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College of Medicine
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Tel and Fax: 964-1-5224368
E-mail: Iraqi_jms_alnahrain@yahoo.com

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Iraqi Journal of Medical Sciences

Aims and Scope

Iraqi Journal of Medical Sciences is published by College of Medicine, Al-Nahrain University. It is a quarterly multidisciplinary medical journal. High quality papers written in English, dealing with aspects of clinical, academic or investigative medicine or research will be welcomed. Emphasis is placed on matters relating to medicine in Iraq in particular and the Middle East in general, though articles are welcomed from anywhere in the world.

Iraqi Journal of Medical Sciences publishes original articles, case reports, and letters to the editor, editorials, investigative medicine, and review articles. They include forensic medicine, history of medicine, medical ethics, and religious aspects of medicine, and other selected topics.

Iraqi JMS FORMAT

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Iraqi Journal of Medical Sciences (Iraqi JMS) is a periodic, peer-reviewed journal published quarterly by College of Medicine, Al-Nahrain University. Iraqi JMS publishes manuscripts in all fields of health and medicine written in English.

Types of Contributions: Original articles, review articles, case studies, editorials, medical education, history of medicine, ethics, practical points, medical quiz, conferences, meetings and letters to the Editor.

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3. Chapter in book: Phillips SJ, and Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

- **Tables**: Each table should be typed on a separate page double-spaced, including all headings, number all tables with English numerals and include a short title. Vertical lines between columns are to be avoided.
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Genetics: The Road to Ancestors

Muthanna A Al-Kaabi *MSc, PhD*

Dept. of Human Anatomy, College of Medicine, Al-Nahrain University

The deCODEme Analysis

It is interesting how medical sciences can show the way to new avenues of information, sometimes far away from medicine itself, an opportunity to pioneer innovative and original disciplines of knowledge.

Recently developed, the deCODEme project is a genetic health scan; it provides early detection of risk that allows the prevention of a disease from occurring. In addition, it reveals the personal genetic origin to uncover one's ancestry. Thus, the history can be explored genetically by tracing the branches of ancestry thousands of years back through time, unlocking the history that is documented in man's genes!

deCODE Your Ancestry

Ancestry is one of the most exciting areas of genetic exploration. The use of genetic data to make inferences about people's ancestors and genealogical relationships allow for some truly remarkable discoveries. Scientific research in this field has revealed that we all descend from a common ancestral group of humans that originated in Africa about 200 thousand years ago. About 70 thousand years ago, the descendants of these first humans began colonizing other parts of the world.

Map of Kinship & Genetic Atlas

Our DNA can tell us how closely we are related to people from all over the world. It can also reveal our ancestral origins. Where did your ancestors come from Africa, Europe, the Americas or Asia?

Genes and Human History

From a genetic point of view we are all unique, but some individuals are more similar than others. Generally speaking, genetic differences reflect geography. People from the same geographic area tend to be more genetically similar than people from distant parts of the world. "Individuals from the same population tend to be genetically similar

From Father to Son – Male Line Testing

If you are male, a Y-chromosome analysis will show you where your ancestors in the direct male or paternal line came from. This analysis can also help identify your relatives through the direct male line. This is possible because the Y chromosome is passed from father to son relatively unchanged through many generations.

"Y chromosome Adam"

Ultimately, all men can trace their Y chromosome through the direct male line to a single male ancestor, playfully named "Y Chromosome Adam". This important male ancestor is thought to have lived in Africa some 190 thousand years ago. Each of the Y-groups in the male line genealogy represents a different line of descent from "Y chromosome Adam". Your Y-group can tell you not only how you are related to other people in the direct

male line, it also provides information about the role your Y-group ancestors played in the colonization of the world thousands of years ago.

From Mother to Child – Female Line Testing

Mitochondrial DNA or mtDNA is inherited only from mother to child. Due to its peculiar mode of inheritance through the mother, mtDNA can be used to determine the genealogical relationship between two or more individuals (men or women) living today through the direct female line.

"Mitochondrial Eve"

Ultimately, all humans can trace their mtDNA through the direct female line to a single female ancestor, playfully named "mitochondrial Eve". This important female ancestor is thought to have lived in Africa some 190 thousand years ago. Each of the mitogroups in the female line genealogy represents a different line of descent from "mitochondrial Eve". Your mitogroup can tell you not only how you are related to other people in the direct female line, it also provides information about the role your mitogroup ancestors played in the colonization of the world thousands of years ago.

Useful link

Further information and details about genetic diseases and ancestry mapping is found at: http://www.decodeme.com/

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Predicting Microvascular Complications in Diabetic Patients

Yousif AR Al Ani, *FICMS/CM*

Dept. of Community Medicine, Al-Kindy College of Medicine, Baghdad University

Abstract

Background Patients with diabetes have an increased risk of developing microvascular complications, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, which if not predicted, early detected and treated, place a significant burden on individual's health and can reduce life expectancy.

Objective To determine the main risk factors (predictors) that associated with microvascular complications in diabetes aiming to construct a module that can detect microvascular complications depending on these predictors.

Methods A cross sectional descriptive study was carried out with 364 diabetic patients. Data about diabetes microvascular complications (retinopathy, clinical peripheral neuropathy, and nephropathy) and their potential risk factors were collected. Primary point was detecting the < 0.01 level of significant association of risk factors with these complications to determine the predictors. These predictors were assessed for each individual's micro vascular complication and also as a composite outcome by logistic regression analysis.

Result Of the examined 364 diabetic cases, 174 (47.80%) patients were found with microvascular complications. Neuropathy, nephropathy, and retinopathy were detected in 66 (18.13%), 62 (17.03), and 46 (12.64%) patients, respectively. Out of 12 potential predictors, only six (age, smoking habit, duration of diabetes, uncontrolled hyperglycemia, hypertension, and macrovascular complications) found to be significantly associated with the presence of microvascular complication (p < 0.01) as compared with patients who had no such complications. Uncontrolled hyperglycemia was the first predictor in neuropathy and nephropathy groups, while diabetic duration was ranking first in retinopathy group.

Conclusions Microvascular complications in diabetic patients can be predicted, and avoided, by detecting their risk factors. Logistic regression equation provide suitable module for evaluation of these risk factors simultaneously.

Key words Microvascular complications, diabetes, logistic regression

Introduction

Diabetes mellitus (DM) is a global health problem, affecting all age groups. Currently, around 177 million people have diabetes worldwide; however, it has been projected that this number will increase to at least 300 million by 2025 [(1)]. This epidemic relates in particular to type II diabetes, which accounts for around 90% of all diabetes cases. The increased prevalence of type II diabetes can be attributed to the ageing population and rising incidence of obesity, besides other factors [(2)].

The greatest rate of rise is predicted to be in the Middle-East [(3)], and as a country of the Middle-East, Iraq is affected by this epidemic, with overall prevalence of 21.8 per 1000 in 2006. Further increases in the rates are seen after age 50, with a prevalence rate of 143.8 per 1000 [(4)].

DM is a disease that is strongly associated with both microvascular complications, (including retinopathy, nephropathy, and neuropathy) and

macrovascular complications, (including ischemic heart disease, peripheral vascular disease, and cerebrovascular disease), resulting in organ and tissue damage in approximately one third to one half of people with diabetes [5]. Although, much of the focus has been made on the macrovascular complications, it is clear that the microvascular complications have a significant impact on both morbidity and mortality amongst diabetic patients [6], and despite the introduction of treatment strategies, diabetes remains a major cause of new-onset blindness, end-stage renal disease, and lower leg amputation [7]. Furthermore, the management of diabetes-related complications generates substantial costs [8].

Diabetic retinopathy is the most common microvascular complication among people with diabetes and results in more than 10,000 new cases of blindness per year. It is slow to develop, and there is some evidence that it can begin to develop as early as 7 years before clinical diagnosis of type 2 diabetes [9]. As much as 90% of blindness due to retinopathy among people with diabetes may be preventable if detected and treated early. Annual dilated eye examinations are recommended for all patients with diabetes [10].

Diabetic nephropathy is defined as persistent proteinuria (more than 500 mg of protein or 300 mg of albumin per 24 hours) in patients without urinary tract infection or other diseases causing the proteinuria [11]. Diabetic nephropathy is a serious and progressive complication in DM. The first manifestation of diabetic nephropathy is typically microalbuminuria, which progresses to overt albuminuria (i.e. increased albumin levels in the urine, indicating more severe renal dysfunction) and eventually to renal failure and it is the leading cause of end-stage renal disease (ESRD) [12].

Diabetic neuropathy is a common complication of diabetes occurring over time in more than half of patients with type 2 diabetes. Nerve conduction studies demonstrate that neuropathy is already present in 10-18% of patients at the time of diabetes diagnosis, suggesting that peripheral nerve injury occurs at early stages of disease [13]. Diabetic neuropathy can be either peripheral (mono or poly) neuropathy or autonomic neuropathy and Physician commonly encounter diabetes associated peripheral neuropathy in the evaluation and treatment because these disorders frequently affect lower-extremity sensation and can cause lower-extremity pain. Loss of lower-extremity sensation coupled with impaired peripheral vascular function can contribute to lower-extremity (commonly foot) ulceration [14].

Although it is clear that diabetes micro vascular complications result from the abnormal metabolic environment engendered by chronic hyperglycemia the actual development of these complications in any individual appeared to be a cumulative function of many factors that specifically affected their occurrence [15]. In order that timely treatment can be given, it is essential that patients at risk for the development of diabetic microvascular complications are identified earlier. Effective evaluation and monitoring of these complications in clinical practice is clearly important, however, it is also important to predict these complications depending on several predictors that might lead to primary prevention of these complications, together with retardation of their progression by tight control of these predictors' "risk factors".

A review of the literature has shown several risk factors (e.g. diabetes duration and glycemic control, blood pressure, ….etc) have consistently been shown to correlate with diabetic retinopathy, neuropathy, and nephropathy, and actually these complications develop and progress in unison and indeed share many common risk factors, but to date, the relationship and the mutual action of these

factors has not been clearly described [16], raised the question that presence or absence of these risk factors simultaneously in diabetic patient might play a summation rule in the pathogenesis of these complication as the role of these risk factors is necessary but not sufficient. This approach appears to be much stronger and beneficial for complications prediction than the sparse of risk factors as in most of time two or more of these factors are existed [17].

The prevalence of microvascular complications has been previously reported in Iraq and was high among diabetic in specialized diabetic centers and in outpatients referring to general hospitals [4,17,18,19], but we did not find any article which assess the risk factors for these complications.

It is essential however, that risk factors associated with the progression and development of diabetic micro vascular complications are detected and treated at an early stage in order to further reduce morbidity and mortality. So, the aim of this study was to determine the main risk factors that associated with micro vascular complications in diabetic patients aiming to construct a module that can predict these complications.

Methods

This was a cross sectional descriptive study set up in a university hospital (Al-Kindy Teaching Hospital) and a diabetic-specialized center (The Specialized Center for Endocrinology and Diabetes 'SCED')- Baghdad, that lasted 2 years (November 2008 – October 2010). The SCED is a referral centre for patients from the greater Baghdad area. The objective of this center is to closely monitor patients to ensure nearly normal life and early detection of any complication of diabetes or other endocrinology disorders. The study was approved by the Al-Kindy Medical College Council and the authority of SCED.

The Study population was diabetic patients attending the above center. The diagnosis was fully established by the specialists in the center and each patient had a file that contains the medical and sociodemographic information. Each registered diabetic patient is supposed to visit the center at least once every month, to be followed up by the caring physician, examined for any complaint or complication, tested for blood sugar, receive his/her drugs or be referred to the hospital for check-up if needed.

The inclusion criteria were patients with DM (type 1 and 2), had recruited in the center for more than one year, with age of 30 years or more, and had disease duration for more than five years. Patient should have file with complete information and regular visits.

Exclusion criteria included gestational diabetes, incomplete laboratory data and follow-up visits requested over the last two years.

The study sample was a convenience one of 364 patients recruited from the above center fulfilled the inclusion criteria.

Data collection:

After explaining the objectives of the study to the patient and taking their verbal consent, the data were collected from the patients and their files by using specially constructed questionnaire. The data obtained about our predictors (risk factors) were age, sex, weight, height, smoking history, type and duration of DM, family history for DM, level of physical activity, presence of uncontrolled hyperglycemia, hypertension, dyslipidemia, microvascular and macrovascular complications.

Dichotomous predictor variables were used to note occurrence of these predictors. We classified and coded the values of variables as 0 and 1. This dichotomous verification is useful for both dummy variables in logistic regression analysis and in interpretation of odds ratio (OR). Age was classified into 30-49 years group (coded 0) and ≥ 50 years' group (coded 1). Sex was marked as 0 for female and 1 for male, type 1 DM was coded as 0 and type 2 as 1. Duration of diabetes in years from time of diagnosis to

enrolment was calculated. Patients with DM of 5-10 years duration were coded as 0 and those with more than 10 years were coded as 1.

Physical activity determination was based on the reported average leisure physical activity per week [20], and classified as active (coded 0), and inactive (coded 1).

Regarding Smoking habit, patients were classified as not smokers (either never smokers or ceased smoking before two years, coded as 0), and smokers (either current smoker, or ex smokers and ceased smoking before less than two years, coded as 1). Patients with no family history of DM were coded as 0 and those with such history coded as 1.

Body mass index (BMI) was calculated for each patient as weight (kg) divided by height squared (meter^2) and was used as the criteria for diagnosis of obesity. Participants with BMI < 30 kg/m^2 considered not obese and coded as 0, while those with BMI ≥ 30 kg/m^2 considered obese and coded as 1 [21].

Presence or absence of uncontrolled hyperglycemia was assessed by measurement HbA1c % level. Measurements for the last 2 years was added together and divided by the number of times were done to get the mean of HbA1c % level (average HbA1c level during 2009-2010).

Patients with mean HbA1c % level 6.5% or less were considered to be with controlled hyperglycemia and coded with 0, and those with level more than 6.5% was considered to be with uncontrolled hyperglycemia and coded with 1 [22].

Definitions Patients were considered hypertensive if already diagnosed and receiving antihypertensive medications. While the criteria for dyslipidemia were according to National Cholesterol Education Program adult treatment panel guidelines. Patients with cholesterol≥ 200 mg/dl, triglycerides > 150 mg/dl, low-density lipoprotein cholesterol (LDL-C) ≥ 160 mg/dl and high-density lipoprotein cholesterol (HDL-C) ≤ 40 mg/dL in the last two years were defined as having dyslipidemia [23].

To assess the presence of diabetic microvascular complications, patients with well established complication were denoted for these complications. Neuropathy was assessed by neurologist through history of symptoms provided by the patient and physical examination with evidence of bilateral decreased pressure sensation by monofilament test. Retinopathy was based on a dilated eye examination by a retinal specialist; moderate to severe non proliferative or proliferative retinopathies were included. Nephropathy was defined as a urinary albumin rate equal or above 300 mg/24 hr in at least two out of three consecutive samples. Patient appeared to have two of these complications were restricted to the one that detected first. To avoid data duplication, Patients with more than two micro vascular complications were restricted to the oldest one [24,25,26].

Macrovascular complications were provided by the patient history and review of the medical chart. A subject was considered to have coronary artery disease (CAD) if there was history of a coronary event including angina, myocardial infarction, cardiovascular intervention, while cerebrovascular disease (CVD) included history of transient ischemic attack or stroke diagnosed by a physician regardless of absence of residual neurological deficit on physical examination [27].

Statistical analysis:

Data were entered and analyzed by MINI TAB software version 14. Statistical analysis was done after examining bivariate associations of predictors and outcomes, Chi square test was used to estimate the association between predictors and outcomes and $p < 0.01$ was required to identify the risk factor as predictor to be included in the logistic equation. So, only the variables that had shown statistically significant p-value when examined individually

were allowed to enter the model. Separate stepwise logistic regression models were conducted in the derivation data set to build the best model to predict each complication depending on associated risk factors predictors. Coefficients for significant predictors were applied to predictor values of the validation data set members. Risk scores for each factor were calculated by summing coefficients across all predictors. We used odds ratio (OR) to find the degree of association of each predictors with absence or presence of risk factors while keeping other constant.

