TOFIQ Journal of Medical Sciences
TJMS
Volume 1, Issue 1, 2014
ISSN: 2377-2808

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TOFIQ Journal of Medical Sciences (TJMS) is published by TOFIQ: an NGO registered at the State of Maryland as a non-profit organization dedicated to helping Iraq Higher Education and Research.

TJMS is devoted to the publication of original research, commentaries on a current topic, reviews, letters to the editor, and editorials in the field of medical sciences. The early focus of the journal is on clinical burden of disease in Iraq: documentation of its nature and extent; clinical patterns and epidemiology; diagnostic findings; and therapeutic strategies. Read more ...

Focus and Scope

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Letter from Editor –in-chief

December 17, 2015

Dear Colleagues,

On behalf of the editorial board of TOFIQ Journal of Medical Sciences, TJMS, it is my pleasure to announce that our newly launched TOFIQ Journal of Medical Sciences (TJMS) has completed its second year.

Colleagues from the U.S., New Zealand, UK and Jordan have contributed to TJMS. That is added to publication of the Iraqi group of scientists and professionals in the medical field, who were the main target of the journal.

TJMS is an international, peer-reviewed open access online journal published by TOFIQ, which is a non-governmental non-profit NGO established by Iraqi American academics and professionals in the United States of America.

TJMS is devoted to the publication of original research, commentaries on a current topic, reviews, letters to the editor, and editorials in the field of medical sciences.

We invite submission of articles on topics pertaining to the science and art of medicine that help fulfill the journal's mission of publishing knowledge in the fields of medicine, dentistry, pharmacy, nursing, bioengineering and all related sciences that help scientists, clinicians and professionals in the medical fields to improve expertise, patient care and public health.

For submission information, please go to the web link:
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I look forward to hearing from you,

With best regards,

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THE CHANGES IN THE INCIDENCE OF GASTRIC VERSUS COLORECTAL CANCER IN IRAQ DURING THE PERIOD BETWEEN 1965-2006

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Abstract

Background:

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer death in the world, while the colorectal cancer (CC) is the third commonest cancer in US and second most common in Europe.

Objective:

This study is aiming to identify the incidence of both GC and CC and their stages in Iraq over a period of forty two years.

Methods:

A retrospective audit study of 2240 cases with 1237 patients with GC and 1003 patients with CC were operated on between 1965-2006 included. The age, sex, stage and the incidence of each cancer every two years for the study period were reported and compared with the Iraqi Cancer Registry and the published data.

Results:

Gastric cancer affected males more than females. The average age for GC was 53.66 for males and 50.47 year for females. The average age for CC was 56.83 year for males and 53.65 year for females. The GC counted for around 2/3 of cases in the cases in the late sixties, while in the early eighties the CC constituted around 3/4 of the total number of cases assessed in this diagnosis. There was a significant increase in earlier cancer stages for both cancers in the later half of the study period. Comparing the first half to the second half with the second one, we found an insignificant rise in the number of cases of GC while there was a significant increase number of CC comparing both periods.

Conclusion:

There was a change in the pattern of gastrointestinal cancer, particularly GC and CC in Iraq, which was attributed to dietary factors. On the other side there was a significant increase number of early staging cancer. These findings mimic the Iraqi Cancer Registry and the western results.

Introduction:

Gastric cancer (GC) is fourth most common cancer in the world with just under one million cases recorded in the year 2008, behind cancers of the lung, breast and colorectal, respectively. It is also the second leading cause of cancer death in both sexes worldwide.

(1) In Iraq, it is the leading gastrointestinal malignancy (2,3) while colorectal cancer (CC) was the third after the oesophagus (3, 4). Gastric cancer include any malignant tumor (excluding gastrointestinal stromal tumours) arising from the region extending from the gastroesophageal junction (GOJ) and the pylorus, it may not be possible determine the site of origin if the cancer involve the GOJ itself; a situation that has become more common in recent years, especially with the rising incidence of Barrett's and its related GOJ cancers. The majority of Gastric Cancers are adenocarcinoma, almost two thirds of them occur in developing countries with peak incidence in the far east regions including China, Korea and Japan.

Colorectal cancer is the third commonest cancer in USA following prostate and lung/bronchus cancer (5) and the second commonest cancer in Europe following breast cancer (6). The incidence of CC incidence in Iraq was increasing gradually within the last three decades (8). The risk of developing CC begin to increase at age 40 years and rises with age (8). Screening for colorectal cancer reduced the number of cases with late stages and increased those with early stage cancer (9).

Aim:

The aim of this study is to present the changes noticed in the rise of CC versus the GC in Iraq during the period (1965-2006), especially in the last three decades of the past century.

THE CHANGES IN THE INCIDENCE OF GASTRIC VERSUS COLORECTAL CANCER IN IRAQ DURING THE PERIOD BETWEEN 1965-2006

Patients and Methods:

A retrospective audit study on 2240 cases, 1237 patients with GC and 1003 patients with CC operated upon by the senior surgeon (Al-Bahrani ZR) at the medical city University hospital and Al-Mustansiria private hospital, Baghdad from 1965-2006, included. The majority of both cancers were adenocarcinoma.

The age, sex, stage and the incidence of each cancer every two years for the study period were reported. The stage of GC and CC were recorded according to American Joint Committee on Cancer (AJCC). (10) We divided the total number of cases between two groups of twenty one years each; period A 1965-1985 and period B 1986-2006. The stages of each cancer were compared between different the two periods. The results were compared between both cancers and with the reports from the Iraqi Cancer Registry.

Results:

Adenocarcinoma of the stomach constituted 90% of all gastric cancers (1117 out of 1237 cases), the rest of the cancers were malignant lymphoma (120 cases, 10%). Gastric cancer affects males more than females with a reducing male: female ratio of 2.3:1 in 1971 to 1.6:1 in 2006. The peak age incidence for GC was in the fifth decade for women while it was in the sixth decade for men. (Figure 1) The average age for GC was 53.66 for males and 50.47 year for females. The males exceeded the females at all age groups except in patients less than 25 years old where the incidence of GC was higher in females than males.

Almost all colorectal cancers were adenocarcinoma (985 cases, 98%); there were only 18 (2%) cases with malignant lymphoma. The same male: female distribution and same age incidence occur for CC with one exception that the incidence of males was higher than females in all age groups. (Figure 2) The average age for CC was 56.83 year for males and 53.65 year for females. It was also noticed that the difference between males and females was minimal (2-5 patients per decade) at age of 54 years and below in CC. In both cancers, the incidence dropped steadily after their peeks in both sexes.

The GC counted for around 2/3 of cases in the cases in the late sixties and continue to increase until reach the peak of around 84% in 1971/1972 where the colorectal reach the lowest incidence of 16% of cases in that period. Then the percentage of both cancers reversed to the original 2/3 for GC and 1/3 for CC by the late seventies where it remained around that figure until mid nineties where the number of CC reach almost same

percentage of GC. After that there were persistent increase in the incidence of CC with continue drop in the percentage of GC until the end of the study period where CC consume 3/4 of cases versus 1/4 for GC in the year 2005/2006. Actually, the number of GC had not dropped that much but the number of CC had increased significantly to reverse the percentage ratio of the total number of cases on that period. (Figure 3)

Comparing the two period groups, we found there was slight increase in the total number of GC from 539 cases in period A to 689 cases in period B, which is statistically insignificant. On contrary, there was a statistically significant increase (over three fold) in the number of cases of CC between the two periods; from 241 cases in period A to 762 cases in period B.

Comparing the stage of each cancer between the two periods, there was an increase in stage I and II and drop in stage IV for GC from period A to period B; this was statistically significant for stage I and a trend toward significance for both stage II and IV. There was no much change in stage III between the two periods. (Table 1)

For CC, there was significant increase from period A to period B for stages II and III, while a significant drop in stage IV was demonstrated in period B compared with period A. (Table 2)

Comparing stage I and II together and stage III and IV together for both cancers during the two periods, there was significant increase in cases with stage I and II (*p:* 0.006) and significant drop in stage III and IV (*p:* 0.006) in the period B versus period A for GC. Similar changes found between both periods in CC but with a trend toward significance for both staging groups (*p:* 0.052). (Table 3)

Discussion:

There was a statistical significant drop in the GC incidence in the USA in the period between 1975-2004 (11). A similar drop was noticed in areas outside the USA (12), even noticed in higher risk countries at the far east of Asia (13). Such drop was reported by the Iraqi Cancer Registry (3) over the periods between 1976-2000; from 3.98% in 1976-1985 to 3.58% of the TBC in 1998-2000 (Table 4) and lately the incidence of GC was reported by AlHasnawi *et al* (7) with further drop to 3.3% of the TBC. A similar drop was found in our report. There is strong evidence for an association between environment and diet with GC. Studies of immigrants have shown that high risk population for GC from Japan or Korea has a dramatic decrease in the risk of GC when migrate to the west and change their dietary habits (8). The essential steps for prevention and control strategy for gastric

malignancy would be focus on control Helicobacter pylori, healthy diet, anti-tobacco campaign, early detection and diagnosis and treatment program (14).

Gastric cancer Affect men more than women with a ratio of male/female about 1.7:1 (8), while in Iraq, we reported a ratio of 2.3 /1 in 1971 (2) and here we reporting a drop up to 1.6/1 in the year 2006. Gastric cancer prevalence increases with advancing age with the peak in the seventh decade of life (8) while in our study; the peak is in the sixth decade in the males and in the fifth decade in females. (Figure 1)

The availability of the endoscopy units with easy access for both national health and private sectors led to early diagnosis and drop in the cancer staging for GC. We reported a significant increase in earlier stages (stage I and II) of GC in the second two decades of the study. (Table 1 and 3)

Colo-rectal cancer is a major problem in western countries, ranked the third cause of death from cancer in both sexes. The environmental, nutritional, genetic and familial factors, in addition to pre-existing disease have been found to be associated with colorectal cancer. Aspirin and Celecoxib (15) appear to decrease the risk of colorectal cancer in those at high risk. Vitamin D, dietary calcium supplement and proper nutrition are important factors in reducing colorectal cancer risk and colorectal adenoma (16-19). We believe that several factors shared causing this rise of CC in Iraq, including Kurdistan region, with change pattern toward western life.

In Iraq, excluding the three Northern provinces (Sulaimanyia, Erbil and Dohouk), CC is the seventh commonest cancer following breast, lung/bronchus, leukaemia, bladder, brain/CNS and lymphoma in the period between 2000-2004 (7). There was an increase incidence of CC over the last three decades from 3.39% of the total body cancer (TBC) in 1976-1985 to 4.6% in 1998-2000. (3) There was a slight drop in CC incidence in the years between 200-2004 to 4.2% of the TBC. (Table 4) This may be due to the Iraq invasion, The Third Gulf War, in 2003 where there was a drop in the registered cancers to the ministry of health. In the Northern Iraq, Kurdistan Region, CC was the fifth commonest cancer in 2009 following leukaemia, breast, lymphoma and lung/bronchus. (20). We reported a constant increase in the number of CC over the last two decades reaching the peak in 2006 (Figure 3). Our findings of the change in the incidence of colorectal versus the gastric cancer was also reported by the data reported by the six Iraqi reports illustrated in Table 3 and by AlHasnawi 2009 *et al* (7).

In Colorectal cancer the mean age at presentation is 60-65 years (2). In our review, the peak incidence occurs at earlier age group; sixth decade in males and the fifth decade in females. (Figure 2)

Type of food, stress of work to both sex, smoking, use of frozen food and appearance of inflammatory bowel disease especially ulcerative colitis in the second half of last century (21) while it was rare before. Diagnostic tools, increase number of specialists trained gastroenterologist after opening several specialized centers in the country and use of flexible scopes, the awareness of the early symptoms of colonic cancer lead to the early diagnosis in the last two decades of the last centaury, on contrary to the silent and late symptom and sign of gastric neoplasm. As a result, there was an increase in number of cases and early diagnosis of CC over the last two decades compared to the first two decades of this study group of patients.

Three main screening tests are available that aided early diagnosis; faecal occult blood testing, flexible Sigmoidoscopy and colonoscopy. These tests became a widely spread and available to the public through the National Health screening programs and public awareness. Screening endoscopy reduced the risk of late stage CC diagnosis, while people lives in rural area with less education and screening facilities associated with increased rate of late stage diagnosis (9). Similar to GC, there was a drop in the late stages of the CC in the period B with trend toward significance. (Table 3) This could be related to the availability of endoscopic units and the education of the general population about symptoms related to CC.

A new screening test is m2-pk test. Tumor pyruvate kinase M2 (tumor M2-PK) is a key enzyme in the altered metabolism of tumor tissue. Tumor M2-PK is elevated in a range of gastrointestinal malignancy, among these is CC (22). It was recommended through a recent meta-analysis that a stool tumour M2-PK to be used as a routine test for CC screening. It closes a gap in clinical practice because it detects bleeding and non-bleeding tumors and adenoma with high sensitivity and specificity (23). We need to introduce these tests in Iraq, including Kurdistan, to improve the early stage diagnosis of CC, in addition to other screening methods.

In conclusion, There is a reduction in the rate of GC and increase that of CC in Iraq over the last two decades of last centaury. This change could be related to westernized diet, stress and the early diagnosis with the availability of endoscopy. The last had led to early diagnosis and significant drop in the stage of both GC and CC.

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Figure 1: Age distribution for Gastric Cancer (1965-2006)

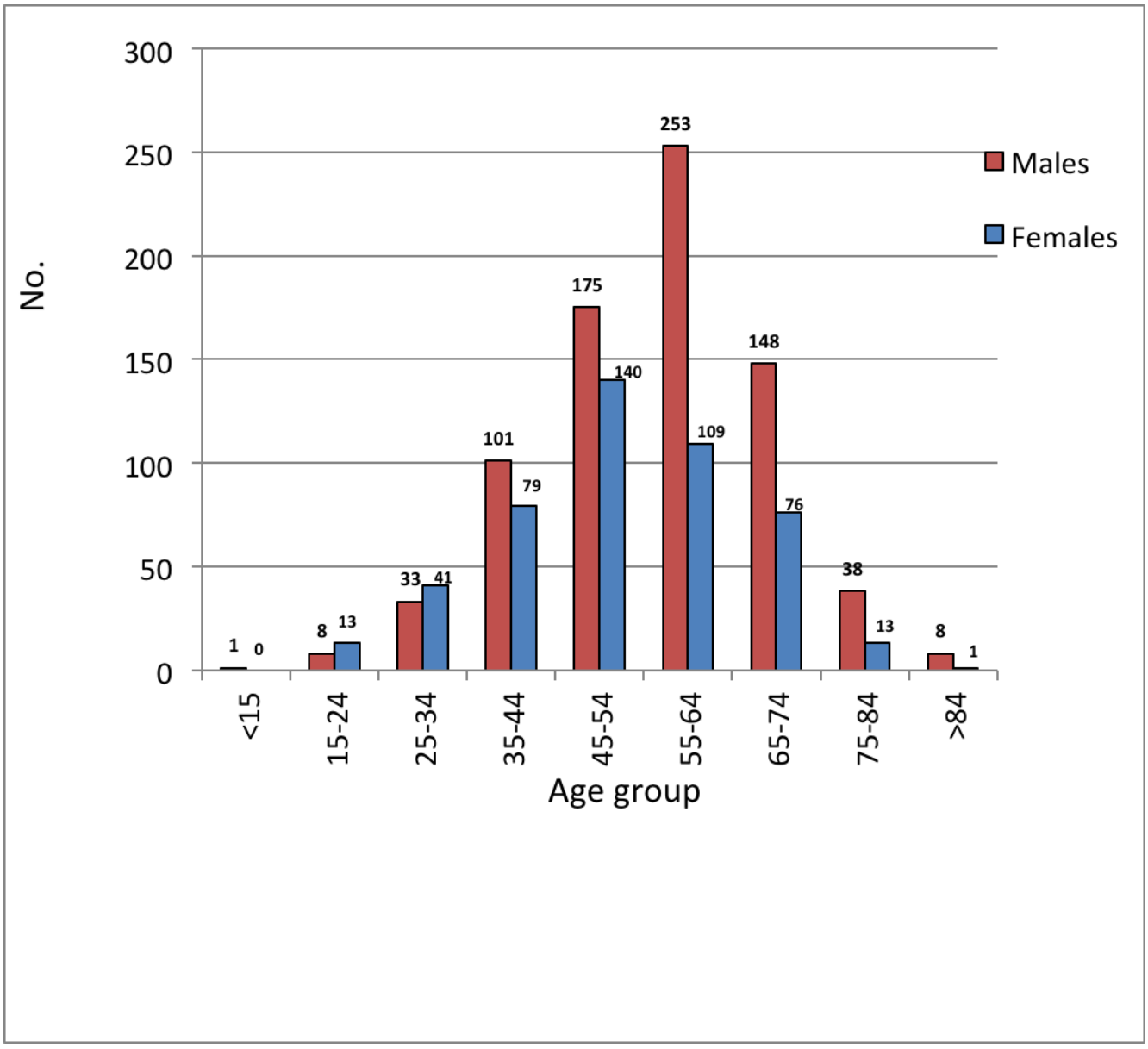

Fig. 1

Figure 2: Age distribution for Colorectal Cancer (1965-2006)

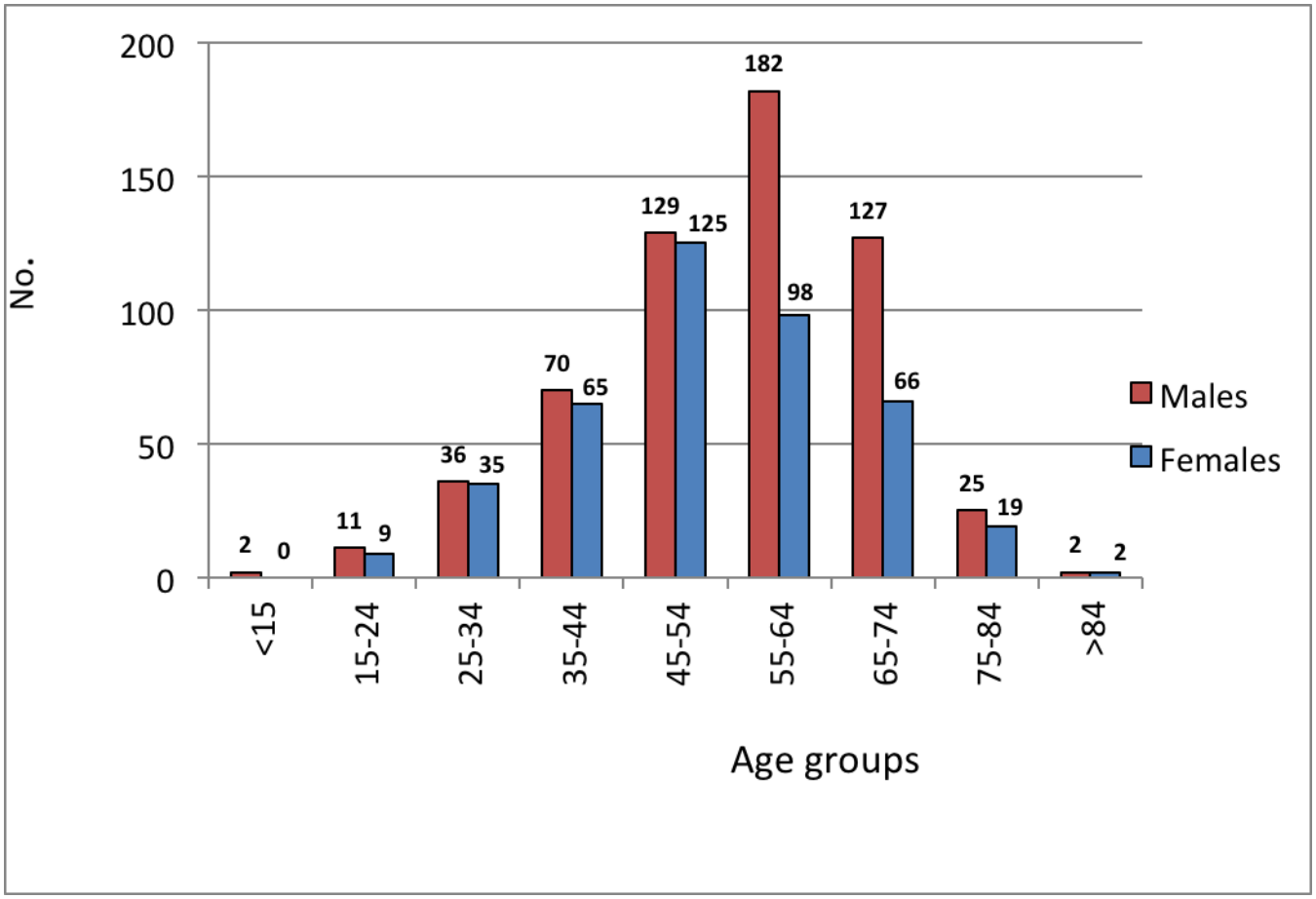

Figure 3: Percentage of gastric and colorectal cancer out of the sum of both cancers (1965-2006)

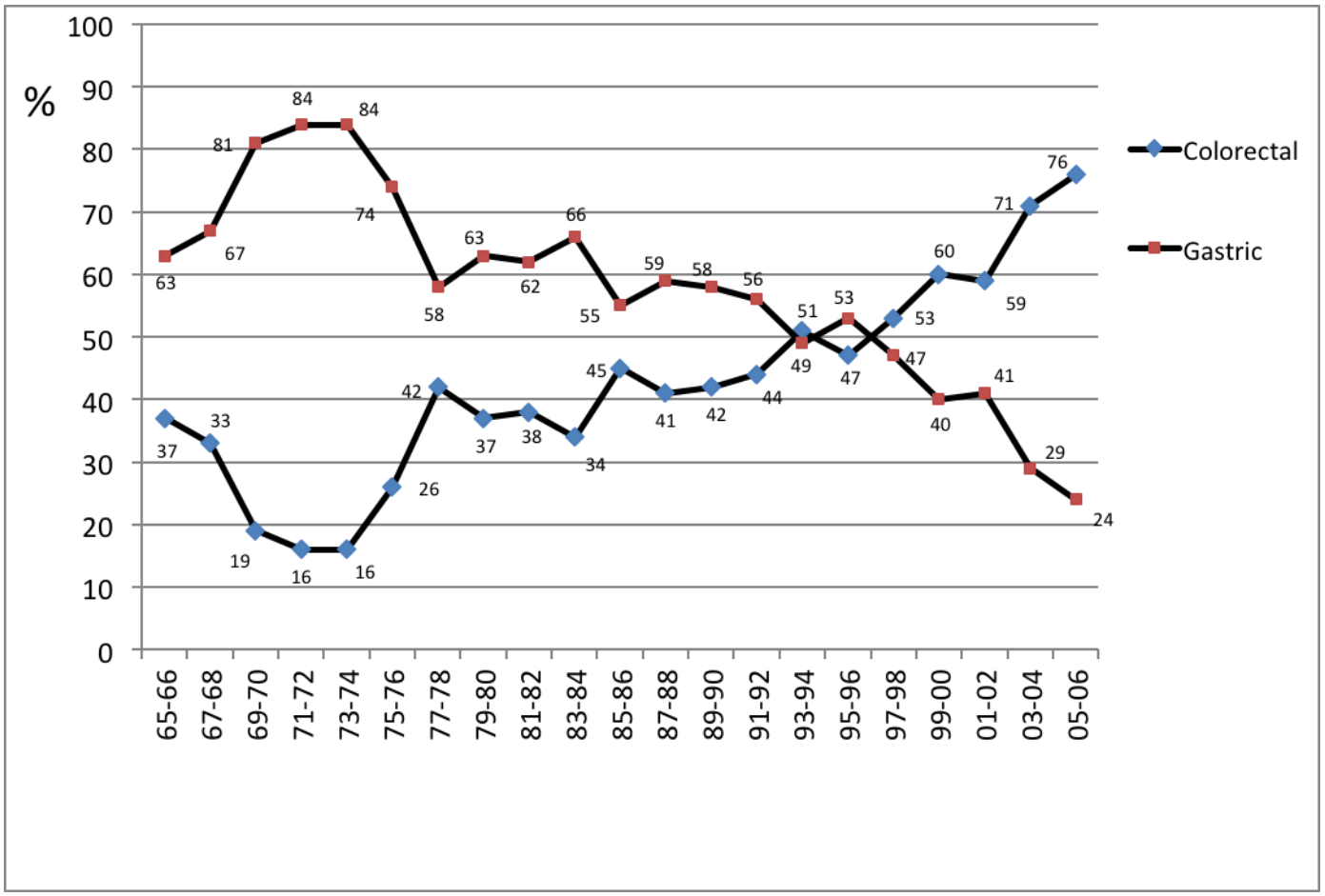

Table 1: Gastric Cancer staging compared between two periods

Stage	Period A (1965-1985)		Period B (1986-2006)		*p value*
	No	%	No	%	
I	1	0.2	14	2	0.004*
II	41	7.6	74	10.6	0.072**
III	168	31.2	222	31.8	0.811***
IV	329	61.0	388	55.6	0.052**
Total	539	100	698	100	

No: number

*: Statistically significant

**: Trend toward statistical significance

***: Not statistically significant

Table 2: Colorectal Cancer staging compared between two periods

Stage	Period A (1965-1985)		Period B (1986-2006)		*p value*
	No	%	No	%	
I	21	8.7	68	8.9	0.920*
II	58	24.1	235	30.8	0.044**
III	65	27.0	270	35.4	0.015**
IV	97	40.2	189	24.8	<0.001**
Total	241	100	762	100	

No: number

*: Not statistically significant

**: Statistically significant

Table 3: Staging groups for both cancers during the two periods

Site	Stage	Period A (1965-1985)		Period B (1986-2006)		*p value*
		No	%	No	%	
GC	I + II	42	8	88	13	0.006*
	III + IV	497	92	610	87	0.006*
CC	I + II	79	33	303	40	0.052**
	III + IV	162	67	459	60	0.052**

GC: Gastric Cancer, CC: Colorectal Cancer, No: number

*: Statistically significant

**: Trend toward statistical significance

Table 4: Percentage of Gastric vs. Colorectal cancers out of total body cancers between 1976-2000

Percent of Gastric & Colorectal Cancer
out of total body cancer in IRAQ (1976-2000)

Years of report *	Gastric	Colorectal
1976 – 1985	3.98	3.39
1986 – 1988	4.1	3.8
1989 – 1991	3.2	3.9
1992 – 1994	3.8	4.1
1995 – 1997	3.2	4.0
1998 - 2000	3.58	4.64

* Report of Iraqi Cancer Registry (Ministry of Health)

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THE USE OF ATORVASTATIN IN THE TREATMENT OF RHEUMATOID ARTHRITIS AND IT'S EFFECTS ON THE DISEASE ACTIVITY AND ACUTE PHASE REACTANTS

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Key Words: Rheumatoid Arthritis, disease activity index, atorvastatin

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Abstract

Background

Rheumatoid arthritis (RA) is a common chronic inflammatory disorder characterized by synovitis, articular destruction, and many systemic extra-articular features**.** It is associated with a high rate of morbidity and mortality due to accelerated atherosclerosis and increased cardiovascular risk. Atorvastatin is a well-known anti dyslipidemic agent that might have valuable anti-inflammatory and immune modulatory functions in RA.

Objectives

The present study was performed to evaluate the anti-inflammatory effect of atorvastatin when used as adjuvant therapy to methotrexate (MTX) and etanercept in Iraqi patients with moderate to highly active RA.

Patients and Methods

A double blind randomized placebo - controlled clinical trial in which 100 patients, both males and females were included. All cases were with active RA who were on MTX and etanercept for at least one month. The cases were divided into two groups to receive either 20 mg atorvastatin or a placebo capsule for three consecutive months. Only 49 patients completed the three months trial, (25 in atorvastatin and 24 in placebo group).

All patients were clinically evaluated by measuring swollen joint count (SJC), tender joint count (TJC), visual analogue scale (VAS) and disease activity score (DAS28).Blood samples of RA patients were evaluated for erythrocyte sedimentation rate (ESR), C reactive protein (CRP) at baseline, monthly and at the end of the study.

Results

RA patients receiving 20mg atorvastatin showed a significant ($p<0.05$) reduction in CRP, SJC and TJC compared to those on placebo. In addition, atorvastatin helped to reduce ESR, VAS and DAS28 more than placebo but without achieving statistical significance ($p>0.05$).

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Conclusions

Atorvastatin 20mg is a safe and well-tolerated drug that has a modest anti-inflammatory effect in patients with moderate to highly active RA.

THE USE OF ATORVASTATIN IN THE TREATMENT OF RHEUMATOID ARTHRITIS AND IT'S EFFECTS ON THE DISEASE ACTIVITY AND ACUTE PHASE REACTANTS

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Background

Rheumatoid arthritis (RA) is a common chronic inflammatory disorder characterized by synovitis, articular destruction, and many systemic extra-articular features[1]. It usually presents with pain, swelling and stiffness affecting the small joints of the hands, feet and wrists[2]. The onset of disease can occur at any age, but the peak incidence occurs within the fourth and fifth decades of life. The average annual incidence of RA in the United States is 0.5 per 1000 persons per year [3]. It is associated with a high rate of morbidity and mortality due to accelerated atherosclerosis and increased cardiovascular risk [4]. Atorvastatin is well known anti dyslipidemic agent that mediates clinically significant cardiovascular risk reduction in patients without inflammatory disease by inhibiting 5-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase enzyme. It has been found that atorvastatin can exert an anti-inflammatory effect in addition to its lipid-lowering actions in animal studies through inhibition of neutrophil infiltration and the local production of pro-inflammatory cytokines (tumor necrosis factor-a (TNF-α),interleukin-1 (IL-1) and interleukin-6 (IL-6)) and chemokines (CCL2 and CCL5) which significantly decrease tissue destruction [5]. Many clinical trials include usage of atorvastatin in rheumatoid arthritis patients as adjuvant therapy to disease modifying

anti-rheumatic drugs (DMARDs), but most of these trials were performed on stable RA patients rather than those with active arthritis [6,7,8].

The present study was performed to evaluate the anti-inflammatory effect of atorvastatin when used as adjuvant therapy to methotrexate and etanercept in a group of Iraqi patients with moderate to highly active RA.

Patients and Methods

Study design

This was a 13-week randomized double blind placebo-controlled single center trial conducted at Rheumatology Unit, Baghdad Teaching Hospital, Baghdad, Iraq from 1st November 2012 until 1st June 2013. One hundred patients of both genders with active RA, who were on MTX and etanercept for at least one month, were divided into two groups to receive 20 mg atorvastatin tablet previously grinded and filled in capsules or capsules prefilled with starch as placebo orally for three consecutive months. The capsules were prepared and packed by a pharmacist who kept a log-book of the coding. The researchers had no way to know the content of the capsules.

Atorvastatin was bought from Micro Company, India under trade name (Avas® 20mg). Patients were evaluated at baseline, monthly and at week 12.

Sample selection

Eligible patients had confirmed RA according to the 1987 American College of Rheumatology (ACR) criteria [12] with moderate to highly active disease defined as disease activity score based on 28 joints and ESR

(DAS28-ESR) greater than 3.2 at baseline. For inclusion, patients also were required to have taken methotrexate (MTX) and etanercept regularly for at least one month. The exclusion criteria included patients who were taking lipid-lowering therapy, had hypersensitivity to statin, pregnancy, breast feeding, renal and liver impairment and patients younger than 18 years old.

Informed consent was obtained from all participants and the ethical committee of Baghdad University, College of Medicine – Medical Department, approved this study.

Clinical and laboratory evaluation

Clinical evaluation of patients for tender and swollen joints was done by a specialized rheumatologist who was blinded to the treatment at the beginning, monthly and after 12 weeks of treatment. The RA disease activity was measured using DAS28-ESR, which is a validated composite [9] and was calculated by the following equation:

$$\mathbf{DAS28 = 0.56\ (TJC)^{0.5} + 0.28(SJC)^{0.5} + 0.70 \ln (ESR) + 0.014(VAS)}$$

-TJC, tender joint count

-SJC, swollen joint counts

-ESR, erythrocyte sedimentation rate

-VAS, visual analogue scale

Blood specimens were collected and laboratory analyzed (at baseline, monthly and after 12 weeks) for the measurement of ESR and CRP. ESR was measured by Westergren method [10] while C-RP was measured by slide agglutination test [11].

Statistical analysis

Statistical software (SPSS version 20) was used for data input and analysis. Continuous variables were presented as mean ± standard deviation (SD) and discrete variables were presented as numbers and frequencies. Chi square test for independence was used to test the significance of association between discrete variables. Continuous variables were tested by the Shapiro Wilk test to determine if they were normally or abnormally distributed. Mann Whitney test was performed to test the significance of difference in the mean of two samples in abnormally distributed continuous variables. Findings with P value less than 0.05 were considered significant whereas P values less than 0.01 considered highly significant.

Results

In this randomized double blind study, 100 patients were included in the study and randomly assigned to receive atorvastatin or placebo. Half of them received atorvastatin and the other half received placebo. Ten patients in the atorvastatin group and five patients in the placebo group withdrew within the first month of the trial and those patients were excluded from the study. Twenty five patients in the atorvastatin group and 26 of patients in the placebo group did not complete the three month treatment course. Patients in the two groups had similar demographic and baseline characteristics. Patients were predominantly middle-aged women (80.25% of the atorvastatin group vs 80% of controls) with disease duration more than eight years . Most of them have positive rheumatoid factor (57.5% of the atorvastatin group vs 68.9% of controls) . C-RP is nearly similar between the two groups . Most of the patients in the two groups are non smokers 87.5% of the atorvastatin group vs 88.9% of controls)). Table 1 shows the mean value of the indices used to evaluate the progress of the patients in the two study groups. It shows a significant difference at pre-treatment ESR level between atorvastatin and placebo group ($p=0.017$). There was a progressive and highly significant decrease in ESR level in atorvastatin groups during the first, second and third month of treatment compared to pre-treatment level. While in the placebo group, the only significant decrease in ESR level was seen after completing three months of treatment.

However, there was no significant difference ($p=0.58$) between the ESR levels of the atorvastatin and placebo at the end of the study.

The CRP levels for patients taking atorvastatin was significantly lower compared to patients receiving placebo after one month of treatment ($p=0.008$).

The SJC of patients receiving atorvastatin for three months was significantly lower compared to patients receiving placebo ($p=0.028$).

The TJC in patients receiving atorvastatin was significantly lower compared to patients receiving placebo after two months of treatment ($p=0.041$).

There was a non-significant difference (p=0.115) in the VAS between the atorvastatin and placebo groups during three months of treatment.

A non-significant difference (p=0.23) in DAS28 between the atorvastatin and placebo groups during three months of treatment.

Table 1 Mean ± s.d. of Indices Used in the Effect of atorvastatin and placebo Groups

Test	Group	Pre treatment	After treatment for		
			1 month	2 months	3 months
ESR	Atorvastatin	58.35±22.9 (n=40) ♣	41.88±20.33 (n=40) **	37.18±20.41 (n=28) **	35±17.18 (n=25) **
	Placebo	48.69±29.75 (n=45)	44.38±25.37 (n=45)	40.42±23.25 (n=33)	33.17±18.0 (n=24) *
CPR	Atorvastatin	38.8±20.17 (n=40)	23.55±16.77 (n=40) **♣	17.79±13 (n=28) **	13.2±6 (n=25) **
	Placebo	40.8±30.74 (n=45)	33.42±28.37 (n=45) *	22.91±14.73 (n=33) *	16.75±11.73 (n=24) *
SJC	Atorvastatin	4.95±4.26 (n=40)	3.13±3.87 (n=40) **	2.25±2.048 (n=28) **	1.6±1.58 (n=25) **♣
	Placebo	5.31±4.15 (n=45)	4.02±3.65 (n=45) *	3.64±3.35 (n=33) *	2.96±2.47 (n=24) *
TJC	Atorvastatin	8.68±5.27 (n=40)	5.43±4.34 (n=40) **	3.5±3.54 (n=28) **♣	2.68±2.41 (n=25) **
	Placebo	6.87±5.26 (n=45)	5.98±5.09 (n=45)	5.36±3.77 (n=33)	4.33±2.94 (n=24)
VAS	Atorvastatin	6.63±1.9 (n=40)	4.93±1.91 (n=40) **	4.25±1.91 (n=28) **	3.6±1.47 (n=25) **
	placebo	6.04±1.93 (n=45)	5.16±1.87 (n=45) **	4.94±2.13 (n=33) **	4.29±1.78 (n=24) **
DAS 28	Atorvastatin	5.80±1.07 (n=40)	4.70±1.16 (n=40) **	4.23±1.15 (n=28) **	3.95±1.01 (n=25) **
	placebo	5.33±1.17 (n=45)	4.91±1.33 (n=45) *	4.77±1.10 (n=33) *	4.43±1.04 (n=24) *

Note: values shown as mean± standard deviation , ***** significantly different compared to pretreatment within the same group ($p<0.05$);****** highly significant difference compared

to pre treatment within the same group (P<0.01), ♣ significant difference of the effect of atorvastatin compared to placebo within same month (P<0.05).

Discussion

This study was designed as a double blind randomized clinical trial, which is compatible with a high rate of agreement and decreases the possibility of bias [13]. Because it is ethically unaccepted to use atorvastatin alone as a separate arm in the trial, adjuvant therapy to methotrexate and etanercept pattern is implemented. Moreover, atorvastatin was not compared alone versus placebo to avoid the unacceptable risks in patients who remain untreated for the required duration of the trial [14].

A high dropout rate was observed in the present study in patients randomized to atorvastatin or placebo but there was non-significant difference between the two treatment groups in this aspect. In the country of Iraq, where the security situation is still volatile, and patient education is low, it is always expected to have a high dropout rate with an impossible task of tracing the patients.

Only 49% of patients completed the three month trial. The other non-completers showed no evidence for the reasons of withdrawal. However , withdrawal may be due to poor compliance , adverse effects of treatment , lack of efficacy , cultural factors or other reasons.

Acute phase reactants ESR and CRP provide reliable means for discrimination between drugs that provide symptomatic relief only and others with a more profound effect in RA [15].

The ESR is sensitive for most types of inflammation, but cannot distinguish if the underlying cause is infectious, inflammatory, or paraneoplastic [16]. The results of this study showed that ESR level in RA patients with active disease was significantly reduced by using 20mg atorvastatin (but less in placebo) over three months of treatment, but with no significant difference between atorvastatin and placebo at the end of the study. Similar result was found in a trial using 80mg atorvastatin for 12 weeks versus placebo [17]. The

same finding was reported when 10mg rusovastatin was used for 38 patients for 8 weeks [18]. Many other studies showed that atorvastatin has the ability to reduce ESR significantly when compared to placebo [19-21]. The different results of those trials from the present findings may be attributed to that this study use 20mg atorvastatin in contrast to 40mg used in other studies. In addition, this study was performed for a shorter duration (3months) compared to 6 months duration in the other studies. One study showed that 20mg simvastatin decrease ESR level significantly when used for 6 weeks [22], which may be attributed to different anti-inflammatory properties of statins [23].

Serum CRP level is the best biochemical indicator of disease activity in RA patients [24]. Serum CRP level changes more quickly than ESR.With sufficient stimulus, CRP can be increased within 4 to 6 hours and normalized within a week [25]. In addition, CRP elevation is directly correlated with RA disease activity [24].

Serum CRP level in the current study decreased significantly by atorvastatin (less with placebo) with a significant difference between the two groups only in the first month. This finding may be due to the decrease in the number of patients in the second and third month. But this result was compatible with many studies using 40mg atorvastatin in RA patients [19-21], and the same result was found after using 20mg simvastatin [22]. In the METEOR study, 40mg rusovastatin reduced CRP level significantly when compared to placebo [26]. While using 10mg rusovastatin for 12 months [27] or 80mg atorvastatin for 12 weeks for 11 RA patients [8] showed no significant improvement in CRP.

Assessment of tender points is considered as the cornerstone during evaluation and treatment decision making in RA [28]. In the present study, TJC was significantly decreased by atorvastatin (-66.16%) compared to pretreatment, while placebo showed non-significant decrease in TJC. Moreover, there was a significant difference between the effect of atorvastatin and placebo at the second month of treatment with no difference at the end of the study which may be due to decreased number of patients at the third month. A similar finding was reported in El-Barbary and co-workers (2011), where 40mg

atorvastatin produced a significant decrease in TJC compared to placebo. Two other studies on simvastatin and statins respectively showed a significant decrease in TJC compared to placebo[22,29]. Other trials showed non-significant decrease in TJC when atorvastatin was used compared to placebo. The difference from our findings may be due to the inclusion of mild RA patients [19] or due to low number of patients [8].

Swelling of joints are considered as the best single variable that detects response to drug therapy in RA patients [30]. In the present study, SJC was significantly reduced by atorvastatin compared to placebo. This finding was confirmed by several trials when atorvastatin as adjuvant therapy was used for six months in rheumatoid arthritis patients [19, 21]. In addition, another large cohort study about using statins in RA patients showed decreased number of swollen joint [29]. Another study using 80mg atorvastatin for 3 months showed non-significance improvement in SJC [17], and this finding may be attributed to small number of RA patient in this study (n=11). One other study where 20mg simvastatin was used showed non-significant decrease in SJC [22]. A similar finding was observed when 10mg rusovastatin was used for 12 months [27] and those different findings may be attributed to different anti-inflammatory potency of statins.

Visual analogue scale (VAS) was shown to be a valid measure of pain intensity in RA patients [31]. In the current study, VAS was significantly reduced by both atorvastatin and placebo, though atorvastatin showed a greater percent of reduction, with no significance difference between them. Several studies confirmed this finding when 40mg atorvastatin was used for RA patients for six months [19, 21] and 80mg atorvastatin for 3 months [8]. While in another cohort which used statin showed a significant difference between the effect of statins and placebo, and this different finding could be due to inclusion of different statins rather than specific one which lead to different anti-inflammatory properties [23].

DAS28 remains the most extensively validated activity index for RA patients [32] to observe the clinical response of the patients to the treatment. Results of the present study

showed that both atorvastatin and placebo produced significant improvement in DAS28 scores after 12 weeks of therapy, though atorvastatin produced a greater percent of the reduction, with no clinical significance between the two groups. Similar results were reported for using atorvastatin 40mg in moderately active RA patients, at which atorvastatin and placebo improved DAS28 but the level of significance was higher in atorvastatin arm [20]. Another large cohort study demonstrated that there is no statically significant difference between placebo and statin on RA disease activity [29]. A third double blind study showed a comparable effect of 80mg atorvastatin and placebo on DAS28 when used for 12 weeks [8]. Two other studies [19,21] reported a significant improvement in atorvastatin group compared to the placebo group. The difference in the results in those trials from the current study may be due to many reasons: The dose dependent pleiotropic effect of statins [33] may be responsible for our finding of no more benefit of atorvastatin than placebo because of the use of low dose atorvastatin (20mg) in the current study whereas in other studies a higher dose of atorvastatin 40mg was used. Moreover, the shorter period of follow up and smaller sample size in the current study may be a potential reason for such difference compared to the two other trials.

Conclusions

It can be concluded that 20mg atorvastatin is a safe and well-tolerated drug that has modest anti-inflammatory effect in patients with moderate to highly active RA. It also has the ability to reduce C-RP level, SJC and TJC but with less effect on ESR, VAS and DAS28 when compared to placebo.

Recommendation

Larger scale multicenter clinical trials are required to support the reported data. In addition, evaluation of the effect of higher doses of atorvastatin in patients with moderate to highly active RA should be taken. Longer period of follow up are required to evaluate long-term benefit of atorvastatin on RA disease activity.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 1, Issue: 1, 2014
Pages: 32-46

HISTORY AND CONCEPT OF ISLET CELL TRANSPLANTATION

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Abstract

Diabetes Mellitus is a chronic disease characterized by a conglomeration of metabolic disturbance of carbohydrate and fat metabolism. Since its recognition around 1500BC, its management was always a challenge for practitioners and has gone through a series of milestones starting with the introduction and use of Insulin in 1921; an established standard for treating Type I diabetes, followed by the introduction of Pancreatic transplantation which was firstly performed in 1966, culminating with the era of Islet cell transplantation which has propagated to a promising pace since 1972. The Edmonton protocol of Islet cell transplantation, which was introduced by Shapiro et al in 2000, adopts an innovative use of immunosuppression drugs with fewer side effects. This paper outlines the historical propagation of treating type 1 diabetes. It highlights the advent of using islet cell transplantation as a newer noninvasive means of providing persistently endogenous insulin for type 1 diabetic patients. This is considered a better accepted way of restoring euglycemia with the merit of avoiding attacks of hypoglycemia which is a well-known side effect of Insulin therapy. Once a dream, islet cell transplantation for treatment of diabetic patients was never as close to being a reality as it is today. We can now see the light at the end of the tunnel through the dramatic discoveries including newer technologies to overcome the prolonged need for immunosuppression, the use of encapsulated islet xenografts, the generation of the unlimited supply of human β cells, and the use of embryonic and adult stem cell.

TOFIQ Journal of Medical Sciences, TJMS, Vol. 1, Issue: 1, 2014
Pages: 32-46

History and Concept of Islet Cell Transplantation

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Introduction

Diabetes Mellitus (DM) is a historical disease that drew the attention of humanity since its recognition around 1500 BC by ancient Egyptians, describing a rare disease in which a person urinates excessively & losses weight. It was not until 1600 years later that the Greek physician Aretaeus, recognized that the urine of the affected persons has a sweet taste, coining it for the first time as Diabetes (Poretsky 2009). According to the World Health Organization (WHO), 347 million people worldwide have diabetes and it is the cause of mortality of 3.4 million every year (Danaei 2011, WHO 2009) . WHO further predicts that deaths from Diabetes would double between 2005 and 2030 (WHO 2013). A formerly labeled rare disease is now announced as a global pandemic posing a challenge to public health agencies worldwide.

Diabetes is a complex heterogeneous disorder characterized by hyperglycaemia caused by a total lack, decrease or diminished effectiveness of circulating insulin. Genetically both Type 1 (T1D) and type 2 diabetes (T2D) are polygenic disorders, and multiple genes and environmental factors contribute to the development of the disease (Ounissi-Benkalha 2008, Stolerman 2009).

T1D occurs predominantly in children and young people and is due to selective auto immune destruction of β cells leading to absolute insulin deficiency (Mathis 2001). On the other hand patients with T2D have a strong family history of T2D, more so than in T1D. Type 2 Diabetes mostly affects older people who consume high calorie diet and with relatively sedentary life style leading to a state of cellular insulin unresponsiveness (Insulin resistance) resulting in increase of pancreatic insulin secretion. The resultant β cell exhaustion necessitates the need of exogenous insulin in the patients' therapeutic regimen (Costa 2002). T1D affects millions of individuals and is associated with multiple medical problems due to the relatively longer life span of the affected persons. Premature atherosclerosis is a cause of substantial morbidity and mortality in T1D and tight control of glycemia was found to reduce the risk of developing cardiovascular disease (Costacou 2007, Nathen 2005). However, intensive insulin treatment is associated with three fold increase in severe hypoglycemia,

an obstacle in attempts to attain good glycemic control (DCCT 1993).

Pancreatic Islet Cells

Identified in 1869 by Paul Langerhans, the islets are the endocrine cells of the pancreas which secrete their hormones directly to the blood (Langerhans 1869). The islets constitute 1-2% of the mass of the pancreas. A healthy adult pancreas contains about one million islets, each islet measuring 0.2 mm in diameter, with a combined mass of the islets of 1 to 1.5 grams (Sleisenger 2009). Islets are distributed among the exocrine tissue of the organ. Each islet is surrounded by thin fibrous connective tissue capsule which is intermingled with the connective tissue of the pancreas. In humans four main cell types are found within the islets; α cells secreting glucagon, β cells secreting Insulin, δ cells secreting somatostatin and γ cells secreting pancreatic polypeptide. Within the islet architecture, human islets display alpha and beta cells in close relationship with each other throughout the cluster (Brissova 2005, Cabrera 2006). The clusters of the islets are embedded in a network of capillaries. The flow of blood in this glomerular-like vasculature is regulated by signals, such as nutrients and hormones from remote tissues. Islets have a portal circulation, with blood flowing from beta to alpha to delta cells (Samols 1988). In addition, islet perfusion determines communication between endocrine and exocrine cells (Ballian 2007).

Implementation of Insulin Therapy in Type 1 Diabetes

Insulin therapy is the gold standard for treating people with T1D. The rationale of intensive treatment of type 1 diabetes was introduced after the establishment of the modern standard of care for the medical management of type 1 Diabetes Mellitus by the Diabetes Control and Complications Trial (DCCT) in 1993 and its follow up study; Epidemiology of Diabetes Interventions and Complications (EDIC) (Nathan 2005, DCCT 1993) . After assigning 1441 patients to either conventional or intensive treatment (by applying multiple daily measurements of blood glucose levels with the combination of daily insulin injections of long, intermediate & short acting insulin in addition to dietary and psychological support) the study showed better glycemic control and less secondary complication rates in the intensive treatment group than in the conventional group, rendering the intensive treatment an accepted norm in Diabetes management. Despite better microvascular outcomes, intensive insulin therapy was hindered by a high rate of frequent and severe hypoglycemia (62 episodes per 100 patient-years of therapy).

Rationale for Insulin Producing β Cells Transplantation

The restoration of β- cell mass and reversing diabetes was the reverie of scientists since the recognition of the role of pancreas in treating diabetes. Logically, this needs to be accomplished by two approaches; either by endogenous regeneration of β cells or by transplantation of β cells from exogenous sources. Advances in both approaches are promising. Regeneration of β cells from embryonic and adult stem cells or pancreatic progenitor cells is work in progress that is beyond the scope of this review (Bonner-Weir 2005).

Transplantation of Insulin producing β cells was performed earlier as a vascular procedure by transplanting the whole pancreas (The world first clinical pancreas transplant was performed at the University of Minnesota in 1966 to treat a uremic diabetic patient) (Kelly 1967) . This approach proved to be a cost effective option that offers independence from exogenous insulin, with favorable glucose levels. The procedure proved to ameliorate the complications of DM and improve the quality of life and patients' life expectancy (Fioretto 1998, Navarro 1997). Since that time more than 15000 pancreas transplants has been performed around the World with 85% one year graft survival rate, and with insulin independent recipient (Sutherland 2001) . The DCCT provided a strong ground for using pancreatic transplantation. It provided evidence that simultaneous kidney and pancreas transplantation is the treatment of choice for most diabetic people with end stage renal failure with a 3- year survival rate of approximately 70- 80 % , simulating the rates for most of other organ transplantation (Gruessner 2001) . Kidney biopsies had shown dramatic reversal of the mesangial accumulation and basement membrane thickening 10 years after the establishment of normal glycemia following pancreas transplantation. Of note is that macrovascular complications as well as the sensory, motor and autonomic neuropathy were stabilized (Fioretto 1998, Fiorina 2001).

Historical Aspects of Islet Transplantation

1. Pancreas Transplantation

The concept of transplanting extracts or pieces from the pancreas to patients with diabetes dates back to 1894 when Williams used minced sheep's pancreas for oral and subcutaneous therapy. This adventurous trial proved a failure, presumably due to using xenografts without immunosuppression (Williams 1894) .

It was not until 1972 that a successful trial by Ballinger and Lacy of transplanting isografts from normal rats to streptozocin induced diabetic rats could reverse diabetes (Ballinger 1970) .

Islets autografts were transplanted successfully in 1980. This was performed on a patient with painful chronic pancreatitis in whom the pancreas was removed, minced, digested with collagenase and unpurified preparation of islets was re-infused into the patient portal vein. Being large in size the islets lodged in the liver without reaching the sinusoids (Sutherland 1980). In the 1990s, some promising reports were documented, with some recipients showing insulin independence for up to one year after the procedure. Wahoff et al. reported insulin independence rate of 74% two years after autologous islet transplantation in 14 patients receiving portal vein infusion of islet cell extracts from their removed pancreas (Wahoff 1995). Trials during the years 1990 to 1995 showed success in only 6 percent of the islet transplants (Hering 1996). The Islet Transplant Registry records of 267 patients who had undergone islet allograft transplantation showed that 12.4% of patients were insulin independent for 7 days. Only 8.2% of them stayed independent for one year (Brendel 1999). It is worth mentioning that these early trials adopted an immunosupression protocol using anti-lymphocyte globulin combined with Cyclosporine, azathioprine and corticosteroids. The use of immunosuppression in transplantation procedures is a considerably challenging issue due to the balance sought between their efficacy and their toxicity to the islet cells. These drugs are diabetogenic as they were found to increase the peripheral insulin resistance. In addition, they have an antiproliferative effect on the engrafted tissue (Ishizuka 1993, Hyder 2005, Lohmann 2000). The high concentration of these drugs in the liver imposes more risk to the engrafted islets at their site of lodgment in the liver sinusoids.

2. Islet Cell Transplantation

Despite the improvement in insulin therapy in treating T1D, many patients are disabled by the refractory hypoglycemia. Compared to pancreas transplantation, introducing cell-based therapy by transplanting islet cells provides minimally invasive means to restoring euglycemia with the avoidance of the complications of surgical procedures. Islet cell transplantation underwent a substantial progress during the last three decades (Hering 1999, Ricordi 2004). Few centers performing the procedure have shown high rates of favorable insulin independence in patients with T1D.

The use of newer immunosuppressive agents was introduced in 2000 at University of Alberta, Edmonton by Shapiro et al who developed a corticosteroid-free

immunosuppressive protocol that includes Sicrolimus, low dose tacrolimus and a monoclonal antibody against interleukin-2 receptor (Daclizumab) for use in trial of islet transplantation in patients with brittle T1D (Shapiro 2000) .

The trial reported 100% success rate of restoring normal glycemia in seven patients during a median follow up of 11.9 months. This was achieved by applying more rigorous criteria to attain sufficient mass of transplanted islets to achieve normal glucose levels. The new approach resulted in sustained freedom from the need for exogenous insulin with better outcome when compared to previous reports (Brendel 1999). However, five years later the results of Edmonton Protocol showed some discouraging trends. Only 15% of the patients were free of exogenous insulin treatment although 85% still showed evidence for the presence of plasma C- peptide which is an indication of endogenous insulin secretion by the transplanted islets (Shapiro 2005).

An international multicenter trial of the Edmonton Protocol was conducted to explore the feasibility of islet transplantation with the use of the Edmonton protocol (Shapiro 2006). Thirty-six subjects with T1D were recruited from 9 centers. The islets were obtained from pancreata of deceased donors and transplanted within two hours after purification. The primary end point was insulin independence one year after the final transplant. Of the 36 recipients, 44% met the primary endpoint (Hemoglobin A1c <6.5; Fasting blood sugar <140 mg/dl; Post prandial sugar <180 mg/dl). Twenty eight percent had partial function of islets (C peptide > 0.3 ng/ml). Twenty eight percent had graft loss one year after the final transplantation and 58% had insulin independence at any point throughout the study. The multicenter study confirmed the former experience with the single center Edmonton protocol and demonstrated the benefit of islet transplantation in patients with brittle T1D. It proved that even in patients with residual islet function, severe hypoglycemic episodes were minimized. The trial also standardized pancreas selection, recipient selection, islet processing and post transplantation care.

Indications of Islet Transplantation:

1. Autologous Islet Transplantation in pancreatectomy- induced diabetes, if the pancreas is affected by trauma, chronic pancreatitis or benign neoplasms.

2. Allogenic Islet Transplantation is indicated in T1D as Islet transplantation alone (ITA), Simultaneous islet and kidney transplantation (SIK) and as Islet after kidney transplantation (IAK) (Pileggi 2004).

3. Allogenic transplantation is indicated in patients with diabetes having other metabolic disorders like cyctic fibrosis and hemochromatosis (Lanng 1992) .

4. Other rare indication is in people with T2D and liver cirrhosis, although advanced patient's age and comorbidity are contraindications to this procedure (Ricordi 1997).

Islet Transplantation Technology:

The modern islets isolation technology includes:

1. Procurement of healthy pancreas from brain dead donor whose heart is beating. Recently cadaveric donors are being used successfully.

2. Cannulation of the pancreatic duct, collagenase infusion to dissociate islets from exocrine, ductal and surrounding connective tissues.

3. Distended pancreas is cut into smaller pieces and transferred into so-called Ricordi's chamber, where digestion takes place to liberate and remove the islets from solution. (A recipient needs more than 12,000 islet equivalents/ kg; IE, the number of islets normalized to an islet of 150 µm of diameter). Around 5000 IE/kg can be obtained from one pancreas. Islet isolation may cost around $10,000 US)

4. Purification of the islets from the enzyme by centrifugation, to minimize the volume of the tissue to be implanted by separating the exocrine tissue and debris, hence decreasing the insult to the liver to prevent portal hypertension.

5. Transplantation; either immediately or after culturing for a short time to assure sterility and to assess in vitro the function of islet preparation. In addition, culturing helps in modulating the islets regarding immunogenicity and induction of cyto-protective molecules, and genetic modification of the islets before transplantation.

These strategies were required to reduce the coagulation/ complement activation and inflammation which may cause islet tissue rejection by the recipient (Villiger 2005). Culturing also helps in preconditioning the recipient regarding immune suppression and reduction of inflammation (Pileggi 2006).

6. Islet infusion to the recipient under conscious sedation, using ultrasonic or fluoroscopic guidance. Islet cells infused though a needle introduced via the skin into the right portal vein. The implants are then engrafted into the liver sinusoids and are ready to function. The islet infusion can be repeated on several occasions to deliver the appropriate amount of islets to achieve the optimal glycemia (Robertson 2004).

7. Other sites for infusion are the kidney capsule, spleen, testes, peritoneal cavity, small bowel, intramuscularly, and subcutaneously.

8. The situation after the infusion may not be favorable and many islets fail to engraft and function. This can be attributed to the prolonged hypoxia during the revascularization period which may take about two weeks. In addition the risk of bleeding when anticoagulation or antiplatelet drugs are used during the infusion as well as the blood mediated inflammatory reaction secreting inflammatory cytokines by the recipient lymphocytes which contribute to apoptosis and necrosis of the newly harbored β cells. The process of rejection peaks in two to three days and ends by two weeks (Hyder 2005). Another challenge that might affect the function and viability of the engrafted islets is the antiproliferative effect of immunosuppressive drugs which hinders angiogenesis for the newly transplanted islets.

Clinical Effect of Islet Cell Transplantation

Few papers discuss the clinical effects of islet transplantation on type 1 diabetic patients regarding morbidity and mortality. One paper studied the effect of islet transplantation on patients' survival. Comparing two groups who had kidney islet transplant; one group showed C peptide secretion > 0.5ng/ ml and the other lost C peptide with early failure of the engrafted tissue. After seven years of follow up, the successful group with sustained restoration of β cell function had significantly higher survival rate (90%) than the other unsuccessful group (51%).

The higher survival in the successful group was accompanied by higher C- peptide levels and lower insulin requirement compared to the unsuccessful group, despite similar glycated hemoglobin levels. The cardiovascular deaths were higher in the latter group with poorer atherosclerotic profile and endothelial function (Fiorina 2005)

Effect of Islet Transplantation on Long Term Diabetes Complications

No controlled studies have addressed the question whether Islet transplantation can halt or delay chronic diabetic complications. This is attributed to the difficulty of performing large clinical trials in the lack of standardized protocols, the variable methods of isolation of the islet tissue and the use of different immunosuppressive measures.

Animal studies had shown that Islet transplantation can improve cases of diabetic cardiomyopathy. An uncontrolled study on a small number of patients showed improvement in cardiovascular function over 3 years follow up period in kidney transplant recipient with functioning islet transplants (Laviola 2001) .

One of the results of this study was the delay in intima medial thickening and improvement in atrial and ventricular function

Nephropathy is one of the most common chronic complications of T1D (DCCT 1993). DCCT demonstrated a reduced incidence of microalbuminuria in type 1 diabetic patients in the intensive group compared to the conventional group. A paper published in 2003 showed that successful islet transplantation in T1 diabetic patients with end stage kidney disease receiving kidney transplant had prolonged graft survival and prevent reduction in vascular function of the graft (Fioina 2003). Better renal vascular function was demonstrated in another group of patients with kidney transplant and functioning islet transplant compared to the group without functioning islets (Fiorina 2005).

Diabetic retinopathy is a well-recognized complication of diabetes (DCCT 1993). It is characterized by new vessel formation and reduced retinal blood flow, hypoxia and distorted endothelial integrity (Miller 1997). A recent case control study on 10 patients who received islet transplantation alone compared with a control group of type 1 diabetic patients used a color-Doppler-imaging to study the effect of ITA after one year on the blood flow velocities of central retinal artery and vein.

A statistically significant increase of blood flow velocities of central retinal arteries and veins was found only in the ITA patients (Frank 2006). An early, significant increase of arterial and venous retinal blood flow velocities was found after ITA .

Peripheral nerve function was assessed with a nerve conduction velocity (NCV) index in islet transplanted patients in a 2 years follow up study. There appeared to be a positive effect of β cell implantation on polyneuropathy. This study did not provide a statistical analysis (Lee 2005). Another report indicated that islet transplantation can induce long lasting stabilization or even improvement of poly neuropathy in type 1 kidney transplanted diabetic patients who received functioning islet transplant (Del Carro 2007).

Conclusion

Islet transplantation is an evolving therapeutic measure for highly selective patients with severe hypoglycemia or labile T1D. It remains at cross roads due to the critical shortage of donor organs, the poor long term results, and the relatively high incidence of adverse effects as well as its high cost. Therefore the procedure is still unsuitable to be expanded to the general population.

Nonetheless the dramatic discoveries and the expansion of the horizon to include newer technologies which overcome the issue of prolonged need for

immunosuppression by using encapsulated islet xenografts, generating an unlimited supply of human β cells, and the use of embryonic and adult stem cell, set the stage for a dream come true in transplanting islets cells to treat diabetic patients.

Conflict of Interest: The author reports no conflict of interest.

The work is original and had not been published or submitted elsewhere.

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MESH REPAIR VERSUS PLANNED VENTRAL HERNIA STAGED REPAIR IN THE MANAGEMENT OF TRAUMA PATIENTS WITH ACUTE ABDOMINAL COMPARTMENT SYNDROME

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Summary

Background: The terms Intra-abdominal hypertension (IAH) and Abdominal compartment syndrome (ACS) are commonly used interchangeably; however it is important to recognize the distinction between these two terminologies. Recently, at the World Society on Abdominal Compartment Syndrome (WSACS), the threshold for IAH and ACS was established as a value ≥ 12 mmHg and > 20 mmHg respectively. Patients with either ACS (defined as a sustained intra-abdominal pressure- IAP) or IAH can be effectively managed via decompression followed by mesh repair (MR) or staged repair (SR) method

Objectives: This research aims to evaluate the results of mesh repair in comparison to planned ventral hernia staged repair for closure of abdominal wall defects after decompression in trauma patients with (ACS).

Patients and methods: All patients with major abdominal trauma and identifiable risk factors for development of ACS presented to the first surgical unit in Baghdad Teaching Hospital between the 1ST OF March, 2005 and the 30th of September, 2008 were enrolled. IAP was measured indirectly using intravesical pressure in most cases.

Patients were screened for IAH/ACS risk factors during the operation and/or upon admission into the surgical ward or ICU. If one risk factor was present, baseline IAP measurement was done but If two or more risk factors were present, an additional serial IAP measurements was performed every 4-6 hr in the first 24 hours. Those with grade IV or ACS on initial measurement or during follow up urgently underwent decompression. Bogota bag was used in the initial phase of closure of abdominal wall defects. Patients were randomized into either the Mesh Repair Group (MRG) or the planned ventral hernia Staged Repair Group (SRG).

Results: A total of 663 patients were enrolled in this study. The mortality rate in the group with normal IAP was 3.5%, contrary to a much higher mortality rate of 19.6% in those with elevated IAP. The mortality rate in the SRG was higher than the MRG. Overall complications incidence was much higher in the SRG (85.7%), in comparison to the MRG (56.3%). The incidence of wound infection, intra abdominal collections, dehiscence and ileus with intestinal obstruction were compared in both groups. The occurrence of intestinal fistulae was higher in the SRG (28.8%) than in the MRG (6.3%) ($P<0.04$). The mean hospital stay was 15.2 days/patient in the MRG but 26.1 days/patient in the SRG (after adding those of the repair of ventral hernias).

Conclusion: ACS is a serious complication in surgical wards but it is often overlooked as a case of subsequent serious complications unless it is suspected early and managed properly. The use of mesh repair to close abdominal defects after decompression for ACS reduces mortality and morbidity in comparison to the use of planned ventral hernia staged repair.

Key words: Intra-abdominal hypertension, abdominal compartment syndrome, mesh repair, staged repair

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INTRODUCTION

The concept that the abdominal cavity can be considered as a compartment had been known since the end of the nineteenth century [(1)] but has only recently become the subject of interest and discussions [(2)].

The terms Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are commonly used interchangeably; however it is important to recognize the distinction between these terminologies. IAH exists when IAP exceeds a measured numeric parameter. This parameter has generally been set between 20 to 25 mmHg [(3,4)]. Recently at the (WSACS), the threshold for IAH was established as a value of 12 mmHg or greater while ACS, defined as a sustained IAP was established as a value > 20 mmHg with or without an abdominal perfusion pressure (APP< 60 mmHg) that is associated with new organ dysfunction or failure. However, most surgeons advocate urgent decompression if IAP exceeds 25 mmHg, in the absence of new organ dysfunction [(5)].

Clinical confirmation of IAH requires either indirect bedside tests that measure IAP from transduction of intravesical, gastric, rectal, vaginal, and caval pressures, or direct measurement by an intraperitoneal catheter [(6,7,8)].

The clinical triad of oliguria, hypoxia, and elevated central venous pressure in association with abdominal distension indicates a late diagnosis with impairment of one or more systems. Decompressive laparotomy, in such occasions should be done as soon as possible to prevent Multiple Organ Failure Syndrome (MOFS), without attempting at measuring IAP [(9)].

Treatment of abdominal wall after decompression is controversial. Some advocate primary repair with mesh, however most surgeons advocate two stage repairs for abdominal wall reconstruction. Firstly, temporary closure of the abdomen is performed; which can be done by different methods such as Bogota bag, the Vacuum Assisted Closure (VAC) technique, Wittmann patch (velcro burr), silo, or absorbable mesh [9,10, 11, and 12]. This study was performed to evaluate the results of mesh repair in comparison to planned ventral hernia staged repair for closure of abdominal wall after decompression in trauma patients with (ACS).

PATIENTS AND METHODS

All patients with major abdominal trauma and identifiable risk factors for development of ACS presented to the First Surgical Unit in Baghdad Teaching Hospital, Iraq were enrolled for this research. The following risk factors were addressed; prolonged pre- and/or intraoperative hypotension (SBP<90mmHg), polytransfusion >10 units blood in 24 hr, massive fluid resuscitation >5L crystalloid and/or colloid in 24hr, retroperitoneal, intraperitoneal, and preperitoneal hemorrhage, abdominal distension (gastric dilatation, ileus, and intestinal obstruction), tight abdominal closure, massive trauma, massive burns, damage control surgery (hypothermia, acidosis, and coagulopathy), prolonged surgery >4 hr, and medical co-morbidity (obesity, ascites, ARDS, and respiratory failure).

IAP was usually measured indirectly using intravesical pressure in most cases while in few patients the measurement was done using nasogastric tube in the stomach to measure the intra-abdominal pressure . First Foley catheter with an appropriate size was positioned in e place, if not already present. The urinary bladder was evacuated, and a 25-50 ml of sterile saline (1 ml/kg for children below 25kg in weight) was injected into the bladder via the aspiration port of the catheter. The sterile tubing of the urinary drainage bag was cross-clamped distal to the drainage port. A hand-held manometer was connected to the Foley catheter, and the pressure in cm H_2O was recorded via the height of the water column in the manometer. This was expressed in mmHg by dividing it by a factor of 1.36 .

The test was performed in supine position, at the end of expiration, and the pressure was zeroed at the iliac crest in the midaxillary line. The pressure was measured every 30-60 seconds after instillation of saline to allow relaxation of the bladder detrusor muscle. There should be no muscle contraction, as this could lead to a falsely high result. Intragastric pressure was occasionally used in patients with genitourinary pathology or trauma.

Patients were screened for IAH/ACS risk factors during the operation and/or upon admission into the surgical ward or ICU. If one risk factor was present, baseline IAP measurement was done but If two or more risk factors were present, an additional serial IAP measurements were performed every 4-6 hr in the first 24 hours. If baseline IAP (i.e. <12 mmHg) or serial IAP records were normal on follow up, a close follow up for development of organ failure was done for another 24 hr. However, on a steady increase of IAP, a serial measurements of IAP were done every 2hr. Urgent decompression was performed if two readings confirmed elevated IAP > 25 mmHg. According to the (WSACS) definition of IAH and ACS, IAH can be graded into four grades; normal (less than 12mmHg), grade I (IAP 12-15 mmHg), grade II (IAP 16-20 mmHg), grade III (IAP 21-25 mmHg) and grade IV (equivalent to ACS).

For patients in grade I, serial measurement of IAP was done every 4-6 hr, but every 2 hrs for grade II. Maintenance of normovolaemia in these two groups was imperative. For grade III patients, additional medical measures were done such as gastrointestinal decompression with NG tube, prokinetics like metaclopromide, analgesics, sedatives, and use of colloids rather than crystalloids to reduce interstitial oedema.

Those with grade IV or ACS on initial measurement or during follow up urgently underwent decompression. Abdominal decompression was also performed primarily as a prophylaxis, when very tight abdominal closure was anticipated, and as a general rule, when the intestines lie above the level of the skin in a fully relaxed patient under neuromuscular blockade of general anesthesia, IAP would be higher than 25 mmHg, if closed primarily.

Once decompression was completed, rapid but thorough examination of the abdominal cavity was performed to identify and control, if possible, any cause of IAH. The initial phase of closure of abdominal wall defects implies the use of temporary closure method. There are multiple methods for temporary closure, but Bogota bag was used in this study. It is either an IV fluid bag or more commonly 2L urinary bags that are cut-open, and sutured to the skin or fascia with a continuous heavy nylon suture. The bag is then covered with Betadine-soaked Laparotomy packs, which are changed daily in the surgical ward or intensive care unit. Occasionally, the bag is changed, if signs of infection are present.

Cardiovascular stability and resolution of sepsis were indication to transfer patient to the theatre for removal of the bag and closure of abdominal wall defect. This phase is normally done five days to three weeks after decompression. Those who require a long period to recover, have their Bogota bag changed on a weekly basis, to reduce the risk of infection.

Patients booked for definitive closures of their abdominal wall defects were randomized into either the Mesh Repair Group (MRG), or the planned ventral hernia Staged Repair Group (SRG). Those who underwent staged repair, had their abdominal wall defects closed with skin undermining to the flanks and closing it in the midline for small defects (< 6cm wide), or secondary meshed split-thickness skin grafting, when healthy granulating tissue had covered the thin peritoneum over the bowels for larger defects (> 6cm wide). The latter was achieved in conjunction with a plastic surgeon. The second stage was performed 3-12 months later; this consists of the repair of any resultant ventral hernia.

Statistical analysis

Statistical analysis was performed using GraphPad InStat 3. Variables were compared using Fisher exact test with two sided P-value.

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Means of continuous variables were compared using Student's t-test. Factors contributing to the development of suture line failure were determined using logistic regression analysis. Statistical significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

A total of 536 male patients (80.8%), and 127 female patients (19.2%), were enrolled in this study. Ages of patients ranged between 10-72 years, with a mean age of 28 years (26 years for males, and 36 years for females). 52% of the patients were within the age range of 10-30 years. Eighty six patients (13%) had blunt trauma to abdomen, while 577 patients (87%) had penetrating trauma. The mean injury severity score (ISS) was 24.

IAP was normal in 566 patients (85.4%). Elevated IAP was noticed in 97 patients (14.6%) while 44 patients (6.6%) had grade IV IAH, and ultimately needed decompression. Twenty three patients (3.5%),14 patients (2.1%) and 16 patients (2.4%) had grade I IAH, grade II IAH and grade III IAH, respectively and were treated with conservative measures.

Out of those who underwent decompression, six patients died before definitive closure of their abdomen. Sixteen patients (43.2%) underwent mesh repair (The MRG). The mesh was covered with secondary skin grafting in six (38%) patients, while primary skin closure over the mesh was done in the other 10 patients (62%).

In contrast, 21 patients (56.8%) underwent staged repair SRG. Skin closure was done primarily in 16 patients (76%), while secondary skin grafting was done for the remaining 5 patients (24%). However, only 13 patients of the SRG had their ventral hernias ultimately repaired, 8 patients could not repair theirs due to either unfitness or/and unwillingness of the patients for multiple procedures. Although there was no mortality among those who underwent ventral hernia repair (VHR), complications of this operation were added to the SRG, as VHR comprises part of the staged repair of the ACS.

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The MRG include 14 male patients (87.5%), and two female patients (12.5%), while the SRG had 17 male patients (81%), and 4 female patients (19%). There was no ender and the surgical procedure (P-value = 0.47).

The mean age of the MRG was 27 years (12-61 years), while the mean age of the SRG was 30 years (10-65 years). There was no statistically significant difference between the two groups (P-value=0.21).

There were no statistically significant differences between the two groups with regards to the mechanism of injury (P-value = 0.52), mean injury severity score, ISS (P-value= 0.61), and the need and duration of ICU admission before the application of definitive treatment (P-value= 0.48, and 0.84 respectively).

The mean duration between decompression and definite treatment was 8.4 days in the MRG, and 9.2 days in the SRG with no statistically significant difference between the two groups in the duration of the initial phase of treatment (P-value= 0.90).

The mortality rate was 3.5% in the group with normal IAP in contrast to a mortality rate of 19.6% in those with elevated IAP (P-value < 0.0001). Those who needed decompression accounted for its major part, as the mortality rate in this group approached 36.4%. Six patients (13.6%) died in the initial phase of the treatment after decompression. Three patients (6.8%) died in the MRG while 7 patients (15.9%) died in the SRG. There was no statistically significant difference between the MRG and SRG as regards to mortality with a P-value= 0.46) .

The overall complications incidence was much higher in the SRG (95.2%) in comparison to the MRG (56.3%), with a P-value < 0.01 but in general there were no statistically significant differences between the two groups regarding wound infection, intra-abdominal collections (pelvic or subphrenic), dehiscence, and ileus with intestinal obstruction (Table 1).

Intestinal fistula is a serious and dreadful complication of exposed abdomen for prolonged periods. It is unavoidable and very difficult to heal. A patient (6.3%) developed intestinal fistula in the MRG while six patients (28.8%) developed intestinal fistulas in the SRG.

Intestinal fistulae were less in the MRG than in the SRG P-value< 0.04). One patient in the MRG and four patients in the SRG died as a result of this complication.

T. Jernigam (???), in his research on 167 patients showed that 14 patients (8.4%) developed fistula amidst various kinds of management; ten out of the 14 were in the MRG. In addition Torrie [21] reported 6(9%) fistula in his study on 68 patients using only mesh repair method.

These differences in results are possibly related to the mechanism of trauma, presence of bowel injury and duration of mesh application prior to coverage of granulating wound which appears to be a major contributor to these results.

Dehiscence, another serious and difficult-to-treat complication associated with exposed abdomen occurred in two patients (12.5%) in the MRG, and was treated by removal of the mesh, refreshing the edges, and reapplication of the Bogota bag, which was changed accordingly, until infection resolved and healthy granulation tissue was gained, when definitive treatment could be reapplied. In contrast, six patients (28.6%) developed dehiscence in SRG; those that occurred as a result of infection (three patients) were treated as previously stated, while those not related to infection, were treated by reclosing the skin (which was done in two patients) and by rectus femoris myocutaneous rotational flap, (done in one patient). There were no statistically significant difference between the two groups (P-value = 0.22). Compared to Torrie (????) who reported dehiscence in 3 cases, many patients in this study had known risk factors for dehiscence; these include raised IAP, wound infection and multiple trauma or malnutrition.

It was generally accepted that wound infection and intra-abdominal collections were mostly related to the more severe injuries, defective management in the wards, incompetent staff and lack of supplies and monitoring devices.

Mean stay-in-hospital was 15.2 days/patient in the MRG and 29.1 days/patient in the SRG (after adding the total days for the repair of ventral hernias) which was statistically significant with P-value <0.05.

Table 1. Comparison of outcome and overall complications between Mesh Repair Group (MRG) and Staged Repair Group (SRG).

Complication	**MRG No. (%)**	**SRG No. (%)**	**P-value**
Total number of cases	16 (43.2%)	21 (56.8%)	…….
Wound infection	2 (12.5%)	4(19%)	0.68
Intraabdominal Collection	2 (12.5%)	2 (9.5%)	0.59
Dehiscence	2(12.5%)	6 (28.6%)	0.22
Fistula	1 (6.3%)	6(28.6%)	<0.05
Ileus/Intestinal obstruction	2(12.5%)	2(9.5%)	0.38
Total complications	9 (56.3%)	20 (95.2%)	<0.01
Mean in-hospital stay day\patient	15.2	29.1	<0.05
Mortality	3 (18.8%)	7 (33.3%)	0.24

The incidence of ACS in all patients admitted to the surgical units during the period of this study was 6.6% as compared to 15% reported by Parsak [(13)] who carried a study on 119 patients which include 68 males and 51 females with a mean age of 55 years. There were 44 deaths for all grades; those who needed decompression which accounted for the major part approached 36.4%. Six patients died at the initial phase of the treatment after decompression, three (18.8%) from the MRG and 7 (33.3%) in the SRG, in comparison to Parsak (15) results, where 40 patients died in total, thirteen (32.5%) from those who needed decompression.

The occurrence of IAH and ACS is on the increase currently, mostly due to improved methods of diagnosis and recognition of the condition. More patients are now being investigated for IAH due to suspicion, as a high index of suspicion is imperative for optimal outcome. If not recognized and treated in time, ACS can result in multi-organ system failure and death. Studies have shown that IAP higher than 20mmHg results in ACS[(5)]. The adverse effects are reversible with the relief of pressure, if done at the proper time.

Traditionally, IAP can be measured indirectly through the urinary bladder using a Foley catheter. This technique was adopted to avoid direct invasive techniques, and was subsequently popularized by Kron et al in 1984[14]. In this study, the intravesical technique was used because it is more applicable and easier to perform than other methods. Further studies are needed to compare their accuracy.

ACS is associated with potentially high mortality that must be recognized early and managed effectively to optimize the outcome. Most deaths associated with ACS are due to sepsis or multiple organ failure. There is a direct correlation between abdominal hypertension and mortality rates[15,16,17]. However, IAP is not the only factor determining survival[18,19]. The presence of co-morbidity is a factor that increases mortality, which was also true for our patient population. Although other factors were not included in this study, the primary etiology, mainly trauma, together with general clinical condition of the patient greatly determine the outcome and prognosis.

Complications encountered during the study include wound infection, intra-abdominal collection, intestinal fistula, dehiscence and intestinal obstruction.

Overall complication incidence in the SRG and MRG was 95.2 and 56.3%, respectively compared to 73.5% in all modality of management used by Parsak (15).

In this study, two patients both in the MRG (12.5%) and in the SRG (9.5%) developed ileus or intestinal obstruction, mostly related to adhesions. The results were comparable to those obtained by Aydin[20], who also clarified no difference in adhesion formation between the two groups.

An open abdominal wound is a great challenge to a surgeon in postoperative management, due to massive fluid loss, heat dissemination and risk of infections. Frequent assessment, care of the wound and replacement of the lost fluids and electrolytes are mandatory. Strict aseptic technique is required for wound care[22].

Mean in-hospital stay in this study was 22 days, 15.2 in MRG and 29.1 in SRG. This is similar to the findings of Torrie (????) and Timothy (????) with a mean in-hospital stay of 20 and 22 days, respectively.

Currently, there are no prospective studies available to show which is the best method or material. Superiority of one material over the other has not been established. The materials advocated are mesh (absorbable, non-absorbable), zipper, adhesive sheets, plastic bag (Bogota bag) and velcro analog[22]. Recent studies have showed that the use of vicryl mesh is preferable, to get rid of several complications encountered with other types.

Polypropylene mesh was used in this study because other types like vicryl, PTFE and polyglactin were not available and our results with this type are comparable to other workers.

However, it is still impossible to specify fixed uniform criteria for preventive use of temporary closure of abdominal cavity, because it will always depend on subjective assessment of the operator regarding the tension of abdominal cavity after approximation of the fascia edges and estimation of the amount by which the content of abdominal cavity may increase or has already increased during tamponade. Nevertheless, the questions still remains, whether it is not more advantageous in hazard patient to carry out the primary closure of abdominal cavity with post-operation IAP monitoring and early ACS diagnostics instead of preventive temporary laparostomy[24].

Conclusions

ACS is not only a serious condition, but also a common scenario in surgical wards and ICU, which is often misdiagnosed as a case of respiratory failure or circulatory collapse. Most residents and some senior doctors are unaware of its lethal complications and how to diagnose it.

The results of this findings showed that the use of mesh repair to close abdominal wall defects after decompression for ACS is associated with reduced mortality and overall complications, in comparison to the use of planned ventral hernia staged repair.

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"MULTIPLE SCLEROSIS IN IRAQ: HISTORY, EPIDEMIOLOGY AND THE FUTURE"

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Introduction:

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) (1). It is the most common cause of disability in young adults after trauma (2). MS is known to show variable prevalence rates, demographic, and clinical manifestations depending on geography and ethnic background. It is generally believed that MS prevalence is low or moderate in Middle Eastern countries (3). The wide variety of clinical manifestations depends on the extent and the anatomical sites of the demyelinating plaques. The functional prognosis of the disease is poor in the late phases of the disease (4).

Common clinical patterns of MS:

1. Relapsing–remitting (RRMS): the most common form (85% of patients). It is characterized by periods of relapses and remissions of neurological symptoms with or without residual disability.
2. Secondary progressive (SPMS): may develop in up to two thirds of patients with relapsing–remitting disease. Patients continue to worsen with or without periods of remission.
3. Primary progressive (PPMS): affects around 10% of patients. Symptoms worsen gradually from disease onset. There are no relapses or remissions although occasional plateaus may occur (1).

Baghdad MS Clinic:

Following the increasing recognition of patients with MS in the nineties seen at different hospitals in Baghdad and the other cities in Iraq and because of the advances in the diagnostic tests for neurological diseases and especially because of the emerging therapies for MS, which used to be perceived as untreatable disease, the idea of establishing a specialized MS clinic started to emerge.

Baghdad MS clinic was one of the first specialized MS Clinics in the Middle East; it was established in 2000 at Baghdad University Hospital (Medical City Teaching Hospital). The clinic was geographically accessible for most of the population in Baghdad and the rest of Iraqi cities. It is a multidisciplinary specialized neurological service with access to neuroimaging, neurophysiological studies and rehabilitation services. The service was virtually free and equally accessible for the entire Iraqi population. One of the main advantages of such service was the collection of data from MS patients which is of great help in setting directions, plans, and research studies. (3) Data from this Clinic was presented at different regional and international conferences.

