lowered umbilical cord and maternal serum leptin levels than women who had normal fetuses and the outcome of such fetuses could be related to the leptin level.
Key words: Intrauterine growth restriction, cord blood leptin, maternal serum leptin.

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