Results

Of the examined 364 diabetic cases, 174 (47.80%) patients were found with microvascular complications. Neuropathy, Nephropathy, Retinopathy were detected in 66 (18.13%), 62 (17.03), and 46 (12.64%) patients, respectively (Table 1). The patients studied were divided into four groups according to the presence of microvascular complications: three with +ve microvascular complications (retinopathy group, nephropathy group, and neuropathy group) and one with -ve microvascular complications group. And when the bivariate analysis was performed to evaluate the predictive qualities for these microvascular complications in cross tabulation with 12 potential risk factors (age, sex, smoking habit, type and duration of DM, family history for DM, level of physical activity, presence of obesity, uncontrolled hyperglycemia, hypertension, dyslipidemia, and macrovascular complications), only six of these risk factors (age, smoking habit, duration of DM, uncontrolled hyperglycemia, hypertension, and macrovascular complications) found to be significantly associated with microvascular complication ($p < 0.01$) as compared with patients with -ve microvascular complications group (Table 1). These six factors were selected to be our predictors and analyze together in logistic regression module. In the stepwise binary logistic regression analysis of these six factors (predictors), the uncontrolled hyperglycemia (measured by HbA1c % level more than 6.5%) found to be the variable with highest association in diabetic group with neuropathy (OR=7.39 and $p = 0.000$). That mean diabetes with uncontrolled hyperglycemia has a risk 7.39-fold greater than that of those with controlled hyperglycemia for neuropathy development after controlling other independent variables. Duration of disease, smoking, age, presence of hypertension and macrovascular complications were following in order with OR values of 5.94, 3.89, 2.86, 1.82, 1.48 respectively (Table 2). In the nephropathy group, the uncontrolled hyperglycemia was also ranking first in prediction with OR = 6.92 and $p = 0.000$, but the subsequent ranking for the other five predictors was duration, age macrovascular complication, hypertension, and smoking with OR values of 5.91, 3.15, 2.36, 2.09, 1.65 respectively (Table 3). The probability that a 30 years old DM patient (male or female) having the disease for more than 10 years, smoker, and presented with HbA1c level > 6.5, HT, and positive macrovascular complications to have retinopathy can be evaluated as follow: -6.024+1.8742 +1.7196 + 1.4728 + (1.1554x0) + 1.0256 + 0.9854 = 1.0539. And by substituting 1.0539 in the equation for the probability ($P_x = 1/1+\exp(-b_0+b_1x_{1+} b_2x_{2+} b_3x_3)$)

Regarding diabetes with retinopathy, duration of the disease (more than 10 years) was the main and number one predictor (OR = 6.52, $p = 0.000$), while uncontrolled hyperglycemia was the second (OR = 5.58, $p = 0.000$). Smoking, age, macrovascular complications, and hypertension were followed in order with OR values of 4.36, 3.18, 2.05, 1.85 respectively.

Table 1. The distribution of the study sample regarding microvascular complications and their potential risk factors

Variable	+ve Microvascular complications n=174						-ve Microvascular complications	Total
	Retinopathy No. (%)	P*	Nephropathy No. (%)	P*	Neuropathy No. (%)	P*	No. (%)	
Frequency	46 (12.64)	-	62 (17.04)	-	66 (18.14)	-	190 (52.20)	364
Gender								
Male	26 (11.40)	0.573	38 (16.67)	0.973	48 (21.05)	0.089	116 (50.88)	228
Female	20 (14.71)		24 (17.65)		18 (13.24)		74 (54.24)	136
Age (years)								
30-49	12 (7.89)	0.000	19 (12.50)	0.000	13 (8.55)	0.000	108 (71.05)	152
≥50	34 (16.04)	S	43 (20.28)	S	53 (25.00)	S	82 (38.68)	212
Type of DM								
1	14 (14.58)	0.233	19 (19.79)	0.173	21 (21.88)	0.115	42 (43.75)	96
2	32 (11.94)		43 (16.05)		45 (16.79)		148 (55.22)	268
Duration of DM								
(years) 5-10	11 (7.54)	0.001	20 (13.70)	0.008	17 (11.64)	0.000	98 (67.12)	146
>10	35 (16.05)	S	42 (19.27)	S	49 (22.48)	S	92 (42.20)	218
Family history of DM								
No	17 (12.23)	0.854	22 (15.83)	0.679	27 (19.42)	0.721	73 (52.52)	139
Yes	29 (12.89)		40 (17.78)		39 (17.33)		117 (52.00)	225
Smoking								
No	13 (7.83)	0.000	18 (10.84)	0.000	23 (13.86)	0.001	112 (67.47)	166
Yes	33 (16.67)	S	44 (22.22)	S	43 (21.72)	S	78 (39.39)	198
Obesity								
No	28 (17.18)	0.762	25 (15.34)	0.861	31 (19.02)	0.446	79 (48.46)	163
Yes	18 (8.96)		37 (18.41)		35 (17.41)		111 (55.22)	201
Physical activity								
Yes	11 (14.10)	0.449	13 (16.67)	0.727	18 (23.08)	0.153	36 (46.15)	78
No	35 (12.24)		49 (17.13)		48 (16.78)		154 (53.85)	286
Uncontrolled hyperglycemia								
No	10 (7.58)	0.001	13 (9.85)	0.000	16 (12.12)	0.000	93 (70.45)	132
Yes	36 (15.52)	S	49 (21.12)	S	50 (21.55)	S	97 (41.81)	232
Hypertension								
No	15 (7.98)	0.000	19 (10.11)	0.000	22 (11.70)	0.000	132 (70.21)	188
Yes	31 (17.61)	S	43 (24.43)	S	44 (25.00)	S	58 (32.96)	176
Dyslipidemia								
No	24 (14.37)	0.841	23 (13.77)	0.066	24 (14.37)	0.047	96 (57.49)	167
Yes	22 (11.17)		39 (19.80)		42 (21.32)		94 (44.72)	197
Macrovasculr complications								
No	14 (7.25)	0.000	17 (8.81)	0.000	19 (9.84)	0.000	143 (74.09)	193
Yes	32 (18.71)	S	45 (26.31)	S	47 (27.49)	S	47 (27.49)	171

**p* value as compared with -ve microvascular complications

S: significant to enter the module

Table 2. Logistic Regression analysis for diabetes with and without neuropathy

Variable	Value	Count
Neuropathy	1	66 (Event)
	0	190
	Total	256

Predictor	Coef	SE Coef	Z	P	Odds ratio	95% CI
Constant	-4.5829	0.6218	-7.73	0.000	-	-
HbA1c level	2.0007	0.3919	5.11	0.000	7.39	3.43-15.94
Duration	1.7810	0.4177	4.26	0.000	5.94	2.62-13.46
Smoking	1.3583	0.36.38	3.69	0.000	3.89	1.89-8.01
Age	0.2821	0.3652	2.56	0.004	2.86	1.56-4.34
Hypertension	0.7335	0.4091	1.98	0.004	1.82	1.59-6.67
Macrovascular	0.1809	0.3982	1.64	0.006	1.48	1.02-7.82

Table 3. Logistic Regression analysis for diabetes with and without nephropathy

Variable	Value	Count
Nephropathy	1	62 (Event)
	0	190
	Total	252

Predictor	Coef	SE Coef	Z	P	Odds ratio	95% CI
Constant	-5.5537	0.8774	-6.33	0.000	-	-
Hb%1c level	1.9344	0.7883	2.45	0.004	6.92	1.48-22.44
Duration	1.7767	0.4320	4.11	0.000	5.91	2.53-13.78
Age	1.1488	0.4426	2.60	0.009	3.15	1.3 2-7.51
Macrovasc	0.8584	0.3938	2.18	0.029	2.36	1.09-5.10
Hypertension	0.8365	0.5337	1. 80	0.049	2.09	0.89-4.89
Smoking	0.9682	0.5909	1.24	0.031	1.65	1.03-4.56

Tabale 4. Logistic Regression analysis for diabetes with and without retinopathy

Variable	Value	Count
Retinopathy	1	46 (Event)
	0	190
	Total	236

Predictor	Coef	SE Coef	Z	P	Odds ratio	95% CI
Constant	-6.024	1.034	-5.82	0.000	-	-
Duration	1.8742	0.5925	3.16	0.002	6.52	2.04-16.81
Hb%1c level	1.7196	0.7002	2.15	0.032	5.58	1.16-18.79
Smoking	1.4728	0.4532	3.25	0.001	4.36	1.79-10.60
Age	1.1554	0.5804	1.99	0.037	3.18	1.02-9.90
Macrovasc	1.0256	0.6340	1.86	0.038	2.05	1.55-6.78
hypertension	0.9854	0.5450	1.18	0.045	1.85	1.24-4.43

Discussion

Diabetic microvascular complications develop sooner or later in most people with type 1 or type 2 diabetes and are associated with clinically significant morbidity and mortality. Individuals may be susceptible to microvascular complications due to many factors such as uncontrolled hyperglycemia, age, disease duration …. etc. However, it has been found that subset of risk factors in patients may give rise to one type of microvascular complications differs from other subsets [28]. Predictors of outcome in critical care are well described and they include clinical, diagnostic, and physiologic factors [29]. To our knowledge, this study is the first that focused on the problem of prediction and avoidability, and thus on the quality of care-related issues in Iraq. This type of study required the involvement of a large number of patients, reflecting different practice styles, so we included 364 consecutive diabetic patients. Furthermore, patients were enrolled from main diabetic center in Baghdad, making the results more generalized to handle the clinical predictors and facilitate the prediction of microvascular complications in our diabetes management.

The results found only six probable risk factors associated with microvascular complications, uncontrolled hyperglycemia was the main factor in neuropathy and nephropathy groups followed by DM duration. But the duration of DM was the first risk factor in retinopathy group. This finding agrees with the results from the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) which convincingly demonstrated the importance of glycemic control for the prevention of microvascular complications of diabetes [30,31]. However, these studies highlighted the importance of other modifiable risk factors such as hypertension, smoking, and presence of macrovascular complications besides the age factor, which is non- modifiable risk factor that may all play a part.

The study showed that smoking is an important predictor for neuropathy, retinopathy, and to a lesser extent nephropathy. This result is surprising as one would assume that hypertension would be more predicting in DM patients for microvascular complication than smoking, but the results of this study found that smoking was associated with neuropathy and retinopathy, but not nephropathy, more than hypertension (In neuropathy group, the OR for smoking was 3.8 versus 1.8 for hypertension, In retinopathy group, the OR for smoking was 4.36 versus 1.85 for hypertension, while in nephropathy group, the OR was 2.09 for hypertension versus 1.65 for smoking). The harmful effects of smoking are now well established, and include a substantial increase not only in cardiovascular and peripheral vascular disease, as well as the best known consequences of lung cancer and chronic obstructive pulmonary disease, but also higher rates of both neuropathy and retinopathy which have been well documented [32].

The importance of arterial hypertension and blood pressure levels to development of diabetic microvascular disease has also been demonstrated in previous studies for the microvascular as well as for macrovascular complications. Tight blood pressure control in patients with hypertension and diabetes achieves a clinically important reduction in the risk of deaths related to diabetes and its complications [33,34].The largest and most comprehensive was the UKPDS, which showed that improved metabolic control and tight blood pressure control reduced the risk of microvascular complication development [35]. In this study, hypertension was important predictors for all diabetic microvascular complications but in different strength. The highest was with retinopathy and the lowest was with neuropathy.

Macrovascular complications, especially CVD are the primary cause of death in people with either type 1 or type 2 diabetes [36]. Although the precise mechanisms through which diabetes increases the likelihood of atherosclerotic plaque formation are not completely defined, the association between the two is profound [37]. And this might explain the existence of both macrovascular and microvascular complications in the same diabetic patient and the presence of complication can predict the other. Other surprising results in this study that neither BMI nor physical activity and dyslipidemia were associated significantly with microvascular complications although many studies had showed this association [38,39]. There are might be many possible explanations for this observation as the social and cultural style of our community differ from that in other communities. The prevalence of obesity as well as dyslipidemia were also high in diabetic group with -ve microvascular complications, but regarding dyslipidemia, the most obvious potential explanation is that quarterly measurement of the lipid profiles in the SCDE did not capture the actual criteria for dyslipidemia. Logistic regression is one of the important methods to perform the statistical modules in epidemiological and medical research It allows the investigators to examine the relationship between a binary dependent variable and a set of continuous and/or discrete independent variables [40]. One advantage of logistic regression analysis is that it requires no assumption about the distribution of the independent variables. Another is that the regression coefficient can be interrupted in term of odd ratio (OR). In other words, the OR of retinopathy in DM patients with disease duration more than 10 years is 6.52 (Table 2). And the OR for those patients with less than 10 years duration is the reciprocal, 1/6.52=0.15; therefore diabetic patient with disease duration of more than 10 years is about six times more likely to have retinopathy than other DM patient with less than 10 years duration after controlling the other important predicting factors. The same interpretations are giving to other predicting factors (HbA1c level, age, smoking, hypertension, and macrovascular complications).

From other hand, the logistic equation can be used to find the probability for any giving DM patient. For example, the probability that a 30 years old DM patient (male or female) having the disease for more than 10 years, smoker, and presented with HbA1c level > 6.5, HT, and positive macrovascular complications to have retinopathy can be evaluated (table 4) [41].the probability result is 0.49. Therefore the chance that this patient has retinopathy is about 49%. In the same manner we can calculate the chance for nephropathy (from table 3) which is 46% and the chance for neuropathy (from table 2) which is 41%.

In summery micro vascular complications are prevalent among diabetes. Earlier diagnosis and improved management of multiple potential risk factors together with the introduction of novel more predictive module may limit their development and progression. Effective evaluation and monitoring of these predictors in clinical practice is clearly important, however, it is also relevant to health education development program addressing the role of these predictors in development of microvascular complications among diabetic patient.

Recommendation

Earlier diagnosis and improved management of multiple potential risk factors together with the introduction of novel more predictive module may limit their development and progression. Effective evaluation and monitoring of these predictors in clinical practice is clearly important, however, it is also relevant to health education development program addressing the

role of these predictors in development of micro vascular complications among diabetic patient.

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Correspondence to: Dr. Yousif AR Al Ani
E-mail: yousifkindi@yahoo.com
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Evaluation of Progesterone and Estradiol in Sera and Tissue of Thyroid Patient

Husam AK Ahmed *FRCS*

Dept. of Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Thyroid cancer is more common in subjects with blood group A and O. Estrogenic receptors in some cases of goiter were observed. Obesity is associated with increased risk of thyroid carcinoma possibly by mechanism of production of estrogenic steroids by adipose tissue.

Objective This study was done to determine and compare the progesterone and estradiol in sera and tissue of female patients with various thyroid disorders.

Methods In this prospective study, Serum and thyroid tissue homogenate were analyzed by measuring progesterone and estradiol in fifty normal healthy women volunteers as a control, in addition to ten patients with malignant thyroid nodules proved by histopathology, and thirty seven patients with benign thyroid nodules from March 2008 to August 2009 in Al Kadimiya teaching hospital and Al Dirgham private hospital in Baghdad.

Results Serum estradiol and progesterone levels for both malignant and benign thyroid nodules patients were less than noticed in healthy control, while tissue estradiol and progesterone levels in malignant thyroid tumor were significantly higher than those in benign thyroid nodules.

Conclusion Tissue estradiol and progesterone levels can be used in the diagnosis and differentiation between malignant and benign thyroid nodules.

Key word progesterone, estradiol, Thyroid nodules

Introduction

The thyroid gland secretes two significant hormones, thyroxin and triiodothyroxine, commonly called T3 and T4. The thyroid secretion is controlled primarily by thyroid stimulating hormone (TSH), (TSH) secreted by the anterior pituitary gland which in turn is under control of thyrotropine releasing hormone secreted by hypothalamus (TRH) [1]

Iraq is an endemic goiter area [2, 3]. Simple colloid goiter is the most common pathology and multinodular goiter is the commonest. Many factors involved mainly environmental, host factors and iodine deficiency [4]. In malignancy, chronic TSH stimulation is held to predispose to neoplastic changes [3].

Body weight is directly proportional; obesity by itself is associated with increased risk of thyroid carcinoma [5] possibly by mechanism of production of estrogenic steroids by adipose tissue. Recently they found the presence of estrogenic receptors in some cases of goiter, in addition to similarities of goiter. [6].

Regularly the type of cancer of thyroid, Follicular carcinoma is more than papilloma cancer [6, 7] and blood group A and O are more affected [8].

Estrogen are secreted by placenta in the pregnant woman, while in non pregnant woman,

ovaries are responsible for secretion of estrogen. Progesterone is secreted in significant amount only during the latter half of each ovarian cycle by the corpus luteum [9].

The aim of this study is to evaluate the progesterone and estradiol in sera and tissue of patient with various thyroid disorders.