Clinical and demographic characteristics of MS in Iraqi patients:

The first account for MS patients among the Iraqi population dates back to the 1950s (5). A study by Hamdi in 1975 found that MS was more prevalent in Kurdish people who live in Northern Iraq (6). In an early publication from Baghdad MS Clinic, the clinical and demographic features of Iraqi MS patients were found to be comparable to the so-called "Western type" of MS as seen in Caucasians. These included: age at onset, clinical course, male predominance and older age of onset in PPMS, frequency of initial presenting symptoms, cumulative neurological signs, disability status, and MRI findings. There was a difference in gender ratio and family history. Female to male ratio of 1.2:1 in one study is somewhat lower than the 2:1 or higher ratio in most Western series, but consistent with reports from neighboring countries like Kuwait, Saudi Arabia, and Jordan (3). A large Iranian study reported increasing disease incidence in females between 2002 and 2008 from 2:1 to more than 3:1 (7).

Family history of MS was reported in up to 5.7% of patients (15% or higher figures reported in Western series). Limitation factors to family history reporting include: lack of careful interviews with 1^{st}, 2^{nd} and 3^{rd} degree relatives of the index case, large family size, and communication difficulties (3). Familial aggregation is a well-known phenomenon in high prevalence areas and it is considered rare in Asia (2).

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A comparison study between familial and sporadic MS in Iraqi patients in 2011 found no significant difference in terms of the demographic patterns, clinical course, and presentation.
Higher percentage of RRMS patients with sporadic MS (41.6%) progressed to SPMS compared with familial cases (around 36.4%). This should be taken with

caution as the numbers were small. There was a statistically significant inverse relation between mean age at onset and lag time to diagnosis ie the younger age, the longer lag time to diagnosis (p=0.007). Also, there was direct correlation between lag time to diagnosis and time to second attack (p=0.05). The individual groups did not vary significantly in this regard (2).

A study looking at the disability and prognosis of 500 RRMS cases from the MS clinic in Baghdad showed that older age at onset, pyramidal and sphincter involvement at the beginning of the illness, and relapse rate in the first two years of the disease were poor prognostic factors. On the other hand, Western studies showed that younger age at onset is a poor prognostic factor, likely due to accumulation of disability with time. Gender was found not to affect outcome in that study, which is in contrast to the widely agreed consensus that female patients have better prognosis than male (4). In 2010, a Lebanese group looked at the prognostic factors of MS in Lebanon found no difference from similar predictors in western countries (8).

MS Epidemiology and North South gradient:

There were no well-conducted MS epidemiological studies from Iraq prior to the establishment of the MS Clinic in Baghdad. An increase in reported cases is noticed possibly due to increasing recognition of the disease, better facilities for the diagnosis, and increasing number of neurologists and other specialties dealing with MS. There is an interesting phenomenon of progressive increase in the number of MS patients over the years in the last three decades according to the age of onset, and the low proportion of patients with long duration of the disease. An increase in incidence in the last three decades is a possibility, but this should be accepted with caution. The significant increase of the number of women with MS compared to men in the last three decades might stimulate the pattern of increasing trend in women in the West. The gender shift is difficult to be explained by a changing social structure that might give women more access to medical services. Changing environmental factors might provide one explanation for increasing incidence; a shift towards a more Western lifestyle has been linked to the increase of MS incidence in Japan. In Iraq, economic and social changes have been apparent, especially since the early 1970s and might be a factor (3).

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Looking at data from neighboring countries, a study from Iran estimated MS prevalence in Tehran to be at least 51.9:100,000 (7). However, a more recent study from Southeastern Iran reported a prevalence of around 14:100,000 which is comparable to countries in the region (9).

MS has been shown to increase in prevalence with increasing distance from the equator. In Iraq, MS patients born in Naynawa were more than six times those born in Basra in one study; both governorates have approximately equal population and are roughly equidistance from Baghdad. There is a latitude difference between the two regions; with Naynawa lying on the 29^{th} and Basra on 35^{th} degree. Also, dietary habits are different between the two areas; people in the North eat animal fat rich diet while South residents eat more seafood. Sun exposure and temperatures are more in the South. Thought the majority of people in both regions are Arabs, it's been observed that Iraqis from Naynawa have fairer skin, lighter eye colour, non-Arab and non-Muslim minorities than those from Basra. These differences raise the possibility of different genetic constitutions between Iraqi patients which in turn may affect the susceptibility to develop MS (3). A study from Israel on Arabic MS patients reported more rapid disease course in Druze and Muslims compared to Christians. High relapse rate, annual disability progression, and MS severity scores were higher among the Druze. These findings support the hypothesis of population specific MS phenotypes (10).

The Future:

MS remains a disabling disease with significant impact on people lives. Advances in research in the last three decades provided new treatments for the relapsing forms of MS. More studies are in progress to find solutions for progressive MS patients.

Health services in Iraq have been suffering as a result of wars, sanctions, and slow recovery after 2003. Many skilled health professionals have migrated to other countries, and new graduates continue to leave. A decade has passed since 2003 but the health infrastructure is not fully restored. The healthcare system is centralised and focused on big hospitals (11). The absence of clear strategies in setting up medical services and financial planning are the main reasons why patients with complex diseases are not receiving ideal medical care. There is a strong need for long term planning for conditions like MS where enthusiastic medical profession and reliable management groups work hand in hand to build the basis for medical institutions where healthcare is provided to patients at excellent level.

Conflict of Interest: none.

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THE EFFECTS OF VAGAL NERVE STIMULATION THERAPY IN REFRACTORY EPILEPSY ON THE ELECTRICAL ACTIVITY OF THE HEART

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Key Words: vagal nerve stimulation, refractory epilepsy

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Abstract:

Background: Refractory epilepsy is a major problem for neurologists and epileptic patients. Vagal nerve stimulation is one of the palliative surgical therapies. Traditionally, the vagus nerve has been considered a parasympathetic efferent nerve (controlling and regulating autonomic functions, such as heart rate and gastric tone). Changes in autonomic nervous system activity affect both depolarization and repolarization phases in addition to heart rate , and thus affect QT interval.

Objective: to evaluate the possible side effect of vagal nerve stimulation therapy on the electrical activity of the heart represented by QT, QTp, TpTe intervals and QT&QTp dispersions.

Patients and method: This study was conducted at Neurosciences hospital in Baghdad ,between September 2008 and march 2010.ECG traces were recorded for 32 pharmacoresistance epileptic patients with a mean age 21.14 $\pm$ 4.4 year, just before and after implantation of the Vagal nerve stimulation device. All QT,QTp and TpTe intervals and QT & QTp dispersion were calculated manually.

Results: there were a differences in both QT and QTp before VNS implantation as compared to that after the implant but it did not reach a significant level . Similarly no significant differences were observed in QT and QTp dispersions. An interesting significant differences in TpTe (before and after the implant) were observed.

Conclusion: Vagal nerve stimulation therapy may alter the cardiac conductivity in some cases.

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THE EFFECTS OF VAGAL NERVE STIMULATION THERAPY IN REFRACTORY EPILEPSY ON THE ELECTRICAL ACTIVITY OF THE HEART

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NTRODUCTION

Approximately one-third of patients with epilepsy continue to have seizures on medication (1). Their seizures are referred to as refractory or drug-resistant. In those with refractory epilepsy, a combination of antiseizure medications may be tried. The combination may help to reduce the total number of seizures. However, polypharmacy often leads to an increased number of side effects. When medications are not enough to control seizures, physicians often turn to nonpharmacologic options: epilepsy surgery, ketogenic diet, and vagus nerve stimulator (VNS) therapy.

The VNS Therapy System was approved as adjunctive therapy for adults and adolescents over 12 years of age whose partial-onset seizures were refractory to antiepileptic drugs. The vagus nerve stimulator (VNS) is a battery-powered device similar to a cardiac pacemaker. Stimulating leads are surgically placed around the left vagus nerve in the carotid sheath and are connected to an infraclavicular subcutaneous programmable pacemaker.

For better understanding of the clinical use & side effect of the VNS; a brief introduction of relevant anatomy, possible mechanisms of action is required,

ANATOMY

the idea of using the vagus nerve in particular stem out of its unique anatomy. It is widely distributed to both sides of the brain and brain stem structures. The majority of vagus nerve fibers 80% are afferent (sending signal towards the brain) rather than efferent 20% (sending signal away from the brain) (2).

If an electrical stimulus is placed on the vagus nerve, 80 percent of the applied stimulation travels back to the brainstem where the vagus nerve originates. The central projections of the vagus nerve (cranial nerve 10) synapse bilaterally on the nucleus of the solitary tract (NTS) in the brainstem. From the NTS, vagal afferent pathways project to many regions in the brain, including pontine and midbrain nuclei, the cerebellum, thalamus, and cortex. In short, electrical impulses, which are applied to one of the vagus nerves, travel to both sides of the brain, and to many disparate regions.

One vagal pathway, perhaps of particular relevance to epilepsy therapy, ascends to the forebrain via the pontine parabrachial nucleus (3). This pathway transmits sensations of visceral origin to the ventroposterior parvocellular nucleus of the thalamus, which then projects to the insular cortex (4). The parabrachial nucleus also projects to other thalamic nuclei, the amygdala, and the basal forebrain. These vagal projections travel to sites that are often found to generate seizures. One way VNS may work is through connections with the seizure generating region.

Another way in which VNS may operate is through the locus coeruleus. The locus coeruleus is another pontine nucleus that receives afferents from the NTS (5.6). While receiving less dense vagal input than the parabrachial nucleus, the locus coeruleus may be essential to the antiepileptic effect of VNS, as suggested by animal studies using ablative and immunolabeling procedures.

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Vagal efferent fibers originate in the dorsal motor nucleus of the vagus and the nucleus ambiguous.

These innervate the heart, vocal cords, and other laryngeal and pharyngeal muscles, and also provide parasympathetic input to the gastrointestinal viscera (2).

Because the right vagus nerve provides more innervations to the cardiac atria than the left vagus nerve (7), electrical stimulation of the left vagus nerve is generally used in clinical practice to avoid adverse cardiac effects.

THERAPEUTIC MECHANISM

Epilepsy is defined clinically as a state of chronic recurrent seizure; the cause may be known (symptomatic epilepsy) or unknown (idiopathic or cryptogenic epilepsy). **Seizure is an abnormal paroxysmal cortical cerebral activity due to hypersynchronus discharge of a group of cortical cerebral cells.**

In the 1960s, studies in animals showed that repetitive vagus nerve stimulation (VNS) would either **synchronize or desynchronize** cortical electrical activity. The effect of stimulation on brain activity depended on the stimulus frequency and the strength of the electrical current. Because epileptic seizures are characterized by **hypersynchronized** cortical activity, **the observation that VNS can desynchronize cortical rhythms suggested a potential antiepileptic effect of VNS (8-10).**

VNS studies in a variety of animal models were then undertaken and have demonstrated that VNS has multiple antiepileptic properties (1-18):

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- VNS can abort an ongoing seizure after seizure onset
- VNS is effective in acute seizure prophylaxis; ie, seizure-inducing insults (eg, strychnine administration) are less effective in inducing a seizure in the presence of VNS

- VNS is effective in chronic seizure prophylaxis, reducing seizure frequency in animal models of epilepsy
- VNS can inhibit epileptogenesis in animal models of seizure kindling

How VNS exerts its antiseizure properties exactly remains unclear.

CLINICAL APPLICATION

In general, VNS is considered a valid treatment option for children and adults with well-documented medically-refractory seizures, who are either opposed to intracranial surgery, are not candidates, or whose medically-refractory seizures were not substantially improved by prior intracranial epilepsy surgery (19-23). Resective surgery for appropriate candidates is preferred over VNS because of the substantially greater potential for complete seizure remission

SAFETY AND TOLERABILITY

Common side effects — side effects that occurred in at least 5 percent of patients receiving high-stimulation vagus nerve stimulation (VNS) were(66):

- Hoarseness (37 percent)
- Throat pain (11 percent)

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- Coughing (7 percent)
- Shortness of breath (6 percent)
- Tingling (6 percent)
- Muscle pain (6 percent)

Hoarseness was the only side effect that occurred significantly more often with high stimulation than with low stimulation.

Shortness of breath and pharyngitis, as well as voice alteration, occurred significantly more often in the high-stimulation group than in the low-stimulation group. Lowering the pulse width of stimulation can alleviate symptoms and allow for higher stimulation intensities. Lowering the frequency can also attenuate side effects related to VNS stimulation (26).

Cardiac events — Physiologic studies have generally found no clinically relevant effects of chronic VNS on cardiorespiratory function (28-30). However, bradycardia followed by transient asystole lasting up to 45 seconds has been reported in association with the lead test conducted during VNS implantation (the initial lead test, which is performed in the operating room when the VNS is being implanted for the first time) in approximately 0.1 percent of cases (31-33). Complete heart block due to atrioventricular nodal block was documented in three patients with no reported adverse effects (34). In some cases, a rechallenge stimulus is uneventful, and the VNS has been implanted successfully without adverse consequences. More often, the procedure is aborted. In general, baseline cardiac conduction disorders are considered a contraindication to VNS.
Two case reports describe VNS-induced episodes of bradycardia and asystole occurring 2 and 9 years after device implantation (35-36).

Other adverse effects

- Surgical complications.
- Electrode failure and lead fracture (37-40).
- Infection of the subcutaneous pocket that holds the VNS generator, usually with Staphylococcus aureus (24,41-43),
- Sleep apnea is a relative contraindication for VNS (44-46). VNS is associated with more frequent apnea and hypopnea episodes in sleep, but this appears clinically relevant only in those with preexisting sleep apnea.

    - Unilateral vocal cord paralysis occurs in about 1 percent of cases, and is attributed to intraoperative manipulation of the recurrent laryngeal nerve]. Most of these recover (24, 25, 27).
- Other cranial nerve palsies that can complicate VNS implant include Horner's syndrome and facial paralysis (47).
- Pneumothorax has been described in at least one patient (24).
- Some patients experience uncomfortable spasm of the left chest wall, which has been demonstrated to be due to collateral spread of stimulation to phrenic nerve, causing contraction of the left hemidiaphragm.
- Contraction of the left anterior sternocleidomastoid muscle may also occur as a result of current stimulating adjacent structures (48). These symptoms are often precipitated by assumption of certain postures or movement and are relieved by changing position.
- While gastrointestinal side effects might be expected with VNS, reports of this are infrequent.
- Forced normalization refers to a phenomenon of psychiatric disturbances that emerge in some patients with long-standing, high-frequency seizures when their seizures are dramatically reduced (49-50).

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TOFIQ Journal of Medical Sciences
TJMS
Volume 2, Issue 1. 2015
ISSN: 2377-2808

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EDITORIAL VIEW-POINT

THE CHALLENGES OF MULTI-MODALITY CARDIAC IMAGING

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Invasive coronary angiography is a great method to visualize the coronary anatomy and determine the presence and severity of coronary stenosis. The assumption has been a stenosis of 50% (diameter narrowing) or greater is marker of 'significant" stenosis implying that it limits normal augmentation of myocardial blood flow (MBF). This threshold was used in determining the accuracy of other tests and to decide on treatment strategies. Decades later it became clear that stenosis severity is a poor marker of the physiology of coronary circulation and specifically MBF because the changes in MBF are dependent not only on the resistance in the focal stenosis but also on downstream resistance due to micro-vascular disease and endothelial dysfunction. These conclusions were derived from elegant studies comparing state-of-the art measurement of stenosis severity with state-of-the art measurement of MBF or pressure gradients across coronary stenoses of different severities.

The MBF is tightly regulated in normal subjects by myocardial oxygen demand (MVO-2) and often remains normal at baseline (resting MBF) despite focal stenosis due to a process of autoregulation. In response to increased demand such as exercise, the MBF increases several folds (peak, or hyperemic MBF). The ratio of the peak to resting is referred to as coronary flow reserve (CFR). In general the peak MBF and CFR are lower in the presence of coronary stenosis than in normal subjects and if the reduction is severe enough, then myocardial ischemia is produced with its clinical manifestations (chest pains or shortness of breath), ECG changes (ST depression or elevation), left ventricular (LV) wall motion abnormalities or perfusion abnormalities. This cascade of changes is the principle of using non-invasive imaging to detect coronary artery disease (CAD).

It was not too long ago that treadmill exercise testing (TET) or its counter-part, the bicycle exercise test in Europe was the only non-invasive stress modality. The TET was used to detect CAD, predict outcome and assess efficacy of new medications and interventions as for approval by the Food and Drug administration (FDA). With decades of experience, we have come to recognize its strengths and limitations, and in the process introduce many modifications, protocols, definitions and scoring systems with the said

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purpose of improving the precision of the test. Obviously back then the ST response was considered the single most important variable of the TET but with time we have come to recognize that though ST response is the best variable for diagnosis, the non-ST variables especially exercise time or METS (metabolic equivalent) are far more important for prognostication.

A number of non-invasive imaging modalities (echocardiography [Echo], nuclear cardiac imaging, cardiac magnetic imaging [CMR] and cardiac computed tomography [CT]) have been introduced to study the structure and function of the heart and coronary circulation at rest and during stress.

The type of stress testing has also changed and pharmacological stress testing is now accounts for almost 60% of the stress tests done with imaging in the United States. The main indication (there are few other exceptions) for pharmacological stress testing is the inability to perform adequate exercise 'sub-maximal exercise" because the sensitivity of any test (TET alone or TET combined with imaging) with submaximal exercise is suboptimal.

The pharmacological stress tests are mainly of 2 types: inotropic agents (dobutamine is the prototype) and coronary vasodilators (dipyridamole, adenosine, regadenoson and adenosine triphosphate). In the United States regadenoson accounts for over 80% of all pharmacological stress tests performed with nuclear imaging and dobutamine for over 90% of the stress tests performed with Echo (the numbers are different elsewhere in the world).

The principles behind stress testing (with or without imaging) is to assess directly or indirectly the ability of the MBF to respond appropriately to an increase in MVO-2 or to a powerful stimulus of primary vasodilation. Both TET and dobutamine increase MVO-2 (increases in heart rate, blood pressure and contractility) and hence the increase in MBF is a consequence to that, while the vasodilators increase MBF irrespective of the change in MVO-2 (uncoupling of demand and MBF). Measurement of MBF in man is difficult during peak exercise but based on many studies in man and animals, the peak is less with exercise than with vasodilators but higher than that achieved with dobutamine.

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The reasons why Regadenoson (Lexiscan in United States and RapidScan elsewhere) became popular in the United states are: ease of administration (it is given as a bolus over 10 seconds rather than an infusion using an infusion pump), the use of a fixed dose regardless of body weight which eliminated errors in dose calculation and it selectivity as A-2 receptor agonist with very little or no A-1 rector activity that is responsible for AV blocks and which explains why bolus adenosine is used to treat some forms of supraventricular tachycardia (adenosine stimulates many receptors A-I, A-2a, A-2b and A-3).

Hybrid stress testing (more than one type) is fairly common. For example the use of atropine with dobutamine in patients who do not achieve adequate target heart rates and the use of handgrip exercise or walking in place in combination with dipyridamole. Regadenoson has made it possible for yet another type of hybrid stress testing, combination of TET and regadenoson in patients undergoing TET but who fail to achieve adequate exercise. Many variants of this hybrid protocol have been used with seemingly no increase in serious side effects and a decrease in the less serious side effects.

Most large university cardiology programs in the United States provide training in all aspects of “multi-modality cardiac imaging” with variable emphasis on one or the other. There are specified training guidelines to become a user (perform, interpret and report) these imaging studies. There is further specialized training for those interested in academic career such as direct imaging laboratories.

Most stress Echo studies for detection of CAD and risk assessment are done with either dobutamine or exercise. The variables of interest are worsening in LV wall motion/thickening and changes in LV volumes and ejection fraction (EF). Myocardial perfusion pattern using microbubbles is another important variable when performed.

Most of stress CMR studies are done with vasodilator stress testing as exercise testing is not feasible. The variable of interest when performed with vasodilators is the myocardial perfusion pattern (scar or ischemia). When performed with dobutamine the variables are similar to those with Echo. CMR could also provide information on coronary anatomy (CMR angiography).

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Cardiac CT provides multiple variables of interest: coronary calcium score, coronary angiography (stenosis severity soft and calcified plaques, remodeling), LV function and potentially MBF.

Nuclear imaging, which is the most widely used imaging modality in the United States, could be performed with single photon emission tomography (SPECT) or with positron emission tomography (PET). Each provides information on perfusion pattern (scar or ischemia), LV function (EF, volumes and wall motion/thickening) and in the case pf PET absolute measurement of MBF (regionally and globally).

The concept of multimodality imaging is still evolving and is centered on the premise that imaging should be personalized and the most appropriate test utilized to answer specific questions. The American College of cardiology, the American Heart Association, the European Society of Cardiology, and multiple imaging organizations have outlined appropriateness criteria for each imaging modality but these guidelines presume that there is equivalent expertise in all imaging modalities in all institutions and across practices, which is unreal. The new generation of trainees who are now being trained in multimodality imaging hold the premise that something like that will happen in the future.

There is probably over use of imaging (studies show regional variations in the use of coronary angiography and all imaging modalities across the United States and yet the mortality rates are very similar!), inappropriate use, less than acceptable quality images and less than ideal reporting of results which when coupled with the decline in mortality due to CAD in developed countries and the decline in the incidence of abnormal test results over the last decade suggests the need for a better system of test selection and patient selection. The developing countries no doubt will have their share of issues to deal with but one is helpful that the experience so far would make it easier to adopt.

Iskandrian AE, Garcia EV (Editors): Nuclear cardiac imaging: principles and applications. 5th edition, 2015. Oxford University press

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 1, 2015

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THE CONCEPT OF DIASPORA
A NEW PARADIGM AND RELEVANCE FOR
IRAQI INTELLECTUAL ELITE IN AMERICAN SOCIETY

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Submitted to TOFIQ
For Consideration As a Possible Publication
in The TOFIQ Online Journal
on August, 22 2015

The Author Extends His Gratitude and Appreciation to
Dr. A. Hadi Khalili for His Encouragement and Support

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ABSTRACT

This paper examines the use of the concept of Diaspora in the field of sociopolitical writings in the past and evaluates its suitability for the systematic analysis of population dispersion in light of the advancements of more rigorous theory building as well as systematic empirical measurement in recent decades. The paper maintains that past usages of the concept of diaspora have been broad, more descriptive and lacking the rigor found in today's academic social research and the more quantitatively-oriented theoretical designs. It suggests the need for a rather "revolutionary" paradigm(to use T.S. (hues publication title) to energize the concept's systematic use in social research and to move it away Tom the rather general trend of lowering its parameters to use(Moynihan terminology)so that it would conveniently fit any social problem resulting from the variety of population dispersion. The paper then proposes a rather elementary new paradigm for the use of the concept in the field and explores its relevance for the Iraqi intellectual elite in the American Society.

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Introduction

One of the numerous theoretical and methodological contributions the late Daniel Patrick Moynihan made to social research, and more particularly to the area of sociopolitical analysis, was his contention that societies' governmental agencies and social writers, when tackling social problems such as disintegration of the family institution, deviant behavior in its different settings and the rise of poverty level, they tend to define them down so as to explain away and make" normal" what a more rigorous and advance empirical researchers would define as deviant. This tendency to destigmatize the behavioral patterns of subjects by lowering their levels of manifested consequences would help a society in general and its collective aggregates and groups to sooth themselves and to assist their members to act normally (Moynihan, 1985).

The process of lowering the parameters of the different social and political phenomena, as advanced by Moynihan has become of late a trendy affair with many social writers in different areas of analysis, especially where the focus of the writing lacks methodological rigor and systematic quantitative measurements (Fiske, 1986; Nachmias and Nachmias, 1996). On the other hand, modern social research has advanced the argument that empirical measurements are necessary to delineate the behavioral of the subjects of the study along with accompanying conceptual frameworks of that particular investigation (Greer, 1989; Nachmias and Nachmias,1996). Without the process of quantification of the variables derived from the proposed conceptual framework, research findings become arbitrary and the validity of their claim border on the questionable side.

One must also make the observation that a great number of these studies with lower parameters tend to be qualitative in nature, rather broad in scope of coverage and have their historical foundations in the past centuries if not in the almost forgotten epoch of human existence. The present-day institutions of these historical phenomena with lower parameters have evolved dramatically such that when compared with similar institutions of the same phenomena of the past they show a drastic departure from them. That being the case, it follows that when delineating the boundaries of the problem under investigation, researchers would have difficulties defining the conceptual frameworks for them. These difficulties when are added to the lack of rigor in the conceptual definitions and in the empirical measurements would result in difficulties in generalizing the findings of these studies across racial and ethnic boundaries and impede their utility in furthering the cause of building deductive frameworks that are necessary for generating more empirically oriented analysis. The net results are stagnated theory building, isolated conceptual frameworks and arrested development of viable and empirically-oriented sociopolitical discipline.

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The Concept of Diaspora

This paper maintains that one such sociopolitical concept that has been lowered in its parameters by social writers and is lacking both conceptual framework and systematic empirical measurements is the concept of Diaspora. Originally, Diaspora (in caps) was derived from the Greek lexicons. At the time of its inception, it meant the HOLISTIC dispersion of the masses of Hebrew population away from their ancient residential communities in Northern Kingdom as a result of the Assyrian invasion during the first part of the seventh century BC and later the mass captivity of the Jewish population in the first part of the seventh century BC and later the mass captivity of the Jewish population in the late sixth century BC by the Babylonians. Some writers add to the preceding waves of dispersion the third Jewish dispersion by the Roman Empire in 70 CE.

The concept then went into a total "hibernation" so to speak and was hardly used in social and political writings for a rather long time. Then suddenly in 1876, The Oxford English Dictionary cited the term in connection with the evangelizing movements among the Protestant churches in Europe. The "reincarnation" of the concept soon would find its way to the writings of a number of discipline and in varied geographical and cultural settings. A survey of literature reveals the use of a variety of adjectives such as trade diaspora, spatial diaspora, ethnic diaspora and even Hurricane Katrina Diaspora. No wonder the eminent scholar, Moynihon reminded scholars with strict methodological concern to avoid attempts at lowering the parameters of their conceptual frameworks and adhere to more rigorous empirical definitions.

It is interesting to point out that the different disciplines of modern social sciences such as sociology, psychology, anthropology, political science and even the different specialties of economics were establishing their quantitative roots at around the times the concept of diaspora was emerging from its inactive stage of use in the literature . However, when examining the early writings in these new disciplines, there is hardly much of use of the concept of diaspora in their coverage of their respective phenomena.

Moynihan's calls to sociopolitical writers to adhere to the more cautious use of the social concepts that explain social problems and to stress theoretical rigor in their analysis have finally found their ways in the mid-nineties and around the turn of last century in the writings of such scholars as Clifford(1994); Cohen, R.(1997) and Cohen and Kennedy(2000). These authors' contributions to the refinement of the concept of diaspora were made within the established social science specialties of sociology and cultural anthropology and probably reflected the more rigorous standards set by the scholarly leaders in their respective disciplines

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Immigration Acts And Policies

While the dynamics of the process of diaspora as advanced by early historians and other social writers may have been appropriate tools to deal with forced and/or voluntary dispersion of population especially in the past centuries, the enactment of series of immigration laws by both European countries and the United States earlier in the twentieth century necessitated the need for a new set of dynamics in handling population movements coming from Eastern Europe, Asia and the Middle East into Western Europe and across the Atlantic to the American shores. The legislative acts especially in the United States set new boundaries for any population movement into its boarders therefore establishing new and powerful restrictions governing the earlier forms of the dynamics of diaspora in the decades following their enactment.

Perhaps the most restrictive of the new legislative acts was the Immigration Act of 1924(The Johnson -Reed Act). It limited the annual number of immigrants who could be admitted to the country to only2% of the number of people from that country who were already living in the United States in 1890. The law was primarily aimed at furthering the restrictions imposed against Southern and Eastern European and immigrants. In addition, it severely restricted immigration of African and prohibited admission of immigrants from Arabia, Eat Asians and Indians. Furthermore, the act set the foundations for a quota system for admitting each immigrant therefore making mass migration from Eastern Europe where the majority of the Jewish diaspora lived virtually impossible.

The Immigration and Nationality Act of 1952, also known as The McCarran-Walter Act abolished racial restrictions found in the United States immigration and naturalization statutes going back to The Naturalization Act of l790, but let the quota system set in the 1924 act stands, insisted on the qualifications of the individual immigrant and excluded the remnants of the Eastern European diaspora from any entry to the United States. President Harry S. Truman vetoed the bill declaring that"...we need to stretch out our helping hand to save those who have managed to flee into Western Europe to succor those who are brave enough to escape from barbarism, to welcome and restore them against the day when their countries will as we hope be free again...", but the House and Senate succeeded in overriding his veto by a comfortable margins thus affirming its restrictive clauses.

Immigration policies in the United States took a radical departure from the legislative acts cited in the preceding paragraphs when the Immigration and Nationality Act of 1965, also known as The Hart-Cetler Act was passed by the Congress and signed into law by President Lyndon B. Johnson at the foot of the Statue of Liberty on October 3,1965 declaring that the old

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system(meaning the 1924 and 1952 acts) violated the principles of American democracy. The new immigration act terminated the use of the quota system and established a new set of criteria for admitting immigrants based on level of knowledge, educational achievement and the need for highly trained and scientifically-oriented skill applicants. The new act opened the doors to immigrants from all over the world and especially from India and the Middle East.

The Refugee Issue

The multiplicity of patterns of refugees throughout human history present a rather high level of complexity for researchers in the field of population dispersion due to the almost infinite circumstances surrounding movements of the refugees themselves, diversity of the geographical locations, political and economic factors, natural disasters and even level of satisfaction with living conditions of the native country. While some of these circumstances are also prominent in cases of migration trends, the latter phenomenon is likely to be governed by the countries' legal restrictions and governmental regulations. By implications, studies of refugees demand different theoretical frameworks, more individualized strategies for empirical analysis and careful plans for generalization of the findings to other refugee cases.

Refugees have received the lion share of the international attention and aid programs. As early as 1921, the League of Nations began its global assistance and more particularly the European refugees through its High Commission organization which proved to be crucial for masses of refugees escaping the Russian Revolution of 1917. Shortly after that, the Commission played a significant role in assisting the Armenian refugees as well as the Anatolian Greeks.

In 1951, The United Nations High Commission of Refugees(UNHCR) was established to assist with flow of refugees following the end of Wold War II throughout the European Continent and other war-affected surroundings in need of immediate assistance. Earlier, following the Arab-Israeli War, the international body created the United Nations Relief and Work Agency(UNRWA) to assist the Palestinian refugees in Palestine and the surrounding countries. Both the First Gulf War of 1991and the invasion of Iraq in 2003 added new waves of refugees to the world displaced population and necessitated more involvement of the UNHCR in assisting them. The war in Afghanistan, Somalia Sudan and lately in Syria and Iraq inflated the total people who are currently in situation of displacement to the figure of some 45 millions. As many as 55% of counted and/or assusted in one fashion or another came from only five countries: Afghanistan, Somalia, Iraq, Syria and Sudan.

The Need for a New Paradigm

The forgoing review of patters of population dispersion in their varied historical and geographical settings clearly shows that if sociopolitical researchers attempt to use the concept of

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diaspora, as it was intended and applied in early years, as their analytic framework, they would soon discover that their choice of the tool of analysis would not be helpful in their efforts to advance an elaborate system of theoretical concepts linked together to form network of relationships supporting their derivation from a larger theoretical construct and providing the necessary tools for their empirical measurements and testing processes. Put differently, the concept of diaspora as it has been used by social writes investigating population movements in almost countless number of historical and geographical cases all over the globe, has reached a point whereby its continued use in its present form would not be conducive to rigorous theory building neither would it be of help in furthering the cause of empirical measurement and meaningful generalization to similar areas of population dispersion. These limitations once added to what Moynihan(1985) had observed regarding the tendency by writers in areas of social problems to lower the parameters of their concepts so that they may be used by them as tools of analysis would in the opinion of this writer necessitate the need for the advancement of a new paradigm. The new paradigm would serve as a path guiding researches in their investigation of problems of population movements and serving as system of logic for deriving the necessary theoretical definitions of the variables of their studies, proposing tools for their empirical measurements and suggesting a set of mechanism necessary for generalizing their findings to similar conditions. Put differently, the time has come to develop a new paradigmatic structure that would assist researchers to elevate their research strategies in their use of the concept of diaspora to a higher level such that they would be able to establish a theoretical foundations for their hypotheses, link its framework to a broader deductive construct, derive the necessary empirical measurements and strive to generalize their findings to other similar cases so that the cause of theory building in the area of population dispersion would be accelerated.

Specifically, what is a paradigm? How does a new paradigm emerge? What are the necessary research conditions that are conducive to its development and maturity? A pioneering scientist of the logic of scientific inquiry, Thomas S. Khun, defined the concept of paradigm as:

> " ...some accepted examples of actual scientific practices — examples which include law, theory, application and instrumentation together — provide models from which spring particular coherent traditions of scientific research...(Khun,1970:10).

Once a paradigm is developed and accepted by practicing researchers, a shared system of values and standard of research would emerge and a broad commitment to its principles become more likely to prevail among members of that scientific community.

> "...That commitment and the apparent consensus it produces are ...the genesis and continuation of a particular research tradition"(Khun,1970: 11)

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In other words, researchers who adhere to a particular paradigm become partisans advocating and defending the principles and practices of the emerging system of logic but not necessarily calling for a stagnated form of scientific methodology. Furthermore,

> "...Acquisition of a paradigm and of a more esoteric type of research it permits is a sign of maturity in the development of any scientific field"(Khun, 1970:24).

It is also maintained that the suggested paradigm for the study of different types of diaspora, much like any other form of systems of logic must serve as a springboard from which scientific propositions and research hypotheses are derived. It must endure continuous evaluation and verification(Scott, 1992). Its composing elements are series of assumptions and measurement operations which should provide researches in the field the theoretical frameworks necessary for the advancement of a particular study of population movement as well as similar acts of dispersion.

The process of building a new paradigm in any discipline must be undertaken with a measure of Paradigm acquire with the passage of time a degree of sacredness due to their effectiveness in past discoveries and the successful applications of the findings of research designs built on them in solving the problems the researchers sought to tackle. These outcomes may lend a particular paradigm certain degree of legitimacy in the eyes of their practitioners and may in fact lead to a measure of rigidity of the use of if. Sociologically, the paradigm may acquire an institution-like status among its practitioners. This is because,

> “in learning a paradigm, the scientist acquires theory, method, and standard together, usually in an inextricable mixture. Therefore, when paradigms change, there are usually significant shifts in criteria determining the legitimacy both of the problem and proposed solutions"(khun, 1962:108)

How does a new paradigm develop? A new paradigm is likely to develop when production of knowledge in a particular scientific discipline is uncertain (Lodhal and Gordon,1972). Put differently, when a discipline reaches a level of stagnation in advancing new research findings, the likelihood of an emerging paradigm becomes strong. Furthermore, an existing paradigm is more likely to maintain its continuity in a discipline if there is sufficient agreement among its practitioners regarding its contribution to the advancement of knowledge. However, when the conceptual tools and methodological techniques of a paradigm loose their cohesion and become segmented, the continuity of a paradigm is threatened (Thompson and Tuden, 1959). Finally, the question of how significant the produced knowledge in the advancement of future research can critically affect the continuity of a paradigm and may encourage the leaders of the discipline to advance a new one.1

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Suggested Paradigm For The Concept of Diaspora

As was pointed out in an earlier section of this paper, the varied uses of the concept of diaspora and the almost absence of a rigorous theoretical underlying its applications to the field of population dispersion make the need for a system of logic that can encompass the properties of the concept in a theoretical construct, which would guide the researchers in their measurement of the variables of the study, collect their data, interpret their findings and generalize them to similar situations, rather urgent and probably justified. Therefore, this paper will include an outline of a preliminary set of components that could enter into the formation of a paradigmatic structure using existing methodological techniques available in research designs literature. The components suggested in the proposed paradigm are not necessarily exhaustive neither are they unique to the field of population dispersion The components are the following:

1. The term diaspora as used in a particular investigation must be defined theoretically in such a way that reflects its attributes (components) as stated in the statement of the problem
    a. the components of the concept must be linked logically and supported by the literature cited in the statement of the problem
    b. empirical definitions of the components of the theory must be operationally established for the purpose of observation
    c. the derivation process of relationships among the variables of the study preferably follows axiomatic format provided that the existing literature include enough verified statements of relationships that can be used by the research to build his/her axiomatic structure
2. A specific type of population that would be suitable for the nature of the problem chosen by the researcher must be secured. The choices of population available may include
    a. an immigrant group
    b. a refugee group
    c. a mixed type of population dispersion
3. Location and legal status of the population
    a. legally established status
    b. undocumented status
    c. transitional status
4. 4.Demographic characteristics of the chosen population
    a. gender differentiation
    b. educational attainment
    c. occupational differentiation d. income levels

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5. Research Design
    a. hypotheses derivation and setting of the equations
    b. identifying the type of sampling
    c. statement of the nature of the model chosen for the study
    d. type and level of the statistical testing and suggested processes of control of the different variables
6. Generalization of the findings
    a. scope and extend of the generalization process to similar dispersion movements
    b. external validity of the generalization of the findings
    c. measurement of the variance between the finding of the study and a normal distribution.

The Case of The Iraqi Intellectual Elite in American Society

A careful review of movements of Iraqi population to other countries and especially to the American shores reveals that a systematic Iraqi dispersion was not in existence following the end of the fist World War. As a matter of fact there was a quite significant non- Iraqis, such as Armenian, Turkic-speaking and Persian-speaking populations migrating to the Mesopotamian urban centers in the north, around soon-to-be the capital city of the newly established nation and other centers of industry and religious worship. Still, one must recognize the individualized and rather irregular out-migration of the Christian minority to the European countries and the United States prior to the war and following its cessation.

The enactment of a number of immigration laws in some Western countries and in the United States served as deterrent to some Iraqis who would have joined waves of population migration following the end of the First World War The passage of the Immigration Act of 1924 (the Johnson-Reed Act) by the Congress with its provision of limiting the annual number of immigrants admitted to the country to only 2% of the number of people from the countries to which immigrants are coming from made it extremely difficult for Iraqis to venture into the act of moving to America. The restrictive provisions continued to be of a significant obstacle in the path of Iraqis aspiring to move to the new world following the end of the second World War especially when the Immigration and Nationality Act of 195! took effect despite President Truman efforts to nullify its provisions against non-Western European immigrants. Still, despite the presence of these restrictive measures, quite a few of the Iraqi Christian minority continued to opt for the challenge of moving to the United States and to take residence in the urban centers of the Eastern coast and in the Midwest.

Iraqi migration took a dramatic upward turn with the enactment of the 1965 Immigration and Nationality Act. Even though the provisions of the act did not go into effect until 1968, the new law opened the flood gates for the vast majority of the Iraqi intellectual elite to migrate to the United States. However, we must be cognizant of the fact that the migration of the Iraqi

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intelligentsia was highly individualized and family-oriented cases of migration rather than the mass dispersion of a population that falls under the rubric of diaspora. Iraqi immigrants took advantage of the provision of the act that stressed the admission of immigrants who possessed high level of education, technical and professional training and legitimate claim claims to scientific achievements. These demographic attributes were by far the most prevalent among Iraqi visa-seeking immigrants throughout the early decades following the years when the provisions of the act were put into effect.

Once the political turmoil of the late 1970's and throughout the 1980's took hold in Iraq, the rise in the Iraqi intellectual elite streaming out the troubled Mesopotamian land to the different European countries and more specifically to the American shores was accelerated so much so that a number of Iraqi communities of intellectual elite sprang up on the Eastern coast, the western coast, the Midwest and the DC metro area. Enter the early years of the Nineties, when the outbreak of the First Gulf War was engulfmg the country of Iraq and you begin to see somewhat different flow of Iraqis seeking refuge in the United States and in a host of European countries. They were mostly refugees who were displaced during the war and shortly after it ended. Even though these waves of refugees included some highly educated and professionally trained Iraqis, they were overwhelmingly dominated by lesser educational attainments. This trend continued throughout the latter part of the last century and the time of the Second Gulf War.

The American invasion of Iraq in the Spring of 2003 and the dragging years of violence that devastated the country triggered new waves of Iraqis seeking refuge in a relatively large number of countries but mostly in the United States where the immigration authorities estimates their number to be over 120,000 and who moved into a large number of US urban centers even though the vast majority of them settled in the already established Iraqi communities of the Western Coastal cities, the Midwest and the DC metro area. Political instability and violence among members of the different religious sects which dragged on long after the invasion kept the stream of out migration growing with the US authorities increasing the total amount of financial assistance to the waves of refugees to over $ 1.7 billion. Here again, the demographic characteristics of the newly arriving refugees indicate a mixture of levels of education and academic achievement with the minority qualify for the elite class.

Since the summer of 2014, population dispersion of Iraqis took a turn to the in-migratory pattern where some million and a half were forced to leave their communities and their possessions to escape the new radical political authority that was controlling a rather significant portion of the northern section of the country. These waves of displaced families were forced to settle in other parts of the country and/or in settlement camps erected by the United Nations High

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Commissioner of Refugees. This agency estimates the total number of refugees since the middle of 2014 to be around a million and a half.

The multiplicity of patterns of Iraqi intellectual elite migrating to the different urban and educational centers in American society did not hinder the eventual creation of rather highly trained communities of scientists, professionally trained physicians, engineers and financial and management personnel who kept one form of contact or another with their native country and built new associations of their own in their adopted homes. Their efforts to build such associations reached a rather critical level when a gathering of a number of their leaders held their pioneering meeting at the Library of Congress in the year of 2008 to set the early foundations for a new organizational structure upon which Iraqi intellectual elite could build a more formal and rather complex organizational entities.

In the spring of 2009 such pioneering efforts reached a new level of maturity and development with the opening of an impressive intellectual gathering of several hundred Iraqi intellectual elite along with a number of Nobel Prize Laureates during the Iraqi-American Academics and Professional Conference hosted by The National Academies in Washington DC. A number of formal awards were presented to the different Iraqi scientists and professional leaders who attended the conference followed by a celebration of the Iraqi musical achievements led by distinguished Iraqi artists and their American collaborators.

The process of formalization of Iraqi intellectual's organizations reached a new level of advancement with the creation of the early formation of Together For Iraq (TOFIQ) at the conclusion of the Iraqi-American Academics and Professional Conference and later when TOFIQ was registered as Non-Governmental Organization (NGO) with the State of Maryland in 2011. The main objectives of the organization are to promote all aspects of higher education in the native country and to coordinate its activities with those of the Iraqi educational institutions and professional organizations. A scholarly journal, TJMS, a biannual Journal of Medical Sciences, was launched by TOFIQ shortly after the establishment of the organization.

The journal is " devoted primarily to Iraqi medical and health communities at academic and health institutions " in the United States and elsewhere where Iraqi intellectuals and scholarly-driven researchers may find a professional outlet for the findings of their research activities hence helping in the process of disseminating their knowledge to other scientific communities and research centers of the world. The Journal had already completed two recent issues which are available online and the expectations are that "sister" journals in other academic endeavors will follow in the near future.

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Since its establishment, TOFIQ have staged a successful conference in March of this year on Addressing Iraq's Current Humanitarian Crisis following the mass displacements of a million and a half Iraqis due to the violence and internal war against the extremism forces. Currently the Organization is pursuing its systematic efforts of recruiting Iraqi and American surgeons to travel to Iraq and assist with performing and training Iraqi professionals at their medical institutions and clinical facilities in the different parts of the native country.

Conclusion

The analysis of the concept of diaspora advanced in this paper shows that the elasticity of its use by sociopolitical writers has transformed its dynamics into a" cure-for-all" mechanism to handle just about any form of population dispersion: forced or voluntary, mass movement or simply a small migratory group, nature-driven act or politically-induced movement. In so doing, they, have lowered its parameters, to use Moynihon terminology, so that it may assist them to explain almost any problem that have resulted from these movements. This "arrested path" of multiple usage stands in contrast to the phenomenal advancement in the theoretical and methodological realms of so many other concepts in modem social sciences. They have been successfully incorporated into networks of highly developed theoretical frameworks and empirically tested conceptual schemes that are found in the leading research journals of their respective disciplines

International laws of immigration coupled with the varied roles played by the different agencies of the United Nations in dealing with problems of refugees, forced or voluntary, have placed considerable other limitations on the methods of description and modes of analysis generally used by writers who rely on the concept of diaspora. These limitations are considerable in the study of mass dispersion or the limited refugee-type movements. Furthermore, the vast majority of diaspora-driven studies are descriptive studies which are not much of help to the modem push for more quantitatively oriented studies that have shown accelerated advancement in the different disciplines of social sciences.

When the preceding limitations are looked at from both theoretical and methodological vantages, one would venture and call for the need to advance a "revolutionary paradigm" composed of a series of methodological steps that would guide researchers dealing with questions of population dispersion in a more systematic way. This paper has taken the first step toward that objective by proposing an elementary paradigmatic scheme.

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NOTES

1. This paragraph was reproduced from an earlier paper by the author titled "A New Japanese business Paradigm : Meaning , Need Assessment and Implications for Organization Analysis " , Dokkyo International Review , Vol. , 9 , 1996 ,371-386

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Editorial

BREACH OF SCIENTIFIC INTEGRITY

Breaches of scientific integrity have been with us for centuries. However, the dependence of societies on scientific integrity has increased with the immense advances and complexities of science especially since Second World War. But scientific misconduct came to the U.S. and the global community's public attention when William Broad and Nicholas Wade in 1982 published a book entitled: *Betrayers of the Truth – Fraud and Deceit in the Hall of Science*. The book recounts historical and recent cases of scientific misconduct by icons of science such as Galileo, John Dalton, Robert Millikan, and Louis Pasteur. More importantly, the authors lamented the indifference of the scientific community to the revelations of recent scientific misconduct case. Shamoo and Resnik (2009) recite dozens of cases of serious misconduct in the past few decades.

The misconduct revelations in public led the U.S. to establish the Office of Research Integrity (ORI). ORI's duties are to establish policies on how to define and deal with scientific misconduct. ORI also proposed educational methods to instruct the scientific community in increasing awareness of issues relevant to scientific integrity. ORI encourages or demands from research institutions to offer educational opportunities to students and trainees to engage with. At present, many countries especially the Europeans have adopted policies and regulations on research integrity.

Scientific misconduct is defined as follows:

Fabrication is making up data or results and recording or reporting them.

Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record.

Plagiarism is the appropriation of another person's ideas, processes, results, or words without giving appropriate record.

Honest errors or differing interpretation are not scientific misconduct.

Misconduct in Research occurs all across the world. The frequency of scientific misconduct is not truly known. Estimates of misconduct vary from 1 % to 10 %. Some estimates goes as high as 30 %. The variation in estimates is the result of the methodology and assumptions used in surveys. The percentages of misconduct are really high. But regardless of the exact frequency of

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misconduct, it represents a serious misdeed in science. Scientific misconduct even if it is a low probability, it has a high impact on science and the public.

The factors leading to misconduct are complex. Many assumes that the following factors contributes to scientific misconduct: societal and cultural acceptance of deviant behavior, pressure to produce, pressure for fame, poor training and education in the scientific method, and poor education in ethics, in general, and of science ethics specifically.

Authorship is the signature credit for years' of the researcher's hard work. The reward system in academia is based on the quality, number, and the impact of publications. More recently the conduct of research and authorship of papers have increased in number of participants, complexity and rewards. The modern environments for research and authorship introduce ethical challenges to maintain high standard of integrity of authorship.

One of the ethical challenges of authorship is plagiarism. There are evidences to indicate that plagiarism is on the increase especially with the age of internet and anonymity. There are major scandals of plagiarism involving major figures all across the globe. Recent data indicate that plagiarism is high in countries entering the status of a developed country such as India and China. These countries have not yet developed a mechanism on how to deal with the issue of plagiarism.

Journal editors have introduced guidelines for authorship but unfortunately they are vague and ineffective. But training and education could raise awareness on the issue and how to deal with it.

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Accountability in Research
Author of the textbook entitled: *Responsible Conduct of Research (3rd edition), Shamoo and Resnik, 2009.*
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كلمة التحرير

خرق الامانة العلمية

لقد رافق خرق الأمانة العلمية المجتمع قرونا عديدة. ولكن المجتمع اعتبر الأمانة العلمية من الاساسيات بتزايد مع التطور العلمي الهائل الذي حدث في العقود الأخيرة وخصوصا بعد الحرب العالمية الثانية.

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في العام ١٩٨٢ أثار ولأول مرة هذه الظاهرة وعلى مستو عال صدور كتاب "خونة الحقيقة: التزوير والخداع في الأروقة العلمية" لمؤلفيه وليام بورد ونيكولاس ويد

"Betrayers of the Truth: – Fraud and Deceit in the Hall of Science؛ William Broad and Nicholas Wade"

أرخ الكتاب حوادث متعددة لخرق الأمانة العلمية من قبل عدد من عمالقة التاريخ العلمي مثل غاليليو، جون دالتن، روبرت ميليكان ولويس باستور. والأدهى من ذلك هو عدم مبالاة المجتمع في حينه عند كشف تلك الخروقات العلمية.

أشار الباحثان شمو ورسنيك في العام ٢٠٠٩ إلى العشرات من حالات خرق وسرقة علمية حدثت في العقود الأخيرة. ومن الحالات المشهورة الاخرى هي سرقة وزير دفاع ألمانيا مما اضطره للإستقالة وهناك العديد من تلك السرقات. وبسبب تعدد الخروقات والسرقات العلمية دعت الولايات المتحدة الاميركية إلى استحداث مكتب خاص يدعى: مكتب النزاهة العلمية (**Office of Research Integrity (ORI** وتكفل هذا المكتب بوضع أسس وسياقات تعريف السرقة العلمية وكيفية التعامل معها.

وكان من أهداف هذا المكتب توعية المجتمع العلمي للإنتباه الى، والاهتمام بموضوع النزاهة العلمية. وشجع القيام بدورات تثقيفية خاصة لأفراد المجتكع العلمي. وفي الوقت الحاضر اعتمدت العديد من الدول وخصوصا الاتحاد الأوربي سياسات وقواعد ثابتة في الأمانة العلمية. .

أن السرقة العلمية وسوء الخلق العلمي يعرف بمايلي:

التلفيق: استحداث بيانات علمية بتوثيقها ونشرها

التغيير: التلاعب في ذكر الأسلوب البحثي، أو المواد المستخدمة، أو طرق البحث، أو تغيير أو حذف نتائج بحثية بما لايمثل حقيقة المعلومات الموثقة في سجلات تلك البحوث .

السرقة العلمية: هي ادعاء ملكية أفكار واساليب ونتائج او استنتاجات باحث آخر بدون الإشارة اليه.

إن الأخطاء غير المتعمدة أو التفسير المغاير لا تعتبر سرقة أو شرخا علميا .

تنتشر السرقة العلمية في كل أنحاء العالم. إن نسبة السرقة العلمية في المجتمعات المتقدمة غير معروف بدقة ولكنه يتراوح بين ١ -- ١٠٪ . وهنالك من يرفع النسبة إلى ٣٠٪. يعود تباين هذه الأرقام إلى الطرق المعتمدة في التقييم. ولكن الحقيقة الواضحة والمؤلمة أن النسبة مهما كانت فهي عالية. وحتى لو كانت النسبة ضئيلة فان تأثيرها على العلم والمجتمع عال جدا وللأسف لم تنج منها أية دولة متطورة أو نامية.

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أن العوامل التي تدعو للسرقة العلمية وسوء التصرف متشابكة. يعتقد الكثيرون بأن العوامل المهمة في ذلك هي مايلي: طبيعة ثقافة المجتمع وقبوله أو تغاضيه، الضغوط على الباحثين لإنتاج البحوث، نزعة حب الشهرة، التدريب السئ وقلة ثقافة الباحثين، وضعف المعرفة والإلتزام بالقيم الأخلاقية بصورة عامة والخلق العلمي بصورة خاصة .

إن امتلاك الجهد العلمي هو الحق الطبيعي الذي يسعى اليه الباحث بعد مسيرة سنين بحثية شاقة. وان أسلوب التقييم العلمي في المجتمع الأكاديمي يعتمد على نوعية وعدد ومدى تأثير واستثمار البحوث المنشورة. وفي عصرنا فإن إجراء البحوث والنشر قد ازداد وتعقد بتزايد المشجعات المتنوعة. إن مناخ البحوث والنشر في عصرنا الحاضر أدخل تحديات عالية لتحقيق مستوى عال من النزاهة.

ومن أهم التحديات الاخلاقية للبحوث والنشر هو السرقة العلمية. هنالك دلائل بان السرقة العلمية في تزايد وخصوصا في عهد الإنترنت الكشف عن المستخدم. وهنالك العديد من الفضائح قام بها أشخاص مهمين في مختلف دول العالم .

وتدل الارقام المتوفرة حول السرقة العلمية في تزايد في الدول التي تخطت التخلف مثل الصين والهند حيث لم تستحدث فيها بعد أساليب تحديد الأمانة العلمية بشكل فعال.

لقد وضع محرروا المجلات العلمية أسسا للنشر ولكن للأسف فإنها في غالبيتها ضبابية وغير فعالة. لذا فان التدريب والتثقيف يرفعانها من مستوى المعرفة بهذا الداء الوبيل وكيفية التعامل معه.

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نائب رئيس منظمة توفيق

.....

تعليق رئيس تحرير مجلة توفيق للعلوم الطبية

نأمل أن تحظى هذه المشكلة الاخلاقية العلمية في مجتمعنا الاكاديمي والبحثي بالاهتمام الواسع ونبدأ بتثقيف الاكاديميين والباحثين أولا ثم توضع آلية صارمة لاكتشاف والتعامل مع الغشاشين وسراق الجهد العلمي كي نحافظ على الخلق العلمي والمستوى الذي نطمح أن نحافظ عليه بما تربى عليه مجتمعنا حينما كان الرائد في العلوم والأمانة العلمية في زمن الاشعاع العلمي.

هنالك العشرات من المواقع المهمة على الانترنت والتي تتعامل مع هذه الظاهرة السيئة ومنها مثلا:

http://www.plagiarismtoday.com/

وكذلك المواقع المتخصصة في ملاحقة السرقة العلمية ومنها:

http://www.ithenticate.com/

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THE IMPORTANCE TO IMPLEMENT AND ENFORCE OF STANDARDIZED GUIDELINES FOR THE CARE AND USE OF LABORATORY ANIMALS IN RESEARCH AND TEACHING IN IRAQI SCIENTIFIC INSTITUTIONS

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 1, 2015

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Abstract

The proposed study was initiated as a result of the first author visit to one of the American higher education institution as a research scholar couple of years ago. He noticed strict measures taken by the IACUC committee (Institutional Animal Care and Use Committee) within the university when he wanted to learn certain procedures. He then started to discuss the issue with his faculty associate of the possibility of adopting some kind of guidelines on laboratory animals to be used in Iraqi higher education institutions for research and teaching. To the knowledge of both authors, there is no committee or guidelines on laboratory animals care and use in Iraqi scientific institutions. There was one committee used to look into the ethics of each research proposal years ago and is not functioning at this time and the authors fail to get on touch with any member. This report was sent to the Ministry of Higher Education and Scientific Research in Iraq through the Iraqi cultural attaché for their consideration to implement or use any part of it to start with when doing research and teaching.

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Introduction

Researchers all over the world may use laboratory animals during their studies and these animals gave and continue to give so much to humanity that we will not be able to pay them back in whatever we will do to them in the future. However, handling and treatment of these laboratory animals during experimentations whether feeding, watering, or injecting certain medications to study their effects will all end up in a great deal of knowledge to humanity. Handling and treatment of animals are under certain rules and regulations depending on where we are in the world. There are so many associations and institutions which have strict rules on the use and handling of laboratory animals in USA, Europe, Japan, Australia and New Zealand (Kagiyama and Nomura, 2004; Houde *et al.,* 2009). Unfortunately, in most developing countries including Iraq, it is up to the researcher or his/her own technician to determine how to use, handle and treat these laboratory animals when they use them in research experiments.

All high impact factor journals all over the world have strict regulations on what are expected when it comes to laboratory animals experimentations (handling, watering, feeding, method of euthanasia at the end of the study and other criteria). Therefore, the reported findings of the research and the manuscript will be rejected without following these rules and regulations when the researcher did the study under no such guidelines or regulations.

The reason behind suggesting these guidelines is to encourage the faculty all over Iraq to publish and be rewarded according to their effort in research. To our knowledge, the Ministry of Higher Education and Scientific Research (MHER) in Iraq demanded that faculty in all universities within Iraq should first try to publish in high impact journals for their better financial reward and promotion. When any faculty submit a manuscript without mentioning certain protocol number and date of obtaining the approval on use and handling of laboratory animals, the manuscript will be rejected by all esteemed scientific journals.

Therefore this report was initiated after a visit of the first author to Western University of Health Sciences (WUHS), California, USA couple of years ago. During that visit, the author was exposed to the strict requirements, rules and regulations imposed by the IACUC (Institutional Animal Care and Use Committee) and suggested to use a shorter version of it to be presented to the Ministry of Higher Education and Scientific Research (MHESR) in Iraq.

Guide for the Care and Use of Laboratory Animals (CULA) in research and teaching

Who Must Apply for CULA Protocol Approval?

Anyone wishing to conduct research or teaching using animals at Baghdad University must file an Animal Use Protocol (AUP) for review and approval by the specific college Animal Care and Use Committee (ACUC) before the activity begins and before any animals are obtained. The use of animals shall include alive or dead animals obtained by the principal investigator for use in a research or teaching exercise.

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Evaluating the Justification for Laboratory Animal Use

If there is no alternative to the use of the specified animals, the ACUC shall evaluate the research and require justification for the number of animals requested. The ACUC will judge the adequacy of the training and skill of the investigator and laboratory personnel and the adequacy of the equipment and facilities. Where appropriate, the ACUC shall enlist the help of consultants in evaluating protocols.

Animal Preparation

All animals must exhibit good health and normal behavior prior to entering a study. Restraint or altered conditions should be planned ahead of time so that the animals will be acclimated to the new conditions prior to conducting the study.
Animals must receive physical examination appropriate to the species prior to being used in a study to determine the presence of preexisting abnormalities or conditions which would impact the study results.

Committee to revise the guide for the care and use of laboratory animals

The goal of this guide is to promote the humane care of animals used in biomedical and behavioral research, teaching, and testing; the basic objective is to provide information that will enhance animal well-being, the quality of biomedical research, and the advancement of biologic knowledge that is relevant to humans or animals. The use of animals as experimental subjects in the 20th century has contributed to many important advances in scientific and medical knowledge (Leader and Stark 1987).

Principles

The principles for taking care of animals for research and teaching encourage:

- Design and performance of procedures on the basis of relevance to human or animal health, advancement of knowledge, or the good of society.
- Use of appropriate species, quality, and number of animals.
- Avoidance or minimization of discomfort, distress, and pain in concert with sound science.
- Use of appropriate sedation, analgesia, or anesthesia.
- Establishment of experimental end points.
- Provision of appropriate animal husbandry directed and performed by qualified persons.
- Conduct of experimentation on living animals only by or under the close supervision of qualified and experienced persons.

In general, the principles stipulate responsibilities of investigators, whose activities regarding use of animals are subject to oversight by an animal care and use committee (ACUC).

Responsibility for directing the program is generally given either to a veterinarian with training or experience in laboratory animal science and medicine or to another qualified professional. At least one veterinarian qualified through experience or training in laboratory animal science and medicine or in the species being used must be associated with the program. The institution is responsible for maintaining records of the activities of the ACUC and for conducting an occupational health and safety program.

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Monitoring the care and use of animals

Animal Care and Use Committee (ACUC)

The responsible administrative official at each college must appoint an ACUC, to oversee and evaluate the college/institute animal program, procedures, and facilities to ensure that they are consistent with the recommendations in this Guide.
ACUC Committee will be consisted of five members elected by their colleagues. The committee members will serve for three years. The first meeting, the committee elects a chair for the committee to serve for one year.
The committee is responsible for oversight and evaluation of the animal care and use program and its components. Its functions include inspection of facilities; evaluation of programs and animal-activity areas; submission of reports to responsible institutional officials; review of proposed uses of animals in research, testing, or education (i.e., protocols); and establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the college.

Animal Care and Use Protocols

The following topics should be considered in the preparation and review of animal care and use protocols:

- Rationale and purpose of the proposed use of animals.
- Justification of the species and number of animals requested.
- Availability or appropriateness of the use of less-invasive procedures.
- Adequacy of training and experience of personnel in the procedures used.
- Housing and husbandry requirements.
- Appropriate sedation, analgesia, and anesthesia.
- Unnecessary duplication of experiments.
- Conduct of multiple major operative procedures.
- Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
- Post procedure care.
- Method of euthanasia or disposition of animal.
- Safety of working environment for personnel.

Personnel caring for animals should be appropriately trained Technical and Professional Education, and the college/institute should provide for formal or on-the-job training to facilitate effective implementation of the program and humane care and use of animals.

Animal Environment, Housing, and Management

Proper housing and management of animal facilities are essential to animal well-being, to the quality of research data and teaching or testing programs in which animals are used, and to the health and safety of personnel. A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results.

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Many factors should be considered in planning for adequate and appropriate physical and social environment, housing, space, and management. These include:

- The species, strain, and breed of the animal and individual characteristics, such as sex, age, size, behavior, experiences, and health.
- The design and construction of housing.
- The project goals and experimental design (e.g., production, breeding, research, testing, and teaching).
- Animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors.

Housing

Primary Enclosures

The primary enclosure (usually a cage, pen, or stall) provides the limits of an animal's immediate environment. Acceptable primary enclosures

- Allow for the normal physiologic and behavioral needs of the animals.
- Allow nonspecific social interaction.
- Make it possible for the animals to remain clean and dry.
- Allow adequate ventilation.
- Allow the animals access to food and water.
- Provide a secure environment that does not allow escape of or accidental entrapment of animals.
- Are free of sharp edges or projections that could cause injury to the animals.
- Allow observation of the animals with minimal disturbance of them.

Primary enclosures should be constructed with materials that balance the needs of the animal with the ability to provide for sanitation. They should have smooth, impervious surfaces with minimal ledges, angles, corners, and overlapping surfaces so that accumulation of dirt, debris, and moisture is reduced and satisfactory cleaning and disinfecting are possible.

All primary enclosures should be kept in good repair to prevent escape of or injury to animals, promote physical comfort, and facilitate sanitation and servicing. Rusting or oxidized equipment that threatens the health or safety of the animals should be repaired or replaced.

Food

Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily or according to their particular requirements unless the protocol in which they are being used requires otherwise.

Water

Ordinarily, animals should have access to potable, uncontaminated drinking water according to their particular requirements. Periodic monitoring for pH, hardness, and microbial or chemical contamination might be necessary to ensure that water quality is acceptable. Water can be treated or purified to minimize or eliminate contamination when protocols require highly purified water.

Watering devices, such as drinking tubes and automatic waterers should be checked daily to ensure their proper maintenance, cleanliness, and operation.

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Bedding

Animal bedding is a controllable environmental factor that can influence experimental data and animal well-being. The veterinarian or facility manager, in consultation with investigators, should select the most appropriate bedding material.

Bedding should be used in amounts sufficient to keep animals dry between cage changes, and, in the case of small laboratory animals, care should be taken to keep the bedding from coming into contact with the water tube, because such contact could cause leakage of water into the cage.

Pain, analgesia, and anesthesia

Minimization of Pain and Distress

The appropriate use of anesthetics and analgesics is important for ethical and regulatory reasons.

Pilot studies, in consultation with the attending veterinarian, may be necessary to assess the compatibility of drugs with the investigation proposed. The PI (Primary Investigator) and the ACUC shall carefully consider any procedures in which alleviation of pain or distress cannot be reasonably assured.

An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative.

Some species-specific behavioral manifestations of pain or distress are used as indicators, for example, vocalization, depression or other behavioral changes, abnormal appearance or posture, and immobility. It is therefore essential that personnel caring for and using animals be very familiar with species-specific (and individual) behavioral, physiologic, and biochemical indicators of well-being. The selection of the most appropriate analgesic or anesthetic should reflect professional judgment as to which best meets clinical and humane requirements without compromising the scientific aspects of the research protocol. Preoperative or intraoperative administration of analgesics might enhance postsurgical analgesia. The selection depends on many factors, such as the species and age of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the length of the operative procedure, and the safety of an agent for an animal, particularly if a physiologic deficit is induced by a surgical or other experimental procedure.

Euthanasia

Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress. In evaluating the appropriateness of methods, some of the criteria that should be considered are ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety; reliability; nonreversibility; time required to induce unconsciousness; species and age limitations; compatibility with research objectives; and safety of and emotional effect on personnel.

Euthanasia might be necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives, or other treatments. Protocols should include criteria for initiating euthanasia, such as degree of a physical or behavioral deficit.

ACUC requires that the methods of euthanasia are consistent with the standardized recommendations used currently among the scientific community in the world. Any deviation from the regular protocol must be justified for scientific reasons and approved by the ACUC.

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Conclusion

It is clear that compliance with world scientific approach to handling and care of laboratory or experimental animals is a must these days in any country in the world. Therefore, we see that Iraq must consider establishing committees at the university levels and college/institute levels to look into the approval of the protocol used in each and every scientific experiment involving laboratory animals. Once these committees are established and start to offer their help to researchers through training and consultations, the researchers in Iraqi institutions will be able to publish their findings in higher impact reputable refereed scientific journals. This proposal can be modified to include not only laboratory animals but all experimental farm animals whenever used in research or teaching by adding certain paragraphs to describe the handling, use and treatment of these animal species.

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Proposed form to be used or modified according to the need of the Iraqi higher education researchers

Ministry of Higher Education and Scientific Research
University of ----------/College of ----------

Animal Care and Use Protocol Application

Protocol #:_____
Type of protocol: [ ] New
Full Renewal; Old Protocol #---------- Expiration Date: ----------
Duration of approval: ---------- to----------

1. Title of Project: ----------
2. Principal Investigator:----------
3. Department/College:----------
4. Office phone: email: Emergency phone number:----------

Declarations
Declarations and Signatures
As the Principal Investigator on this protocol, I acknowledge full responsibility to abide by this protocol and provide assurances for the following:
1. All persons participating in the following approved activities are assuming responsibility for the well-being of all animals involved in these activities.
2. The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless deviation is specifically approved by the IACUC.
3. Safety issues have been addressed and all personnel understand the rules and regulations regarding radiation protection, bio-security, animal handling, etc.
4. All personnel involved in animal handling described herein are technically competent, have been specifically trained to appropriate methods, will be supervised to the appropriate degree, and agree to comply with this protocol.
5. This protocol accurately reflects the description of animal use provided in all appropriate funding

Principal Investigator Signature---------- Date----------
Dean/Dept Chair Signature---------- Date----------

6. For each person, including the PI and off campus personnel, involved in this study that will have animal contact, provide a) their name, email address and phone number,
b) Their specific duties in this project, c) A description of their animal training relevant to their duties in this project.

7. Summary of:
Animal species ----------
Number of Animals Requested: Species/Strain ----------
Number Requested ----------
Gender ----------

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Age or Weight ----------
Housing Location----------
Vendor/Source ----------
If additional species are requested, provide information here:

a) Estimate the total (mothers, fathers, and offspring) number of animals on hand at any one time during breeding.
b) Describe any special care or monitoring that may be required.
c) What will be done with any surplus offspring?
d) If transferring animals from an off-campus investigator, an animal transfer form and health report must be submitted and approved prior to protocol approval.

8. For what scientific purpose are these animals being bred?
9. Provide a detailed description of how the animals will be housed and grouped for breeding.
10. Reduction and Replacement of Animals

a. How does the experimental or course design assure the use of the fewest animals? Justify the numbers of animals requested. This should agree with the numbers in the table requested in 7.

b. Can the project/course for which these animals are being bred be done using a lower species or a non-animal model? Yes; explain why it is not being used No; how was this determined?

11. Please fill the blank spaces with appropriate responses:

a. Provide the names, titles and off campus emergency phone numbers of anyone other than the Animal Care Facility personnel who will provide daily animal care, including weekends and holidays.

b. What will be the criteria used to warrant euthanasia or premature removal from the study/course? ----------
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c. Who will monitor morbidity and serve as a contact person if problems arise?
---

d. If euthanizing animals, the IACUC requires pentobarbital, 100 mg per kg, or isoflurane 32%. If not using the required methods, justify.----------
---

12. Transferring Animals

a. If you will be transporting animals between different colleges or facilities or between institutions, explain.
---

13. Final Disposition of Animals - Mark all appropriate items and provide the required information.

The animals are under the care of their private owners.----------

For euthanizing animals, the ACUC requires pentobarbital, 100 mg per kg, or isoflurane 32%. If not using one of the required methods state the method and justify.

(i) How is death confirmed? ----------

(ii) How will you dispose of the carcasses? ----------

Transferring to another protocol. Provide the protocol number to which these animals will be transferred. ----------
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If none of the above, explain----------.

Thank you

This protocol was modified and adopted with permission from Western University of Health Sciences/California/USA

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MALIGNANT LYMPHOMA OF THE SMALL AND LARGE BOWEL IN IRAQ (MIDDLE EAST LYMPHOMA)

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Presentation:

Podium Presentation at the XXVI ISURS Biennial Congress, Cape Town, South Africa, September 4-7, 2014.

Financial Disclosure:

None reported

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ABSTRACT

Background and Objectives:

Primary gastrointestinal tract (GIT) is the most common site in extra nodal Lymphoma which accounts for 5-10% of all non-Hodgkin's lymphomas. The most common organs involved are the stomach, followed by the small intestine and ileocecal region of the colon. It can also be secondary to wide spread nodal disease with pathological appearance of bulky lymph node. We report the experience of 227 cases of Small Bowel primary Lymphoma (SBPL) and 18 cases of Colorectal Primary Lymphoma (CRPL) that spans a period from 1965-2000 reported at the Medical City Hospital, Medical School of Baghdad, Iraq operated by one surgeon (ZRB).

Patients and Methods:

From 1965 to 2000, there were 245 patients collectively with Primary Lymphoma of the Small Intestine and Colorectal area. There were 227 small bowel lymphoma and 18 colorectal cases that underwent surgery by resection, bypass or exploration. The most frequent symptoms in (SBPL) were weight loss, abdominal pain, diarrhea, fever, vomiting, melena in addition to abdominal mass, clubbing, ascites, hepatomegaly. It was complicated by malabsorption syndrome, peritonitis, obstruction, jaundice. The most frequent symptom of (CRPL) was intestinal obstruction, abdominal mass, dyspepsia, rectal bleed- ing, diarrhea and weight loss. The most frequent site in (SBPL) was a tumor located in the duodenum in 10 cases, the jejunum in 37 cases, and the ileum in 53 cases with multicentric in 127 cases. While the location in the (CRPL) were the cecum 6, ascending colon 7, transverse colon 1, sigmoid colon 2 and rectum 2. Histologically, there were 178 high-grade and 49 low-grade non-Hodgkin's lymphoma and in the (CRPL), 8 were high-grade, 9 low-grade non-Hodgkin's lymphoma and one Burkitt's lymphoma. The most common pathological type in small bowel lymphoma is Lymphoplasmacytic while in the large bowel was intermediate and high grade large B cell lymphoma.

Results:

Radical surgery was performed on 201 patients of all stages in (SBPL) with 179 patients having post-operative chemotherapy, 8 had radiation treatment with 14 having the combination of chemotherapy/radiation therapy. Survival rate for (SBPL) was 92 cases (42%) for 2 years, 55 cases (28%) for 5 years. The (CRPL) revealed 14 cases (52%) survival of 2-4 years and 12 cases (48%) survived an average of 10 years.

Conclusion:

Intestinal Lymphoma differs significantly from the gastric lymphoma. The most common pathological type in the small bowel was lymphoplasmacystic type and in the large bowel was the diffuse intermediate and high grade, Large-B Cell lymphoma.

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Our experience shows improvement in the prognosis with the combination of treatments in the early localized lesion. It supports the efficacy of surgery combined with chemotherapy/radiation in obtaining a good remission

Introduction:

Primary gastrointestinal lymphomas are uncommon, accounting for 1%-4% of all gastro-intestinal tumors. They represent the largest group of all extra nodal non-Hodgkin's lymphoma 5-10% (1, 2). GI Lymphomas are predominantly located in the stomach, 60%. Whereas intestinal lymphoma are more infrequent and appear in the small bowel 30% and in the colon and rectum 10% (1, 2, 3, 4). Intestinal Lymphoma differs from the gastric one in pathology, clinical features, treatment and prognosis. 90% of the primary intestinal lymphomas are of B-cell type with a few T-cell lymphoma and Hodgkin's Lymphoma. Subtypes of lymphoma noted with the predilection site as mucosa-associated lymphoma tissue (MALT). Mantle cell lymphoma (MCL) in terminal ileum, jejunum and colon, as well as enteropathy-associated T-cell lymphoma (EATL) in jejunum (5), also immunoproliferative small intestine disease (EPSID) viewed as alpha chain disease (5,6). The risk factors have been implicated in the pathogenesis is H-Pylori infection, human immunodeficiency virus (HIV), celiac disease, Campylobacter jejuni, Epstein-Barr virus (EBV), hepatitis B virus (HBV), human T-cell lymphotrophic virus-1 (HTLV), inflammatory bowel disease and immunosuppression (7).

Dawson's criteria used for primary gastrointestinal lymphoma include (A) absence of peripheral lymphadenopathy; (B) lack of enlarge mediastinal lymph nodes; (C) normal total and differential white blood cell count; (D) predominance of bowel lesion at the time of laparotomy with only lymph nodes affected in the immediate vicinity; and (E) no lymphoma involvement of the liver and spleen(8).

Ann Arbor staging with Musshoff modification were used for extra nodal lymphoma (9) (Table 1).

Presentation: Clinical presentation of the primary small intestinal lymphoma is nonspecific (5). Abdominal pain, vomiting, weight loss and rarely obstruction. Primary small intestinal lymphoma are more heterogeneous, including MALT, EATL, MCL, immunoproliferative small intestinal disease (IPSID). IPSID manifested by infiltration of the plasma cells that secrete monotypic immunoglobulin and affects old children and young adults (10). Symptoms present are usually diarrhea and abdominal mass. MCL lymphoma affects individual at age over 50 years and involves the terminal ileum and called multiple lymphomatosis polyposis (11). Burkitt's lymphoma affect children and associated with EBV and HIV virus. T-Cell Lymphoma accounts for 10% as enteropathy. Follicular Lymphoma is rare.

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Primary Colorectal lymphoma has increasing incidents with age and affects patients in their fifth to seventh decades of life. The affected male to female ratio is 1.5% to 1%. The most common symptoms are weight loss, abdominal pain and rectal bleeding (12, 13). Half of the patients had abdominal pain and bowel obstruction is rare. The majority of the lesions are found in the cecum and ascending colon 72%. The tumor of the descending and rectosigmoid colon account for 28%. The lesion can be local or diffuse nodular or polypoid circumferential.

Colorectal Lymphoma is mostly B-Cell type and comprised of low or high grade B-Cell arising from MALT, MCL, EATL and others.

The diagnostic procedure used for staging includes endoscopic ultrasound, endoscopic biopsies, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), or molecular marker, Hybrid PET CT Scan Imaging (14, 15).

Treatment: Treatment for primary small intestinal lymphoma is usually surgery, chemotherapy, and radiotherapy. It has a different modality for its management. Intestinal Lymphoma warrants surgical removal. Low Grade B-Cell Lymphoma stage IE requires only surgical resection, radiotherapy is not beneficial due to the multifocal involvement. MALT lymphoma if local can be excised. Follicular Lymphoma needs surgery with standard treatment and dosage of chemotherapy (CHOP) (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone, and Bleomycin) or radiation. MCL has a poor prognosis and treated with Stem-Cell transplant (SCT). IPSID in early stages responds to antibiotics like tetracycline or Metronidazole and Ampicillin. Most of the lymphoma will require surgical intervention. However, Burkitt's lymphoma requires chemotherapy.

Management for Colorectal Lymphoma: In the absence of diffuse disease, surgical resection is preferred for colorectal lymphoma, especially the diffuse B-Cell lymphoma. Low Grade Lymphoma such as MALT associated lymphoma is treated by surgery which serves as a definite treatment. About 14%-24% are Stage IE, 62%-86% with regional lymph involvement, Stage II-E, is an indication for regional lymphadenectomy (16, 17).

Prognosis: Primary Small Bowel Lymphoma (5, 17, 18, 19, 21), especially Stage IE and IIE is the surgical resection and will have a good prognosis. Palliative resection is preferred to avoid obstruction or perforation. Chemotherapy offered to those of high risk, Stage IIIE or IVE treated with multi-agent chemotherapy and radiation. The five year survival ranges from 45% for stage IE to 19% for stage IIE. Relapse occurs 5-10 years after resection for patients with nodal involvement. The prognosis for colorectal lymphoma, the median survival range is from 24-36 months (12). Zighelbein, et al. (13) reported improved survival from 36 to 53 months who underwent adjuvant chemotherapy. Aviles, et al. (16) noted some change in rate of relapse with local or disseminated disease in stage IE for those treated with surgery alone. This result emphasizes the importance of chemotherapy. The recurrent rate ranges from 33% to 75% that occurs within the first five years after resection who had diffuse disease.

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Patients and Methods:

From 1965 to 2000, there were 245 patients collectively with primary lymphoma of the small intestine and colorectal area treated at the Medical-City Teaching Hospital in Baghdad University, Iraq (18, 19, 20, 21, 23, 24). There were 227 primary small bowel lymphomas and 18 colo-rectal lymphoma. The patient characteristics were 126 males, 91 females for a total of 227. The age range is between 10 to 60 and the highest incidence between 20-60 years old followed by children. (Table 2) Pathologically there were 3 types of disease; diffuse, localized and the Burkitt's type. The average age for the diffuse type was 31, for localized was 22 and for the Burkitt's was 6.7 years. (Table 3) The ratio from males to females was 1.5% to 1%. The race incidence of Arabs to Kurds, was 5.5 to 1 ratio. Clinical type of lymphoma showed there was 65% diffuse, 21% localized, 14% Burkitt's lymphoma. The most common clinical presenting symptoms are listed in (Table 4) with the highest incidence of malabsorption, intestinal obstruction and abdominal mass. The relevant clinical data comparing all three types of lymphoma regarding weight loss, diarrhea, clubbing and abdominal mass with fever, vomiting, edema, ascites, melena, and hepatomegaly noted in (Table 5).

Distribution and Histology: The most frequent site of primary small bowel primary lymphoma was in the duodenum 10 cases, jejunum 37 cases, ileum 53 cases and multicentric 127 cases. Histologically, there were 147 high grades and 49 low grade non-Hodgkin's Lymphoma and 31 Burkiitts type. Pathologically the majority of the small bowel exhibits the Lympho-plasmacytic type that strongly expresses type alpha heavy chain (figure 1). The B cell lymphoma of the large bowel (figure 2) and the Burkitts type shows the classic starry sky appearance (figure 3, 4). Serum Electrophoresis studies showed alpha heavy chain disease (AHCD) as a lympho-proliferative process involving the IGA secretary system and the production of immunoglobulin molecule consistent mainly of incomplete alpha chain (6, 10, and 20).

Treatment and Prognosis:

SBPL staging showed the highest number of cases was in stage IV, 42%, followed by stage III B at 35.7%, stage III A 12.8% and stage II 8.8%. Histological Type showed the non-Hodgkin's Lymphoma low grade 4%, intermediate grade 22%, high grade 18%, Burkitt's type 19% and the Mediterranean type was 26%.

Surgical procedures were done with a total of 123 patients had resection, 27 underwent bypass surgery and 77 had exploration only with the highest mortality.

Multi-agent chemotherapy was used in 179 patients and external beam radiotherapy in 8 patients and the combined chemo-radiation was used in 14 patients. The 2-5 year survival rate showed 62% for two years and 28% five years.

Colorectal Primary Lymphoma. Age range was 6-65 years with a mean of 26.5, 12 males and 6 females with 14 Arabs and 4 Kurds. Clinical presentation was intestinal obstruction, abdominal mass, dyspepsia, rectal bleeding and diarrhea. Tumor location was in the cecum, ascending colon and transverse colon 72% and the sigmoid colon and rectum 28%. Gross appearance

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showed fungating tumor in 9, infiltrative in 5, ulcerative in 4 for a total of 18 patients. Histopathological staging showed non-Hodgkin's Lymphoma: 2 low grades, 7 intermediate, 8 high grades and 1 Burkitt's lymphoma according to the old international working formulation (IWF) (table 6a), and classification of gut lymphoma, updated-Kiel, a modified from the European association for hemato-pathology (table 6b)(18).

Surgical procedures performed were colectomy 13, Anterior Resection 2, Abdominoperineal Resection and Bypass Exploration 1 each. The majority received chemotherapy. Follow-ups and results showed 78% survived 2-4 years, 67% survived 5-34 years.

Discussion

The incidence and location of primary gastrointestinal lymphoma varies around the world. The stomach is the most common type of primary GI lymphoma in western countries. But, in the Middle East, most primary gastrointestinal lymphoma arises in the small intestine followed by the stomach (19, 20, 22, 23). Burkitt's lymphoma in Iraq and Primary Intestinal Lymphoma in Iraqi children were reported by Dr. A. Al-Attar, et al. (24) and Z. Al-Bahrani, et al. (25) Intestinal lymphoma differs significantly from the gastric counterpart in pathology and clinical features, management and prognosis. Surgical resection should always be the first choice for localized disease. The management of extensive intestinal lymphoma remains controversial. Conservative approach consists of limited resection of the obstructive or perforated segment followed by whole abdomen radiation. However, some surgeons prefer to do surgical debulking of the intestinal lymphoma including those of Stage III and IV. Recent data, Aviles, et al. (16), suggests that extensive resection may improve local control and improve mortality and morbidity during adjuvant therapy. Complications rate account for 22% to 78% of treatment failure and the trend towards using chemotherapy and radiotherapy after surgery. Current studies showed the localized lesion has much better prognosis than diffuse and Burkitt's type of lymphoma. Radical tumor resection with polychemotherapy in early stage improves survival and it was an early determinate of prognosis. The major prognostic factors for survival were early stage and radical resection ability. In the literature the recommendation for surgical treatment ranging from aggressive resection to limited surgical approach. In regards to prognosis, patients with low grade histology had general response better than those with high grade tumor.

Conclusion

While colorectal lymphoma in Iraq maintained the same low incidence, small bowel lymphoma has subsided gradually while gastric lymphoma is on the rise. The incidence of primary small bowel lymphoma in children and adults during the period of 1965 to 1981 and 1982 to 2000

Pages: 21-29

showed a rise of lymphoma in children 16% to 44% and subsided in adults from 84% to 56%. In Iraq the most common pathologic type in primary small bowel lymphomas is the lymphoplasmacytic type while in the colorectal were the intermediate and high grade Lymphoma, diffuse large B cell (MALT) type, while in the West the majority were low grade lymphoma. The data confirmed the findings of other authors, that surgical resection is the main course of treatment for localized primary intestinal and colorectal lmphoma. In addition, radiochemothereapy positively improved survival. Thus our observation, early stage primary intestinal lymphoma is to adapt resection in such a way as to make it as radical as possible with the role of selective postoperative chemo radiotherapy for advanced cases after surgery. Low grade lymphoma had a better prognosis than high grade lymphoma.

STAGE	**SITE OF INVOLVEMENT**
Ann Arbor- Musshoff Modification	
I or IE	GI tumor alone
IIE1	GI tumor with regional nodal involvement
IIE2	GI tumor with extra regional sub diaphragmatic nodal involvement
IIIE	GI tumor with nodal involvement on both sides of the diaphragm
IVE	GI tumor with other extra nodal involvement (i.e., bone, liver)

Table 1

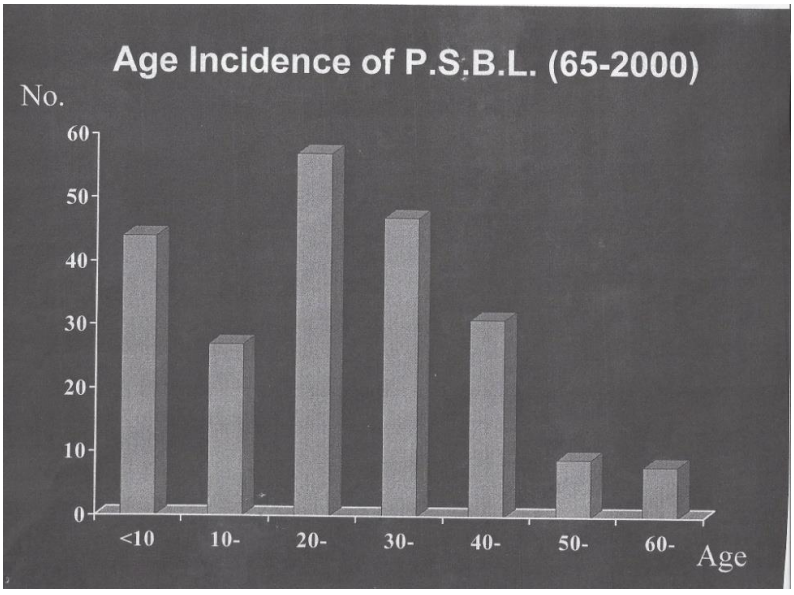


Table 2

Pages: 22-29

Clinical types of S.B Lymphoma (65-2000)

Type	Total	
	No.	%
Diffuse	148	65
Localized	48	21
Burkitt's	31	14
Total	227	100

Table 3

Clinical presentation of P.S.B.L.(65 - 2000)

	D	L	B	Total
Malabsorption	64	5	1	70
Int. Obst.	28	22	9	59
Abd. Mass.	25	17	17	59
P. U. Dyspepsia	18	1	2	21
Peritonitis	5	2	1	8
Obst. Jaundice	1	1	-	2
Melena	6	1	1	8
Total	147	49	31	227

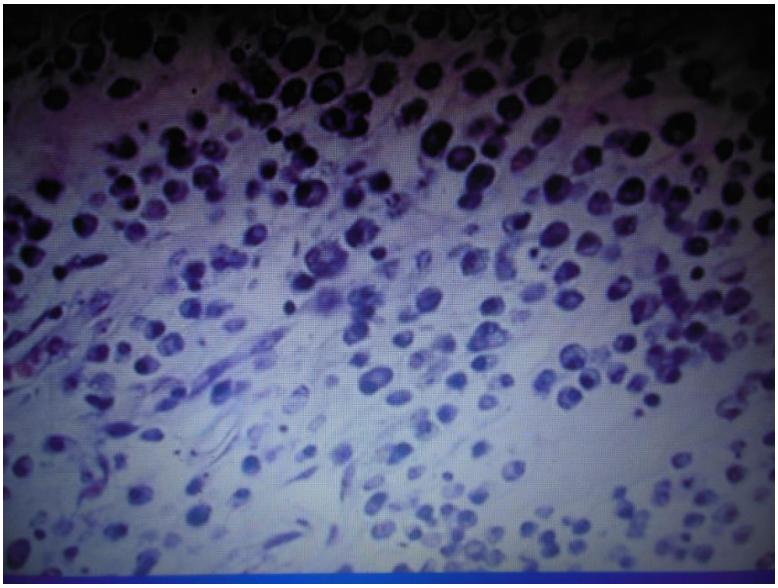
Table 4

Pages: 23-29

Relevant Clinical Data in P.S.B.L 227 Cases (65-2000)

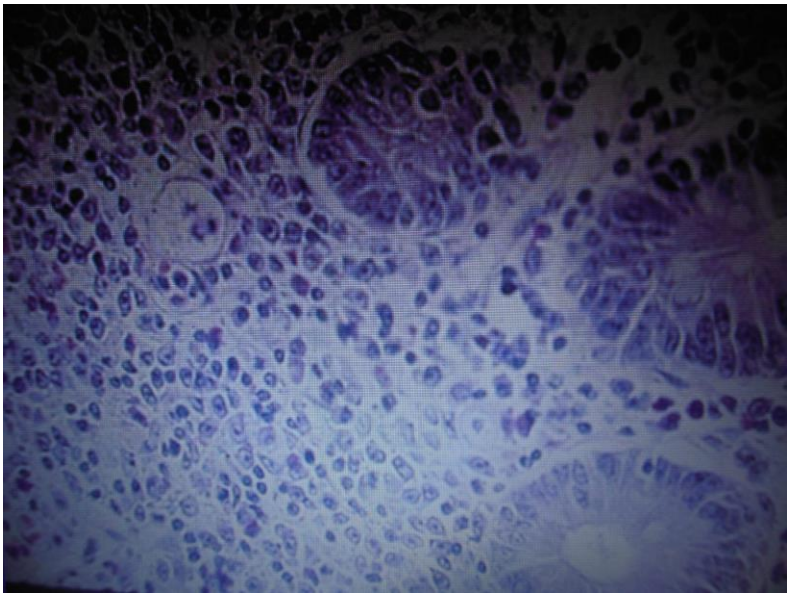
	D (147)	L(49)	B(31)
Wt. Loss	145	46	30
Abd. Pain	142	44	29
Diarrhoea	96	11	7
Clubbing	85	10	3
Abd. Mass	80	44	27
Fever	67	14	11
Vomiting	67	30	16
Oedema	24	3	2
Ascites	19	2	6
Melena	18	5	1
Hepatomeg.	8	3	1

Tabe 5

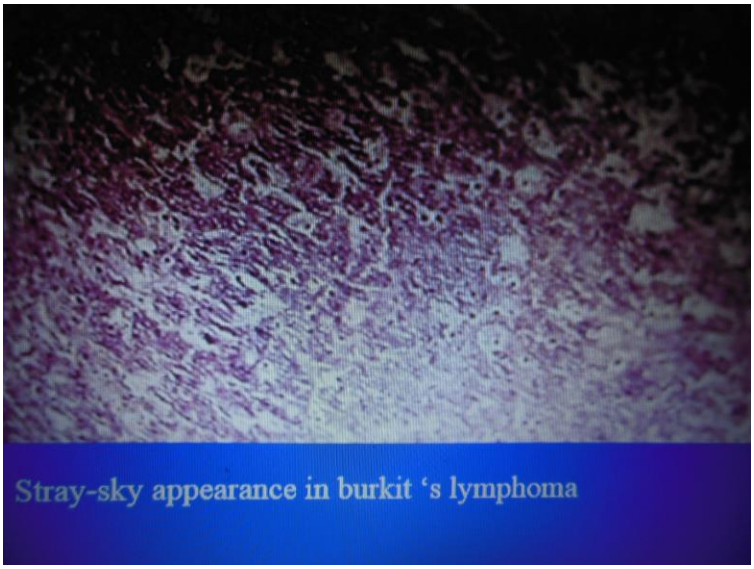


Lymphoplasmacytic Lymphoma
Small bowel
Figure -1-

Pages: 24-29



B cell Lymphoma of the
Large bowel
Figure -2-



Starry sky pattern
Burkitt's Lymphoma
Figure -3-

Pages: 25-29

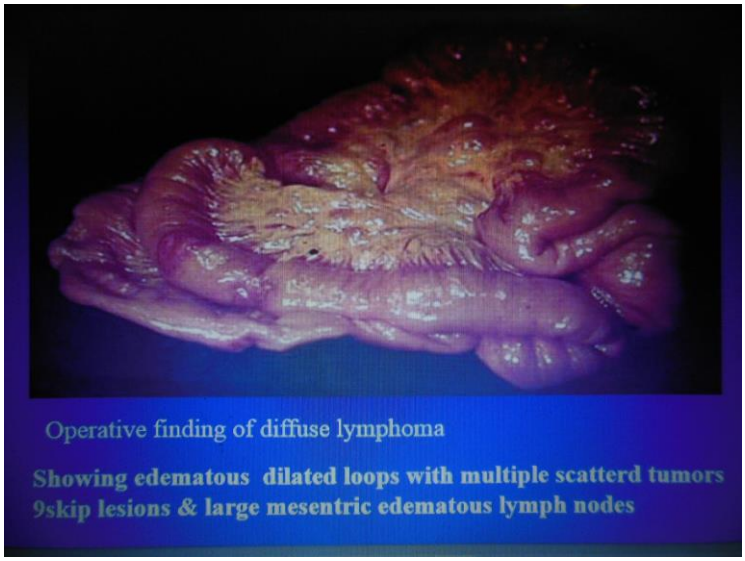


Figure -4-

International Working Formulation

LG:ML small lymphocyic, plasmacytoid.	HG:ML large cell (immuno-blastic, plasmacytoid, clear cell) with epithel-oid cell component.
ML follicular with diffuse area area & sclerosis predominant small cleaved or mixed small cleaved and large cells.	ML lymphoblastic convoluted and non-convoluted.
IG:ML follicular with diffuse area & sclerosis predominantly large cells.	ML small noncleaved cell, Burkitt's with follicular areas.
ML diffuse & sclerosis small cleaved or mixed small and large or large cleaved & noncleaved cell.	ML composite,histio-cytic,extramedullary plasmacytoma, unclassifiable.....

Old classification table -6 A-

Pages: 26-29

Primary gastrointestinal non-Hodgkin's lymphoma (Isaacson) (221)

- B cell
  - I. MALT type
    - a. Low grade
    - b. High grade with or without low-grade component
    - c. Immunoproliferative small intestinal disease
      1. Low grade
      2. High grade with or without low-grade component
  - II. Mantle cell (lymphomatous polyposis)
  - III. Burkitt's and Burkitt's-like
  - IV. Other—nodal equivalent
- T cell
  - I. Enteropathy associated
  - II. Other types unassociated with enteropathy
  - III. Rare types

MALT, mucosa-associated lymphoid tissue.

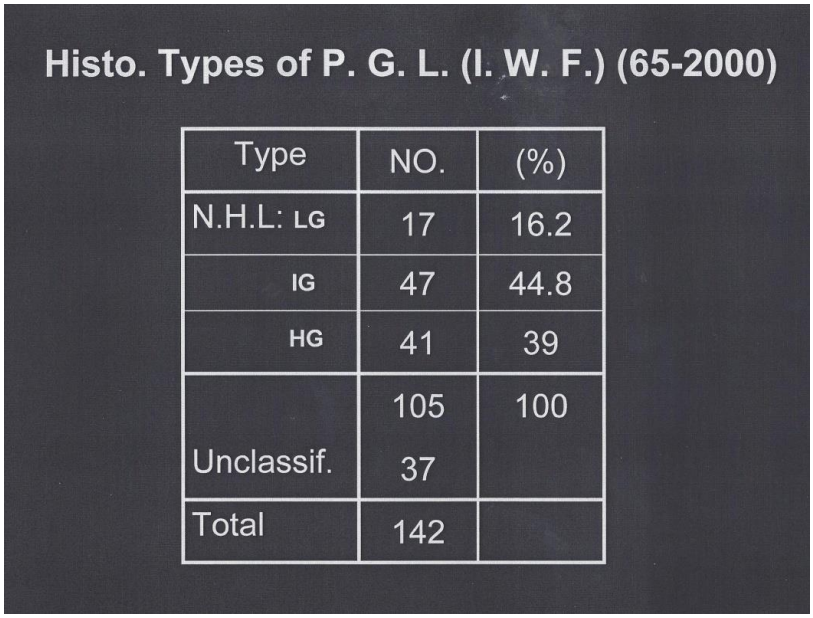
Classification of gut lymphomas, updated—Kiel (modified from the European Association for Hematopathology) (222,223)

B cell	T cell
Low grade	Low grade
Low-grade lymphoma of MALT	Pleomorphic small cell
Immunocytoma	
Alpha-chain disease	
Centroblastic/centrocytic	
Centrocytic with MLP	
Plasmacytic	
High grade[a]	High grade[b]
Centroblastic	Pleomorphic medium and large cell immunoblastic
Classical	Large cell anaplastic (Ki − 1 positive)
Polymorphic	Unclassifiable
Centrocytoid	
Multilobated	
Burkitt's lymphoma	
Lymphoblastic	
Immunoblastic	
Large cell anaplastic (Ki − 1 positive)	
Unclassifiable	

MALT, mucosa-associated lymphoid tissue, MLP, malignant lymphomatous polyposis.
[a] With or without a low-grade component.
[b] Any of which can be with enteropathy or eosinophilia

Two new classifications
Table -6 B-

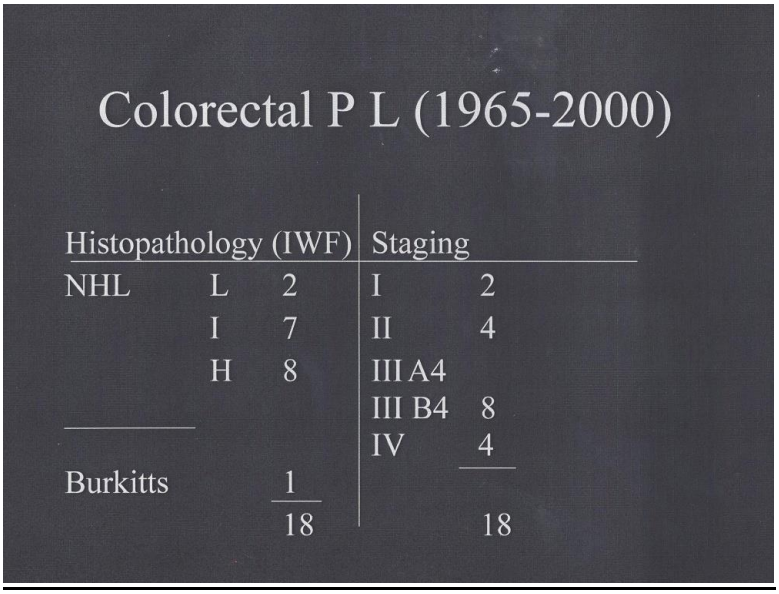
Pages: 27-29



Histo. Types of P. G. L. (I. W. F.) (65-2000)

Type	NO.	(%)
N.H.L: LG	17	16.2
IG	47	44.8
HG	41	39
	105	100
Unclassif.	37	
Total	142	

Table-7-



Colorectal P L (1965-2000)

Histopathology (IWF)			Staging	
NHL	L	2	I	2
	I	7	II	4
	H	8	III A4	
			III B4	8
			IV	4
Burkitts		1		
		18		18

Table-8-

Pages: 28-29

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Acknowledgement:

We would like to thank Professor Najib Haboubi for reviewing the article and my nurse Teresa shield for helping in typing the article.

Pages: 49-50

LETTER TO THE EDITOR

DO NOT FORGET: SNOW WHITE AND THE SEVEN "Ps".

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Acute limb ischemia (ALI) is a common emergency that can lead to loss of life or limb if not treated swiftly.

It occurs in 14 out of every 100,000 people per year. Despite a well-known acronym (the six "Ps") which describes the clinical picture of ALI (Pain, Pallor, Perishing cold, Pulselessness, Paresthesia and Paralysis) it is commonly mistaken for acute disc prolapse, Baker`s cyst, and deep venous thrombosis, or a complete failure for its possibility to be considered, especially in a young patient with no history of atrial fibrillation or current smoking.

The purpose of this letter is to reflect on the current acronym and to emphasize few points which might help the first line doctors. ALI can affect patients at any age and should be considered in the differential diagnosis of all patients presenting with leg pain of sudden onset, irrespective of age and risk factors. The mere presence of sudden severe pain, pallor and coldness of the limb, should alert the clinician that he is dealing with ALI. Loss of muscle power and sensation are signs of advanced ischemia .
Examination of peripheral pulses is not a reliable tool in this situation and hand held Doppler should be used.

To prevent propagation of thrombosis in to the small vessels which results in high vascular bed resistance and in failure of the graft, 5000 unit of HEPARIN (unfractionated) should be given IV immediately,

Pages: 50-50

The management of ALI depends on the presence and the severity of reduced muscular power and reduced sensation:

1)The limb is not at an immediate threat If the patient can wiggle the toes and retains intact sensation .The surgeon might ask for imaging & proceeds with surgery.

2) If the patient cannot wiggle the toes but still has some reduced sensation, the limb is in an immediate threat and an emergency surgery is the answer.

3) Presence of profound paralysis, anesthesia of the foot and fixed mottled skin .Sadly, the limb is unsalvageable and the only option is to perform primary amputation to save the patient's life (life is more precious than a limb).

I propose a new acronym which is easy to memorize "Snow White and the Seven "Ps". Number 7 is prevention of propagated thrombosis by heparin.

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TOFIQ Journal of Medical Sciences

TJMS

Volume 2, Issue 2, 2015

ISSN: 2377-2808

TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 2, 2015,

Contents Vol, 2, Issue 2, 2015

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BEHAVIORAL AND MOLECULAR IMPACT OF CHRONIC PSYCHOSOCIAL STRESS IN A NOVEL PRE-CLINICAL MODEL OF ALZHEIMER'S DISEASE

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Keywords: rat AD model, amyloid-beta, learning and memory, signaling molecules, synaptic plasticity

Disclosures: The author has no conflict of interest to declare

Abstract:

Because of the extensive individual variations in the time of onset and severity of the prevalent sporadic type of Alzheimer's disease (AD), a patient-related external factor must be assumed to play a significant role in the development of the disease. Since stress is increasingly recognized as an external factor in the development of AD, a number of labs, including ours, have shown that chronic stress or corticosterone administration worsens the AD phenotype in both transgenic and non-transgenic animal models of the disease. Recently we develop a novel rat model that correlates with seemingly normal individuals who are predisposed to develop AD. This review is a summarized recount of the findings we have reported on the effect of chronic psychosocial stress in this at-risk model of AD. Behavioral (learning and memory tests), electrophysiological (evoked LTP) and molecular (establishing protein levels of signaling molecules) studies indicated that even mild psychosocial stress clearly transforms this seemingly normal rat model to a full-fledge AD phenotype.

1. Introduction

Alzheimer's disease (AD) is an insidious gradual deterioration of intellectual and emotional wellbeing. The disease is hallmarked by extracellular accumulation of high levels of neurotoxic amyloid-beta (Aβ) peptides, followed later by intracellular aggregation of hyperphosphorylated tau protein, leading to neuronal death and gradual loss of mental abilities (Tanzi and Bertram, 2005; Castellani et al., 2008). The early

symptoms of the disease are a slow and insidious personality changes with loss of memory and cognitive skills.

While the early-onset familial form of AD, which is due to mutations in the genes for amyloid precursor protein (APP), is rare, the overwhelming majority of AD cases are of the late onset, sporadic type. The sporadic nature of the disease indicates an environmental connection that may hasten the appearance of AD symptoms. In addition to its late-onset, the variation in susceptibility to and time of onset of the disease suggests that, aside from genetic factors, environmental determinants, such as chronic stress, may play a critical role in the severity of sporadic AD (Srivareerat et al., 2009, 2011, Alkadhi et al., 2010 a, b).

It is widely thought that the cognitive deficits result from progressive synaptic dysfunction and irreversible neurodegeneration are probably initiated by soluble small oligomeric Aβ peptides, in particular the $A\beta_{1-42}$ form. Progressive failure of synaptic transmission occurs in the course of development of AD, which begins as a localized reduction in synaptic function and gradually progresses to large-scale impairment of neurotransmission in the brain (Mesulam 1999; Rowan et al., 2003; Selkoe, 2004). The original amyloid cascade hypothesis has been substantially revised in order to explain the weak correlation of the severity of dementia with the degree of neuronal loss and amount of fibrillar Aβ in the AD brain. The new thinking now is that soluble Aβ can cause cognitive impairment in the absence of neurodegeneration (Shankar et al., 2008). Soluble Aβ species can disrupt all forms of synaptic plasticity, including long-term potentiation (LTP), the cellular correlate of learning and memory (Sakono and Zako, 2010).

Chronic stress is known to aggravate the destructive changes associated with various brain disorders including schizophrenia (Walker et al., 2008), Cushing's disease (Whitworth et al., 2000), hypothyroidism (Gerges et al., 2004 a,b; Alzoubi et al., 2008), and AD (Srivareerat et al., 2009, 2011, Alkadhi et al., 2010 a,b). Reports of hypercorticostroidism in AD patients (Elgh et al., 2006; Hartmann et al., 1997) and from animal studies (Budas et al., 1999; Islam et al., 1998) imply that glucocorticoids may participate in the regulation of APP levels suggesting involvement of the stress hormones in the pathogenesis of AD. In support of this proposition epidemiological studies show that individuals under chronic stress are more liable to develop mild cognitive deficits, or even AD, than non-stressed individuals (Wilson et al., 2003; 2007, 2011). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis develops as a result of exposure to severe and/or prolonged mental stress, which causes adverse changes in the brain morphology and function (Aleisa et al., 2006a; McEwen, 2008).

The hippocampal formation is particularly vulnerable to the harmful effects of stress and is among the first brain regions to succumb to the assault of AD as indicated by

considerable impairment of the hippocampus-dependent cognitive abilities. Reports from my laboratories have shown that chronic intracerebroventricular infusion of pathogenic doses of Aβ peptides in chronically stressed rats impairs learning and memory and negatively impacts long-term potentiation (LTP) in the hippocampal area CA1 (Srivareerat et al., 2009; 2011, Alkadhi et al., 2011). The combination is decidedly more harmful than either chronic stress or Aβ infusion alone. We then wanted to ascertain that chronic stress accelerates or reveals symptoms of AD in normal individuals who are at risk for developing the disease. To answer this question, we have devised a novel rat model meant to exemplify normal individuals who are at-risk for or being predisposed to developing AD but are not yet displaying AD symptoms.

2. The "at-risk" Aβ rat model of AD.

Both transgenic and exogenous Aβ administration models of AD have limitations in that both models do not fully imitate the complexity of the human AD pathology. Exogenous administration of Aβ may cause neurodegeneration, inflammation and microglial activation (Pepeu et al., 1996; Nabeshima and Itoh, 1998, Giovannini et al., 2002). Injection of Aβ peptides may produce some injury at the site of injection, which may contribute to the inflammatory processes. The confounding effects of the procedure can be surmounted to a significant extent by adjusting the infusion rate, the vehicle, the volume of injection, and the recovery time.

Amyloid β effect is dose-dependent both in vitro (Goodman and Mattson, 1994; Green et al., 1996; Lambert et al., 1998; Assis-Nascimento et al., 2007) and in vivo (Lue et al., 1999; Ingelsson et al., 2004; Carrotta et al., 2006), which suggests that the neurotoxic effects of Aβ appear only after it reaches a threshold concentration. We developed a unique at-risk AD rat model by icv infusion of a sub-pathogenic concentration of Aβ (subAβ). Various tests indicated that this model of AD was not significantly different than control rats. This is the first non-transgenic rat AD model to reproduce a condition where there is a heighten vulnerability to AD with no observable cognitive impairment. However, when the at-risk AD rat model is generated in chronically stressed rats, they show AD phenotype similar to that previously reported in a full-fledged model of AD (Srivareerat et al., 2009). This model may represent individuals who seem normal but can develop AD dementia when exposed to stressful conditions.

3. Animal groups and treatments.

To study the impact of chronic psychosocial stress on the at-risk AD model, Wistar rats were assigned into four experimental groups: control, stress, subAβ, and stress/subAβ. The stress and stress/subAβ groups were subjected to psychosocial stress for 6 weeks. In addition, the subAβ and stress/subAβ groups were infused, icv, with $A\beta_{1-42}$ (sub-pathogenic concentration 160 pmol/day) during the fifth and sixth weeks using 2-

week osmotic pumps. This dose was determined by constructing a dose-response relationship (dose-performance in the radial arm water maze). The inactive reverse peptide, $A\beta_{42-1}$ (160 pmol/day) was infused in the control and stress groups (Tran et al., 2010, 2011).

For chronic stress, we used a form of ‘‘intruder’’ psychosocial stress (Aleisa et al., 2006c). After one week of acclimation to establish a social hierarchy in the cages, rats in the stress groups were chronically stressed by daily random switching of two rats from each cage to other cage for a period of six weeks. This type of chronic psychosocial stress was shown to markedly increase blood corticosterone level (Gerges et al., 2001) and elevate blood pressure (Alkadhi et al., 2005).

4. Behavioral tests: the radial arm water maze task.

The radial arm water maze (RAWM: Fig. 1 inset) is a black circular pool filled with water at room temperature with six V-shaped structures arranged so as to create six swim paths (arms) radiating from one open central area as described (Gerges et al., 2004a). The RAWM is a reliable and sensitive behavioral test for analyzing hippocampus dependent spatial learning and memory. Being a hybrid of the radial arm maze and the Morris water maze, it combines the spatial complexity of the radial arm maze with the motivated fast learning of the Morris water maze while reducing their shortcomings (Buresova et al., 1985; Hodges, 1996; Diamond et al., 1999; Alamed et al., 2006).

The RAWM training protocol for this project involved an acquisition phase of four one-minute successive learning trials. Then, 20 min later a short-term memory test was given followed 24 hr after the last learning trial by a long-term memory test. The rat had to find a platform hidden 1 cm below the water level at the far end of one of the six swim arms. Each time the rat entered an arm other than that containing the hidden platform an error was scored. This procedure was repeated for a minimum of 8 consecutive days (Alkadhi et al., 2010a, b; Srivareerat et al., 2009, 2011;Tran et al., 2010, 2011).

4.1. Learning, Short-term memory and Long-term memory

The ability of rats in all groups to learn the location of the survival platform during the four acquisition phase trials was initially similar throughout the initial days of testing as rats learned the location of the hidden platform. However, based on the number of errors in locating the hidden platform, the learning ability of the stress/subAβ rats was markedly impaired as days went by, particularly in the fourth trial, compared to the other three experimental groups (figure 1A) (Tran et al., 2010a).

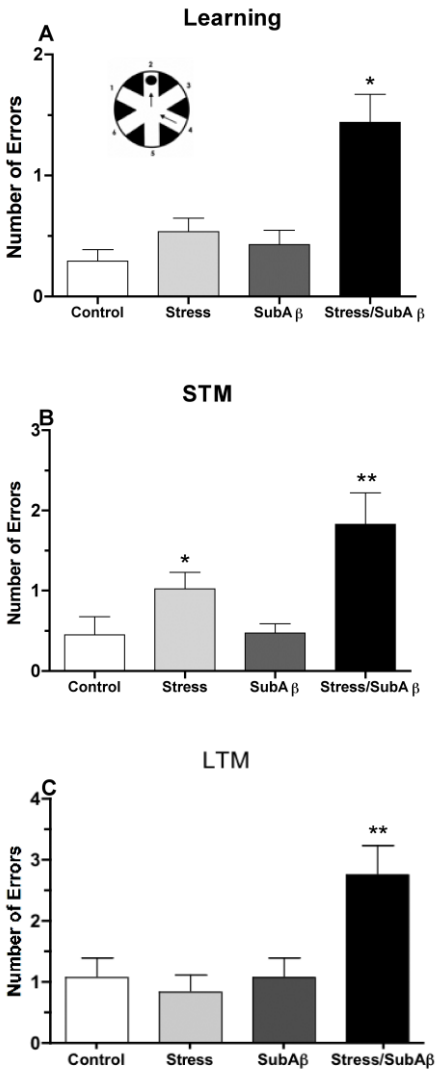

Figure 1: Performance in the radial arm water maze (inset at panel A) task is impaired in stress/subAβ rats. (A) The number of errors made by all groups in learning trial number 4 (days 6–8). The stress/subAβ group made significantly more errors than all other groups. (B) The short-term memory (STM, measured 20 min after the last learning trial) was significantly more severely impaired in the stress/subAβ rats than in the other three groups. (C) The long-term memory (LTM) test was conducted 24 h after the last learning trial, shows severe impairment in rats of the stress/subAβ group. (*= $p<0.05$, ** $p<0.001$, n=12 rats/group; mean±SEM). Note that subAβ rats were cognitively normal in all these tests and were not significantly different than control rats.

Rats were tested for short-term memory 20 min after the last learning trial. Again, the subAβ group performance was not significantly different than that of control rats. As expected, chronically stressed rats showed impaired short-term memory by making significantly higher number of errors compared to both control and subAβ groups (Fig. 1B). Dramatically, the stress/subAβ rats continued to commit significantly higher number of errors than all other groups, including the stress group. These findings were further confirmed by another parameter, the days to criterion (DTC), which showed that the stress/subAβ rats required significantly ($p<0.05$) more days to reach a criterion (number of days in which the rat commits no more than one error in three consecutive days) than the other three experimental groups (Tran et al. 2010a).

In the long-term (24 hr) memory test, the stress/subAβ animals committed significantly more errors than the control, stress, and subAβ groups (Fig. 1C). Note that our 6-week chronic psychosocial stress did not affect long-term memory confirmed our earlier reports (Aleisa et al., 2006b, c). In confirmation, the DTC test showed that the stress/subAβ rats required significantly more days to reach the criterion than the other three experimental groups (Tran et al. 2010).

4.2. Summary

Behavioral testing in the RAWM showed that the cognitive ability of the novel preclinical model of AD was normal in that it was not different from that of the control rats. However, when these rats were under chronic stress they exhibited clear symptoms of impaired cognitive ability similar to those seen in rats infused with a full pathogenic dose (300 pmol/day) of Aβ (Srivareerat et al., 2009, 2011, Alkadhi et al., 2010 a, b). Together these findings validate the subAβ as a model for seemingly normal (pre-clinical) individuals who may be at-risk for developing AD.

5. Basal levels of cognition-related signaling molecules

To clarify the molecular mechanisms by which stress accelerates impairment of cognitive ability in the at-risk (subAβ) rats and to correlate these findings with the results from the learning and memory studies, we assessed, by immunoblot analysis, the basal levels of cognition related signaling molecules in this preclinical AD model and the effects of chronic psychosocial stress.

5.1. Calcium calmodulin kinase II (CaMKII)

Interestingly, certain forms of stress may upregulate CaMKII expression. For example, stress caused by postnatal maternal deprivation and pubertal immobilization

upregulates CaMKII (Novak et al., 2013). The reason for this differential response is not well understood.

Long-term potentiation (LTP) and other forms of synaptic plasticity are believed to be the cellular correlate of learning and memory. The critical role of calcium calmodulin kinase II (CaMKII) in the memory processes and synaptic plasticity is well known. Experimentally, the putative molecular cascade of events leading to the formation of the active phosphorylated CaMKII (p-CaMKII) and eventually expression of LTP is experimentally initiated by high frequency stimulation of the synaptic pathway. It is widely accepted that high frequency stimulation causes presynaptic release of glutamate, which activates glutamate receptors on the postsynaptic membrane, causing calcium influx through n-methyl-d-aspartate (NMDA) glutamate receptor (Malenka et al., 1988; Nicoll et al., 1989; Fukunaga, 1993). This transient elevation of intracellular calcium leads to activation of protein kinase C gamma (PKCγ), which phosphorylates neurogranin, causing dissociation of the neurogranin-calmodulin complex (Feng, 1995; Kim and Yoon, 1998; Gerendasy and Sutcliffe, 1997). The free calmodulin forms a calcium/calmodulin complex, which binds to and activates CaMKII. Activation of CaMKII enables it to phosphorylate itself (Wang and Kelly, 1995; Holmes, 2000). Autophosphorylation results in a constitutively active CaMKII (p-CaMKII), which phosphorylates and activates α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) glutamate receptors and the synaptic vesicle-specific protein, synapsin, both of which are important for LTP expression and memory formation (Fukunaga et al., 1993; 1996; Nayak et al., 1996; Barria et al., 1997a). The activation of these substrates by p-CaMKII is continuous, even when calcium concentration returns to normal levels, and stops only when it is dephosphorylated by protein phosphatases including calcineurin (Fukunaga et al., 1996; Wang and Kelly, 1996; Fukunaga and Miyamoto, 2000). It is suggested that activated CaMKII works as a molecular switch that changes temporary Ca^{2+} signals into long-term biochemical changes that generate synaptic plasticity and memory (Lisman et al. 2002).

Chronic psychosocial stress or pathogenic doses of Aβ peptides markedly reduced the basal levels of p-CaMKII without affecting the levels of total CaMKII (Srivareerat et al., 2009). In the subAβ rats, the basal levels of both p-CaMKII and total CaMKII were not significantly different than those in control rats (Tran et al., 2010). However, in both the stress and stress/subAβ rat groups, the p-CaMKII levels were markedly reduced compared to control and subAβ groups (Fig. 2A) whereas levels of total CaMKII remained unchanged (Tran et al., 2010a) suggesting impairment of the mechanism of CaMKII phosphorylation and/or enhanced dephosphorylation. The finding that the basal levels of the dephosphorylating enzyme, calcineurin are markedly increased in the stress

and stress/subAβ groups compared to control and subAβ groups (Fig 2C; Tran et al, 2010) provides support to the notion of enhanced dephosphorylation.

5.2. Cyclic AMP response element binding (CREB) protein:

Active CREB signaling may play a central role in mediating the effects of chronic stress on neurogenesis, LTP and calcium currents in the DG area (Ecke et al., 2011; Datson et al., 2012). Findings from this lab consistently showed that moderate chronic psychosocial stress in rats did not significantly affect the basal expression of p-CREB or total-CREB (Alkadhi et al., 2010; Alkadhi, 2011; Alkadhi 2012).

Active CREB is essential for hippocampus-dependent long-term memory formation in mammals (Bourtchuladze et al., 1994; Alberini, 2009). The role of CREB in AD-associated impairment of memory and synaptic plasticity has been suggested by a number of studies. For example, nuclear translocation of pCREB is blocked by Aβ resulting in decreased CRE-mediated responses (Arvanitis et al., 2007). Moreover, CREB phosphorylation is reduced by Aβ through decreasing NMDA receptor activation (Snyder et al., 2005).

Although normal in control, stress and subAβ rat groups, the basal levels of p-CREB and total CREB in brain area CA1 were significantly reduced in the stress/subAβ group (Fig 2B). This suggests curtailed synthesis of the CREB protein in area CA1 by chronic stress in the presence of subAβ concentrations. Infusion of full pathogenic dose of Aβ caused a marked decrease of p-CREB levels in area CA1 of rats (Alkadhi et al., 2010; Alkadhi, 2012). CREB plays a critical and highly conserved role in the generation of protein synthesis-dependent long-term changes required for long-term memory and synaptic plasticity in the brain (Bourtchuladze et al., 1994; Lonze and Ginty, 2002). Therefore, the reduced basal levels of CREB may explain the compromised long-term memory in the stress/subAβ animals. These findings further establish that chronic psychosocial stress can hasten the emergence of AD symptoms in at-risk individuals.

5.3. Calcineurin:

Activated signaling molecules including p-CaMKII, p-CaMKIV and p-CREB are typically dephosphorylated by a protein phosphatase, mainly calcineurin. Calcineurin dephosphorylates inhibitor 1 protein, the natural regulator of protein phosphatase 1 (Mulkey et al., 1994), which is efficient in the dephosphorylation of CaMKII (Strack et al., 1997). The hippocampus contains various phosphatases; of which calcineurin is thought to be the principal phosphatase involved in the regulation of synaptic plasticity.

Calcineurin diminishes postsynaptic activity in hippocampal neurons (Wang and Kelly 1997) and is implicated in the induction and maintenance of long-term depression (LTD) (Thiels et al., 2000). Furthermore, upregulation of calcineurin in the hippocampus inhibits LTP (Winder et al., 1998) and weakens hippocampus-dependent memory formation in mice (Mansuy et al., 1998).

Chronic psychosocial stress causes over-expression of calcineurin in area CA1 of the hippocampus (Gerges et al., 2004a; Aleisa et al., 2006c; Srivareerat et al., 2009; Alkadhi et al., 2011). Other types of stressors may have different effects on the levels of calcineurin. For example predator exposure stress produced no significant effect on the expression of calcineurin in hippocampal area CA1, amygdala or prefrontal cortex medial prefrontal cortex (mPFC) of rats (Zoldaz et al., 2012). Interestingly, calcineurin expression in the hippocampus of maternally deprived rats seems to be sex-specific in that it is decreased in male but not female animals (Takase et al., 2012). Remarkably, within the hippocampal formation, the DG area seems to be immune to the effect of moderately chronic stress in that chronic stress produced a significant decrease, rather than increase, in calcineurin levels. Thus, the DG of chronically stressed rats seems to have a compensatory mechanism whereby calcineurin levels are reduced in order to maintain normal P-CaMKII levels, which may be responsible for the normal early LTP of the DG in chronically stressed rats (Gerges et al., 2003).

Elevated calcineurin levels have a negative impact on cognitive function and appear to be involved in AD pathogenesis. For instance, cognitive impairment in Tg2576 mice is reversed on treatment with calcineurin inhibitor tacrolimus (Taglialatella et al., 2009). The involvement of calcineurin in AD pathology is well recognized (Gong et al., 1994; Billingsley et al., 1994; Berridge, 2010). Examination of AD brains showed that mRNA of calcineurin was upregulated in pyramidal neurons of the hippocampus, suggesting that calcineurin may play a key role in the pathogenesis of AD (Hata et al., 2001). Experiments on cultured cortical neurons showed that disruption of amyloid precursor protein (APP) function, which resulted in neurite degeneration, oxidative stress, nuclear condensation and cell death, was due to a calcineurin-dependent breakdown of CaMKIV and its nuclear target CREB (Mbebi et al., 2002). In addition, calcineurin activation in astrocytes was associated with the formation of reactive inflammatory processes related to AD (Norris et al., 2005).

Calcineurin levels in area CA1 of subAβ at-risk rats were not significantly different than those in the control rat group. However, area CA1 of both the stress and the stress/subAβ rats showed significantly increased levels of calcineurin (fig 2C). Similar increases in calcineurin levels were seen in the CA1 area of rats treated with a full pathogenic dose of Aβ peptides (Srivareerat et al., 2009).

5.4. Brain derived neurotropic factor (BDNF):

Brain-derived neurotrophic factor (BDNF) exists at high levels in hippocampal neurons, and its expression is controlled by neural activity. It is a member of a family of neurotrophic factors that includes nerve growth factor, neurotrophin-3, and neurotrophin-4/5, which activate various isoforms of tropomyosin kinase (Trk) receptors. BDNF supports synaptic plasticity and promotes survival of existing and nascent neurons in the central nervous system. BDNF supports survival of cholinergic neurons of the basal forebrain (Knusel et al., 1992), and other neurons in the hippocampus (Lindholm et al., 1996; Lowenstein and Arsenault, 1996) and cortex (Ghosh et al., 1994). It can act as an activity-dependent modulator of neuronal structure, and its release after tetanic stimulation modulates the induction and maintenance phases of LTP in the hippocampus (Bramham and Messaoudi, 2005; Soule et al., 2006; Tran et al., 2011). In addition to its action on neuronal survival and differentiation, BDNF has a role in the regulation of synaptic strength.

Experimental evidence supports the role of BDNF in memory processes. For example, expression of BDNF mRNA is correlated with performance in various cognition tests (Thoenen, 2000; Huang and Reichardt, 2001;Yamada et al., 2002). The critical role of BDNF in memory and synaptic plasticity, may justify the assumption that its loss may contribute to memory deficits associated with neurodegenerative diseases including AD. For example, inhibition of activity-regulated cytoskeleton-associated gene (ACR) protein synthesis has been reported to impair both the maintenance of LTP and consolidation of long-term memory, hence may contribute to the memory deficits in AD (Wang et al., 2006; Echeverria et al., 2007). This was substantiated by showing marked downregulation of BDNF mRNA and protein in the hippocampus and temporal cortex of autopsied AD brains (Connor et al., 1997). Additionally, It has been reported that exposure to various stressors both acute (Smith et al., 1995a; Ueyama et al., 1997; Lee et al., 2008) and chronic (Murakami et al., 2005; Tsankova et al., 2006; Nair et al., 2007; Rothman et al., 2012) can significantly down regulate both BDNF mRNA expression and protein levels in the hippocampus. There is, however, seemingly conflicting reports concerning the levels of BDNF in the brains of animal models of AD. The reason for this inconsistency is unclear; it may be the outcome of an assortment of factors including different phases of the disease at which the levels of BDNF was determined, the type of AD model used, the dose level of Aβ administered etc.

We determined the protein levels of BDNF and showed that its basal levels were not significantly changed in stress and subAβ animals compared to control animals. However, when chronic stress was coupled with subAβ infusion (in the stress/subAβ group), there was a significant ($p < 0.05$) decrease in the basal level of BDNF compared to the control and stress groups (Fig 2D; Alkadhi, 2012). In cultured cortical neurons comparable findings have been reported on the effect of sub-lethal dose of Aβ (Tong et

al, 2001, 2004). Another publication from our lab reported significantly ($p<0.05$) higher levels of BDNF in rats infused with a pathogenic dose of Aβ (Alkadhi et al., 2010a), which may be explained as an attempt by the brain to effect repair at this stage of the disease.

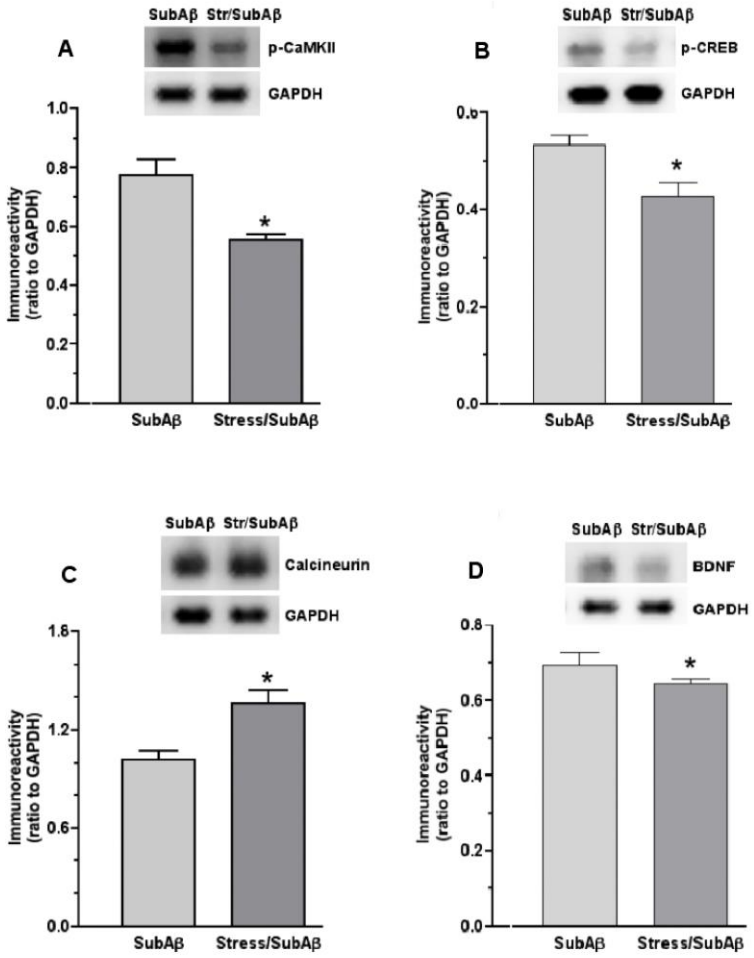

*__Figure 2__: Western blot analysis comparing the basal levels of memory-related signaling molecules in subAβ and stress/subAβ rats. A significant decrease in the basal levels of the short-term memory related phosphorylated calcium/calmodulin-dependent protein kinase II (p-CaMKII) (A). (B) The long-term memory related phosphorylated cyclic AMP response element binding protein (p-CREB) in the hippocampal area CA1 of stress/subAβ rats. (C) Basal levels of the phosphatase calcineurin in area CA1 of the hippocampus were significantly increased in stress/subAβ rats. (D) Basal levels of brain-derived neurotrophic factor (BDNF) in the CA1 area of the hippocampus were significantly decreased in stress/subAβ rats. (*) indicates significant difference from control values ($p<0.05$, n=6–8

rats/group; mean±SEM). Insets are representative bands.

5.5. Summary

Testing the effects of stress at the molecular levels by determining the amounts of signaling molecules involved in memory and synaptic plasticity (table 1) provides further evidence of the deleterious impact of chronic psychosocial in subjects prone to developing AD by accelerating the progress of the symptoms of the disease. These experiments also show that these signaling molecules are normal in the unstressed subAβ rat model, which correlates with our behavioral findings.

6. Alzheimer's disease-relate proteins

Amyloid precursor protein (APP) is a transmembrane protein with its Aβ domain partly embedded in the plasma membrane. Cleavage and processing of APP can occur through two major pathways: a non-amyloidogenic pathway, which produces harmless Aβ peptides, and an amyloidogenic pathway that produces the pathogenic peptides (Lue et al., 1999; Thinakaran and Koo, 2008; La Ferla et al, 2007; Querfurth and LaFerla, 2010). Cleavage of APP is achieved by cutting of APP by three enzymes: α, β, and γ secretases. The predominant non-amyloidogenic pathway is initiated by α-secretase cleavage of APP within the Aβ domain, thereby precluding the formation of pathogenic Aβ and releasing a large soluble APP fragment (soluble alpha-amyloid precursor protein; α-sAPP) (Querfurth and LaFerla, 2010). In the amyloidogenic pathway, production of Aβ peptides begins with the proteolytic cleavage of APP just above the Aβ domain by β-secretase releasing soluble beta-amyloid precursor protein (β-sAPP) fragment and yielding the C-terminal fragment 99 (C99), which is then cleaved by γ-secretase to release the pathogenic Aβ peptides (La Ferla et al, 2007; Querfurth and LaFerla, 2010). Most β-secretase activity originates from an integral membrane aspartyl protease called β-site APP cleaving enzyme 1 (BACE1), which functions as the rate limiting step in the pathogenic production (Vassar et al., 1999).

We used immunoblot analysis to determine the basal levels of APP in hippocampal area CA1 of the four experimental groups. There was no significant change in the APP levels in the stress, subAβ or stress/subAβ compared with those of the control group (Fig. 3 left panel, Table 1). We also analyzed the basal levels of BACE in area CA1 and showed normal levels in stress or subAβ rats. However, chronic stress significantly ($p < 0.05$) increased the levels of BACE in subAβ-infused (stress/subAβ) animals compared with control, stress, and subAβ rats (Fig. 3 right panel, Table 1). The increase in basal levels of BACE in stress/subAβ rats suggests that in these rats the processing of APP though the pathogenic pathway is enhanced. Congruent with that we

also reported that full pathogenic Aβ dose (300 pmol/day produced a significant elevation of BACE in area CA1 (Srivareerat et al., 2011).

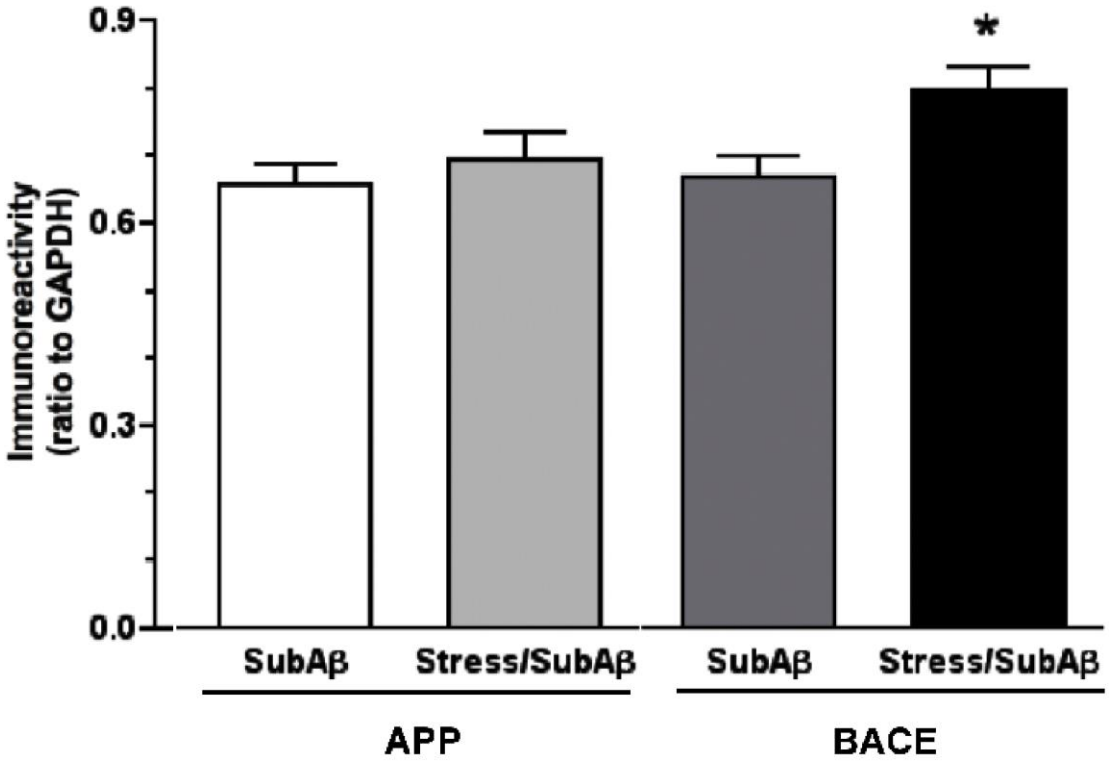

Figure 3: Western Blot analysis of the basal levels of Alzheimer's disease-related molecules. No significant changes in the basal levels of amyloid precursor protein (APP) among the four experimental groups were observed in the hippocampal area CA1. Basal levels of beta-site amyloid precursor protein-cleaving enzyme (BACE) in the CA1 area of the hippocampus were significantly increased in stress/subAβ rats. Results are expressed as mean ± SEM. *Significant difference ($p < 0.05$, $n = 6–7$ rats/group) from control, stress, and subAβ groups.

	STRESS	SUBAβ	STR/SUBAβ
p-CaMKII	⇓	⇔	⇓
t-CaMKII	⇔	⇔	⇔
Calcineurin	⇑	⇔	⇑
BDNF	⇔	⇑	⇑
p-CREB	⇔	⇔	⇓
t-CREB	⇔	⇔	⇔
CaMKIV	⇔	⇔	⇓
Learning	⇔	⇔	⇓
Short-term memory	⇓	⇔	⇓⇓
Long-term memory	⇔	⇔	⇓
Early LTP magnitude	⇓	⇔	⇓
Late LTP magnitude	⇔	⇔	⇓
LTD magnitude	⇑	⇔	⇑⇑
BACE	⇔	⇔	⇑
APP	⇔	⇔	⇔

Table 1

Summary of the effects of chronic psychosocial stress (STRESS) on cellular and molecular neuronal functions in a pre-clinical AD rat model (SUBAβ: 160 pmol/day; sub-pathogenic dose) in area CA1 of the hippocampus. ⇓ decreased, ⇑ increased, ⇔ no change compared to control rats.

7. Likely mechanisms of the deleterious effects of stress

Abnormal levels of Aβ peptides disrupt phosphorylation of CaMKII and interfere with LTP induction (Srivareerat et al., 2011; Townsend et al., 2007; Zhao et al., 2004).

We have shown previously that chronic stress reduces the magnitude of LTP and decreases basal levels of p-CaMKII in hippocampal area CA1 (Gerges et al., 2004a; Srivareerat et al., 2009). Because of the critical role of CaMKII in learning and memory it is likely that disruption of the CaMKII-dependent protein phosphorylation by stress may accentuate the subthreshold loss of p-CaMKII caused by Aβ, thus contributing to the mechanism by which chronic stress impairs memory in this model of AD.

In general, activation of glucocorticoid (type-II) receptor by high levels of corticosteroids during stressful conditions boosts Ca^{2+} influx, which results in inhibition of CA1 pyramidal cell excitability (Conrad et al., 1999; Pavlides et al., 1995). Previous reports have suggested that Aβ interferes with intracellular Ca^{2+} signaling (Dong et al., 2004; Liang et al., 2007; Liu et al., 2005) and inhibits Ca^{2+}-dependent post-translational protein phosphorylation (Jarret and Lansbury, 1992). Furthermore, acute application of $A\beta_{1-42}$ during repetitive stimulation showed inhibition of LTP in the dentate gyrus, accompanied by reductions in p-CaMKII levels (Zhao et al., 2004). Thus, it is not surprising that stress worsens Ca^{2+} dependent signaling processes in Aβ rats.

Brain levels of neurotrophic factors, including BDNF, are elevated in certain brain areas in reaction to various types of insults, including ischemia, seizure, traumatic brain injury, and neurotoxins (Durany et al., 2000; Lindvall et al., 1992). Brain-derived neurotrophic factor (BDNF), in particular, is known to plays a major role in neuronal health and survival (Barde, 1989; Zuccato and Cattaneo, 2009). In early stages of AD, a protective mechanism may be activated to counter the Aβ-induced neurotoxicity. This is suggested by earlier reports that showed an increase in the mRNA of BDNF in the hippocampus (Tang et al., 2000) and in the protein levels of BDNF in the forebrain in APPsw mice model of AD (Hellstrom et al., 2004) as well as area CA1 in the full dose Aβ-treated rats (Srivareerat et al., 2011). In contrast, chronic stress has been reported to significantly decrease BDNF levels in area CA1 of the hippocampus (Aleisa et al., 2006). Therefore, by reducing the availability of BDNF, stress interferes with the repair process and consequently augments the effect of Aβ.

Mental stress and AD seem to interfere with mitochondrial function by a similar mechanism. A mitochondrial enzyme that can bind to Aβ peptide directly is 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD) or Aβ-binding alcohol dehydrogenase (ABAD) (Yan et al., 1997; Grimm et al., 2012). Other reports showed that mental stress impaired mitochondrial function and increased ABAD expression in the brain (Seo et al., 2011; Yao et al., 2011). Therefore, it is likely that effect of behavioral stress in increasing ABAD brings mitochondrial dysfunction to a critical threshold level required to show clear AD phenotype.

Stress seems to be able to modify the processing and production of various proteins involved in the expression of AD. Exposure to stress or glucocorticoids has been shown to increase the concentrations of APP and BACE proteins, suggesting that stress may be pushing the processing of APP toward the pathogenic pathway. This may explain the enhanced levels of pathogenic Aβ protein (Catania et al., 2009; Srivareerat et al., 2009, 2011) and the increased quantity of plaque formation (Lee et al., 2009) that are also reported with stress.

In summary, the presence of chronic stress can have a profound effect on the course of development of AD. Using various experimental approaches in a number of studies, this lab has reported the negative impact of chronic psychosocial stress on the development of dementia in a novel rat model that correlates to seemingly normal individuals predisposed to develop AD (at-risk for AD). Findings of these studies imply that the concurrence of chronic stress in these subjects accelerate the development of AD.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 2, 2015

Pages: 34-56

CHOLERA EPIDEMIC IN IRAQ DURING 2015

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Key words: Bacteria, cholera, dehydration, shock, Vibrio cholerae

TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 2, 2015

Pages: 34-56

Summary:

Background: Cholera became an endemic disease in Iraq, strikes in epidemic form nearly every ten years, but with irregular outbreaks. Iraq is facing great disasters of destruction of the infrastructures with shortage of electricity and safe water for drinking especially in the poor districts and the refugee camps that helps for the appearance of water-borne diseases including cholera. Also the sewage disposal systems has greatly damaged or obstructed.

Aim: It is to shed light on the epidemiology, the pattern of cholera cases that appeared suddenly during the thirty eighth week of October in Baghdad districts and spread to many other governorates. The outcome of cases was also mentioned. It is also a descriptive analysis for the additive cases on daily bases through addition or discarding of cases of suspected cholera according to the reference laboratory testing.

Subjects, Methods, and Materials:

The study is a descriptive analysis; is that the data (officially supplied from the Center of Disease Control/ Ministry of Health) is dynamic and every day and week there are new cases and discarded cases till at the end of month the final report was done. As Iraq is an endemic country for cholera, the case definition in the endemic countries according to the WHO is: (Acute watery diarrhea for more than three bowel motions with or without vomiting in a patient aged 5 years or more).

Accordingly, cases with the above complaints who attended the hospitals and health facilities are examined for stool cultures and sero- typing for Vibrio cholerae in the Central Public Health Laboratory (CPHL). Those who are stool culture- positive will be reported here as cholera cases. Follow up of the hospitalized cases for their outcome are reported; diarrheal cases reported from all governorates of Iraq together, with the positive cases of cholera are mentioned also. Epidemiological curves for cholera and acute diarrheal diseases for the years 2013, 2014 and 2015 are presented for comparison.

Results:

The number of acute diarrhea cases together with cholera cases for the year 2015, their ages and gender are reported according to the distribution by weeks. The trend of cholera in Iraq since the year 1990- 2014 is reported. The total number of admitted cases of

cholera from the 23rd September till 6/11/2015 that were laboratory confirmed are 2651 cases comprising of 1477 males and 1174 females, with all age groups were included. The sero typing of the isolates show that the Vibrio cholera was O1 sero type Inaba with few cases only of Ogawa sero type. This final figure was corrected every day through the addition of new cases and discarding of others; the number includes all cholera cases in Baghdad and fourteen other governorates. There were only two cases died with CFR of 0.075%. It appeared, the trend of attack of cholera in Iraq was classical i.e. during the months of September and October and not in the hot months of Sumer as that appeared during the years of 1990 and 2007.

Conclusion and Recommendation:

Communicable diseases surveillance needs an immediate notification, daily, weekly and monthly reports, with Syndromes Surveillance when the situation is complex. Epi- info soft ware is to be used to collect surveillance data via fixed systems and submitted to the center by using the internet. Health education campaigns should be adapted to the local cultures and their habits; they should promote practices of personal hygiene with concentration on hand-washing with soap and detergents. The storage and preparation of food should be safe, together with encouraging of breastfeeding for lactating women. Innovative WASH interventions are needed to prevent cholera, together with the surveillance and diagnostics through laboratory net work; the use of rapid diagnostic tests (RDTs) is needed. Oral cholera vaccine (OCV) should always be used as an additional public health tool with mass vaccination campaigns to be launched for people especially in the camps or when facing disasters of mobilization or facing flood and sewage contamination.

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Introduction:

Cholera is an acute diarrheal bacterial disease that attacks in an epidemic and outbreak forms; it remains a major public health problem in many parts of the world, causing an enzymatic diarrhea.1-5 If not treated, it might result in severe dehydration and hypovolemic shock. Two sero groups, O1 and O139, cause outbreaks of cholera; the O1 causes the majority of outbreaks, while O139 was first identified in Bangladesh in 1992, and is confined to South-East Asia 6-9; it appeared in Iraq for the first time during the epidemic of 199910. In severe form, cholera is characterized by sudden onset, profuse painless watery stool (rice-water stool), nausea and profuse vomiting early in the course of illness. In untreated cases, rapid dehydration, acidosis, circulatory collapse, hypoglycemia in children, and renal failure can rapidly lead to death. Resuscitation by the proper fluid and electrolytes is the key role in saving lives; while the antibiotics role is important in decreasing the time of the diarrhea, shortening the hospitalization period and decreases the percentage of microbial carriage 8-10. The diagnosis of cholera is done by the conventional culture method and this would need for no less than two days for isolation and identification of the microbe. The rapid diagnostic tests (RDTs) can be widely used and help in the quick identification and reporting of the cholera cases even in the absence of laboratory facilities.1-3

Cholera remains especially an overlooked disease in the developing countries and in the endemic areas. The real number of cholera cases worldwide is underestimated, is that 1.3-4.0 million cases are occurred each year with 21- 143 thousands of deaths. The case fatality rate (CFR) according to the epidemic of 2014 has ranged between 0.01- 25.71%. The highest CFR are reported mainly from the poor countries due to a confusion of the case definition where the severe cases only are regarded as cholera cases 11-15, also due to a delayed or poor medical care and management 1, 3, 6. Unsanitary environment and conditions and poor water supply with unhealthy water available for drinking in the year 2015 is estimated to be present among 2-4 billion people all over the world 11, 13-15. Lack of the security conditions impedes the implementation of an appropriate surveillance and control activities especially now in many Middle East countries including Iraq and Syria. There were a total of 190 549 cholera cases reported by the WHO from forty two countries during the year 2014, with CFR of 1.17% 16. There might be an under estimated reporting of the real number of cholera cases because of its negative reflect on travel, trade and food export from the affected countries. The lack of laboratory facilities for the diagnosis and absence of expert health teams dealing with the disease might result in over reporting of acute diarrheal cases as cholera because of

confusing case definitions 16-19. The oral cholera vaccine (OCV) is now available, its efficacy and protection time are still not finally addressed, and it can be given for people at any age including one year old 15.

Despite Iraq was free of cholera during the year 2014, in the year 2015, it has faced an epidemic of cholera started during the mid of September till 6/11/2015 (time of reporting this paper, and the number is still increasing). The total number of laboratory confirmed case of cholera was 2651cases with two deaths only.

The work reported here has shed light on this epidemic, together with the laboratory results and the surveillance data of information, together with the epidemiological curve on cholera since 1990.

Subjects, Methods, and Materials:

The study reported here is a descriptive Analysis is that the data is dynamic and every day and week there are new cases and discarded cases according to the CPHL results till at the end of month there will be a final report. The data is officially supplied from the Center of Disease Control/ Ministry of Health) and presented in the Sub-Regional Meeting on Scaling Up preparedness and response to current outbreak of cholera in Iraq and neighboring countries, 16-17 October 2015, Beirut, Lebanon. As Iraq is an endemic country for cholera, the case definition of the disease is followed for its detection. The case definition in the endemic countries by the WHO is mentioned above and was followed carefully.

Accordingly, cases with the above conditions who attended hospitals and health facilities are examined for stool cultures and sero- typing for Vibrio cholerae in the Central Public Health Laboratory (CPHL). Those who are stool culture- positive are reported here as cholera cases. Follow up of the hospitalized cases for their outcome, and the health facilities they managed in are reported. Diarrheal cases reported from all governorates of Iraq together, with the positive cases of cholera are mentioned also. Epidemiological curves for cholera and acute diarrheal diseases for the years 2013, 2014 and 2015 are presented for comparison.

The number of acute diarrhea cases together with cholera cases for the year 2015, their ages and gender are reported according to the distribution by weeks. The trend of cholera in Iraq since the year 1990- 2014 is reported.

Results:

In the year 2015, Iraq by all its governorates has faced an epidemic of cholera started during the third week - on of September. The total number of admitted cases of cholera till 6/11/2015 that were laboratory confirmed are 2651 cases, and two reported deaths only. The deaths were one male from Baghdad and one female from Babylon; their ages were 50 years and thirty years respectively; they died because of irreversible circulatory collapse and renal failure due to their delay in reaching the hospital. The number of cases included in this analysis was 1691 till the eleventh of October 2015, added to it through the official daily record by the MoH, and addition of confirmed cases of 960 cases of cholera till the sixth of November 2015 to bring the total to 2651 confirmed cases. As it is shown in the Fig. 1, the epi curve of acute diarrheal cases for the all ages has started to sharply increasing from the 23rd of September (week 37); the index case was from Abu Graib province of Baghdad. The peak number of cases was in the week 39 to decline steadily after that. The epi curve for diarrheal cases for the years 2013- 2015 is comparable in Fig. 2 that shows sharply increasing during the week 39 for the year 2015. The number of stool tested for cholera for the year 2015 was increased when cholera was suspected as it is shown in the Fig. 3; this was sent to the CPHL for confirmation of the presence of vibrios. It appeared from Fig. 4 and 5 that all age groups were involved by the acute diarrhea and those below five years old are parallel to those exceeding this age.

The same thing was seen for the gender that both males and females were affected by the disease without significant difference (1477 males and 1174 females), Fig.6.

All the Iraqi provinces and governorates are attacked by the cholera and the disease was reported from all Baghdad areas, Babylon, Najaf, Karbala, Muthanna, Nasireyah, Basra, Diwaneyah, Wasit, Misan, Kirkuk and the Anbar governorates, (Fig.7). Figures 8 and 9 show the distribution of suspected cholera cases by weeks of hospitalization and the confirmed cases of cholera according to the different provinces. This shows the peak number of the admitted and confirmed cholera cases all over Baghdad and the other Iraqi governorates was during the week 39, starting from the week 38 and then started to steadily escalating down in the weeks 40, 41 and afterword's. The laboratory – confirmed cases of cholera among all the acute diarrheal cases from all the governorates is shown in the Fig. 10 and in the Table 1. The sero types of the isolates were of O1, sero type Inaba, with very few cases of Ogawa.

The epidemiological trend of cholera in Iraq since the year 1990 till 2014 has shown in the figure 11, that shows the years of 1999 and 2007 were of the major epidemics but with the absence of cases during the year of 2014 before the epidemic of 2015.

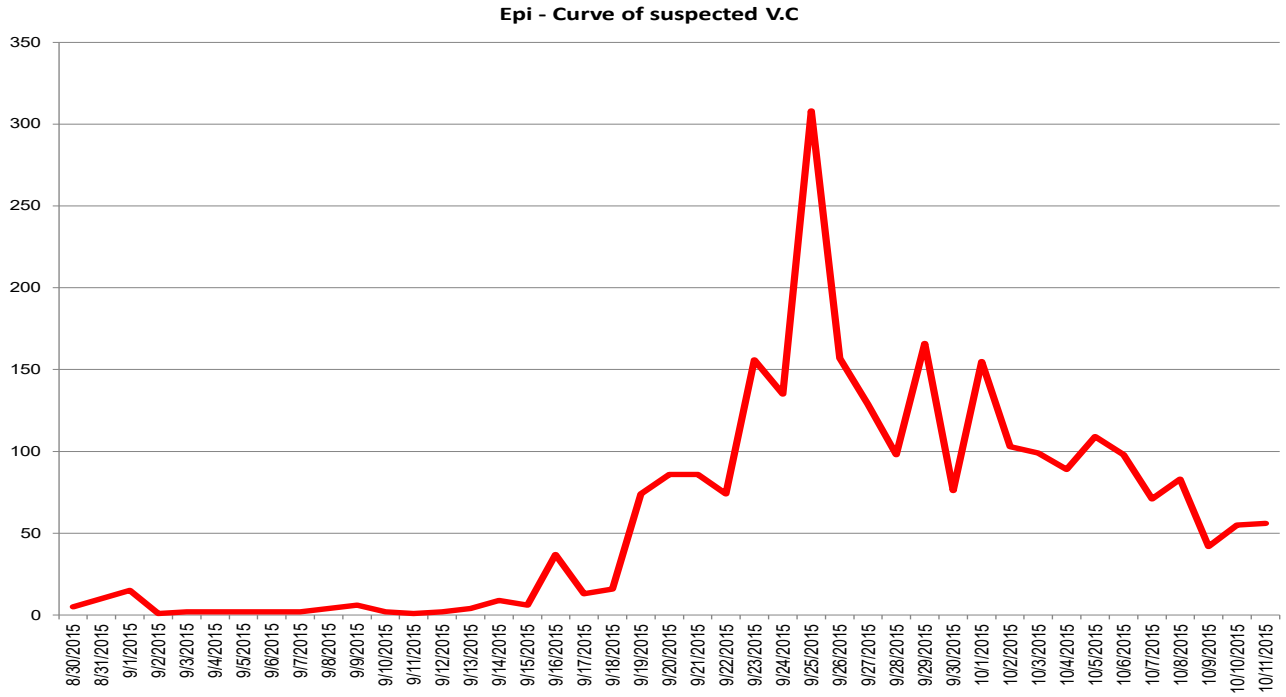

Figure (1): EPI- Curve of suspected cases of cholera in Iraq during 2015 (Ref. no.22, MoH, Directorate of Preventive Medicine, Section of Control of Infectious diseases)

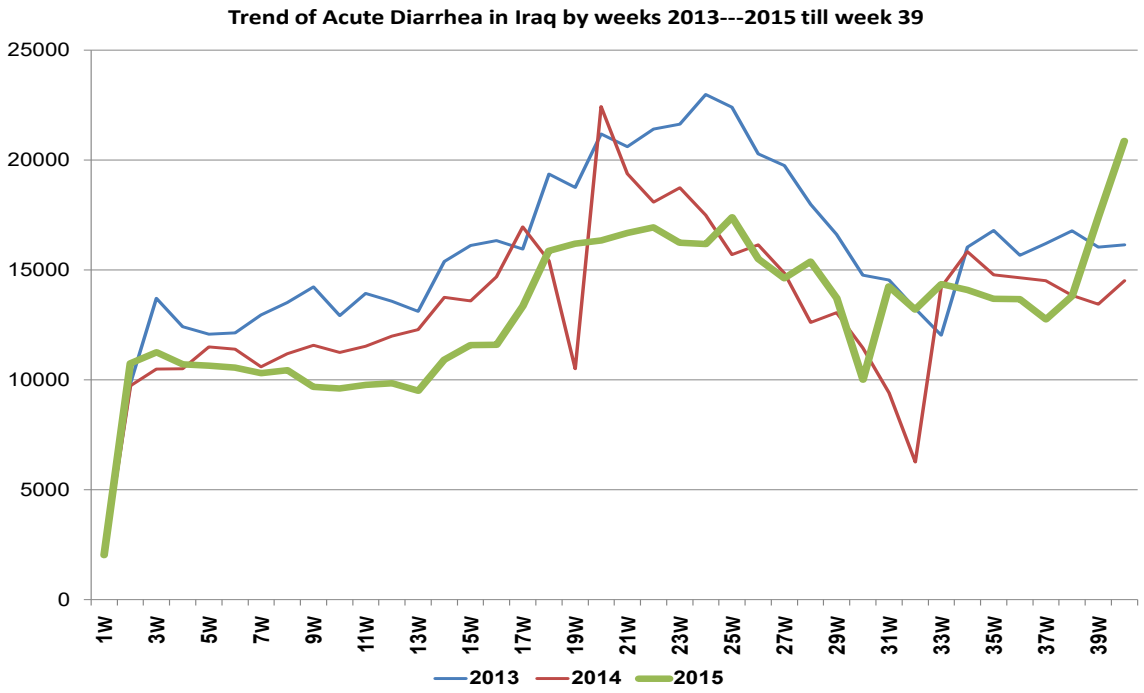

Figure (2): Trend of acute diarrhea cases in Iraq by weeks for years 2013-2015 till week 39, (Ref. no. 22)

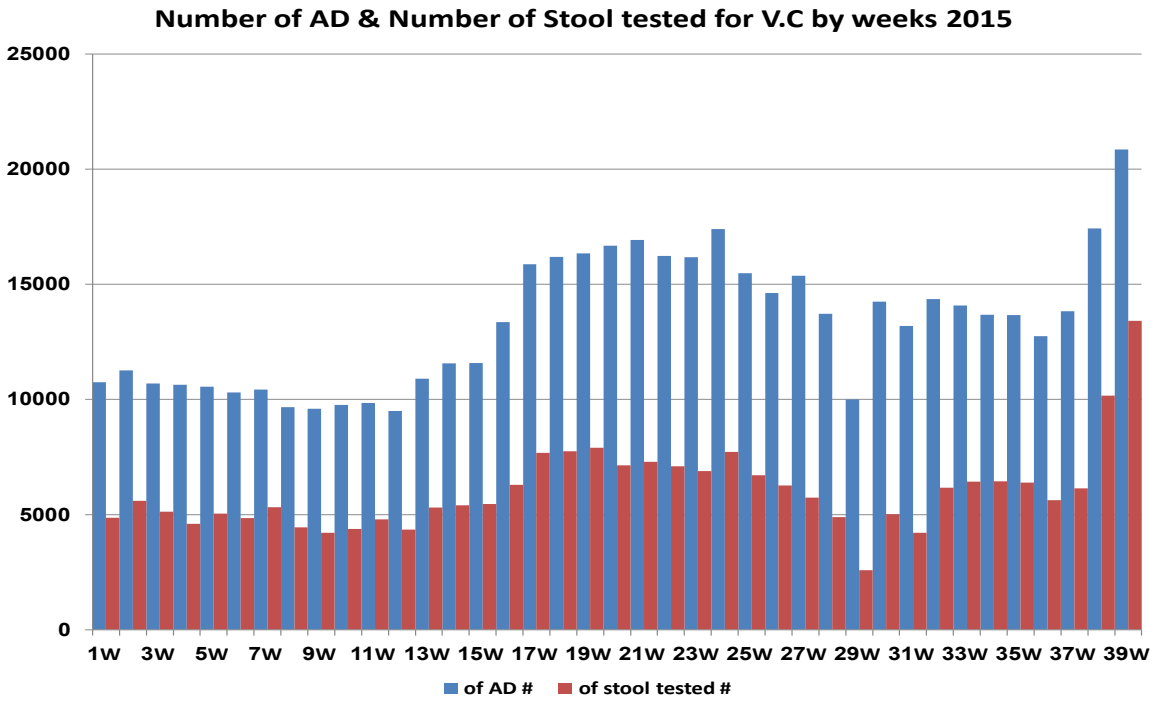

Figure (3): Number of diarrhea cases with number of stool samples tested for cholera till the week 39, (Ref. no. 22)

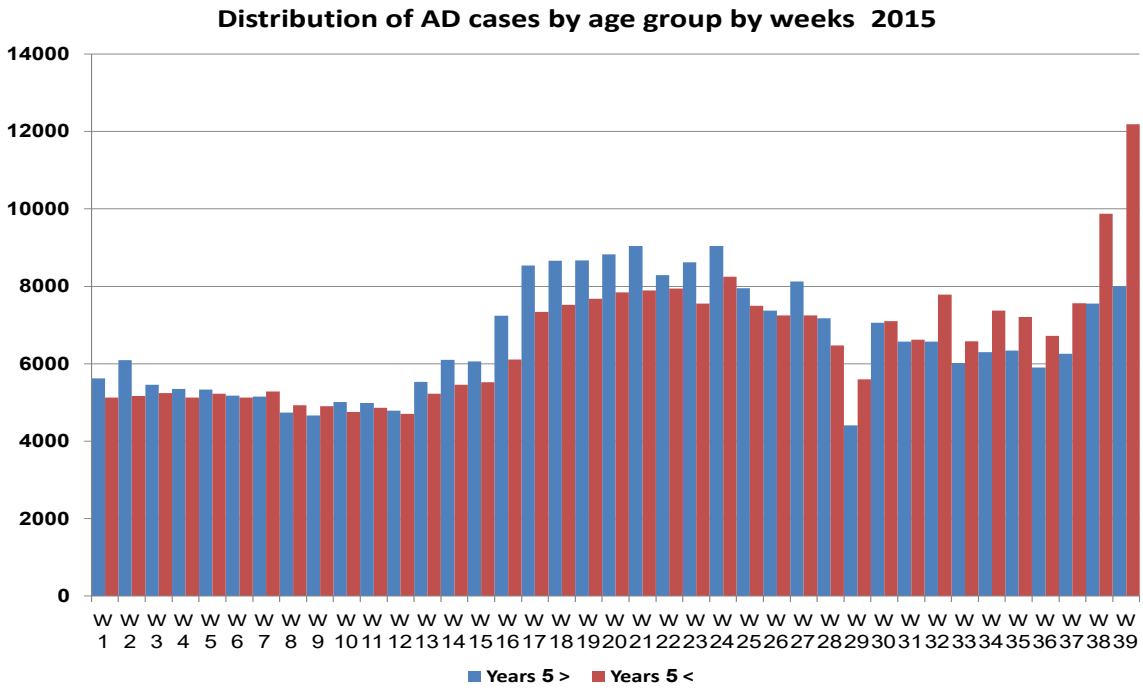

Figure (4): Distribution of cholera cases by age group by weeks of their hospitalization, (Ref. no. 22)

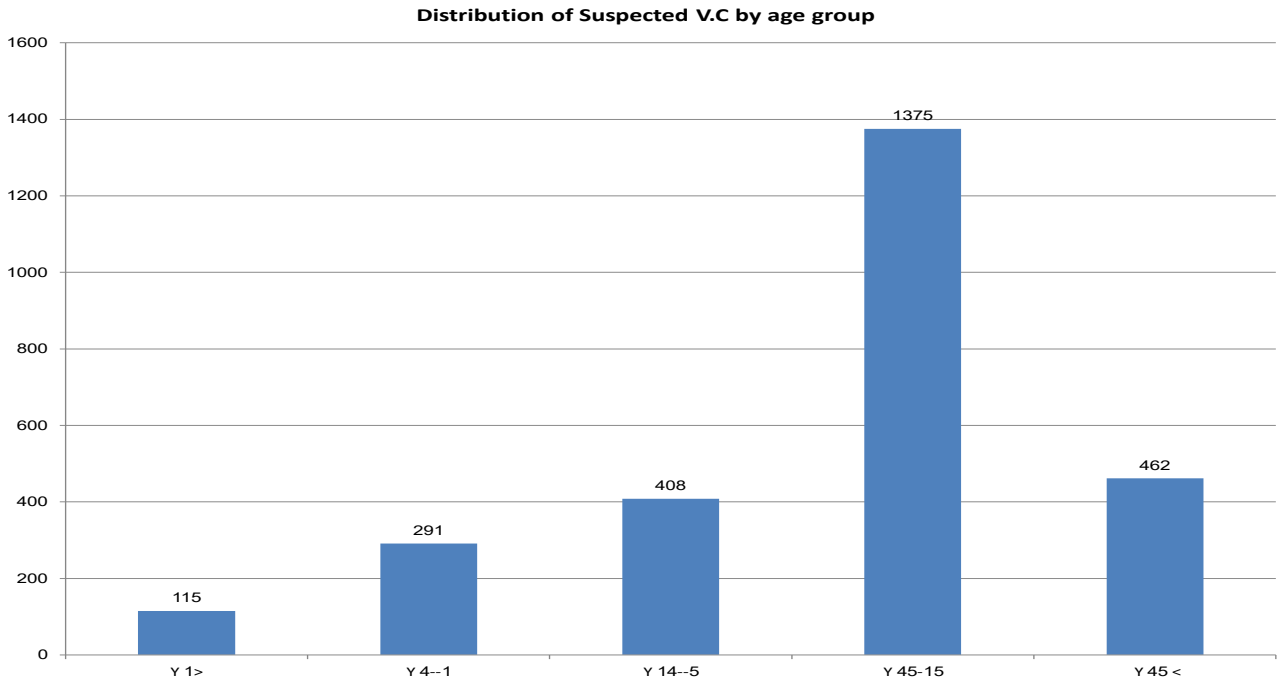

Figure (5): Distribution of suspected cholera cases by age group, (Ref. no. 22)

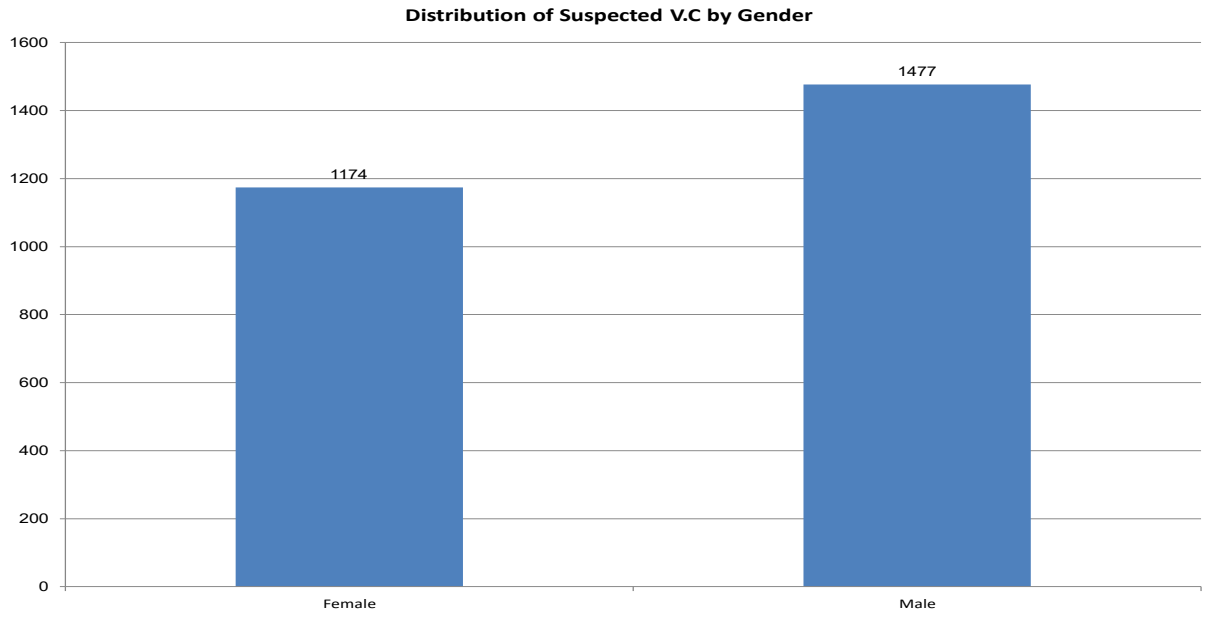

Figure (6): Distribution of suspected cholera cases according to their gender, (Ref. no. 22)

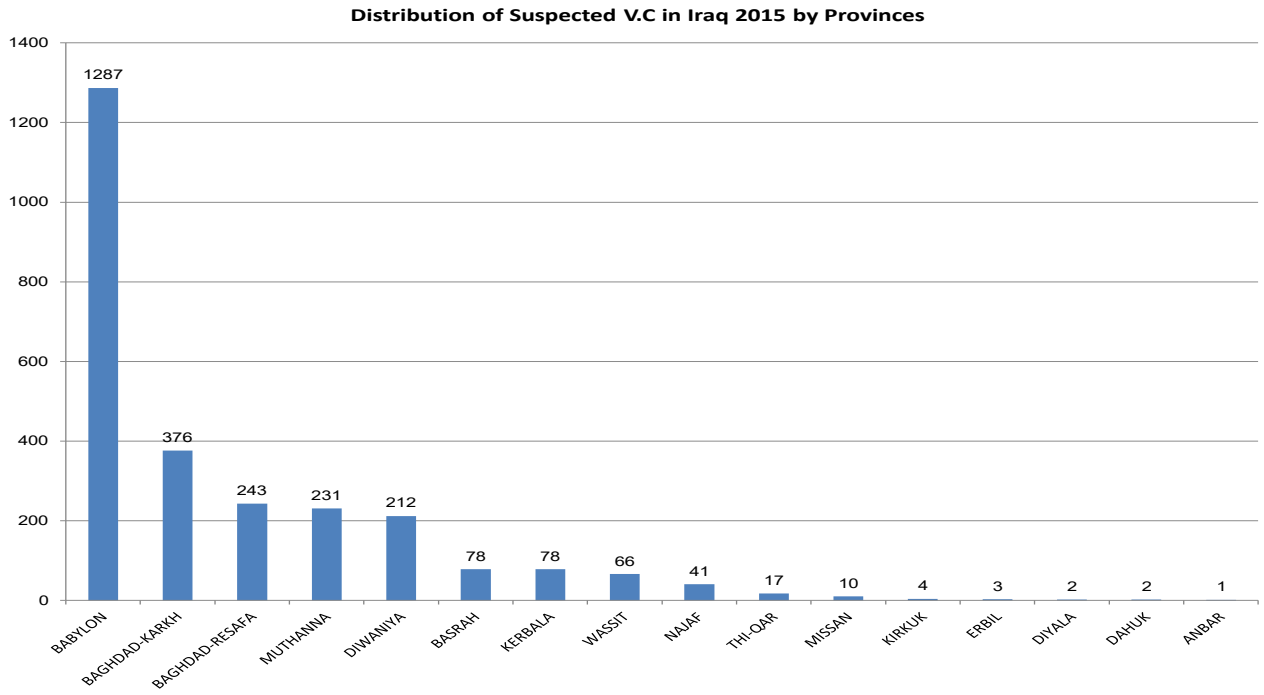

Figure (7): Distribution of suspected cholera cases according to the provinces & governorates, (Ref.22)

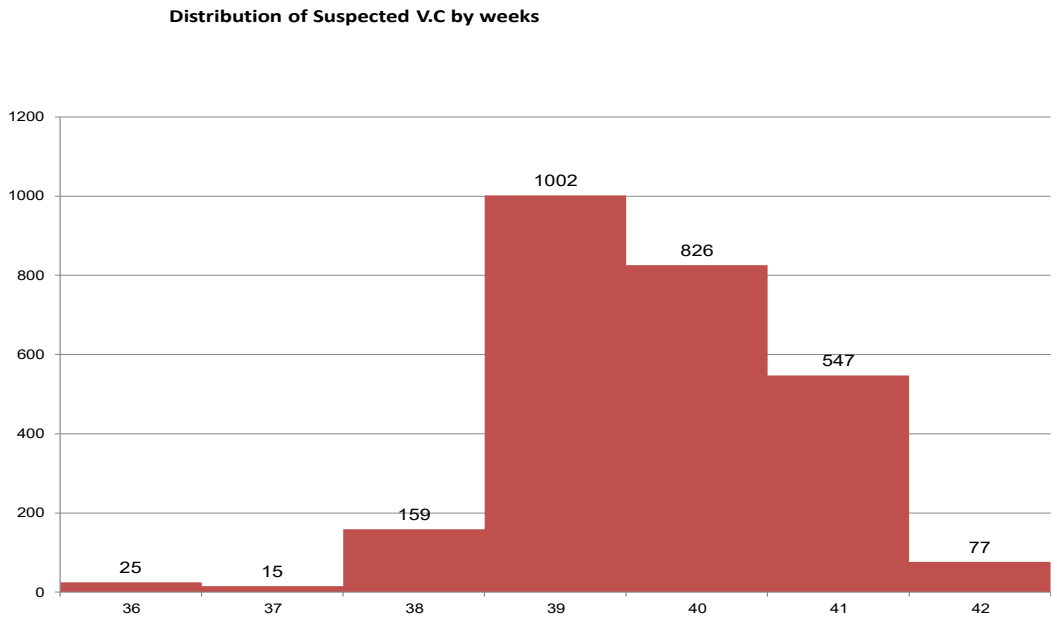

Figure (8): Distribution of suspected cholera cases by weeks of hospitalization, (Ref. no. 22)

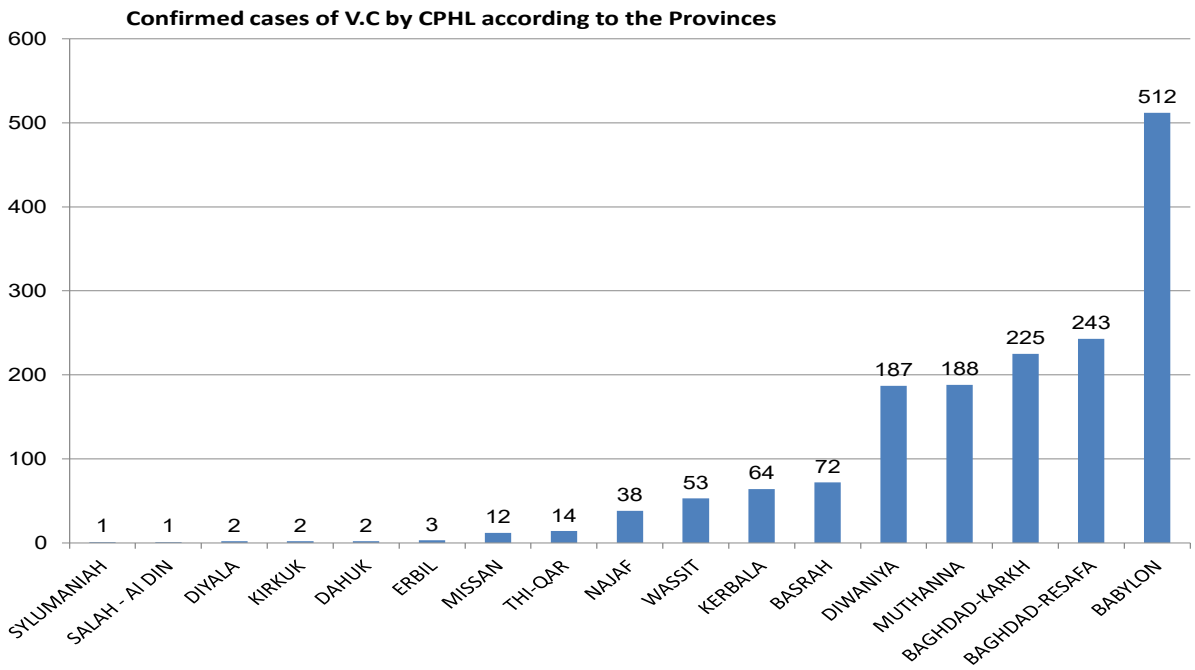

Figure (9): Confirmed cases of cholera according to the provinces, (Ref. no. 22)

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CONFIRMED CHOLERA CASES

DOH	CASE	%	DOH	CASE	%
KERGH	225	13.8	WASIT	53	3.2
RESAFA	243	15.1	THIQAR	14	0,9
NAJAF	38	2.3	DIALA	2	0.1
DIWANYIA	187	11.5	SULAIMANYIA	1	0.06
BABIL	512	31.6	ERBIL	3	0.1
MUTHANA	188	11.5	SALAHDIN	1	0.06
BASRA	72	4.4	DUHOK	2	0.1
MISAN	12	0.7	KIRKUK	2	0.1
KERBLA	64	3.1	TOTAL	1691	

Table (1): Distribution by percentages of the confirmed cholera cases according to the provinces, (Ref. no. 22)

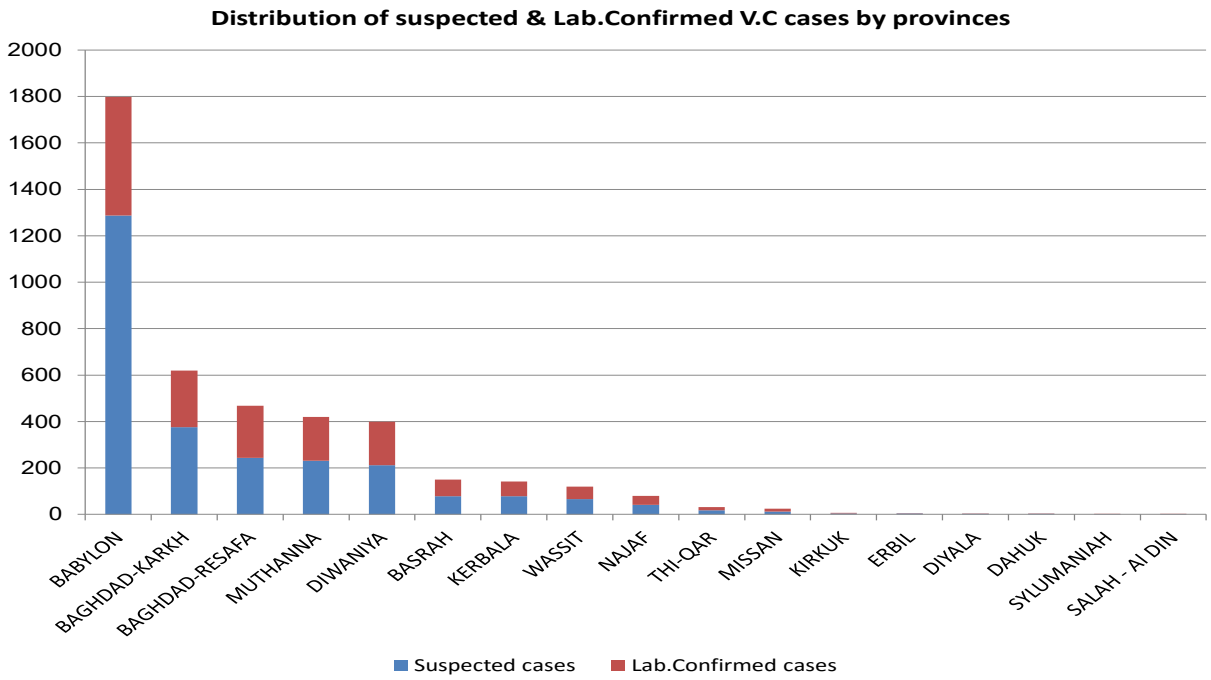

Figure (10): Distribution of suspected and confirmed cases of cholera, (Ref. no. 22)

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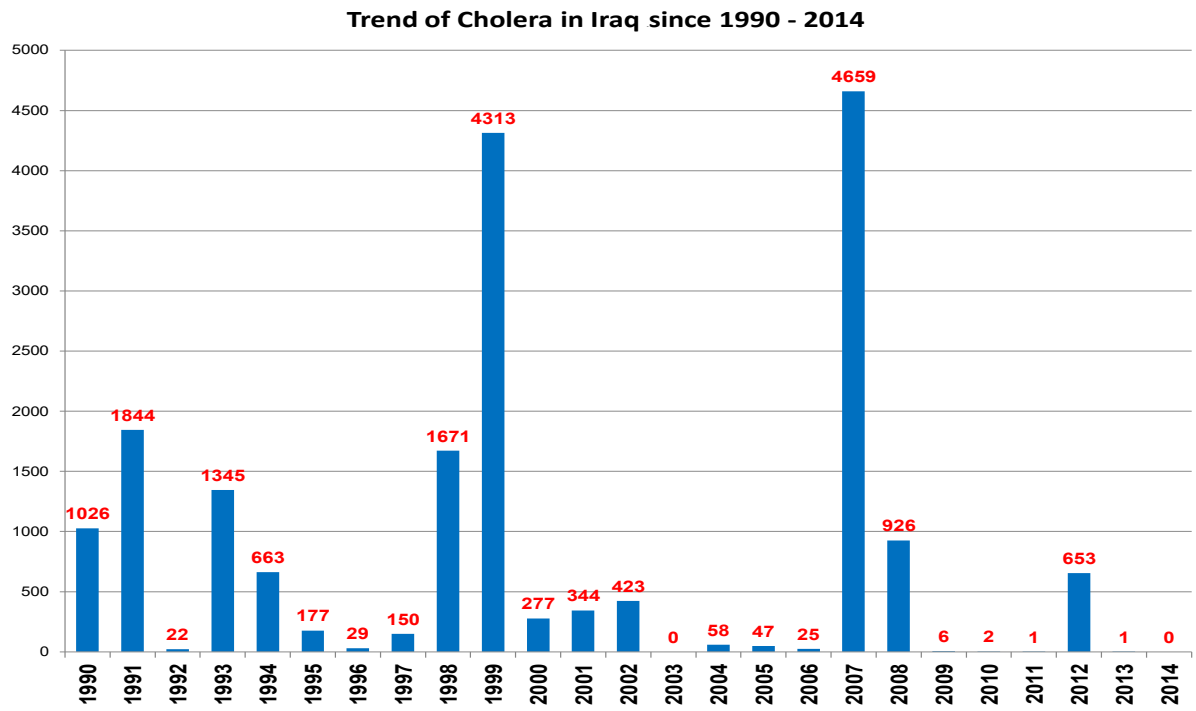

Figure (11): Epidemiological trend of cholera in Iraq between 1990- 2014 (Ref. no. 22)

Discussion:

Surveillance on cholera must be shared on the regional and global levels through an integrated disease surveillance system. The information should be dealt with in feedback at the local and global levels of sharing data 1-7, 11, 17, 19. The impact on cholera strikes and transmission was significant in all emergency settings and endemic outbreaks.