Methods

Sera of fifty normal healthy women volunteers as a control, fifty nodules were taken from ten patients with malignant thyroid nodules proved by histopathology, and thirty seven patients with benign thyroid nodules were taken. The patients were admitted to al Kadimiya teaching hospital and Dirgham private hospital –Baghdad, from March 2008-August 2009.

The thyroid tumors tissues were immediately immersed in ice-cold saline solution after recording their dimensions, types and localized their positions in thyroid gland. Tissue samples were kept at -20 ^{o}C before processing up to two weeks. They were weighted and sliced with clean scalpel in Petri dish standing on ice. Slices were thawed and minced with the scissors then homogenized with 0.02M Tris buffer pH 7.4 with a ratio of 1:3 (W:V) tissue to buffer solution using a mechanical homogenizer [10]. The homogenate was filtered through ten layers of nylon gauze and centrifuged at 4 ^{o}C in order to precipitate the remaining intact cells and nuclei at 4000 xg for 30 minutes [11].

Sera and tissue homogenates were analyzed by measuring progesterone and estradiol [12].

Results

The mean plus minus SD of serum estradiol was 66.8±16.43 pg/ml, 57±10.3 pg/ml and 110.7±7.8 pg/ml for malignant, benign and controlled patients respectively. While the results for serum progesterone was 0.35±0.07 ng/ml, 0.4±0.05 ng/ml and 0.27±1.1 ng/ml for malignant, benign and controlled patients respectively.

The values of estradiol in thyroid tissue were 85.07±19.4 pg/ml and 30.7±19.3 pg/ml for malignant and benign thyroid patients respectively. While progesterone level in thyroid tissues was 1.73±0.7 ng/ml and 0.44±0.3 ng/ml in malignant and benign patients respectively.

Discussion

The results presented in this study revealed a significant decrease in serum estradiol and progesterone level in both malignant and benign thyroid tumor patients compared to normal control. This result was agreeing with Kologlu et al [13] and disagrees with Son et al [14].

In this study, the estradiol and progesterone level in thyroid tissues was significantly increased in malignant thyroid tumors when compared with benign thyroid nodules. This indicates that tissue estradiol and progesterone level may be used in the diagnosis and differentiation between malignant and benign nodules. It is known that no other authors have studied human thyroid nodules estradiol and progesterone level.

Conclusion

Estimation of estradiol and progesterone level in thyroid tumor tissues can be used in both diagnosis & differentiation between malignant and benign tumors.

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Correspondence to Dr. Husam AK Ahmed
E-mail: dr_hussam44@yahoo.com

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The Effects of Dexamethasone on the Histology and Histochemistry of Thyroid Gland in Female Rabbits

Khalida I Shaya *MSc*
Dept. Anatomy, College of Medicine, Al-Mustansiriya University.

Abstract

Background The adverse effects of corticosteroids are widely recognized but there are few qualitative data on which they adversely act on the tissue of thyroid gland, in this paper we scrutinize how these corticosteroids affect the thyroid tissues.

Objective To investigate the histological and histochemical changes, due to the effects of dexamethasone sodium phosphate, in the thyroid gland of female rabbits using a light microscope.

Methods Two groups, each one with seven female rabbits were used in this study. The control group received 0.9% saline solution intramuscularly and the treated group received a daily intramuscular injection of dexamethasone sodium phosphate (1.5 mg/kg b.w.) for 15 days, the thyroid glands obtained from these animals were dehydrated, cleared and embedded in paraffin and then sectioned and stained by Haematoxylline and Eosine and histochemically were stained by periodic acid- Schiff reagent, periodic acid- Schiff reagent with enzyme diastase, Toludine blue and Masson's trichrome.

Results Marked changes were observed in the thyroid glands treated with dexamethasone. Histolgically, these changes include a decrease in the height of the follicular cells to become low cuboidal and even squamous, and the follicles distended with colloid accumulation. These changes affected both central as well as peripheral follicles. Histochemically, the thyroid follicles showed a low positive reaction to glycoprotein which might indicate a decrease in the activity of the follicular cells of dexamethasone treated thyroid glands.

Conclusion Dexamethasone causes morphological changes in the thyroid gland consistent with a decrease in thyroid activity and is considered as side effects of this drug.

Key words Dexamethasone, histochemistry of thyroid gland, rabbits

Introduction

A host of pharmacological agents can influence the function and activity of thyroid gland, and among these agents are the glucocorticoids [5,9,24]. Michael *et al.* (1976) [22] observed that serum T_3 decrease significantly 24 hours following administration of dexamethasone in euthyroid subjects and there was little further reduction in serum T_3 with continued dexamethasone administration. However, T_3 briskly rose and it overshot within 48 hours after dexamethasone withdrawal.

In 1977, Westegren and coworkers [28] observed that dexamethasone may partially divert the deiodination of T_4 from the activating T_4 to T_3 to the inactivating T_4 to rT_3 (reverse T_3) pathway.

Maes *et al.* (1990) [19] found that dexamethasone has a pronounced suppressive effect on basal TSH and free T_3 levels. It has significant stimulating effect on rT_3 levels.

The effects of multiple injections of prednisone on thyroid morphology and on plasma T_4 and T_3 concentration in dogs were determined by Woltz *et al.* (1983) [29], plasma T_4 and T_3 concentration decreased significantly after repeated injections of prednisone. Histological examination of thyroid tissue revealed

accumulation of colloid droplets in follicular cell cytoplasm of dogs treated with prednisone. This indicates that prednisone may interfere with basal thyroid hormone secretion by inhibition of lysosomal hydrolysis of colloid in the thyroid follicular cell.

Chopra *et al.* (1975) [4] found that acute administration of glucocorticoids was associated with rapid and persistent decrease in serum concentration of T_3 and less marked decrease in T_4 in Gravis disease patients. However, in hypothyroid patients receiving treatment with synthetic T_4, cortisol causes decrease of T_3 but not accompanied by appreciable decrease in serum T_4 and thyroglobulin. These results suggested that corticosteroids may affect the peripheral conversion of T_4 to T_3. However, the conversion of T_4 to metabolically inactive rT_3 was enhanced.

Loebenstein *et al.* (1983) [16] found that administration of dexamethasone induced a fall in total T_3 and a rise in rT_3 concentration, while the withdrawal of glucocorticoid led to an increase in serum concentration of total T_3 and decrease of serum rT_3, basal plasma TSH concentration was unchanged by glucocorticoid withdrawal and it fell during subsequent dexamethasone therapy.

Methods

Healthy white New Zealand female rabbits weighing between 1000- 1250 grams were used and kept in separate plastic cages and fed *ad- libitum*. The animals were divided into two groups, seven animals in each. The first group was treated daily for 15 days with (1.5 mg/kg b.w.) intramuscular injection of dexamethasone sodium phosphate (ZMC import- export GmbH Germany) in the thigh muscle. The second group considered as a control animals, they received equal amounts of 0.9% saline solution as intramuscular injections.

Twenty- four hours after the last injection, the animals were anaesthetized with chloroform. After dissection of the neck, the two lobes of thyroid gland were removed from the side of superior part of trachea.

The glands were fixed in 10% formaline solution for 24 hrs., dehydrated, cleared, and embedded in paraffin and the blocks obtained were sectioned and stained by Haematoxylline and Eosine stain (H&E), Alcholic periodic acid-Schiff stain (PAS**)**, Alcholic periodic acid- Schiff stain with diastase digestion method (PAS-D**)**, Toluidine blue (TB) and Masson's acid Fuchin Aniline blue Trichrome stain (MT**)**.

Staining methods and techniques were done on the basis of Humason (1972) [12] and Luna (1968) [18].

Results

Histological Study:

Control Group:

The thyroid gland was encapsulated by a moderately thick layer of a well developed connective tissue. From this capsular connective tissue septa extended inward dividing the parenchyma of the gland into lobules which were incompletely separated from each other. So the thyroid gland was not truly lobulated. The interlobular stroma consists mainly of connective tissue, blood vessels, nerve fibers, and a group of cells which not surround follicular lumen (Figure 1a).

The thyroid gland is composed of an aggregation of spherical or oval cyst like follicles or acini of variable sizes, each follicle lined by a secretory epithelium composed of a single layer of cuboidal to low columnar cells with clear cytoplasm and distinct, mostly rounded nuclei, parafollicular cells sometimes were observed between the epithelial cells. The lumen of the thyroid follicles were filled with colloid material which in some follicles was vacuolated at the periphery (Figure 2a).

Generally, two types of follicles were found in the thyroid gland, the follicles located in the periphery of the gland had larger diameter than those in the central part. Furthermore, the peripheral follicles had a small cuboidal epithelial cells compared to those of the central follicles (Figure 3).

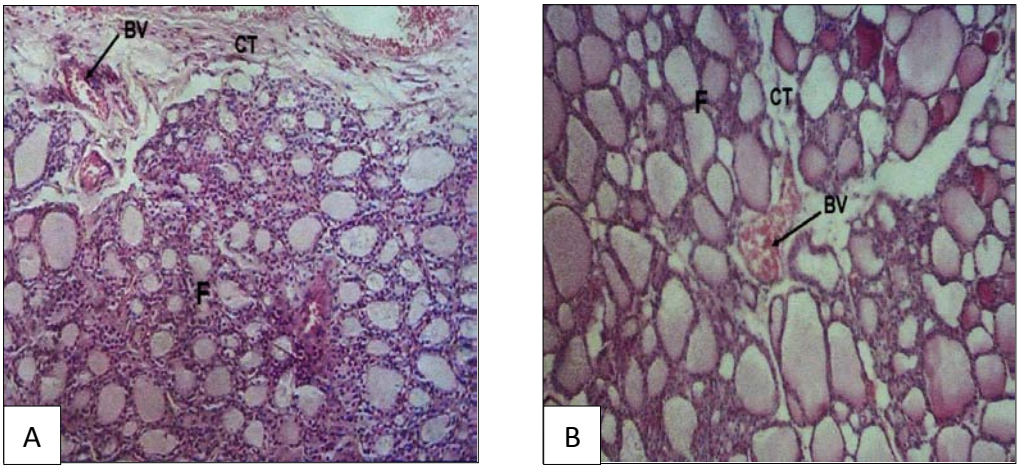

Figure 1. Light micrographs of the thyroid gland section in control (A) and treated (B) rabbits, showing the general structure of the gland. The connective tissue (CT) containing blood vessel (BV) and dividing the glandular tissue into incomplete lobules. H&E (100 X) for A & (150 X) for B.

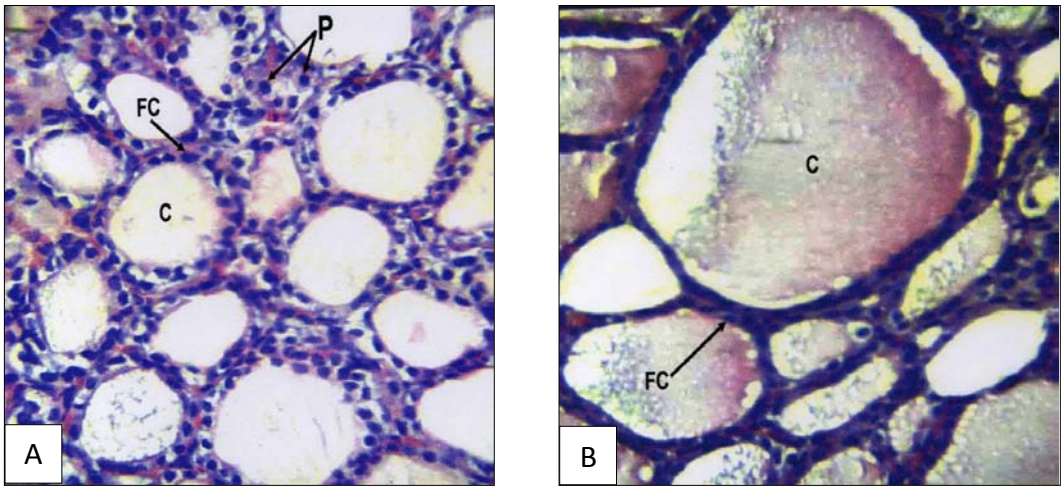

Figure 2. Light micrographs of the thyroid gland section in control (A) and treated (B) rabbits, showing the thyroid follicular cell (FC), colloid (C) and parafollicular cell (P). H&E (450 X) for A & (675 X) for B.

Treated Group:

The histological appearance of the thyroid gland in rabbits treated with dexamethasone sodium phosphate showed changes detected mainly in the follicular components. These changes differed from one animal to another and even from one lobule to another within the same animal. Generally the follicular epithelial cells became low cuboidal or even squamous, the nuclei were mostly flat and the cytoplasm was not distinct. The thyroid follicles were large in most parts of the glands, due to distention with colloid material (Figures 1 and 2b).

Dexamethasone treatment brought about almost same changes in both types of thyroid follicles, peripheral and central follicles (Figure 4).

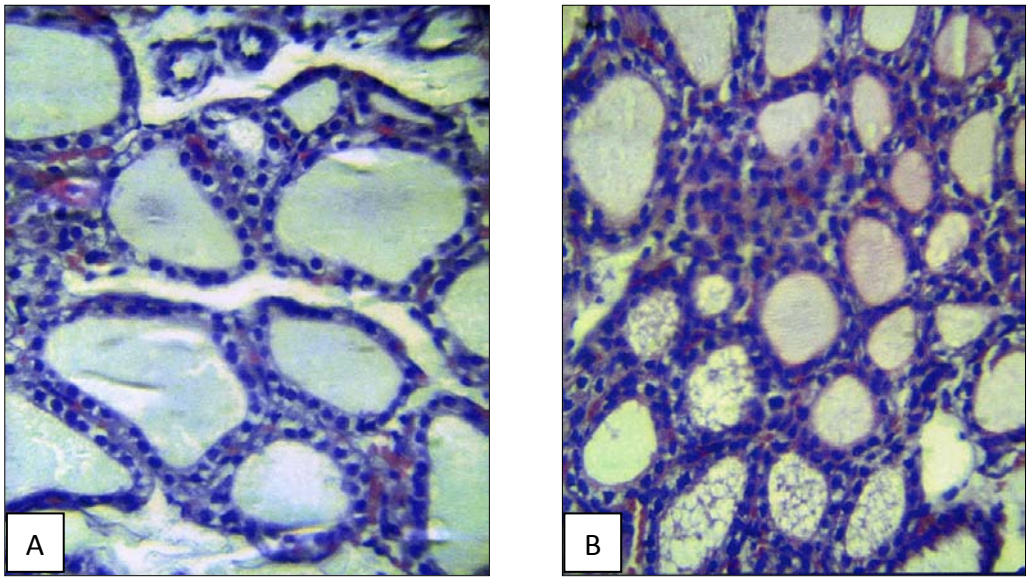

Figure 3. Light micrographs of the thyroid gland section from the peripheral (A) and central (B) areas in control rabbits. H&E (450 X).

Histochemical Study:

Control Group:

The intensity of the stains reaction in thyroid gland was summarized in table (1).

Table 1. The intensity of the stains reaction in the thyroid glands of control and treated rabbits.

Stains	*Control Group*	*Treated Group*
PAS	+++	++
PAS-D	+++	++
TB	-	-
MT	++	+

(+++) strong positive reaction
(++) moderate positive reaction
(-) negative reaction

The thyroid follicles stained strongly with PAS (Figure 5a) and PAS-D (Figure 6a) but they showed no metachromasia with TB (Figure 7a) which indicated that the secretory product of the thyroid follicle is mainly glycoprotein in nature.

Masson's trichrome staining (Figure 8a) showed a good amount of collagen fibers in the interfollicular stroma of thyroid gland.

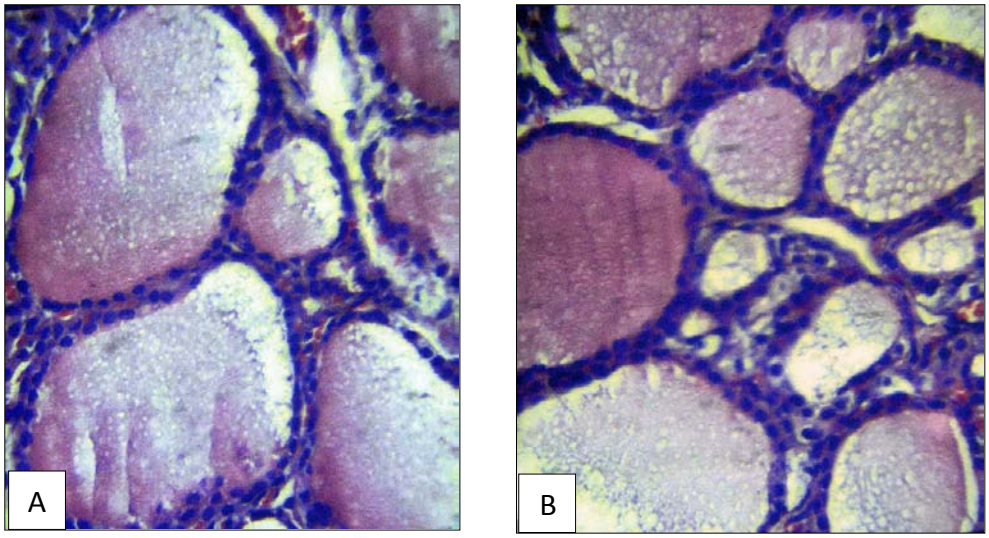

Figure 4. Light micrographs of the thyroid gland section from peripheral (A) and central (B) areas in treated rabbits. H&E (675 X).