Notification of all cases of cholera is no longer mandatory under the International Health Regulations, even though, public health issues related to cholera must continuously be assessed against the criteria of the Regulations to draw a conclusion if an official notification is needed 17, 19, 20.

It is to strengthen the Local capacities for collecting, and analyzing data, also to improving the diagnosis are need for comprehensive control activities. This will help the populations living in high-risk areas to be identified in order to benefit from the control measures. Iraq is one of the EMR countries, located in the South West of Asia and has borders with Turkey, Iran, Kuwait, KAS, Jordan and Syria 21, 22.

The WHO Global Task Force on Cholera Control act to help to support the global strategies to share the capacity building for the global prevention and control of cholera. It is to provide a forum for coordination, exchange of technical activities and cooperation on cholera and water- borne related diseases to strengthen countries' capacity for prevention and control of these diseases 11, 16-19.

Also the WHO Global Task Force on Cholera Control helps to support the development of research ideas with emphasis on creation of innovative protocols to cholera prevention and control in the invaded countries (Weekly Epid. Rec. WHO, 2 Oct. No. 40. 2015. 90, 517-544.)

Iraq has 18 Provinces and the health authority is organized by the Ministry of Health that controls all health facilities (public & private sectors) on central and peripheral levels. The total population is estimated to be 35 million; it has 19 Directorates of Health, 135 Health Districts and 1350 Health Surveillance Sites 22.

Social mobilization:

Iraq is suffering of repeated external and internal wars since more than three decades; unstable life, destruction of homes and mobilization of its people are dynamic processes. Unsafe life, shortage of electricity, lack of safe water for drinking among wide

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communities besides, the destruction of it's infra structures hasn't been corrected. The ISIS attack on the country since more than one year and its occupation and demolishing of the standards of life for nearly four governorates had led to a huge mobilization of people for temporary camps mostly of tents. In these camps there is great shortage of facilities and requirements for an ordinary life especially a clean water and sewage disposal.

The heavy rain during the end of October 2015 had led to flooding of most of the streets and houses in Baghdad and many of other cities all over Iraq due to a bad or closed or not maintained sewage systems. Another problem facing the country and the health authorities is the masses of people in millions of pilligrims during the days of Ashura and other million visitors to the holy shrines in Karbala and the Najaf governorates especially during the month of Moharram. The food processing in hundreds of tons for these pilligrims in different stations of movement and their water supply for drinking are not an easy process and might disseminate the disease for further locations (The Sub-Regional Meeting on Scaling Up preparedness and response to current outbreak of cholera in Iraq and neighboring countries, 16-17 October 2015, Beirut, Lebanon).

Cholera has became an endemic disease in Iraq; the above mentioned destruction of the infra structures and the mobilization of people had made the outbreaks and epidemics of the disease to happen in the country during the hot months and classically during September. The epidemics were striking nearly every ten years, but with irregular outbreaks 10, 22. Despite cholera stroked 42 countries during the year 2014, with a total number reported to the WHO of 190 549 cases with 2231 deaths but few countries had carried the heaviest burden (Democratic Republic of Congo, Ghana, Nigeria from Africa, Afghanistan, from Asia and Haiti from the America); the overall CFR was 1.17% (Weekly Epid. Rec. WHO, 2 Oct. No. 40. 2015. 90, 517-544). The risk factors involved for the dissemination of cholera and contributing to its long lasting outbreak are inadequate supply of safe water, poor sanitation, inadequate disposal of waste and poor personal hygiene. The clinical expression of cholera is ranging from severe florid picture to asymptomatic infection 10; the ratio of infection to clinical disease status ranges from 1:3 to 1:100. The asymptomatic infection during epidemics will help to spread the disease rapidly, to stop the outbreak from spread to the remote areas; an early identification of the initial cases is needed. The prevention of cholera can be done through the application of the control measures on the disease as the rate of cholera carriers varies from 18% - 22%among household contacts and 0.34% - 1.3% through the community. These rates are present even in the absence of clinical disease 18-20.

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OCV is included among the strategy of control of cholera; it is promising to be effective to give immunity for six months to five years on revaccination. In 2013; the GAVI board approved a strategic aid to the global cholera vaccine stockpile for endemic and epidemic settings for the years 2014-2018.

Conclusion and Recommendation:

Cholera as it is acute communicable diseases, immediate notification surveillance on it needs, daily, weekly and monthly reports and syndromic surveillance when the situation is complex. Epi- info soft ware and internet services are used to collect and distribute the surveillance data to the center with a feedback of the dynamic information. Health education campaigns can be distributed to local and remote cultures to help for the practicing of sound personal hygiene as hand-washing with soap, safe preparation and storage of food and encourage breastfeeding. Innovative WASH interventions can be practiced to prevent cholera, so as the use of rapid diagnostic tests (RDTs) for the diagnosis of suspected vibrios in stool.

Currently there are two WHO oral cholera vaccines (OCVs) (Dukoral® and Shanchol®); both vaccines were used in mass vaccination campaigns with WHO support. OCV can be used as a public health tool in protecting populations at high risk of cholera.

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ACADEMIC RESEARCH (MSc research)

Ovarian reserve of infertile women having polycystic ovarian syndrome: Chronological based factor

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Abstract

Backgrounds: Patients with polycystic ovarian syndrome (PCOS) are more likely to be presented with infertility and hormonal imbalance. Ovarian reserve in PCOS patients is often high and age factor effect in the counting of this reserve is a matter of controversy.

Objectives: This study was intended in a small sample of women to assess the effect of age on the female reproductive hormones and ovarian ultrasound markers, also, to find any relation between the chronological age and the ovarian age (represented by ovarian reserve); of infertile women having polycystic ovarian syndrome (PCOS), in comparison to a group of fertile female, in Iraqi society.

Patients and methods: A total number of forty infertile women presented with infertility having no any infertility cause other than PCOS; by clinical, hormonal and ultrasonic assessment (Group I) and another forty fertile women with no any clinical, hormonal or ultrasonic evidence of PCOS (Group II) which was regarded as the control group. Serum hormonal assay was taken account of FSH, LH, E2 and testosterone. The ultrasound determinants included estimation of ovarian volume, numbers and diameters of follicles.

Results: In women belonged to Group I when compared with Group II; there was significant low serum FSH level in patients aged ≥ 30 years with significant high LH hormone level in both age groups. Also, Group I had significant low serum E2 level at age < 30 year but high level at age ≥ 30 year. Significant inverse correlation between mean ovarian volume and the numbers of antral follicle with the age factor in Group I was noticed.

Conclusions: Ovarian reserve in PCOS clients is significantly elevated and chronological factor is an independent factor for alterations of markers that related to ovarian reserve in a sample of Iraqi women.

Key words: Polycystic ovarian syndrome, Ovarian reserve, Hormonal assay, Aging.

Introduction

Adulatory causes of infertility comprise about 30.65% of Iraqi female infertility, while tubal infertility is present in about 38.66%. However, cervical, uterine, endometriosis, male factor, and unexplained causes involve 10-15%, 9.76%, 5.43%, 21% and 15.5% respectively of checked couples [(1)]. The causes of infertility worldwide may be ovulatory dysfunction, impaired oocyte class, uneasy endometrial receptiveness, endometriosis, tubal and peritoneal infection, elevated abortion incidence and high chromosomal abnormality rate [(2)]. Polycystic ovarian syndrome (PCOS) is the commonest endocrine disorder in women worldwide, at reproductive age, associated with metabolic disturbance, fundamental to which is the peripheral insulin resistance and compensatory hyperinsulinemia. These metabolic abnormalities have implications both as reproductive dysfunction and long term health disorder [(3)]. Oligomenorrhea, amenorrhea, irregular, few or absent menstrual period and heavy bleeding are also an early warning sign of endometrial cancer, for which women with PCOS are at high risk [(4)]. Pregnancy in women aged over 40 years as a rule is exceptional, since older women has a low reproductive ability [(5)]. They have defective oocyte quality often with increased risk of fetal chromosomal anomalies as well as great risk of spontaneous miscarriage. Although it is expected that the ovary grow old in a certain time, organic aging of the ovaries may occur independently of chronological age. Hence viewing for ovarian reserve is fundamental in primary infertility assessment. Antral follicle count is done by counting the resting follicles within the ovary at the start of each menstrual cycle. They are about 2 – 9 mm in size. It can be a hint of the ovarian reserve for a woman. An elevated antral follicle count points out that a woman has a great number of eggs remaining in her ovary, or at times PCOS.

Ovarian reserve usually assessed by hormonal assay, ultrasound image and ovarian biopsy [(5 & 6)]. Nevertheless, ultrasound examination could detect so many data for assessment of infertility [(7)].

Therefore, the current study intended to assess the ovarian reserve in respect to the age in small sample of Iraqi women presented with PCOS. The assessment included clinical examination, hormonal assay and ultrasound measurements.

Materials and methods

This study was conducted at the Department of Anatomy, Histology and Embryology in cooperation with the Department of Medical Biochemistry, College of Medicine, Al-Mustansiryia University, in Baghdad, Iraq and was extended from December, 2008 to June, 2009. It was approved by the local scientific committee of institute. A verbal consent was obtained from each patient prior to the study .These patients were recruited from the Center of Infertility at Al-Yarmouk teaching hospital in Baghdad, Iraq. Polycystic ovarian syndrome was diagnosed when two of the following criteria were present: Oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries [(8)]. Modified Ferryman-Galway by single observer was used to assess the degree of hirsutism score of at least 8 of 36 was taken as significant [(9)]. The criteria of inclusion included; infertile or fertile women, known cases of polycystic ovarian syndrome, age ≥ 40 years old. Women with infertility that not related to polycystic ovarian syndrome, other causes of androgen excess or related disorders, and patients on hormonal therapy were excluded. A total number of forty known cases of PCOS presented with infertility (Group I) and forty women without clinical, laboratory or ultrasound evidence of PCOS (Group II) were enrolled in this study. The clinical records of patients were obtained and the anthropometric measurements including body weight (kg), height (cm) and the

calculated body mass index (kg\m^2) were determined. The patients were categorized according to the body mass index (BMI) into overweight-obese if the BMI ≥25 kg/m^2 [10].

By ultrasound technology [Vaginal ultrasound (Esata) hospital type (Europe)], on 3rd day of menstrual cycle the ovaries were assessed for ovarian volume and follicle number per ovary.

Ovarian volume was calculated using the formula for an ellipsoid (0.5 x length x width x thickness). Each ovary was scanned in both longitudinal and transverse cross-section from the inner to the outer margins to count the total number of follicles of 2-9 mm diameter from both ovaries [7].

Venous blood sample was obtained from each patient (Day 3 of cycle) for hormonal assay, using Addendum-Mini VIDAS apparatus (VIDAS, BioMerieux, France), through an enzyme linked fluorescent assay (ELFA) technique. This test was used for the estimation of E2 and testosterone, while the principle of estimation of follicle stimulating hormone (FSH) and luteinizing hormone (LH) combine a one step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). Each studied group was sub-grouped into patients < 30 years and ≥ 30 years to elucidate the ovarian reserve on chronological bases.

Statistical analysis

The results were expressed as mean ± SD and percent. The data were analyzed using two tailed, unpaired student's t test, difference between proportions test and simple correlation test taking $p \leq 0.05$ as the lowest limit of significance [11]. All calculations were made using Excel 2003

Results

Table 1 showed the characteristic of patients and subjects enrolled in this study. There were non-significant differences in distribution of cases according to the age factor. Obstetric history revealed significant delayed menarche age, high percent of irregular menstrual cycle, history of dysmenorrhea, and history of abortion in patients with PCOS (Group I); when they were compared with patients without PCOS (Group II). ***Table 2*** showed the clinical and hormonal assay employed here, for diagnosis of PCOS. Significant high serum testosterone level observed in Group I which approximated two folds of corresponding level of Group II. Hormonal assay that related to ovarian reserves showed imbalance of FSH, LH and E2 hormones in PCOS as shown by ***table 3***. Follicular stimulating hormone level was significantly less in patients with PCOS aged ≥ 30 years, while LH hormone tended to increase significantly, irrespective to age factor in PCOS in comparison with those without PCOS. There was significant lower E2 hormone level at age < 30 year and higher E2 hormone level at age ≥30 year in patients with PCOS; again when they were being compared to those without PCOS. Ultrasound findings included significant high ovarian volume (> 10ml) was observed in 37 out of 40 patients with PCOS and all patients have more than 12 follicles of 2-9 mm diameter in PCOS patients, as shown by ***table 4.***

Table 1 Characteristics of clients in this study

	Group I (n=40)	% within Group I	Group II (n=40)	% within Group II
Age (year): **< 30**	18	45	16	40
Age (year): **≥ 30**	22	55	24	60
Housewives	27	67.5	29	72.5
Employee	13	32.5	11	72.5
Menarche age: **< 13 year**	14	35	33	82.5
Menarche age: **> 13 year**	26	65*	7	17.5
Irregular menstrual cycle:	36	90*	18	45
Dysmenorrheal menstrual cycle:	31	77.5**	19	47.5
History of abortion:	24	60*	10	25

The results expressed as number (%).* $p < 0.001$, ** $p < 0.05$ compared with corresponding data of Group II.

Table 2: Characteristic features of polycystic ovarian syndrome in this study

	Group I (n=40)	% within Group I	Group II (n=40)	% within Group II
Body mass index (kg/m^2) ≥ 25	36	90*	18	45
Hirsutism	34	85*	4	10
Serum testosterone (ng/ml)	0.85±0.19*		0.43±0.16	

The results expressed as number (%) and mean± SD. * $p < 0.001$ compared with corresponding data of Group II.

Table 3: Selected Hormonal assay to indicate ovarian reserve:

	Group I (n=40)		Group II (n=40)	
Selected Hormone	< 30 year (n=18)	≥ 30 year (n=22)	< 30 year (n=16)	≥ 30 year (n=24)
Follicular stimulating hormone(FSH) (mIU/ml)	4.65±1.6	4.36±1.32*	4.71±0.8	7.89±1.68
Lutinizing hormone(LH)(mIU/ml)	9.77±4.63*	7.81±1.91*	3.29±0.84	4.04±1.52
E2 (pg/ml)	47.4±4.13*	42.0±7.34**	54.22±4.67	39.35±10.57

* $p < 0.001$, ** $p < 0.05$ compared with corresponding data of Group I.

Table 4: Ultrasound findings: Mean ovarian volume and antral follicle count:

Ultrasound findings	Group I (n=40)		Group II (n=40)	
	No	percentage	No.	percentage
No. of women having increased mean ovarian volume (>10 ml)	37	92.5%	zero	zero%
No. of women having mean antral follicle count =>12	40	100.0%	5	12.5%

The Pearson chi-square statistic is significant at the 0.05 level.

Discussion

Patients of group I in this study had late onset of menarche which might be linked to genetic and/or environmental factors [12, 13 &14]. Also, patients of group I had elevated rate of abortion; most likely due to the effect of PCOS, which is well known to cause repeated abortion [15]. The most likely explanations of these events are the changes in the follicular constituents including androgens and insulin which ultimately lead to disruption of the interface between the endometrium and the placenta, leading to placental insufficiency and miscarriage [16 & 17]. The high BMI value seen in PCOS patients within this study was in agreement with the results of others when they stated the obesity effect on reproduction in different ways; interferes with induction of ovulation [18 & 19], increases the incidence of gestational diabetes, preeclampsia, premature labor, neonatal macrosomia, and stillbirth [17]. The results of this study showed that patients of group I had high E2 levels in same patients were not being related to chronological factor [20]. The FSH level changes in women of group I; seemed to be influenced by age factor. They had significant high FSH level at age ≥ 30 years compared with subjects without evidence

of PCOS features, which may be attributed to the disturbances in hypothamic-pituitary-ovary (HPO) axis as a result of ovarian failure, present usually in PCOS [21].

This study showed that the changes in LH hormone were not related to age factor. Therefore aging may be considered as independent additive factor to the ovulation failure in PCOS. Despite its specification in triggering ovulation, but certain mutations of LH and LH receptors may lead to changes in bioactivity of this hormone, as in PCOS, rendering it inactive, even with its high serum level; according to previous studies [22]. Also, as a result of normal aging process the menstrual cycle becomes irregular and could be anovulatory which may be responsible for high serum level of LH due to the negative feedback mechanism [23]. Another cause of high level of LH in a woman with PCOS may be attributed to the elevated insulin levels, which contributed to the abnormal hypothalamic-pituitary-ovarian axis related to PCOS, so hyperinsulinemia increases the GnRH pulse frequency and leads to augment in LH level [24].

As it was expected, according to previous studies; in all age groups the testosterone hormone in the PCOS patients was higher than the control women [24 & 25]. Insulin resistance occurred in majority of PCOS subjects and it causes thickening of the ovarian theca and thereby increasing androgen production, also, insulin resistance basically can lead to the hormonal imbalance seen in PCOS, as the ovaries are particularly sensitive to insulin, and when insulin is thrown off balance, the ovaries try to compensate by producing to many of certain hormones, something that can throw the entire hormonal system off balance [14 & 24].

The counting of the antral follicle in women of group I was considered as a radiological marker of ovarian reserve which is usually of high integer in women with PCOS [23 & 26]. Women of group I showed enlarged ovaries and increase antral (resting) follicles count of 2-9 mm diameter which was linked to ageing process; that could be caused by PCOS effect [19 & 23]. The large ovarian volume in PCOS might be because the ovary contains 2-3 times the number of antral follicles and enlarged stroma [7 & 23]. The ultrasound findings in this study were in agreement with others [7, 13 & 23]. Positive correlation between antral follicles count and age was reported in this study as well as in other studies which may give a clue to anticipate the clinical symptoms of the PCOS that tended to regress or disappear when some of these follicles are lost [27]. It might be concluded here; the ovarian reserve in Iraqi PCOS patients is significantly increased and the chronological factor is an independent factor to alter the clinical markers of ovarian reserve.

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TOFIQ Journal of Medical Sciences

STUDY OF SERUM OXIDANT-ANTIOXIDANTS STATUS IN ADULT BRONCHIAL ASTHMATICS

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Abstract

Asthma is a chronic inflammatory airway disorder associated with recruitment of inflammatory cells. Asthmatic patients produce reactive oxygen species damaging the antioxidant defense system and forming a state of oxidative stress in asthmatics. The present study included 50 patients [25 males and 25 females] and 50 supposed healthy subjects (control) [25 males and 25 females]. The results show a presence of a significant increase ($p \leq 0.05$) in malondialdehyde (MDA) and ceruloplasmin (Cp) levels in both sexes of bronchial asthmatic (BA) patients in contrast with the control group. On the other hand, transferrin (Tf) showed a significant decrease ($p \leq 0.05$) in male BA patients compared with the control group, whereas, serum Tf levels demonstrated a non-significant decrease among the female BA patients in comparison with the control group. Correlation coefficient (r) test is used to describe the association between lipid peroxidation products and different antioxidants. This study revealed a significant negative correlation between MDA and the levels of Cp in both male and female BA patients ($r = -0.74$ and $r = -0.37$ respectively). Similarly, there was a significant negative correlation between MDA and Tf in male patients ($r = -0.89$), whereas the comparison between MDA with Tf revealed non-significant correlation in female BA patients. Asthma leads to a considerable an imbalance between antioxidants and oxidants. Both sexes of BA patients undergo an extraordinary degree of reactive oxygen species (ROS) development triggering considerable oxidative stress indicated by high levels of lipid peroxidation marker MDA and antioxidant Cp and low level of the antioxidant Tf in both male and female BA patients.

Keywords: *Bronchial Asthma, Malondialdehyde, Ceruloplasmin and Transferrin*

Introduction

Asthma may be observed as a diffuse obstructive lung disease with hyper-reactivity of the airways to a range of stimuli and a high degree of reversibility of the obstructive process, which may follow either spontaneously or as a result of treatment (1). When people talk about bronchial asthma, they are definitely talking around asthma, a common chronic inflammatory disease of the airways that causes the airway of the lungs to swell and narrow leading to coughing, shortness of breath, wheezing, chest tightness and excess mucus production (2). Asthma is a complex condition involving biochemical, autonomic, immunologic, infectious, endocrine, and psychological factors in variable degrees in different tpersons; it can be prevented by avoiding causes, such as irritants and allergens (3). Most persons can develop a severe exacerbation of asthma from various triggering agents. Bronchial asthma triggers may include smoking and secondhand smoke; infections such as flu, colds, or pneumonia; allergens such as food, mold, pollen, mites, dust and pet dander; exercise; air pollution and toxins; weather, mainly great changes in temperature; drugs (such as aspirin and beta-blockers); food additives; emotional tension and anxiety; singing, crying, or laughing; perfumes and fragrances and acid reflux. Both virus and bacterial infections of the upper respiratory tract infection can worsen asthma (4).Several mechanisms operate in cellular damage and death, lipid peroxidation caused by free radicals being one of the most important mechanisms. A free radical is an atom or molecule that has one or more unpaired electrons, they are two types; a reactive oxygen species (ROS), such as superoxide anion($O_2^{\bullet -}$),peroxide anion($O_2^{\bullet -2}$) , hydrogen peroxide (H_2O_2), hydroxyl radical($HO^{\bullet}$), and hydroperoxyl radical ($HO_2^{\bullet}$); and a reactive nitrogen species (RNS), such as nitric oxide ($NO^{\bullet}$) nitrogen dioxide ($NO_2^{\bullet}$), peroxynitrite ($OONO^-$) (5) . Because ROS are so reactive, they can inflict considerable damage on living cells if formed in significant quantities. These damage results primarily form enzyme inactivation, polysaccharide depolymerization, DNA breakage and membrane destruction (6, 7). Oxidative stress doesn't only cause direct harmful effects in the lungs but also activates molecular mechanisms that recruit lung inflammation (8). Thus, an imbalance between oxidants and antioxidants is considered to play an essential role in the pathogenesis of asthma. A lot of oxygenated compounds, particularly aldehyde such as MDA, are produced during the attack of free radicals to membrane lipoprotein and polyunsaturated fatty acid, products of lipid peroxidation formed in the primary site. These products reach the other organs and tissues via the blood stream provoke other lipid peroxidation and cause cellular and tissue damage (9). The Increase of lipid peroxidation could possibly play a role in the complication of cardiovascular disease, chronic pulmonary disease, cataract and cancer (10,11). Oxidative stress occurs when this balance is disrupted by extreme production of reactive oxygen species and / or by insufficient anti oxidative defenses, including superoxide dismutase, catalase , vitamins C and E, β-carotene, uric acid, glutathione and trace elements such as zinc , selenium , magnesium , cupper , iron , which are cofactor for many biochemical reactions (12, 13,14). Ceruloplasmin (E.C.1.16.3.1) is a multicopper enzyme carrying around 95% of circulating copper (15). Cp is an important extracellular antioxidant through ferroxidase activity, it also scavenges superoxide anion radical ($O_2^{\bullet -}$). Ceruloplasmin with its ferroxide activity can oxidize ferrous ions to the less toxic ferric form without releasing reactive oxygen species, causing protect the body from the potentially damaging effects of various oxidizing agents (16, 17, 18). Increased plasma ceruloplasmin levels are associated with the generations of oxidation products, i.e., $O_2^{\bullet -}$and H_2O_2 (19).

The aim of this study is to investigate the oxidative stress by evaluating MDA in patients with BA disease, and to determinate serum antioxidant status (by measurement Cp and Tf) compared with the control group, then to shed a light on the possible correlation between MDA and each of Cp and Tf.

Materials and Methods

Design of Study

This study was conducted at AL-Hussein Teaching Hospital in AL-Muthanna governorate (Iraq) at the period 10/3/2014 to 15/7/2014. The study includes 100 subjects, fifty patients with bronchial asthma [25 males and 25 females] with an age range [15- 65] years for male and female. The control group included 50 subjects [25 males and 25 females] with an age range [15-68] years, healthy, non-smokers without any history of lung disease and had normal pulmonary function tests.

Blood Sample Collection

From the patients with bronchial asthma and the control groups, 5mL blood sample was taken. Blood samples were collected from each subject by vein puncture, centrifuged at 3000 rpm for 10 min after permitting the blood to clot at room temperature. The serum was divided into two equal parts in sample tubes, serum MDA were tested within 24 hours, whereas the others were stored at -20°C till estimating Cp and Tf.

Lipid peroxidation Marker in Bronchial Asthma (Serum MDA)

Lipid peroxidation as serum MDA level was estimated in terms of thiobarbituric acid reactive species (TBA) *(*20). In this investigation, the chromogen is formed by the reaction of one molecule of MDA with two molecules of TBA. The process involves warming the sample (serum) with TBA and trichloroacetic acid, then reading the absorbance of (MDA-TBA) adduct at 532 nm.

Serum Antioxidants

Serum Cp concentration was measured by the method of Menden at 525 nm using para-phenylenediamine as a reagent (21). Transferrin (Tf) is the most important extracellular iron transport protein in humans (22). Transferrin concentration was measured by colorimetric method includes saturation of Tf with an excess predetermined iron concentration, removal of unbound iron by magnesium carbonate as a precipitating agent, then measurement of the iron-binding Tf (23). After centrifugation at 3000 rpm for 10 min, the iron in the supernatant is determined at 600 nm (24). The total iron binding capacity (TIBC) of human serum is considered to be a useful estimation of its transferrin concentration. The serum Tf concentration was calculated by the following equation (25).

(Serum Tf (gm/L) = (0.094 × TIBC (µg/dL) – 32.8)

Statistical Analysis

Statistical analysis was performed using Statistical Package Social Science SPSS version 15.0**.** Quantitative data were summarized as mean ± standard deviations values obtained for AP patients and controls. The one way analysis of variance (ANOVA) is a technique used to determine whether there are any significant differences between the means of different studied group. P-values ($P \leq 0.05$) are taken as the level of significance. Further, Pearson's correlation (r) was used to correlate between the different parameters in each patient group.

Results and Discussion

Asthma is a chronic inflammatory disease of the respiratory tract of unknown etiology. According to the world health organization, asthma is regarded a serious public health problem with over 100 million sufferers worldwide, death from this condition has reached over 180,000 annually (26,27). Oxidants-antioxidants balance is essential for the normal lung function. Both, an increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant stability in favor of oxidants, leading to lung injury. A number of diseases involving the lung, such as emphysema, bronchiectasis and bronchial asthma have balances (28). The present study revealed a highly significant elevation ($P \leq 0.05$) of MDA among all BA patients studied compared to healthy control in both males and female patients (Table 1), indicating thereby the relation of serum MDA level with that of the underlying inflammatory process in bronchial asthma. These imply that patients during asthmatic attack are wide-open to a great degree of lipid peroxidation. This result is consistent with the observations of others (11). MDA is a pointer of lipid peroxidation, it has a strong correspondence with atopic asthma, proposing that oxidative stress occurs simultaneously on lipid peroxidation. Oxidative stress can have differnt special effects on the function of airway, including contraction of airway smooth muscle, induction of airway hyper responsiveness, hyper secretion of mucus, vascular exudation and epithelial shedding. Moreover, ROS can induce the production of cytokine and chemokin, through the initiation of oxidative stress-sensitive transcription on nuclear impact in bronchial epithelial cells includes the consumption of oxygen and cosecutive release of ROS into adjacent cells (29). During the respiratory burst, the inflammatory cells have released high concentration of $O_2^{\bullet -}$, $OH^{\bullet}$, HOCl and H_2O_2 that may leak into adjacent cells resulting in increased amounts of free radicals in airway tissues. Moreover, the inflammatory cell of asthmatics has an increased capacity to create free radicals, which further contributes to high concentrations of ROS. One marker of airway inflammation in the asthmatics is the excess generation of reactive nitrogen species (RNS) (30). Cytokines may excite increased production of nitric oxide radical ($NO^{\bullet}$) which reacts with $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$), a cytotoxic species that has many destructive effects, including lipid peroxidation. Therefore, the additional amounts of ROS and RNS that are produced by asthmatics may overcome the host antioxidant defenses and cause oxidative stress (31, 32).

Table 1: Serum level of malondialdehyde in BA patients and healthy control classified according to sex.

Groups	n	MDA(nmol/mL) Mean ± SE	
		Female	Male
Control	25	14.63 ±0.35 [b]	13.72 ±0.49 [b]
Patients	25	338.00 ±10.38 [a]	405.50 ±9.14 [a]

The above values represent mean± SD values with non-identical superscript (a, b) were differ significantly ($p \leq 0.05$).

Ceruloplasmin

The oxidant/antioxidant imbalance has been proposed to play important role in the pathogenesis of asthma. However in the current study, serum ceruloplasmin level was increases significantly ($p \leq 0.05$) in both males and females BA patients as compared with normal subjects (Table 2). The present study is in agreement with another study (33). Increased ceruloplasmin levels were demonstrated in asthma patients (34). Levels of certain proteins in plasma rise through acute inflammatory state or according to certain types of tissue damage. These proteins are named acute phase proteins or reactants. They are synthesized by the liver in response to the mentioned cases. Following stimulus through infection or injury, the macrophages liberate monokine and interleukin-1, which induce the liver to secrete a lot of acute phase proteins. These reactants have been shown to serve different effective roles during tissue rehabilitation of injury or inflammation in various mechanisms of host immune defense. Cp is recognized to keep considerable ferroxidase activity and capable of suppression superoxide radicals. It is responsible for restricting the damage caused by these radicals (35).

Table 2: Serum level of ceruloplasmin in BA patients and healthy control classified according to sex.

Groups	n	Ceruloplasmin (g/L) Mean ± SE	
		Female	Male
Control	25	0.275 ±0.13 [b]	0.284 ±0.13 [b]
Patients	25	1.899 ±1.02 [a]	2.437 ±1.64 [a]

-Legend as in table (1)

Transferrin

The presence of inflammatory cells such as eosinophils, mast cells, macrophages and lymphocytes has been observed in different specimens of asthmatic patients. These inflammatory cells secrete several kinds of cytokines (36). Therefore, asthma and allergic inflammation include wide cytokine network. This cytokine and their complex relationship have an important role in inflammation and immune. The acute - phase reaction is a general response to inflammation and probably triggered by interleukins, released from the site of injury or inflammation. Interleukin-2, interleukin-6 and tumour necrosis factor-α are an important mediator in the synthesis of amyloid precursor protein by liver (37). In the present study, transferrin level was significantly lower ($p \leq 0.05$) in male BA patients compared with the control group. A similar result was observed by Mukadder (33). Similarity, Bakkeheim (38) found that there was a significant decrease ($p \leq 0.05$) in transferrin levels in asthmatic patients. In consistence with other observations showed no difference was observed in transferrin levels between the groups (34). Our study revealed non-significant decrease of Tf serum level among the BA female patients in comparison with the control group (Table 3).

Table 3: Serum level of transferrin in BA patients and healthy control classified according to Sex.

Groups	n	Transferrin (g/L) Mean ± SE	
		Female	Male
Control	25	3.39 ±0.12 [a]	3.52 ±0.13 [a]
Patients	25	2.61 ±0.25 [a]	1.74 ±0.39 [b]

-Legend as in table (1)

Correlation coefficient (r) between oxidant and antioxidant was studied for both male and female patient groups. The results of this study illustrate the presence of a significant negative correlation between Cp and MDA ($P<0.01$) in a male patient ($r = -0.74$) (Figure 1), also there was a significant negative correlation between Cp and MDA ($P<0.05$) in female patients ($r = -0.41$) (Figure 2). However, there was a significant negative correlation between transferrin and MDA ($P<0.01$) in a male patient ($r = -0.89$) (Figure 3). Similarity comparison between transferrin with MDA revealed a significant negative correlation ($P<0.01$) in female (BA) patients ($r = -0.86$) (Figure 4).

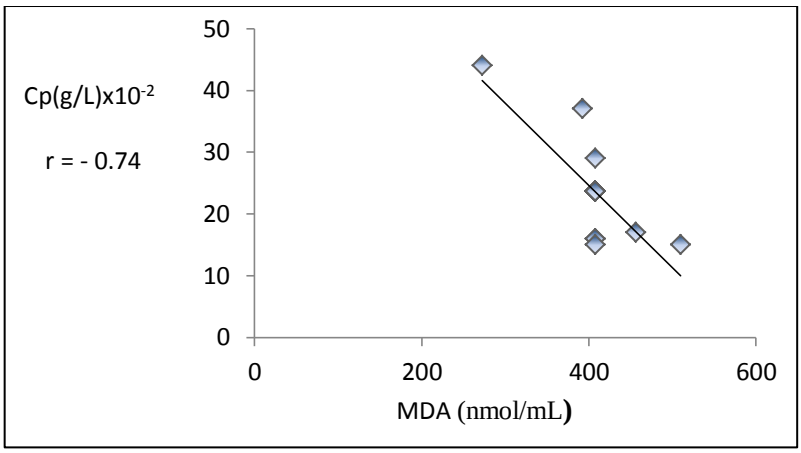

Figure 1: Correlation between MDA and Cp in male BA patients

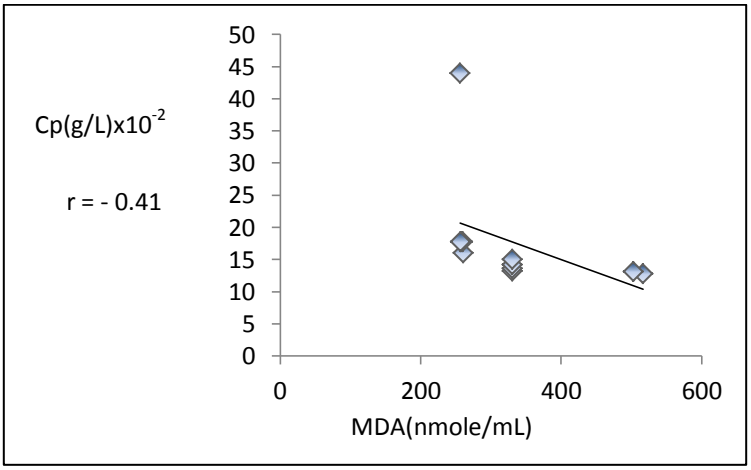

Figure 2: Correlation between MDA and Cp in female BA patients.

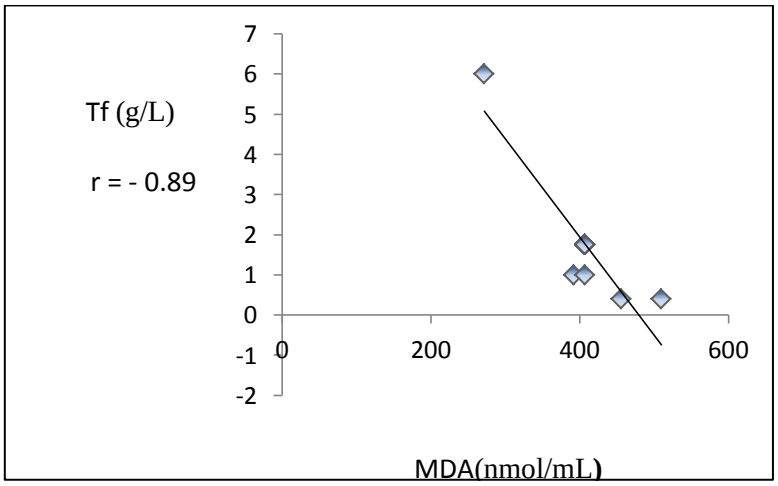

Figure 3: Correlation between MDA and Tf in male BA patients.

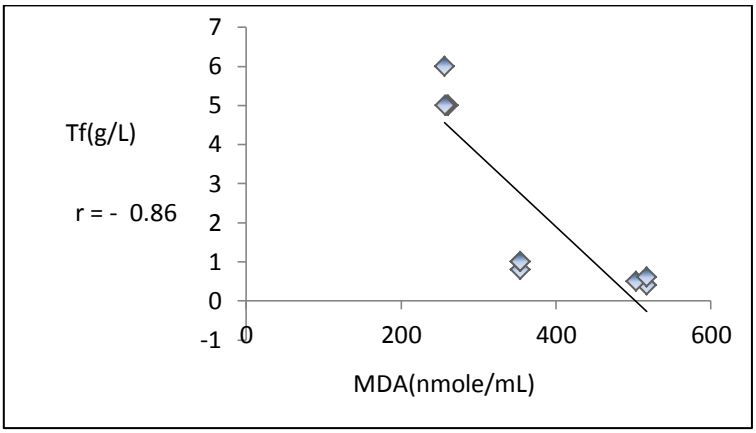

Figure 4: Correlation between MDA and Tf in female BA patients.

Conclusion

Bronchial asthmatic patients have increased lipid peroxidation level as detected by the increase in serum MDA level, which was an important factor in increasing the production of free radicals that developed bronchoconstriction. As a result of continuous production of reactive oxygen species the antioxidant Tf level was significantly decreased, while antioxidant Cp increased.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 2, Issue 2, (2015), 62-71 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

SYNTHESIS AND SPECTRAL STUDY, THEORETICAL EVALUATION OF NEW BINUCLEAR CR(III), FE(III) AND ZN(II) METAL COMPLEXES DERIVED FROM PYRANE-2-ONE AND ITS BIOLOGICAL ACTIVITY

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Abstract

The new acyclic Schiff base)L) ligand: 3,3'-(1E,1'E)-1,1'-(2,2'-azanediylbis (ethane-2,1-diyl)bis(azan-1-yl-1-ylidene))bis(ethan-1-yl-1-ylidene) bis(4-hydroxy-6-methyl-2H-pyran-2-one) derived from condensation of one mole diethylene diaminetriamine(dien) with two moles of dehydroacetic acid have behaved tetra dentate dibasic chelating agent with all metal ions under study.

Three bimetallic Cr(III), Fe(III) and Zn(II) acyclic polydentate complexes $(M_2L_2Cl_2)Cl_4$,M=Cr(III) , Fe(III) and $)M_2L_2Cl_2)$, M=Zn(II) have been prepared and fully characterized by UV-Vis., FTIR, micro-elemental analysis , as well as the magnetic moments of solid complexes and the measurements of molar conductance in DMSO solution helped us in investigate the chemical structure of bimetallic models . From the results obtained by different techniques, it was found that the proposed structures of the prepared complexes have tetrahedral structure.

A theoretical treatment of the formation of complexes in the gas phase was studied, this was done using the HYPERCHEM-6 program for the Molecular mechanics and Semi-empirical calculations.

The synthesized compounds were tested for antimicrobial activity by cup plate diffusion method. The results indicate the enhanced activity of metal complexes over the parent ligands.

Keywords: *Synthesis, Spectral Study, Binuclear Complexes, Pyrane-2-one.*

1. Introduction

The binuclear metal complexes possessing heterocyclic rings are one of the most widely used as antibiotics, antifungal and semiconductor sensors)1-4). Now days, there exists interest in the development of new and more effective antifungal compounds (5), urged infection by the increasing importance of opportunistic provoked by factors that depress or destroy the immune system , like chemotherapy of cancer, the use of drugs to avoid organ rejection in transplanted patients and discussed like AIDS(6). According to that, the development of new systemic fungicides is of prime importance in modern medicinal chemistry. 3-acetyl-6-methyl -2H-pyran-2,4(3H)-dione)I)a commercially available compound usually obtained through the on to condensation of ethyl acetate) (7) has been shown to posses modest antifungal properties (8). Also, it has been shown that the complexes of dehydroaceticacid with zinc and several transition metal cat ions, are fungi static (9). This has motivated our study on the synthesis and structural characterization of new bimetallic complexes of Cr(III), Fe(III) and Zn(II) with new acyclic Schiff base)L) derived from condensation of diethylenetriamine with dehydroacetic acid

2. Experimental

2.1 Physical measurements

Electronic spectra of the new ligand and its metal complexes were recorded in the region 800-200nm on Shimadzu 670 spectrophotometer. IR-spectra were recorded on PC. Shim adz FT-IR spectrophtometric –Japan model as KBr and CsI-disic in the range 400-4000cm^{-1}. Magnetic moments studies were carried out on magnet-Bruker balance MG. On Al-Nahrain university Laboratories. The conductivity of solution of 10^{-3}Mcomplexes in DMSO were done on digital conductivity meter (HPG system, G-3001).

As well as the microanalysis of C.H.N, were recorded at Carolo Erba 1108 elemental Vario in Laboratories of chemistry Depart in Al-Mustanisrya university .As well as %M in solid complexes were estimated using standard methods (10).

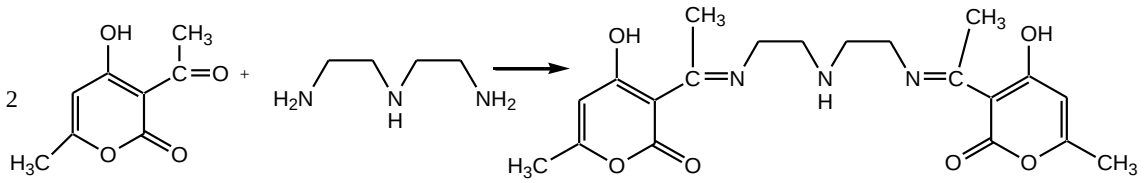
2.2 Reagents and solvents

Dehydroacetic acid and diethylenetriamine (dien) obtained from Merk , was used as supplied , and the metal chloride hydrated $CrCl_3.6H_2O$,$FeCl_3.6(H_2O)$ and $ZnCl_2.6H_2O$ of an. grade , were used for synthesis of complexes without any purification .

2.3 Synthesis of Ligand 3,3'-(1E,1'E)-1,1'-(2,2'-azanediylbis(ethane-2,1-diyl)bis(azan-1-yl-1-ylidene))bis(ethan-1-yl-1-ylidene)bis(4-hydroxy-6-methyl-2H-pyran-2-one) (L)

The ligand (L), was prepared by refluxing(2g,0.011moles)of dehydro acetic acid in 30ml absolute ethanol with(0.613,5.9x10^{-3}moles) of diethylenetriamine for about 9 hrs on water bath . The polydentate acyclic ligand (L), thus formed upon cooling the mixture to room temperature over night. m. p. 44-46C^{o} . The yield was ca 60% .

Exact mass 403.17 g/ml, of $C_{20}H_{25}N_3O_6$ formula with good solubility in methanol and ethanol, DMF and DMSO, to give clear off white solutions. The micro elemental analysis %calc (found)%C 59.54 (58.69), %H 6.25 (5.81), %N 10.42(11.61). as shown in scheme (1).



Scheme (1) Synthesis of Ligand (L)

2.4 Synthesis of Metal complexes

(0.65 g, 2 mole) of ($CrCl_3$. $6H_2O$) in (20 mL) methanol was added to (0.5g, 1mol)of (L) ligand dissolved in methanolic solution of potassium hydroxide(5%) to keep the pH of the solution for(≈8). The resulting mixture was refluxed under nitrogen atmosphere for 3 hrs. until its solution has become green in color, then cooled to room temperature, a green precipitate formed, filtered off, washed several times with 15ml of diethyl ether ,and dried under vacuum to afford(0.51g, 61%) yield ,scheme(2),

A similar method was used to prepare Fe(III) and Zn(II) complexes Scheme(2), Table (1) shows some physical properties and reactant amount of the prepared complexes.

2.5 Study of biological activity for ligands and their metal complexes:

The biological activity of the ligands and their metal complexes were studied against two selected type of bacteria which included *Escherichia coli*, as gram negative (-Ve) and *Staphylococcus aureus* as gram positive (+Ve) to be cultivated and as control for the disc sensitivity test (11). This method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plate. The plates were incubated for 24 hours at 37C°, the zone of inhibition of bacteria growth around the disc was observed.

3. Result and Discussion

The new acyclic Schiff base (L) ligand derived from condensation of one mole diethylenetriamine with two moles of dehydroacetic acid have behaved tetra dentate dibasic chelating agent with all metal ions understudy. The stochiometric of the ligand and its complexes were confirmed by their elemental analysis. Table (1).

3.1 Infrared Spectra:

The IR spectrum of the (L) ligand shows bands in the region at 3400$cm^{-1}$and 1656cm^{-1}, which assignable to υ(OH) and azomethane group υ (C=N))12**) .**As well as the work absorption at 1695cm^{-1} attributed to the υ(C=O)of the pyrane group . The IR showed no bands around at 3571 and 3357cm^{-1} assigned to the υ_{asy} (N-H) and υ_{sy} (NH) respectively which indicate the absent of υ_{asy} (N-H) and υ_{sy} (NH) (13). The negative shift generally in υ(C=N) further suggested the coordination to metal ions through nitrogen atom of (-C=N-)

Schiff's base (12) of the ligand.
The observations indicate the coordination of the ligand L through the carbonyl group stretching frequency decreases to 1650-1640 cm^{-1} compared to the free ligand at 1695 cm^{-1}, due to the charge transfer from the ligand to the metal)13). The band assigned to the phenolic OH group in the same wave number comparing with that of the free ligand, proving it's not involve coordination. (12) (Table 2). New bands which appeared at low frequencies in the spectra of the prepared complexes were probably due to (metal- nitrogen), and (metal- oxygen), (14) (table 2).

3.3 Electronic Spectra And Conductivity Measurements:

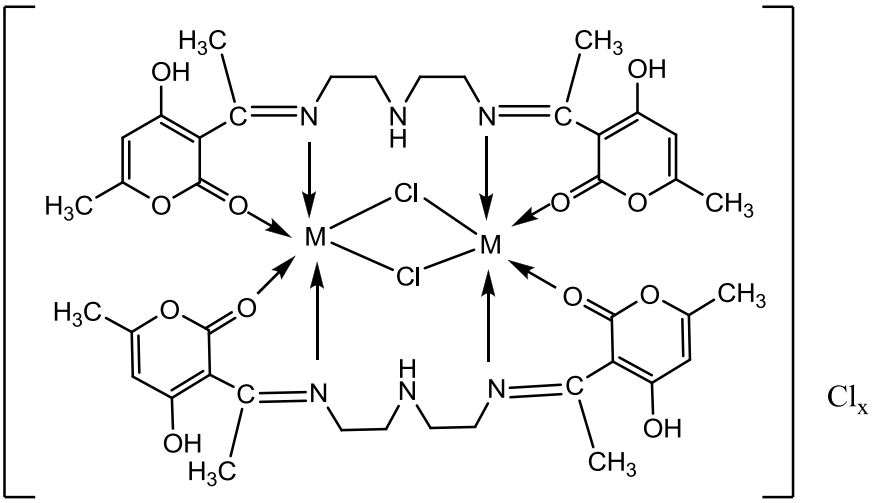
The free ligand (L) in methanol spectral exhibits three distinct absorptions in the range 42735, 37878 and 31847cm^{-1} which are assigned to π-π* of benzenoid and E1, E2 and n-π* of C=N, and C=O chromospheres (15) . The green solution of Cr (III) complex in DMF exhibits absorption in the range 22250, 25666 and 37017 cm^{-1}, which resemble these reported to be octahedral. Thus, assuming the effective symmetry to be Duh, and the various bands can be assigned to $^4A_2g(F)\rightarrow{}^4T_2g(p)$, $^4T_1g(F)\rightarrow{}^4T_1g(P)$ respectively (15). The Zn(II) complex solution exhibit a well weak absorption in visible region at 22645 cm^{-1}which can be assigned to charge transfer (16) . The measurements of molar conductance in DMSO , Λm 90 $S.cm^2$ ml^{-1} for Zn(II) showed that these are electrolytic and 115, 125 $S.cm^2$ ml^{-1} for Cr(III) and Fe(III) respectively which showed electrolytic (10).

3.4 The Magnetic Measurement :

The magnetic moment of Cr(III)(~ 3.35*BM*) revealed the present of three unpaired electrons which agree with octahedral environments around Cr(III) ion (11). The magnetic moment of Fe(III)(~ 3.34*BM*) revealed which agree with octahedral environments around Fe(III) ion(17). Table (3).

3.5 The Proposed Structure:

Based on various physiochemical studier like elemental analyses, conductively measurements, magnetic moments , UV-Visible, and IR spectral studies , a distorted octahedral geometry may be proposed for Cr(III), Fe(III) and Zn(II) complexes, as shown in scheme (2).



M = Zn , X=Cl_2
M = Cr,Fe , X=Cl_4

Scheme (2): proposed structure of metal complexes, M= Cr(III),Co(II) and Fe(III)

3.6 Theoretical Study:

The ball and cylinders and some of selected structural parameters (bond length and angles) of the optimized geometries are shown in Fig.(1) Table (4). As shown in this figure, there is no obvious trend for the variation of these parameters. The values of the bond length and angles of the optimized geometries are quite similar to the experimental results of the corresponding compounds

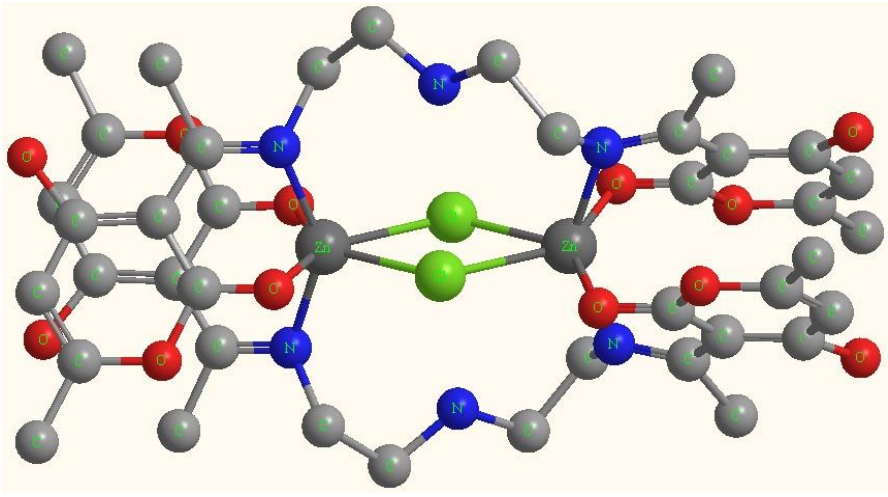


Figure.(1): The optimized structural geometry of Zn(II) complex

3.7 Biological Activity: .(18).

The antibacterial activity of the Schiff bases and its complexes were tested on Gram positive bacteria, Staphylococcus aureus and Gram negative, E. coli. The antibacterial activities of the samples were evaluated by measuring the inhibition zone observed around the tested materials as shown in Figure.(2).

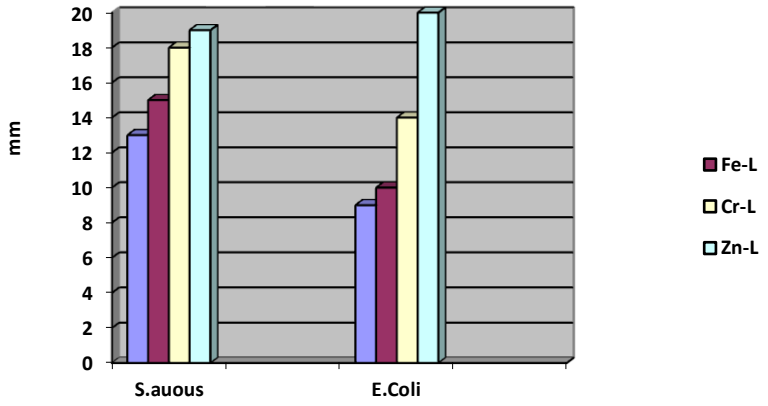


Figure.(2): The effect of ligand and their metal complexes toward bacteria

Conclusion

The presence of nitrogen and oxygen atoms in the structure of an organic chelating agent has led to interesting results in the studies of coordination compounds. Prepared type of Schiff base ligand derived from pyrane-2-one and their complexes. The synthetic and structural study of above new type of tetrahedral complexes have bidentate ligands. The study of biological activity of the ligands and their metal complexes against two selected type of bacteria which included Escherichia coli, as gram negative (-Ve) and Staphylococcus aureus as gram positive (+Ve) to be cultivated and as control for the disc sensitivity test shows that different activity of inhibition on growth of the bacteria.

Table (1): Analytical data for Ligand and their complexes

compound	Formula υ(cm^{-1})	Molecule weigh	color	Calc. (Found)				
				C%	N%	H%	Cl%	M%
L	$C_{20}H_{25}N_3O_6$	403	Off white	58.98 (58.94)	10.97 (10.92)	5.76 (5.72)	-	-
C1	$[Cr_2L_2Cl_2]Cl_4$	1123	green	42.76	7.48	4.49	18.93	9.26
C2	$[Zn_2L_2Cl_2]Cl_2$	1066	brown	45.05	7.88	4.73	13.30	11.05
C3	$[Fe_2L_2Cl_2]Cl_4$	1131	brown	42.47	7.43	4.45	18.80	9.87

*Analysis of metal percentage via F..A.A.S.

Table (2): IR spectrum data for Ligand and their complexes

compound	υ(C=O)	υ(OH)	υ (C=N)	υ (C-H) aromatic,υ (C-H)alaph.	υ M-N, υ M-O
L	1695(s)	3400(br)	1656	2990 , 2920	-
C1	1640(m)	3399(br)	1606	2995 , 2925	455,535
C2	1650(m)	3401 (br)	1600	2985,2925	478, 529
C3	1645 (s)	3400(br)	1605	2995, 2920	475,545

Br=Broad. S=strong, m=medium, and w=weak

Table (3) : (UV-Vis) spectra data, magnetic moment and molar conductivity measurement for the prepared complexes

compound	absorption $\upsilon(cm^{-1})$	Assignment	Geometry	magnetic moments μ eff. (BM)	* Scm^2ml^{-1}
L	42735 37878 31847	INCT π-π* n-π*	-	-	-
C1	37017 25666 22250	LF $^4A_2g(F)\rightarrow{}^4T_2g(p)$ $^4T_1g(F)\rightarrow{}^4T_1g(P)$	octahedral	3.35	115
C2	22645	CT	octahedral	-	90
C3	23250 20530	$^1A_1g\rightarrow 1B1g$ $^1A_1g\rightarrow{}^1B_2g$	octahedral	3.34	125

INCT=Intra-ligand charge transfer, *Molar conductance measurement were carried out in 10^{-3} M solution of DMSO

Table (4) : Structural Parameters, Bond Length (°A) and angles(°) of the $[Zn_2L_2Cl_2]Cl_2$ complex.

Bond lengths (°A)		Bond angles(°)		Bond angles(°)	
Cl(62)-Z(n60)	2.1500	H(98)-N(35)-C(36)	120.4029	Cl(62)-Zn(60)-Cl(61)	0.0000
Cl(61)-Z(n60)	2.1500	H(98)-N(35)-C(34)	120.4035	Cl(62)-Zn(60)-O(46)	141.3880
Cl(62)-Zn(59)	2.1500	C(36)-N(35)-C(34)	119.1935	Cl(62)-Zn(60)-N(32)	89.9999
Cl(61)-Zn(59)	2.1500	H(97)-C(34)-H(96)	109.5000	Cl(62)-Zn(60)-O(27)	51.3879
C(53)-C(48)	1.3370	N(32)-C(47)-C(31)	129.9443	Cl(62)-Zn(60)-N(9)	90.0002
C(48)-C(49)	1.3510	H(104)-O(45)-C(44)	120.0001	Cl(61)-Zn(60)-O(46)	141.3880
O(46)-Zn(60)	0.6000	O(45)-C(44)-C(43)	120.0002	Cl(61)-Zn(60)-N(32)	89.9999
O(45)-H(104)	0.9720	O(45)-C(44)-C(39)	120.0003	Cl(61)-Zn(60)-O(27)	51.3879
C(39)-C(40)	1.3787	O(41)-C(40)-C(39)	123.0011	Cl(61)-Zn(60)-N(9)	90.0002
N(38)-Z(n59)	1.8360	C(47)-C(43)-C(44)	118.4178	O(46)-Zn(60)-N(32)	90.0004
N(38)-C(55)	3.1983	C(47)-C(43)-C(42)	85.1247	O(46)-Zn(60)-O(27)	90.0001
N(32)-Zn(60)	1.8360	C(44)-C(43)-C(42)	117.6004	O(46)-Zn(60)-N(9)	89.9996
N(32)-C(47)	1.2600	C(42)-O(41)-C(40)	112.0000	N(32)-Co(60)-O(27)	90.0004
N(32)-C(33)	1.4700	O(46)-C(42)-C(43)	123.0004	N(32)-Co(60)-N(9)	179.9999
C(28)-H(82)	1.1130	O(46)-C(42)-O(41)	112.6989	O(27)-Co(60)-N(9)	89.9997
O(27)-Zn(60)	0.6000	C(43)-C(42)-O(41)	124.2984	C(19)-C(26)-N(9)	103.9373
O(25)-H(81)	0.9720	O(21)-C(20)-C(19)	124.2988	C(19)-C(26)-C(1)	128.0312
C(24)-O(25)	1.3550	Zn(60)-O(46)-C(42)	179.9993	O(56)-C(49)-C(48)	123.0000
O(17)-Zn(59)	0.6000	Zn(60)-N(32)-C(47)	120.0002	O(50)-C(49)-C(48)	124.2987
O(16)-H(79)	0.9720	Zn(60)-N(32)-C(33)	120.0003	Zn(60)-Cl(62)-Co(59)	90.0000
C(15)-O(16)	1.3550	C(47)-N(32)-C(33)	107.9998	Zn(60)-Cl(61)-Co(59)	90.0000
C(14)-C(18)	1.3370	Zn(60)-O(27)-C(20)	179.9994	Zn(59)-O(56)-C(49)	179.9996
C(14)-C(15)	1.3370	Cl(62)-Zn(59)-Cl(61)	0.0000	Zn(59)-N(38)-C(55)	72.0407
C(13)-O(17)	1.9625	Cl(62)-Zn(59)-O(56)	90.5730	Zn(59)-N(38)-C(37)	120.0000
N(9)-Zn(60)	1.8360	Cl(62)-Zn(59)-N(38)	90.0000	C(55)-N(38)-C(37)	107.9999
N(9)-C(26)	1.9830	Cl(62)-Zn(59)-O(17)	90.0000	Co(59)-O(17)-C(13)	146.3428
N(3)-Co(59)	1.8360	Cl(62)-Zn(59)-N(3)	90.0000	H(84)-C(28)-H(83)	109.5199
N(3)-C(18)	1.2600	Cl(61)-Zn(59)-O(56)	90.5730	Cl(61)-Zn(59)-O(17)	90.0000
		Cl(61)-Zn(59)-N(38)	90.0000	Cl(61)-Zn(59)-N(3)	90.0000
		N(38)-Zn(59)-O(17)	90.0006	O(56)-Zn(59)-N(38)	90.0005
		N(38)-Zn(59)-N(3)	180.0000	O(56)-Zn(59)-O(17)	179.4270
		O(17)-Zn(59)-N(3)	89.9994	O(56)-Zn(59)-N(3)	89.9995

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THE INDIAN CATFISH, *Heteropneustes fossilis* (BLOCH, 1794) ENVENOMATION- A REPORT OF FOUR CASES FROM BASRAH AND MAYSAN, SOUTH OF IRAQ

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Abstract

Venomous catfish stings are a common environment hazard worldwide. Although these stings are often innocuous, significant morbidity may result from stings, including severe pain, retained foreign bodies, infection, respiratory compromise, arterial hypotension, and cardiac dysrhythmias. Treatment included hot water immersion, analgesia, wound exploration, and prophylactic antibiotics. In this article, three cases of stings by the Indian catfish, *Heteropneutes fossilis* were referred to the emergency Department, Republican Hospital at Basrah and Mysan Provinces, south of Iraq and their treatments have been reported.

Keywords: catfish, stings, venomous animals, poisonous glands, frenzy feeding

1. Introduction

The Indian stinging catfish, *Heteropneustes fossilis*, is belong to the bony fish family Heteropneustidae with four nominal species included in the genus *Heteropneustes* namely *H. fossilis* Bloch (1794) from Tamil Nadu of India, *H. microps* Günther (1864) from Dambuwa of Sri Lanka, *H. kemratensis* Fowler (1937) from the Chao Praya River of Thailand, *H. longipectoralis* Devi & Raghunathan (1999) from Western Ghats of India, and *H.nani* Hossain et. 2013 from Noakhali, Bangladesh (Ali et al., 2015).

The Indian catfish is found throughout south and southeast Asian countries including Bangladesh, India, Laos, Myan-mar, Nepal, Pakistan, Sri Lanka and Thailand (Talwar and Jhingran, 1991). It has been introduced into Iraq in the late 1950s (FAO, 1997) and recorded from the freshwaters of southern Iraq in 1960 by Khalaf (1961) and from the marine waters of Khor Al-Zubair, northwest Arabian Gulf (Al-Hassan and Muhsin, 1986). The control of the snail *Bulinus truncatus*, the vector for the human parasite causing schistosomiasis, was the aim of its introduction into Iraqi freshwater system, but which proved to be ineffectual (Jawad, 2003). This species was reported from other parts of the Middle East like the rivers and marshes in Khuzestan, Iran (Coad and Abdoli, 1993; Coad, 1996; Abdoli, 2000), the Tigris River watershed in Turkey (Ünlü et al., 2011), and recently it was recorded from the first time from Al-Khabur River, Al-Hasaka, Syria (Ali et al., 2015).

Indian catfish has an elongate and laterally compressed body with depressed head and terminal mouth. The dorsal fin is very small, with no leading spine and located in the anterior third of body, almost coinciding with the pelvic fin level. Presence of 4 pairs of barbels. Eyes are small and lateral. The body color of the fish is gray through to brown or black. Presence of two tubular air sacs extending from the gill cavity almost to the caudal peduncle. It uses two breathing systems and can respire aerially by taking in air at various intervals when oxygen content of the water reaches minimum level of requirement (Munshi, 1993). As a result, this catfish can live in conditions not suitable to other fishes.

Although it has not been taken as food species in Iraq and the Middle East, *H. fossilis* is commercially and aquaculturally is considered a significant fish in many Asian countries (Hossain et al., 2015). It is heavily fished for its energizing meat quality that includes taste, nutritional, and medicinal values (Jha and Rayamajhi, 2010). There are high amounts of protein

in its muscles, iron (226 mg/100g) and calcium (Saha and Guha, 1939; Alok et al., 1993). Being a lean fish (fat content only 2.57 ± 0.24%), it is conducive to people on low-fat diets (Rahman et al., 1982).

This species of catfish is dangerous to human. A venom glands are present near the spine of the pectoral fin of the males. Fishermen are aware of the dangerous stings of this fish and if they catch it in their nets, they remove it with a piece of cut-off net, with great care (Satora et al., 2015). Several instances where this fish became in contact with human delivering a single sting were reported from the inland water of Iraq and the other countries (Zakaria, 1964; Coad, 1979; Berra, 2001).

In the present cases, a report for the first time about four cases (5 persons) of severe attack by the fish giving multiple stings to the same victim from Shatt Al-Arab River, Basrah Province and Majar Al-Kabir River, Mysan Province, Iraq.

2. Cases Reports

Cases of Basrah Province-

1. A 30-year-old male was admitted to the Emergency Department (ED) of the Republican Hospital at Basrah City, Iraq in July 2004 with a painful leg wound caused by a Stinging Catfish (*Heteropneustes fossilis*). According to the victim, a group of the stinging catfish, *Heteropneustes fossilis* actively gave him four stings in his thigh and two in his leg during swimming in Shatt Al-Arab River at Al-Ashar area. There were medium bleeding and severe pain that prevent the victim from normal walk and received assistant from friends. The victim declared that he was swimming after throwing pieces of bread into the water. For the next 15 minutes there was a serious progression of the pain and there was no response to the nonsteroidal anti-inflammatory agent (ibuprofen) which he had taken.

On admission the patient was alert, with good verbal response. The heart rate varied from 92 to 104 b/min. and the arterial blood pressure was 120/70 mmHg. All biochemical results as well as ECG did not show any abnormalities.

On the arm, hand and leg there were a puncture wounds about 1-3 mm in diameter with medium edema and erythema at the position of each sting. After irrigating and debriding of

retained spine fragments and necrotic tissue the patient was given tetanus anatoxin (AT) and prophylactic antibiotic course (ciprofloxacine). The X-ray of the upper and lower limbs did not reveal any abnormalities and residual spine. The opioid analgesia (dolargane) and local anesthesia with lidokaine had to be provided to relieve the pain.

After 24 hours of observation the patient was discharged. The follow up after 2 weeks showed healed wound of the hand and no subjective complaints of the patient.

2. A 10 years old young male was taken to the Emergency Department of the Republican Hospital at Basrah City in June 2005 suffering from severe multiple stings in his bottom and on his back. These stings were caused by a stinging catfish (*Heteropneustes fossilis*). The victim admitted that he swam across a large number of stinging catfishes feeding on the surface on pieces of bread. The fishes seem to turn to attack frenzy when they found the young man in the middle of their feast. Due to the large number of stings, the victim lost consciousness and he was taken to the hospital by friend's car. There were five stings on his bottom and another three on his back. The patient complained of numbness, dizziness, and quite intense pain at the site of the injury progressively extending into the surrounding areas. At the base of each sting, there was a puncture wound about 2 mm in diameter with medium edema and erythema. On admission, his temperature was 37.8˚C, with a heart rate of 96 and blood pressure of 110/
70. Tests were made for the following: ESR, complete blood count, electrolytes, enzymes, and coagulation profile. All of them were normal. The area of wound was infiltrated with 1% lidocaine and examined carefully. Any remains of the broken spines were removed. The wound was thoroughly irrigated and cleansed with antiseptic solution and left open. A plain radiographic study of the injured area confirms the absence of any foreign material. The affected area was covered with pads wetted with hot water (45˚C) for approximately 45 minutes to inactivate venom in the wound. The patient received a prophylactic short course of oral antibiotic therapy with Trimethoprim-sulfamethoxazole. After 24 hours of observation, the patient was discharged from the hospital at his own request under the care of his father. Over the period of three weeks, the wound healed slowly and after two months, the wound appeared to be completely healed without any complication.

Cases of Mysan Province

1. The affected persons were two young women age 25 and 27 years old, and envenomation occurred during washing some cutlery on the Majar Al-Kabir River Bank in rural area on 23 May 2004. The two young women taken to the Emergency Department at the Republican Hospital in Mysan. Each victim received six stings in each leg. The wounds were near the ankles. The injury was accompanied by intense pain, numbness of the site, dizziness, local oedema and erythema. In addition, symptoms such as tachycardia, weakness, arterial hypotension, loss of consciousness, respiratory distress and unusual sensations (tingling, pricking) were observed. I consider these envenomation as moderately serious accidents. The first aid treatment for stinging catfish stings was removal of all foreign material and irrigating the site with whatever clean liquid is available, warm water immersion and pain management. Radiographs aimed at the inspection for foreign bodies was used in all cases. The patients received a prophylactic short course of oral antibiotic therapy with trimethoprim-sulfamethoxazole. Tetanus prophylaxis was given when indicated. They were observed for 24 hours. No residual deficits in motor or sensory functions were noted. In 6 weeks' time, the wounds healed slowly.

2. A 35-year-old fisherman operate at the edge of the marsh area in Mysan Province presented to the emergency department 2 hrs. after tolerating an injury to his hand while trying to clear the fishing net from the unwanted catfish, *Heteropneustes fossilis.* There was a persistent cutaneous oedema, erythema, intense burning or throbbing pain that appeared at the wound site. Paresthesias, weakness, localized sweating, and muscular fibrillation can be accompanied by cyanosis and inflammation around the puncture site. The attack was performed by at least 10 fishes based on the number of spines found inside the wounds. The spines were imbedded in the volar aspect of the hand, and after numerous failed attempts to shake the offending fishes loose, the victim broke the spines with his other hand. He felt numbness to the elbow, and slight dizziness. Initial examination revealed a 2 X 2-mm foreign bodies imbedded in the soft tissues midway between the fourth and fifth metacarpal bones. Motor and sensory functions were grossly normal and capillary refill was preserved. A plain radiograph of the hand showed a 3 mm Iong foreign bodies with dense retroflexed barbs projecting perpendicular to the shaft. Treatment

consisted of warm water immersion for 30 min, tetanus prophylaxis, pain management with meperidine/promethazine, and I g intravenous cefazolin.

The spine was successfully removed after a minor operation and the patient was discharged on a 7-day course of cephalexin 500 mg. The wound was healing well but slowly. Wound cultures obtained on initial presentation grew no organisms. No residual deficits in motor or sensory functions were noted.

3. Discussion

In order to evaluate the attack of the Indian catfish, *H. fossilis*, it is important to know the structure of the poison gland and the pectoral fin spine of this species. There are two toxicity mechanisms in the Indian catfish: the first is related to sting penetration and rupture of the venom glandular tissue surrounding the sting, whereas the second, called crinotoxicity, is associated with the production of toxins in the entire fish skin (Al-Hassan et al., 1986). There are several components in the the venom contained in the poisonous gland of this catfish such as complex composition of hemolytic, dermonecrotic, oedema-producing, and vasospastic factors in addition to the presence of several amino acids, 5HT, 5-nucleotidase, and phosphodiesterase (Helm, 1976), whose potency is largely inversely proportional to the fish size and is a defensive mechanism (Al-Hassan et al., 1986). The Crinotoxins are proteinaceous substances found in the epidermal secretions coating the entire catfish body surface, not just the spines, and are released when the catfish is excited or threatened. If exposed to open skin, these toxins can cause similar symptoms of throbbing pain, tissue necrosis, and, possibly, muscle fasciculation (Shepherd et L., 1994; Das et al., 1995).

The transverse section of the spine showed a central canal running through the longitudinal axis of the spine. From this canal, a number of canaliculi are radiated outwards reaching the outer edge of the spine. The main canal contains fine blood vessels, lymphatics and connective tissues. The poison gland is composed of large rounded cells found on each side of the spine. These cells are derived from the basal columnar cells of the skin epidermis. This gland has no duct to convey the poison to the outside. Instead, a pressure is excreted on the wall of this gland due to the erecting of the spine and the excretion of the gland comes out through temporary rupture of the skin at the tip of the spine.

Due to the structural nature of the spine, its extraction and debridement can be difficult, as was the case with the presenting patient. The angled serrated edges prevent easy extraction of the spine and often remain behind if the spine is extracted using excessive force (Howard and Burgess, 1993).

The inflection of spine in the body of a victim comes as defense mechanism acquired by the fish. During the fast swimming, the fins and spines are lowered and they are erected when fish moves slowly (Fernando and Fernando, 1960). When the fish is disturbed, it moves quickly forward with head and trunk are displaced sideways and caudal region involved in an undulating movements. In such position, the spine becomes in a well position for stabbing (Zakaria, 1964).

All the cases reported in this study are common in one issue in that the attack of the fish became as a result of the fish has been alarmed or disturbed. For the 1st case from Basrah Province, the fishes were alerted to the presence of food and dash to the area and at the same time the victim was swimming. In the 2nd case, the food of the fish became threatened by the victim finding himself in the middle of bunch of feeding fishes. For the 1st case of Mysan Province, the fishes were alarmed by the presence of large amount of food remains thrown by women washing their cooking pans, while the 2nd case the fishes were disturbed as they were removed from the fishing nets. On the other hand, the attack of the Indian catfish in all cases discussed in this report, except for the 2nd case from Mysan province, have a feeding frenzy in nature. This feeding habit is not unusual and it is documented in several fish species (Atema, 1980; Leong and O'Connell, 1969; Powell, 2003). More interested, Olaifa et al. (2004) found that when the catfish, *Clarias gariepinus* becomes irritable and show a frenzy feeding behavior when expose to high concentration level of copper. Such feeding habit is reported for the first time for the Indian catfish, *H. fossilis* in the present study. Both Shatt Al-Arab River and Majar Al-Kabir are shown to have a polluted level of copper in their waters (Thummarukudy et al., 2012; Ghalib and Söğüt, 2014; Yasser and Naser, 2011; Al-Saad et al., 2009).

Fish stings facilitate the penetration of microorganisms across the skin into the body that are susceptible to infection (De Haro and Pommier, 2003; Harrison, 2000). Usually, freshwater catfish visit areas with standing and dirty water hereby potentially increasing the risk of infection. The bacterial flora found on the skin of the stinging fish will be the causative agent for an infection that accompany fish-inflicted wounds (De Haro and Pommier, 2003).

Antibiotic therapy is recommended in all cases of wound infections caused by fish; the choice should be based on the most probable etiological factor related to the specific fish species (De Haro and Pommier, 2003).

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TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 1, 2016 ISSN: 2377-2808

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Vol 3, No 1 (2016)

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Vol 3, Issue 1 (2016)

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1. TJMS is a high quality, biannually, peer-reviewed, electronic medical sciences journal, publishing original research and scholarly review articles, letters to the editor, and editorials.
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3. An outlet for the current research and an academic product in Iraq in the fields of medicine, dentistry, pharmacy, nursing and related disciplines that supports recognition and academic advancement in Iraq and beyond.
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Vol 3, No 1 (2016)

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 1, (2016), 1-16 ISSN: 2377-2808

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EXPLOSION BLAST INJURIES: PHYSICS, BIOMECHANICS AND PATHOPHYSIOLOGIC EFFECTS: A UNIQUE PATTERNS AND MASKED KILLING EFFECTS

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Abstract

Growth of improvised explosive devices IEDs and barrel bombs that fall down like rain will continue to put the world at a unique war, as the number of nonconventional conflicts is near epidemic globally. This necessitates to share our experience and lessons learned of the primary blast wave physics, biodynamic, pathophysiologic effects and management of unique primary blast injury patterns and masked killing effects which are unlike injuries caused by shrapnel or bullets. The types of blast wave manifestations can be termed implosion, acceleration-deceleration, spalling, and pressure differentials.

The author has contributed to the vocabulary of maxillofacial blast injuries for each of the above manifestations: biodynamic, hypothesis, diagnosis and new procedures, including lifesaving airway compromise management. Evolving of a new finding as impact of a spherical blast wave inflicts transverse lines of fractures on the mandibular body, and may be associated with

transverse shearing of teeth at the cementoenamel junction. Fractures of the mandible caused by blast are different from those in the same region caused by any type of civilian or ballistic injury.

Primary blast wave induces facial skin shredding, extensive contusions, lacerations, multiple puncture wounds, partial scalping, flash and thermal burns, thermal inhalation, and toxic fume injuries. Additionally, collateral injuries to the lung and/or brain are the initial and immediate care of related airway compromise resulting in life-threatening conditions.

The implosion and miniature re-explosion of compressed air sinuses leads to skeletal crush injury to the nasal-orbital-ethmoidal, maxillary sinuses, and nasal bones. A variety of surgical approaches were used successfully under conditions of war.

keywords; blast injuries; Improvised explosive devises; IEDs; facial-skin blast injury; explosion blast injuries; blast physics; air containing cavities blast injuries.

Introduction

Nonconventional war acts are worldwide especially if we know that a shocking new study has revealed only 11 countries in the world are currently at peace. Out of 162 countries, 151 of the world's nations are involved in some form of conflict. The five least peaceful countries were, in order: Somalia, Iraq, South Sudan, Afghanistan and, in last place, Syria.[1]

This significant, near epidemic increase in the number of conflicts is contrary to logical expectations when contrasted with civilization's achievements during the same period. Since the invention of black powder by China more than 1000 years ago, blast injuries have consequently occurred. The blast wave producing injuries implies those detrimental changes occurring in an exposed to the effects of changes in air pressure produced by an explosion.

Explosive blast injuries within the current geopolitical environment, terrorist-related explosions, and barrel bombs are universally the primary human disasters that continue to challenge both civilian and military medical/surgical personnel.

Blast injuries inflict a unique pattern and masked killing effects, with a high mortality rate and many serious multiple injuries in general and specifically in the maxillofacial region which are neglected or not fully described in the medical literature.

Improvised explosive devices (IEDs) are bombs or explosive devices used to destroy or incapacitate a massive number of people. The term, IED, came into common usage in 2003 during the Iraq War.[2] They are widely recognized as among the weapons of choice for terrorists throughout the world due to their availability, simplicity, and effectiveness. Now blast waves of large amount of explosive materials are being used more than ever before as frontline conventional weaponry, or as terrorist weapons against civilians to inflict high mortality and destruction rates. Explosive devices as IEDs, booby traps, rocket-propelled grenades (RPG-7 to -29), thermobaric, enhanced-blast explosives, explosive-formed projectiles, and 500- to 2000-lb barrel bombs are falling from the sky. Consequently, it became difficult even to those in uniform to count the number of weapons that can cause high blast and fragmentation. Fig. 1

Fig.1

IEDs explosion in Baghdad showing many cars at scene catch fire, produce a great deal of hot dust, particles, debris, smoke, toxic fumes, and gases which inhaled by the victims.

Blast physics

The detonation of solid or liquid explosive material releases a large amount of heat and gaseous products that are transmitted as a blast (shock) wave, a pressure pulse a few millimeters thick that travels at supersonic speed outward from the point of the explosion.[3-5] The physical processes involved in the body's response to explosion blasts occur in 1/1,000 of 1 second, with consequent exposure to ambient pressure changes, rapid winds, and the heat wave. Fig. 2

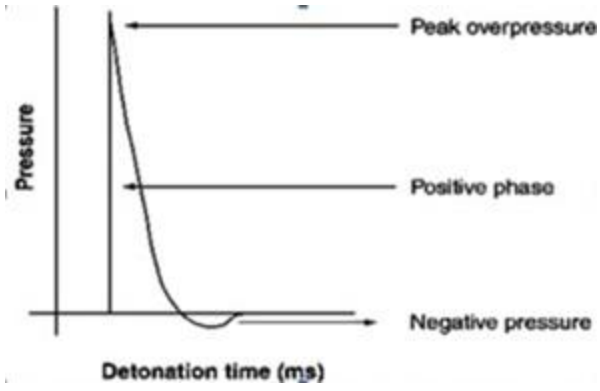

Fig.2
Air blast shockwave positive and negative phases (pressure/time).

Clinical findings have revealed how the spherical leading edge of the blast wave exercises a severe crushing, shattering, and shearing effect resulting from the increase in ambient pressure. The molecules of gas in the atmosphere around us are in constant thermal motion. On average at sea level, there are 30 million billion molecules in every cubic millimeter of air, moving at the speed of 300 m/s and bumping into one another 100 million times each second, after traveling only 0.001 mm. This continual bombardment of gas molecules against any solid surface exerts a force on every part of that surface and is most appropriately expressed as a force per unit area, or a pressure.[6]

These air molecules are compressed to such a density that the pressure wave itself acts more like a solid object striking a tissue surface. This thin layer of compressed air forms a shock front that is propagated spherically in all directions from the explosion's epicenter. A longer negative under pressure (relative vacuum) follows the peak positive overpressure. Because the intensity of the blast (the peak overpressure) decreases rapidly proportionate to the distance from the detonation, persons must be very close to an explosion to sustain primary blast injuries.[7] Fig.2

Slotnick [8] stated that this "blast wave" (positive wave) moves in all directions, exerting pressures of up to 700 tons per square inch on the atmosphere surrounding the point of detonation at velocities of up to 13,000 miles per hour or 29,900 fps. Shock waves possess the quality of brisance (shattering effect). [9,10]

Blast Injury Mechanism

Explosions release energy and produce a large volume of gaseous product, which induces 4 categories of tissue injury. The first is the primary blast effect changes potential energy to kinetic energy induced by the spherical front blast wave consisting of a few millimeters of over pressurized air. This results in a discontinuous increase in pressure, density, and high temperature, known as a shock front.

The second is caused by different kinds and shapes of objects ranging from conventional shell fragments to car fragments or other components that can cause devastating damage to the body. Third is the tertiary blast effect, which results from the propelling of the body against walls or objects, crush injuries, or blunt trauma. Finally, the quaternary blast effect causes asphyxia by inhalation of toxic fumes, burned materials, and burns by high thermal temperatures generated by the explosive effects.

To better understand how high explosives possess such shattering power one can compare this to the small changes in atmospheric pressure that can lead to high-velocity winds. For instance, a peak pressure of as little as 0.25 psi can generate winds as great as 125 mph. The temperatures from the explosive gases can reach 3,000°C in the center zone, particularly in a thermobaric explosion.[11]

PHYSICAL AND PATHOPHYSIOLOGIC EFFECTS

Blast biodynamic effect of primary blast wave is a complex event and can cause complex injuries to living tissue and several organ systems, with different tissue distortion. Thus the destruction can be a manifestation of one type of blast injury or some combination of more than one type. These can be termed implosion, acceleration-deceleration, "spalling fragmentation," and pressure differentials.[12]

Primary blast wave causes injury by its sudden loading on a facial surface that is oriented toward the blast front wave. Furthermore, the resulting stress within the impact region may be concentrated at certain locations called stress points. Clinical presentation of primary blast wave facial injuries are mostly due to the direct impact of the shock wave and far less due to secondary and tertiary effects of the blast. The primary blast injury may be only one of the problems in a poly-traumatized patient. Ears, upper respiratory tract and lungs are the structures that are most sensitive to a primary blast injury. Haemoptysis and chest pain or tightness are frequent, as are tachypnoea, evidence of pulmonary consolidation. Pneumothorax may present as unilateral hyper-resonance and decreased breath sounds, and arterial air emboli pose the most immediate threat to life. Fig. 3,4,5

Fig. 3

Sketch figure demonstrate a spherical blast front wave consists of hot compressed air and secondary small and large fragments that cause soft tissue shredding, contusions, and flash burn

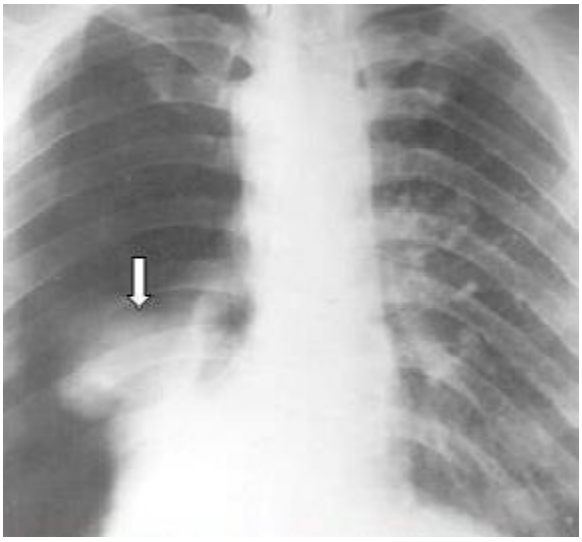

Fig.4

Chest radiograph revealing blast collapsed right lung

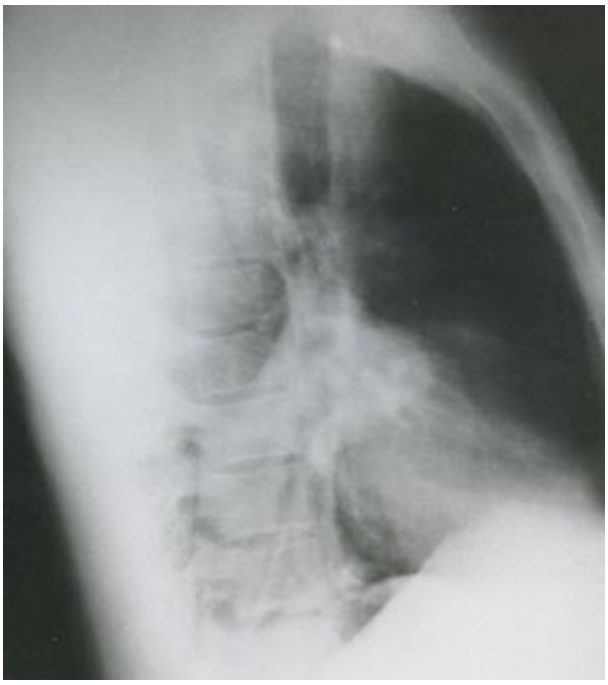

Fig.5

Lateral chest radiograph showing blast collapsed lung

Primary blast injuries

a. The architectural conception and anatomical variation of the face lead to different types of "implosion, acceleration-deceleration, spalling fragmentation, and pressure differentials " injury when exposed the enface or the lateral side to explosion shock wave as:

Implosion damage is limited to the gas-containing structures of the lungs auditory canal, paranasal sinuses and gastrointestinal tract. A rapid displacement of the chest wall causes local compression of the lung parenchyma that cannot be relieved through the airways, thus stressing the lung tissue. In the maxillofacial region, the implosion mechanisms of the primary blast cause the most dramatic effects, observed in the fractures to the midface. The middle third facial skeleton consists of large, skeletal, air-containing cavities that are second in size to the lungs, the largest air-container in the body.[13,14] The blast wave causes injury by its instant, rapid, external loading from an explosive detonation that manifests by compressing the air-containing paranasal sinus walls. These parts of the skeleton are similar to an egg shell or a crystal ball that is crushed, splinters into fragments, with the exception that the periosteum membrane preserves the bone fragments' connection to their location. Because the pressure differential across the sinus wall cannot be balanced by airflow through the sinus ostia, this, in turn, causes significant compression that stresses the sinuses' thin bone plate walls with air that originally filled the sinus cavities. This is followed by a miniature re-explosion or expansion of the compressed air sinuses[15] (Figs. 6-9)

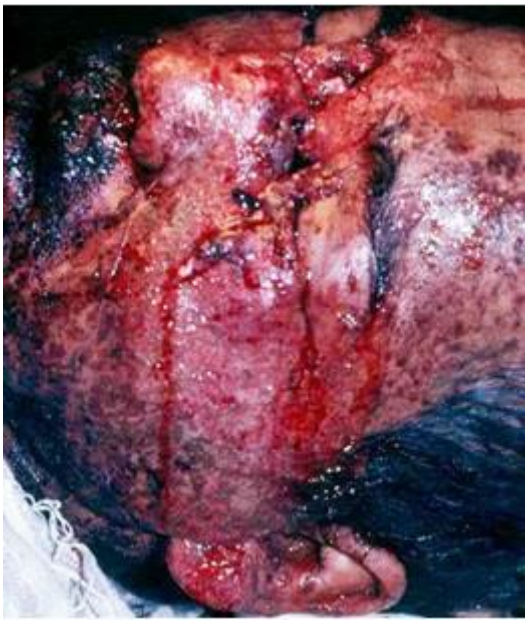

Fig.6

Blast mid-face implosion blast injuries showing typical secondary fragments cases laceration and eggshell crushed intercanthal space.

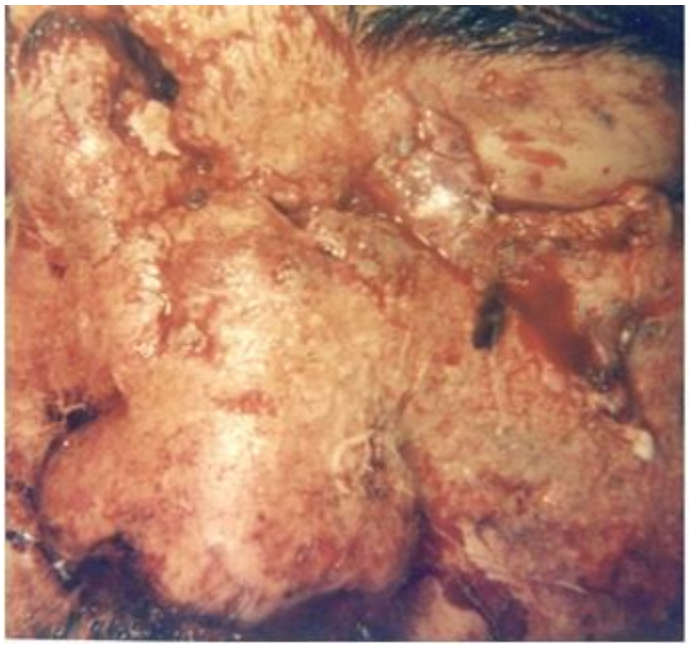

Fig.7

Closer view showing Implosion effects showing 5-6 cm wide intercanthal space plus tattered tissue.

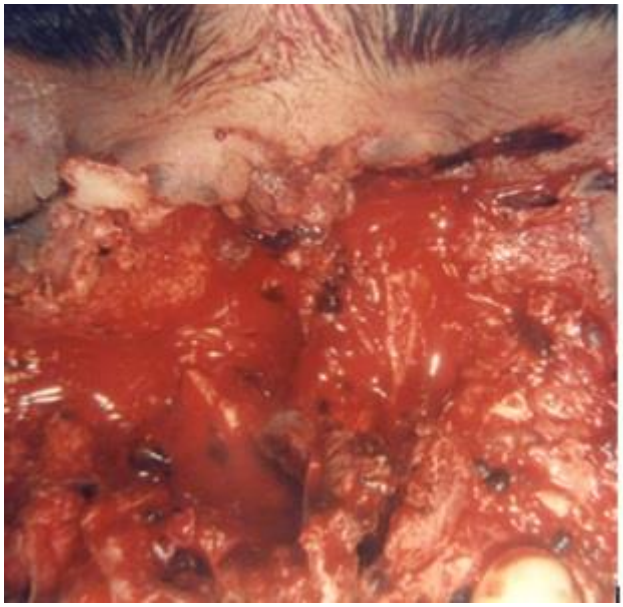

Fig.8

Photo showing severely crushed shredded nasal pyramidal and intercanthal tissue.

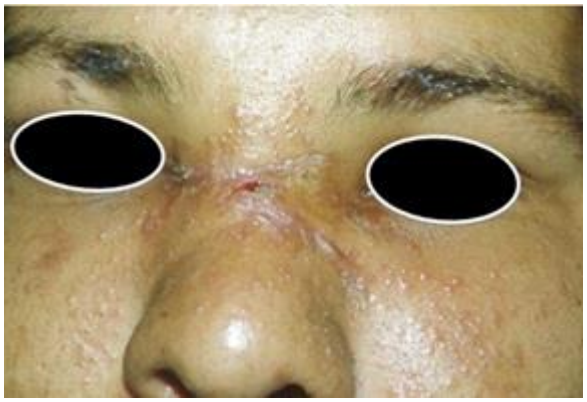

Fig.9
Good result by utilizing modified tracheostomy portex tube as intranasal cavity stent technique.

b. Facial skin presented different types of injuries by primary blast wave when tension exceeds the tensile strength, collagen fibers will fracture and tissue will break. Lacerations of facial skin Shuker[16] presented a unique analysis of different types of and intense flash burns that will assist in the early diagnosis of life-threatening airway compromise due to the inhalation of hot gases and toxic fumes. IEDs most likely result in blast injuries and severe incendiary situations, which create and provoke new challenges in lifesaving techniques and procedures. Because skin has strong resistance to the primary blast wave effect, most injuries seen on the cheeks, eyelids, and lips are due to the combination of primary and secondary biophysical effects.

Contusions have been seen when the impacts of the primary wave hit over bony processes such as the zygomatic process or mandibular body and symphysis, energy is released that will crush the soft tissue between the compressed air wave in front, which hits the skin surface, and behind that, at the internal bone surface, causing skin and subcutaneous contusion. These wounds are characterized by ragged, tattered, ecchymosed edges. (Figs 10 -12).

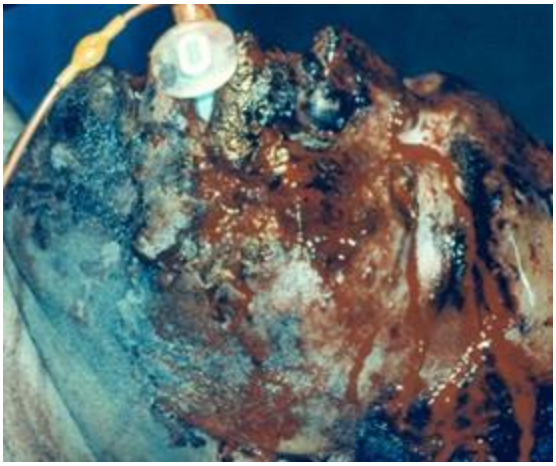

Fig.10.
Severe blast facial injury and fumes inhaled air compromise survived because of immediate oro- tracheal intubation.

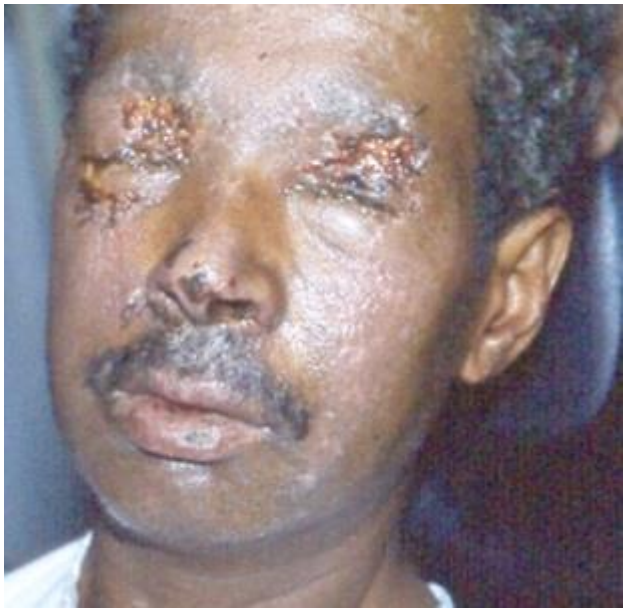

Fig. 11

One month later showing the victim lost both eye.

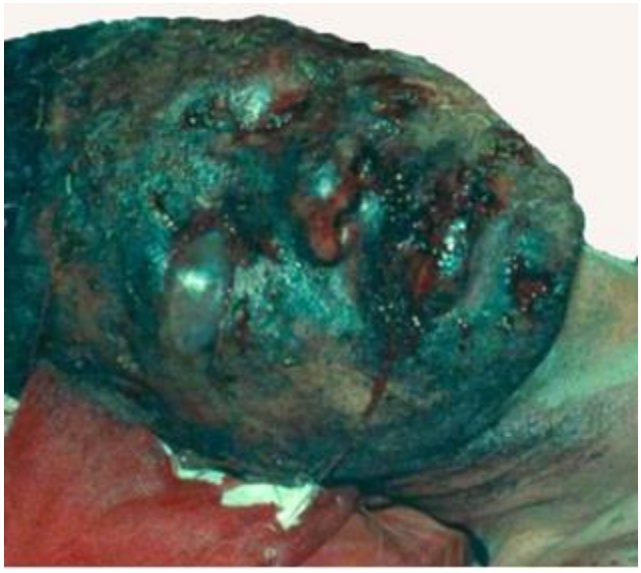

Fig. 12

Facial skin penetrating small fragment lacerations and intense *flash burns*

c. Mandibular and teeth transverse fractures inflicted by acceleration-deceleration are another primary blast wave pathophysiological effect initiated by movement of the body tissue in the direction of the blast wave. Adjacent structures with different physical properties will accelerate at different rates, resulting in the shearing or disruption of tissues.[5] Solid parts of the body simply vibrate as the blast wave passes through them, this has caused a new type of fracture seen only in the teeth and mandible caused by blasts and typically showing the succession movement of the transverse wave. Because not all the teeth and mandibular bony tissues will be equally capable of withstanding the forces, blasts will lead to transverse mandibular shearing fractures at the points of weakness as described by Shuker.[17]Fig. 13

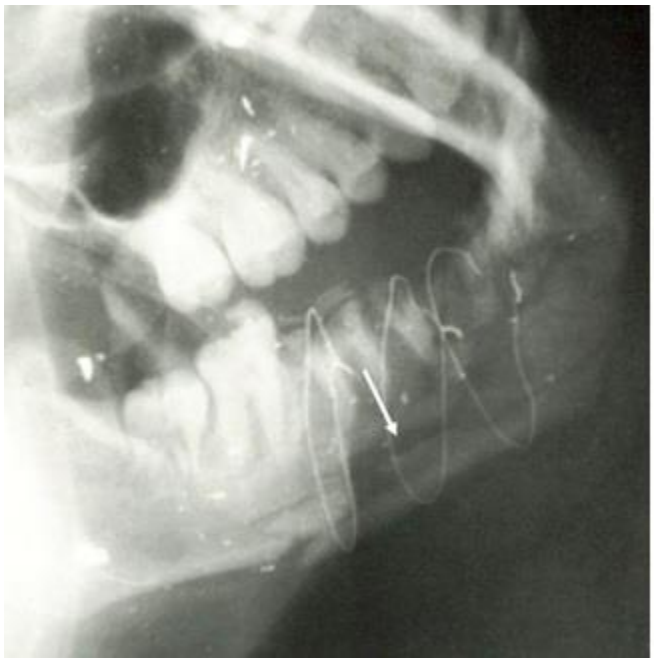

Fig.13

Lateral mandibular radiograph showing typical horizontal line of fractures caused by a primary blast wave (arrow).

d. Spalling or broken fragments can occur at the interface of 2 different media when the shock waves move from a high density to a lower density medium. For example, when air meets water, the water surface is broken up into showers of droplets. The primary blast wave impacts on the weakened eye wall, facilitating a rupturing of the eyeball through the mechanism of spalling and pressure differentials of different eye and orbital medium, sustained stress of high pressure force may be enough to rupture the eyeball.[18]. fig. 14

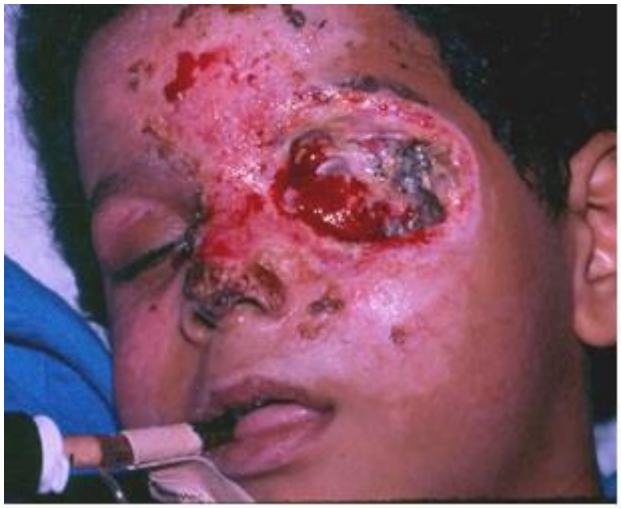

Fig. 14

The primary blast wave impacts ruptured the eyeball and spalling out the orbital tissue.

A bomb blast may cause the full severity range of traumatic brain injury (TBI), from mild concussion to severe, penetrating injury. The pathophysiology of blast-related TBI is distinctive, with injury magnitude dependent on several factors, including blast energy and distance from the blast epicenter. [19] TBI is an important cause of morbidity and mortality following a high-order explosive event. The severity and nature of brain injuries that occur depend partly upon the nature and quantity of the explosive used.[20] Fig. 15

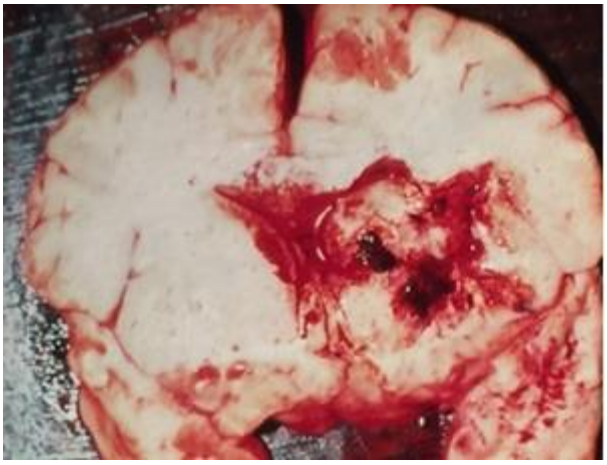

Fig. 15

Blast wave brain injury showing by brain coronal section (courtesy Dr. A Hadi Al Khalili)

Discussion

Scientific discourse on primary blast injury PBI is a twentieth-century phenomenon. Although only a limited number of clinical findings on PBI has been published in articles in the second half of the twentieth century; most of that was on gas containing organs (ears, lungs and intestines).

Biodynamic blast tissue injuries are different from region to region, as seen in Lungs, brain, eye/orbital, mandibular body, and air-containing cavity blast trauma. When the blast wave interacts with a person's facial soft tissue and when the tension exceeds the tensile strength, collagen fibers will break and tissue will sever. These are dissimilar and they depend on tissue locations. Implosion damage is limited to the gas-containing structures of the auditory canal, paranasal sinuses, gastrointestinal tract, and lungs. In the maxillofacial region, the implosion mechanisms of the primary blast cause the most dramatic effects, observed in the fractures to the middle third of the face.

Primary blast wave effects have created new types of trauma that differ from civilian and ballistics injuries in general; mandibular fracture is one of them. Mandibular transverse shearing fractures (Shuker`s mandibular fracture) caused by blast waves, were confirmed in between the line at the apices of roots and just above the cortical bone of the lower border. It gives a better understanding of the biophysics of blast, and the transverse tensile strength of alveolar bone because of the cylindrical shape of the roots and the cross-section of the sockets. So civilian fractures are at the point of weakness vertically, they also spread between the roots.[17, 21]

Tearing of the skin by blast effects differs from the sharp cut of the surgeon's scalpel. Wave impacts hit over bony prominences, which inflict ragged wound edges and tattered tissue. Shredded wounds are found more often on flexible soft tissues such as the lips, cheeks, eyelids, and neck. These injuries are not totally due to air overpressure impact but are compounded by primary blast and secondary fragments of small ground elements, sand, or other components that inflict penetrating injuries and/or deep abrasion of the skin. Immediate wound closure in victims with blast injuries to the facial area was successful despite the reduction in the capillary blood supply. This is significantly different from extremity injuries or in other parts of the body, where immediate closure is not advised when the blood supply is diminished or not rich.

The threat to life is not in the appearance of severity of the injury that is initially observed on the external facial tissue on admission. The potentially lethal injuries could be overlooked and not discovered. These include airway compromise, which requires aid and resuscitation immediately. The upper respiratory system and lungs should be checked in all maxillofacial blast injuries. Low oxygen flow to tissue should not be misdiagnosed.

Protection of the head and face is necessary to protect individuals against the primary blast of IEDs injuries, because any solid object interposed between an individual and the source of an explosion reflects much of the excess pressure and deflects the known dynamic pressure of blast wave. A helmet with facial protection attached to it, in a half circle around the face, of a strong transparent material will protect a great deal against injuries. Reinforcement of external walls of

special thickness sand or ceramic built around the armored vehicle may be of value. Wearing protective eye gear and wearing ceramic vests can diminish the extent of IED injuries, while creating typical patterns of injuries to be treated.

In conclusion a picture of two years old girl holding tightly her milk bottle two days after sustaining facial blast injury, sends a message to stop using explosive device against children. Fig. 16

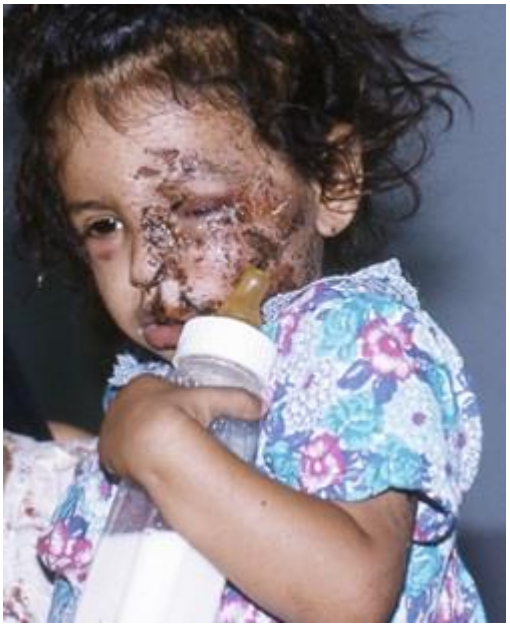

Fig.16.

A two years old girl holding tightly her milk bottle two days after sustain facial blast injury

Disclosure:

No financial and material support for this research and work. No other sources of support that require acknowledgment.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 1, (2016), 17-25 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

APPLICATION OF ND: YAG LASER TO INDUCE SHOCK WAVE STONE FRAGMENTATION FOR LITHOTRIPSY

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Abstract

Laser induced shock waves have found many medical applications such as, lithotripsy, photo-disruption and nano-surgery. Laser is capable of inducing localized effects in inaccessible places, encapsulated materials or out-of-the-way places. It is an automated, non-contact and much faster than conventional methods. Artificial stone samples were fabricated from mixing water and cement. They were ablated and laser hammered by high intensity Nd: YAG laser pulses of different laser energy fluence. The stone ablation and fragmentation in water showed better results than in air, due to the better shock wave confinement which resulted in higher knocking pressure. Higher laser pulse energies produced higher intensity which ablated more material. In this work, complete Nd: YAG laser fragmentation of stones was accomplished in two conditions: the use of 200 ($2.5x10^{11}$ w/cm^2) laser pulses in ambient air, and in the use of 14 ($2.5x10^{11}$ w/cm^2) pulses in water.

Key words: laser lithotripsy, photo-disruption, Laser-induced breakdown (LIB),

Introduction

Laser lithotripsy is a localized, automated, non-contact and very fast technique. It can replace conventional surgery and abolish patients' post-operation suffering. Shock waves, induced by high intensity laser beam, can successfully hammer and fragment urinary and kidney stones. First attempts started in late sixties using ruby lasers[1] but later were replaced in the 80's by Ho: YAG laser[2] because of its final clearness of stones and less complications. In 2008, better results were obtained when using frequency doubled Nd: YAG laser; operating at 532nm. In 2013, the composition effect of stones, immersed in water, on fragmentation efficacy after irradiating them with Ho: YAG laser was investigated.

Urinary and kidney stones formation is a result of precipitating dissolved salts in the urine[5]. They are painful and require certain medical procedure to solve the problem[4,5]. Small diameter stones (≤ 4 mm) always come out without surgical operation by drinking water and taking medicine to increase urine[4,7]. If the problem remains, surgery is used to remove them out. Until recently, surgical intervention was the most prominent procedure to remove kidney stones[8]. Laser lithotripsy of stones is a technique that can be implemented to remove difficult bile duct stones[9] and retrograde stones using an endoscope without the need of anesthesia[10]. Choosing any medical technique will depend on the size and location of the stones[11, 12]. Intra corporeal laser lithotripsy is now recommended to treat the very hard calcium oxalate monohydrate calculus[13]. Nd: YAG (1064 nm), Ho: YAG (2,100 nm), and Alexandrite (750 nm) are some examples of the lasers used in laser lithotripsy today. For efficient stone disintegration and minimum damage of the surrounding tissue, low thermal energy and high mechanical effects are needed. Stone hammering by pulsed lasers is materialized when high-temperature plasma is reached. A fully ionized gas around the stone expands at a very fast rate and generates an acoustic shock wave[14]. Short pulse Nd: YAG laser induced shock wave can break down gallstones into small pieces[15].

The use of combined 532 nm and fundamental 1064 nm Nd: YAG laser wavelengths are very beneficial. The 532 nm laser beam generates the plasma at the stone surface, while the 1064 nm beam heats this plasma and causes relaxation and contraction, which disintegrate the stones[16]. Direct endoscopic vision is always used with laser lithotripsy to avoid accidental damages of the bile duct wall[17]. Laser pulses from Nd: YAG or Ho: YAG lasers hit the stone's surface in the urine and form transient cavity after rapid vaporization and plasma formation[18]. The interaction of laser with electrons in the stone could generate quasi-free electrons with energies that could be enhanced by the electric field and generate more free electrons[20]. Risk of tissue damage with laser lithotripsy is less than other lithotripsy techniques[14, 21]. Fabricating artificial kidney stones is vital for experimenting lithotripsy and obtaining standard data. To investigate this issue, similar stones in terms size, mass, shape, and material need to be prepared. Ultracal 30 gypsum cement, ceramic materials and BegoStone dental plaster are well-known material having very close physical properties to those of natural stones[22, 23].

Experiment

The artificial stones that mimic the natural urinary stones were made from mixing water with cement by 2:1 ratio and left to dry in ambient air for 10 days. The stones were ablated by high intensity Nd: YAG, 9 ns laser pulses using different values of laser pulse energy (1.57, 1.96, 2.2, 2.5 and 2.7) x 10^{11} MW/cm^2 and different number of pulses. The idea was to study the effect of these two parameters and finalizing the optimum ablation conditions. The laser beam was focused by (50mm) focal length lens for the purpose of obtaining high laser intensity levels. The experiment was performed in air and in water to investigate the effect of the processing environment. Stones were weighed before and after laser irradiation, and the weight loss was calculated by 4-digit digital balance. SP-3000 Plus, OPTIMA, (500-1070nm), spectrophotometer was utilized to investigate the spectral absorption of the artificial urinary stones.

Results and Discussion

To study the effect of pulse laser energy and the number of pulses, 4-digit balance was used. The results obtained shows that the weight of the stones mass was decreased with the increase of the number of pulses. The proceeding pulses lose more of their energies than the preceding ones because of the greater attenuation at deeper points in the substrates. The material is ablated but ejecta are immediately re-condensate on the wall of the hole. Figure (1) shows the weight loss measurements of an artificial stones ablated in ambient air.

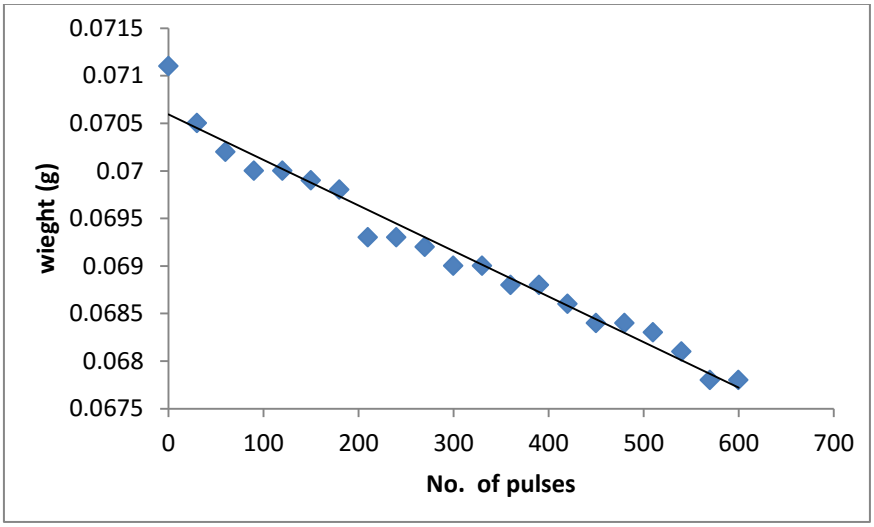

Figure (1): Weight loss rate against laser pulses number in air.

The stone ablation in water showed better results than in air due to the better shock wave confinement, and therefore the stronger shock wave generated. This induces higher mechanical stress on the stone surface, and as a result, greater stone ablation rate for smaller number of laser pulses. Figure (2) shows the weight decreasing as a function of number of pulses.

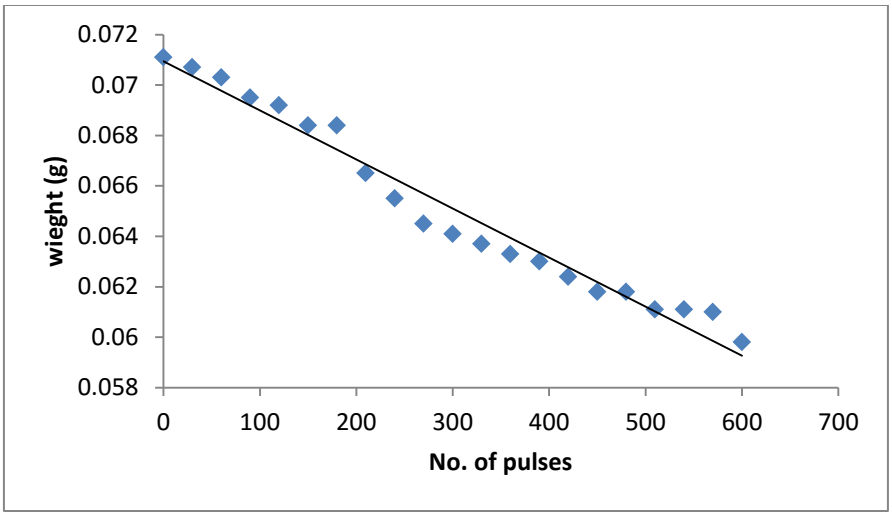

Figure (2): Weight loss rate change with pulses number in water.

The effect of pulse energy on stone ablation or weight loss necessitated the fabrication of artificial stones were irradiated by 30 pulses at different energies (1.57, 1.96, 2.2, 2.5 and 2.7) x 10^{11} mW/cm^2 per pulse. The stone weight before and after laser ablation was recorded to calculate the amount of weight loss (ablation). The stone weight loss after being irradiated with (2.7x 10^{11} mW/cm^2) pulse was larger ($0.7x10^{-3}$g) than the weight loss after irradiating the stone with (1.57x 10^{11} mW/cm^2) pulse ($0.5x10^{-3}$g). Higher laser pulse energy produces higher intensity that ablates more material [4, 13]. Stone material weight loss measurement ablated in water shows more effective results than in air. The ablation rate, and therefore the material loss rate were much higher when ablation took place in water because of the higher generated stress. Using (2.7x 10^{11} mW/cm^2) laser pulses caused a weigh loss of ($5.9x10^{-3}$g) in water and $0.7x10^{-3}$ g) in air as shown in figures (3) and (4).

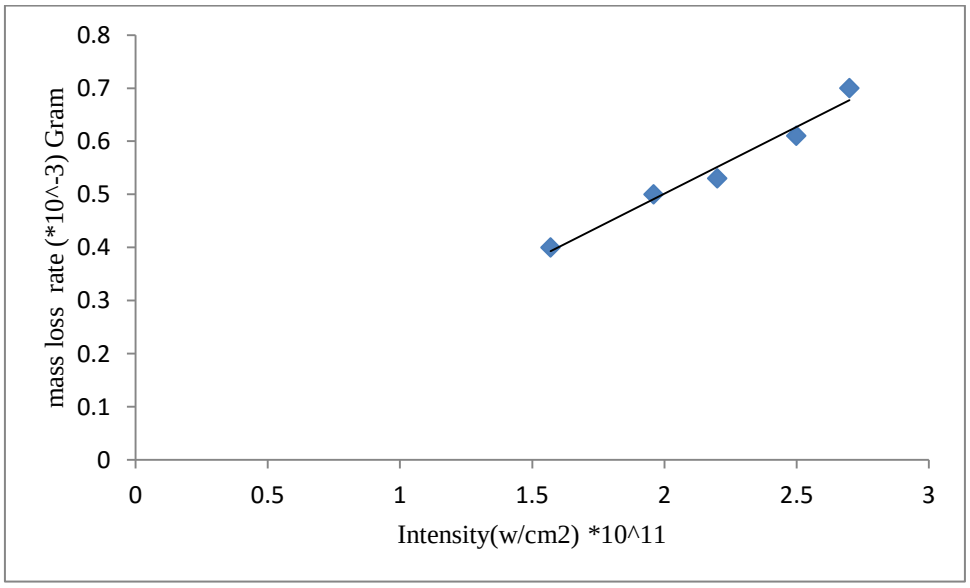

Figure (3): Weight loss as a function of laser pulse energy (in air) using 30 pulses.

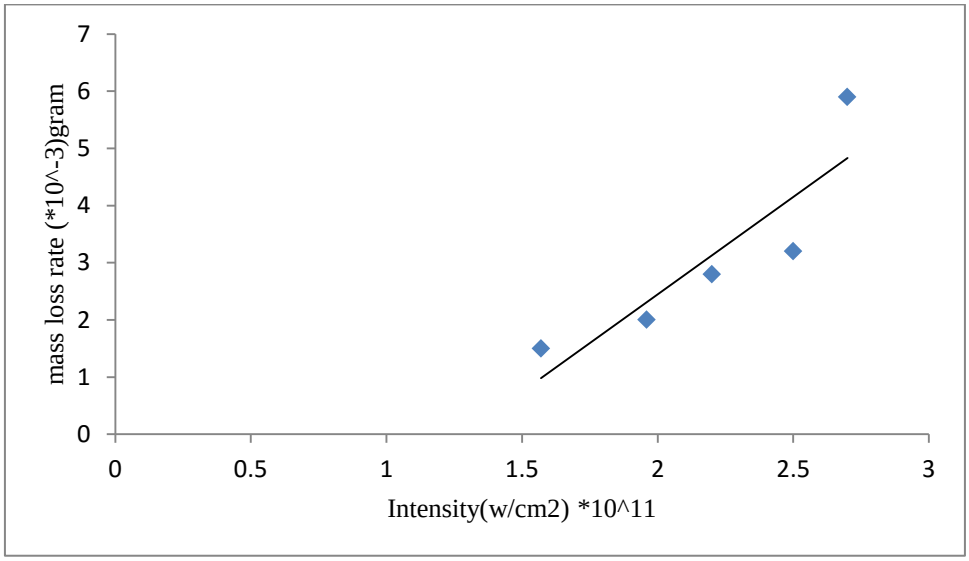

Figure (4): Weight loss against laser energy (in water) using 30 pulses.

Complete fragmentation of stones was reached in two conditions by using Nd: YAG laser; In the first condition, 200 laser pulses; each of ($2.5x10^{11}$ w/cm^2) were used in ambient air, while in the second condition, only 14 pulses, each of 300 mJ energy in water. Figure (5) shows that the laser ablated stone broke at its weakest points by using large number of pulses, while figure (6) shows that the stone broke into two pieces after only 9 pulses; and fragmented more by using 5 additional pulses.

Figure (5): Complete fragmentation of artificial stone by Nd: YAG laser using 200 pulses; each of ($2.5x10^{11}$ w/cm^2) (in air).

Figure (6): Complete fragmentation of artificial stone by Nd: YAG laser using 19 pulses; each of ($2.5x10^{11}$ w/cm^2) in water.

Weight loss measurement showed higher weigh loss values with 1064 nm than the 532 nm and the (1064 nm + 532 nm) wavelengths. The weight loss in water was greater than in air due to the stronger shock wave in water. Tables (1) and (2) outline the results of stone fragmentation in air and water.

Table (1): Weight loss dependence of samples in air on laser wavelength in air; using $2.5x10^{11}$ w/cm^2 and 60 pulses

sample	Wavelength (nm)	Weight loss (g)
1	1064	$0.4x10^{-3}$
2	530+1064	$0.2x10^{-3}$
3	530	$0.1x10^{-3}$

Table (2): Weight loss dependence of samples in water on laser wavelength; using $2.5x10^{11}$ w/cm^2 and 60 pulses

sample	Wavelength(nm)	Weight loss(gram)
1	1064	$1.5x10^{-3}$
2	530+1064	$1.3x10^{-3}$
3	530	$1.2x10^{-3}$

The results of UV-Visible Spectrometry showed that the absorption of artificial stone was higher at 1064 nm, (83.2%) than at 532 nm, (81.1%), see figure (7). This result is in harmony with other experimental data in which the 1064 nm wavelength was more effective than the 530 nm and causes more stone mass loss.

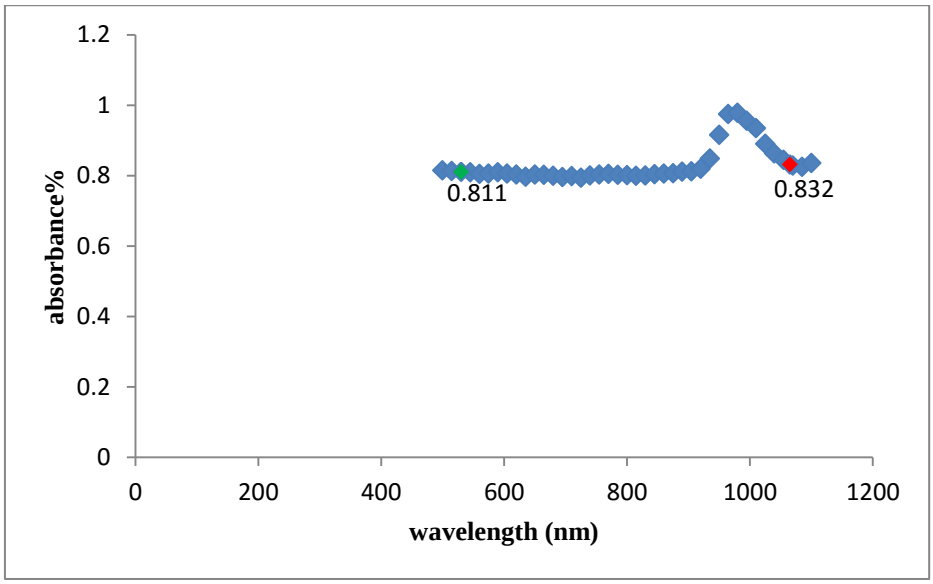

Figure (7): UV-visible spectrometry analysis of cement colloidal.

Conclusion

Laser hammering of stone was accomplished in both; air and water. The minimum laser intensity required to fragment a stone (laser lithotripsy) was 15.7 x 1010W/cm^2. Due to the better shock wave confinement, stone ablation and fragmentation in liquid is more effective than in air, and requires lower number of laser pulses. Greater ablated mass was achieved with (1064nm) laser wavelength than with the (530nm). For the future work, the body fluid will be simulated chemically and repeat the experiment to be much closer to real effect. Laser hammering of stones in rabbits will also be attempted at later stage

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 1, (2016), 26-38 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

EVALUATION OF IL-2, IL-1 EVALUATION OF IL-2, IL-10 AND IFN-GAMMA IMMUNE EXPRESSION IN LIVER AND SPLEEN AFTER TREATMENT OF EXPERIMENTAL CYSTIC ECHINOCOCCOSIS

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Summary:

We have evaluated IL-2; IL-10 and IFN-ɣ protein expression in both liver and spleen of BALB/c mice experimentally induced secondary hydatidosis and treated with different drugs. Following Intraperitonial inoculation of Echinococcus granulosus-protoscolices, following the infection, mice were treated for 6 months with different drugs. The sera of the mice were screened for the biochemical enzymes. Liver and spleen were processed and paraffin embedded tissue sections were examined by immunohistochemistry for IL-2, IL-10 and IFN-gamma from different study groups. Treatment with Oxfendazole shows 93.75% efficacy, but it's more effective in combination with Praziquantel (96.7%), but it lower in combination with Albendazole 82.5% or Albandazole combined with Praziquantel 77.5%. After treatment, liver and spleen express an increased IL-2, IFN-ɣ and IL-10 proteins in treated groups and positive control group compared with control negative group.

Conclusion: After successful treatment of Cystic Echinococcosis (CE) will increase IL-2 and IFN-ɣ with IL-10 reduction, this would support the hypothesis that restoration of the host cell-mediated response occurs after elimination of *E. granulosus*.

Key words: hydatid cyst**,** IHC, IL-2, IFN-ɣ, IL-10

Introduction:

Hydatid cyst or Cystic Echinococcosis (CE) is a widely endemic helminthic disease caused by infection with metacetodes (larval stage) of the tapeworm *Echinococcus granulosus*. In mammalian species, the liver is the most frequent site of hydatid cyst followed by the lungs and, less usually in spleen, kidneys, heart, bones and central nervous system[1]. The immature cyst has to overcome host, mainly cell-mediated immune responses, especially the infiltration of macrophages and eosinophils and low level polarized T helper (Th1) responses. Which may benefit both host and parasite; pronounced pathologic changes in the liver and spleen[2]**.**In chronic stage CE, cyst growth is maintained and complex echinococcal antigens are released from the cyst stimulate complex immune responses[3]. These include polarized Th2 responses balanced with Th1immune responses. Th1 responses are responsible for damaging the parasite whereas Th2-promoting cytokines for parasite growth in host[3]. It may facilitate the parasite survival in the intermediate host but results in chronic granulomatous reactions and fibrosis. Nevertheless, little is known about the arrangement of resident and non-resident cells in chronic responses to hydatid cyst in the human liver and spleen[4]**.**

Very few studies addressed that drugs can bias the local immune response, the majority used ELISA to evaluate serum level of cytokines. In human subjects undergoing chemotherapy treatment, an increasing Th1 cytokine profile, rather than a Th2[5]. It may be one of the proposed killing mechanisms that set in during the later stages of infection[5].

Significantly, increased production of IL-4 and IL-10 in hydatid disease patients corresponds to high levels of IgE and IgG4**.** Therefore, both IL-4 and IFN- regulate the IgE and IgG4 responses[6]. AE patients experiencing a relapse of the disease have a tendency to increased production of IL-5 but lower IFN- production accompanied by significantly higher levels of IgE and IgG4 compared to patients with a primary infection[7].

It can be hypothesized that the effective treatment will support cellular composition of inflammatory cytokines in liver and spleen. Accordingly, this first study was aimed to characterize the tissue expression of IL-2, IL-10 and IFN-gamma proteins by immunohistochemistry.

Materials and methods:

1-Animals: sixty white males' white mice (*Mus musculus*) strain Balb / c, aged 4-5 weeks, their weight ranged from 20 ± 5 g, were bred and adapted at the animal house of the college of pure science / Ibn-Al-Haitham for 2 weeks before starting the experiment by rearing in separated, clean and disinfected cages ,they were fed on commercial assorted pellets and clean water.

2-Preparations of antigen: Hydatid cysts of infected sheep were collected to prepare HCFAg according to[8], and select concentration (3.36 mg/ml) according to the method of[9], and isolated protoscolices (PSCs) estimated viability and their number by[10], a single dose challenge (2000PSCs±5).

3-preparation of drugs: Three **drugs** prepared according to[11] used in an attempt to treat hydatid cysts in mice are- :

-Oxfendazole (OFZ) a concentration of 30 mg/kg of body weight, equivalent to 0.04 mg / ml[12] were obtained on the property locally (Synanthic ®, Fort, Dodge, Mexico).

-Praziquantel (PZQ) a concentration of 40 mg/kg of body weight, equivalent to 0.06 mg / ml 1.

-Albendazole (ABZ) a concentration of 10 mg/kg of body weight, equivalent to 0.01 mg / ml.

Drugs given to mice groups in single dose or mixed, as follows- :

1- OFZ. **2-** OFZ + ABZ. **3-** OFZ + PZQ. **4-** ABZ + PZQ.

4-Experimental designs: Sixty mice were **immunized** at day 0 with 0.2 ml of HCFAg S/C after mixed with an equal volume of incomplete Freund adjuvant, after 21 days given **booster dose** consists of hydatid fluid 0.2 ml with an equal volume of complete Freund adjuvant, and with immunization the mice injected with 0.2 ml antioxidants (Pharmaton R, Switzerland) daily/orally for month.The **challenge dose** (2000 PSCs ± 5) I / P in day 30 of the first day of immunization and at the same time injected a group of positive control (15 mice) were injectedwith 0.2 ml phosphate buffer saline. The immunized group's mice administered 0.2 ml of the above drugs orally, one dose a week for four months. Four months post challenge dose sacrificed all the animals.

5-Examination of therapeutic index: Each group of animals were sacrificed by cervical dislocation, and examined their internal organs (such as Liver, spleen, lung, stomach intestine and etc.) of mice were removed under sterile conditions by abdominal incision. Hydatid cysts were collected for evaluation of their diameters and weight as well as their number. Therapeutic index based on calculation of mean number of cysts in control group-mean number of treated group/mean number of control group x100[13].

6-Histopathology and immunohistochemistry staining for IL10, IL2 and IFN-gamma: Liver and spleen were preserved in Bounis solution for 24 hrs, then dehydrated and embedded in paraffin for routine hematoxylin and eosin staining.

Sections of 5 μm were obtained and mounted on positive charge glass slides. Before labeling, sections were deparaffinized in xylene and rehydration by graded series of ethanol baths. Endogenous peroxidase activity in deparaffinized sections was blocked with 0.3% of H_2O_2. Sections were then incubated in phosphate buffered saline (PBS), pH 7.1, for 10 minutes at room temperature (20-25 ºC).

Incubation with the primary antibody after dilution to 0.3microgram/ml of each primary antibody (rabbit anti-mouse IL 2, I7663-27M6, IL 10, I8432-13F and IFN-γ, I7662-16N6) was carried out

in a wet chamber for one hour at 37C temperature.

Following incubation with the primary antibody, tissues were washed with PBS, pH 7.1, incubated with a biotin link anti-rabbit IgG for one hour at 37°C in humid chamber, and then washed with PBS. Streptavidin peroxidase coupled applied on tissue section for one hour at 37C, and then re-washed with PBS, pH 7.1.

Subsequent localization of proteins was revealed by reaction with 3'-diaminobenzidine tetrahydrochloride (DAB) substrate freshly prepared. Sections were then counter-stained with hematoxylin for 1-2 min. Finally, sections were dehydrated in ethanol and xylene and mounted by DPX on cover slips. Negative controls included incubation with PBS without the primary antibody. Immunostaing was analyzed using an Olympus BX45 microscope.

Data analysis: Results of IHC test were evaluated by applying a semi- quantitative assessment: each slide was counted under light microscope for three times at x400 magnification and about 5 fields were randomly selected in each round. Thus, the number of immunolabeled cells was counted in 5 fields under a fixed focus for each slide and value of mean for positive count in total number of all counted cells (each tissue section) for each sample group. Data were expressed as mean ± standard deviation (SD) and Analysis of Variance (ANOVA) test was used for differences between groups. Values $p \leq 0.05$ was regarded as statistically significant.

Results:

Estimation of therapeutic index among treated groups:

Necropsy finding showed that mice from positive control group have secondary hydatid cysts with irregular shapes, single or multiple arranged in oval or irregular forms. However, mice treated with OFZ+PZQ showed higher percentage of therapeutic affect (96.7%), followed by mice treated with OFZ (93.75%), and mice treated with OFZ+ABZ (82.5%) and the lowest rate found in mice treated with ABZ and PZQ (77.5%) (Table- 1).

Table- 1: relative percentage of shrinkage cysts, number of cysts and therapeutic index among treated groups.

Groups	Percentage of shrinkage cysts	Number of cysts	Therapeutic index
OFZ	50±14	2.5±1.2	93.75%
OFZ+PZQ	75±15	1.3± 0.94	96.7%
OFZ+ABZ	28.4±14	7±1.3	82.5%
ABZ+PZQ	37.4± 8	9± 2	77.5%
Negative control	0	0	
Positive control	0	29±8.3	

Immunohistochemical expression of IL-2, IL-10 and IFN-γ in liver and spleen:

The results in this study describe the protein expression of some cytokines in both liver and spleen of mice treated with different therapeutic regimens. In table 2, it has been shown that there is highly statistical increase in IL-2 protein expression (Figure 1) in liver from OFZ (23.4%), OFZ+BZQ (34.2%) and OFZ+ABZ (54.1%) treated groups in comparison with control negative and control positive groups. While ABZ+PZQ treated group showed that there is no statistical significant difference with negative and positive control groups.

IL-10 protein (Figure 2) have been found to be increase in mice treated with OFZ+PZQ in comparison with negative control group while its not significant with positive control group. Mice treated with ABZ+PZQ were statistically different from negative and positive control groups. In contrast, mice treated with OFZ+PZQ have lowered IL-10 protein expression when compared with positive control group.

Interferon gamma protein expressions (Figure-3) were generally higher expression among treated groups than those negative and positive control groups. Except that OFZ+PZQ treated group was not significantly different from negative control group.

Table 2: Immunohistochemical expression of IL-2, IL-10 and IFN-γ in liver among study groups.

Groups	**IL-2**	**IL-10**	**IFN-γ**
OFZ	23.4±3.2 a**,b**	10.2±6.4a^{NS},b^{NS}	35.8±12.9 a**,b**
OFZ+PZQ	54.1±8.3 a**,b**	8.2±6.9 a^{NS},b*	23.2±7.1 aNS,b**
OFZ+ABZ	34.2±9.2 a**,b**	24.1±7.9 a**,b^{NS}	54.7±12.3 a**,b**
ABZ+PZQ	13.2±8.5 a^{NS},b^{NS}	32.1±6.9 a**,b**	28.7±8.8 a*,b**
Negative control	6.4±2.6	6.4±3.2	17.2±8.4
Positive control	12.7±8.3	18.2±8.5	8.3±5.4

a; comparison with negative group. b; comparison with positive group.

***** Significant differences on (p≤ 0.0). ******Significant differences on (p0≤.001).

NS: No significant (p>0.05).

In spleen, IL-2 protein expression showed that there is higher expression among all treated groups when compared with control negative group.

Table- 3: Immunohistochemical expression of IL-2, IL-10 and IFN-γ in spleen among study groups.

Groups	IL-2	IL-10	IFN-γ
OFZ	41.3±8.3 a**,b^{NS}	24.1±5.3 a**,b**	12.7±6.5 aNS,b*
OFZ+PZQ	65.1±21.4 a**,b**	29.7±12.2 a**,b**	24.7±9.2 a*,b**
OFZ+ABZ	63.2±12.9 a**,b^{NS}	37.3±11.8 a**,b^{NS}	35±8.3 a**,b**
ABZ+PZQ	45.2±17.2 a**,b**	45.7±7.5 a**,b^{NS}	23.4±6.2 a*,b**
Negative control	5.1±3.3	8.2±2.8	12.9±9.33
Positive control	34.2±12.8	43.8±15.2	5.3±2.2

a; comparison with negative group. b; comparison with positive group.

***** Significant differences on ($p \leq 0.05$). ******Significant differences on ($p \leq 0.001$).

NS: No significant ($p > 0.05$).

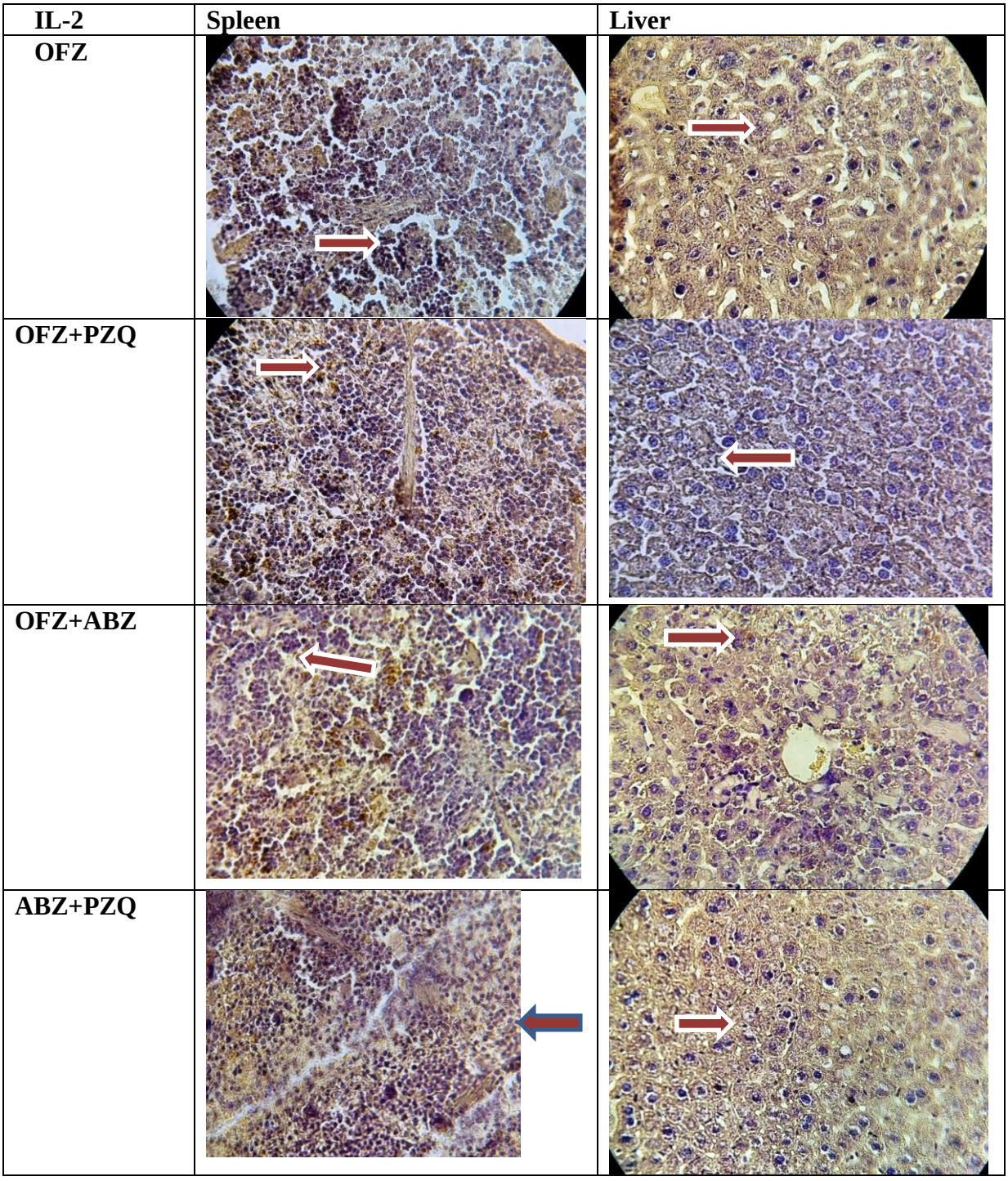

Figure 1: Immunohistochemical staining of IL-2 protein in mice spleen and liver using anti-IL-2 and visualized by peroxidase anti-rabbit. Stained cell appears as dark brown color especially in white pulp of spleen while infiltrating lymphocytes in the liver stained moderately to faint brown staining. Original photos were captured at 400X resolution.

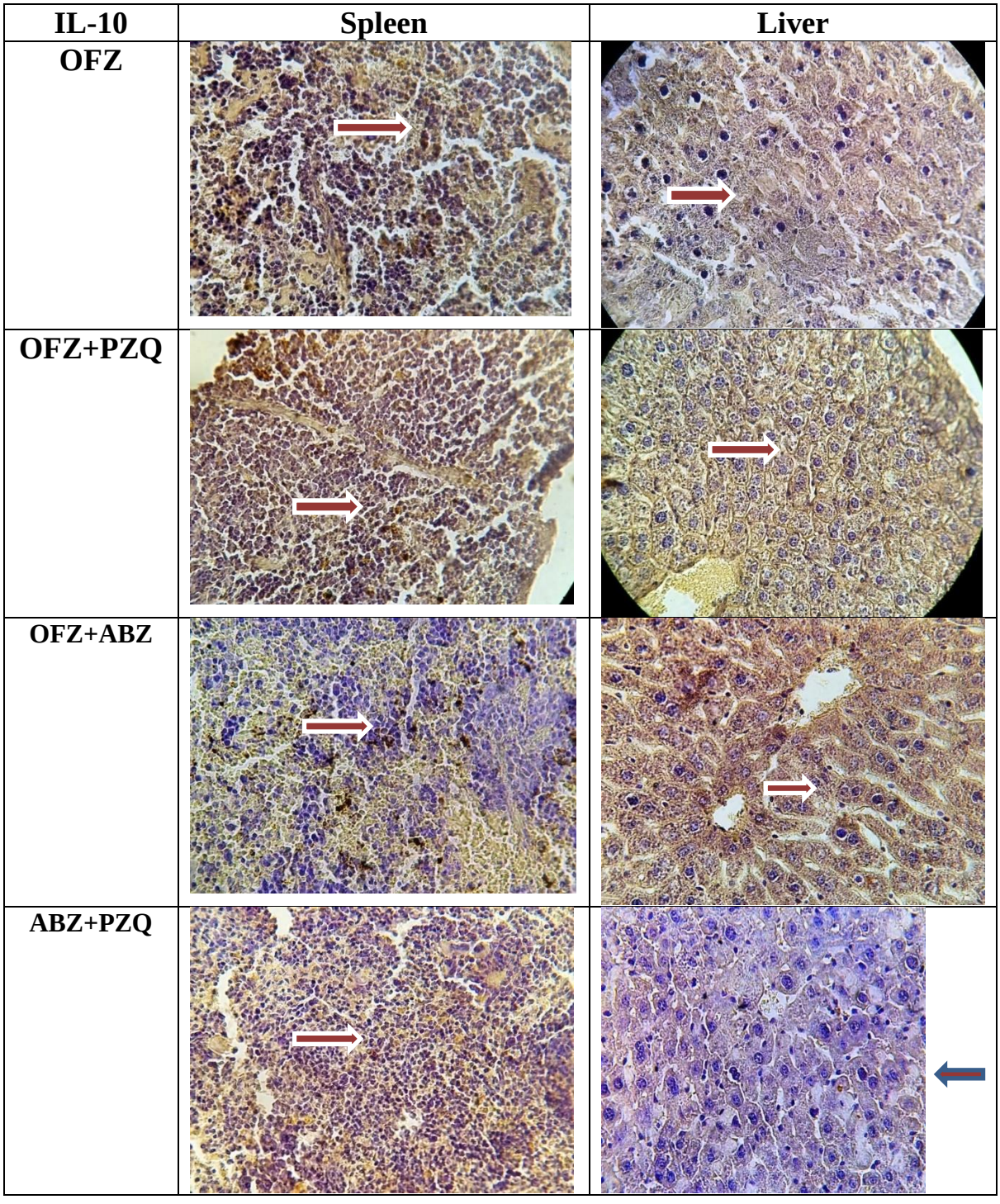

Figure- 2: Immunohistochemical staining of IL-10 protein in mice spleen and liver using anti-IL-2 and visualized by peroxidase anti-rabbit. Stained cell appears as dark brown color especially in white pulp of spleen while infiltrating lymphocytes in the liver stained moderately to faint brown staining. Original photos were captured at 400X resolution.

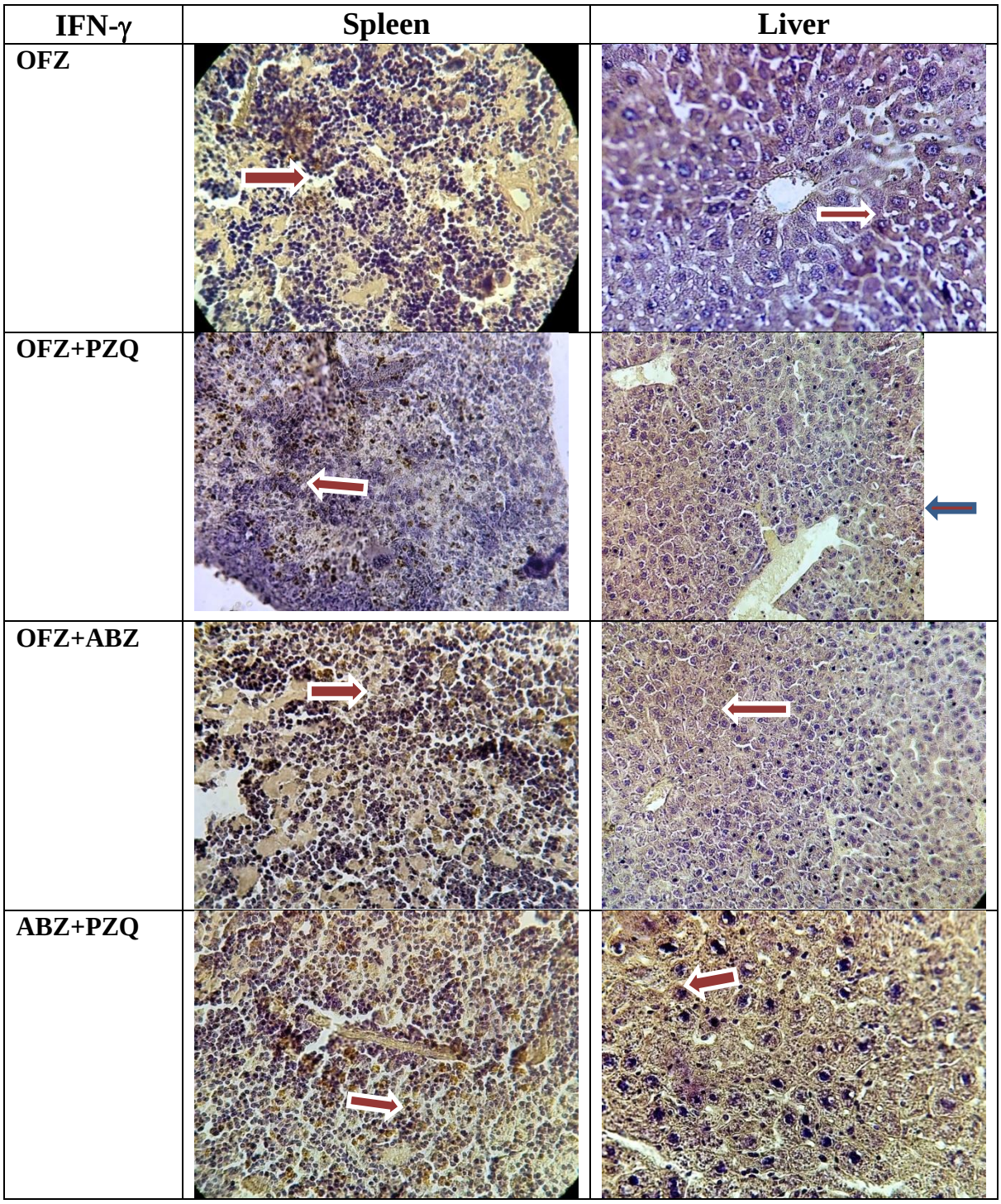

Figure- 3: Immunohistochemical staining of IFN-γ protein in mice spleen and liver using anti-IL-2 and visualized by peroxidase anti-rabbit. Stained cell appears as dark brown color especially in white pulp of spleen while infiltrating lymphocytes in the liver stained moderately to faint brown staining. Original photos were captured at 400X resolution.

Discussion:

This is the first study in Iraq that describes the cellular IL-2, IL-10 and IFN-γ expression in liver and spleen of mice treated with different anthelminthic drugs regimens after experimental cystic Echinococcosis infection. We reported an increased expression of these cytokines in both hepatic and splenic inflammatory cells in all treated groups that possessed different therapeutic efficacies. These results "in particular" highlight the importance of use anthelminthic drugs in combination form to treat Echinococcosis in animals.

Studies were described pronounced elevation of both Th1 and Th2 in both peripheral blood lymphocytes[14], cytokines[15] or in their infected tissues like spleen and liver[16]. Others highlighted the importance of cytokine responses in the pathogenesis of CE was previously studied in animal models[17, 18] . It has been reported that both cellular and humeral immunity evoked during *Echinococcus granulosus* infection[19]. There is no severe inflammatory process and cysts are surrounded by a fibrous layer which separates the laminated layer from the host tissues. The exception is brain where no fibrous layer surrounding the cyst as observed in histopathological reports (data not shown) of liver and spleen in positive control groups.Both IL-2 and IFN-γ were Th1 cytokines required to mount an effective inflammatory action against CE infection, the higher percentage of expression was found in OFZ+PZQ treated group (the highest therapeutic efficacy=96.7%) may explain the successful of this therapeutic combination in treating CE. More than other therapeutic regimens (Table 2, 3) that indicate changing the microenvironment into more protective immunity against CE. In another hands, IL-10 showed mild reduction in the same group suggesting a reduced susceptibility to the disease as the anthelminthic drugs been effective against CE.

These results have been argued by[20] who reported same results. In more details, the host-cytokines is manifested by parasite hepatotoxins that would induce hepatocyte proliferation or apoptosis[21]. The Pulp hepatotoxins are capable of decreasing the transcription of pro inflammatory cytokines (IL-2, IL-6, and TNF-α, IFN-ɣ). Pulp decreases the metabolic activity of peritoneal macrophages and Kupffer cells.As results in a local immunosuppression[22]. The hydatid antigen B would induce immunosuppression by eliciting a non-protective Th2 response (IL-10, IL-4 and IL-13). Additionally, the antigen B inhibits the PMN chemotaxis. This effect is neither due to cytoskeleton impairment, nor to toxic effect[23]. However, it is possible that IL-4, IL-10 and IL-13 would affect the chemotaxis, by reduction of certain chemokines[24, 25]. In addition to that, the counter regulatory effect of immune suppression will inhibit IFN-ɣ and the TNF-α [26] secretions. This mechanism is observed in other parasitic diseases such as Leishmaniasis[27]. Considering the findings of the present studies, we were able to propose that TGF-β induce local immunosuppression; such a mechanism would help to spreading of *E. granulosus* on the liver.

Conclusion:

In general, our results were argued by recent study done by Naik *et al*.,[28] they confirm that effective treatment against will significantly reduce serum IL-10 among human cases**.** We suggest that effective treatment against *E. granulosus* would induce *in situ* immune-activation. Probably such a mechanism is mediated by increased IL-2 and IFN-gamma with IL-10 reduction; this would support the hypothesis that elimination of the parasite will restore the host cell-mediated response.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 1, (2016), 39-46 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

MEDICAL EDUCATION IN IRAQ:

THE FIRST INTERNATIONAL MEDICAL EDUCATION CONFERENCE AT BABYLON UNIVERSITY

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Key words: medical education, medical curriculum, Iraq, quality, assessment, ethics, professionalism

Introduction

Medical education in Iraq, as in other progressive nations, is being rethought and revised to improve the education experience, student skills, and provide for better quality of care for patients. Twenty-first century medical education relies less on memorization and authoritarian – one might even say dictatorial – teaching methods, and more on active learning, critical thinking, and synthesis of knowledge, skills, and attitudes.

Toward those ends the University of Babylon hosted the First International Conference on Medical Education in February, 2015. The University of Babylon, in conjunction with Baghdad University and Kufa University, invited all the Iraq universities to evaluate their curricula. Faculty, students, and invited international guests participated in the process.

The conference, the schedule of which is attached, addressed principles and their applications in a series of plenary presentations and workshops.

Historical Background

The Medical College in Babylon University was established in the 1993 adopting traditional curriculum split mainly into a basic sciences phase and a clinical phase. The old curriculum was subject and discipline based and was heavy on knowledge with a large number of formal lectures. It was focused more on the presentation of information than on the students' learning, and there were no dedicated modules on research, audit or professionalism.

However after 2003, the leadership at the medical college became more aware of the significant developments in medical education during the last two decades in response to the

significant advances in medicine and communications. In line with increasing interest in Iraq in modernising medical curricula, Babylon Medical College explored opportunities for collaboration with medical schools in the developed world. A visit was organised by delegation from the medical school to Leicester medical school in 2012 to have better understanding of development in medical education. This was followed by number of visits and workshops to update staff at the Babylon medical college on medical education. This effort culminated in medical education conference that took place in February, 2015.

Babylon University Medical Education Conference February, 2015

This conference was an important milestone in the journey of medical education development at Babylon Medical college for number of reasons:

1. It was the first international medical conference for the university that focused purely on medical education.
2. It provided an important gathering for large number of Iraqi Medical Colleges to share their progress in the field of medical education and review of their curricula.
3. There was a special session on medical ethics and its role in improving standards of care.
4. There was a session dedicated to the college council considering what changes were needed to the current curriculum and why.
5. There was a general agreement that the medical curriculum in Babylon university would benefit from a complete revision of its philosophy, vision, aims, objectives and methods.
6. Following on from the conference, the Medical College decided to revise its curriculum from a traditional to an integrated one.

The next step was a workshop on medical education that took place in Istanbul October, 2015

for the staff of the medical education unit at the college. Staff had further opportunity to enhance their understanding of adult learning, course design, and principles of assessment in medical education. In addition, there was clarity of future steps and task allocation among the staff of the unit.

The characteristics of the new medical curriculum are:

1. Shift the focus from subject / discipline based to system and service based

2. Promote integration of basic and clinical knowledge early in the course.

3. Encourage student-based learning and improving the reflective abilities of the students using more interactive teaching methods.

4. Review the assessment system benefiting from advances in assessment methods

5. Add research methodology and a critical appraisal module to promote a culture of research.

Challenges & Opportunities

- Resistance to change which comes with any similar project. This is being addressed through engagement and commitment from leadership of the college and the university.
- Financial difficulties that Iraq is going through which make it difficult to have additional support from government to this ambitious program. However, staff of the medical college are showing great determination by exploring alternative sources of funding for the conference and workshops.
- Limited understanding of the new curriculum and its requirement by staff. This is

addressed through increasing number of workshops and educational events on medical education. In addition, encouraging the medical college staff to enroll in courses/ degree in medical education to increase local expertise in the field and support future development.

Future plan

The Medical College is collaborating with other Iraqi universities as well as benefiting from the Memorandum of Understanding (MoU) signed with University of Leicester to introduce new integrated curriculum for the academic year 2017-2018. Republic of IRAQ

Babylon University

College of Medicine

First International Conference for Medical Education

15-16 February, 2015

Conference Schedule

Sunday 15 February, 2015

9-10 am	Registration	
10-	Formal opening session	
11.0	Break	
	Scientific session	
12.0 pm	Overview of medical education	Dr Mohammed ALUZRI
12.20	Ethics and Professionalism in medical education	Prof Dr Allen DYER
12.40	Quality in medical education	Dr Mohammed ABBAS
1.0	Medical education progress in Baghdad medical college	Dr H. ALSAFFAR
1.20	Medical education progress in Kufa medical college	Dr M. ABDULZAHRA
1.40	Iraqi medical graduates -competencies required to meet the global standards.	Dr H. ALSAFFAR
2.0	Lunch Time	

Monday 16 February, 2015

Workshops

930-11 am	Concurrent sessions	
	1-Designing a curriculum	DR Mohammed Al-Uzri
	2-Incorporation Ethics and Professionalism in curriculum	Prof Allen Dyer
	3-Quality in medical education	Prof Allen Dyer
	4-Working with local legislation to approve revised curriculum	DR Hilal
11.0-11.30	Break	
12-1.30 pm	Concurrent sessions	
	1-Being a facilitator of small group	Prof Allen Dyer
	2-Developing assessment model	Dr Mohammed Saeed
	3-Skill-based assessment	Dr Mohammed Abbas
	4-Problem solving medical education	Dr Mohammed Al-Uzri
1.45 pm final session	(conference recommendations)	
2.30	lunch	

We are the Change

While international news focus on the war in Iraq, military and political problems, the everyday stories of civil society, people working to improve their communities and their country, receive much less attention. An important component of the medical education improvements come from the students themselves, who is recent years have had opportunities to travel abroad and learn firsthand about innovations elsewhere.

The group pictured here, students at the University of Babylon College of Medicine, delivering food and blankets to displaced persons has formed a Non-Governmental Organization (NGO) called we are the change, echoing Mahatma Gandhi's admonition to be the change in the

world that you wish to see. With the encouragement of their dean, Dr. Moshtak Witwit, they are actively involved in the process of curriculum reform and in realizing humanistic goals of the medical profession. They express the idea that "We have an Iraqi society which seeks to develop and grow and help each other". And we appreciate the role of youth in the estimated change for a better future.

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 1, (2016), 47-63 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

DEPRESSION & TREATMENT ADHERENCE AMONG IRAQI IMMIGRANTS WHO IMMIGRATED TO UNITED STATE

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Acknowledgements

Drs. Jamil and Arnetz were partly supported by Award Number R01MH085793 from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of the Mental Health or the National Institutes of Health. The authors thank Pfizer Corporation for providing financial support to collect the original survey data (Health Assessment of Iraqi People Residing in Southeast Michigan 2004. Thanks goes to Pierre Yaldo who reviewed the manuscript grammar.

Abstract

The study objective was to estimate the rate of adherence to treatment among depression patients, and to determine the possible factors in non-adherent. We sampled 337 random participants of Iraqi descent, who live in Michigan. Of all the participants, 93 reported forms of depression. Of those 93 people, 36.6% are non-adherent to anti-depressants. There were significant differences among those who are adherent with treatment and those who were not. We noted that Participants with chronic disease were adherence to treatment of chronic illness more than adherence to antidepressants. A non-adherent participant tends to have poorer health compared to his or her health in the previous year. In conclusion the prevalence of medication adhering participants was 63.4%.