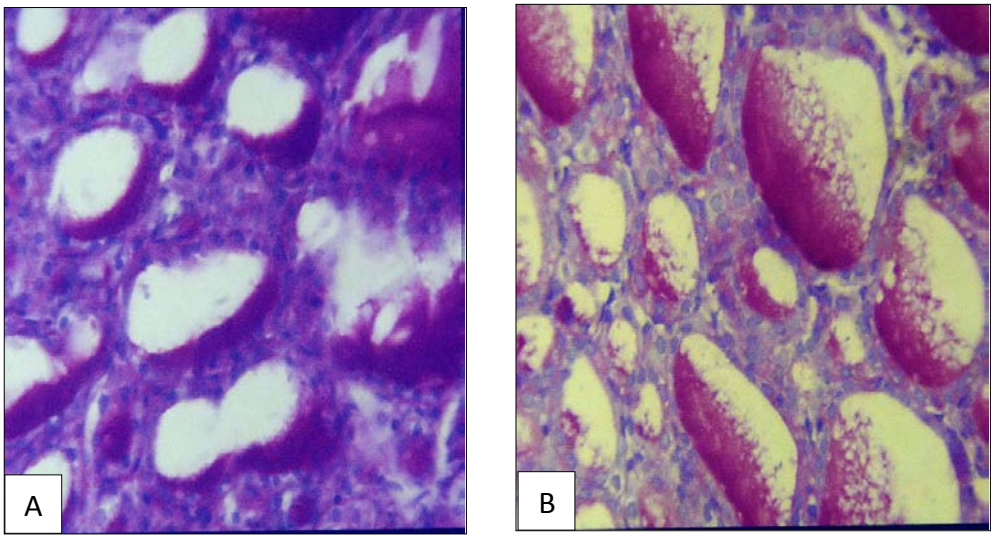

Figure 5. Photomicrographs of control (A) and treated (B) thyroid section stains with PAS. Shows a strongly positive staining of control group and moderately staining of treated group. (450 X).

Treated Group:

Intensity of the stains reaction in the thyroid glands of dexamethasone treated animal was summarized in table (1).

The thyroid follicles stained moderately with PAS (Figure 5b) and PAS-D (Figure 6b) while TB showed no metachromasia (Figure 7b). This indicates that the secretory product (glycoprotein) of the gland was reduced.

Masson's tri chrome (Figure 8b) staining showed that collagen fiber in the interfollicular stroma in the thyroid gland of treated animal is much less than that of control group.

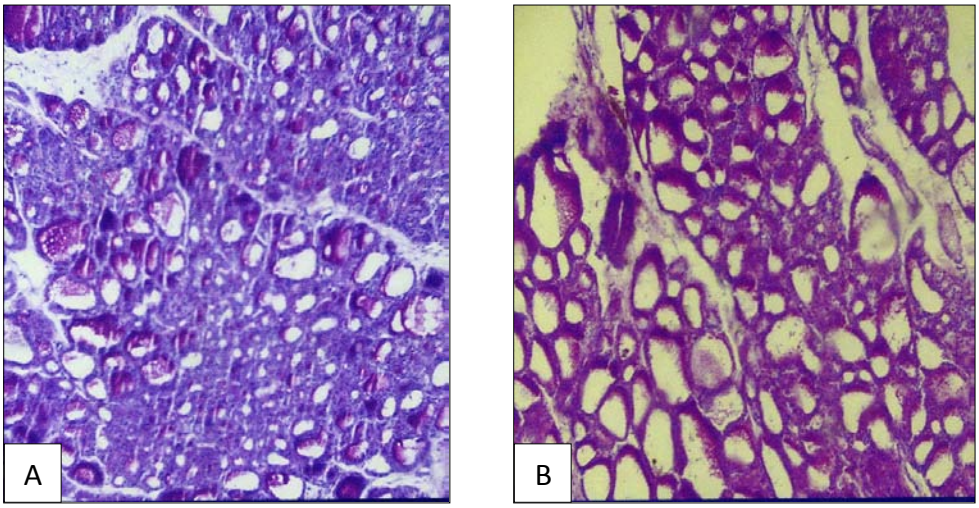

Figure 6. Photomicrographs of control (A) and treated (B) thyroid sections stains with PAS-D. Shows a strong positive staining of control group and moderate staining of treated group. (100 X).

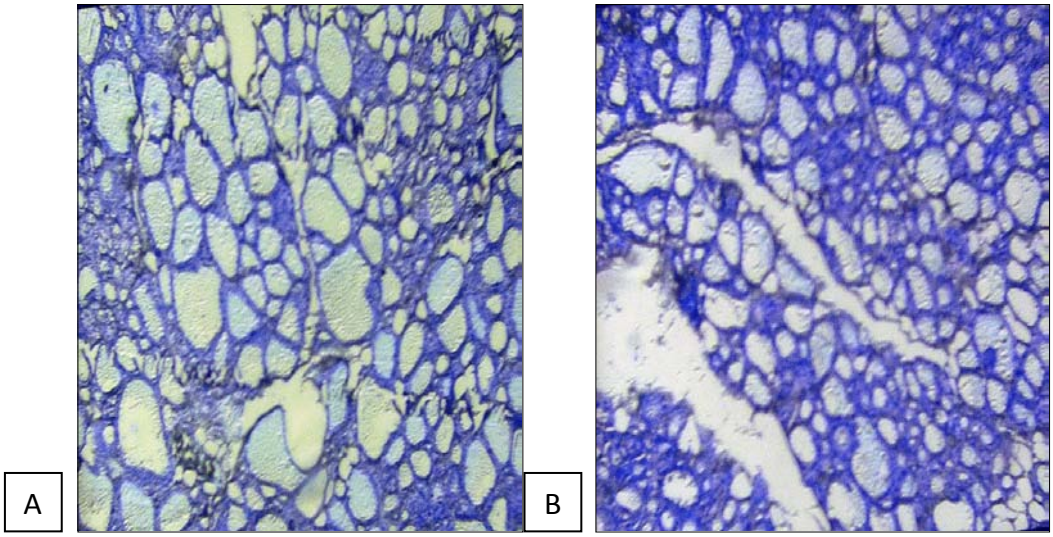

Figure 7. Photomicrographs of control (A) and treated (B) thyroid section stained with TB. Show no metachromasia of both groups. (100 X).

Discussion

Control Group:

The histological appearance of the control thyroid gland obtained in this study were similar to that described by other authors on the rat thyroid gland [20], on the mouse thyroid gland [7], and on dolphin thyroid gland [11]. Harach (1987) [10] described mixed follicles in human which are composed from squamous like epithelium and the lumen of these follicles contains an esonophilic and positive colloid like material which contains acid mucins and cell debris.

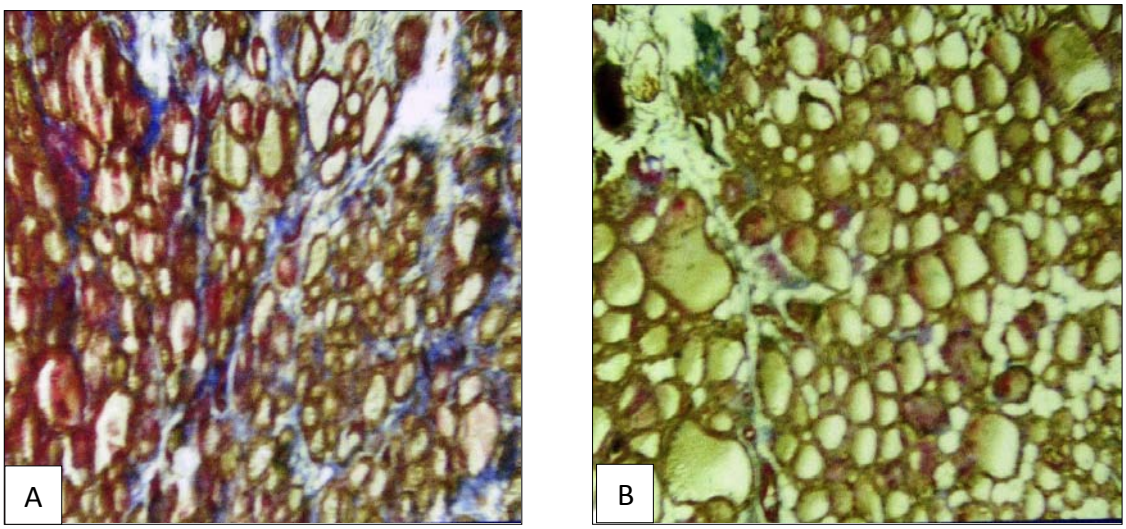

Figure 8. Photomicrographs of control (A) and treated (B) thyroid section stained with Masson's Trichrome. Showing a decrease amount of the stroma of treated group. (100 X).

In the present study, the parafollicular cell or C-cells which are large polygonal cells with clear cytoplasm were observed in a few number scattered between the follicular cell or in the interfollicular stroma. These cells are also described in dolphin thyroid gland [(11)], and on human thyroid gland [(3)].

Two types of follicles were found in rabbit thyroid gland, the follicles which are found in the periphery of the gland are larger than those found in the center. Furthermore, the peripheral follicles had a small cuboidal cells and large lumen as compared to the central follicles. These findings were in agreement with Maiti (1980) [(20)] who divided the follicle of rat thyroid gland into peripheral and central. Low (1988) [(17)] showed that in rabbit thyroid gland the larger follicles were found in the outer regions with low epithelial cells.

Our histochemical investigations showed that the colloid of the thyroid follicles react strongly with PAS and PAS-D. This finding suggested that the secretory products of follicular cells which are stored in the follicular lumen are mainly glycoprotein in nature. While staining with TB showed a negative reaction (no metachromasia) which indicated that the thyroid follicle might not contain mucopolysacchoride or the content was insufficient to produce a demonstrable staining with this technique. These findings were in agreement with Berndorfer *et al.* (1996) [(2)] who demonstrated that thyroglobuline was a complex molecule of glycoprotein extracellulary stored in the follicular lumen for future liberation of thyroid hormones. Similar observations were found in many other mammalian thyroid glands [(8,11,14)].

Our study also revealed that there was a good amount of collagen fibers in the connective tissue of the interfollicular space which reacts positively with masson's trichrome staining.

Treated Group:

This study showed that treatment of female rabbits with dexamethasone resulted in marked morphological changes in the structure of thyroid gland which might be due to the effect of dexamethasone on the thyroid function. Messer *et al.* (1995) [(21)] observed that the administration of dexamethasone in a large dose was associated with depression of many aspects of the thyroid function.

Although many previous studies indicated numerous dysfunction of the thyroid gland attributed to the treatment by dexamethasone

[1,9], few studies have been carried out to demonstrate histopathological changes in thyroid gland of the dexamethasone treated animals.

The response of thyroid follicles to dexamethasone treatments was not uniform and there were distinct differences in the response from rabbit to rabbit and even from lobule to lobule within a single gland. The thyroid follicles did not respond uniformly to dexamethasone treatments, as shown by the fact that there were areas of thyroid follicles which were indistinguishable from those of the control rabbits.

The general effects of glucocorticoids led to the shrinkage of thyroid follicular cells, the height of these cells decreased so they become low cuboidal or even squamous with flat nuclei. Consequently, it is difficult to point out the occasional presence of C-cell. The thyroid follicles in most cases were large due to distension with colloid. This histological finding may indicate a decrease in the activity of thyroid gland.

In contrast to this study, Kalamars *et al.* (1981) [13] observed that the height of follicular cells increased significantly while the nuclear volume decreased in the thyroid gland of rats that treated with hydrocortisone for one month. This finding might be explained by the observation of Tapioranta (1975) [27] who stated that the effect of glucocorticoid was a dose and time related. In contrast to our study, Sarria *et al.* (1994) [26] showed that adrenalectomy resulted in an increase in percentage and complexity of pituitary thyrotrophic cells which led to an increase in TSH secretion, so the thyroid gland showed some histological evidences of activation.

Assisting our histological study by histochemical investigation which shows an alteration in the intensity of staining of follicular component of thyroid gland in the rabbit treated with dexamethasone. There is a decrease in synthesis of thyroglobulin in follicular cells due to the action of dexamethasone; as a result, there is a decrease in the intensity of PAS and PAS-D. These histochemical results confirmed our histological results in that the secretory activity of follicular cell may be decreased by dexamethasone treatment.

In the present study, we found changes in the intensity of staining of connective tissue. There was a decrease in collagen fibers in the interfollicular spaces thus; there was a decrease in the intensity of connective tissue staining with MT. This finding was in agreement with the finding of Saitoe *et al,* 1997 [25] who stated that glucocorticoid was a fibroblast growth inhibitor factor.

Some compounds such as glucocorticoids have multiple effects and thus influence thyroid physiology at various levels. The physiological doses as well as pharmacological doses of glucocorticoids influenced the thyroid function; their effects were variable and multiple depending on the dose and on the endocrine status of the individual. The type of glucocorticoid and the rout of administration might also influence the magnitude of the effect [6,9,15,23].

From these results, we conclude that glucocorticoids may cause a decrease in the thyroid activity and that both structure and function of thyroid gland are affected by glucocorticoids.

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Correspondence to Dr. Khalida I Shaya
E-mail: kanarsawa@yahoo.com
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Glutathion, Glutathion Reductase and Gama-glutamyl Transferase Biomarkers for Type 2 Diabetes Mellitus and Coronary Heart Disease

Zainab AA Al-Shamma[1] *MSc*, **Hedef D El-Yassin**[2] *PhD*

[1]Dept. of Clinical Pharmacy and Therapeutics, [2]Dept. of Physiological Chemistry, College of Medicine, Baghdad University

Abstract

Background Hypercholesterolemia, one of the major risk factors of atherosclerosis, is a major health problem in the world that enhances the free radical generation in various ways. The level of antioxidants was decreased in hypercholesterolemic patients. This depletion of antioxidants may increase in type 2 diabetic patients with hypercholesterolemia, which also may increase the risk of complications from the most common form of diabetes mellitus.

Objective To evaluate serum reduced glutathione and glutathione reductase as an antioxidant, and gamma-glutamyl transferase as a marker of oxidative stress in both hypercholesterolemic and diabetic-hypercholesterolemic patients.

Methods The study involved 33 diabetic hypercholesterolemic patients, 37 hypercholesterolemic and 54 healthy control subjects. Ten ml of blood were collected from each patient and normal control subject after an overnight fast for the measurement of gluathion (GSH) glutathione reductase (GR) and gamma-glutamyl transferease (GGT), glucose, lipid profile, urea, creatinine and glycated Hb (HbA1c). The last was for the diabetics only.

Result Showed a significant decrease in GSH and GR in diabetic-hypercholesterolemic patients compared with hypercholseterolemic patients and a significant increase in GGT in both groups compared with controls. There was a negative correlation between cholesterol with GR in both groups of patients involved in this study and a negative correlation between HbA1c and each of GSH and GR in the diabetic-hypercholesterolemic patients.

Conclusions High levels of oxidative stress and reduced antioxidants in people with coronary heart disease, previously thought to be markers of the heart condition, could also, indicate a condition of glucose abnormality, such as overt type 2 diabetes.

Key words hypercholestermia, type 2 diabetes, antioxidants, oxidative stress, GGT.

Introduction:

Oxygen free radicals (OFRs) play a significant role in the pathogenesis of many diseases like atherosclerosis, cancer, neuro-degeneration and inflammation. Their production may be greatly enhanced by exogenous factors like environment pollutants, drugs, radiation and pathogens [(1)]. Hypercholesterolemia, one of the major risk factors of atherosclerosis, is a major health problem in world [(2,3)], enhances the free radical generation in various ways [(4)]. Prime targets of OFR attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function [(5)]. Decomposition of peroxidized lipids yields a wide variety of end products. The protective efficiency of antioxidants in hypercholesterolemic patients would depend on the balance between OFR and the availability of antioxidants themselves [(6)]. The

organism's susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defense. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [7-9]. Diabetes is usually accompanied by increased production of free radicals [8,10] or impaired antioxidant defenses. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C [11,12].