Key Words:

Depression**,** Adherence, Chronic diseases, Self-rated health, Arabs, Chaldeans

Introduction:

Depression is next only to hypertension as the most common chronic medical illness seen in daily medical practice. In a study done by the Global Burden of Disease Study, it has been shown that the fourth leading cause of disability worldwide is major depression (Finley, 2002, WHO, 2011). Studies have shown that up to 70% of participants who have been diagnosed by a physician as depressed and are treated with antidepressants are not adherent with taking their antidepressants in one way or another (Katon, 1992, Lin, 1995). Depression is one of the diseases that is frequently coupled with patient reports of forgetting to take medications (Gehi, 2005, Gonzalez, 2007). Response to treatment can be affected by many factors, such as side effects and medications activity; in addition, patient's preference to method of treatment can affect the response to treatment (Churchill, 2009). Adherence to the treatment can play a role in the response to treatments; "adherence" is the degree of correspondence between the patient's genuine dose history and the prescribed treatment.

The model of adherence denotes a joint interactive patient-doctor relationship. When the patient is a passive responder to the authorized physician, orders will be adherent (Beena, 2011).

Controlling chronic medical conditions such as mental health disorders like depression needs active decision making and promise to be adherent to the medication regime (Donovan, 1992, Meredith, 1999, Beena, 2011). About 30% to 50% of participants who are using antidepressants, stop taking their treatment prematurely (Lin, 1995, Melfi, 1998, Peveler, 1999, Bultman, 2002). A study by RAND (2011) showed that depression participants have fewer adherences to their depression medication than chronic illness medications. Non-adherence to treatment regimens has a significant effect on raising the cost of health care in the United States of America (McDonnell, 2002) from 33% to 69% of hospital admission due to medical cause are namely medications non adherence, which costs about $100 billion a year (Senst, 2001).

In the developing countries where no insurance coverage is available, this cost has been studied. A study in Pakistan showed that 16.32% participants stopped their treatment due to lack of finances. As a result information about the causes that affects the participant's adherence and non-adherence with the medications are important (Cramer, 1998). Non–adherence to medication limits the advancements in the treatment of mental disorders as it decreases the beneficial effect even from the best and most effective medicine. The World health organization published a guide to improve the methods of treatment adherent (Sabaté, 2003). Low adherence to medications may be found among refugees and immigrants population in the United States, Lee found that only 12% of the south east Asian refugees adhere to their prescribed medications (Lee, 1993), while Kinzie (2007) found that 15% of south east Asian refugees are adherent to their anti-depressant medication. Sleath (2003) found that Hispanics are significantly non-adherent to their anti-depressants when compared to non-Hispanic whites.

Many factors can cause non-response to treatment regarding refugees. Considering the medicine as stigma (cultural belief), medication shape, patient questioning the dose, no access to medications, are all factors. Gender preference is also a factor; males prefer male doctors and females prefer female doctors. Illiteracy and low literacy, low health literacy, time, Interpretation, trust and Communication (Avery, 2008)… The factors are very numerous. As treatment progresses depressed participants will have different causes to justify their non-adherence to these medications. Whether the patient will inform the physician that he/she will discontinue the medication depends on the approach of the physician and what information he or she provided the patient about that medicine (Demyttenaere, 2001). Maintenance of adherence can be developed through giving strong verbal and written educational explanations about the medications (CIGNA, 2011). In addition the presence of medical or mental comorbid disease, drug abuse or alcohol abuse (Kaplan, 2000) lack of English language proficiency may be a cause of the patient to not adhere with their medications (Lanouette, 2009). A study by Katon (2001) showed that educating participants, frequent meetings with depression specialists, telephone follow-ups and frequent monitoring over a period of at least one year drastically enhanced adherence to anti-depressants.

Ethnic variation can affect the preference of the treatment modality and thus will affect the treatments response; a study by Givens showed that ethnic and racial differences greatly affect responses and choice. Ethnic minorities prefer counseling for the depression treatment much more that what has been observed in white Americans (Givens, 2007). Ten Doesschate (2009) found that higher education level and high level of personality pathology are baseline predictive factors for non-adherence to depression treatment.

Since the US civil war, syndromes in veterans have appeared but are not well understood. Syndromes include forgetfulness, impaired concentration, depression and more. These can all be causes of non-adherence to medicine (Hyams, 1996). Regarding the relationship of demographic profiles of the patient and the risk of non-adherence, some studies demonstrated some characteristics correlate with non-adherence, one of which is age; younger participants are usually at greater risk of non-adherence than other participants (Sirey, 2001, Sirey, 2001, Goethe, 2007). While other studies showed the impact of gender on adherence in such way that women are more adherent compared to men (Melartin, 2005, Goethe, 2007, Chakraborty, 2009), other studies showed that the difference is not significant.

A study by Sirey (2001) found that race, marital status and living arrangements were not significant predictors of adherence. Goethe (2007) reported that non-adherence was more common in employed than non-employed individuals, but that it was not associated with race, ethnicity, marital status or education. High cost service utilization is an important cause of medication non-adherence problems in depressed participants (Julian, 2010). A 77% increase in the relapse risk and recurrence has been shown in a study and it contributes to potentially increasing health care costs (Melfi, 1998). Different interventions directed toward improving patient adherence to the anti-depressants have been tried worldwide. Most of them are not successful; however, counseling about the medication showed significant improved adherence, while the informational leaflets related to the participant's anti-depressant therapy had a very non-significant effect on the adherence of the patient to the anti-depressants (Peveler, 1999). Depressed patients who are at risk of non-adherence will need to understand the cause for poor adherence to these medications (Stephenson, 1993).

The aim of this study is to compare those self-reporting that they have depression and are adherent with their medication versus those who have depression but are not adherent to their medication (from a random sample of two different ethnic groups sharing same background). The study also aims to predict the risk factors that affect medical adherence.

Methods:

In 2004-05 this study was conducted as part of large survey that targeted Iraqi immigrants who are residents in the metropolitan Detroit area. Ethical clearance for this study was granted through Wayne state university, Human Investigation Committee [WSU/IRB/HIC# 086903B3E]. This cross sectional study was announced among the Iraqi community through different channels e.g. media, flyer and social community meeting. A comprehensive standardized questionnaire was used. This questionnaire initially developed through the collaboration between IOWA Persian Gulf war study group and the Center of Disease Control. Data of this study was used in several studies (Koslowe, 1998, Jamil, 2006, Jamil, 2007). The final random sample was driven from a list of 5,490 residents in the metropolitan Detroit area. The sample was drawn from 24 cities, which includes 55 zip codes within the metropolitan Detroit area. From this study sample we excluded those who were not Arab or Chaldean because their numbers were very small (13 participants). The end sample was 337. From the 337 only 93 have self-reported depression, from them only 59 reported that they are adherent to the treatment and the other 34 self-reported that they were non-adherent. A structural interview was planned to administer the questionnaire. The questionnaire was written in Arabic and English, and if the participant can't read Arabic or English, the questions were verbally addressed during the interview. The questionnaire included different types of questions, some examples follow: demographic characteristics, medical conditions, and self-rated health of the participants.

Questions regarding the demographic characteristic are presented in table 1 & 2, e.g.: age , gender, date of arrival to United States (US). Also questionnaires include data on environmental exposure (chemical and non-chemical/stressors) variables, as well as questionnaires on depression and other medical condition such as hypertension, diabetes mellitus, heart disease & asthma. The study questionnaires were based on self-reported medical conditions; so to classify a participant as depressed or having a chronic disease, the person had to state that he/she both had been diagnosed by physician and that he/she received treatment. The questionnaire also included questions about adherence with treatment, based on the fact that adherence is a group of actions taken by patients, such as diet restrictions, medications intake, healthy life style changes, etc. corresponding with health care provider recommendations (CDC, 2013, WHO, 2013), the questionnaire asked the patient if they really and correctly followed the medical advice. In addition, self-rated health status was attained (SRH) by asking questions and comparing the current SRH to health one year ago.

The SRH question was based on a Liker t-type scale which ranging from 5, excellent health to 1, poor health. The SRH scale is well used to predict future health and mortality in currently healthy person (Templin, 2003, Skrondal, 2008). Data analysis was obtained by using the SPSS 21.0 for windows. To see the difference between the categorical variables (E.g. age, gender, occupation), Chi Square was used for categorical variables while t-test was used for the continuous variables. Binary logistic regression was used to predict the significant risk factors for those who were adherent to treatment or not and also for the current SRH.

Results:

Result showed that 36.6% of 93 participants who reported depression were not adherent to treatment of anti-depressants. Tables 1 show the prevalence of participant who have and do not have depression by different variables. It is of interest to find significant differences between the two groups in subcategory of each demographic variable, e.g. ethnicity, years in the US, gender, marital status, employment status and income.

Table 1: Prevalence of participants who have and do not have depression by demographic variables

Variable		No Depression (n=244)	Have Depression (n=93)	Total (n=337)
Age Group	32-44 Y	107(43.9)	42(45.2)	149(44.2)
	40-49 Y	137(56.1)	51(54.8)	188(55.8)
Ethnicity ***	Chaldean	137(56.1)	16(17.2)	153(45.4)
	Arab	107(43.9)	77(82.8)	184(54.6)
Years in USA ***	2-5 Years	12(4.9)	13(14)	25(7.4)
	6-9 Years	47(19.3)	27(29)	74(22)
	10 + Years	185(75.8)	53(57)	238(70.6)
Gender ***	Male	150(61.5)	40(43)	190(56.4)
	Female	94(38.5)	53(57)	147(43.6)
Gender ***	Single	37(15.2)	4(4.3)	41(12.2)
	Married	207(84.8)	89(95.7)	296(87.8)
Employment Status ***	have work	25(10.2)	33(35.5)	58(17.2)
	Don't have	219(89.9)	60(64.5)	279(82.8)
Health insurance	Don't have	86(35.2)	21(22.6)	107(31.8)
	Have	158(64.8)	72(77.4)	230(68.2)
Annual Income ***	$10,000 or less	21(21.2)	14(58.3)	35(28.5)
	$19,000 +	78(78.8)	10(41.7)	88(71.5)
Smoking Status	Do not smoke	160(65.6)	66(71)	226(67.1)
	Current smoker	84(34.4)	27(29)	111(32.9)

*** P < 0.05 ; ** P < 0.01 ; *** P < 0.001**

Table 2 shows the prevalence of participants who have and do not have treatment for depression by different variables. It also shows that there is similar pattern among those who adhere to their treatment and those who do not adhere to it, irrespective of gender, age, education, smoking status & years in US. It also shows that older participants were have more tendency to adhere to the medications. It also shows that for those who had treatment for depression, ethnicity, education and employment status were significant. Being married, female gender and being in us for more than 10 years showed higher prevalence.

Table 2: Prevalence of participants who have and do not have treatment for depression by demographic variables.

Variable		No treatment (n=34)	Have treatment (n=59)	Total (n=93)
Age groups	32-44 Y	20(55.6)	22(37.3)	42(44.2)
	40-49 Y	16(44.4)	37(62.7)	53(55.8)
Ethnicity **	Chaldean	11(32.4)	5(8.5)	16(17.2)
	Arab	23(67.6)	54(91.5)	77(82.8)
Years in USA	2-5 Y	2(5.6)	11(18.6)	13(13.7)
	6-9 Y	11(30.6)	16(27.1)	27(28.4)
	10 + Y	23(63.8)	32(54.2)	55(57.9)
Gender	Male	16(44.4)	24(40.7)	40(42.1)
	Female	20(55.6)	35(59.3)	55(57.9)
Marital Status	Single	3(8.3)	2(3.4)	5(5.3)
	Married	33(91.7)	57(96..6)	90(94.7)
Education ***	< High School	27(75)	19(32.2)	46(48.4)
	High School & >	9(25)	40(67.8)	49(51.6)
Employment Status **	Don't have	7(19.4)	27(45.8)	34(35.8)
	have work	29(80.6)	32(54.2)	61(64.2)
Health insurance *	Don't have	13(36.1)	9(15.3)	22(23.2)
	Have	23(63.9)	50(84.7)	73(76.8)
Annual Income	$10,000 or less	4(40)	10(71.4)	14(58.3)
	$19,000 +	6(60)	4(28.6)	10(41.7)
Smoking Status	Do not smoke	25(69.4)	42(71.2)	67(70.5)
	Current smoker	11(30.6)	17(28.8)	28(29.5)

* P < 0.05 ; ** P < 0.01 ; *** P < 0.001

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Table 3a shows binary logistic regression analysis for that likelihood for having depression; the predicted risk factors are: being females and unemployed (higher likelihood of having depression), females were about 7 times more likely to have depression, on the other hand, being unemployed increased the depression likelihood by about 6.5 times.

Also table 3b shows binary logistic regression analysis for that likelihood for those having treatment for Depression; the predicted risk factors are those being (After excluding all non-compliant participants from those who takes treatment for depression) female and unemployed.

Table 3: Binary logistic regression analysis to predict factors for those (a) with depression (b) having treatment for depression

Binary logistic regression analysis *	**Sig.**	**Odds Ratio**	**95% C.I.f or OR**	
			Lower	**Lower**
(a) **Likelihood for having Depression**				
Male (Reference)	**0.006**	**6.968**	**1.732**	**28.035**
Have work (Reference)	**0.018**	**6.453**	**1.385**	**30.074**
(b) **Likelihood for those having treatment for Depression**				
Male (Reference)	**0.022**	**20.693**	**1.544**	**277.413**
Have work (Reference)	**0.007**	**40.07**	**2.771**	**579.464**

***Adjusted for:: Age, Years in US, Ethnicity, Marital, Education, health Insurance, Income, Smoking, chemical and non-chemical(stressor) exposure.**

Table 4 shows the prevalence rate of chronic diseases among participants who reported depression. In this table we see that the percentage of participants who were adherent to their chronic illness medicine but non adherent to the depression medicine is much higher than the percentage of the participants who are not adherent to medicine of depression and chronic illnesses. So it is quite obvious from table 4 that adherence to treatment of chronic illness is much higher than adherence to treatment of depression. It also shows that people who are adherent to their chronic illness medications are also adherent to their medications in depression. This pattern was significantly observed in diseases like asthma & diabetes.

Table 4. Prevalence of chronic diseases among participants who reported depression

Chronic disease		Participants with		
		No Depression [n=244]	Depression but compliance with treatment [n=59	Depression but do not compliance treatment [n=34]]
		No.(%)	No.(%)	No.(%)
Hypertension	No	208(85.2)	44 (74.6)	29(85.3)
	Yes with treatment	30(12.3)	15(25.4)	4(11.8)
	Yes without treatment	6(2.5)	0(.0)	1(2.9)
heart disease	No	238(97.5)	54(91.5)	33(97.1)
	Yes with treatment	5(2.0)	5(8.5)	1(2.9)
	Yes without treatment	1(.4)		
Asthma (P < 0.01)	No	236(96.7)	48(81.4)	33(97.1)
	Yes with treatment	7(2.9)	10(16.9)	0(.0)
	Yes without treatment	1(.4)	1(1.7)	1(2.9)
Diabetes (P < 0.01)	No	220(90.2)	43(72.9)	28(82.4)
	Yes with treatment	20(8.2)	16(27.1)	3(8.8)
	Yes without treatment	4(1.6)		3(8.8)

Table 5a shows that about 70% of those who have self-reported depression and non-adherent to their medicine reported their health as poor or fair compared to 60% one year ago (table 5b). While about 84% (table 5a) of those who have depression and they are adherent to their medication reported their health as fair or poor compared to about 56% one year before (table 5b).

Table 5: Self-rated Health (SRH) by those who reported depression

SRH / Liker Scale	No treatment for depression [n=34]	Have treatment for depression [n=59]	Total [n=93]
	No. (%)	No. (%)	No. (%)
(a) Currently SRH ***			
excellent	1(2.9)		1(1.1)
very good	4(11.8)	1(1.7)	5(5.4)
good	5(14.7)	8(13.6)	13(14.0)
fair	19(55.9)	24(40.7)	43(46.2)
poor	5(14.7)	26(44.1)	31(33.3)
(b) Compared SRH today to one year ago			
Much better than one year ago,	1(2.9)	1(1.7)	2(2.2)
Somewhat better now,	5(14.7)	10(16.9)	15(16.1)
About the same,	7(20.6)	15(25.4)	22(23.7)
Somewhat worse now,	16(47.1)	-30.5	34(36.6)
Much worse now than one year ago	5(14.7)	15(25.4)	20(21.5)

***p < 0. 001

A binary logistic regression analysis for current SRH among those who reported depression to predict risk factors after adjusting to demographic variables and chronic diseases was conducted. Result show that only those who have more years in US reported their health as excellent/good 1.16 times more than those who reported their health as poor.

Discussion:

This study is different from most of published studies in that its population was randomly selected, and all the participants originally emigrated from Iraq. All participants also share similar background and culture. However, the percentage of non-adherence of depression participants with their treatment is about 36.6%, which is in agreement with other studies that showed 30-50% of participants who are using antidepressants, stop taking their treatment prematurely (Lin, 1995, Melfi, 1998, Peveler, 1999, Bultman, 2002). The study shows that there is similar pattern among those who adhere to their treatment and those who do not adhere to it. Such adherence patterns can be seen in the adherence to treatment of chronic illness e.g. is much higher than adherence to treatment of depression which is similar to a RAND study (2011) which showed that participants of depression are less adherent to their depression medication than chronic diseases medication. Another pattern of non-adherence was clearly seen in that the older the participant's age, the more they tend to comply with treatments. Interestingly, the longer participants have lived in the US, the less compliant they tend to be. We also found that Arabs are more non-adherent than Chaldeans on average, which is similar to the findings in a *Givens* study (Givens, 2007). These patterns of non-adherence could be explained by the fact that Arabs of this study arrived to the U.S more recently as refugees after the Gulf War. On the other hand, Chaldeans mostly started their immigration much earlier as immigrants and not refugees, which is in agreement with a Lanouette study (2009) which showed that being refugees and an ethnic minority made them preferring of counseling rather than medication use alone. The Givens study (2007) showed that ethnic minorities prefer counseling for the depression treatment with medications rather than medications alone; more so than what has been observed among white Americans.

In this study, participants with health insurance were more non-adherent to their medication than those who lack health insurance; this could be explained by the lower direct cost of the medicine. Melfi (1998) disagrees with this study and shows that increasing health care cost and lack of health insurance causes 77% of relapses cases. This study shows that about 70% of those who have self-reported depression and are non-adherent to their medicine reported their health as poor or fair compared to 60% one year ago. About 84% of those who have depression and adhere to their medication reported their health as fair or poor compared to about 56% one year before. This might be due to the fact that depression participants may have different levels

of their illness according to different seasons of the year; this is in agreement with a Loy study (2011) which shows that episodes of depression occur more often in late fall and winter that alternate with normal mood periods for the rest of the year.

The deterioration in health may be due to lack of health education, lack of follow up visits, and non-frequent meetings with patients, which is similar to findings in a Katon study (2001) which shows that educating participants, frequent meetings with a depression specialist, and telephone follow-up and frequent monitoring over a period of at least one year drastically enhanced adherence to antidepressants. This study also shows that those who have higher educational level were 6 times more adherent than those who do not have higher education, which disagrees with the Ten Doesschate study (2011), which showed that higher education is a prediction sign of non-adherence. This study also showed that married people who have depression have much higher prevalence than non-adherent to their medication compared to singles that have depression, which can be explained by the marital stress and complexity of life which make people often feel hopeless. On the other hand our study did not show marital status as a predictor, which put us in agreement with what was shown by a Sirey study (2001) that showed that marital status has no significant effect as predictor for adherence. This study found that those who are unemployed have about 6.5 times more likelihood to have depression but about 16 % higher adherence than the employed, which shows that education and employment decrease the adherence of participants to their medications. However, employed people's ability to work tends to give them feelings of strength and feelings that cause them to think they do not need to use the medications, which is similar to a Goethe study (2007) that showed that employed people are more non adherent to their medication. Females showed more likelihood to have depression which may be due to hormonal swings as well as lack of support system for females in the Arabic and Chaldean cultures.

Conclusions:

The prevalence of depression patients who were adherent to treatments is 63.4%. Adherence to chronic diseases is higher than adherence to depression medications. In addition depressed but employed participants reported lower adherences to their medications. Therefore we recommend further studies to assess the effect of habits such as smoking, with the adherence to the medication in depression patients, and the effect of depression being a stigma on the adherence. We look forward to study ethnic minorities toward the goal of generalizing the results and improving accuracy.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 64-75 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

SYNTHETIC, SPECTROSCOPIC AND ANTIBACTERIAL STUDIES OF Co(II),Ni(II),Cu(II),Zn(II),Cd(II)AND Hg (II),MIXED LIGAND COMPLEXES OF TRIMETHOPRIME ANTIBIOTIC AND ANTHRANILIC ACID

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Abstract

Mixed ligand complexes of bivalent metal ions, viz ; M= Co(II),Ni(II),Cu(II), Zn(II), Cd (II), and Hg(II) of the composition $[M(Anth)_2(TMP)]$ in 1:2:1 molar ratio, (where . AnthrH= Anthranilic acid ($C_7H_7NO_2$) and Trimethoprime (TMP) = ($C_{14}H_{18}N_4O_3$) have been synthesized and characterized by repeated melting point determination, Solubility, Molar conductivity ($\mathbf{\Lambda_m}$),determination the percentage of the metal (M%) in the complexes by (AAS), FT-IR, magnetic susceptibility measurements [µeff (BM)] and electronic spectral data. The two ligands and their metal complexes have been screened for their bacterial activity against selected microbial strains (Gram +ve) & (Gram -ve).

Key words: Trimethoprim, , Complexes, Anthranilic Acid and Antimicrobial

1. Introduction

Mixed ligand complexes plays an important role in numerous medicine, chemical and biological systems like antioxidant,water softening, ion exchange resin, photosynthesis in plants, removal of undesirable and harmful metals from living organisms electroplating, dying also great importance in the field of environmental chemistry[1-7]. Development of antimicrobial drugs was wide as one of the great medical success story of the twentieth century[8].

Research are being undertaken in fields such as cancer[9], diabetes[10], metal-mediated antibiotics, antibacterial, antiviral, antiparasitic and radiosensitizers[11], In continuation of our efforts[12, 13] to progress metal-based therapeutics agents, the synthesis, characterization, and antibacterial studies of Anthranilic acid and trimethoprim are presented.

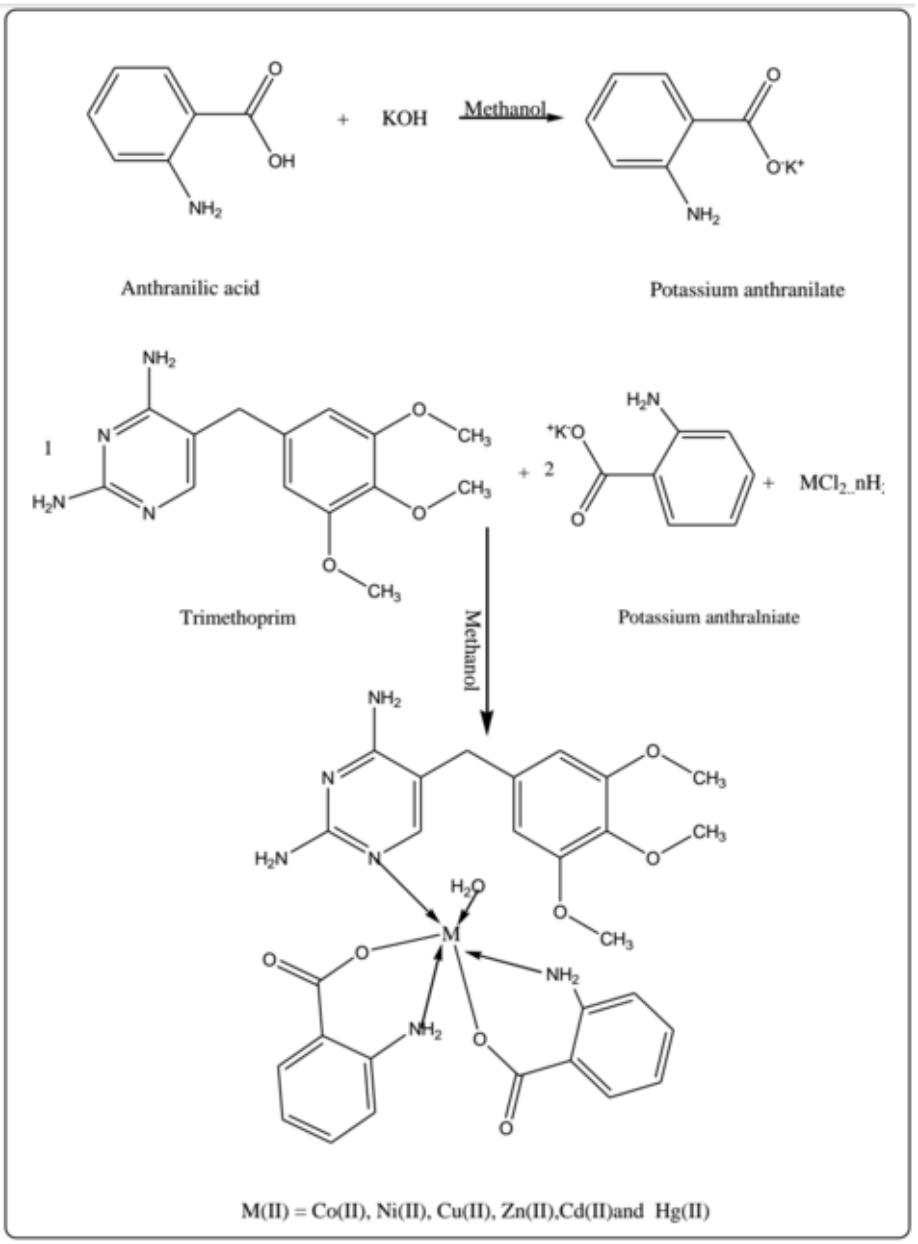
2. Experimental[12, 13]

2.1. Chemicals

All chemicals used were of reagent grade and were used as received.$CoCl_2$.6H, NiCl2.6H2O,CuCl2. $2H_2O$, $CdCl_2.H_2O$,$HgCl_2$, $ZnCl_2$, Na OH (supplied by either Merck or Fluka) ethanol, methanol, dimethylforamaide, dimethyl sulfoxide and KBr, from (B.D.H). Trimethoprime powder DSM (Spain) and Anthranilic acid from Riedial- Dehaen.

2.2 Synthesis of (Mixing ligands) complexes with some metal ions

A solution of the metals containing[0.237g, 0.237g, 0.170g, 0.136g, 0.201g and 0.271g (1 mmol)] of $CoCl_2.6H_2O$,$NiCl_2.6H_2O$,$CuCl_2.2H_2O$,$ZnCl_2$,$CdCl_2.H_2O$ and $HgCl_2$ in methanol (10ml) respectively was added gradually with stirring to methanolic KOH solution (0.112g,2mmol) of the anthranilic acid.(0.290g ,1mmol) of Trimethoprime (TMP) was added to the mixture in each case by using stoichiometric amount (1:2:1) Metal:K^+ $Anth^-$:(TMP) moler ratio. The mixture was refluxed with constant stirring for an hour. The mixture was cooled at room temperature pale precipitate was formed, filtered and recrystallized from ethanol dried at room temperature according to the following reaction :(scheme 1)



Scheme (1): Schematic representation preparation of the Complexes [M(Anth)$_2$(TMP)]

3. Results and Discssion

3.1. Characterization of Metal Complexes.

The complexes were prepared by reacting the respective metal Chloride with the ligands using 1:1:2 mole ratios, [TMP: M: 2 Anth] , i.e. one mole of Trimethoprim [TMP] , one mole of metal Chloride and two moles of Anthranilic acid [Anth.H][12, 13]. The formula weights and melting points are given in Table (1). It was found that all the complexes were appeared as powders and stable in air at room temperature with higher melting points revealing that the

complexes are much more stable than their [AnthH & TMP] ligands indicating formation of complexes.

All these complexes are colored solids, insoluble in common organic solvents but soluble in DMF and DMSO. The conductivity values for the complexes (in DMSO,10^{-3} M,25°C), ranging in the (3.1-19.9) $\Omega^{-1}mol^{-1}cm^{2}$ region, indicate that the complexes are non electrolytes[11]. The test for Chloride ion (Cl) with $AgNO_3$ solution was (-negative)[11-13]. The calculated and experimental values of (M%) in each complex are in fair agreement as shown in Table (1).

Table (1): The Physical Properties & Atomic Absorption Results of the [(TMP- Metal-Anth)] Complexes

Compounds Chemical Formula)	M. wt Calc	Color	Yield %	M .p°c (de) °c	Λ_m Ω^{-1} cm^2 mol	Metal% Theory (exp)
Anth.H	137.14	Pale-yellow	–	146	6.2	–
[Co(Anth) $_2$(TMP)(H_2O)]	620.96	Brown	66	280	3.66	9.49 (9.01)
[(Ni(Anth)$_2$(TMP)(H_2O)]	621.27	Blue	63	265	13.3	9.47 (10.50)
[(Cu(Anth) $_2$(TMP)(H_2O)]	625.57	Green-blue	75	285	19.9	10.15 (10.13)
[(Zn(Anth) $_2$(TMP)(H_2O)]	627.96	Yellow	60	290	11.3	10.41 (10.56)
[(Cd(Anth) $_2$(TMP)(H_2O)]	674.99	White	70	240	3.66	16.65 (16.11)
[Hg(Anth) $_2$(TMP)(H_2O)]	763.16	Yellow	59	112	5.0	26.28 25.73

3.2. FT-IR spectra of [(TMP), (Anth)] ligands & [Co(Anth)$_2$(TMP) (H_2O)] (1), [Ni(Anth)$_2$(TMP)(H_2O)] (2), [Cu(Anth)$_2$(TMP)(H_2O)] (3), [Zn(Anth)$_2$(TMP)(H_2O)] (4), [Cd(Anth)$_2$(TMP)(H_2O)] (5), and [Hg(Anth)$_2$(TMP)(H_2O)] (6) complexes.

The I.R spectrum of the Trimethoprim (TMP) which used as a primary ligand exhibits strong bands at (3471, 3319) cm^{-1} ascribed to stretching vibration of primary amine υ(NH_2) asym &sym respectively[12, 13]. A sharp very strong frequency band at 1633 cm^{-1} & 1508cm^{-1} in (TMP) assigned to the pyrimidine nitrogen ν(C=N). The absorption bands at 1263 and 1236 cm^{-1} which account for

C-O-C str. (asym.) and C-O-C str. (sym.) respectively[14, 15]

The FT-IR spectrum of the [Anth.H] which used as a secondary ligand Table (3-25) exhibits bind the metal ion as a bidentate monobasic fashion through (COO-) & (NH_2) donors, while (TMP) bind the metal ion as a mono dentate ligand through the (N) atom[14]. The FT-IR spectra assignments bands for compounds (1), (2), (3), (4), (5) and (6),. are summarized in Table (4). The assignments have been carried out based on comparison of the spectra data with of similar compounds (6,11)..New weak intensity bands were observed in the regions (509-586) cm^{-1} & (424-478) cm^{-1} may be ascribed to M-N and M-O vibrations, respectively[15, 16].

The FT-IR spectra of all the complexes exhibited peaks around (3305- 3213) cm^{-1} and in the range (1627-1623) cm^{-1}. These peaks can be appointed to OH (stretching & bending) vibration, which indicate the presence of coordinated water molecule in the complexes. The coordinated between the (H_2O) molecules and the (M^{+2}) resulted in the appearance of vibrational bands at range (756-759) cm^{-1} ($M-OH_2$) in the all complexes[12, 13] and all complexes adopt an octahedral geometry as proposed.

Table (2): Infrared spectral data(wave number ύ) cm^{-1} for the Trimethoprim

υ (N-H) $_{asym}$	υ (N-H) $_{sym}$	υ (C=N) Pyrimidine(N)	υ (C-O-C)$_{asym}$ Str	υ (C-O-C)$_{sym}$ Str	υ (-OCH_3)
3471vs	3319	1633vs 1508vs	1263s	1236s	1128vs

Table (3): FT-IR Spectrum Data of the L-Anthranilic acid

(NH_2) $_{asym,sym}$ Str	υ ($N-H_3^+$)	υ C=O Str (carbox.)	υ(-COO^-)$_{asym.}$	υ(-COO^-)$_{sym.}$	Δυ (-COO^-)$_{asmy-smy}$
3321 s 3240	3101s	11716	1662s	1485s	177

Table (4): Infrared spectral data(wave number ύ) cm^{-1} for the mixed $[M(Anth)_2(TMP)(H_2O)]$ complexes

Compounds	υ (OH-H_2O)	υ(N-H_2) asym & sym	υ (C-H) arom.	υ(C-H) aliph	Pyrimidine Nitrogen (C=N)	υ_{asy} COO υ	□υ υ_{as} COO- - υ_{as} COO	υ(C=C) arom.	υ(C-C) aliph.	υ –*OH* H_2O	υ (M-N)	υ(M- N)	(M-O)
1) Co	3410	3305 3226	3136	2935,	1616	1538	211	1492 s	1242	756	586	516	470
2) Ni	3448	3305 w 3217 w	3028	2939, 2835s	1605	1543	216	1492	1237v s	756	586	516	478
3) Cu	3406	3275 3236	3124	2936 2835	1600	1550	227	1500	1238v s	759	567	516	424
4) Zn	3406	3298 3213	3128	2935s 2835	1616	1543	226	1492	1238	756vs	563	513	428
5) Cd	3425	3329 3224	3140	2935 s 2835	1616	1589	189	1531	1238	756vs	567	509	455
6) Hg	3420	3352 s 3217	3065	2939, 2835 Vs	1627s	1593	220	1492 s	1238s	759	578	528	451

3.3 .The (UV-Vis spectra) of the free (TMP) and ((Anth.H)) and [M(Anth) $_2$(TMP)(H_2O)] complexes were carried out as DMSO (10^{-3} M) solutions and corrected magnetic moment (μeff) in Bohr magneton units are given in Table 5. The μeff for the all M(II) in this study as expected for six coordinated M (II) species suggest an octahedral geometry. These employments are in agreement with the literature values[12, 13, 17, 18].

Table5: Electronic Spectral data, magnetic moment μ_{eff} (BM)of the [M(Anth)$_2$(TMP)(H$_2$O)]complexes

Compound	λ_{max} nm	ύ cm^{-1}	ε_{max} $Mol^{-1}.L.cm^{-1}$	Assignments	μ_{eff} (BM)
TMP	257	38910	2431	$\pi \rightarrow \pi^*$	-
Anth.H	245 332	41322 30120	1857 1924	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	-
[Co(Anth)$_2$(TMP)(H$_2$O)]	301 773 821	33222 12936 12180	1651 76 66	Charge transfer $^4T_1g \rightarrow T_1g^{(p)}$) υ_3 $^4T_1g \rightarrow {}^4A_2g^{(f)}$ υ_2	4.34
[(Ni(Anth)$_2$(TMP)(H$_2$O)]	276 624 663 706 821	36231 16025 15082 14164 12180	1950 81 68 55 37	Ligand Field $^3A_2g^{(F)} \rightarrow {}^3T_1g^{(p)}$ υ_3 $^3A_2g^{(F)} \rightarrow {}^3T_1g^{(f)}$ υ_2 $^{3A2g(F)} \rightarrow {}^{3T2g(F)}$ υ_1	2.73
[(Cu(Anth)$_2$(TMP)(H$_2$O)]	289 345 813	34602 28985 12300	1484 1198 15	Ligand Feild CT 2Eg → 2T2g	1.75
[(Zn(Anth)$_2$(TMP)(H$_2$O)]	325	30769	1934	C.T	Diamagnetic
[(Cd(Anth)$_2$(TMP)(H$_2$O)]	318	31446	2245	C.T	Diamagnetic
[Hg(Anth)$_2$(TMP)(H$_2$O)]	247 343	40485 29154	2399 1824	C.T C.T	Diamagnetic

3.4 . The Proposed Molecular Structure for Studying [M(Anth)$_2$(TMP)(H$_2$O)] Complexes :

Studying complexes on bases of the above analysis, spectral observations suggesting the octahedral geometry for all the prepared complexes which exhibited coordination number six and may be formulated as:[M(Anth)$_2$(TMP)(H$_2$O)],

M=Co(II),Ni(II),Cu(II),Zn(II),Cd(II)and Hg(II).

The general structure of the complexes is 3D as is shown in Figure (1) .Accordingly, we can deduce that the ($Athr^-$) binds the M(II) as bidentate fashion (NO^-) .The bonding sites are the Nitrogen (amine group) & Oxygene the carboxylato group ,while (TMP) binds the M(II) as mono dentate. through the (N) atom of the pyrimidine group .

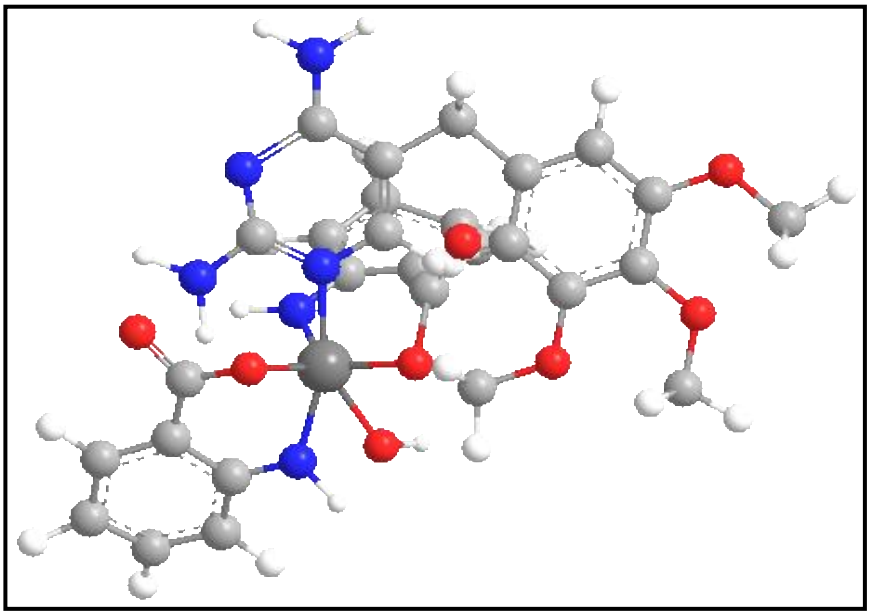


Figure (1): 3D molecular modeling proposed $[M(Anth)_2(TMP)\ (H_2O)]$ complexes

3.5. Bacterial activities of the $[M(Anth)_2(TMP)(H_2O)]$complexes :

M=Co(II),Ni(II),Cu(II),Zn(II),Cd(II)and Hg(II)

Antimicrobial activity were expressed in terms of millimeter (mm) by measuring inhibition zone diameters (ZI) and contrasted with the DMSO solvent (as control) and the values have been tabulated. Tables (6), Chart (1) and Figure (2)

Compounds (ligads & complexes), were screened for their in vitro antibacterial activity against (2Gram-negative (-) =,*Escherichia coli, , and Pseudomonas aeruginosa*) and (2 Gram-positive (+) =*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains[20, 21.]
Generally the (ZI) mm compounds were in the following order;
Metal complexes > Anth.H > TMP = DMSO.

The (ZI) of the(TMP) show inactive to weak active against the growth of three bacteria but (Anth.H) show moderately active to highly active.The $[Co(Anth)_2(TMP)(H_2O)]$show highly activity against 3-organisms uses except *Pseudomonas*.
All complexes show highly antibacterial activity against *Bacillus.*

$[M(Anth)_2(TMP)(H_2O)]$, M=Ni(II),Cu(II),and Zn(II)]complexes ,shows active antibacterial against *E-coli* and *Pseudomonas*.

$[Cd(Anth)_2(TMP)(H_2O)]$ and $[Hg(Anth)_2(TMP)(H_2O)]$ shows very good antibacterial activity against all bacteria .

The increased inhibition activity of the metal complexes can be explained on the basis of Tweedy's chelation theory[13,14].In metal complexes, on chelation the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital[20, 21].

Table (6):Biological activity of the mixed $[M(Anth)_2(TMP)(H_2O)]$ complexes (ZI) (mm)

Compound	N_o in Petri dish	*E-coli*	*Pseudomonas*	*Staphylococcus aureus*	*Bacillus*
Control(DMSO)	c	5	7	5	6
TMP	_	4	0	5	10
Anth.H	_	18	9	21	13
$[Co(Anth)_2(TMP)(H_2O)]$	1	11	0	12	37
$[Ni(Anth)_2(TMP)(H_2O)]$	2	0	0	11	26
$[Cu(Anth)_2(TMP)(H_2O)]$	3	0	0	22	30
$[Zn(Anth)_2(TMP)(H_2O)]$	4	0	0	12	35
$[Cd(Anth)_2(TMP)(H_2O)]$	5	20	33	0	36
$[Hg(Anth)_2(TMP)(H_2O)]$	6	27	18	39	40

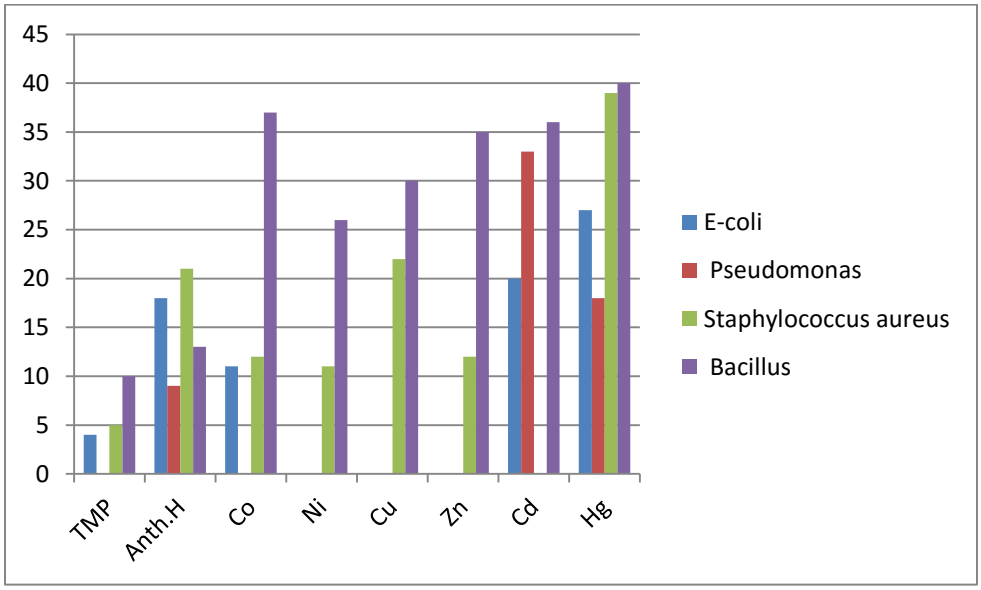


Chart (1) : The (ZI) mm of mixed

[Trimethoprim-Metal Chloride–Athranilic acid]Complexes

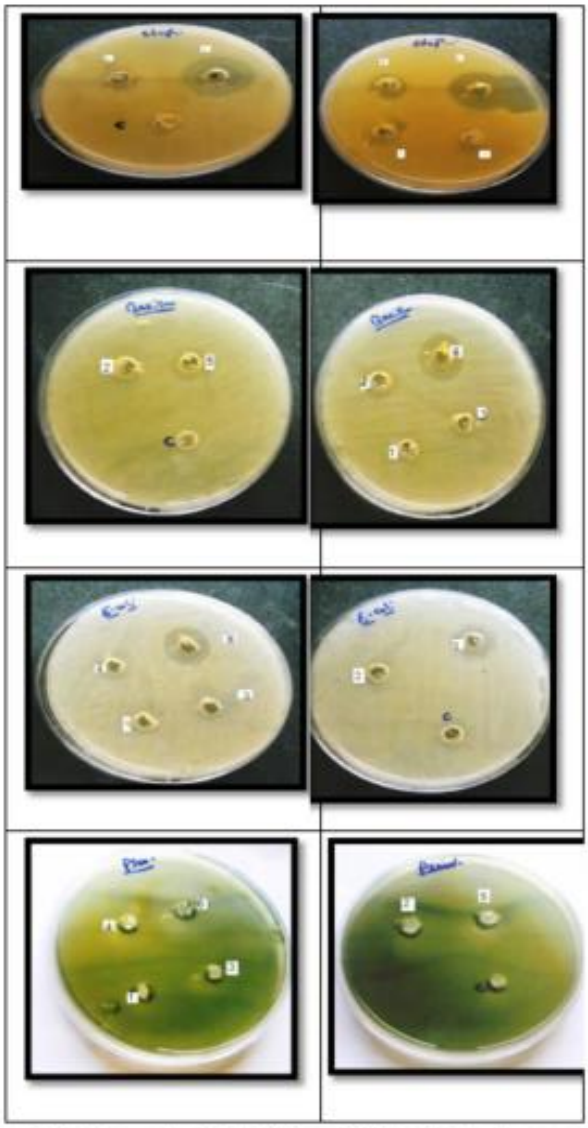


Figure (2) Photograph of Antimicrobial Activity of compounds

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TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 1, 2016 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 2, 2016 ISSN: 2377-2808

TOFIQ
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Vol. 3, No. (2), (2016)

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TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 2, 2016 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

TOFIQ Journal of Medical Sciences (TJMS)

Vol 3, Issue 2 (2016)

Issued by:
TOFIQ Office,
2405 Carey lane,
Vienna, VA 22181
USA

TJMS.tofiq.org

Indexing

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

Indexing

TOFIQ Journal of Medical Sciences
ISSN: 2377-2808

Dear TJMS readers,

It is my great pleasure to present to you the Volume 3 (2016) of our TJMS.

We would like to announce that TJMS has been accepted into DOAJ. Being included in DOAJ will raise the profile of the journal, and the metadata we supply to DOAJ will be searchable on their web site and via many libraries who take our data feed..

The current issue (Issue 2, Volume 3) contains a variety of scholarly articles which have already attracted many communications from different disciplines all over.

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Thank you again,

A Hadi Al Khalili, MBChB, MPhil, FRCSE, FACS

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Mission:

1. TJMS is a high quality, biannually, peer-reviewed, electronic medical sciences journal, publishing original research and scholarly review articles, letters to the editor, and editorials.
2. TJMS focuses on the disease burden in Iraq.
3. An outlet for the current research and an academic product in Iraq in the fields of medicine, dentistry, pharmacy, nursing and related disciplines that supports recognition and academic advancement in Iraq and beyond.
4. TJMS will lay the groundwork for creation of sister journals in other disciplines relating to Iraq (engineering, agriculture, science and technology, social sciences and humanities).

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1. Single editor-in-chief, working in a full-time, compensated capacity.
2. Editorial board consisting of experts in the various branches of medicine, dentistry, pharmacy, nursing and related disciplines.
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5. Articles can be submitted by any individual/group, or can be solicited (invited reviews and discussions). Decisions for publication will be blinded to author or region of origin. The criteria on which the submissions are evaluated for acceptance will be heavily weighted on their applicability to the burden of disease in Iraq.
6. Publication will be biannual however, approved articles will be released continuously.

7. Funding: will attempt to obtain corporate sponsorship through unrestricted educational grants. Sponsorship will be acknowledged in compliance with ACCME and ICMJE guidelines.
8. The publication would be the official medical sciences journal of TOFIQ, and we would encourage other medical organizations to consider collaborating with.

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TOFIQ Journal of Medical Sciences ISSN 2377-2808

Supplement, No 2, (2016)

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 1-16 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

NEBIVOLOL HYDROCHLORIDE LOADED NANOSTRUCTURED LIPID CARRIERS AS TRANSDERMAL DELIVERY SYSTEM:-PART 2:- HYDROGEL PREPARATION, EVALUATION AND PERMEATION STUDY

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Abstract:

Nebivolol hydrochloride (NEB), is a third generation highly selective β_1-blocker, it has an antihypertensive properties. Its elimination half-life is around 10 hrs while its oral bioavailability is about 12%. The objective of the current study was to develop nanostructured lipid carriers (NLCs) for transdermal delivery of (NEB). Through the preparation, characterization and conducting of an in vitro study for (NEB) loaded (NLCs), the formulation of NEB-NLCs based hydrogel using different types of gelling agents was introduced. Moreover, the incorporation of lipid nanoparticles into carbapol 934 as hydrogel base in different formulations was described in this study. The optimized formula (350 mg Glyceryl monostearate, 150 mg oleic acid, 2% (W/W) span 80, and 2% (W/W) Cremophor EL) was tested for entrapment efficiency, particle size and loading capacity then incorporated into hydrogel for expedient transdermal application. A number of measures were implemented for the NEB-NLCs based hydrogel, the results for the optimized formula were found to be as the following: particle size 228 nm, polydispersity index 0.3, zeta potential -29mV, pH 7.05 viscosity 7210cps, spreadability 6 cm, drug content 95%, and Ex Vitro skin permeation 90.8%. The transmission electron microscopy (TEM) and the optical microscope study revealed almost spherical shaped nanoparticles. Nebivolol based hydrogel demonstrated no skin irritation and showed a prolong release for up to 24 hrs. The flux for the permeation study through rat skin was found to be (143µg/cm^2/hr). Carbapol 934 was used as a gelling agent; the obtained formula gave evidence for good spreadability, homogeneity and rheological behavior. In conclusion, the data

obtained from this study illustrated a successful development of NEB-NLCs-based hydrogel in the increase of the encapsulation efficiency of colloidal lipid carriers. The advantages of the colloidal lipid carriers of the improved performance were in terms of stability and provides a sustaining NEB transdermal effect.

Keywords: Nebivolol hydrochloride; Nanostructured lipid carriers, Hydrogel, Transdermal delivery

Introduction

In the last decade, the prefix "nano" has an increasing application to various fields of knowledge. Nanoscience, nanotechnology and nanomaterials or nanochemistry, all represent examples of few terms that occur repeatedly in scientific reports, books as well as in daily newspapers, it has been recognized for a wide range of audience even for non experts. International system (IS) of units is used to indicate a reduction factor of 10^9 times[1]. Transdermal. Nebivolol hydrochloride (NEB) is a lipophilic β_1-blocker, devoid of intrinsic sympathomimetic and membrane stabilizing activity. Clinically, (NEB) is administered as a racemic mixture of equal proportions; d-isomer ((SRRR)-nebivolol a potent cardioselective β1 adrenoceptor blocker) and L-isomer ((RSSS)-nebivolol with favorable hemodynamic profile[2,3]. These two enantiomers possess unequal potency regarding to β-receptor blocking activity and nitric oxide mediated vasodilation. Moreover, the blend of (SRRR and RSSS) has a greater antihypertensive activity than either enantiomer alone[4]. Nebivolol hydrochloride is an official drug in the British & Indian Pharmacopoeia[5]. The molecular weight for NEB is 441.9 while for the free bases are 405.4[6]. The lipid nanoparticles, such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLCs) are stable colloidal systems with notable compensations such as drug delivery systems, i.e. biocompatibility, biodegradability, physicochemical stability, versatility and controlled drug release. [7,8] Furthermore, they are providing controlled release profiles for many substances. Aqueous dispersions of lipid nanoparticles are being investigated as drug delivery systems for different therapeutic purposes. Their most interesting characteristic is the possibility to be used topically and transdermally, while other systems have to be incorporated into commonly used dermal carriers, such as creams or hydrogels, in order to have a proper semisolid consistency[7,8].

Materials and Methods

Nebivolol (NEB) and transcutol P were purchased from ProvizerPharma, India. Oleic acid was supplied by Riedel De Haen AGHonnover, German, Cremophore EL (Polyoxy l35 Castor oil) was purchased from HiMedia Lab Pvt. Ltd, India. Span 80 was provided by Hopkin&Williams LTD, England. Poloxamer 188 (Pluronic F-68) Lutrol® and Lecithin (Phosphatidylcholine purity 72.7%) were purchased by Sigma-Aldrich, Chemie GMBH, Germany. Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Diethyl ether Carbapol 934 and Sodium alginatewere provided by BDH Chemicals Ltd., Poole, England. Glycerylmonostearate was supplied by BDH Chemicals Ltd. Poole, England.Myverol™18-04K was provided by Gattee fosse, France. Chitosan , HPMC was purchased from Fluka AG. Chem.

1: Preparation of Nebivolol-loaded NLCs based hydrogel

According to the previous work[9], 100 mg NEB was incorporated into combination of different solid and liquid lipids, surfactants and co-surfactants (Table 1). Accordingly, four formulas were selected for hydrogel preparation. The optimized (NEB-NLCs) formula (F3) was selected based on the evaluation of characteristics like: particle size, entrapment efficiency, and in vitro release. Different gelling agents such as: carbopol 934, sodium alginate, chitosan, and hydroxyl propylmethyl cellulose (HPMC) were used for the conversion of NLCs dispersion into NLCs based hydrogel formulation (Table 2). Based on the compatibility with NLCs dispersion and for easier spreadability, carbopol 934 as gel forming polymer was selected (F3B). The hydrogel base was prepared by dispersing (1%w/w) carbapol 934 in distilled water, stirred for 10 mins at 1500 rpm to obtain a homogeneous gel base, and then immediately neutralized by adding drops of triethanolamine (0.5%V/V) to promote gelation. The pH of gel was adjusted to 7.4. Hydrogel was further allowed to stand overnight at a room temperature to remove any entrapped air. The hydrogel was used to disperse a freshly prepared NLCs suspension. The aqueous NLCs dispersion and hydrogel were stirred and mixed under a mechanical stirrer at a speed of 1000 rpm/15 min.

Table (1); Preparation of Different Formulas of Nebivolol - Loaded Nanostructured Lipid Carriers

Formula Code	Materials								
	GMS (mg)	OA (mg)	F68 (mg)	TCp (%)	T80 (%)	Le (%)	S80 (%)	CR (%)	Myv (%)
F1	350	150	150	5					
F2	300	200			0.8	0.4			
F3	350	150					2	2	
F4	350	150	200						0.3

Table (2); Different Nebivolol Nanostructured Lipid Carriers based Hydrogel formulations

Formula Code	Cabapol 934 (%w/w)	Chitosan (%w/v)	Sod. Alginate (mg)	HPMC (%w/v)
F3A	0.5			
F3B	1			
F3C		0.5		
F3D		1		
F3E		2		
F3F		3		
F3G			2	
F3H			3	
F3I				1
F3J				2

2: Evaluation of the NEB-NLCs based hydrogel

1. 2. Physical Examination of NEB-NLCs based hydrogel

The prepared NEB-NLCs hydrogel formulation was inspected visually for its color, appearance and consistency.

2.2. Determination of pH, Viscosity and Spreadability

The pH of the NLCs loaded hydrogel was determined using digital pHmeter (France and Hanna instruments type), standardized using standard buffer solutions (pH 4.0 and 7.0). One gram of hydrogel was dissolved in 100 mL of distilled water and stirred for 10 min then stored for 2 hrs. Results were taken in triplicate[10]. The viscosity of NLCs based hydrogel was measured using Brookfield DVII+Prodigital viscometer at 25◦C. A formulation weight of 20 gm was utilized and exposed to speed of 50 rpm. The measurements were attained in triplicate. The rheological nature of the disperse system was assayed by plotting shear stress against shear rate in a rheogram to determine if the systems are thixotropic. The readings were performed in triplicate[11].

For determination of the spreadability of hydrogel 0.5 g of gel was placed in a circle with a radius of 1cm premarked on a glass plate on top of another glass plate. A

weight of 500 gm was allowed to rest on the upper glass plate [10]. The extension in the diameter due to spreading of the hydrogels was measured with a linear scale. Experiments were done in triplicate.

3.2. Drug Content Determination

The NEB content in the hydrogel was determined by taking required quantity of the prepared gel which is equivalent to 10 mg of NEB and transferred to 100 ml of volumetric flask containing phosphate buffer (pH 7.4). Then, it was sonicated and filtered. Later, it was suitably diluted and analyzed at λ_{max} of NEB. The content of NEB was determined using UV-visible spectrophotometer at 281nm against blank [11].

4.2. Measurement of Particle Size and Polysisperisty Index of NLCs based Hydrogel

Light dynamic light scattering (LDS) was used. The aqueous NEB-NLCs was dispersed in a fixed amount of filtered distilled water (1:50) dilution of all formulations was made and placed in 1cm diameter disposable cuvette to yield a suitable scattering intensity. The mean particle size and polydispersity index PDI (The measurement of the width of size of distribution) for NEB-NLCs based hydrogel was calculated using Brookhaven Instruments Corp90 PLUS (ZetaPlus Particle Sizing, NY, Software, Version 5.34). Experiments were carried out in triplicate, and standard deviations (±SD) were calculated at a fixed scattering angle of 90°at (25°C)[12].

5.2. Zeta Potential determination of NEB- NLCs based Hydrogel

Zeta potential of NLCs based hydrogel was measured using (NanoBrookZeta PLAS) Zetasizer. The NanoBrookZetaPALS determines zeta potential using Phase Analysis Light Scattering technique which is up to 1000 times more sensitive than traditional light scattering methods based on the shifted frequency spectrum. Samples were placed in disposable zeta cells

and the NLC suspensions were diluted with distilled water (1:100) of all the formulations to get a uniform dispersion prior to analysis. The conductivity of the diluted sample was measured to choose the detection model. The whole measurement was carried out at 25°C[11].

6.2. Visualization by Optical Microscope

Optical microscope (BX51M Model) offered a reflected light illumination. One drop of each prepared (NEB) dispersions was examined under the optical microscope using an (1000x) magnifying power. Particle behavior, shape and morphology were investigated.

7.2. Visualization by transmission electron microscope (TEM)

The size and morphology of the selected formula was examined using TEM (PHILIPS CM 10), with an accelerating voltage of 100 K_{VA}. The work was conducted by placing one drop of the sample on a copper grid coated with a formvar carbon film and allowed to stand at room temperature for 90 sec to form a thin film. Excess of the solution was wicked away with the aid of filter paper. The grid was allowed to thoroughly dry in air, the sample was viewed and ready for analysis and photomicrographs were taken at suitable magnification

3. In-vitro Skin Permeability of NEB-NLCs based hydrogel

1.3. Preparation of Rat Skin (Diffusion Membrane)

Albino rats (4-6 week old males) were euthanized. Then the abdominal skin was shaved lightly with an electrical clipper taking care to prevent any damage to the surface of the skin. A rectangular section of abdominal skin several centimeters in each dimension were excised from the animal using a sharp blade. The skin was lifted easily from the animal after incision was made. **The defatting procedure:** the skin was defatted by wiping with a cotton tip soaked in diethyl ether to remove the subcutaneous fat and scraping the dermal side to remove the muscle and blood vessels. The skin was wiped again with a cotton tip soaked in ether to prevent any adhering fats and kept in a phosphate buffer pH 7.4 for about 2 hrs in a water bath at constant temperature of 37°C to allow water soluble UV absorbing material to leach out. The buffer was change three times during this period with fresh amounts. Then the prepared skin for diffusion study was stored in a phosphate buffer for 24hrs in the refrigerator at 2°C before use[13].

2.3. Permeation Study of NLCs based hydrogel

Franz diffusion cell Equipment-MCF10, was used in all diffusion studies. It consists of six Franz diffusion cells arranged in a water jacket with a heater to obtain a constant experimental temperature of 32 ± 1°C, on magnetic stirrers at equal speed of rotation, temperature and rotation were equilibrated electronically. All Franz diffusion cells used in the experiment consisted of two compartments: Upper donor compartment and lower receptor compartment. Phosphate buffer solution pH 7.4 was used as a receptor medium with volume of 30 ml for all the release studies of NEB-NLCs to assure sink condition. Firstly, all receptor compartments were filled

with phosphate buffer solution pH 7.4 and the assembled set up left in instrument to equilibrate to experimental temperature as to get rid of air bubbles for at least half an hour. Then the rat skin membrane with surface area of 3.97 cm^2 were mounted between the two compartments of the diffusion cells in such a way that the SC layer was facing the donor compartment and the dermis facing the receptor compartment, and fastened with an O-ring. The solution in receptor compartment was agitated with a magnetic stirrer at 100 rpm[14]. After assembling the described set up, samples of 1 ml were taken periodically through the sampling port from the receptor compartment at pre determined time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 10, 15, 20 and 24 hours), and replaced with an equal volume of fresh receptor solution at temperature of 32 ± 1^oC to maintain a constant volume of the receptor phase. Samples were analyzed for NEB content using UV-visible spectrophotometer. A cumulative amount of drug diffused was calculated[15]. All results were repeater in triplicates and presented in mean values ± standard deviations.

4. Skin irritation study

The primary skin irritation test was very important to estimate the irritancy of substances that applied repeatedly to the skin of humans. Irritancy test was done using healthy male albino rat weighing approximately 200 gm. Firstly, the left dorsal surface of the rat was shaved carefully with an electrical clipper then cleaned with rectified spirit then distilled water and left for drying. The optimized NEB-NLCs hydrogel was placed on the left dorsal surface of the rat, and skin irritation from the formulation was determined by observations for 7 days for any skin sensitivity and reactions such as redness, erythema, edema, and skin rash [10].

Results and Discussion:

1. Preparation of NEB-NLCs based Hydrogel

Based on the particle size, the entrapment efficiency and the in Vitro release profiles of NEB based hydrogel (F 1, 2, 3 and 4) which showed optimum physicochemical properties, were selected for the formulation of the hydrogel for transdermal NEB delivery by incorporation into (1%W/W) carbapol 934. However, (F3B) was chosen as the selected formula as it exhibited the best release profile, viscosity, spreadability, pH, and % drug content.

2. Evaluation of NEB-NLCs based hydrogel

1.2. Visual Inspection

The visual inspection of NEB-NLCs based hydrogel was found to be off-white in color homogenous and showed smooth texture. Hydrogel loaded with NEB-NLCs (F43B) was stored in a tight closed glass container, protected from light. The physical properties of the selected formulas after two months of storage was examined, no changes in the original off-white color or unpleasant odor were observed, which indicates no probability of microorganism growth and hence, the physical stability of the selected formula.

2.2. pH, Viscosity and Spredability Determination

The pH of the prepared NLCs loaded hydrogel was between 6.9±0.02 to 7.05±0.05 that lies in normal range of skin pH (4.5-7) as shown in (Table 3). Viscosity of the prepared hydrogel is mainly dependent on the gelling agent used. In the current study, when carbapol 934 (1%W/W) being under hydration condition it can form a physically bounded structure that is crucial for providing the proper mechanical strength to the hydrogel. Accordingly there is noticeable difference in viscosity values of the control hydrogel and NLCs based hydrogel which can be justified as the participation of nanostructured lipid carriers on the viscosity of the formulation. Spredability is a crucial factor to uniform and ease the application of topical and transdermal preparations from the patient`s compliance point of view. The application of the formulation to the inflamed skin is more comfortable if the base spreads easily, enhancing maximum slip and drag[16].Spreadability was found to be 6±1 cm for the optimized NEB-NLCs hydrogel formula (F3B) which indicates excellent spreadability as the large diameter signifies better spreadability.

3.2. Drug Content Evaluation in the Prepared Hydrogel

Drug content of the optimized NEB-NLCs based hydrogel(F3B) was found to be 95.0%±0.6 and gave better drug loading capacity of formulation as shown in (Table 3).

Table (3); pH, Viscosity, Spreadability and % Drug Content Evaluation of the different NEB-NLCs Based Hydrogels

Formula Code	pH	Viscosity (cp)(50rpm)	Spredability (cm)	%Drug Content
Control	6.02±0.02	3521±3.2	2.9±1.5	80.3±0.6
F1B	6.10±0.03	7622±1.4	3.5±1.0	87.1±0.1
F2B	6.78±0.11	7834±2.5	4.9±0.5	86.0±0.5
F3B	7.05±0.05	7210±1.2	6.0±1.0	95.0±0.6
F4B	7.20±0.12	7243±2.3	5.2±1.5	94.1±0.7

4.2. Particle Size, Polydispersity Index and Zeta potential evaluation of NEB-NLCs based Hydrogel

The average particle size, Polydispersity index and zeta potential of NEB-NLCs based hydrogel (F3B) was found to be 228nm, 0.3, -29.0 respectively. Zeta potential value of the freshly prepared hydrogel (F3B) is illustrated in figure (1). Meanwhile, figures (2 A, B, C and D) for the NEB-NLCs based hydrogel (F3) under TEM. TEM micrographs showed nearly rounded nanoparticle surrounded by a homogeneous shading with a rather uniform distribution and no prominent sign of aggregate remained in the hydrogel. However, there was no drug detected in the blank NLCs (figure 3), whose particle size was smaller than the NEB-NLCs. Such results

were confirmed by optical microscope images (Figure 4) which suggests that the optimized formula (F3) resembled the drug-enriched core model (i.e. the drug occupies the core of the particles), (NEB) was dispersed well due to its miscibility in the lipid matrix. In such a model, the core is surrounded by a practically drug-free lipid shell and homogenous hydrophilic polymer[17,18].

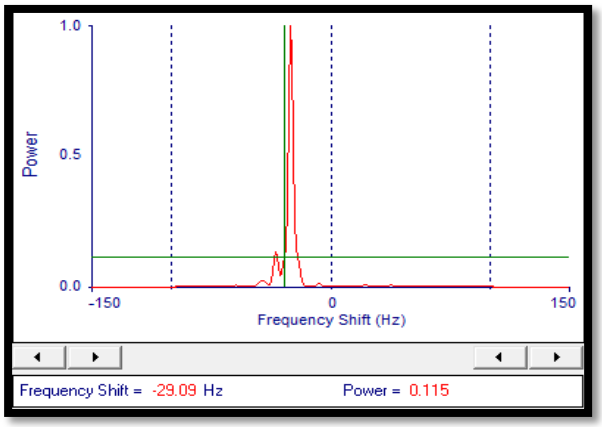

Figure (1); Zeta Potential of the freshly prepared (NEB-NLCs) based hydrogel (F3B)

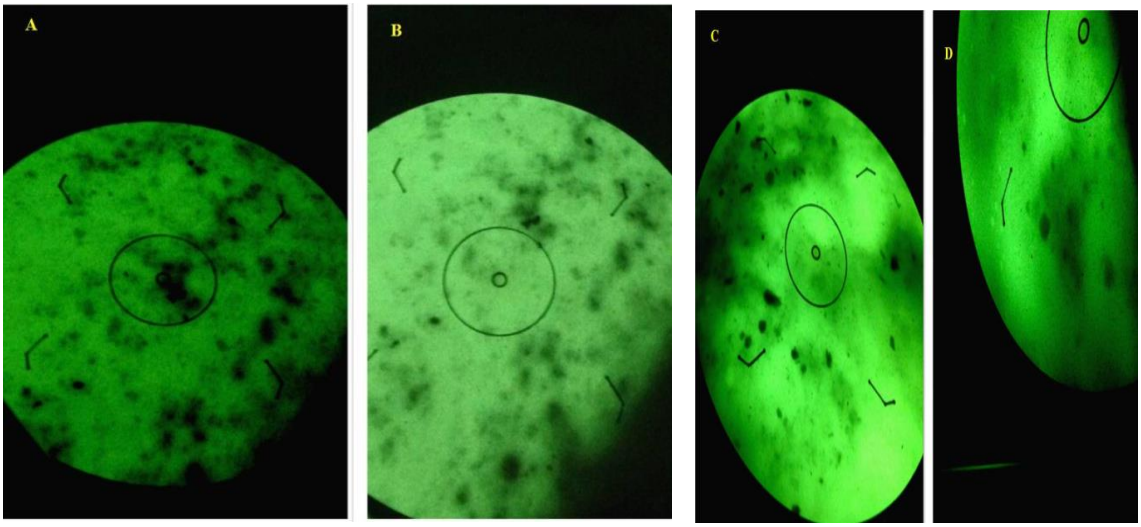

Figure (2); TEM Micrographs of A, B, C and D of NEB-NLCs, and D/Blank NEB-NLCS

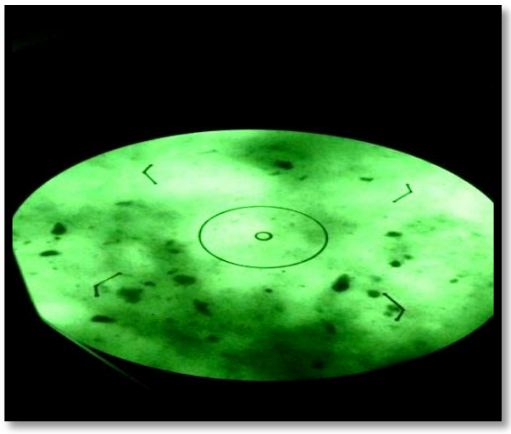

Figure (3); TEM image of NEB-NLCs based hydrogel (F3B)

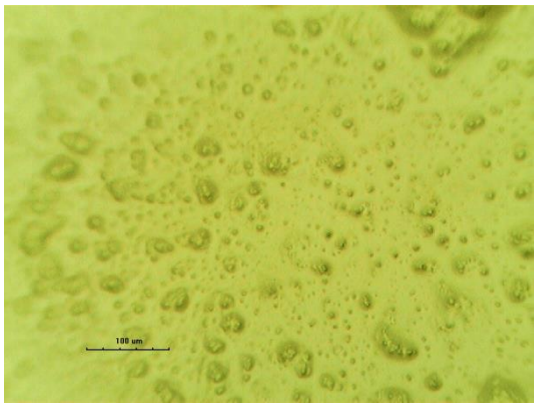

Figure (4); NEB-NLCs based hydrogel (F3B) under optical microscope

3. Skin Permeation Study of Prepared NEB-NLCs based hydrogel

Drug permeating across the skin in the vitro status represents the amount available for skin absorption for some anti hypertensive drugs such as carvidolol, propranolol and labetolol[19]. Hence, drug flux may be an indicator of the drug absorption to the targeted skin tissue. The kinetics of drug permeation which is helpful to explain the mechanisms of drug absorption via the skin. Release of drug from the vehicle must occur before the permeation of the drug into the skin. In vitro permeation study was performed to compare the permeation ability of NEB to the various NLC gel formulations (NLC-F1, NLC-F2, NLC-F3, and NLC-F4) using Franz diffusion cells. The in vitro permeation profile is portrayed in figure (5). Cumulative permeation profile of the different formulations revealed that the permeation of NEB from the NLCs hydrogel formulation (NLC-F3) is significantly high ($p<0.05$) as compared to the permeation of NEB from hydrogel formulations (NLC-F1, NLC-F2, and NLC-F4) which confirms the selection of (NLC-F3) to be the best formula. It was found that 90.8% of NEB permeated within 24 hrs of the study

as depicted in (Figure 5). The diffusion parameters such as lag time, permeation coefficient and (NEB) flux of the optimized formula were: 0.5 hr, 141 (cm/hr) × 10^{-3} and 143 (µg/cm^2.hr), respectively.

In this study, it has been proven that release rate of NEB is close to the flux value which indicates that NEB can easily penetrate into the stratum corneum. This suggests that partitioning into the skin and subsequent penetration are predominant steps in transdermal delivery of NEB. The mechanism for this finding is not clear, however different hypotheses have been placed forth. These include enhancement of solubility and the large surface area due to the nanoparticulate size[19]. Generally, intact particles are unable to permeate the stratum corneum. Lipid nanoparticles can deliver substances by interactions of the lipids used to construct the particles, and skin surface lipids[20]. Since NEB shows high lipophilicity thus might have a greater affinity for the SC. Nebivolol might be transported with lipid. A partitioning of NEB-NLC (F3) into the stratum corneum would lead to high accumulation of the drug. A rise in the concentration gradient leads to an elevation in the diffusion pressure of the drug into the skin. An Increased adhesiveness to skin surfaces is a general feature of nano particles. Lipid nanoparticles adhering to the skin form an adhesive film which leads to occluding the skin surface[18], thus increasing the amount of drug reaching the site of action. This effect should be meaningful for NEB since the therapeutic response to NEB can be increased by occlusion with a polyethylene film. Due to the adhesion effect, hydration of the stratum corneum was raised by reducing keratinocyte packing, and widening of the intercellular bilayers helping in facilitating drug penetration into deeper strata[19]. Generally, decreasing particle size results in increasing the adhesion effect due to disruption of the permeability barrier in the skin.

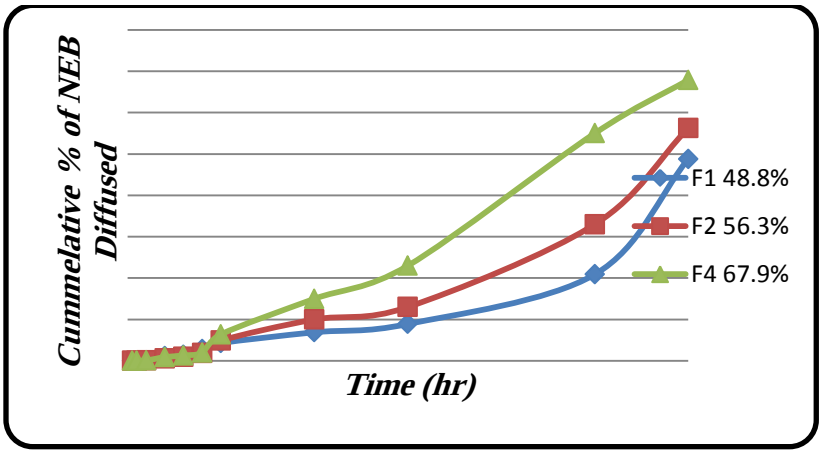

Figure (5); Cumulative % of NEB-NLCs loaded hydrogel (F1, 2, and 4 B) diffused through rat skin at 32◦C.

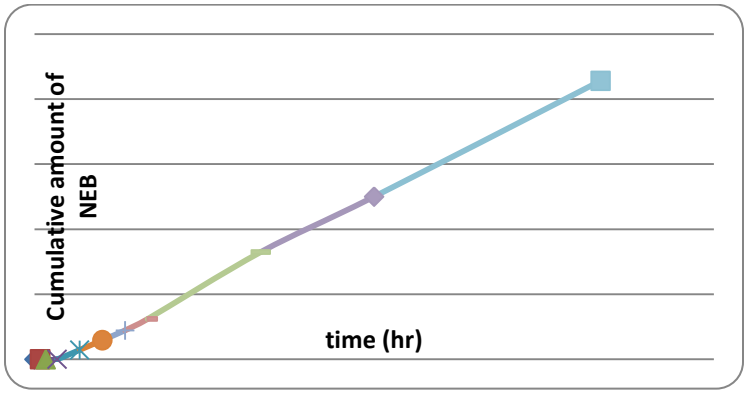

Figure (6); Cumulative % of NEB-NLCs (F3B) loaded hydrogel diffused through rat skin at 32◦C

4- Rheological Study

The rheological property of semisolid drug carriers is a very important physical parameter for its cutaneous application[17]. The aim of the current investigation is the rheological behavior of hydrogel when nanostructured lipid carriers are entrapped into their network. Therefore, rheological behavior of the NEB-NLCs based hydrogel was evaluated. According to this, analysis has been performed for NEB-NLCs based hydrogel formula (F43) and blank gel base as shown in figure (7 A, B and C) respectively, the obtained results were recorded after two months of hydrogel storage at 4 ºC,25 ºC and 40ºC. According to the results, the shear stress was not proportional to the shear rates in NEB-NLCs based hydrogel systems. The unique concavity of the rheogram toward the shear rate axis indicates that all developed formulations exhibited pseudoplastic flow. Such pseudoplasticity results from a colloidal network structure that aligns itself in the direction of shear, thereby decreasing as the shear rate increases the viscosity as well as will be increased[19].During all the rhological work, the temperature has been maintained at 25±0.1ºC using a thermostated water bath in order to avoid obtaining false positive results in the test for thixotropy. Figure (7) indicates that all systems show thixotropy, which in turn can be defined as an isothermal and comparatively slow recovery on standing of a material, of a consistency lost through shearing. Such result is in agreement with Sanap and Mohanta study in which, complex systems such as NLCs-loaded hydrogels exhibit a loose network connects together the sample, thixotropy proceeds from structural breakdown and re-aggregation[20].

5-Determination of Irritancy Test

Materials used in skin pharmaceuticals and cosmetics can be primary irritants, which induce irritation after a single contact or accumulative irritants that produces a fatigue reaction produced only after the application on successive days [21]. To ensure the transdermal preparations are innocuous, it is important to study the irritation effect of NEB-loaded hydrogel. The NEB-NLCs based hydrogel (F3B) was subjected to the irritancy test previously mentioned. After 7 days of application on the dorsal shaved skin of albino rat, there was visual redness developed on the skin (figure 8 and 9). In this present preliminary safety test, findings suggest

skin tolerance to the NEB-loaded hydrogel. In summary, the selected formula is considered as a nonirritant

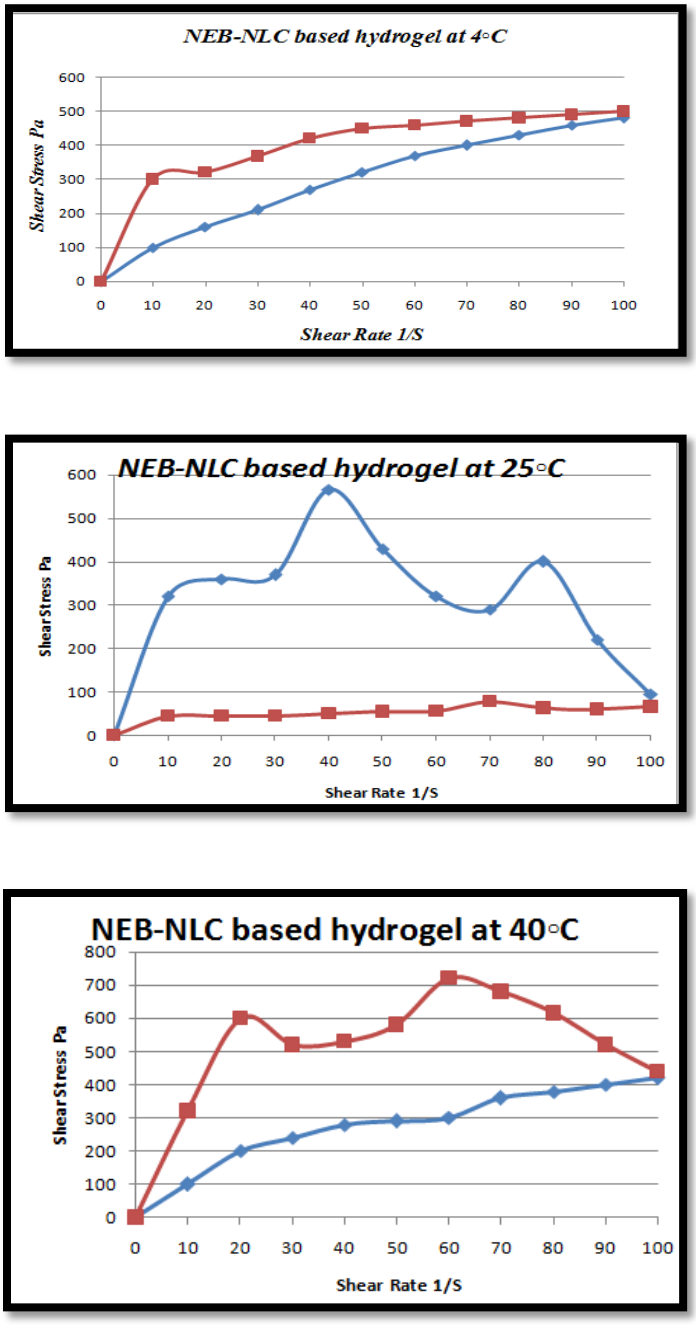

Figure (7); A,B, and C Represent Shear Rate (1/S) Vs Shear Stress (Pa) of NEB-NLCs based hydrogel after two months of storage at different temperatures (4, 25, 40◦C) respectively.

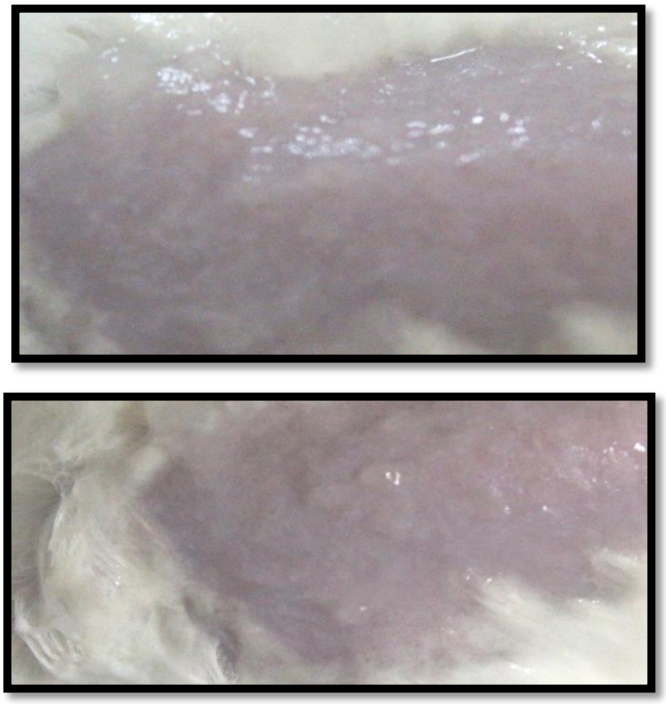

Figure (8); A and B of the applied NLCs based hydrogel of NEB on the dorsal area of rat at different magnification power

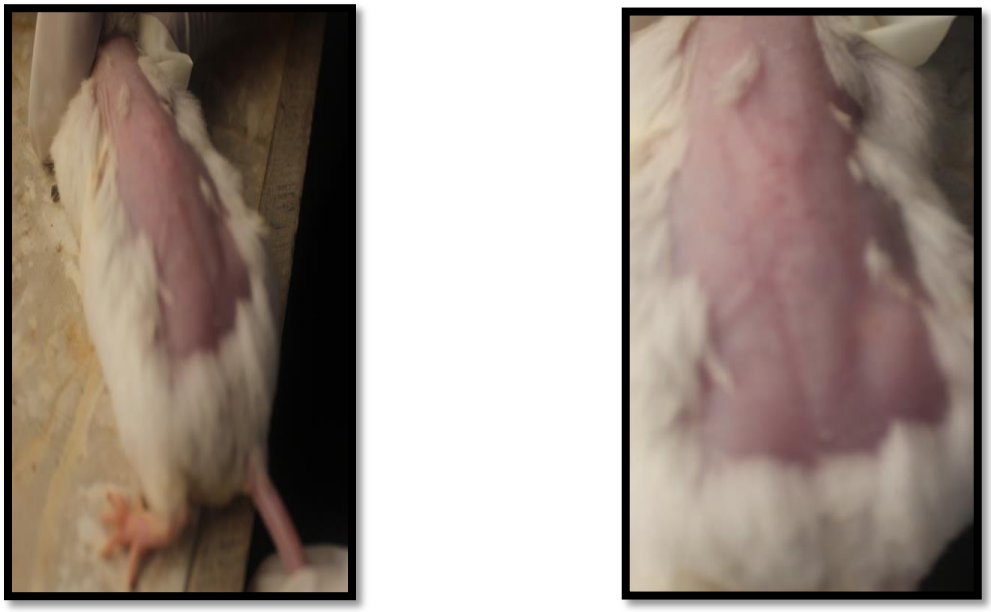

Figure (9); After 7 days of NEB-NLCs based hydrogel application

Conclusion

Nebivolol hydrochloride as a model drug was used for the preparation of nanostructured lipid carriers.The NEB-loaded NLCs could be fabricated and successfully incorporated into hydrogel for transdermal application. The Ex -Vitro skin permeation data indicated that NEB-NLCs bearing hydrogel provided sustained release of NEB. The results reflected the potential of NLC as a carrier for transdermal administration of NEB and that would demonstrate greater drug deposition into the skin. The NEB-NLCs system considered as a promising alternative drug carriers for transdermal pharmaceutics. The data presented indicated the successful development of NEB-NLCs-based hydrogel in increasing the encapsulation efficiency of colloidal lipid carriers. The advantage for the colloidal lipid carriers is in the improved performance in terms of stability and providing a sustained NEB transdermal effect.

Acknowledgement: Authors are very grateful to A. Prof. Dr. Abdul Wahab Al-Shekhly, the head of the department of pharmacy/ Al-Rasheed University College for providing lab facilities, A. Lecturer Nahida A. Al-Jubury and A. Lecturer Zena Qaragholi, Dept. of pharmacognosy and Medicinal plants /college of pharmacy/ University Of Baghdad for the language editing.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 17-25 ISSN: 2377-2808

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INTERLEUKIN-18 AND CAROTID THICKNESS IN *HELICOBACTER PYLORI* POSITIVE PATIENTS WITH DYSPEPSIA IN SULAIMANI- KURDISTAN

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection stimulates the production of proinflammatory cytokines associated with the development of atherosclerosis. Levels of circulating interleukin-18 (IL-18) have been positively correlated with carotid intima-media thickness (CIMT) and coronary plaque area and have been identified as important predictors of coronary events and cardiovascular mortality. This study aimed to examine the relationship between serum IL-18, carotid intima thickness and *H. pylori*-IgG antibody as a sign of *H. pylori* infection in dyspeptic patients.

Methods: This cross sectional- case control study was conducted in Kurdistan Teaching center for Gastroenterology and Hepatology (KCGH) in Sulaimani city from January 2014 to March 2015. One hundred dyspeptic patients with positive *H. pylori* infection and 100 apparently healthy asymptomatic volunteers with negative *H. pylori* tests were enrolled in this study. Sera were tested for *H. pylori* IgG & IgA antibodies at Sulaimani Central lab., using ELISA tests. Interleukin 18 (IL-18) was measured based on immunoenzymometric assay. All participants

were evaluated for both internal carotid (IC) and common carotid (CC) arteries thickness by using high resolution grey-scale Doppler ultrasonography.

Results: A significant difference (P<0.01) was found between patients and controls in the mean serum IL-18. A significant correlation between *H. pylori* IgG level and IL-18 was found ($p < 0.01$), but not with *H. pylori* IgA level. A significant correlation was found between IL 18 level and ICA, CCA thickness in *H. pylori* positive patients ($p < 0.01$).

Conclusion: *H. pylori* infection was significantly associated with higher serum IL-18. There was significant correlation between IL-18 and Carotid intima- media thickness.

Keywords: H. pylori, IL-18, Carotid Thickness, Sulaimani.

Introduction

The immunoinflammatory response plays an important role in the development, progression, and complication of atherosclerotic disease (1,2). Several studies have demonstrated the association between H. pylori infection and coronary heart disease (CHD) (3, 4), while some studies indicated that there was no correlation between H. pylori infection and coronary atherosclerosis (5 , 6).

Interleukin 18, previously known as IFN-γ inducing factor, is a proinflammatory member of IL-1 super family which plays role in the initiation and progression of atherosclerosis (7, 8). Carotid artery intima-media thickness (CIMT) measured by ultrasound are predictive of cardiovascular disease in individuals without clinically evident disease. CIMT is now widely used as an early marker for atherosclerotic disease (9).

This study aimed to examine the relationship between serum IL-18, carotid intima thickness and H. pylori-IgG antibody as a sign of chronic H. pylori infection in dyspeptic patients.

Materials and Methods

This cross sectional case control study was conducted in Kurdistan Teaching center for Gastroenterology and Hepatology (KCGH) in Sulaimani city during the period of January 2014 to March 2015. One hundred dyspeptic patients with positive H. pylori infection (IgG) and 100 apparently healthy asymptomatic volunteers with negative H. pylori tests were enrolled in this study. Both groups were comparable in age distribution and gender.

Exclusion Criteria: Pregnant women, smokers, patients previously treated for H. pylori infection and who had received antibiotics; proton pump inhibitors or bismuth compounds in the preceding 4 weeks. This study was approved by the Ethics Committee of Faculty of Medicine, University of Sulaimani and Directory of Health in Sulaimani. Written informed consents were obtained from all the participants.

A special form used to obtained demographic data (name, age, gender, history of dyspepsia, and drug history).

After overnight fasting, 10 ml venous blood aspirated then centrifuged at 5000 r/min for 5 min. Sera were tested for H. pylori IgG & IgA antibodies at Sulaimani Central lab., using ELISA tests (Nova Lisa, NovaTec, Germany), according to the standard operating procedures. That has a sensitivity of 97% and a specificity of 98.8%.

Interleukin 18 (IL-18) , using RayBio_ Human IL-18 ELISA Kit (ELH-IL18BPA-001), USA was measured based on immunoenzymometric assay. Subjects were evaluated for both internal carotid (IC) and common carotid (CC) arteries and plaque occurrence by using high resolution grey-scale Doppler ultrasonography (Philips, En visor, Version C.1.3, 2007) , In a semi-dark room, the subject lay supine with slightly hyperextended neck and rotated away from the imaging transducer. Both carotid arteries were scanned. CIMT was defined as the distance between the leading edge of the lumen intimal interface and the leading edge of the media adventitia interface of the far wall.

Statistical Analysis

All data were analyzed using Excel and SPSS (Version 20 software) computer program. To assess the correlation between different variables, bivariate correlation coefficient analysis was performed. In this analysis, the statistical significant association was determined. All p values were based on 2-sided tests and $p < 0.05$ was considered statistically significant.

Results

Both groups of H. pylori seropositive and seronegative groups were comparable in mean age and gender (p>0.05).

The mean serum Interleukin 18 among patients and controls were (19.7 + 8.9) and (8.9 + 1.96) pg/mL respectively, with highly significant difference (P< 0.01), Table 1.

Table 1. Interleukin 18 in study population.

Investigations	Patients	Controls	p value
	Mean +SD	Mean+ SD	
Interleukin 18 (pg/mL)	19.7+8.9	8.9+1.96	< 0.01

A moderate correlation between H. pylori IgG level and Interleukin 18 was found (r = 0.341), which is statistically significant (p < 0.01), Figure 1

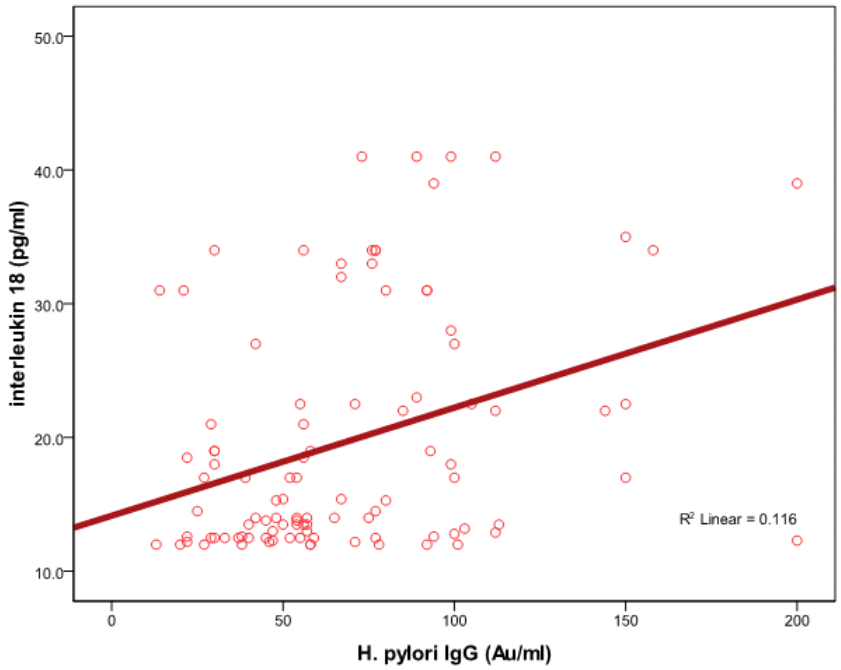

Figure 1. Relationship between H. pylori IgG level and Interleukin 18 in patients

A negative correlation between H. pylori IgA level and Interleukin 18 was found (r = - 0.084), (p >0.05), Table 2.

Table 2. Relationship between H. pylori IgA level and IL-18 in patients.

H. pylori IgA (Ndx)	Correlation coefficient (r)	p value
Interleukin 18 (pg/mL)	- 0.084	>0.05

Correlation between serum interleukin 18 level and ICA, CCA thickness was found (r = 0.545), (r = 0.502) respectively, which is statistically significant (p < 0.01), Figure 2 & 3.

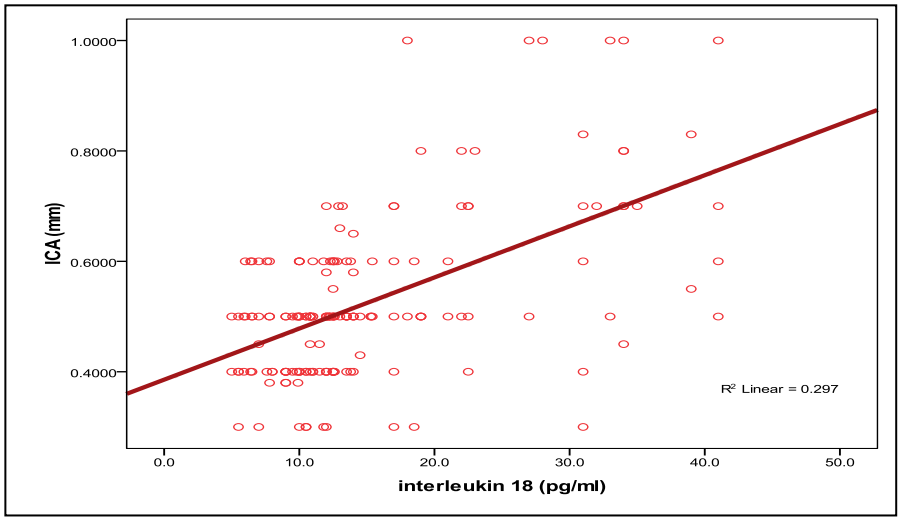

Figure 2. Relationship between interleukin 18 level and ICA thickness in patients.

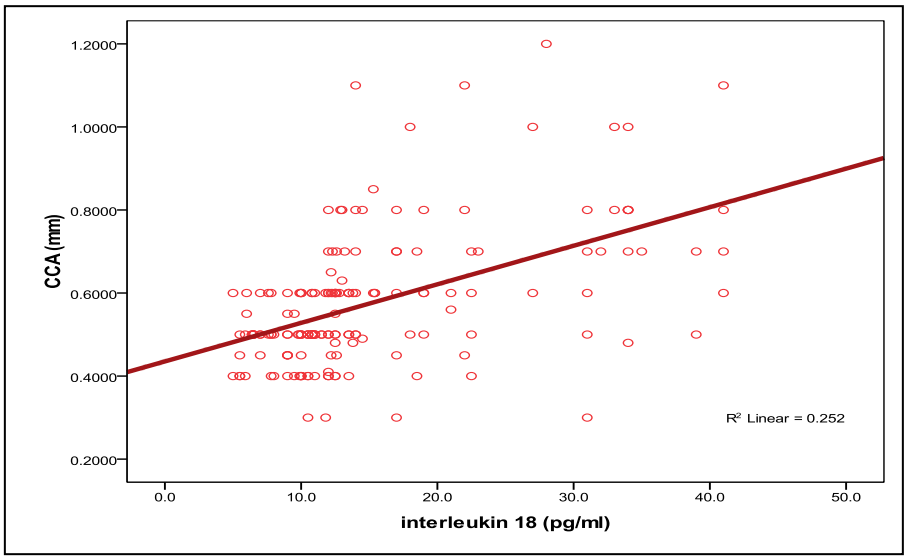

Figure 3. Relationship between interleukin 18 level and CCA thickness in patients.

Discussions:

In the present study the mean serum Interleukin 18 concentration was higher in H. pylori-infected patients than in control group, this observations is consistent with findings of other studies(10, 11, 12, 13). A significant correlation between Interleukin 18 and H. pylori IgG level was found, but not with H. pylori IgA level .Those may be explained by H. pylori infection induces IL-18 in the gastric mucosa (11). IL-18 may play an important role in the inflammatory response and promote the chronic and persistent inflammatory changes in the stomach. This may ultimately influence the outcome of H. pylori -associated diseases that arise within the context of gastritis. (14). Also H. pylori have been shown to induce a strong cytokine response in both human gastric epithelial cells and gastric epithelial cell lines. Chronic infection with H. pylori is associated with gastric IFN- γ producing T cells and increased mucosal IL-12, indicating a predominant Th1 response. IL-12 mRNA levels are increased in infection with cag-positive H. pylori . This results in up-regulation of the expression of IL-18 receptors in both Th1(T-helper 1) and NK cells. It seems that in chronic H. pylori infection, mucosal production of IL-18, together with IL-12, would be important in promoting Th1 responses and IFN- γ secretion. Moreover, it has been demonstrated that Th cells respond to H. pylori antigen by secreting high levels of IFN- γ. (15).

A statistically significant correlation between interleukin 18 and ICA, CCA thickness was found. (16) found that elevated serum IL-18 levels are associated with increased carotid IMT(Intima media thickness) as evaluated by B-mode ultrasound, suggesting their link with carotid atherosclerosis. However IL-18 is highly expressed in human carotid atherosclerotic plaques predominantly co localized with macrophages. Thus, increased IL-18 production from severe atherosclerotic lesions could contribute to the higher IL-18 (7). Also, experimental studies have shown that IL-18 enhances atherosclerosis through release of interferon- γ (17) and induces expression of IL-6 in vascular endothelial and smooth muscle cells (18). Inversely, IL-18 deficiency reduces the extent of atherosclerosis in apolipoprotein E–knockout mice. These findings are in accordance with the studies that IL-18 plays a key role in atherogenesis, supporting the link between IL-18 and carotid atherosclerosis (19).

Conclusion: H. pylori infection was significantly associated with higher serum IL-18. There was significant correlation between IL-18 and Carotid intima- media thickness.

The auther disclose that, they have no conflict of interest.

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 64-80 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

RETROSPECTIVE STUDY OF POSTERIOR DORSOLUMBAR FIXATION IN BAGHDAD: A CLINICAL STUDY OF 100 PATIENTS

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Abstract :

Background: Spondylolisthesis describes a condition of a forward slippage of one vertebra over another, which may or may not be associated with demonstrable instability. Spinal fixation is a neurosurgical procedure in which two or more vertebrae are anchored to each other through a synthetic "vertebral fixation device"

Objective: To determine the demographic distribution of different patient factors and the most commonly vertebra undergo fixation in the thoracolumbar instrumentation.

Patients and Methods: one hundred patients were evaluated during the period of this study in a retrospective manner from January 2013 to January 2015 in four hospitals in Baghdad

(Neurosurgical Teaching Hospital, Neuroscience hospital, Al-Kahdymia Teaching Hospital, Medical City\Ghazy AL-Hariri Hospital). The patients' data regarding the etiology of instability, mechanism of injury for trauma patients, gender, age, segments undergoing instrumentation were identified.

Results: The study revealed female predominance over male: female ratio of 1:2.7**,** the age distribution was highest from 3rd to 7th decades of life, the etiology of instability was either degenerative or traumatic, the degenerative instability was 65% while traumatic cases was 35%. The neurological status of the patients was assessed by neurological examination and revealed 75% with incomplete deficit and 25% with complete neurological deficit, the most common pathologically involved vertebra was the L4, the most common vertebrae used in fixation were the L4 and L5 levels, the most common type of fixation used was the short segment fixation.

Conclusion: Posterior spinal fixation with pedicle screws and rods system is an effective and safe method in maintaining the stability of spine. The intraoperative imaging is important in maintaining safe trajectory of screws. Short segment fixation using the posterior approach with pedicle screw-rod fixation devices achieve good stabilization. The ideal candidates for undergoing posterior spinal fixation are patients with unstable fractures & incomplete neurological deficit.

Recommendation: The use of intraoperative neuro-monitoring, use of navigation system, use of fluoroscopy and the O-arm in spinal fixation surgery. Bone fusion is recommended for each patient.

Keywords: Thoracolumbar spine, spondylolisthesis, pedicle screw fixation

Introduction:

Spinal instrumentation basically means the implantation of more or less rigid metallic or non-metallic devices which are attached to the spine. These devices function to provide spinal stability and thus facilitate bone healing leading to spinal fusion. [1]

Types of instrumentation[2]

1.Metallic Pedicle Screw-Rod Systems

2. Polyetheretherketone (PEEK) Rods

Goals and indications of spinal instrumentation:

1. Trauma

2. Non trauma

a. Tumor
b- Infection
c. Degenerative changes and spondylolisthesis[2]

Indications for fusion fall into two broad categories:

A. Preoperative structural problems that predispose to instability after decompression:
   1. Degenerative spondylolisthesis or lateral listhesis.
   2. Progressive scoliosis or kyphosis.
   3. Recurrent spinal stenosis requiring repeat decompression at the same level.
B. Intraoperative structural alterations that warrant consideration of a fusion:
   1. Excess facet joint removal 50%
   2. Pars interarticularis fracture or removal.
   3. Radical disc excision with resultant destabilization of the anterior spinal column.
C. Trauma that predispose to unstable spine

Measures for Correct Screw Placement

1. Navigation , CT, and fluroscope Guidance[3,4]
2. Electromyographic Monitoring[4]

Complications:

1. Pedicle Fracture[2]
2. Cerebrospinal Fluid Fistulae[5]
3. Infection[10]
4. Hardware Failure (Screw Breakage , Screw Pull-out, Screw Loosening or Plate or Rod Breakage, Loss of Correction, Wound Breakdown.)[5,6,7,8]
5. Nerve root or cord injury.

Free –hand technique

Free-hand pedicle screw placement relies on an intricate appreciation of the relationship of various anatomical landmarks at each level of the thoracolumbar spine. Analogous entry sites guided by differential anatomy are utilized for both the thoracic and lumbar spine.[9,10]

Accuracy of pedicle screw placement

Criteria of pedicle screw placement were**:** [11]

(1) Relation of pedicle screws to the pedicle.
(2) Relation of pedicle screws to the vertebral body.

Aim of the study:

- To determine the demographic distribution of different patient factors
- To identify the most common vertebra levels to undergo fixation in the thoracolumbar spine.

Patients and Methods:

One hundred patients were evaluated during the period of this study in a retrospective manner from January 2013 to January 2015 in four hospitals in Baghdad (Neurosurgical Teaching Hospital, Neuroscience hospital, Al-Kahdimiyya Teaching Hospital, Medical City/Ghazi AL-Hariri Hospital).

Demographic, Admission complaints and imaging were obtained for 100 patients through chart review. On Admission the following parameters independently reviewed: gender, age, chief complaint, etiology of instability, for the trauma patients the mechanism of injury was identified, the level of pathology, neurological examination, type of neurological deficit (whether complete or incomplete), level of fixation, number of screws used, outcome before discharge and a 6 months follow up.

The patients' data regarding the etiology of instability, mechanism of injury for trauma patients, segments undergoing instrumentation were identified.

All patients' undergone hematological investigations in the form of complete blood, ESR, C-reactive protein, renal function test, fasting blood sugar, blood group & Rh.

Only 16 patients have NCS and EMG prior to surgery, so the neuro-electro-physiological studies can't be analyzed in correlation to other clinical factors.

The findings of the patients' pre-operative imaging including thoracolumbar X-rays, CT-scan and MRI were reviewed.

The systems used were Medtronic and Aesculap.

Results:

The gender analysis revealed female predominance over male: female ratio of 1:2.7 and that 27% were males while 73% were females. (Figure1)

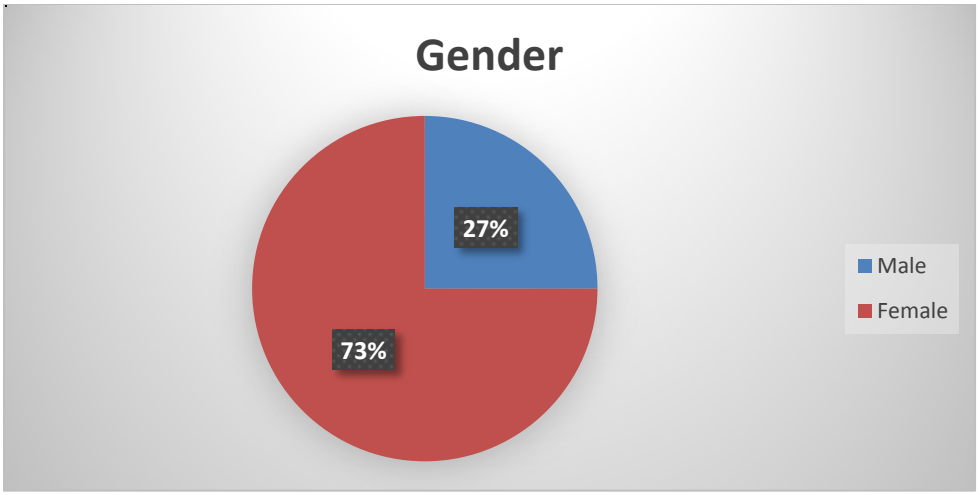

Figure .1 Gender distribution

The age distribution of spine instability for both the traumatic and non-traumatic cases was highest from 3rd to 7th decades of life, it's very low in the first two decades of life and in the 7th decade of life.

Table (2) Age distribution

AGE	
10-19 years	2%
20-29 years	13%
30-39 years	23%
40-49 years	36%
50-59 years	21%
60-69 years	5%
Total	

The etiology of instability was either non traumatic or traumatic, the non-traumatic instability was 65% while traumatic cases was 35%. In the non-traumatic group the

spondylolisthesis was predominant 89.2% while the pathological fractures were 11.8% of the pathological fractures 71.4% were due to infections while 28.6% were due to tumors. In the traumatic patients the FFH as a cause was 51.5% while the RTA as a cause to the instability was 48.5%.

Table (3) Etiology of instability

<table>
<tr><th colspan="5">Etiology of instability</th></tr>
<tr><td colspan="2">Traumatic 35%
(35 patient)</td><td colspan="3">Non-Traumatic 65%
(65 patient)</td></tr>
<tr><td>Road trrafic accident
51.5%
(17 patient)</td><td>Fall from height
48.5%
(18 patient)</td><td>Spondylolisthesis
89.2%
(58 patient)</td><td colspan="2">Pathological #
11.8%
(7 patients)</td></tr>
<tr><td colspan="2"></td><td></td><td>Infection
71.4%
(5 patients)</td><td>Tumor
28.6%
(2 patients)</td></tr>
</table>

The neurological status of the patients was assessed by neurological examination and revealed 75% with incomplete deficit and 25 % with complete neurological deficit, of the incomplete injury 20% were incomplete motor deficit, while 93.3% were incomplete sensory deficit and 12% sphincter disturbance. Such deficits (motor, sensory and sphincters) occurred either in isolation or together.(Fig.2)

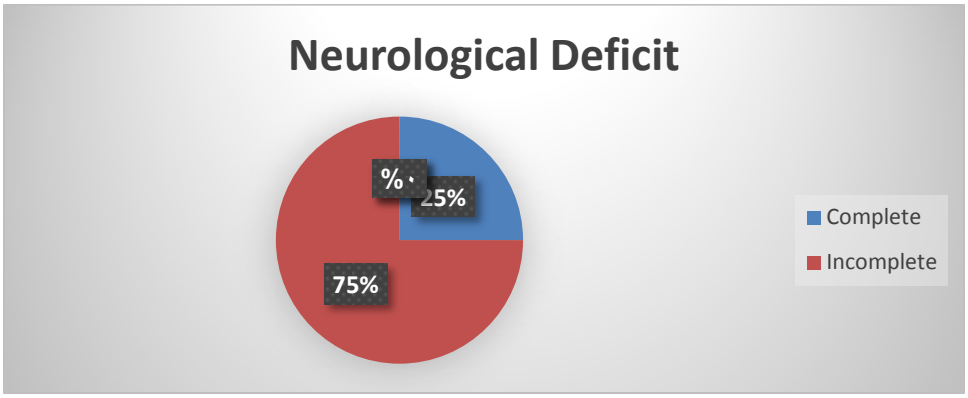

Figure .2 Neurological deficit

The Neurological deficit according to the etiology was reviewed and revealed a complete neurological deficit of 23 patients in traumatic spondylolisthesis while only 2 patients with complete neurological deficit in non-traumatic cases. The incomplete neurological deficit was predominant in non-traumatic spondylolisthesis of 63 patients while only 12 patients having incomplete neurological deficit in the traumatic cases.

Table (4) Linking the Neurological Deficit to Etiology

Linking the Neurological Deficit to Etiology		
Etiology of Instability	**Complete Neurological Deficit**	**Incomplete Neurological Deficit**
Traumatic	66% (23 patient)	34% (12 patient)
Non-Traumatic	3% (2 patient)	97 % (63 patient)

The most common pathologically involved vertebra was the L4, followed by L5, L3 and L2. The least involved vertebrae were D10 and D11. Table (5) illustrates whether the pathological vertebra involved single or multiple levels (there were 2 cases with multiple fracture).

Table (5) Level of Pathology

Level of Pathology	
D10	**2**
D11	**2**
D12	**10**
L1	**5**
L2	**16**
L3	**15**
L4	**37**
L5	**15**

The most common type of fixation used was the short segment fixation using 4 pedicle screws while the least common was the long segment fixation using 8 pedicle screws.

Table (6) No. of screws used in a single fixation

Type of segment	No. of patients	No. of screws
4 screws (short segment fixation)	**67**	**268**
6 screws (intermediate segment fixation)	**27**	**162**
8 screws (long segment fixation)	**6**	**48**
Total No. of screws		**478**

The type of intraoperative guidance for screw placement was either by fluoroscopy (C-arm X-ray) 77 cases or by anatomical landmarks (free hand fixation) 23 cases.

Table (7) Type of Intraoperative Guidance for screw placement

Type of Intraoperative Guidance for screw placement		
Type of Guidance	**Fluoroscopy Guided Fixation**	**Free Hand Fixation**
No. of patients	77	23
Accuracy	98%	91.3%

The complications occurred was either intraoperative or postoperative. The intraoperative complications include dural tear (3 patients), screw misplacement (4 patients). The postoperative complications include infection (7 patients), screw fracture (4 screw fractured in 3 patient as in Figure10), CSF leak (3 patients), and exposure of the system (1 patient).

Table (8) Intraoperative Complications

operative Complications	
Screw misplacement	4 patients
Dural Tear	3 patients

Table (9) Postoperative Complications

Postoperative Complications	
Infection	7 patients
Screw fracture	3 patients
CSF leak	3 patients
Exposure of the system	1 patient

Regarding follow up and outcome: 42 patient were followed for 6 months of which 39 patients show improvement of symptoms (1 patient with complete neurological deficit show improvement in motor function), 2 patients show worsening of symptoms and 1 patient died.

Table (10) Follow up and Outcome

Follow up and Outcome	
Improvement of symptoms	39 patients
Worsening of symptoms	2 patients
Death	1 patient

Discussion:

A significant number of patients suffer spinal diseases around the world, and a high percentage of them experience pathology in thoracolumbar region. Of those patients, a number of vertebral instability disorders need surgical stabilization.

Different surgical modalities are used in treating thoracolumbar spinal instability. Of these, the most common being used is posterior spinal fixation with different instruments.

The most modern and commonly used fixation is the pedicle screw and rod system. Other methods include anterior spinal fixation with cage, kyphoplasty, vertebroplasty, posterior interbody fusion and posterior intertransverse fusion. Of the posterior spinal fixation, the various instruments used are pedicle screws and rods system.

The neurological recovery is dependent on the degree of stability provided by fixation, and on initial instability and neurological loss. So, if stability is provided to spine then neurological status of some patients can improve.

Regarding the gender 73% of our study group was female and 27% was male with a male/female ratio of 1:2.7, while in Muralidhar B.M. et al Study [12] there was male predominance of 80% and female of 20%, Shailendra et al study [13] the male percentage was 76% and female was 24%. This difference is due to that 65% of our patient complaining of instability due to non-traumatic causes of which degenerative spondylolisthesis more commonly occurs in elderly females because of the postmenopausal osteoporosis.

About age group, in our study 36% of our patient are located in 50-59 age group which is the commonest age group while in Muralidhar B.M.et al study most of the patient are in 50 -59 age group, in Shailendra et al study the commonest age group is 16-25 years followed by 26-35 years, this difference because the largest group of our patient had degenerative diseases other than trauma which prevalence increased with age.[12, 13]

Concerning etiology, 35% are due to traumatic injury (17 patient RTA, 18 patient FFH), 65% are due to non-traumatic causes (58 patient spondylolisthesis, 7 patient pathological fracture {pathological fracture 5 patient infection, 2 patient tumor}) , while in study done by Alp Ozgun BOrcek et al [15] the non-traumatic cases was 63.9% while the traumatic cases was 36% which is close to our result.

The relation between etiology and neurological deficit in traumatic cases 66% had complete neurological deficit and 34% incomplete deficit while in non-traumatic cases 3% had complete deficit and 97% had incomplete deficit, this difference is due to two factors first is that traumatic injury which cause vertebral fracture and instability means sever trauma while in degenerative cases it is usually a chronic process and patient seek medical management before complete deficit occur, the other factor is that trauma mostly cause injury to thoracolumbar junction because the transition between the more rigid thoracic spine and the mobile lumbar spine concentrates bending and axial loads at the thoracolumbar junction where there is spinal cord in the spinal canal while in degenerative cases are mostly in L4 level where there is cauda equina and no cord.

Regarding the level of pathology: in 37% of cases, the pathology involving L4 followed by 15% for L3 and L5, in Muraldhiar B.M. et al study 70 % at L1 level and 20|% at L2 level in our study the higher percentage of L4 are due to that most of our cases are degenerative which is more common in the lower lumbar region leading to L4 and L5 levels to be the most commonly instrumented level. [12]

Regarding type of pedicle screw fixation, the short segment was used 67 times, while the long segment fixation was used 6 times only because it is used in lesions involving the thoracolumbar junction vertebrae, while in a study done by Kashif Mahmood Khan et al[16] on 50 patient with thoracolumbar fixation, four screws with 2 rods were used in 38 patients (76%) and 8 screws were used in 12 patients (24%).

As regards the intraoperative guidance, 23% of operations done by free hand procedure without intraoperative fluoroscope, the accuracy was 91.3%, while in Parker et al study[17] on free hand fixation for degenerative cases (964 patients) the accuracy was 98.3%. In Gertzbein et al study[21] for traumatic patient (171 patients) the accuracy was 71.9%. The accuracy of free hand screw insertion is related to the experience of the surgeon, and possibly to change in anatomical landmark in traumatic cases.

The intraoperative complications, screw misplacement in 4 patients 4%, while in - et al study [38] 3 patients 3.2% had screw misplacement, dural tear occurred in 3 patients 3% in our study, while is study done by Faraj et al 4 patients 4.4% had unintended dural tears.

The postoperative complications in our study included infection in 7 patients (7%) all of them had removal of the system, the patients with secondary neoplasia were receiving concurrent chemo- and radiotherapy for their malignant diseases which rendered their immunity low. In Sanford et al study[19] the infection rate was 3.2%. The CSF leak occurred in 3 patients 3% in our study, while in Faraj et al study[38] it was 2.1%. Screw fracture occurred in 4 screws in 3 patients out of 478 screws used in our study (1.2%), while 9 screw fractures out of 296 in Ohlin et al study.[20] Exposure of the system occurred in 1 patient only as a complication of being bed ridden in a complete neurological deficit, this patient died of septicemia and pneumonia.

Regarding six-month follow-up and outcome: 42 patients were followed (we couldn't follow all the patients because of their residency in far provinces), 39 patients showed improvement in their neurological status (1 patient with complete neurological deficit show improvement within 3 weeks), 2 patient show worsening of symptoms (increased pain) and 1 patient died as mentioned above . While in a study done by Roop Singh et al[21] 41% of patients improved and 27% did not improve (all of them are paraplegic) with the difference possibly related to severity of injury and timing of surgery.

Patients who experience worsening of symptoms feel so due to misplacement of the screw with pressure on the nerve root or due to pathology in other levels.

Conclusion:

Posterior spinal fixation with pedicle screws and rods system is an effective and safe method in maintaining the stability of spine, by improvement in all the scores and parameters including the pain and mobility, range of motion of spine, and deformity.

1. The intraoperative imaging in coronal and sagittal planes is important in maintaining safe trajectory of screws, although free hand screw insertion is applicable with more experienced hands after thorough knowledge of anatomical landmarks.
2. Short segment fixation using the posterior approach with pedicle screw-rod fixation devices with or without bone grafting achieves good stabilization and fair enough neurological recovery in patients with unstable thoracolumbar fractures
3. By applying the transpedicular screw fixation on the unstable fractures of the thoracolumbar spine, a stable fracture fixation can be achieved. So early ambulation, rapid return to work and less rehabilitation costs could be achieved. This kind of fixation prevents secondary spine deformities.
4. The ideal candidates for undergoing posterior spinal fixation are patients with unstable fractures & incomplete neurological deficit.
5. In patients with complete neurological deficit, spinal fixation done to aid to achieve stabilization and easy nursing care .
6. Non-Traumatic cause in 40-49 years old females represent the most common etiology.

Recommendation:

- Use of intraoperative neuro-monitoring in spinal fixation surgery.
- Use of a navigation system to guide to the intraoperative manipulation.
- Use of fluoroscopy and the O-arm as an intraoperative guiding tool.
- Bone fusion is recommended for each patient with degenerative changes to improve stability of the spine and to prevent fixation failure.
- Use of short segment is better than long segment to decrease blood loss, timing of surgery, risk of vascular, neural and visceral injury, lost of motion, and cost.

Intraoperative & postoperative Imaging:

Figure 3. Screw fracture indicated by the red arrows

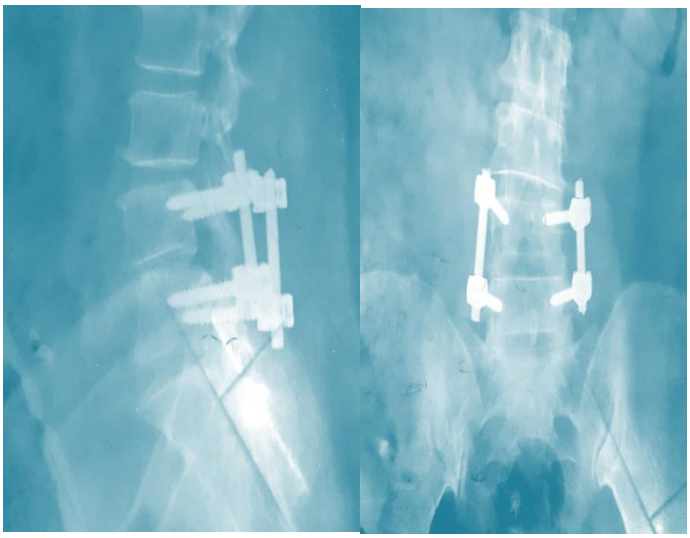

Figure.4 Free Hand Fixation lateral and AP views showing the 4 screws does not span 80% of the vertebral body AP diameter

Figure.5 Intraoperative C-arm AP view showing the upper right screw directed too medially

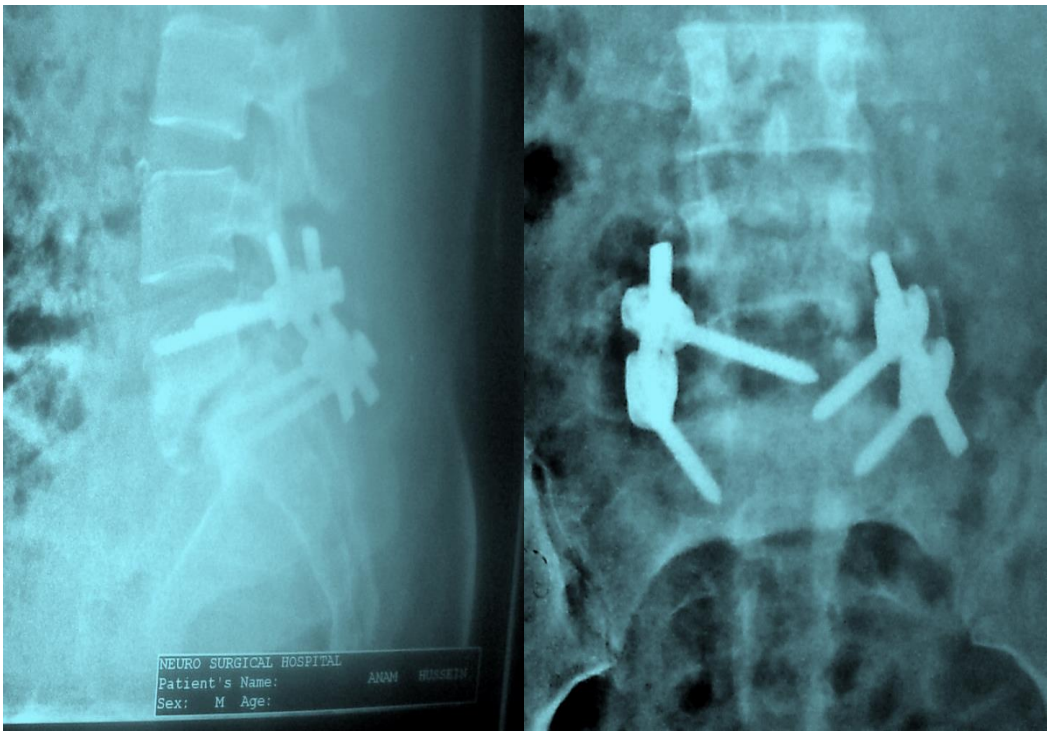

Figure . 6 Postoperative lateral and AP views of lumbosacral vertebrae showing 4 screw system fixation the upper left screw in the AP view is directed too medially.

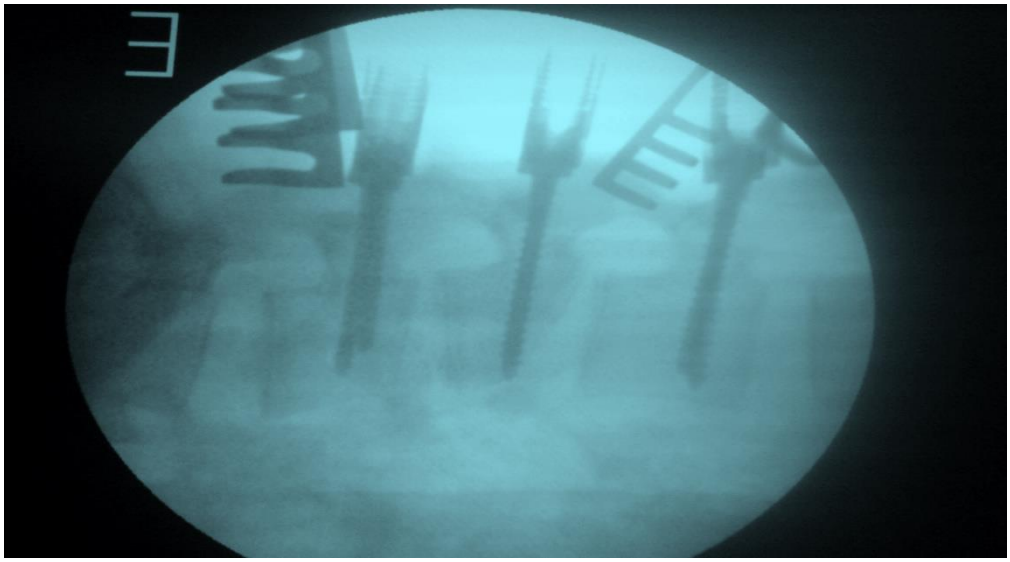

Figure.7 Intraoperative C-arm lat.view

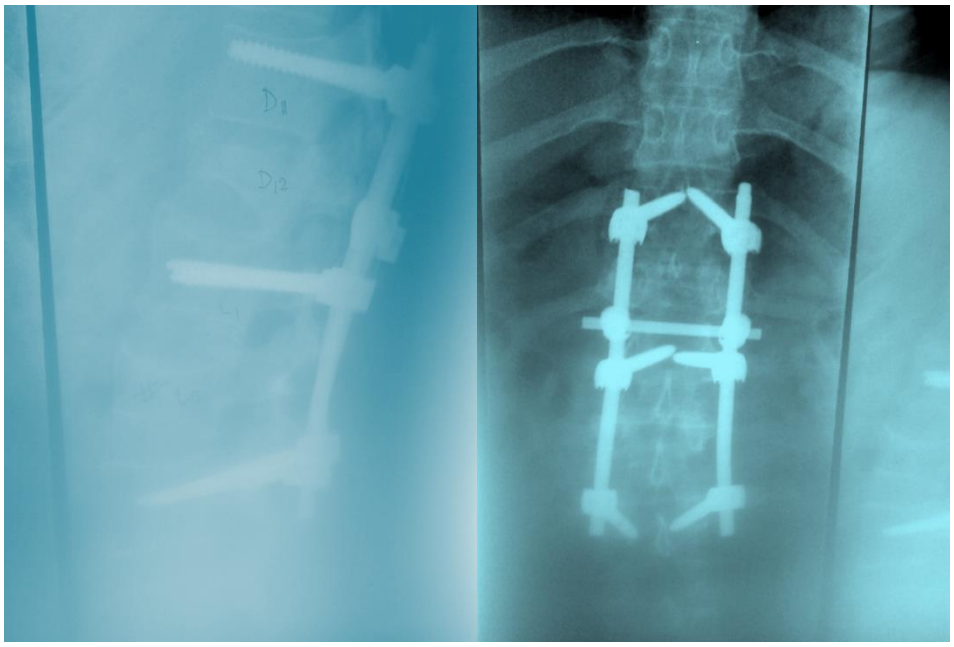

Figure 8 . Postoperative dorsolumbar lateral X-ray to the left and dorsal X-ray AP view showing 6 screw fixation system

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 81-92 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

REAL TIME -PCR FOR QUANTITATIVE DETECTION OF *TOXOPLASMA GONDII* IN ABORTED WOMEN BEFORE AND AFTER TREATMENT

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Abstract:

Background: Human *Toxoplasmosis* is a parasitic disease caused by a *Toxoplasma gondii*. Toxoplasma gondii was the subject of disease burden estimations as a result of the severe clinical symptoms and lifelong implications. Primary infections with T.gondii acquired during pregnancy are usually asymptomatic for the pregnant woman but can lead to serious neonatal complications, isolation of parasite despite it is considered as a golden standard parameter to detect infection,

the repetitive B1 gen is the most target gene used in conventional PCR for molecular detection of *T.gondii.*

Objective: Detection of *T. gondii* DNA using Real- time PCR seems to be a reliable method in diagnosis and follow up of patients.

Methods: Thirty patients (aborted women), their range age between (20− 46) years, were included in this study. All patients were assessment of Real time Polymerase chain reaction from the same patients was taken before and after spiramycin therapy to confirm the infection of the *T.gondii* and to evaluate the parasite load (copy number).

Result: Data of this study revealed that there is significant difference(P=0.002) in the mean of the copy numbers in aborted women's, the mean of parasite copy number in patients samples before treatment was (4.24E+06) and the mean of copy number after treatment was (7.53E+05).

Conclusion: The diagnosis of toxoplasmosis is mainly based on serological tests detection of *T. gondii* DNA using Real time- PCR seems to be a reliable method in diagnosis and follow up of patients.

Keywords: *Toxoplasma gondii*, Real- time PCR, B1-gen

Introduction:

Toxoplasmosis is a worldwide infection that may cause severe disease and is regarded as a serious health problem in world. It is one of the well-studied parasites because of its medical and veterinary importance, and its suitability as a model for cell biology and molecular studies with a unicellular organism [1]. Toxoplasmosis is also a major opportunistic infection in immunocompromised individuals, often resulting in lethal toxoplasmic encephalitis .If a woman was infected for the first time during pregnancy the parasite may be transmitted transplacentally to the fetus, this can result in death of the fetus, central nervous system abnormalities, or eye disease and affecting the quality of life of the child throughout its life time [2].

Toxoplasma gondii was the subject of disease burden estimations. As a result of the severe clinical symptoms and lifelong implications, the disease burden of *T.gondii* is high .Primary infections with *T.gondii* acquired during pregnancy are usually asymptomatic for the pregnant woman but can lead to serious neonatal complications [3].

Toxoplasma infection stimulates both humeral immune response characterized by antibody production of (IgM and IgG) and cell mediated immunity (CMI) which are essential

for the host control of intracellular infections , so the protection against Toxoplasmosis is mediated by cellular defense [4].

The seroprevalence varies from 5% to 90% depending on geographical location, age, habit of eating raw meat or unwashed fruit and vegetables, and general level of hygiene, the incidence of infections is higher in warmer and humid climate and increases with age [5].

Serological tests including the detection (and quantification) of *T.gondii* antibodies in serum are used to establish whether a pregnant woman has been infected and, if so, to determine whether the infection was acquired recently or in the distant past. If serological test results suggest a recently acquired infection, an effort is made to determine whether the infection was likely acquired during gestation or shortly before conception. If so, the fetus is at risk [3].

In Iraq, many studies were accomplished concerning the sero prevalence of Toxoplasmosis by using different techniques including indirect haemagglutination test (IHA), Indirect Fluorescent Antibody Technique (IFAT), and Enzyme-linked Immunosorbent Assay (ELISA) [6]. Real time- PCR can be used as an additional diagnostic tool for the rapid detection of *T.gondii* in various clinical materials [7].

The use of the macrolide antibiotic *Spiramycin* has been reported to decrease the frequency of vertical transmission [8] .However, carefully designed; prospective studies that demonstrate this effect have not been recorded. The protection has been reported to be more distinct in women infected during their first trimester [9].

Material and methods:

Thirty women with spontaneous miscarriage were included in the study all have had curettage operation at the Obstetrics and Gynecology Department of Al-Kadhimyia Teaching in Baghdad and private clinic during the period from November 2012 to April 2013. Their age ranged from (20- 45) years old. Ten healthy pregnant women with a history of a normal pregnancy attending the outpatient clinics for routine gynecologic checking enrolled in this study as control group, their age were ranging between (21-39) years old. From each patient two blood samples were collected pre and post treatment with oral spiramycin , 4 capsules (at 500mg) two times a day , three ml. were placed in EDTA tubes for DNA extraction, and all samples were stored at -20 °C DNA was extracted from the whole blood samples using a commercial purification system (ExiPrepTM DNA/ RNA Prep Kit).

The DNA concentration was determined by using the spectrophotometer (Bioneer), 10 µL of each DNA sample were added to 990 µL of D.W. and mixed well. The spectrophotometer was used to measure the optical density (O.D) at wave length of 260 nm and 280 nm. An O.D of one corresponds to approximately 50 µg / ml for double stranded DNA. The concentration of DNA was calculated according to the following formula: DNA concentration (µg/ml) = O.D 260 nm x 50 x Dilution factor

The spectrophotometer was used also to estimate the DNA purity ratio according to this following formula: DNA purity ratio = O.D 260 nm / O.D 280 nm.

Amplification of B1-gene by Real-Time Polymerase chain reaction

Principle

TaqMan probes are hydrolysis probes that are designed to increase the specificity of quantitative PCR. The principle of this technique relies on the 5´–3´ exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophores-based detection [10].

Toxoplasma gondii Real-TM PCR kit uses "hot-start", (Anatolia geneworks- Turkey) which greatly reduces the frequency of nonspecifically primed reactions. The forward and reverse sequences were (GCATTCCCGTCCAAACT and AGACTGTACGGAATGGAGACGAA) respectively [11].

The control positive DNA copy number was determined as follows: copy number (copies /ml) =(C x Avogadro constant) / (660 x control positive DNA in bp 10^9) Where C (copy) is given in ng/ml, Avogadro constant (N_A) = 6.022 140 857(74) $\times 10^{23}$ /mole.

Preparing the PCR:

Real-time PCR assays were carried out in duplicate and performed following manufactured protocol in a total volume of (41µl) for one reactions as follow PCR Mix (20µl), (1.6µl) from detection Mix 1 which contains forward and reverse primers and dual-labeled probe, (1.2µl) from detection mix 2 which contains internal control-specific forward and reverse primers and dual-labeled probe, Internal control (0.2µl).Twenty three microliter of this master mix was added in the PCR tube with 18µl of DNA (sample, positive or negative control). Tubes were closed well and spin, The PCRs were performed with the Agilent Real-time PCR (Techne-UK). After initial activation of hotStar Taq DNA polymerase at 94°C for 2 min, 40 PCR cycles of 93°C for 10 s , 60°C for 10 s and 72 for 30s were performed.

Calculation of results:

Data were analyzed by smart-cycler software using arithmetic baseline adjustment, *T.gondii* DNA copy number was estimated from the cross point threshold relative to positive standard. The standard curve correlates each copy number with a particular Ct, the copy number value of the unknown samples are driven from the standard curve (According to this study).

Statistical Analysis:

Experimental data were presented in terms of observed numbers and percentage frequencies, and then analyzed by using Chi- square ($\chi 2$) test. P value ≤ 0.05 was considered statistically significant

Results:

The detection of B1 gene of *T.gondii* in whole blood specimens from aborted women's and control group by Real-time PCR:

In this study, the absolute quantification method was used for data analysis to determine the actual copy number before and after treatment, the standard curve was generated, in which concentration is fixed to a specific Ct value as the Ct value is inversely related to the amount of starting template.

Standard curve generation:

A standard curve was generated using a 10-fold dilution of a template amplified on the Cycler - real-time system (Figure 1), the copy number and CT value correlation was 0.9987.

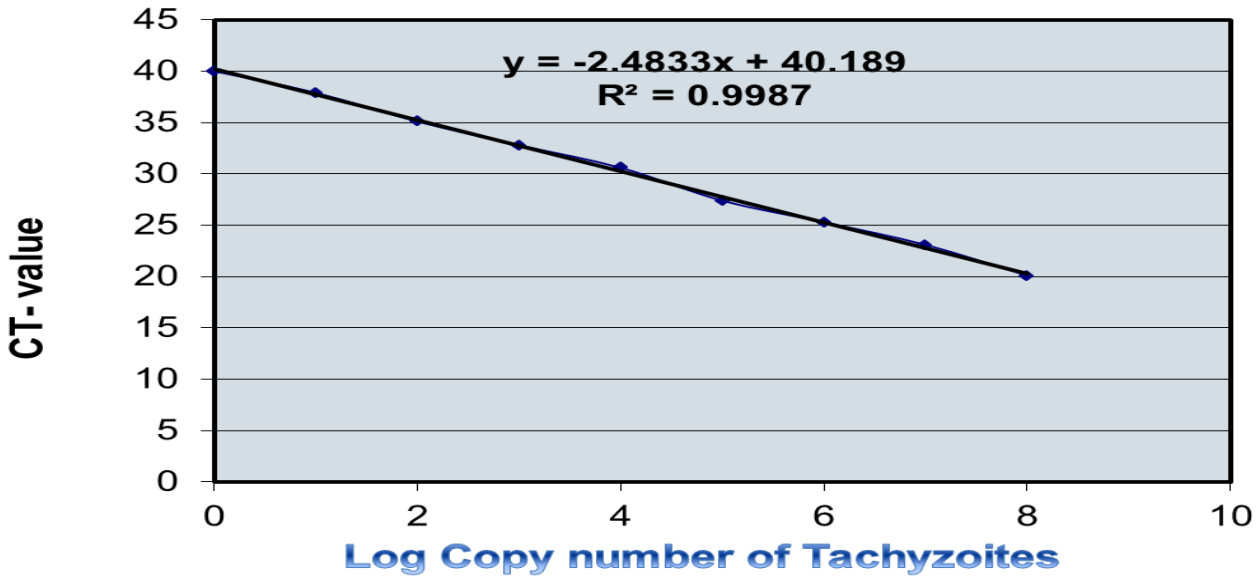

Figure (1): Standard curve for quantitation of *T.gondii* tachyzoites. (Serial dilution ranging from (100– 108) copies used as template for real time PCR analysis)

Parasite load in abortive women's as determined by Real- time PCR:

The range of *T.gondii* load in abortive women's under study before and after treatment with oral spiramycin , 4 capsules (at 500mg) two times a day in table (1) which is found to be ($0.0192X10^{8}$ – $0.110X10^{4}$) before treatment and ($0.156X10^{7}$- $0.036X10^{0}$) after treatment,(P value =0.002). Data of this study revealed that there is significant difference(P=0.002) in the mean of the copy numbers in aborted women's, results in our study revealed that the mean of parasite copy number in patients samples before treatment was (4.24E+06) and the mean of copy number after treatment was 7.53E+05) figure(2)

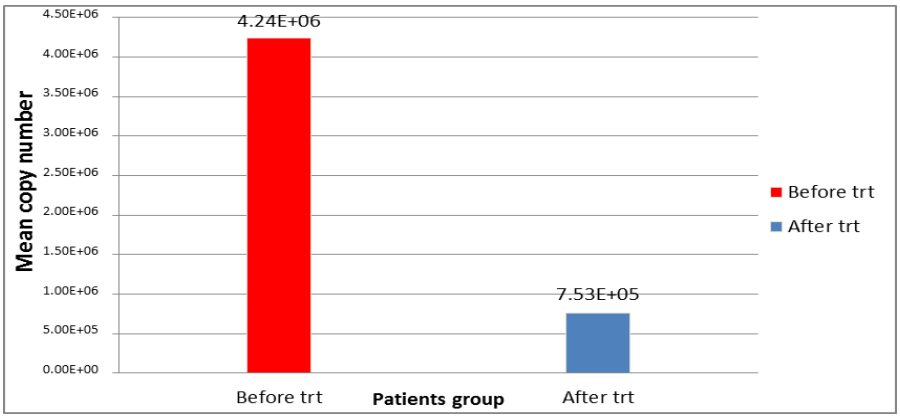

Figure (2): Mean of copy number parasite /ml of patients' blood samples before and after treatment

Table (1): *Toxoplasma gondii* copy numbers screening in abortive women's as determined by Real- time PCR

Sample Name	CT-pre treatment	Copy no. pre treatment	CT-post treatment	Copy no. post Treatment
P 1	20.8	0.192 X 10^7	25.81	0.187X10^5
P2	27.1	0.0624X10^5	38.61	0.151X10^0
P3	20.1	0.0192X10^8	23.65	0.156X10^6
P4	21.4	0.129X10^7	24.11	0.110X10^6
P5	25.6	0.208X10^5	21.17	0.156X10^7
P6	23.7	0.151X10^6	37.12	0.057X10^1
P7	21.5	0.122X10^7	23.18	0.201X10^6
P8	19.4	0.086X10^5	22.19	0.057X10^7
P9	26.4	0.129X10^5	24.16	0.108X10^6
P10	22.7	0.007X10^7	35.55	0.206X10^1
P11	20.9	0.182X10^7	26.9	0.084X10^5
P12	20.5	0.220X10^7	37.12	0.055X10^1
P13	21.9	0.084X10^7	32.7	0.002X10^3
P14	26.4	0.129X10^5	22.41	0.036X10^7
P15	29.1	0.110X10^4	37.12	0.057X10^1
P16	22.4	0.036X10^7	33.1	0.204X10^2
P17	21.8	0.093X10^7	29.1	0.110X10^4
P18	22.5	0.028X10^7	35.1	0.009X10^2
P19	23.7	0.086X10^6	38.6	0.153X10^0
P20	22.4	0.038X10^7	32.1	0.062X10^3
P21	22.8	0.237X10^6	39.6	0.055X10^0
P22	28.6	0.158X10^4	30.1	0.014X10^4
P23	26.9	0.084X10^5	32.9	0.223X10^2
P24	25.1	0.014X10^6	39.8	0.036X10^0
P25	24.1	0.112X10^6	37.9	0.220X10^0
P26	23.5	0.170X10^6	31.8	0.088X10^3
P27	21.6	0.112X10^7	39.1	0.105X10^0
P28	26.4	0.132X10^5	31.8	0.088X10^3
P29	23.5	0.170X10^6	36.7	0.096X10^1
P30	27.9	0.225X10^4	33.7	0.146X10^2
Negative control	NTC		NTC	

Regarding response to treatments toxoplasmosis infected women in this study were divided to the following groups:

Group I include patients had high parasite load ($0.062X10^{5}$-$0.112X10^{7}$**)** which decrease completely after completion the course of treatment ($0.036X10^{0}$-$0.223X10^{0}$).

Group II include a patients with high parasite load ($0.225x10^{4}$ -$0.036x10^{7}$) at time of diagnosis and decreases after completion the course of treatment ($0.055x10^{1}$ -$0.223x10^{1}$).

Group III includes patients with high parasite load at diagnosis ($0.225x10^{4}$ -$0.036x10^{7}$**)** which still with high parasite load or slightly lower ($0.002x10^{3}$ – $0.201\ x10^{6}$**)**.

Group IV includes patients with high parasite load ($0.158x10^{4}$ – $0.129x10^{5}$**)** at time of diagnosis which showed elevated parasite load after treatment ($0.014x10^{4}$ -$0.156x10^{7}$**)**.

Discussion:

Firstly DNA was successfully extracted from whole blood samples for all subjects, Menotti, et al [12] showed that the parasites are as likely to be found in the plasma or red cell fractions, which might indicate that the most suitable sample is a whole blood extract. However, in patient's blood *T.gondii* present might be intracellular in leukocytes [13]; in that case the white cell fraction would be as appropriate as whole blood.

Parasitemia in pregnant women may be due to the toxoplasmosis reactivations occur during immunosuppressive states in pregnancy. Tachyzoites or *T.gondii* DNA release from the cyst in the body tissues may be the source of parasitemia or *T.gondii* DNA in blood [14].

Quantitative detection of pathogen by real time PCR has been successfully applied to a variety of specimens and the finding of *T.gondii* DNA in peripheral blood by PCR might help in the early diagnosis of invasive diseases [15].

The qRT-PCR that have been applied in this study to diagnose and for the determination of copy number of *T.gondii* DNA in whole blood, it gives reproducible diagnostic and quantitative results over conventional PCR with the risk of false positive due to contamination with previously amplified products since in qRT-PCR there is no risk of hand manipulation and the use of internal control will exclude the false negative which due to inhibiters in amplified mixture, reproducibility of qRT-PCR quantification was tested by the testing of the 10 fold dilution.

In the present study a qRT-PCR based B1 gene – specific Taq man assay have been developed for quantitative detection of *T.gondii* which extremely sensitive to (0.168 parasite / reaction) and its ability to quantify infection load of clinical specimen and its long linear range of 7 logs of DNA concentration with CT value as shown in figure (1) where R2=0.998 which is indicative of a significant correlation between logarithmic value of each PCR product of copy number amplified and CT value. These results were in agreement with Mei-huilin, [(16)]. In this assay an increase of the fluorescent signal above the preset threshold within 40 PCR cycle was considered positive (CT< 40), Table (1) where CT for negative control was 40. Quantification of

parasite load in pregnant women under study has been used to ass's disease severity and the treatment outcome.

The relative quantity of tachyzoites in each DNA sample was determined using the standard curve presented in Figure (2) which showed a range between (0.110X10^^4 to 0.0192 $\times 10^{8}$) before treatment and (0.036×10**0** to 0.156×10^{7}) after treatment .In addition figure (2) revealed that there is significance difference (P<0.05) of in the mean of the copy number before and after treatment.

According to their response to treatments, Group 1 patients which represent, **5** (16.66%) % of patients having high parasite load at time of diagnosis with a range of (0.062X10^{5}-0.112X10^{7}) copies /ml , they show complete response to treatment with decline in parasite load to undetectable value , these results were in accordance with Costa , et al [17] who recorded that the parasite count decreased sharply in patients having cerebral toxoplasmosis symptoms , when treated with anti-toxoplasma therapy which was efficient and reducing the parasite load and they conclude that Light Cycler PCR provide precise evaluation of parasite load in immunocompromised patients and PCR is useful in monitoring of treatments .

In addition, Kupferschmidt, [18] conclude that decreasing in parasite load in patient's blood sample demonstrated that treatment used was effective. Contini **,** et al [19] recorded that patient under specific treatment did not show detectable B1 DNA levels in samples collected after many days of therapy . So that *T.gondii* DNA load is a valuable tool for monitoring of toxoplasmosis. Group Π patients which represent 10(33.33 %) of patients having high parasite load at time of diagnosis with a range of (0.225x10^{4}-0.036x10^{7}**)** copies /ml, they show lower parasite load with a range of (0.055x10^{1} -0.223x10^{1}**)** copies /ml after treatment. These results observed by Menotti, et al [12].

They consider that prolonged persistent detection of *T. gondii* DNA in blood justifies the maintenance of anti *T.gondii* treatment and close follow up by PCR. These results were in agreement with Romand, et al [20] who used quantitative Real time PCR to evaluate *T. gondii* concentration in amniotic fluid and conclude that quantitative PCR may help the clinician to form prognosis of congenital infections with regard to fetal and neonatal outcome. Other study by Christianne, et al [21] reported that high parasite load in amniotic fluid was found in cases of severe congenital toxoplasmosis as determined by quantitative PCR.

Group III which represent 11(36.66 %) include patients with high parasite load at time of diagnosis with a range of (0.038x10^{7} –0.019x10^{8}) copies/ml, they did not show any response to treatment, parasite load was remained with undetectable decrease. After treatment it's found to be (0.002x10^{3} – 0.201 x10^{6}) copies/ ml. These results were in agreement with Kupferschmidt, et al [(18)] .who used Tag man PCR to monitor parasite load in the blood of an immunocompromised patients after allogenenetic bone marrow transplantation. They found that at day 199 after therapy with pyrimethamine the detected parasite load remained unchanged and then decreased after prolonged therapy.

Regarding group IV of patients which represent 4(13.33 %) having high parasite load at time of diagnosis with a range of ($0.158x10^4$ – $0.129x10^5$) copies /ml , they showed elevated parasite load after treatment with a range of ($0.014x10^4$ -$0.156x10^7$) copies /ml .This increase of parasite load seems to be correlated to progression of disease or to poor response to treatment. These results were in agreement with Menotti, et al [12] who recorded evolution in parasite load in patients under treatments and those with slow decrease in parasite load after initiation of treatments with pyrimethamine and sulfadiazine on day 43 post transplantation. They give explanation to the prolonged presence of *T.gondii* DNA that could be due to disseminated form of disease with a peak of parasitemia (29000 tachyzoites/ ml) of blood buffy coat.

Real time-PCR can be used as an additional diagnostic tool for the rapid detection of *T.gondii* in various clinical materials. Studies have documented that Real time- PCR can actually detect *T.gondii* in blood specimens of women before or during pregnancy. Nimri, et al [22] and Khan et al [23] showed that a small numbers of parasites may be detected in circulating blood from some patients with acute or recurrent toxoplasmosis.

Several studies have reported that, PCR could detect parasitemia a few weeks prior to the appearance of any clinical signs or symptoms .PCR is highly sensitive and specific because a single tachyozoite can be detected in a clinical sample [24].

The most important records in this study is that the parasite load could be determined and correlated with treatment. The parasite quantification in some treated subjects become lower and in others, *T.gondii* load disappeared when spiramycin was given, this exhibit that the anti-toxoplasma therapy was efficient in reducing the parasite load.

The quantitative real-time PCR assay developed in this study can be used not only to detect the presence of *T.gondii* DNA but also to provide precise evaluations of the parasite load in pregnant women's. This PCR test should be useful for the monitoring of treatment efficacy and should help provide an understanding of the pathogenesis of *T.gondii* reactivation.

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TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 2, 2016 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 64-80 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

RETROSPECTIVE STUDY OF POSTERIOR DORSOLUMBAR FIXATION IN BAGHDAD: A CLINICAL STUDY OF 100 PATIENTS

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Abstract :

Background: Spondylolisthesis describes a condition of a forward slippage of one vertebra over another, which may or may not be associated with demonstrable instability. Spinal fixation is a neurosurgical procedure in which two or more vertebrae are anchored to each other through a synthetic "vertebral fixation device"

Objective: To determine the demographic distribution of different patient factors and the most commonly vertebra undergo fixation in the thoracolumbar instrumentation.

Patients and Methods: one hundred patients were evaluated during the period of this study in a retrospective manner from January 2013 to January 2015 in four hospitals in Baghdad (Neurosurgical Teaching Hospital, Neuroscience hospital, Al-Kahdymia Teaching Hospital, Medical City\Ghazy AL-Hariri Hospital). The patients' data regarding the etiology of instability, mechanism of injury for trauma patients, gender, age, segments undergoing instrumentation were identified.

Results: The study revealed female predominance over male: female ratio of 1:2.7**,** the age distribution was highest from 3rd to 7th decades of life, the etiology of instability was either degenerative or traumatic, the degenerative instability was 65% while traumatic cases was 35%. The neurological status of the patients was assessed by neurological examination and revealed 75% with incomplete deficit and 25% with complete neurological deficit, the most common pathologically involved vertebra was the L4, the most common vertebrae used in fixation were the L4 and L5 levels, the most common type of fixation used was the short segment fixation.

Conclusion: Posterior spinal fixation with pedicle screws and rods system is an effective and safe method in maintaining the stability of spine. The intraoperative imaging is important in maintaining safe trajectory of screws. Short segment fixation using the posterior approach with pedicle screw-rod fixation devices achieve good stabilization. The ideal candidates for undergoing posterior spinal fixation are patients with unstable fractures & incomplete neurological deficit.

Recommendation: The use of intraoperative neuro-monitoring, use of navigation system, use of fluoroscopy and the O-arm in spinal fixation surgery. Bone fusion is recommended for each patient.

Keywords: Thoracolumbar spine, spondylolisthesis, pedicle screw fixation

Introduction:

Spinal instrumentation basically means the implantation of more or less rigid metallic or non-metallic devices which are attached to the spine. These devices function to provide spinal stability and thus facilitate bone healing leading to spinal fusion. [1]

Types of instrumentation[2]

1.Metallic Pedicle Screw-Rod Systems

2. Polyetheretherketone (PEEK) Rods

Goals and indications of spinal instrumentation:

1. Trauma
2. Non trauma

a. Tumor

b- Infection

c. Degenerative changes and spondylolisthesis[2]

Indications for fusion fall into two broad categories:

A. Preoperative structural problems that predispose to instability after decompression:
   1. Degenerative spondylolisthesis or lateral listhesis.
   2. Progressive scoliosis or kyphosis.
   3. Recurrent spinal stenosis requiring repeat decompression at the same level.

B. Intraoperative structural alterations that warrant consideration of a fusion:
   1. Excess facet joint removal 50%
   2. Pars interarticularis fracture or removal.
   3. Radical disc excision with resultant destabilization of the anterior spinal column.

C. Trauma that predispose to unstable spine

Measures for Correct Screw Placement

1. Navigation , CT, and fluroscope Guidance[3,4]
2. Electromyographic Monitoring[4]

Complications:

1. Pedicle Fracture[2]
2. Cerebrospinal Fluid Fistulae[5]
3. Infection[10]
4. Hardware Failure (Screw Breakage , Screw Pull-out, Screw Loosening or Plate or Rod Breakage, Loss of Correction, Wound Breakdown.)[5,6,7,8]

5. Nerve root or cord injury.

Free –hand technique

Free-hand pedicle screw placement relies on an intricate appreciation of the relationship of various anatomical landmarks at each level of the thoracolumbar spine. Analogous entry sites guided by differential anatomy are utilized for both the thoracic and lumbar spine.[9,10]

Accuracy of pedicle screw placement

Criteria of pedicle screw placement were**:** [11]

(1) Relation of pedicle screws to the pedicle.
(2) Relation of pedicle screws to the vertebral body.

Aim of the study:

- To determine the demographic distribution of different patient factors
- To identify the most common vertebra levels to undergo fixation in the thoracolumbar spine.

Patients and Methods:

One hundred patients were evaluated during the period of this study in a retrospective manner from January 2013 to January 2015 in four hospitals in Baghdad (Neurosurgical Teaching Hospital, Neuroscience hospital, Al-Kahdimiyya Teaching Hospital, Medical City/Ghazi AL-Hariri Hospital).

Demographic, Admission complaints and imaging were obtained for 100 patients through chart review. On Admission the following parameters independently reviewed: gender, age, chief complaint, etiology of instability, for the trauma patients the mechanism of injury was identified, the level of pathology, neurological examination, type of neurological deficit (whether complete or incomplete), level of fixation, number of screws used, outcome before discharge and a 6 months follow up.

The patients' data regarding the etiology of instability, mechanism of injury for trauma patients, segments undergoing instrumentation were identified.

All patients' undergone hematological investigations in the form of complete blood, ESR, C-reactive protein, renal function test, fasting blood sugar, blood group & Rh.

Only 16 patients have NCS and EMG prior to surgery, so the neuro-electro-physiological studies can't be analyzed in correlation to other clinical factors.

The findings of the patients' pre-operative imaging including thoracolumbar X-rays, CT-scan and MRI were reviewed.

The systems used were Medtronic and Aesculap.

Results:

The gender analysis revealed female predominance over male: female ratio of 1:2.7 and that 27% were males while 73% were females. (Fig. 1)

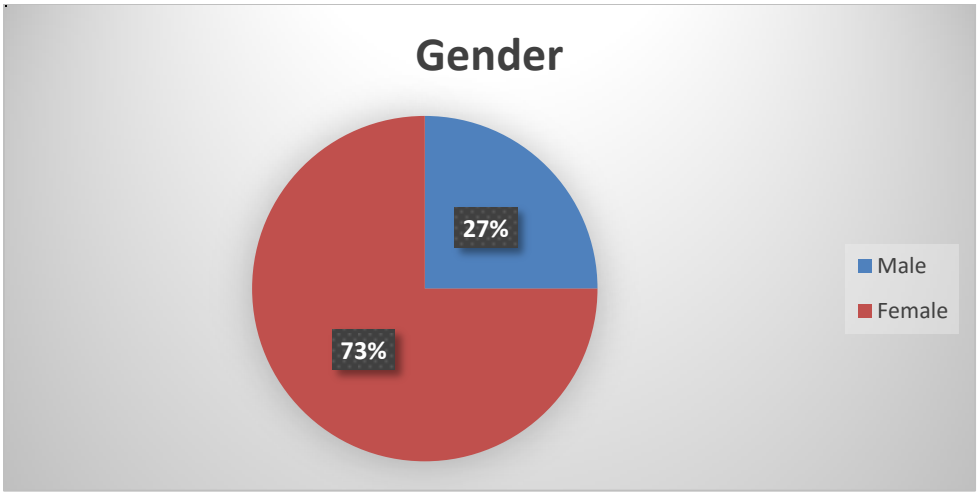

Fig. 1 Gender distribution

The age distribution of spine instability for both the traumatic and non-traumatic cases was highest from 3rd to 7th decades of life, it's very low in the first two decades of life and in the 7th decade of life.

Table (1) Age distribution

AGE	
10-19 years	2%
20-29 years	13%
30-39 years	23%
40-49 years	36%
50-59 years	21%
60-69 years	5%
Total	

The etiology of instability was either non traumatic or traumatic, the non-traumatic instability was 65% while traumatic cases was 35%. In the non-traumatic group the

spondylolisthesis was predominant 89.2% while the pathological fractures were 11.8% of the pathological fractures 71.4% were due to infections while 28.6% were due to tumors. In the traumatic patients the FFH as a cause was 51.5% while the RTA as a cause to the instability was 48.5%.

Table (2) Etiology of instability

<table>
<tr><th colspan="5">Etiology of instability</th></tr>
<tr><td colspan="2">Traumatic 35%
(35 patient)</td><td colspan="3">Non-Traumatic 65%
(65 patient)</td></tr>
<tr><td>Road trrafic accident
51.5%
(17 patient)</td><td>Fall from height
48.5%
(18 patient)</td><td>Spondylolisthesis
89.2%
(58 patient)</td><td colspan="2">Pathological #
11.8%
(7 patients)</td></tr>
<tr><td colspan="2"></td><td></td><td>Infection
71.4%
(5 patients)</td><td>Tumor
28.6%
(2 patients)</td></tr>
</table>

The neurological status of the patients was assessed by neurological examination and revealed 75% with incomplete deficit and 25 % with complete neurological deficit, of the incomplete injury 20% were incomplete motor deficit, while 93.3% were incomplete sensory deficit and 12% sphincter disturbance. Such deficits (motor, sensory and sphincters) occurred either in isolation or together.(Fig.2)

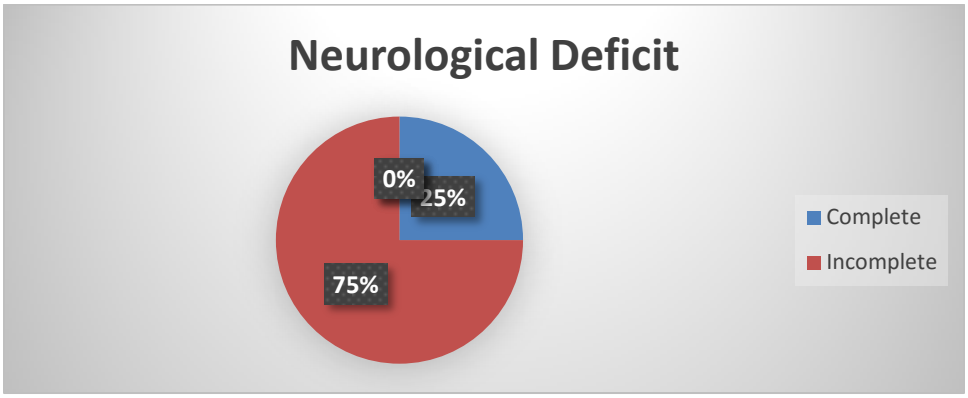

Fig. 2 Neurological deficit

The Neurological deficit according to the etiology was reviewed and revealed a complete neurological deficit of 23 patients in traumatic spondylolisthesis while only 2 patients with complete neurological deficit in non-traumatic cases. The incomplete neurological deficit was predominant in non-traumatic spondylolisthesis of 63 patients while only 12 patients having incomplete neurological deficit in the traumatic cases.

Table (3) Linking the Neurological Deficit to Etiology

Linking the Neurological Deficit to Etiology		
Etiology of Instability	**Complete Neurological Deficit**	**Incomplete Neurological Deficit**
Traumatic	66% (23 patient)	34% (12 patient)
Non-Traumatic	3% (2 patient)	97 % (63 patient)

The most common pathologically involved vertebra was the L4, followed by L5, L3 and L2. The least involved vertebrae were D10 and D11. Table (5) illustrates whether the pathological vertebra involved single or multiple levels (there were 2 cases with multiple fracture).

Table (4) Level of Pathology

Level of Pathology	
D10	**2**
D11	**2**
D12	**10**
L1	**5**
L2	**16**
L3	**15**
L4	**37**
L5	**15**

The most common type of fixation used was the short segment fixation using 4 pedicle screws while the least common was the long segment fixation using 8 pedicle screws.

Table (5) No. of screws used in a single fixation

Type of segment	No. of patients	No. of screws
4 screws (short segment fixation)	**67**	**268**
6 screws (intermediate segment fixation)	**27**	**162**
8 screws (long segment fixation)	**6**	**48**
Total No. of screws		**478**

The type of intraoperative guidance for screw placement was either by fluoroscopy (C-arm X-ray) 77 cases or by anatomical landmarks (free hand fixation) 23 cases.

Table (6) Type of Intraoperative Guidance for screw placement

Type of Intraoperative Guidance for screw placement		
Type of Guidance	**Fluoroscopy Guided Fixation**	**Free Hand Fixation**
No. of patients	77	23
Accuracy	98%	91.3%

The complications occurred was either intraoperative or postoperative. The intraoperative complications include dural tear (3 patients), screw misplacement (4 patients). The postoperative complications include infection (7 patients), screw fracture (4 screw fractured in 3 patient as in Fig. 10), CSF leak (3 patients), and exposure of the system (1 patient).

Table (7) Intraoperative Complications

operative Complications	
Screw misplacement	4 patients
Dural Tear	3 patients

Table (8) Postoperative Complications

Postoperative Complications	
Infection	7 patients
Screw fracture	3 patients
CSF leak	3 patients
Exposure of the system	1 patient

Regarding follow up and outcome: 42 patient were followed for 6 months of which 39 patients show improvement of symptoms (1 patient with complete neurological deficit show improvement in motor function), 2 patients show worsening of symptoms and 1 patient died.

Table (9) Follow up and Outcome

Follow up and Outcome	
Improvement of symptoms	39 patients
Worsening of symptoms	2 patients
Death	1 patient

Discussion:

A significant number of patients suffer spinal diseases around the world, and a high percentage of them experience pathology in thoracolumbar region. Of those patients, a number of vertebral instability disorders need surgical stabilization.

Different surgical modalities are used in treating thoracolumbar spinal instability. Of these, the most common being used is posterior spinal fixation with different instruments.

The most modern and commonly used fixation is the pedicle screw and rod system. Other methods include anterior spinal fixation with cage, kyphoplasty, vertebroplasty, posterior interbody fusion and posterior intertransverse fusion. Of the posterior spinal fixation, the various instruments used are pedicle screws and rods system.

The neurological recovery is dependent on the degree of stability provided by fixation, and on initial instability and neurological loss. So, if stability is provided to spine then neurological status of some patients can improve.

Regarding the gender 73% of our study group was female and 27% was male with a male/female ratio of 1:2.7, while in Muralidhar B.M. et al Study [12] there was male predominance of 80% and female of 20%, Shailendra et al study [13] the male percentage was 76% and female was 24%. This difference is due to that 65% of our patient complaining of instability due to non-traumatic causes of which degenerative spondylolisthesis more commonly occurs in elderly females because of the postmenopausal osteoporosis.

About age group, in our study 36% of our patient are located in 50-59 age group which is the commonest age group while in Muralidhar B.M.et al study most of the patient are in 50 -59 age group, in Shailendra et al study the commonest age group is 16-25 years followed by 26-35 years, this difference because the largest group of our patient had degenerative diseases other than trauma which prevalence increased with age.[12, 13]

Concerning etiology, 35% are due to traumatic injury (17 patient RTA, 18 patient FFH), 65% are due to non-traumatic causes (58 patient spondylolisthesis, 7 patient pathological fracture {pathological fracture 5 patient infection, 2 patient tumor}) , while in study done by Alp Ozgun BOrcek et al [15] the non-traumatic cases was 63.9% while the traumatic cases was 36% which is close to our result.

The relation between etiology and neurological deficit in traumatic cases 66% had complete neurological deficit and 34% incomplete deficit while in non-traumatic cases 3% had complete deficit and 97% had incomplete deficit, this difference is due to two factors first is that traumatic injury which cause vertebral fracture and instability means sever trauma while in degenerative cases it is usually a chronic process and patient seek medical management before complete deficit occur, the other factor is that trauma mostly cause injury to thoracolumbar junction because the transition between the more rigid thoracic spine and the mobile lumbar spine concentrates bending and axial loads at the thoracolumbar junction where there is spinal cord in the spinal canal while in degenerative cases are mostly in L4 level where there is cauda equina and no cord.

Regarding the level of pathology: in 37% of cases, the pathology involving L4 followed by 15% for L3 and L5, in Muraldhiar B.M. et al study 70 % at L1 level and 20|% at L2 level in our study the higher percentage of L4 are due to that most of our cases are degenerative which is more common in the lower lumbar region leading to L4 and L5 levels to be the most commonly instrumented level. [12]

Regarding type of pedicle screw fixation, the short segment was used 67 times, while the long segment fixation was used 6 times only because it is used in lesions involving the thoracolumbar junction vertebrae, while in a study done by Kashif Mahmood Khan et al[16] on 50 patient with thoracolumbar fixation, four screws with 2 rods were used in 38 patients (76%) and 8 screws were used in 12 patients (24%).

As regards the intraoperative guidance, 23% of operations done by free hand procedure without intraoperative fluoroscope, the accuracy was 91.3%, while in Parker et al study[17] on free hand fixation for degenerative cases (964 patients) the accuracy was 98.3%. In Gertzbein et al study[21] for traumatic patient (171 patients) the accuracy was 71.9%. The accuracy of free hand screw insertion is related to the experience of the surgeon, and possibly to change in anatomical landmark in traumatic cases.

The intraoperative complications, screw misplacement in 4 patients 4%, while in Faraj et al study [38] 3 patients 3.2% had screw misplacement, dural tear occurred in 3 patients 3% in our study, while is study done by Faraj et al 4 patients 4.4% had unintended dural tears.

The postoperative complications in our study included infection in 7 patients (7%) all of them had removal of the system, the patients with secondary neoplasia were receiving concurrent chemo- and radiotherapy for their malignant diseases which rendered their immunity low. In Sanford et al study[19] the infection rate was 3.2%. The CSF leak occurred in 3 patients 3% in our study, while in Faraj et al study[38] it was 2.1%. Screw fracture occurred in 4 screws in 3 patients out of 478 screws used in our study (1.2%), while 9 screw fractures out of 296 in Ohlin et al study.[20] Exposure of the system occurred in 1 patient only as a complication of being bed ridden in a complete neurological deficit, this patient died of septicemia and pneumonia.

Regarding six-month follow-up and outcome: 42 patients were followed (we couldn't follow all the patients because of their residency in far provinces), 39 patients showed improvement in their neurological status (1 patient with complete neurological deficit show improvement within 3 weeks), 2 patient show worsening of symptoms (increased pain) and 1 patient died as mentioned above . While in a study done by Roop Singh et al[21] 41% of patients improved and 27% did not improve (all of them are paraplegic) with the difference possibly related to severity of injury and timing of surgery.

Patients who experience worsening of symptoms feel so due to misplacement of the screw with pressure on the nerve root or due to pathology in other levels.

Conclusion:

Posterior spinal fixation with pedicle screws and rods system is an effective and safe method in maintaining the stability of spine, by improvement in all the scores and parameters including the pain and mobility, range of motion of spine, and deformity.

1. The intraoperative imaging in coronal and sagittal planes is important in maintaining safe trajectory of screws, although free hand screw insertion is applicable with more experienced hands after thorough knowledge of anatomical landmarks.
2. Short segment fixation using the posterior approach with pedicle screw-rod fixation devices with or without bone grafting achieves good stabilization and fair enough neurological recovery in patients with unstable thoracolumbar fractures
3. By applying the transpedicular screw fixation on the unstable fractures of the thoracolumbar spine, a stable fracture fixation can be achieved. So early ambulation, rapid return to work and less rehabilitation costs could be achieved. This kind of fixation prevents secondary spine deformities.
4. The ideal candidates for undergoing posterior spinal fixation are patients with unstable fractures & incomplete neurological deficit.
5. In patients with complete neurological deficit, spinal fixation done to aid to achieve stabilization and easy nursing care .
6. Non-Traumatic cause in 40-49 years old females represent the most common etiology.

Recommendation:

- Use of intraoperative neuro-monitoring in spinal fixation surgery.
- Use of a navigation system to guide to the intraoperative manipulation.
- Use of fluoroscopy and the O-arm as an intraoperative guiding tool.
- Bone fusion is recommended for each patient with degenerative changes to improve stability of the spine and to prevent fixation failure.
- Use of short segment is better than long segment to decrease blood loss, timing of surgery, risk of vascular, neural and visceral injury, lost of motion, and cost.