The one of major classes of antioxidant enzymes is Glutathione reductase (GR). It is a key enzyme of the antioxidative system that protects cells against free radicals. The enzyme catalyzes the reduction of gutathione (GSSG) to GSH by the NADPH-dependent mechanism. Decreased GSH/GSSG ratio contributes to oxidative stress [13-14]. Due to its important role, this enzyme is more stable than the other cytosolic enzymes and can protect its activity at high temperatures. In the inhibition of GR and disturbance in the cellular prooxidant-antioxidant balance, intracellular GSSG accumulates, and the loss of thiol redox balance may cause loss of cellular homeostasis and numerous diseases [15,16].

Reduced glutathione (GSH) is a major intracellular redox buffer glutathione functions as a direct free-radical scavenger, as a co-substrate for glutathione peroxidase activity, and as a cofactor for many enzymes [17].

The physiological function of Gamma Glutamyl transpeptidase (GGT) enzyme activity as a source of peptide precursors for intracellular GSH re-synthesis, as well as the current clinical concept of its serum activity as the consequence of a compensatory overexpression in response to hepatobiliary dysfunction or alcohol toxic effect, is challenged. The evidence is growing in favor of a detrimental role, Glycated triggering a prooxidant action within the atherosclerotic plaque. Additional investigation would permit the identification among subjects with higher GGT value those with a higher risk of developing clinical disease, allowing definition of the interrelationships with iron metabolism alterations, markers of inflammatory process, of glucose and metabolic disease, and with presence, features, and extent of atherosclerotic vessel disease, to better define the most risky combination for the vulnerable plaque and the best medical strategies for the stabilization of lesions, rather than percutaneous or surgical procedures [18, 19].

Methods

This study included 33 patients with T2DM aged between (30-70) years (19 females and 14 males) and disease duration (1-8 years) with hypercholesterolemia, and 37 patients with hypercholesterolemia alone (23 females and 14 males), who were attending the Diabetic Clinic and Internal Clinic at Al-Kadhymia Teaching Hospital. The patients attended the hospital between (9.00-12.00 am) after an overnight fast. The study also included 54 age-matched normal volunteers (30 female, 23 male) type 1 DM and gestational diabetes were excluded from the study.

Ten milliliters (10 ml) of venous blood were withdrawn from both patients and controls. One milliliter (1 ml) of the blood was added to EDTA tube for HbA1c measurement. The rest of the blood sample was collected in plain tube and centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were divided into aliquots and stored frozen at (4 ˚C) for determination of glutathione reductase (GR) and gamma- glutamyl transferease (GGT). Fasting blood glucose, lipid profile, urea and creatinine were done immediately after separation of the serum by the available routine methods. Glycated hemoglobin (HbA1c) was measured by variant HbA1c program which is based on ion-

exchange high performance liquid chromatography (HPLC). Serum GSH concentration was determined by modified method of Elleman, 1959, serum GR by a kit supplied by Randox laboratories Ltd., and serum GGT by a kit from Human Geseli Schaft fur Biochemica and Diagnostica mbH.

Results

From table 1 there are highly significant differences in the mean values of both groups of patients (diabetic hypercholesterolemic DM+HC and hypercholesterolemic HC) and the control group in the following serum biochemical parameters: cholesterol, TG, LDL-c/HDL-c ratio, GSH, GR, GGT (p < 0.001), while a highly significant difference in the mean values of fasting blood glucose between DM+HC patients and both HC patients and control group is seen.

There are highly significant differences in the mean values of GSH and GR between the diabetic hypercholesterolemic patients (DM+HC) and the hypercholesterolemic patients (HC).

Serum cholesterol correlated negatively with both s.GSH and s.GR in the diabetic hypercholesterolemic patients as shown in figures 1 and 2 respectively and negatively with s.GR and positively with s.GGT in the hyper-cholesterolemic patients as shown in figures 3 and 4 respectively. Figures 5 and 6 shows the presence of negative correlations between HbA1c and antioxidants parameters (s.GSH and s.GR) in diabetic hypercholesterolemic patients.

Table 1. Serum parameters involved in the study of hypercholesterolemic patients (HC) and diabetic hypercholesterolemic (DM+HC) patients as (mean±SD)

Group	HC	DM + HC	C
Glucose (mg/dl)	91.75±12.96	177.72±53.51**†	85.30±13.23
Cholesterol (mg/dl)	263.08±23.66†	265.24±16.64	165.20±23.22
TG (mg/dl)	193.78±82.55†	224.33±72.26†	127.05±31.21
LDH/HDL	5.07±1.25†	5.32±1.07†	2.2±0.64
GSH (mmol/L)	0.33±0.031†	0.30 ±0.042*	0.38±0.02
GR (U/L)	60.39 ±6.03†	54.24±5.75**	70±5.2
GGT (IU/L)	56.75 ±11.0†	58.15±8.30†	27.94 ±3.71
HbA1c %	----------	8.25±0.62	----------

GSH: reduced Glutathion, GR: Glutathion reductase, GGT: Y- glutamyl transpeptidase, HbA1c: glycated Hb.

* Significant at p < 0.05 ** significant at p < 0.001 when DM with HC patients compared to HC patients

† significant at p < 0.001 when HC patients and DM with HC patients were compared to their control subjects.

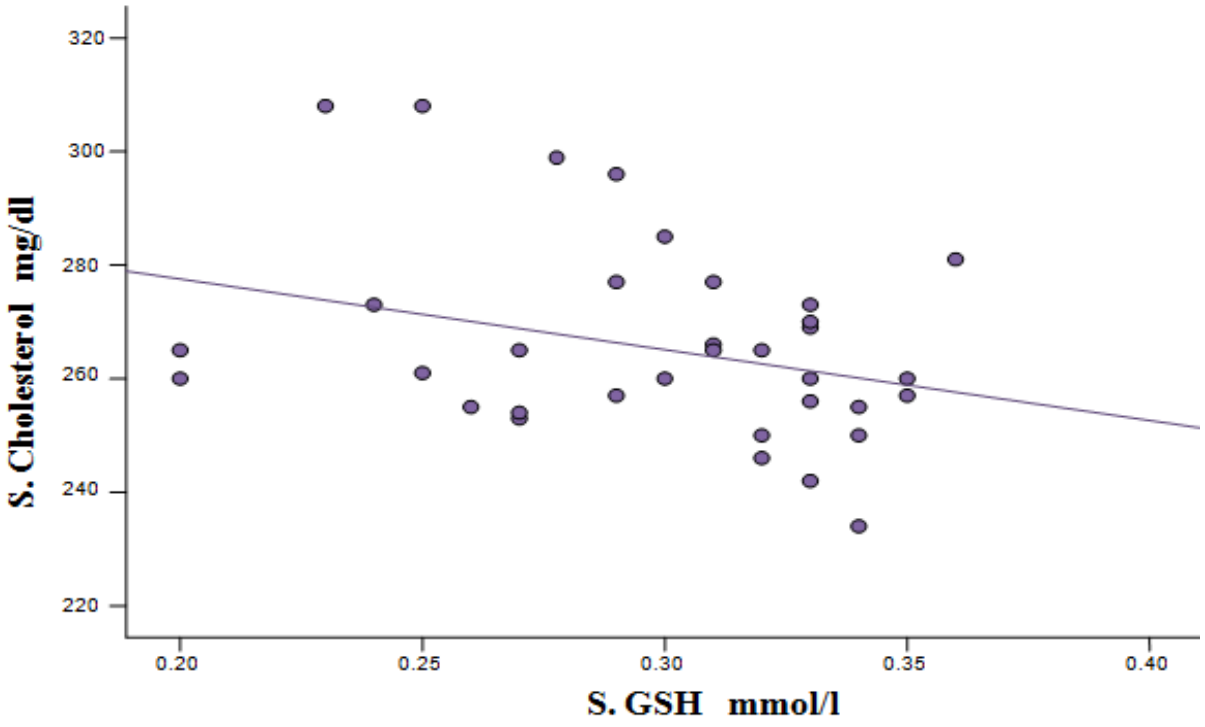

Figure 1. Correlation between serum cholesterol and glutathione (GSH) in diabetic hypercholesterolemic patients (r= -0.34, *p* < 0.05, n= 33).

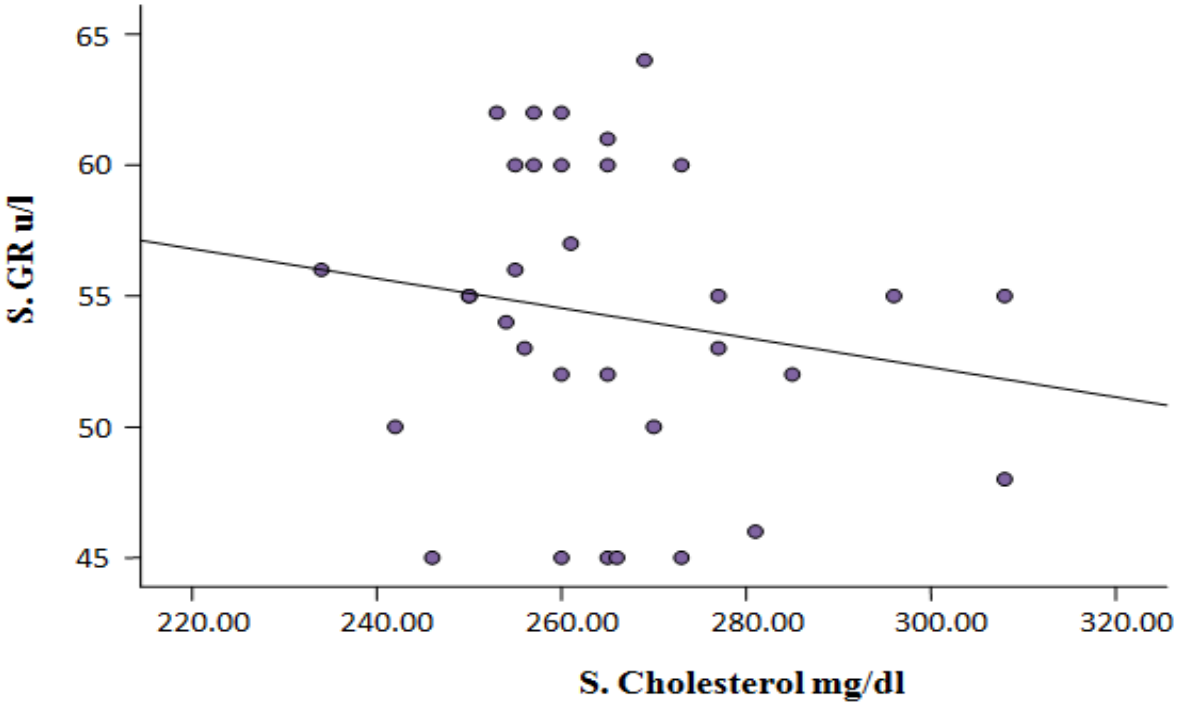

Figure 2. Correlation between S. cholesterol and S. GR in T2DM with hypercholsetermic patients (r= -0.16, *p* < 0.05, n=33)

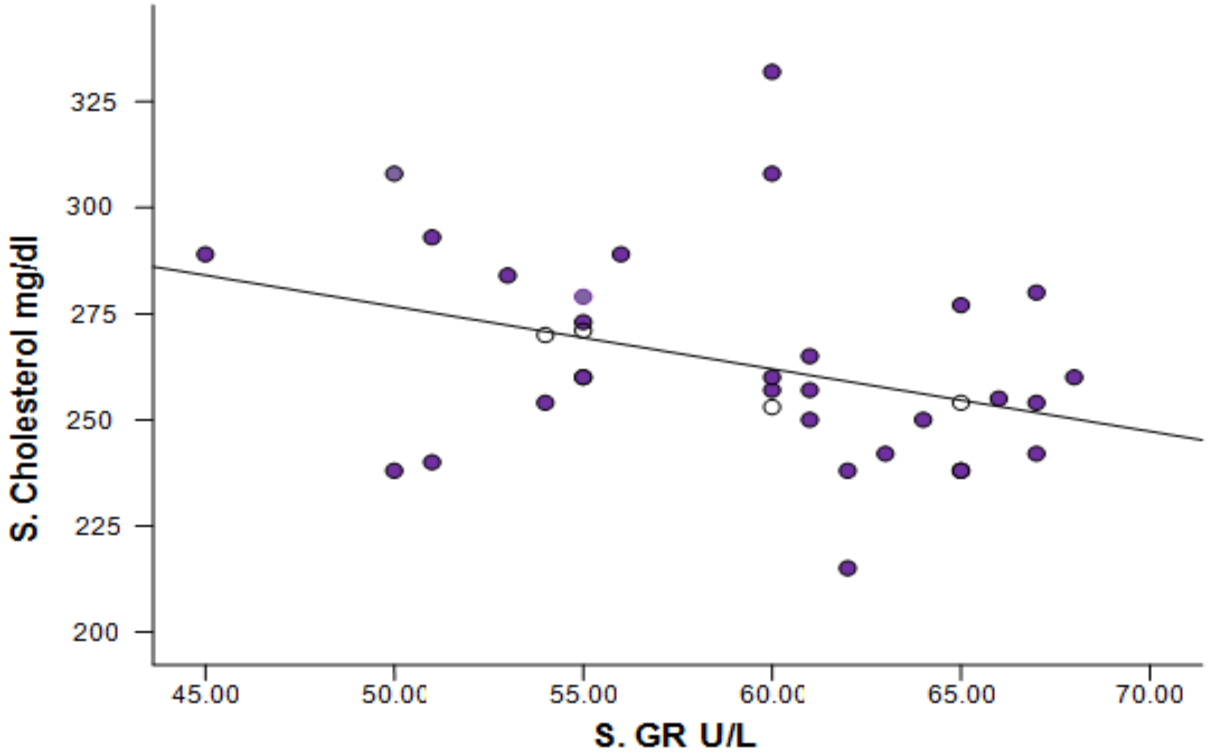

Figure 3. Correlation between S. cholesterol and S. GR in hypercholestrmic patients (r= -0.36, $p < 0.05$, n=37)

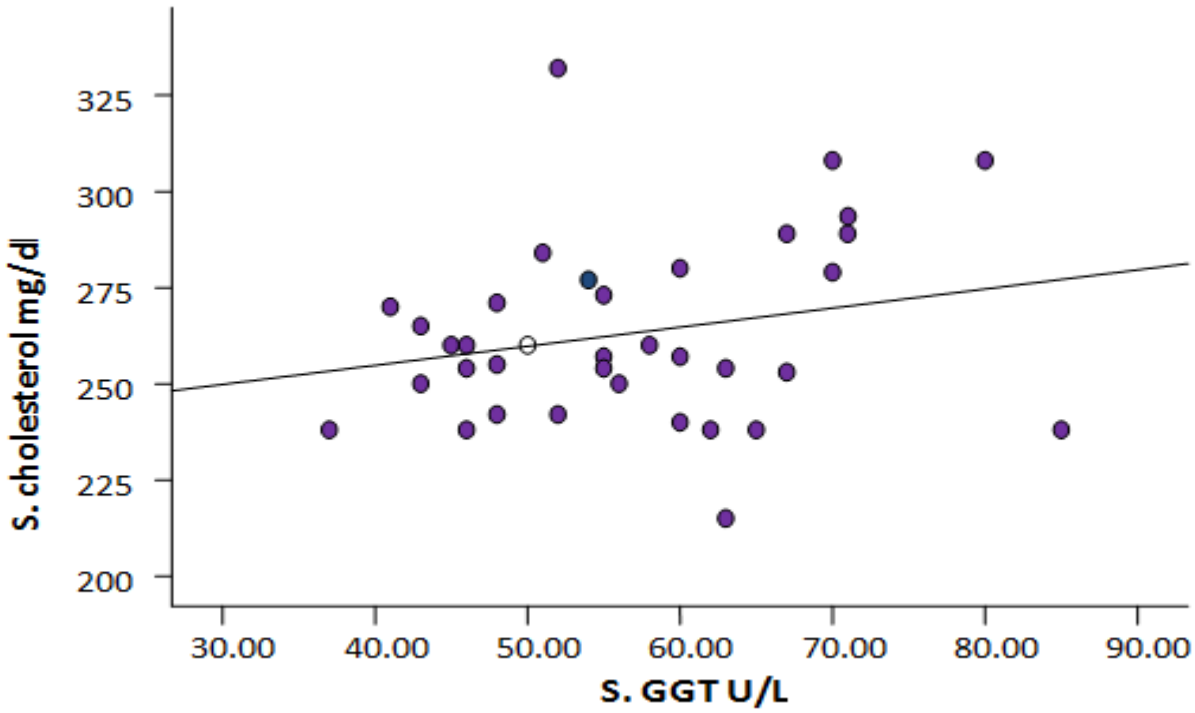

Figure 4. Correlation between S. cholestrol and S. GGT in hypercholsetermic patients (r= 0.23, $p < 0.05$, n= 37)