Intraoperative & postoperative Imaging:

Fig. 3 Screw fracture indicated by the red arrows

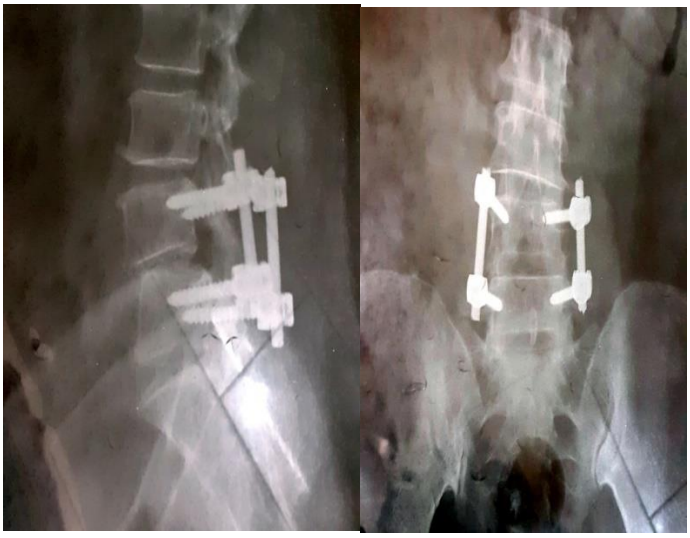

Fig. 4 Free Hand Fixation lateral and AP views showing the 4 screws does not span 80% of the vertebral body AP diameter

Fig. 5 Intraoperative C-arm AP view showing the upper right screw directed too medially

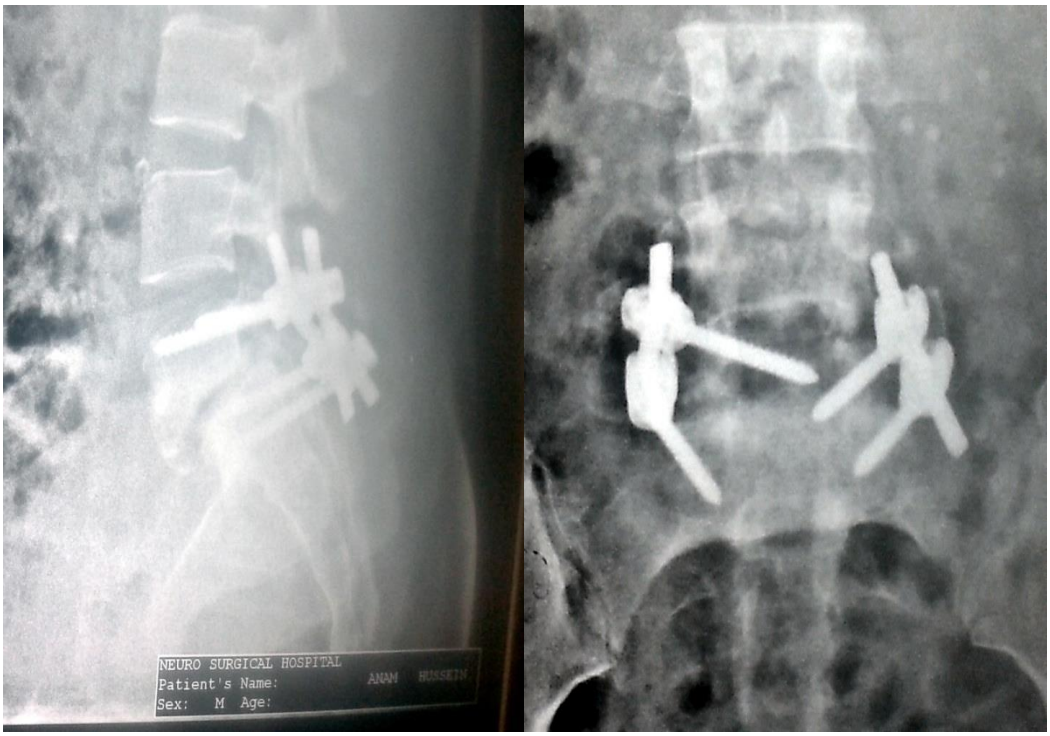

Fig. 6 Postoperative lateral and AP views of lumbosacral vertebrae showing 4 screw system fixation the upper left screw in the AP view is directed too medially.

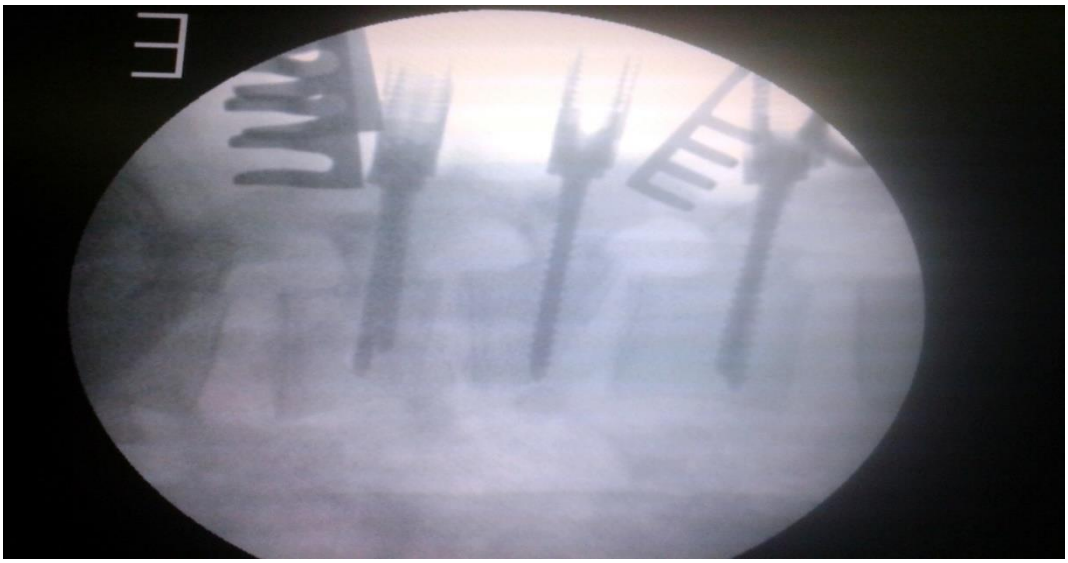

Fig. 7 Intraoperative C-arm lat.view

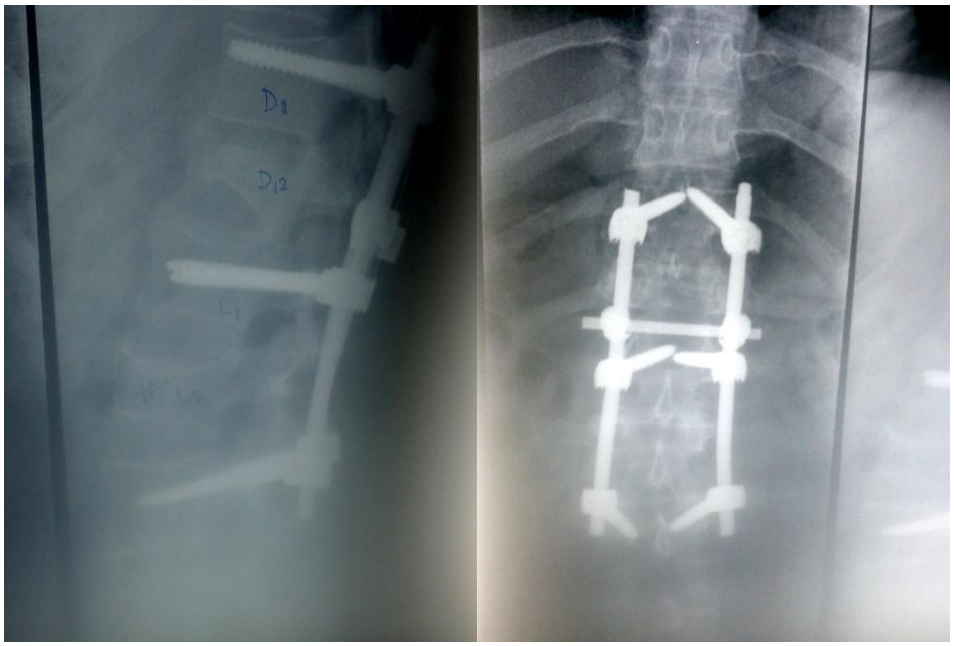

Fig. 8 Postoperative dorsolumbar lateral X-ray to the left and dorsal X-ray AP view showing 6 screw fixation system

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TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Issue 2, (2016), 81-92 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

REAL TIME -PCR FOR QUANTITATIVE DETECTION OF *TOXOPLASMA GONDII* IN ABORTED WOMEN BEFORE AND AFTER TREATMENT

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Abstract:

Background: Human *Toxoplasmosis* is a parasitic disease caused by a *Toxoplasma gondii*. Toxoplasma gondii was the subject of disease burden estimations as a result of the severe clinical symptoms and lifelong implications. Primary infections with T.gondii acquired during pregnancy are usually asymptomatic for the pregnant woman but can lead to serious neonatal complications, isolation of parasite despite it is considered as a golden standard parameter to detect infection,

the repetitive B1 gen is the most target gene used in conventional PCR for molecular detection of *T.gondii.*

Objective: Detection of *T. gondii* DNA using Real- time PCR seems to be a reliable method in diagnosis and follow up of patients.

Methods: Thirty patients (aborted women), their range age between (20− 46) years, were included in this study. All patients were assessment of Real time Polymerase chain reaction from the same patients was taken before and after spiramycin therapy to confirm the infection of the *T.gondii* and to evaluate the parasite load (copy number).

Result: Data of this study revealed that there is significant difference(P=0.002) in the mean of the copy numbers in aborted women's, the mean of parasite copy number in patients samples before treatment was (4.24E+06) and the mean of copy number after treatment was (7.53E+05).

Conclusion: The diagnosis of toxoplasmosis is mainly based on serological tests detection of *T. gondii* DNA using Real time- PCR seems to be a reliable method in diagnosis and follow up of patients.

Keywords: *Toxoplasma gondii*, Real- time PCR, B1-gen

Introduction:

Toxoplasmosis is a worldwide infection that may cause severe disease and is regarded as a serious health problem in world. It is one of the well-studied parasites because of its medical and veterinary importance, and its suitability as a model for cell biology and molecular studies with a unicellular organism [1]. Toxoplasmosis is also a major opportunistic infection in immunocompromised individuals, often resulting in lethal toxoplasmic encephalitis .If a woman was infected for the first time during pregnancy the parasite may be transmitted transplacentally to the fetus, this can result in death of the fetus, central nervous system abnormalities, or eye disease and affecting the quality of life of the child throughout its life time [2].

Toxoplasma gondii was the subject of disease burden estimations. As a result of the severe clinical symptoms and lifelong implications, the disease burden of *T.gondii* is high .Primary infections with *T.gondii* acquired during pregnancy are usually asymptomatic for the pregnant woman but can lead to serious neonatal complications [3].

Toxoplasma infection stimulates both humeral immune response characterized by antibody production of (IgM and IgG) and cell mediated immunity (CMI) which are essential

for the host control of intracellular infections , so the protection against Toxoplasmosis is mediated by cellular defense [4].

The seroprevalence varies from 5% to 90% depending on geographical location, age, habit of eating raw meat or unwashed fruit and vegetables, and general level of hygiene, the incidence of infections is higher in warmer and humid climate and increases with age [5].

Serological tests including the detection (and quantification) of *T.gondii* antibodies in serum are used to establish whether a pregnant woman has been infected and, if so, to determine whether the infection was acquired recently or in the distant past. If serological test results suggest a recently acquired infection, an effort is made to determine whether the infection was likely acquired during gestation or shortly before conception. If so, the fetus is at risk [3].

In Iraq, many studies were accomplished concerning the sero prevalence of Toxoplasmosis by using different techniques including indirect haemagglutination test (IHA), Indirect Fluorescent Antibody Technique (IFAT), and Enzyme-linked Immunosorbent Assay (ELISA) [6]. Real time- PCR can be used as an additional diagnostic tool for the rapid detection of *T.gondii* in various clinical materials [7].

The use of the macrolide antibiotic *Spiramycin* has been reported to decrease the frequency of vertical transmission [8] .However, carefully designed; prospective studies that demonstrate this effect have not been recorded. The protection has been reported to be more distinct in women infected during their first trimester [9].

Material and methods:

Thirty women with spontaneous miscarriage were included in the study all have had curettage operation at the Obstetrics and Gynecology Department of Al-Kadhimyia Teaching in Baghdad and private clinic during the period from November 2012 to April 2013. Their age ranged from (20- 45) years old. Ten healthy pregnant women with a history of a normal pregnancy attending the outpatient clinics for routine gynecologic checking enrolled in this study as control group, their age were ranging between (21-39) years old. From each patient two blood samples were collected pre and post treatment with oral spiramycin , 4 capsules (at 500mg) two times a day , three ml. were placed in EDTA tubes for DNA extraction, and all samples were stored at -20 °C DNA was extracted from the whole blood samples using a commercial purification system (ExiPrepTM DNA/ RNA Prep Kit).

The DNA concentration was determined by using the spectrophotometer (Bioneer), 10 µL of each DNA sample were added to 990 µL of D.W. and mixed well. The spectrophotometer was used to measure the optical density (O.D) at wave length of 260 nm and 280 nm. An O.D of one corresponds to approximately 50 µg / ml for double stranded DNA. The concentration of DNA was calculated according to the following formula: DNA concentration (µg/ml) = O.D 260 nm x 50 x Dilution factor

The spectrophotometer was used also to estimate the DNA purity ratio according to this following formula: DNA purity ratio = O.D 260 nm / O.D 280 nm.

Amplification of B1-gene by Real-Time Polymerase chain reaction

Principle

TaqMan probes are hydrolysis probes that are designed to increase the specificity of quantitative PCR. The principle of this technique relies on the 5´–3´ exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophores-based detection [10].

Toxoplasma gondii Real-TM PCR kit uses "hot-start", (Anatolia geneworks- Turkey) which greatly reduces the frequency of nonspecifically primed reactions. The forward and reverse sequences were (GCATTCCCGTCCAAACT and AGACTGTACGGAATGGAGACGAA) respectively [11].

The control positive DNA copy number was determined as follows: copy number (copies /ml) =(C x Avogadro constant) / (660 x control positive DNA in bp 10^9) Where C (copy) is given in ng/ml, Avogadro constant (N_A) = 6.022 140 857(74) $\times 10^{23}$ /mole.

Preparing the PCR:

Real-time PCR assays were carried out in duplicate and performed following manufactured protocol in a total volume of (41µl) for one reactions as follow PCR Mix (20µl), (1.6µl) from detection Mix 1 which contains forward and reverse primers and dual-labeled probe, (1.2µl) from detection mix 2 which contains internal control-specific forward and reverse primers and dual-labeled probe, Internal control (0.2µl).Twenty three microliter of this master mix was added in the PCR tube with 18µl of DNA (sample, positive or negative control). Tubes were closed well and spin, The PCRs were performed with the Agilent Real-time PCR (Techne-UK). After initial activation of hotStar Taq DNA polymerase at 94°C for 2 min, 40 PCR cycles of 93°C for 10 s , 60°C for 10 s and 72 for 30s were performed.

Calculation of results:

Data were analyzed by smart-cycler software using arithmetic baseline adjustment, *T.gondii* DNA copy number was estimated from the cross point threshold relative to positive standard. The standard curve correlates each copy number with a particular Ct, the copy number value of the unknown samples are driven from the standard curve (According to this study).

Statistical Analysis:

Experimental data were presented in terms of observed numbers and percentage frequencies, and then analyzed by using Chi- square ($\chi 2$) test. P value ≤ 0.05 was considered statistically significant

Results:

The detection of B1 gene of *T.gondii* in whole blood specimens from aborted women's and control group by Real-time PCR:

In this study, the absolute quantification method was used for data analysis to determine the actual copy number before and after treatment, the standard curve was generated, in which concentration is fixed to a specific Ct value as the Ct value is inversely related to the amount of starting template.

Standard curve generation:

A standard curve was generated using a 10-fold dilution of a template amplified on the Cycler - real-time system (Figure 1), the copy number and CT value correlation was 0.9987.

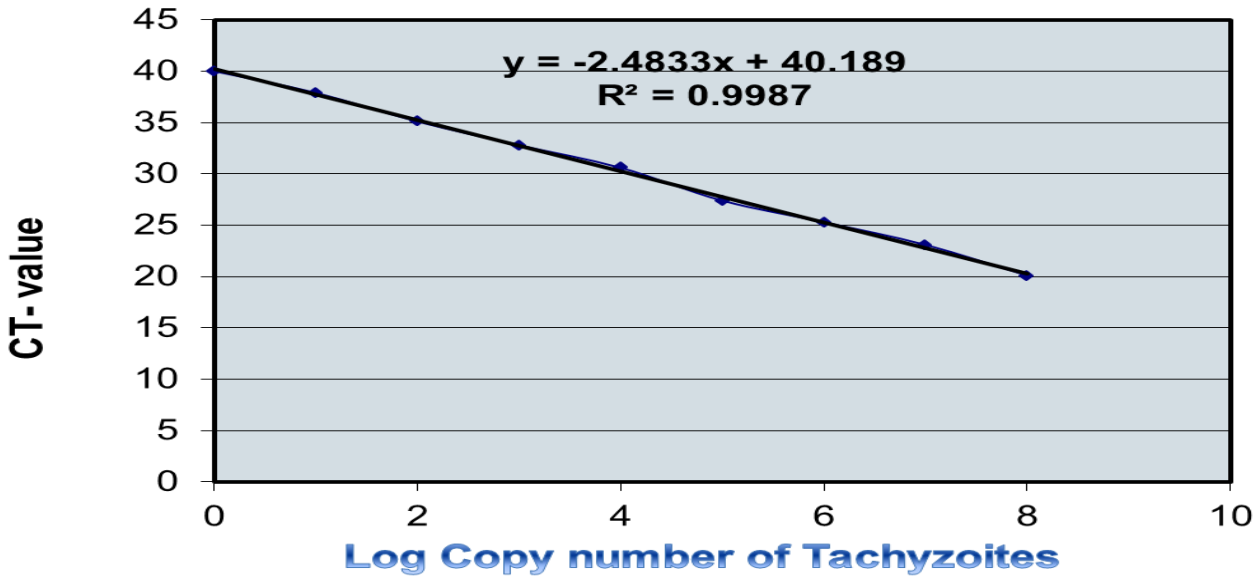

Figure (1): Standard curve for quantitation of *T.gondii* tachyzoites. (Serial dilution ranging from (100– 108) copies used as template for real time PCR analysis)

Parasite load in abortive women's as determined by Real- time PCR:

The range of *T.gondii* load in abortive women's under study before and after treatment with oral spiramycin , 4 capsules (at 500mg) two times a day in table (1) which is found to be ($0.0192X10^{8}$ – $0.110X10^{4}$) before treatment and ($0.156X10^{7}$- $0.036X10^{0}$) after treatment,(P value =0.002). Data of this study revealed that there is significant difference(P=0.002) in the mean of the copy numbers in aborted women's, results in our study revealed that the mean of parasite copy number in patients samples before treatment was (4.24E+06) and the mean of copy number after treatment was 7.53E+05) figure(2)

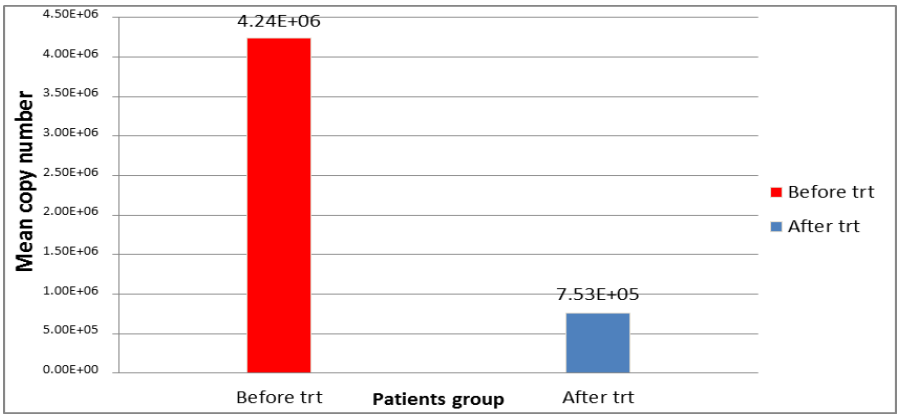

Figure (2): Mean of copy number parasite /ml of patients' blood samples before and after treatment

Table (1): *Toxoplasma gondii* copy numbers screening in abortive women's as determined by Real- time PCR

Sample Name	CT-pre treatment	Copy no. pre treatment	CT-post treatment	Copy no. post Treatment
P 1	20.8	0.192 X 10^7	25.81	0.187X10^5
P2	27.1	0.0624X10^5	38.61	0.151X10^0
P3	20.1	0.0192X10^8	23.65	0.156X10^6
P4	21.4	0.129X10^7	24.11	0.110X10^6
P5	25.6	0.208X10^5	21.17	0.156X10^7
P6	23.7	0.151X10^6	37.12	0.057X10^1
P7	21.5	0.122X10^7	23.18	0.201X10^6
P8	19.4	0.086X10^5	22.19	0.057X10^7
P9	26.4	0.129X10^5	24.16	0.108X10^6
P10	22.7	0.007X10^7	35.55	0.206X10^1
P11	20.9	0.182X10^7	26.9	0.084X10^5
P12	20.5	0.220X10^7	37.12	0.055X10^1
P13	21.9	0.084X10^7	32.7	0.002X10^3
P14	26.4	0.129X10^5	22.41	0.036X10^7
P15	29.1	0.110X10^4	37.12	0.057X10^1
P16	22.4	0.036X10^7	33.1	0.204X10^2
P17	21.8	0.093X10^7	29.1	0.110X10^4
P18	22.5	0.028X10^7	35.1	0.009X10^2
P19	23.7	0.086X10^6	38.6	0.153X10^0
P20	22.4	0.038X10^7	32.1	0.062X10^3
P21	22.8	0.237X10^6	39.6	0.055X10^0
P22	28.6	0.158X10^4	30.1	0.014X10^4
P23	26.9	0.084X10^5	32.9	0.223X10^2
P24	25.1	0.014X10^6	39.8	0.036X10^0
P25	24.1	0.112X10^6	37.9	0.220X10^0
P26	23.5	0.170X10^6	31.8	0.088X10^3
P27	21.6	0.112X10^7	39.1	0.105X10^0
P28	26.4	0.132X10^5	31.8	0.088X10^3
P29	23.5	0.170X10^6	36.7	0.096X10^1
P30	27.9	0.225X10^4	33.7	0.146X10^2
Negative control	NTC		NTC	

Regarding response to treatments toxoplasmosis infected women in this study were divided to the following groups:

Group I include patients had high parasite load ($0.062X10^{5}$-$0.112X10^{7}$**)** which decrease completely after completion the course of treatment ($0.036X10^{0}$-$0.223X10^{0}$).

Group II include a patients with high parasite load ($0.225x10^{4}$ -$0.036x10^{7}$) at time of diagnosis and decreases after completion the course of treatment ($0.055x10^{1}$ -$0.223x10^{1}$).

Group III includes patients with high parasite load at diagnosis ($0.225x10^{4}$ -$0.036x10^{7}$**)** which still with high parasite load or slightly lower ($0.002x10^{3}$ – $0.201\ x10^{6}$**)**.

Group IV includes patients with high parasite load ($0.158x10^{4}$ – $0.129x10^{5}$**)** at time of diagnosis which showed elevated parasite load after treatment ($0.014x10^{4}$ -$0.156x10^{7}$**)**.

Discussion:

Firstly DNA was successfully extracted from whole blood samples for all subjects, Menotti, et al [12] showed that the parasites are as likely to be found in the plasma or red cell fractions, which might indicate that the most suitable sample is a whole blood extract. However, in patient's blood *T.gondii* present might be intracellular in leukocytes [13]; in that case the white cell fraction would be as appropriate as whole blood.

Parasitemia in pregnant women may be due to the toxoplasmosis reactivations occur during immunosuppressive states in pregnancy. Tachyzoites or *T.gondii* DNA release from the cyst in the body tissues may be the source of parasitemia or *T.gondii* DNA in blood [14].

Quantitative detection of pathogen by real time PCR has been successfully applied to a variety of specimens and the finding of *T.gondii* DNA in peripheral blood by PCR might help in the early diagnosis of invasive diseases [15].

The qRT-PCR that have been applied in this study to diagnose and for the determination of copy number of *T.gondii* DNA in whole blood, it gives reproducible diagnostic and quantitative results over conventional PCR with the risk of false positive due to contamination with previously amplified products since in qRT-PCR there is no risk of hand manipulation and the use of internal control will exclude the false negative which due to inhibiters in amplified mixture, reproducibility of qRT-PCR quantification was tested by the testing of the 10 fold dilution.

In the present study a qRT-PCR based B1 gene – specific Taq man assay have been developed for quantitative detection of *T.gondii* which extremely sensitive to (0.168 parasite / reaction) and its ability to quantify infection load of clinical specimen and its long linear range of 7 logs of DNA concentration with CT value as shown in figure (1) where R2=0.998 which is indicative of a significant correlation between logarithmic value of each PCR product of copy number amplified and CT value. These results were in agreement with Mei-huilin, [(16)]. In this assay an increase of the fluorescent signal above the preset threshold within 40 PCR cycle was considered positive (CT< 40), Table (1) where CT for negative control was 40. Quantification of

parasite load in pregnant women under study has been used to ass's disease severity and the treatment outcome.

The relative quantity of tachyzoites in each DNA sample was determined using the standard curve presented in Figure (2) which showed a range between (0.110X10^^4 to 0.0192 $\times 10^{8}$) before treatment and (0.036×10**0** to 0.156×10^{7}) after treatment .In addition figure (2) revealed that there is significance difference (P<0.05) of in the mean of the copy number before and after treatment.

According to their response to treatments, Group 1 patients which represent, **5** (16.66%) % of patients having high parasite load at time of diagnosis with a range of (0.062X10^{5}-0.112X10^{7}) copies /ml , they show complete response to treatment with decline in parasite load to undetectable value , these results were in accordance with Costa , et al [17] who recorded that the parasite count decreased sharply in patients having cerebral toxoplasmosis symptoms , when treated with anti-toxoplasma therapy which was efficient and reducing the parasite load and they conclude that Light Cycler PCR provide precise evaluation of parasite load in immunocompromised patients and PCR is useful in monitoring of treatments .

In addition, Kupferschmidt, [18] conclude that decreasing in parasite load in patient's blood sample demonstrated that treatment used was effective. Contini **,** et al [19] recorded that patient under specific treatment did not show detectable B1 DNA levels in samples collected after many days of therapy . So that *T.gondii* DNA load is a valuable tool for monitoring of toxoplasmosis. Group Π patients which represent 10(33.33 %) of patients having high parasite load at time of diagnosis with a range of (0.225x10^{4}-0.036x10^{7}**)** copies /ml, they show lower parasite load with a range of (0.055x10^{1} -0.223x10^{1}**)** copies /ml after treatment. These results observed by Menotti, et al [12].

They consider that prolonged persistent detection of *T. gondii* DNA in blood justifies the maintenance of anti *T.gondii* treatment and close follow up by PCR. These results were in agreement with Romand, et al [20] who used quantitative Real time PCR to evaluate *T. gondii* concentration in amniotic fluid and conclude that quantitative PCR may help the clinician to form prognosis of congenital infections with regard to fetal and neonatal outcome. Other study by Christianne, et al [21] reported that high parasite load in amniotic fluid was found in cases of severe congenital toxoplasmosis as determined by quantitative PCR.

Group III which represent 11(36.66 %) include patients with high parasite load at time of diagnosis with a range of (0.038x10^{7} –0.019x10^{8}) copies/ml, they did not show any response to treatment, parasite load was remained with undetectable decrease. After treatment it's found to be (0.002x10^{3} – 0.201 x10^{6}) copies/ ml. These results were in agreement with Kupferschmidt, et al [(18)] .who used Tag man PCR to monitor parasite load in the blood of an immunocompromised patients after allogenenetic bone marrow transplantation. They found that at day 199 after therapy with pyrimethamine the detected parasite load remained unchanged and then decreased after prolonged therapy.

Regarding group IV of patients which represent 4(13.33 %) having high parasite load at time of diagnosis with a range of ($0.158x10^4$ – $0.129x10^5$) copies /ml , they showed elevated parasite load after treatment with a range of ($0.014x10^4$ -$0.156x10^7$) copies /ml .This increase of parasite load seems to be correlated to progression of disease or to poor response to treatment. These results were in agreement with Menotti, et al [12] who recorded evolution in parasite load in patients under treatments and those with slow decrease in parasite load after initiation of treatments with pyrimethamine and sulfadiazine on day 43 post transplantation. They give explanation to the prolonged presence of *T.gondii* DNA that could be due to disseminated form of disease with a peak of parasitemia (29000 tachyzoites/ ml) of blood buffy coat.

Real time-PCR can be used as an additional diagnostic tool for the rapid detection of *T.gondii* in various clinical materials. Studies have documented that Real time- PCR can actually detect *T.gondii* in blood specimens of women before or during pregnancy. Nimri, et al [22] and Khan et al [23] showed that a small numbers of parasites may be detected in circulating blood from some patients with acute or recurrent toxoplasmosis.

Several studies have reported that, PCR could detect parasitemia a few weeks prior to the appearance of any clinical signs or symptoms .PCR is highly sensitive and specific because a single tachyozoite can be detected in a clinical sample [24].

The most important records in this study is that the parasite load could be determined and correlated with treatment. The parasite quantification in some treated subjects become lower and in others, *T.gondii* load disappeared when spiramycin was given, this exhibit that the anti-toxoplasma therapy was efficient in reducing the parasite load.

The quantitative real-time PCR assay developed in this study can be used not only to detect the presence of *T.gondii* DNA but also to provide precise evaluations of the parasite load in pregnant women's. This PCR test should be useful for the monitoring of treatment efficacy and should help provide an understanding of the pathogenesis of *T.gondii* reactivation.

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TOFIQ Journal of Medical Sciences

TJMS

Volume 3 Issue 2, 2016 ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

TJMS

Supplement (1), Vol.3, 2016 ISSN 2377-2808

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

TOFIQ Journal of Medical Sciences (TJMS)

Supplement, 1,Vol 3 (2016)

Issued by:
TOFIQ Office,
2405 Carey lane,
Vienna, VA 22181
USA

TJMS.tofiq.org

Indexing

TOFIQ
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TOFIQ Journal of Medical Sciences ISSN: 2377-2808

TOFIQ Journal of Medical Sciences
ISSN: 2377-2808

July 27, 2016

Letter from the Editor-in Chief

Supplements to TJMS

To our distinguished readers,

It is our pleasure to tell you that this is the third year of publication of Tofiq Journal of Medical Sciences, TJMS. Our contributors are mainly the blossoming Iraqi medical professionals and scientists. Also we have a rich share by leading Iraqi scholars from the U.S., U.K., New Zealand and Jordan with contribution from our American colleagues.

As part of our objectives and aims in helping Iraqi higher education, the Editorial Board have decided to publish the abstracts of the dissertations and theses of Iraqi postgraduate scholarship students at the United States Universities in supplements to help researchers all over the world to learn about the good work and achievements of our students.

Our appreciation goes to Prof. Tahani Al Sandook, the Iraqi Cultural Attaché in Washington D.C. and her team for their kind support.

We thank our beloved students for their hard and successful work and for being a great potential to promote science and technology in Iraq.

It is our aim and hope that this project will continue and expand to promote higher education in Iraq.

Please accept my best wishes,

Prof. A Hadi Al Khalili,
Editor-in Chief, TJMS

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

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TOFIQ Journal of Medical Sciences (TJMS) is published by TOFIQ: an NGO registered at the State of Maryland as a non-profit organization dedicated to helping Iraq Higher Education and Research.

TJMS is devoted to the publication of original research, commentaries on a current topic, reviews, letters to the editor, and editorials in the field of medical sciences. The early focus of the journal is on clinical burden of disease in Iraq: documentation of its nature and extent; clinical patterns and epidemiology; diagnostic findings; and therapeutic strategies. Read more ...

Focus and Scope

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Mission:

1. TJMS is a high quality, biannually, peer-reviewed, electronic medical sciences journal, publishing original research and scholarly review articles, letters to the editor, and editorials.
2. TJMS focuses on the disease burden in Iraq.
3. An outlet for the current research and an academic product in Iraq in the fields of medicine, dentistry, pharmacy, nursing and related disciplines that supports recognition and academic advancement in Iraq and beyond.
4. TJMS will lay the groundwork for creation of sister journals in other disciplines relating to Iraq (engineering, agriculture, science and technology, social sciences and humanities).

Structure:

1. Single editor-in-chief, working in a full-time, compensated capacity.
2. Editorial board consisting of experts in the various branches of medicine, dentistry, pharmacy, nursing and related disciplines.
3. Open-access, electronic-only journal
4. Peer-reviewed publication supported by an online submission, review, and decision for articles.
5. Articles can be submitted by any individual/group, or can be solicited (invited reviews and discussions). Decisions for publication will be blinded to author or region of origin. The criteria on which the submissions are evaluated for acceptance will be heavily weighted on their applicability to the burden of disease in Iraq.
6. Publication will be biannual however, approved articles will be released continuously.

7. Funding: will attempt to obtain corporate sponsorship through unrestricted educational grants. Sponsorship will be acknowledged in compliance with ACCME and ICMJE guidelines.
8. The publication would be the official medical sciences journal of TOFIQ, and we would encourage other medical organizations to consider collaborating with.

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TOFIQ Journal of Medical Sciences ISSN 2377-2808

Supplement, No 1, (2016)

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TOFIQ Journal of Medical Sciences

OVARIAN RESERVE OF INFERTILE WOMEN HAVING POLYCYSTICOVARIAN SYNDROME: CHRONOLOGICAL BASED FACTOR

Ahmed A. Hussein

.A Thesis submitted to the Department of Earth, Ocean and Atmospheric Sciences in partial fulfillment of the requirements for the degree of Master of Science

Degree Awarded: Fall Semester, 2014

ABSTRACT:

Clouds play an important role in the earth's energy budget, and changes in their properties can remarkably impact the amount of warming in response to greenhouse gas increases. In this study, we applied the Coupled Feedback Response Analysis Method (CFRAM) to estimate the contributions of cloud property changes to the magnitude of the annual mean-surface temperature response in a transient simulation where CO2 increases at rate 1% , using the NCAR Community Climate System Model, version 4 (CCSM4). To examine closely the contributions of changes in cloud properties to the annual mean-surface temperature, the full-cloud level is divided into three levels in terms of the cloud-top pressure (CTP). This study found that the annual and global mean-surface temperature response is a warming of (+0.175 °K) due to the net cloud feedback that comes mainly from the positive SW cloud feedback. The medium (400 < CTP < 700 mb) clouds changes are the dominant contributors (+0.175 °K) to the surface warming due to their magnificent positive SW cloud feedback (+0.33 °K). High (CTP < 400 mb) clouds changes cause a weak negative contribution (-0.0218 °K) to the surface warming because of the close cancellation between their large negative SW and large positive LW high-cloud feedbacks. Low (CTP > 700 mb) cloud changes are the least contributors to SW and LW cloud feedbacks (positive SW and negative LW); however, they still contribute positively (+0.0217 °K) to the net cloud feedback with an absolute magnitude that is almost equal to the contribution of the high-cloud changes. Furthermore, this study found that the annual mean-surface

temperature increases in the Polar Regions (60°-90° in both hemispheres) are due to the positive LW cloud feedback from the changes in the three cloud levels, mostly due to positive LW medium-cloud feedback. However, the annual mean surface warming for the region covered between (60° S-60° N) is due to the positive SW low- and medium-cloud feedbacks.

TOFIQ Journal of Medical Sciences

FABRICATION AND CHARACTERIZATION SILICON NANOWIRES BASED HETEROSTRUCTURE SOLAR CELL DEVICES

Alaa Abdul-Halim AL-Hilo

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of
DOCTOR OF PHILOSOPHY in Applied Science Emphasis in Applied Physics "Nanotechnology-in the Department of Applied Science
and the College of Art,Letters, and Sciences December 2014

ABSTRACT:

Silicon nanostructures are good semiconductor materials candidates for replacing bulk silicon because the nanostructure may act as antireflective layer on Si wafer when used in a solar cell device. Silicon nanostructures, whether nanowires or nanorods, has a direct path for charge transport to enhance the efficiency of solar cell devices depends on the nanostructure size and quality. It also has some properties different from that of bulk silicon. For example, the thermal conductivity of silicon nanostructures is lower than that of the bulk silicon because the diameter of the silicon nanowires is smaller in size than the bulk sample. In addition, the Si nanowires provide a large surface area and the band gap of silicon nanowires is tunable through changing the diameter of nanowires. Furthermore, the reflection of incident light on silicon nanowires is lower than that of bulk silicon and thus increases its absorption of the light, which improves the performance of the solar cell device. In this dissertation, we used chemical etching method to grow silicon nanowires on a planar silicon substrate. This simple technique provides a rapid and facile way to grow large-area of nanowires. However, low temperature is needed for growing vertically aligned silicon nanowires. In this method the length of nanowires can be controlled by controlling the growth time, on the other hand the diameter of nanowires can't be control through controlling the growth time. By using this technique we have succeeded in growing n-type and p-type nanowires on planer silicon substrates to build our solar cells devices in low cost.

TOFIQ Journal of Medical Sciences

FUNCTIONAL ANALYSIS OF GENES INVOLVED IN RESPONSE TO SHEATH BLIGHT DISEASE USING VIRUS-INDUCED GENE SILENCING SILENICNG

Elaf Osamah Mohammed Ali

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of
DOCTOR OF PHILOSOPHY in Applied Science: Biosciences
in the Department of Biology of the College of Art, Letters, and Sciences
December 2014

ABSTRACT:

Phytotoxins (RS toxin) play a crucial role in the virulence of the necrotrophic phytopathogenic Rhizoctonia solani. This pathogen is highly invasive and has an extremely wide host range that causes economic loss; moreover, this pathogen is both soil and water-borne and can produce lesion symptoms that rapidly develop into a disease known as sheath blight disease. Susceptibility to this disease and sensitivity to phytotoxins depend on the plant's ability to defend itself against the pathogen attack and induce an active defense mechanism. There are several gene candidates that have been suggested as possible host-plant sensitivity genes associated with R. solani infection. In this study, we identified genes that involved in the induction of necrosis in plants affected by RS toxin from R. solani. Based on published study of Costanzo et al. (2011), we selected two candidates gene in rice, Cytokinin-O- -glucosyltransferase familyl and Cytokinin-O-glucosyltransferase-2 that are possibly involved in RS toxin recognition and sensitivity of rice cultivars to sheath blight disease. A reverse genetic approach was taken for clarification functions of both genes. Particularly, we used Virus-Induced Gene Silencing (VIGS) for gene functional analysis. V IGS is a mechanism based on the anti-viral plant RNA interference phenomenon initiated by the presence of double-stranded RNAs. In this work, we applied VIGS to regulate/disrupt the in planta response to biotic stress by selectively silencing genes-targets that could be involved in sensitivity to a crude phytotoxin from the culture filtrate of R. solani. VIGS is a very complicated method for functional characterization of rice genes. However, VISG is very well developed

for Solanaceae plants including tomato and tobacco. We have searched and identified the tomato functional homologues for rice Cytokinin-O-glucosyltransferase family l and Cytokinin-O-glucosyltransferas-2 genes. Using VIGS and tomato as a model system we worked on to elucidation functions of both tomato functional homologues (UDP-glucosyltarnsferase 1 and UDP-glucosyltransferase 2 genes) that are possibly involved in toxin recognition and necrosis-inducing response. We have optimized conditions of VIGS methodology for the selected phytotoxin-sensitive tomato cultivars and determined suitable concentrations of R. solani culture filtrate for implementing the screening procedures. Infiltration tests have identified tomato cultivars (cv. Money Maker; cv. MoteIla) that are sensitive to the R. solani phytotoxin and exhibit sheath blight symptoms that are similar to those observed in infected rice plants. As the next step, vector constructions for silencing were established using the Gateway cloning system. To examine the effectiveness of the silencing constructed vectors on susceptible cultivars, vectors were delivered to the RS toxin-sensitive tomato cultivars using Agro-inoculation. The information obtained from measuring the expression level for the silenced and non-silenced plant leaves showed a decrease in the gene expression level in plants infiltrated with silencing construct vectors carrying both target tomato genes (UDP-glucosyltarnsferase 1 and UDP-glucosyltransferase 2). Decrease of expression of both genes were in a positive correlation with a significant reduction of sheath blight disease symptoms caused by application of RS toxin to the tomato leaves. Our study contributed in identification of genes involved in plant response to sheath blight disease.

Established data will open perspectives to establishment rice cultivars resistant to sheath blight disease using traditional (breeding) or genetic (transformation) approach.

TOFIQ Journal of Medical Sciences

CHARACTERIZATION OF *SALMONELLA* ENTERICA ISOLATES FROM FOOD AND CLINICAL SAMPLES: FUNCTIONALITY OF CYTOLETHAL DISTENDING TOXIN (CDT) FROM NON-TYPHI SALMONELLA ENTERICA

Ezat Hussain Mezal

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of DOCTOR OF PHILOSOPHY in Applied Science in the Department of Applied Science of the College of Science May 2014

ABSTRACT:

Salmonella enterica is recognized worldwide as a significant cause of both human and animal disease. Recently, many random outbreaks of infections associated with the pathogens have been documented. Thus, state of the art molecular tools are necessary to conduct effective surveillance that would aid monitoring and prevention of disease. In this study, 110 Salmonella enterica serovars (Enteritidis & Javiana) strains isolated from food, environmental and clinical samples were analyzed for genetic diversity, antibiotic resistance, presence of virulence genes, plasmids, and plasmid replicon types. To assess the genetic diversity, pulsed-field gel electrophoresis (PFGE) fingerprinting, multiple-locus variable-number tandem repeat analysis (MLVA1 and plasmid profiles were performed. Most of the isolates were sensitive to chloramphenicol, gentamicin, kanamycin, nalidixic acid, and sulfisoxazole, whereas some of the isolates were resistant to ampicillin and tetracycline. The isolates were screened by PCR for 19 virulence genes. Our data show that S. *Javiana* and S. *Enteritidis* isolated from foods and environmental samples carry the same virulence genes as clinical isolates. Furthermore, cdtB, pltA and pltB genes were found in all 50 S. Javiana isolates, suggesting that the CdtB toxin may contribute to its pathogenesis. In this study, some of the S. *Javiana* isolates carried one or more large plasmids of approximately 30, 38, 58 and 80 kb. However, all S. *Enteritidis* isolates carried a 58 kb plasmid, type Inc/FHA. The PFGE

fingerprinting of 50 S. *Javiana* indicated several foods isolates that clustered with clinical isolates. However, PFGE was not helpful in discriminating the most common S. *Enteritidis.* MLVA was powerful technique to genotype S. *Enteritidis* isolates from poultry house and clinical sources, with 18 MLVA patterns detected among the 60 isolates. This study provides data that support the potential transmission of S. *Javiana* and S. *Enteritidis* virulence factors from food and environmental sources to cause infections in humans. The presence of cdtB, pltA and pltB genes in S. Javiana suggests the importance of these genes in these serovar and it may play an important role in the virulence of the pathogens. To determine the function of cytolethal distending toxin genes in S. Javiana wild type and mutants of cytolethal distending toxin genes, cloning of these genes in expression vector, expression and purification of S. Javiana recombinant CdtB, PltA and PltB proteins were undertaken. For in-vitro studies, HeLa cells were infected with a wild type S. Javiana, its isogenic AcdtB, ApltA and ApltB and purified recombinant proteins CdtB, PltA and P1tB to determine cell cycle arrest, cytoplasmic distension, and DNase activity. These results indicated that HeLa cells infected with S. *Javiana* wild type strain caused G2/M cell cycle arrest and distension of cytoplasm and nuclei. However, cells infected with AcdtB and ApltA showed no distension. The complemented AcdtB and ApltA Salmonella strains showed activity like wild type. We demonstrated that purified His6- tagged CdtB alone is capable of inducing typical signs of cytolethal distension toxin such as cell cycle arrest, cytoplasmic distension and DNa se activity. These results indicate that S. Javiana CdtB is likely to play an important role during host infection and disease.

TOFIQ Journal of Medical Sciences

ENGINEERING GRAPHENE FOR NANOELECTRONICS

Haider Sahib Al-Mumen

A Dissertation Submitted to Michigan State University in partial fulfillment of the requirements for the degree of Electrical Engineering - Doctor of Philosophy 2014

ABSTRACT:

Graphene has attracted research interest since its discovery in 2004 and professor Geim's receipt of the Nobel prize in 2010. It has been used for constructing a variety of electronics and sensors due to its unique electrical, mechanical, thermal, and optical properties.

In this dissertation, we developed several technologies to control the electronic properties of graphene, which will pave the way for the future development of graphene Nano electronics. First, since graphene properties vary depending on its number of layers, we identified a method for engineering the number of graphene layers using fine-tuned oxygen plasma etching. With this technique, a single layer of graphene can be removed at a time. In addition. we demonstrated a template-less nanofabrication technique for batch production of graphene nanomeshes and multiribbons, and explored the feasibility of using these nanopatterns to construct field effect transistors (FETs). By introducing nanopatterns into pristine graphene, we could effectively open the band gap of graphene and convert it from semimetal into semiconductor. Furthermore, we studied a doping method for making n-type graphene with long-term chemical stability in air and stability at wide range of temperature. Highly stable n-type graphene with minimal defects was achieved using photo acid generator (PAG) mixed with SU-8 epoxy resin as an effective electron dopant and encapsulation. The electronic properties of the as-doped n-type graphene were confirmed by measuring its current transport characteristics and Fermi level shifts.

Building on the aforementioned engineering techniques, we proposed a new Metal-SU8-,graphene (MSG) technology, which is compatible with the conventional CMOS fabrication technology. MSG FETs were fabricated on both rigid and flexible

substrates. A graphene invertor was also constructed as a proof of concept. Finally, we explored the potential applications of graphene in nano sensors, including chemical, temperature and flow sensors. We studied the possibility of using an inter-layer graphene nano configuration to detect the absorption/desorption of different chemical molecules. Our results show a remarkable enhancement in graphene surface sensitivity, which can be attributed to extra edges and inter-sheet tunneling effects. We also demonstrated the capability of using graphene nanowires in temperature and flow rate sensing.

TOFIQ Journal of Medical Sciences

EFFECTS OF HEALTH EXPENDITURES ON POPULATION AGE DISTRIBUTION AND LABOR FORCE PARTICIPATION RATES: EMPIRICAL AND COMPARATIVE ANALYSIS

Jassim M. H. Al-Jebory

A thesis submitted to the Graduate College in partial fulfillment of the requirements for the degree of Master of Arts Economics Western Michigan University August 2014

ABSTRACT:

Baby boom and population aging are the main features of the world population that are leading to child and elderly people in the labor force. Categorizing the world into low and high-income countries, the baby boom and child labor can be found in low-income countries, while population aging and elderly people in the labor force can be found in high-income countries. The cause of these features is declining rates of population and labor force ages 15-64, which is the most productive and active proportion. Health expenditures is one of the main factors that is associated with undesired trends of population and labor force through the high correlation with the rates of fertility, birth, death, and mortality. Population and labor force can be controlled by these rates and these rates can be controlled by health expenditures. Therefore, the allocation of health expenditures negatively influences population and labor force participation rates. In my thesis, I am going to investigate the trends of population and labor force participation rates in low-income and high-income countries over the period 1996-2010. The investigation will be done by using the statistics that are related to population and labor force such as the rates of population ages 0-14, ages 15-64, and ages 65+, and the rates of labor force in terms of ages and gender.

TOFIQ Journal of Medical Sciences

ENHANCED LIGHT TRAPPING BY METALLIC NANOROD ARRAYS FOR ORGANIC PHOTOVOLTAIC CELLS

Rosure Borhanalden Abdulrahman,

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of DOCTOR OF PHILOSOPHY -in Applied Science in the Department of Applied Science of the College of Science- May 2014

ABSTRACT:

Metallic nanostructures can exhibit different optical properties compared to bulk materials mainly depending on their shape, size, and separation. Vertically aligned arrays of aluminum (Al) nanorods of different lengths and morphologies were fabricated by DC sputtering using the glancing angle deposition (GLAD) method. Detailed structural and optical properties of GLAD Al nanorod were investigated and compared to those of conventional flat Al thin film coatings. Optical characterization results show that total reflectance is inversely proportional to nanorod length in the wavelength range 200-1800 nm, due to the enhanced surface plasmon resonance and light trapping among the nanorods. In addition, we present the results from an optical modeling study on ordered arrays of Al nanorods with a hexagonal periodic geometry. We used a finite-difference time-domain (FDTD) method to solve the Maxwell's equations and predict the reflectance of the nanorod arrays. In FDTD simulations, height and nanorod center-to-center periodicity were varied and reflectance profiles were calculated in the wavelength range 200-1800 nm. In addition, we calculated spatial electric field intensity distributions around the nanorods for some wavelengths. Our results show that average reflectance of Al nanorods can drop down to as low as ~50%, which is significantly lower than the ~90% reflectance of conventional flat Al film at similar wavelengths. In addition to the overall decrease in reflectance,

Al nanorod arrays manifest multiple resonant modes (higher-order modes) indicated by several dips in their reflectance spectrums (i.e. multiple attenuation peaks in their absorption profiles). Positions of these dips in the reflectance spectrum and spatial EM field distribution vary with nanorod height and diameter. Multiple reflectance peaks are explained by cavity resonator effects. Spatial EM field distribution profiles indicate enhanced light trapping among the nanorods, which can be useful especially in optoelectronic and solar cell applications. Preliminarily results from organic solar cells that utilize GLAD Al nanorods are presented in this study.

TOFIQ Journal of Medical Sciences

THE FABRICATION AND CHARACTERIZATION OF HIGH-QUALITY In„Gai_xN NANORODS (0.28<x<0.36)

Samir M. Hamad

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of DOCTOR OF PHILOSOPHY in Applied Science Concentration in Applied physics in the Department of Applied Science of the College of Science- May 2014

ABSTRACT:

Wurtzite In.Gai, N (0001) nanorods have been grown on Si (111) substrate by plasma-assisted molecular beam epitaxy (PA-MBE) methods without any heterocatalyst. The In,Gai_, N (—0.3 < x< — 0.4) is a unique material as high-brightness light emitting diodes (LEDs), laser diodes (LDs), which may emit/detect the green/blue light. However, the growth of the high quality of In„Gai, N with a tunable bandgap, having a 30-40% of In content, is not formidable due to thermodynamic & kinetic limitations in the alloy formations. For instance, the miscibility gap induced phase separation and In segregation on the growth surface are the cases. In this dissertation, we will report the growth of high quality In„Gal.. N nanorods in relation to the growth parameters., substrate temperature, In/Ga ratio, group-III (In+Ga) flux, and Group V flux. with understanding the physics behind. Using in-situ reflection high-energy electron diffraction, the surface crystal structure was monitored. The morphological and optical properties of In,Gai_x N nanorods were characterized by scanning electron microscopy and dual-light room-temperature photoluminescence methods. In addition, the nitrogen species from plasma source, which contribute to the growth, was studied by measuring optical emission spectroscopy.

TOFIQ Journal of Medical Sciences

ECOLOGY OF INSECT PESTS OF COTTON AND THEIR NATURAL ENEMIES

Soolaf Abud Kathiar

A Dissertation Submitted to the Graduate School University of Arkansas at Little Rock in partial fulfillment of requirements for the degree of DOCTOR OF PHILOSOPHY in Applied Biosciences "Insect Ecology" in the Department of Applied Science of the College of Science -May 2014

ABSTRACT:

This thesis represents a combination of three projects, all related to cotton agriculture, their pests, and natural enemies of those pests. In the first project, I compared the abundance of soil-surface arthropods in cotton fields with three different tillage methods (conventional and two conservation tillage methods). Cotton plants produce extrafloral nectar, an adaptation of many plants that attracts natural enemies of plant pests and reduces feeding injury from herbivores. Therefore, in the second project, I analyzed the sugar and amino acid concentrations of the extrafloral nectar of five lines of cotton. Green lacewings are natural enemies that are often used in biological control of agricultural pests and are known to feed on extrafloral nectar. In the third project, I tested preferences of green lacewings (Chrysoperla rufilabris) for artificial mimics of two cotton extrafloral nectars and the nutritional effects of five diets on adult green lacewings. Adoption of conservation tillage systems, including cover crop and no-till practices, have reduced environmental and economic costs compared to conventional tillage systems. Impact of conservation tillage on conservation biological control and insect pests in mid-south cotton (Gossypium hirsutum L.) is largely unknown. I examined effects of three tillage treatments (conventional tillage, cover crop, and no-till) on cotton arthropod herbivores and their predators in a two year study, using pitfall traps to survey soil-surface arthropods. In the first year (2010), three tillage systems with three pesticide treatments were used. In the second year (2011), these tillage systems with two different fertilizers at different application times were used. Repeated Measures Analysis of Variance was

used for statistical analyses. The conservation tillage systems did not increase cotton pest populations on the soil surface. Conservation tillage systems resulted in significantly higher predator populations (carabids in Year 1 and spiders in Year 2) than conventional tillage. The abundance of both herbivores and predators changed between years, possibly because of different weather conditions or because of differences in the cotton cultivars grown in the two years. Conservation tillage did not lead to increase in pest arthropods. Pest risks should not pose a barrier to adoption of conservation tillage practices for farmers in northeast Arkansas. Extrafloral nectar produced by many plants attracts natural enemies (predators and parasitoids) that then reduce herbivore abundance. The two most concentrated solutes of extrafloral nectar are sugars and amino acids; both compounds contribute to natural enemy attraction and nutrition. I measured the sugar and amino acid composition of extrafloral nectar of five different cotton lines, including three conventional lines (Ark 0102-48, Ark 0222-12, and Ark 0219-15), and two Roundup Ready®, Bt transgenic varieties, Stoneville 5288 B2F and Deltapine 0912 B2RF. Extrafloral nectar sugar and amino acid compositions and concentrations were analyzed by gas chromatography-mass spectrometry (GC-MS). Sugars and amino acids were quantified by constructing a calibration curve for each individual chemical using linear regression. The sugar concentrations of the lines differed significantly. The highest and lowest sugar concentrations were measured in the two commercial cultivars. Stoneville 5288 B2F produced nectar with the highest sugar concentrations while Deltapine 0912 B2RF produced nectar with the lowest sugar concentrations. The three conventional lines (Ark 0102-48, Ark 0222-12, and Ark 0219-15) were intermediate in sugar concentrations.

Although six amino acids (alanine, proline, valine, serine, leucine, and glutamic acid) were identified and quantified in the extrafloral nectars of all five cotton lines, no significant differences occurred in the concentrations of individual amino acids among lines. The differences in sugars among cotton lines were genetic because all were grown simultaneously under the same conditions. If extrafloral nectar can be manipulated genetically, we might be able to improve the attraction and nutrition of natural enemies in the field and improve natural biological control. Biological control, the use of natural enemies, is widely recommended to decrease the need for

pesticides. Because green lacewings (Chrysoperla rufilabris) can play an important role in biological control, they are of great interest to many farmers and scientists. In order to produce baseline data that could improve the reproductive output of green lacewings in the field, I experimentally tested the ability of C. rufalabris to detect amino acids in extrafloral nectars, their preferences for extrafloral nectars of different cotton cultivars Stoneville 5288 B2F and Deltapine 0912 B2RF, and the nutritional value of five different adult diets. Using mimics of an ecologically unrealistic extrafloral nectar with a high concentration of amino acids (Passiflora menispermifolia, a tropical canopy vine), both male and female C. rufilabris preferred sugar-amino acid nectars over sugar-only nectars. Thus, amino acid-rich nectars could be important in attracting green lacewing adults. However, neither male nor female C. rufilabris discriminated between sugar-only and sugar-amino acid mimics of cotton extrafloral nectars (Stoneville 5288 B2F and Deltapine 0912 B2RF), probably because the amino acid content of the nectar mimics was too low. In nutrition tests of five adult diets ' (Stoneville 5288 B2F extrafloral nectar only, Deltapine 0912 B2RF extrafloral nectar only, Stoneville 5288 B2F extrafloral nectar plus pollen, Deltapine 0912 B2RF extrafloral nectar plus pollen, and an artificial diet), females fed only nectar produced the fewest number of eggs. The highest fecundities were produced when adults received high-protein diets (artificial diet or nectar plus pollen). If cotton cultivars can be developed with high levels of amino acids in the extrafloral nectars, they might contribute to increased reproduction by C. rufalabris. Alternatively, planting pollen sources on the edges of agricultural fields could also help improve reproduction by C. rufalabris.

TOFIQ Journal of Medical Sciences, TJMS, Supplement Vol 3 (1), (2016), 17-18

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SCALABLE PULSED MODE COMPUTATION ARCHITECTURE USING INTEGRATE AND FIRE STRUCTURE BASED ON MARGIN PROPAGATION

Thamira Hindo

A DISSERTATION Submitted to Michigan State University in partial fulfillment of the requirements for the degree of Electrical Engineering- Doctor of Philosophy 2014

ABSTRACT:

Neuromorphic computing architectures mimic the brain to implement efficient computa-tions for sensory applications in a different way from that of the traditional Von Neumann architecture. The goal of neuromorphic computing systems is to implement sensory de-vices and systems that operate as efficiently as their biological equivalents. Neuromorphic computing consists of several potential components including parallel processing instead of synchronous processing, hybrid (pulse) computation instead of digital computation, neuron models as a basic core of the processing instead of the arithmetic logic units, and analog VLSI design instead of digital VLSI design. In this work a new neuromorphic computing ar-chitecture is proposed and investigated for the implementation of algorithms based on using the pulsed mode with a neuron-based circuit. The proposed architecture goal is to implement approximate non-linear functions that are important components of signal processing algorithms. Some of the most important sig-nal processing algorithms are those that mimic biological systems such as hearing, sight and touch. The designed architecture is pulse mode and it maps the functions into an algorithm called margin propagation. The designed structure is a special network of integrate-and-fire neuron-based circuits that implement the margin propagation algorithm using integration and threshold operations embedded in the transfer function of the neuron model. The integrate-and-fire neuron units in the network are connected together through excitatory and inhibitory paths to impose constraints on the network firing-rate. The advantages of the pulse-based, integrate-arid-fire margin propagation (IFMP)

algorithmic unit are to im-plement complex non-linear and dynamic programming functions in a scalable way; to im-plement functions using cascaded design in parallel or serial architecture; to implement the modules in low power and small size circuits of analog VLSI; and to achieve a wide dynamic range since the input parameters of IFMP module are mapped in the logarithmic domain. The newly proposed IFMP algorithmic unit is investigated both on a theoretically basis and an experimental performance basis. The IFMP algorithmic unit is implemented with a low power analog circuit. The circuit is simulated using computer aided design tools and it is fabricated in a 0.5 micron CMOS process. The hardware performance of the fabricated IFMP algorithmic architecture is also measured. The application of the IFMP algorithmic architecture is investigate for three signal pro-cessing algorithms including sequence recognition, trace recognition using hidden Markov model and binary classification using a support vector machine. Additionally, the IFMP architecture is investigated for the application of the winner-take-all algorithm, which is important for hearing, sight and touch sensor systems.

TOFIQ Journal of Medical Sciences

TJMS

Supplement (1), Vol.3, 2016 ISSN 2377-2808

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

TOFIQ Journal of Medical Sciences (TJMS)

Supplement, 2 (2016)

Issued by:
TOFIQ Office,
2405 Carey lane,
Vienna, VA 22181
USA

TJMS.tofiq.org

Indexing

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

Indexing

TOFIQ Journal of Medical Sciences
ISSN: 2377-2808

September 1, 2016

Letter from the Editor-in Chief

Supplement 2 to TJMS

To our distinguished readers,

Following the positive response we have to our first TJMS supplement it is our pleasure to publish this second supplement. It is TOFIQ's objective to help our students anywhere inside and outside Iraq.

This second issue is dedicated to our scholarship PhD students in Canada.

Our appreciation goes to Prof. Asaad T. Omaran, Cultural Attaché in Otawa who just finished his post and also thanks to Mr. Sabah W. Ali who is in charge of the cultural office, for their kind support.

We thank our beloved students for their hard and successful work and for being a great potential to promote science and technology in Iraq.

It is our aim and hope that this project will continue and expand in support of the great efforts of the Iraqi academic and official leaders to promote higher education in Iraq.

Please accept my best wishes,

Prof. A Hadi Al Khalili, MBChB, MPhil, FRCSE, FACS
Editor-in Chief, TJMS

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

TOFIQ Journal of Medical Sciences

TOFIQ Journal of Medical Sciences (TJMS) is published by TOFIQ: an NGO registered at the State of Maryland as a non-profit organization dedicated to helping Iraq Higher Education and Research.

TJMS is devoted to the publication of original research, commentaries on a current topic, reviews, letters to the editor, and editorials in the field of medical sciences. The early focus of the journal is on clinical burden of disease in Iraq: documentation of its nature and extent; clinical patterns and epidemiology; diagnostic findings; and therapeutic strategies. Read more ...

Focus and Scope

TOFIQ Journal of Medical Sciences (TJMS) is published by TOFIQ: an NGO registered at the State of Maryland as a non-profit organization dedicated to helping Iraq Higher Education and Research.

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TOFIQ Journal of Medical Sciences ISSN: 2377-2808

Mission:

1. TJMS is a high quality, biannually, peer-reviewed, electronic medical sciences journal, publishing original research and scholarly review articles, letters to the editor, and editorials.
2. TJMS focuses on the disease burden in Iraq.
3. An outlet for the current research and an academic product in Iraq in the fields of medicine, dentistry, pharmacy, nursing and related disciplines that supports recognition and academic advancement in Iraq and beyond.
4. TJMS will lay the groundwork for creation of sister journals in other disciplines relating to Iraq (engineering, agriculture, science and technology, social sciences and humanities).

Structure:

1. Single editor-in-chief, working in a full-time, compensated capacity.
2. Editorial board consisting of experts in the various branches of medicine, dentistry, pharmacy, nursing and related disciplines.
3. Open-access, electronic-only journal
4. Peer-reviewed publication supported by an online submission, review, and decision for articles.
5. Articles can be submitted by any individual/group, or can be solicited (invited reviews and discussions). Decisions for publication will be blinded to author or region of origin. The criteria on which the submissions are evaluated for acceptance will be heavily weighted on their applicability to the burden of disease in Iraq.
6. Publication will be biannual however, approved articles will be released continuously.

7. Funding: will attempt to obtain corporate sponsorship through unrestricted educational grants. Sponsorship will be acknowledged in compliance with ACCME and ICMJE guidelines.
8. The publication would be the official medical sciences journal of TOFIQ, and we would encourage other medical organizations to consider collaborating with.

TOFIQ Journal of Medical Sciences ISSN: 2377-2808

Editorial Team

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TOFIQ Journal of Medical Sciences ISSN 2377-2808

Supplement, No 2, (2016)

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Characterization of The Viable but Non-Culturable *Legionella pneumophila* in Water and the Role of 3-Hydroxybutyrate Dehydrogenase in Its Formation

Badii H. Q. Al-Bana

A Dissertation Submitted -partial fulfilment of the requirements for the degree of Doctor of Philosophy at Dalhousie University .Halifax, Nova Scotia September 2013

ABSTRACT:

Legionella pneumophila, the causative agent of Legionnaires' disease (LD), is an intracellular pathogen of freshwater protozoa that can also persist in the environment as a free-living bacterium. *L. pneumophila* has many morphological forms that fit within a developmental cycle. In water, *L. pneumophila* enters into a viable but non-culturable (VBNC) state that is largely uncharacterized. VBNC cells were produced from two developmental *L. pneumophila* forms, stationary phase forms (SPFs) and mature infectious forms (MIFs) by suspension in double deionized (dd) or tap-water at 45°C. Electron microscopy results showed that VBNC cells have a unique morphology and that in tap water they lose their poly 3-hydroxybutyrate inclusion bodies. Both SPFs and MIFs lost culturability faster in dd- than in tap water, and addition of salts to dd-water prolonged *L. pneumophila* culturability and enhanced viability. However, MIFs retained higher viability in dd- and tap water (85% and 51%, respectively) than SPFs (5% and 20%, respectively) as determined by the BacLight vital stain. Only ~1 VBNC cell out of 105 of those produced from SPFs in tap water regained culturability via infection of *Acanthamoeba*. All VBNC cells, except for those produced from SPFs in dd-water, resisted both digestion inside *Tetrahymena* spp. and detergent-mediated lysis. SDS-PAGE analysis and shotgun proteomics revealed a number of VBNC cell specific

proteins; one of these was 3-hydroxybutyrate dehydrogenase (BdhA), which is involved in the metabolism of poly 3-hydroxybutyrate inclusion bodies. A *bdhA* mutant showed an early loss of culturability and a dramatic decrease in viability as compared to the parent strain, and complementing the mutant with a functional *bdhA* gene restored the parent's strain phenotypes. In conclusion, VBNC *L.pneumophila* has a distinct morphology and physiology

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that varies according to the developmental stage and the environmental conditions used to produce such VBNC cells.

VBNC cells have a different protein profile and morphology than the culturable cells,suggesting that this state constitutes a distinct differentiated form within the developmental cycle of *L. pneumophila.* BdhA seems to influence *L. pneumophila* survival and hence VBNC cell formation. Collectively, the results from this study provide a better understanding of *L. pneumophila* VBNC form and the factors influencing its formation.

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Effects of Endothelin in Integrated Venous Function in Health and Disease

Afrah Al-Najeer

A Thesis Submitted To The University of Guelph In Partial Fulfilment Of Requirements For The Degree Of Doctor of Philosophy In Biomedical Sciences Guelph, Ontario, Canada, 2014

Advisor:

Dr. Ron Johnson

CO advisor Dr. Jonathan LaMarre

ABSTRACT:

The endothelin system and integrated venous function are both under the influence of sex steroids. However, there is limited understanding of the influence of gender and acute deprivation of steroid hormones on venoconstrictor responses to ET-1. Unpressurized mesenteric vessels of gonadally intact, or neutered male and female rats were used to assess vascular responses to (Endothelin) ET-1 or the selective endothelin B receptor (ETBR) agonist, sarafotoxin 6c (S6c) in presence and absence of the selective endothelin A receptor (ETAR) antagonist (BQ-610) and/or and the selective ETBR antagonist (BQ-788). There were no differences in ET-1 contractile responses between intact male and female rats. However, S6c produced significantly greater responses in the veins of intact male compared to intact female. In intact rats, blockade of ETARs revealed gender related differences in ETAR mediated venoconstriction, whereas blockade of both ETARs and ETBRs eliminated these differences. Ovariectomy enhanced the contractile responses to S6c in veins. Blockade of ETARs by BQ-610 was reduced in veins following ovariectomy and castration. These results suggest that intact male veins produce greater ETBR mediated contractions, estrogen can modulate ETBR mediated contractions, and ETARs are under the influence of both sex steroid hormones.

HF is associated with elevated plasma ET-1 levels and an increase in cardiac preload due to venoconstriction. The role of elevated plasma ET-1 levels in altered venous functions in HF has not been evaluated yet. Therefore we aimed to identify the changes in ET-1 vascular contractile responses and concomitant alterations in the ET-1 receptors mediated vascular contractile responses together with changes in receptors mRNA and protein expression levels in mesenteric veins. At 4, 8 and 16

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weeks post sham operation or Myocardial infarction (MI), venoconstrictor responses were recorded using a pressure myograph. RT-qPCR and Western blotting were used to quantify mRNA and protein expression of receptors. In Mesenteric veins, vasoconstrictor responses to ET-1 and S6c were progressively reduced in the period following MI. BQ-788 significantly reduced ET-1 responses in 4 and 16wk sham but not HF mice. BQ-610 abolished ET-1 responses in 4wk HF and sham mice, whereas in 16 wk mice, BQ-610 caused greater inhibition in ET-1 responses in HF than sham mice and co-application of BQ-788/BQ-610 completely inhibited ET-1 responses in sham and HF mice. ETBR but not ETAR mRNA and protein expression was decreased in HF mesenteric veins compared to those of sham mice. In conclusion, a reduced venoconstrictor response to ET-1 in HF is associated with the functional and molecular down regulation of venous ETBR post-MI.

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Adaptive Approaches for Medical Imaging Security

Ahmed Badr Mahmood

A Thesis Submitted To The University of Guelph In partial fulfilment of requirements for the degree of Doctor of Philosophy in Engineering .Guelph, Ontario, Canada . 2015

Advisor:

Professor Robert Dony

ABSTRACT:

Securing medical images is important to protect the privacy of patients and assure data integrity. Consequently, encryption and watermarking were explored to improve the security of medical images.

A novel encryption method is introduced to reduce the implementation time and maintain the robustness of algorithms such as AES and 3DES. This method uses selective encryption in an adaptive way. The medical image was divided according to threshold criteria into the region of interest (ROI) and region of background (ROB). If ROB is large, the two-region algorithm is used.

The criteria used to determine the threshold values are the K-means or region growing. If ROB is small, multi-region algorithm is used. The multi-region algorithm is a novel adaptive approach based on the variation of information. The threshold values of the regions are obtained using genetic algorithms (GA) tuned with selected parameters. A modified Gold code is designed as an encryption algorithm for background regions. A new algorithm based on the Chinese remainder theorem is modified to encrypt a medium information region. The high information region is encrypted using the AES. The results showed that implementation time is reduced by an average of 20% and the robustness is maintained in most cases. In some cases, the correlation coefficient is high; therefore, an adaptive stopping-criterion permutation algorithm is designed.Non-reversible watermarking is utilized to achieve ownership verification as well as integrity of medical images. To avoid an incorrect diagnosis, the watermark should not modify the informative region. Image segmentation is applied to identify the lowest information region. Embeddingmaps based on location

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and entropy are built to reduce cropping attack effect. The DCT domain is used to achieve better invisibility and robustness. GA-based adaptive embedding algorithm is designed to select the frequency coefficients among three DCT models. The GA fitness function is designed using the variance and the SSIM. Invisibility improved to obtain better security for various modalities.

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Supplement (2), (2016), 7-8 ISSN: 2377-2808

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Towards Efficient Packet Classification Algorithms and Architectures

RAWA ABDUL REDHA A. FINJAN

A Thesis Submitted To The Faculty of Graduate Studies
of The University of Guelph. 2011

Advisors :
Dr. Keith Warriner
Dr. Tatiana Koutchma
Dr. Joseph Odumeru

ABSTRACT:

Disinfection of milk by a novel UV reactor based on Taylor-Couette vortex flow was evaluated as part of an effort to develop non -thermal processing technology for pasteurizing milk. By using Bacillus subtilis endospores and bacteriophages as model microbes. it was demonstrated that level of inactivation was dependent on the flow -pattern of the milk passing through the reactor.

The transition from laminar wavy vortices (WV) to laminar modulated wavy vortices (MWV) achieved 4.5log pfu reduction of Salmonella phages. Turbulent wavy vortices (TWV) achieved 5.8log cfu reduction of *Bacillus* subtilis endospores PS346 . The stability of the generated Taylor-Couette vortices could be enhanced by increasing the viscosity of the milk although this did not translate to higher levels of microbial UV inactivation. Tryptophan and cysteine provided protection to the microbes by absorbing UV photons. Although the actual UV dose required to inactive Escherichia co// in milk was comparable to the efficacy

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of other reactors, only a Ilog cfu reduction in numbers could be achieved under optimal operating conditions. The results from this study would suggest to re -design Taylor Couette reactor to decrease the force of the flow rate and to generate stable vortices that could support efficient mixing. hence higher level of microbial activation.

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Supplement (2), (2016), 9-10 ISSN: 2377-2808

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Efficient Scheduling, Mapping and Resource Prediction for Dynamic Run time Operating Systems

Ahmed Al-Wattar

A Thesis presented to The University of Guelph In partial fulfilment of requirements for the degree of Doctor of Philosophy in Engineering , Guelph, Ontario, Canada 2015

Advisor:

Professor Shawki Areibi

ABSTRACT:

Several embedded application domains for reconfigurable systems tend to combine frequent changes with high performance demands of their workloads such as image processing, wearable computing and network processors. Time multiplexing of reconfigurable hardware resources raises a number of new issues, ranging from run-time systems to complex programming models that usually form a Reconfigurable hardware Operating System (ROS). In this thesis a novel ROS framework that aids the designer from the early design stages all the way down to the hardware implementation is proposed. An efficient reconfigurable platform was implemented along with several novel scheduling algorithms. The algorithms proposed tend to reuse hardware tasks to reduce reconfiguration overhead, migrate tasks between software/hardware to efficiently utilize resources and reduce computation time. A framework for efficient mapping of execution units to task graphs in a runtime reconfigurable system is also designed. The framework utilizes an Island.

Based Genetic Algorithm flow that optimizes several objectives including performance, area and power consumption. The proposed Island based GA framework achieves on average 55.2% improvement over a single GA implementation and 80.7% improvement over a baseline random allocation and binding approach. Finally, we present a novel adaptive and dynamic methodology based on a Machine Learning approach for predicting and estimating

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the necessary resources for an application based on past historical information. An important feature of the proposed methodology is that the system is able to learn and generalize and, therefore, is expected to improve its accuracy over time.

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Fabrication Of Nanofibers Reinforced Polymer Microstructures Using Femtosecond Laser Material Processing

Mohammed-Amin Alubaidy

A dissertation Presented to Ryerson University in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the program of Mechanical Engineering
Toronto, Ontario, Canada.2012

ABSTRACT:

A new method has been introduced for the formation of microfeatures made of nanofibers reinforced polymer, using femtosecond laser material processing. The Femtosecond laser is used for the generation of three-dimensional interweaved Nanofibers and the construction of microfeatures, like microchannels and voxels, through multi photon polymerization of nanofiber dispersed polymer resin. A new phenomenon of multiphoton polymerization induced by dual wavelength irradiation was reported for the first time. A significant improvement in the spatial resolution, compared to the two photon absorption (2PA) and the three photon absorption (3PA) processes has been fabricated through this method . The mechanical properties of nanofiber reinforced polymer microstructures has been investigated by means of nanoindentation and the volume fraction of the generated nanofibers in the nanocomposite was calculated by using nanoindentation analysis. The results showed significant improvement in strength of the material. The electrical conductivity of the two photon polymerization (TPP) generated microfeatures was measured by a two -probe system at room temperature and the conductivity- temperature relationship was measured at a certain temperature range. The results suggest that the conductive polymer microstructure is reproducible and has a consistent conductivity- temperature relation. The magnetic strength has been characterized using Guassmeter To.

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Supplement (2), (2016), 12-13 ISSN: 2377-2808

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Non-parametric Spatial-Domain Algorithm for Analysis and Mapping of Urban Scenes Using 3D Point Clouds

Hussein Al-Gurrani

A Thesis Submitted To The Faculty Of Graduate Studies In Partial Fulfilment of The Requirements For The Degree Of Doctor Of Philosophy Graduate Program In Geomatics Engineering Calgary. 2016

ABSTRACT:

The world population is expected to grow to nine billion by 2050. With half of the current seven billion people living in urban areas, urbanization most certainly will continue in the future. Such a steep urbanization growth curve requires and justifies the growing interest not only in urban sustainability but also in sustainable development in general, which explains the growing interest as well in spatial information as it can provide sound support for decision-making. Geomatics engineering, which is the core of spatial information collection and processing, provides a multitude of tools for spatial information end-users, such as planners.

The research of this dissertation developed a new geomatics-based tool for the urban data collection process, which is the first and most time-consuming step in any urban planning project. Using 3D point clouds obtained using state-of-the-art surveying equipment, which consisted of stationary lasers scanners, a mobile mapping platform, and a UAV, an automatic comprehensive classification algorithm was developed for urban scenes. This algorithm identifies and provides essential information about basic features that can be used in analyzing and understanding a surveyed urban scene, including 1) building locations, dimensions, and other design details; 2)road inventory (e.g., traffic signs, overhanging traffic signals, poles, and powerlines); 3) greenery details (e.g., individual trees, separated canopies, and bushes); and 4)identification of parked cars.The algorithm developed in this dissertation is comprised of four modules to process the input data. In Module 1 conducts ground detection and removal. Then, points clustering and characterization using principal components analysis is accomplished in Module 2. The data are further classified in Module 3 into four main categories: buildings, cars, road inventory, and iii greenery. Fine classification and analysis of the candidate classes and information extraction is conducted in Module 4, which consists of several subroutines for each process.

The new algorithm was evaluated using four different datasets from different types of sensors and different urban sites. The experimental results revealed that the algorithm successfully classified the input datasets into the targeted classes and provided accurate information about the recognized objects. The algorithm also proved to be consistent over different types of sensors and urban sites, which validated the research hypotheses. As an example of how the extracted information can be used, wind flow analysis was conducted around complex of buildings in one of the datasets using Star software. The analysis was implemented by Center for Design Research at the University of Kansas, USA. The GIS layers were also updated using the extracted information and a 3D digital building model was created for one of the buildings in the datasets.

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Clash Of The Barbarians: The Representation Of Political Violence In Contemporary English And Arabic Language Plays About Iraq

AM1R A. AL-AZRAKI

A Dissertation Submitted To The Faculty Of Graduate Studiesin Partial Fulfillment Of The Requirements For The Degree Of Doctor Of Philosophy Graduate Program In Theatre York University. Toronto, Canada.2011

ABSTRACT:

The history of Iraq. especially since the establishment of the modern nation in 1920. has been a conspicuously violent one. The yoking together of Sunni, Shia and Kurds under an imported monarchy was a recipe for civil conflict, as was the corruption, authoritarianism and British backing of the monarchy itself A succession of revolutions and coups d'etat, each bloodier than the last, culminated in the ruthless dictatorship of Saddam Hussein, ushering in brutal repression, torture, a devastating war with Iran. the invasion of Kuwait. Saddam's overthrow in the invasion of 2003, and the carnage and destruction that has followed. Given such a history, it is not surprising that Iraqi playwrights would find themselves drawn to reflect the trauma of these decades in their plays.

The aim of this study is to analyze and appraise the ways in which the endemic political violence of recent decades in Iraq has been represented in drama, both by Iraqi playwrights writing in Arabic, and by English -language playwrights, mainly in the UK and the US, where the issues surrounding the justification and conduct of the 2003 invasion have generated intense political debate.

How do Iraqi and non -Iraqi playwrights differ in their representations of political violence, in terms both of critical perspective and of dramatic strategy? For analytical convenience, political violence has been subdivided into three categories: resistance violence against tyranny and foreign occupation: revolutionary violence: and the violence of terror. whether by the state or by sectarian elements within society. While the culminating focus has been on drama since 2003. the study has sought to situate the contemporary output in a tradition of Iraqi drama

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Supplement (2), (2016), 15-16 ISSN: 2377-2808

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Mode of Action of Daptomycin, a Lipopeptide Antibiotic

Jawad Kadhum Muraih

A thesis presented to the University of Waterloo in fulfillment of the thesis requirement for the degree of Doctor of PhilosophyIn Chemistry(Biochemistry)Waterloo.Ontario. Canada. 2012

ABSTRACT:

Daptomycin is a lipopeptide antibiotic that contains 13 amino acids and an N -terminally attached fatty acyl residue. The antibiotic kills Gram-positive bacteria by membrane depolarization. It has long been assumed that the mode of action of daptomycin involves the formation of oligomers on the bacterial cell membrane: however, at the outset of my studies. this had not been experimentally demonstrated. In the work described in this thesis. I have used fluorescence energy transfer (FRET) between native daptomycin and an NBD-labeled daptomycin derivative to demonstrate that the antibiotic indeed forms oligomers on bacterial cell membranes. In a liposome model, oligomer formation depends on calcium and on phosphatidylglycerol (PG). The oligomer forms rapidly and is stable for a length of time longer than required for the bactericidal effect. Through variation of the ratio of FRET donor (native daptomycin) and acceptor (NBD-daptomycin). I have determined that the oligomer consists of approximately 6-7 molecules, or. depending on the structure of the oligomer. possibly up to twice that number.

Oligomer formation on liposomes and on bacterial membranes was confirmed using excimer fluorescence of a perylene-labeled daptomycin derivative.

Excimer fluorescence was also used to demonstrate a stoichiometric interaction between daptomycin and PG. It has previously been shown that the bactericidal activity of daptomycin requires calcium and correlates with the concentration of PG in the bacterial cell membrane: these requirements mirror those observed here for oligomer formation.

Furthermore. membrane permeabiliza tion is selective, and electron microscopy of bacterial membranes exposed to daptomycin has revealed no discontinuities or accretions of electron density. Both of these findings suggest formation of a small membrane lesion, which is compatible with the small size of the oligomer that was determined here.

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In conjunction with these previous findings, the experiments contained in my thesis strongly suggest that the oligomer is the bactericidal form of daptomycin

TOFIQ Journal of Medical Sciences, TJMS, Vol. 3, Supplement (2), (2016), 17-18 ISSN: 2377-2808

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Towards Efficient Packet Classification Algorithms and Architectures

Omar Ahmed

A Thesis presented to The University of Guelph In partial fulfilment of requirements for the degree of Doctor of Philosophy in Engineering Guelph, Ontario, Canada .2013

Advisor:
Professor Shawki Areibi

ABSTRACT:

Packet classification plays an important role in next generation networks. Packet classification is important to fulfill the requirements for many applications including firewalls, multimedia services, intrusion detection services, and differentiated services to name just a few. Hardware solutions such as CAM/TCAM do not scale well in space. Current software-based packet classification algorithms exhibit relatively poor performance, prompting many researchers to concentrate on novel frameworks and architectures that employ both hardware and software components.

In this thesis we propose two novel algorithms, Packet Classification with Incremental Update (PCIU) and Group Based Search packet classification Algorithm (GBSA), that are scalable and demonstrate excellent results in terms of preprocessing and classification.

The PCIU algorithm is an innovative and efficient packet classification algorithm with aunique incremental update capability that demonstrates powerful results and is accessible for many different tasks and clients. The algorithm was further improved and made more available for a variety of applications through its implementation in hardware. Four such implementations are detailed and discussed in this thesis. A hardware accelerator based on an ESL approach, using andel-C, resulted in a 22x faster classification than a pure software implementation running on a state of the art Xeon processor. An ASIP implementation achieved on average a 21x quicker classification.

We also propose another novel algorithm, GBSA, for packet classification that is scalable, fast and efficient. On average the algorithm consumes 0.4 MB of memory for a 10k rule set. In the worst case scenario, the classification time per packet is 2 μs, and the pre-processing speed is 3M Rule/sec, based on a CPU operating at 3.4 GHz. The proposed algorithm was evaluated and compared to state-of-the-art techniques, such as RFC, HiCut, Tuple, and PCIU, using several standard benchmarks. The obtained

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results indicate that GBSA outperforms these algorithms in terms of speed, memory usage and pre-processing time. The algorithm, furthermore, was improved and made more accessible for a variety of applications through implementation in hardware. Three approaches using this algorithm are detailed and discussed in this thesis. The first approach was implemented using an Application Specific Instruction Processor (ASIP), while the others were pure RTL implementations using two different ESL flows (Impulse-C and Handel-C). The GBSA ASIP implementation achieved, on average, a 18x faster running speed than a pure software implementation operating on a Xeon processor. Conversely, the hardware accelerators (based on the ESL approaches) resulted in 9x faster processing.

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Supplement (2), Vol.3, 2016 ISSN 2377-2808