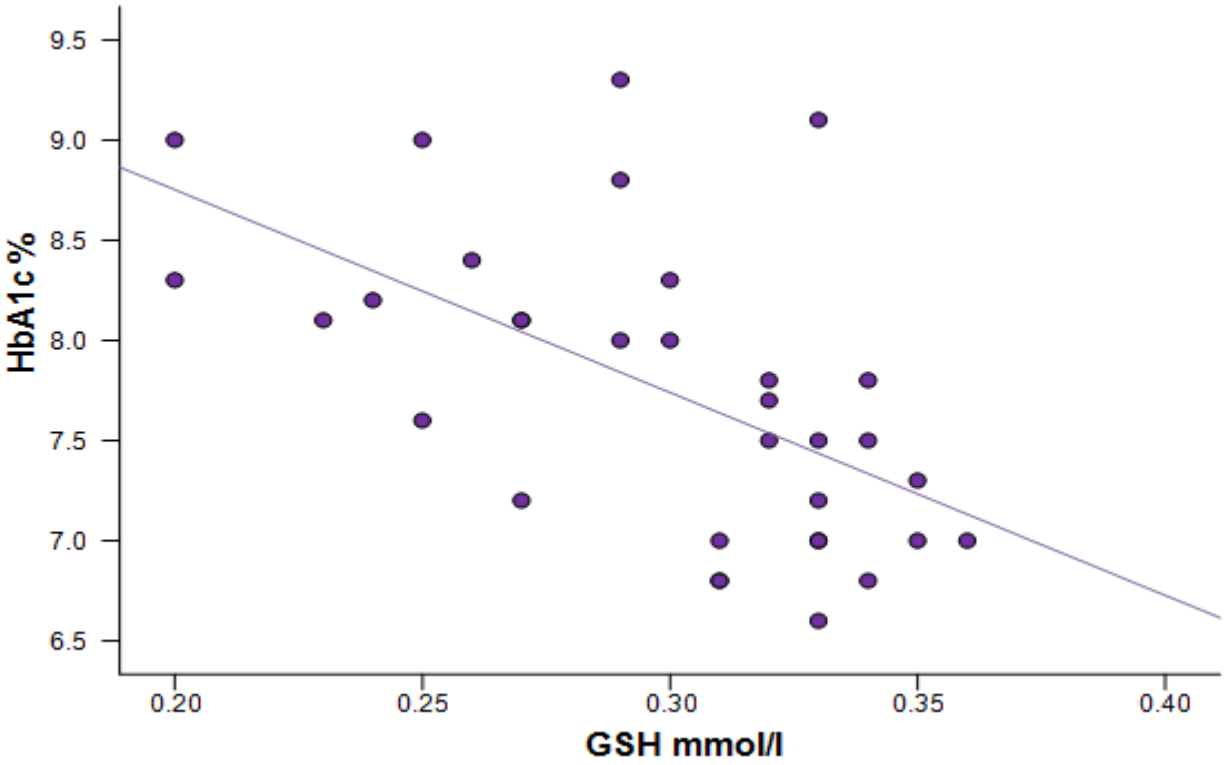

Figure 5. Correlation between serum glutathione (GSH) and HbA1c in diabetic hypercholesterolemic patients (r=-0.35, $p < 0.05$, n=33)

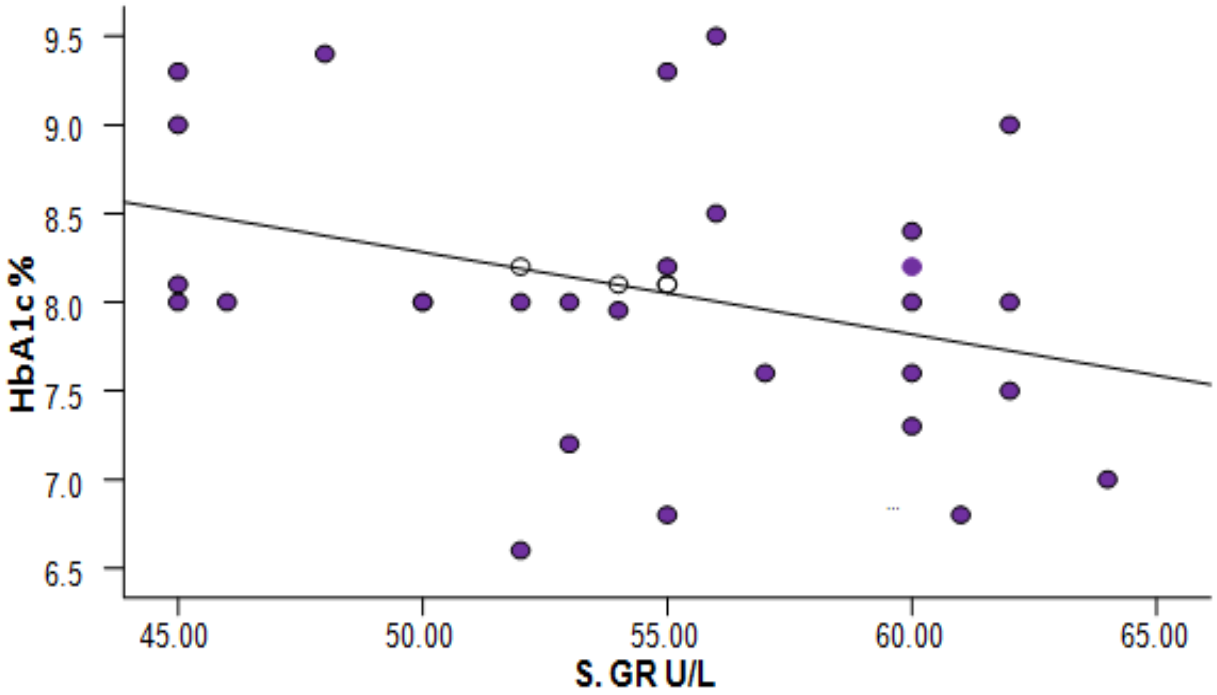

Figure 6. Correlation between serum GR and HbA1c in diabetic hypercholesterolemic patients (r=-0.4, $p < 0.05$, n= 33)

Discussion

The primary findings of this study reveal that there were significant differences in serum glutathione concentration and highly significant differences in glutathione reductase activity between the hypercholsetermic patients and T2DM patients with hypercholestermia. Studies showed high level of oxidative stress in subjects with hypercholesterolemia (risk factors for coronary heart). Researchers at the University of Warwick (UK) have suggested diabetes to be a hidden condition for some patients with coronary heart disease [20]. They found that high levels of oxidative stress in people with coronary heart disease, previously thought to be a maker of the heart condition, could instead indicate a condition of glucose abnormality, such as overt type 2 diabetes [20,21].

The lower GSH, GR and protein with the rise in GGT (as compared to the controls) of the present study reveal decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid profile in these patients (tables 1, 2, and 3).

Sailaja et al in 2003 reported that diabetic humans have shown increased lipid peroxidation and decreased levels of reduced glutathione, glutathione reductase and glutathione peroxidase activities [22].

Glutathione concentration was found to decrease in the liver [23,24]. Kidney, pancreas, plasma, red blood cells, nerve, and precataractous lens of chemically induced diabetic animals [25-28].

Emerging evidence has shown that serum GGT is more than a marker of alcohol consumption. Population-based studies have observed a strong association between serum GGT levels and many cardiovascular disease risk factors [29,30].

After adjusting for alcohol consumption, the factors show a positive association with elevated serum GGT level in the population studies including: old age, male gender, body mass index, smoking, lack of exercise, high blood pressure, heart rate, high blood cholesterol, high blood fasting triglycerides, high blood LDL cholesterol, low blood HDL cholesterol, high fasting glucose, and, among women, menopause and use of oral contraceptive [31]. These relationships have been shown to be strong even within the normal range of GGT levels. In addition, several prospective studies have shown that baseline serum GGT level is an independent risk factor for the development of heart disease, hypertension, stroke, and type 2diabetes, regardless of alcohol consumption [29,30]. Experimental findings that cellular GGT changes its role from an antioxidant to a pro-oxidant in the presence of a transition metal such as iron are of interest because iron can increase cellular GGT during oxidative stress [30]. The prospective study done by Ruttman and colleagues in 2005 on 163 944 Austrian adults studied for 17 years showed that GGT was independently associated with cardiovascular mortality. This was found to be true in both sexes, with a clear dose-response relationship, and with a stronger prognostic significance of GGT in younger participants [33]. Prospective cohort studies have revealed that plasma GGT activity exhibits a positive association with coronary artery disease [34].

Another three-year follow up study has shown that GGT increases in type 2 diabetes in middle aged men and women [35].

Increased oxidative stress as measured by markers of oxidative stress has been shown to be increased in type 2 diabetes mellitus. Despite strong experimental evidence indicating that oxidative stress may determine the onset and progression of late-diabetes complications, controversy still exists about whether the increased oxidative stress is merely associative rather than causal in DM [36,37] .

The negative correlation between serum cholesterol and each of serum GSH and GR in both groups of patients (Figures 1 and 3) may confirm the fact that both hypercholesterolemia and diabetic hyper-cholesterolemia will lead to a

decrease in the levels of antioxidant enzymes and an increase in the levels of free radicals , being more obvious in the later [36].

Alteration in antioxidant defenses in diabetes might lead to the development of diabetic induced complications. Evidence has accumulated indicating that oxidative stress may play an important role in the etiology of diabetic complications. The poor glycemic control in diabetic patients was associated with decreased free radical scavenging activity, something confirmed by the presence of a negative correlation between the levels of HbA1c and each of GSH and GR of the present study (Figures 5 and 6). In hyperglycemia, glucose undergoes auto-oxidation and produces free radicals that in turn lead to peroxidation of lipids in lipoproteins. Elevated levels of lipid peroxidation, hydroperoxide and conjugated diene seen in diabetic patients are clear manifestations of excessive formation of free radicals resulting in tissue damage [37,38], and this may lead to the speculation that elevated oxidative stress in coronary heart disease is not a marker for the heart condition only, but may indicate a condition of glucose abnormality similar to that of type 2 diabetes mellitus.

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Correspondence to: Zainab A.A.Al-Shamma
E-mail: z.alshamma@gmail.com
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Local *Staphylococcus aureus* Phage Groups

Imad Sh Mahmoud[1] *PhD*, **Abdul Munim N Mohammed**[1] *MSc*, **Sabiha S Sharif**[2] *MSc*

[1]Dept. of Microbiology, College of Medicine, Al-Mustansiriyah University, [2]Dept. of Bacteriology, Al-Sulaimanyiah Teaching Hospital

Abstract

Background *Staphylococcus aureus* isolates distributed into 3 groups according to their sources, 10 isolates from each source. Each of the 30 isolates produced phage lysate. Based on our results, it has been found that these phages obtained from all isolates can be classified into 3 groups (A, B and C).

Objective To produce local phage groups from locally isolated *Staphylococcus aureus* strains to be used for epidemiological purposes.

Methods A total of 60 specimens were obtained from three different source locations, surgical theaters (instrument, walls, floor and masks), nurses and inpatients, were enrolled in a study based at Al-Sulimanyiah Teaching Hospital from December 2008 to November 2009. Each specimen was subjected to well known established microbiological. Methods for isolation and identification of *Staphylcoccus aureus.* All isolates were tested for the presence of phage employing heat method and detected by spotting method, also based on resistance or sensitivity of each isolates to give phage lysates by application of the cross-lysis technique.

Result *Staphylococcus aureus* isolates distributed into 3 groups according to their sources, 10 isolates from each source. Phages were induced from thirty Staphylococcus *aureus* isolate. Based on results obtained of the isolates, it has been found that these phages obtained from all isolates can be classified into 3 groups. Group (A) revealed that 1 and 6 phage lysates originally from isolates 1 and 6 were able to lyse all isolates in group 1 except 1 and 6 isolates and those in other groups which were unlysed. A strain was phage typeable (at least one phage produced 20 or more plaques of lysis). Isolates 15 and 16 produced phage lysates 15 and 16 in group (B) which were able to lyse all isolates in group 2 except 15 and 16 isolates and the remaining isolates in other groups which were unlysed. phage lysates 23 and 26 in group (C) which were induced from isolates 23 and 26 were able to lyse all isolates in group 3 except isolates 23 and 26 and the remaining isolates in other groups which were unlysed also.

Conclusions It is detected that 3 local phage groups from *Staphylococcus aureus* are presented to be used for epidemiological purposes in case of *Stapylococcus aureus* epidemic.

Key words *Staphylococcus aureus*, phages, epidemiology.

Introduction

Staphylococcus aureus is one of the most common agents causing infections of a wide spectrum of clinical conditions ranging from simple to life-threatening causes [1,2,3].

Staphylococcus aureus can survive on dry surfaces, increasing the chance of transmission and it is implicated in toxic shock syndrome in which some transpose allowed the rapid growth of this bacteria to release toxin that were absorbed into the blood stream [4].

Nosocomial infection is a significant epidemiological problem resulting in prolongation morbidity. *Staphylococcus aureus* species is the leading cause of nosocomial infection a methicillin resistant *Staphylococcus aureus* (MRSA). MRSA strains in the hospital are diresistant [4,5,6].

Problematically, Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) has become a major cause of hospital-

acquired infections and being recognized with increasing frequency in community acquired infections [1,5,7]. This necessitates the need for programs to prevent the spread of anti-microbial resistant microorganisms and control the use of anti-microbial drugs in health-care settings [7].

Phage typing is currently used for typing of Staphylococcus *aureus* strains in epidemiological studies. Since 1952 this technique has found widespread use, and an international system has been established for typing human strains [8, 9].

It also provides a useful information about other genera which has been particularly significant for *Staphylococci* which are difficult to distinguish on any other basis [10].

Methods

Collection of specimens:

The study was carried out between December 2008 to November 2009 at Al-Sulaimanyiah Teaching Hospital. A total of 60 specimens were collected from three different source locations; surgical theaters (instrument, walls floor and masks), nurses and inpatients. Swabs were processed for isolation of *Staphylococcus aureus*.

Specimens were screened by preliminary Gram stain and were inoculated on 10% blood agar and MocConkey's agar. *Staphylococcus aureus* was identified by conventional techniques [11].

Phage induction:

Phages were induced from each isolates using the heat method in which 10 ml of the isolate which were incubated for 16-18 h. at 37 °C, held in water bath for 2 h. at 56 °C, centrifuged or 15 minutes at 3000 RPM.

Supernatants were collected to represent the phage lysates. Filter sterilized through 0.45 ul filters. Each phage lysate was labeled to carry the number of the original isolates. [12]

Phage detection:

Cross lysis technique was followed in which each phage lysate was crossed against all isolates on plates seeded confluent by 0.1 ml of culture of 16-18 h. at 37 °C by spotting a drop (0.01 ml) of phage lysate on designated position on plaque plate of trypticase soy agar incubated for 6-8 h. and examined for plaque formation which demonstrate the sensitivity of certain isolate to certain phage lysate [8,12]. 3 phage typing of all strains were performed according to standard methods [8,9].

Initially, strains were typed in routine test dilution (RTD, the highest dilution producing confluent lysis). In the cases of negative reactions, 100 concentration (100 RTD) was also used. The lytic reactions ×RTD were read as follows: + + = more than 50 plaques (strong lysis); + = 20-50 plaques (moderate lysis); ± = less than 20 plaques (weak lysis); - = no plaques (no lysis). A strain was considered phage typeable when it was lysed strongly (+ +) or moderately (+) by at least one phage.

Results

In this study 60 samples were collected from three different source locations, surgical theaters (instrument, walls, floor and masks), nurses and inpatients in Al-Sulaimanyiah Teaching Hospital, yielded 30 *Staphylococcus aureus* isolate grouped into 3 groups according to their sources and distributed as followed:

Group 1: 10 isolates (1-10) from surgical theaters.

Group 2: 10 isolates (11-20) from nurses.

Group 3: 10 isolates (21-30) from inpatients.

Based on results obtained of the isolates, it has been found that these phages obtained from all isolates can be classified into 3 groups in which isolate 1 and 6 represent phage group (A) which is able to lyse all isolates in group 1 except isolate 1 and 6.

Lysis is indicated by the formation of plaques, a strain was considered phage type able when it was lysed strongly or moderately by at least one phage (at least one phage produced 20 or more plaques of lysis). It has been found also that all other isolates in the other groups were resistant to this phage group.

Phage group (B) which represents the isolates 15 and 16 has been found to lyse all isolates in

group 2 except isolates 15 and 16 which were resistant also.

In this work it has been found also that phage group (C) which is represented by isolates 23 and 26 was able to lyse all isolates in group 3 except isolates 23 and 26 which were resistant in addition to all other isolates and the remaining groups of isolates were resistant to this phage group.

Discussion

Several workers around the world agreed that phage typing for *Staphylocoocus aureus* to trace the source of an epidemic is a very important technique [13,14,15,16].

Our study seems to confirm this approach and it could be the first attempt in our country during the period from December 2008 to November 2009, to present 3 phage groups of *Staphylococcous aureus* which can be used locally for epidemiological purposes. This fact coincides with results of other authors [17,18], while our results were higher than those reported by others [13,19,20].

It is possible that some variations could be present due to changes in phage lytic patterns which can be affected by geographical distribution or also the abuse of antimicrobials which lead to drug resistance which in turn lead finally to change the behavior of both the *staphylococcus aureus* isolates and their phages [13,14].

The reason behind taking equal number of isolates from each source is to reduce any chance of variation in lytic pattern between phage lysates.

It is a fact that a bacterium is resistant to its own phages [21], this can be observed in our results in which the isolates 1, 6, 15, 16, 23 and 26 were resistant to their own phages. This could be either that the specific locus on the DNA donor bacterium is already occupied by another phage particle [22,23,24] or the receptors are missed.

Lysis as shown by the agar method of spotting supernatant materials on lawns can be caused by (i) high phage multiplicity's leading to strongly lysis, (ii) cell wall enzymes. Lysis sometimes found in phage containing crude supernatant fluid, (iii) infection by low phage multiplicities followed by lytic cycles and release of phage progeny that are mutated or defective. [25,26,27,28].

It is concluded that 3 local phage groups from *Staphylococcus aureus* are presented to be used for epidemiological purposes in case of *Stapylococcus aureus* epidemic.

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Correspondence to: Dr. Abdul Munim N Mohammed
E-mail: drwaleedarifalani@yahoo.com
Mobile: +964 7702871054
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Colo-rectal Cancer Risk After Cholecystectomy in Al-Khadymia Teaching Hospital

Hussam AK Ahmad, *FRCS*

Dept. of General Surgery, College of Medicine, Al-Nahrain University.

Abstract

Background Cholecystectomy is one of the most commonly performed operative procedures, on the other hand, the wide acceptance of laparoscopic cholecystectomy for sure led to the increased rate of cholecystectomies. The data assessing relationship between cholecystectomy and colo-rectal carcinoma is limited; therefore the relationship of whether prior cholecystectomy modifies the natural history of colo-rectal carcinoma is worth assessing in Al-Khdymia Teaching Hospital.

Objective To estimate the risk of colo-rectal carcinoma in cholecystectomized patients and to assess the feasibility of screening in these patients.

Methods Retrospective evaluation of a total of 123 patients with approved colo-rectal carcinoma over a period of eleven years in a wide district drainage referral Hospital (Al-Khadymia Teaching Hospital) in Baghdad-Iraq.

Result The total number of patients included is 123 from June 1999 to June 2010. The whole group approved to have a colo-rectal carcinoma. The age range is from 17-90 years, the females constitute 55 patients of the total and the rest 68 patients were males. The results were not significant regarding the gender of cholecystectomized patients and the risk of colo-rectal carcinoma. While on the other hand there were slightly increased statistically significant relative risk of colo-rectal carcinoma specially involving the proximal colon in cholecystectomized patients.

Conclusions There is an increased risk of incidence of colo-rectal carcinoma in cholecystectomized patients regardless the gender of the patient, and the risk is higher for the proximal colon than the distal colon.

Key words cholelithiasis, cholecystectomy, colo-rectal carcinoma, screening of cholecystectomized patients.

Introduction

Cholelithiasis affects approximately 20% of the female and 10% of the male population. Although most of these patients remain asymptomatic for many years or even for a life time, therefore cholecystectomy is one of the commonly performed operative procedures; in addition to that the wide acceptance of laparoscopic cholecystectomy may have led to the increased rate of cholecystectomies. It is estimated that more than 600,000 cholecysetectomies are performed each year in the United States according to the annual reports issued by the health care services in the US [(1)].

The first clinical report relating the possible correlation between cholecystectomy and colo-rectal carcinoma appeared in 1978 [(1)]. Since then, more than 70 studies have been designed to confirm whether cholecystectomy could increase the risk of colon cancer with controversial or partially inconsistent results. Most of these studies report a slightly increased risk of colo-rectal carcinoma in cholecystectomized patients.

Bile acids are of two types [(2)]:

1- Primary bile acids; formed in the liver from cholesterol. These are the cholic and chenodeoxycholic acids.
2- Secondary bile acids; formed in the distal ileum and colon by bacterial action which causes deconjugation and hydroxylation which convert the cholic acid to deoxycholic acid and the chenodeoxycholic to lithocholic acid.

The proposed pathogenic mechanisms is the increased exposure of the intestinal epithelium to secondary bile acids, which may promote carcinogenesis shown in several clinical and experimental studies [3,4]. However, this possible correlation should lead to screening of cholecystectomized patients for colo-rectal cancer remains questionable. The incidence and risk have never been assessed in major Hospitals in Iraq therefore the incidence is also questionable.

The hypothesis behind the increased risk of colo-rectal carcinoma is that post cholecystectomy changes in the composition and secretion of bile salts affect enterohepatic circulation and exposure of the colon to bile acids [5,6] which may promote carcinogenesis. Some studies suggested the risk of carcinoma is higher in women and mainly at the right side of the colon [7].

The aim of this study is to evaluate the possible correlation between cholecystectomized patients and the risk of colorectal carcinoma in a wide district drainage referral teaching Hospital in Baghdad-Iraq (Al-Khadymia Teaching Hospital) and to answer a question about whether cholecystectomized patients should be put on screening program for early detection of colonic cancer.

Methods

Retrospectively evaluated a total of 123 patients' records with colo-rectal carcinoma that were diagnosed and approved through histopathology study of the specimens obtained after resection of their tumors or through endoscopy if the patient were beyond surgery in Al-Khadymia Teaching Hospital for the period from January 1999 to January 2010.

The following parameters were recorded;

1- The total number of patients.
2- The age.
3- The gender (male or female).
4- Site of the tumor.
5- History of cholecystectomy if present (Yes/No) and the history is approved during staging of the tumor by ultrasonography, CT scan or during the course of surgery. The cholecystectomized groups of patients were further subdivided into a female and a male group.

Results

The total number of patients with colo-rectal tumor is (123). The age range was from 17-90 years and the females constitute (55) patients from the total number and the rest were (68) male patients as shown in the demographic (Table 1).

Table 1. Demographic characteristics of patients enrolled

Patient	Information
Total no. of patients	123
Males	68
Females	55
Age range	17-90 years

According to our results (101) of the patients had colonic tumors and only (22) of the patients had rectal tumors. Patients with colonic tumors are further subdivided to those involving the proximal colon (60 out of 101) and the distal colon (41 out of 101) as summarized in the information (Table 2).

Table 2. Site of Tumor

Site of tumor	No. of patients	%
Colon	101	82.15
Rectum	22	17.85
Ascending colon	60	59.4
Descending colon	41	40.6

The other important parameter is the history of cholecystectomy in which we found that the majority were non cholecystectomized (102 from a total of 123) and the cholecystectomized were only (21 from a total of 123), in addition to that the cholecystectomized patients are categorized according to their gender which showed that only (11) females were cholecystectomized out of (55) and only (10) males were cholecystectomized out of (68) male patients included in the study as shown in (Table 3).

Table 3. Colo-rectal carcinoma and cholecystectomy

History of Cholecystectomy	No.	%
Non-cholecystectomized	102	82.9
Total cholecystectomized	21	17.1
Cholecystectomized Females	11	20
Cholecystectomized Males	10	14.7

Discussion

The relation between cholecystectomized patients and the subsequent risk of development of colo-rectal cancer has been thoroughly studied in recent decades in many parts of the world and the relation has been controversial; therefore we were very much interested to find out the risk and relation of cholecystectomy with colo-rectal carcinoma in our community.

Al-Khadymia Teaching Hospital is a referral hospital draining a wide range of patients from large district in our country, and over an extended period of time from June 1999 till June 2010 which is around eleven years hoping that we end up with real results reflecting the exact nature and the possible risk and relation.

Studying the relation between cholecystectomized patients and colo-rectal carcinoma is a critical issue because cholecystectomy is one of the most commonly performed elective surgeries especially after the introduction of laparoscopic surgery; this is its self raises a reasonable concern if this increase in the number of performed cholecystectomies had led to an increase incidence of colo-rectal cancer. A further concern came after the fact that not only cholecystectomy but also gall stones themselves to a lesser extent have increased biliary and fecal concentration of secondary bile salts[4], this concern came with increased reported missed pathologies of the

Intraperitoneal organs especially the colo-rectal region in laparoscopic surgery over the advantage of open cholecystectomy through which you can see and palpate the whole colon and rectum in the concomitant presence of gall stones, but it seems that the risk is not that important if certain safety rules are followed [3,8,9], which include proper preoperative patient assessment and preoperative careful examination of the colon and rectum prior of removing the gall bladder which should be highly recommended.

The role of secondary bile acids as an endogenous colon carcinogens as we mentioned earlier has been also shown in a number of clinical and experimental studies [9,10]. Narisawa et al also proved that secondary bile salts can promote colonic epithelial cell proliferation in animal models but he did not give much attention to time factor for exposure [11]. Mannes et al, who reported a significantly increased incidence of large bowel adenoma after cholecystectomy [12].

Our data regarding the relation of gender and cholecystectomy with the development of colo-rectal cancer were not significant in accordance to Chi Square ($X2=0.6$, $p < 0.05$). On the other

hand our results regarding the risk of history of having previous cholecystectomy, the possibility of having increased risk of colo-rectal carcinoma and the results regarding the site of predilection in the colon were very close to other large studies and even meta-analysis; therefore we statistically compared our results with those groups and in order to- reach this we applied the t-test for proportions equation that judge about the closeness of results, for the results to be similarthe end figure result should be less than 1.96 and the equation is (t= P1-P2/√P1q1/n1+P2q2/n2) where P1;is sample one;P2;is sample two;q1;is the rest of percentage of first sample;n1;sample size one;q2; rest of percentage of second sample;n2;sample size two.

We applied this method of comparing results for the relation of cholecystectomy and colo-rectal carcinoma risk and for the most common involved site. All our group results were below 1.96 ($p < 0.05$), which means that there is no difference between the results. Our data were compared with very large extended studies and even meta-analysis [7,13,15,20,21,22], they all agree that there is slight increase in relative risk of developing colo-rectal cancer in cholecystectomized patients, further more they noted as we do an increase risk of proximal colon carcinoma over the distal and rectal carcinoma.

We found that there is an increased relative risk of developing colo-rectal carcinoma and increased risk of carcinoma of the proximal colon rather than the distal or rectal regions which is strongly supported by the following large studies; retrospective, prospective and even meta-analysis [13,14,15]. Also we had no significant results between the gender and risk of colo-rectal carcinoma despite the fact that cholecystectomy is performed more in female patients than in male patients supported by our results in which the number of female patients who gave a history of cholecystectomy is higher than in male patients, also we lack the long term follow up for patients with cholecystectomy, however other authors suggested a more than 15 years screening [14,15].

The most reliable evidence we comparing our study with is supplied by Giovanucci who showed slightly increased relative risk for colo-rectal cancer after cholecystectomy with risk slightly higher in women in addition to that he noted a prevalence for the proximal colon [13]. In two large cohort studies, Ekbom et al followed patients up to 23 years after cholecystectomy and he observed only an increased risk among women for right sided colon cancer 15 years after operation [14], while Johansen et al followed up patients for less than 15 years and showed only a border line significant association between cholelithiasis and colon cancer [15].

After all this regarding the question that we raised at the aim of the study about involving cholecystectomized patients in screening program for early detection of colon cancer, almost all authors with results similar to ours agree that there is no evidence to suggest the need for this [4,16,17,18,19].

Conclusion

1- Our study demonstrates a slightly increased relative risk which is statistically significant of colo-rectal cancer after cholecystectomy in the area drained by our referral Teaching Hospital (Al-Khadymia) in Baghdad/Iraq.
2- The site of predilection for carcinoma in cholecystectomized patients is the proximal colon rather than the distal colon and the rectum comes second after the proximal colon.
3- The screening program for the possibility of developing colo-rectal carcinoma after cholecystectomy is not recommended since the elapsed time needed for surveillance is around 23 years.
4- There is no significant relation between the gender of cholecystectomized patient and the future risk of colo-rectal carcinoma.

5- With the increased number of operations for cholecystectomy after the introduction of laparoscope we suggest to follow safety measures and rule to at least inspection of colon and rectal area specially the proximal colon for the fact even cholelithiasis is a relative risk in the incidence of colo-rectal carcinoma.

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Correspondence to: Dr. Hussam AK Ahmad
E-mail: dr_hussam44@yahoo.com
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Assessment of Autonomic Neuropathy in Patients with Diabetes Mellitus by Measurement of Heart Rate Turbulence and Heart Rate Variability

Abbas F Al-Hashimi[1] *MSc*, **Najeeb H Mohammed**[2] *Ph D*, **Hilal B Al-Saffar**[3] *FRCP*

[1]Dept. of Physiology, College of Medicine, Al-Nahrain University, [2]Dept. of Physiology, [3]Dept. of Medicine, College of Medicine, Baghdad University

Abstract

Background Heart rate variability (HRV) and heart rate turbulence (HRT) illustrate regulation of the heart by autonomic nervous system (ANS). The autonomic nervous system plays an important role not only in physiological situations, but also in various pathological settings such as diabetic neuropathy Diabetic autonomic neuropathy is a serious and common complication of diabetes.

Objective To determine the association between HRT, HRV and diabetes control monitored by concentrations of HbA1c.

Methods In 52 patients with diabetes mellitus type II of either sex attending Cardiac Care Unit (CCU) in Al-Kadhimya Hospital, 24-hour Holter ECG monitoring was performed to evaluate time domain HRV parameters (SDNN, SDNNI, SDANN, rMSSD, pNN50) and HRT parameters (TO and TS). HBA1c was measured in all patients. Regression analysis was performed to evaluate the association between tested parameters.

Results Significant correlation has been observed between TO and SDNN, SDNNI and SDANN. TS correlated significantly with SDNN, SDNNI, SDANN, rMSSD, pNN50. We noted no correlation between HbA1c and HRV or HRT parameters.

Conclusion We concluded that HRV time domain parameters correlate with HRT in patients with diabetes mellitus. Diabetes control estimated on basis of HbA1c value did not show correlation with HRV and HRT.

Key words Heart rate turbulence HRT, Heart rate variability HRV, Diabetic Autonomic Neuropathy. HbA_1c.

Introduction

Heart rate variability (HRV) and heart rate turbulence (HRT) reflect the functional status of the autonomic nervous system [1,9]. In recent years noninvasive techniques based on the electrocardiogram (ECG) have been used as markers of autonomic modulation of the heart, these include HRV and heart rate turbulence (HRT), a new method based on fluctuations of sinus rhythm cycle length after a single premature ventricular contraction. Diabetic autonomic neuropathy is a serious and common complication of diabetes mellitus (DM) [12]. Dysfunction of the autonomic nervous system is associated with increased risk of mortality in patients with diabetes.[6] Autonomic neuropathy is characterized by early and widespread neuronal degeneration of small nerve fibers of both sympathetic and parasympathetic tracts [3,5]. Its clinical manifestations are ubiquitous

with functional impairment and include postural hypotension and persistent tachycardia [3]. Once clinical manifestations of diabetic autonomic neuropathy (DAN) supervene, the estimated 5-year mortality is approximately 50% [3,5,6,10]. Thus, early subclinical detection of autonomic dysfunction is important for risk stratification and subsequent management [3]. Analyses of short-term and/or long-term HRV have been proven useful in detecting DAN [9-12]. In neuropathy associated with DM characterized by alteration of small nerve fibers, a reduction in time domain parameters of HRV seems not only to carry negative prognostic value but also to precede the clinical expression of autonomic neuropathy [5,7,13]. In diabetic patients without evidence of autonomic neuropathy, reduction of the absolute power of low frequency (LF) and high frequency (HF) during controlled conditions was also reported [3,11]. Thus, the initial manifestation of this neuropathy is likely to involve both efferent limbs of the autonomic nervous system [1].

In the present study we analyze correlations between HRV and HRT parameters and HbA1c in patients with diabetes mellitus.

Methods

The study group consisted of 52 patients with non-insulin depended DM, attending Cardiac care Unit in Al-Kadhimia Hospital. Clinical characteristics of the studied patients are shown in table 1.

Patients without sinus rhythm, after myocardial infarction and those with left ventricular systolic dysfunction in echocardiography (EF<50%) were excluded. All patients underwent 24-hour Holter monitoring (3-channel recorders Schiller MT 101, Swiss). Holter ECG was firstly analyzed automatically, followed by manual correction by the operator. HRV was analyzed in the time domain in accordance with standards all artifacts, arrhythmias, pauses and conduction disturbances were eliminated. The following HRV parameters were analyzed:

- SDNN: Standard deviation of all NN (normal-normal) intervals
- SDNNI: Mean of the standard deviations of all NN intervals for every 5-minute segments of the entire recording
- SDANN: Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording
- rMSSD: The square root of the mean of the sum of the squares of differences between adjacent NN intervals
- pNN50: NN50 (50% of NN) count divided by the total number of all NN intervals

In patients with premature ventricular beats (PVC) we analyzed HRT parameters, which are the turbulence onset (TO) and the turbulence slope (TS).

The TO is the percentage difference between the heart rate immediately following PVC and the heart rate immediately preceding PVC. It is calculated using the equation:

TO = ((RR1 + RR2) - (RR-2 + RR-1)) / (RR-2 + RR-1) * 100 with RR-2 and RR-1 being the first two normal intervals preceding the PVC and RR1 and RR2 the first two normal intervals following the PVC.

The TS is the steepest slope of a linear regression line through five consecutive measurement points in the averaged tachogram. We used filters which exclude RR intervals with the following characteristics were excluded from the HRT calculation using special filters:

1. R-R < 300 ms
2. R-R > 2000 ms
3. Difference to the preceding sinus interval > 200 ms
4. Difference to the reference interval (mean of the 5 last sinus intervals) >20%

In addition, we limit the HRT calculations to PVCs with:

a. a minimum prematurity of 20% and

b. a post-extrasystole interval which was at least 20% longer than the normal interval.

Glycosylated hemoglobin (HbA1c) level was measured in all patients for evaluation of glycaemic control.

Table1. Demographic features of the patient group.

Parameter	N(%) or mean ± SD
Number of patients (n)	52
Gender (females/males) (n%)	34 (65%) M / 18 (35%) F
Age (years) (mean±SD)	62.6 ± 9.2 years
Duration of diabetes (mean±SD)	7 ± 3.6 years
Hemoglobin A1c concentrations (mean±SD)	8.64% ± 2.84%
Insulin therapy (n %)	17(32%)
Oral antidiabetic agents: sulphonylureas (n %)	35(32%)

Results

The HbA1c level was from 5.9% to 10.2% (mean 8.64% ± 2.84%) In 11 (20%) patients the HRT parameters could not be computed (appear ventricular beats did not or ventricular beats did not meet criteria of HRT analysis). HRV parameters calculated in subgroup in whom it was not been possible to calculate HRT did not differ significantly from remaining studied group.

Correlation coefficients between HRT parameters and HbA1c are shown in table 3.

A significant correlation between TO and SDNN, SDNNI and SDANN was observed. On the other hand, TS correlated significantly with SDNN, SDNNI, SDANN. rMSSD, pNN50 (Table 2). No correlation was noticed between HbA1c (table 1) and HRV or HRT parameters (Table 2).

Table 2. HRV values and correlation coefficients between HRV and TO, TS, HbA1c

HRV parameter	Value ±SD	TO		TS		HbA1c	
		r	p	r	p	r	p
SDNN	130±33	-0.33	0.005	0.52	0.001	-0.12	NS
SDNNI	46±16	-0.21	0.01	0.48	0.003	-0.14	NS
SDANN	122±29	-0.26	0.003	0.42	0.001	-0.12	NS
rMSSD	31±13	-0.12	NS	0.36	0.005	-0.18	NS
pNN50	9±8	-0.14	NS	0.32	0.005	-016	NS

Table 3. HRT values and correlation coefficients with HbA1c.

HRT parameter	Value	HbA1c	
		r	p
TO	-1.1 ±1.4	0.18	NS
TS	12.1 ±9.5	-0.12	NS

Discussion

HRT is the physiological, biphasic response of the sinus node to premature ventricular contractions. The underlying mechanisms of HRT are an autonomous baro-reflex. It consists of a short initial acceleration followed by a deceleration of the heart rate causes a brief disturbance of the arterial blood pressure. If the autonomic control system is impaired, this reaction is either weakened or entirely missing [1,9].

HRV describes variations of both instantaneous heart rate and RR intervals. Depressed HRV can be used as a predictor of risk after acute myocardial infarction (MI) and as an early warning sign of diabetic neuropathy [10,12]. Correlations between HRT and HRV parameters was observed in large populations of patients after myocardial infarction [7,13]. Our study confirms this significant correlation between HRT and time domain HRV in patients with diabetes mellitus. Interestingly is the lack of correlations between time domain HRV parameters or HRT and HbA1c level. Previous studies have shown decreased heart rate variability in diabetic patients as a result of diabetic neuropathy, but frequency of occurrence of neuropathy has not correlate directly withHbA1c [8,10,12, 13].

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Correspondence to Dr. Abbas F Al-Hashimi
Email: abbasalhashimi04@yahoo.com
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Risk Factors for Ischemic Heart Disease among Patients Admitted to Coronary Care Unit (CCU) in AL-Hussain Hospital in Karballa

Najlaa F Jamil [1] *FICMS/CM*, **Jalal Abdul Gahni**[2] *FIBMS/CM*

[1]Dept. Community Medicine, College of Medicine, Al-Mustansiriyah University,[2]Ministry of Health, Karballa Health Directory

Abstract

Background Adequate control of cardiovascular disease risk factors and health habits are important for preventing ischemic heart disease, but it has been reported that many patients remain uncontrolled despite regular care.

Objective To identify some of the risk factors of ischemic heart diseases among patients admitted to coronary care unit (CCU).

Methods A hospital based case-control study was conducted in AL-Hussain Hospital in Karballa, from January 2007 to October 2007. The study included 300 cases of ischemic heart disease admitted to coronary care unit (CCU) of the hospital during the study period, and 300 age & sex matched controls, who attended the outpatient clinic of the same hospital. All the participants were interviewed with a special questionnaire form. In addition the weight and height were measured for both cases and controls to determine their body mass index (BMI).

Result 73 (24.3%) of the cases were in the age group (60-69) years. The females accounts for two third of cases, 199 (66.3%) in comparison to males 101 (33.7%).
The study showed a statistical significant association between the following risk factors and admission to CCU: smoking, alcohol consumption, obesity, hypertension, diabetes, and positive family history of various cardiovascular diseases among first degree relatives of the cases.

Conclusions The admission to CCU was more common in the age group (60-69) years and the females were more likely than males need for CCU admission, patients with the following risk factors were in more need to CCU admission than others: smoking, elevated body mass index (BMI), hypertension, diabetes and positive family history of various cardiovascular diseases among first degree relatives of the cases.

Key words risk factors, CCU admission

Introduction

Risk factors increase the chances of developing a disease or having it worsen. Over 300 risk factors have been associated with coronary heart disease and stroke. The major established factors meet three criteria: a high prevalence in many populations, a significant independent impact on the risk of coronary heart disease or stroke; and their treatment and control result in reducing the risk [(1)].

Risk factors for coronary heart diseases categorized into three groups: controllable, non controllable and predisposing factors. The controllable or life style factors include tobacco use, diet and physical activity.

Non controllable factors include gender, age, and family history. Predisposing risk factors include diabetes mellitus, hypertension, obesity, and hypercholesterolemia [(1,2)].

The prevalent behavioral risk factors reflect the underlying major social, economic and cultural driving forces like low education, unemployment, poor income, unhealthy environmental conditions, in addition to stressful events [3,4].

In the EMRO region of WHO, cardiovascular diseases (CVD) are emerging as a major health problem, the proportion of deaths from CVD range from 25-45 % [5].

In Iraq, CVD represent the main cause of hospital admission and account for around 40% of all causes of deaths in the country [6].

Heart disease is preventable, many action can be taken to reduce the risk of heart disease by focusing on human lifestyle and habits, also there is much that can be done to control the risk factors for heart disease, prevent heart attack, and increase the chances for a long and vital life [2,5].

The present study was conducted to determine the risk factors of ischemic heart diseases among patients admitted to coronary care unit (CCU) in AL-Hussein hospital in Karballa.

Methods

This is a hospital based case-control study conducted in the Coronary Care Unit (CCU) of AL- Hussain Hospital in Karballa, over a period of ten months, from January 2007 till the end of October 2007.

The patients who were admitted to the CCU due to ischemic heart disease during the study period were considered as cases according to the following inclusion criteria (any patient admitted to CCU with ECG evidence of angina or myocardial infarction), while the control group was collected from people attending the outpatient clinic of the same hospital (other than the internal medicine clinic), who were matched by age and gender with no history of any ischemic heart disease. A control to case ratio of 1: 1 was aimed.

All the participants were interviewed with a special questionnaire form consisting of sociodemographaic data which include age, sex, years of education, occupation, marital status, health habits (smoking habit was assessed among the smokers by asking them whether they smoke on daily basis or not and the average daily number of cigarette smoked and duration of smoking, alcohol consumption, eating habits was assessed by asking about eating fatty foods, type of fat used for cooking and methods used for cooking foods and number of eggs eating per week).

The physical activity was assessed according to the following definition (walking 30 minutes a day , five days or more in atypical week) [6].

Family history (1st degree relatives) for some of the medical conditions and medical health history for hypertension and diabetes mellitus, in addition the weight & height measurements for both cases and control were taken to determine their body mass index (BMI), the BMI was calculated and classified for each individual [7].

Data analysis

SPSS version 15 was used for data entry and analysis. The Pearson Chi-square test was used to assess the statistical significance of observed differences in proportion; in addition Odds ratio & 95% confidence interval were calculated [8]. A *p* value less than 0.05 was considered significant.

Results

The total sample studied included 300 cases and 300 controls. The females accounted 199 (66.3%) of the total cases admitted to CCU in comparison to males 101 (33.7%) cases. 24.62% of the female cases were in the age group 50-59 years, while the largest proportion among males (25.74%) were in the age group 60-69 years, Table 1.

Table 2 illustrates the distribution of the study groups according to some demographic characteristics, the years of education, marital status and occupation all shows statistical significant association with CCU admission.

The results in table 3 shows that smoking and alcohol consumption both increase the risk for CCU admission as the odds ratio were: 1.443 and 2.434 respectively. While the eating habits

revealed no statistical significant association with the CCU admission (*p*= 0.562).

The distribution of the cases and controls according to their BMI, shows that higher proportion of the cases (49%) were obese and extreme obese in comparison to their controls (43.4%) (*p*= 0.013), Table 4.

The history of hypertension and diabetes both increase the risk of CCU admission as the odds ratio were 1.563 & 1.528 respectively, Table 5.

Table 6 revealed statistical significant associations between the family history of hypertension, diabetes, ischemic heart diseases and death from heart diseases and the CCU admission.

Table 1. The age and sex distribution of cases

Age group (years)	Females		Males		X^2; df; *p*
	No	%	No	%	
20-29	2	1	-	-	5.526; 5;0.478
30-39	16	8.04	7	6.93	
40-49	44	22.11	22	21.78	
50-59	49	24.62	18	17.82	
60-69	47	23.61	26	25.74	
70-79	27	13.56	22	21.78	
80-	14	7.03	6	5.94	
Total	199	100	101	100	

Table 2. The distribution of the study groups according to some demographic characteristics

Demographic character	Cases (N=300)		Control (N=300)		X^2; df; *p*
	No.	%	No.	%	
Years of education					
0-6	191	63.7	99	33	60.924; 2; 0.0001
7-12	69	23	101	33.7	
>12	40	13.3	100	33.3	
Marital status					25.473; 3; 0.0001
Married	197	65.7	199	66.3	
Unmarried	40	13.3	20	6.7	
Widow	32	10.7	67	22.3	
Divorced	31	10.3	14	4.7	
Occupation					
Governmental employee	42	14	67	22.33	8.206; 3; 0.04
Self-employee*	87	29	87	29	
House wife	124	41.33	111	37	
Retired	47	15.67	35	11.67	

*Self- employee [self-employee, daily payment seeker, farmer].

Table 3. The health habits of cases and controls

Health Habits	Cases (N= 300)		Controls (N=300)		X^2; df; *p*
	No.	%	No.	%	
Smoking					4.751;1,0.029
Yes	129	43	1.3	34.3	OR= 1.443,
No	171	57	137	65.7	95% CI= 1.037-2.007
Alcoholic					5.053;1;0.025
Yes	21	7	9	3	OR= 2.434;
No	279	93	291	97	95% CI= 1.096-5.405
Eating fatty foods					0.3361; 1;0.562
Yes	171	57	178	59.3	OR= 0.909;
No	129	43	122	40.7	95% CI= 0.657-1.257
Physical activity					572.38;1;0.0001
Yes	2	0.7	295	98.3	OR=8791
No	298	9..3	5	1.7	95% CI=1521.1-22026.5

Table 4. The BMI distribution of cases and controls

BMI	Cases (N= 300)		Controls (N=300)		X^2; df; *p*
	No.	%	No.	%	
Under- Weight (BMI< 18.5)	3	1	-	-	10.7; 3; 0.013
Normal weight (BMI 18.5-24.9)	85	28.3	79	26.3	
Overweight (BMI 25-29.9)	65	21.7	91	30.3	
Obese (BMI 30- 39.9)	126	42	122	40.7	
Extreme obese (BMI=>40)	21	7	8	2.7	

Table 5. The medical health history for the cases and controls.

Medical Health history	Cases (N= 300)		Controls (N=300)		X^2; df; *p*
	No.	%	No.	%	
Hypertension					7.3381; 1; 0.007
Yes	151	50.3	118	39.3	OR= 1.563
No	149	49.7	182	60.7	59% CI= 1.131-2.161
Diabetes mellitus					6.121; 1; 0.013
Yes	121	40.3	92	30.7	OR=1.528
No	179	59.7	208	69.3	95% CI= 1.091-2.140

Table 6. The distribution of cases and controls according to their family history of medical conditions

Family History	Cases (N= 300)		Controls (N=300)		X^2; df; *p*
	No.	%	No.	%	
Hypertension					11.4470; 1; 0.001
Yes	87	29	52	17.3	OR= 1.984
No	213	71	248	82.7	95%CI= 1.320-2.875
DM					19.103; 1; 0.0001
Yes	78	26	36	12	OR= 2.577;
No	222	74	264	88	95% CI= 1.670-3.974
Ischemic Heart disease					25.158; 1; 0.0001
Yes	36	12	5	1.7	OR= 8.045;
No	264	88	295	98.3	95% CI=3.111-20.804
Death from heart diseases					5.042; 1; 0.025
Yes	36	12	20	6.7	OR=1.909
No	264	88	280	93.3	95% CI= 1.078-3.382

Discussion

Adequate control of cardiovascular disease (CVD) risk factors and health habits are important for preventing heart problems, but it has been reported that many patients remain uncontrolled despite regular care [(1)].

The study results revealed that cardiovascular events were more common in the age group (60-69) years than in other age groups, a similar finding was also reported in previous study [(8)], this finding could be explained by that, the incidence and prevalence of heart diseases increases dramatically with advancing age may be due to changes of cardiovascular structure and function [(8)].

The current study showed that about two third of cases were women, and this disagree with study conducted in Tunis [(9)], were men affected more than women. There is no accurate model for heart disease in women, until now most researches were done in men, women have been under represented in heart studies, under investigated, under diagnosed and under treated, but heart attacks are often more severe in women and they are more likely than men to die of it [(5,8,10)].

Statistical significant association was observed between years of education and admission to CCU, which coincided with study done in Oman[(11)], this might be attributed to the fact that individuals of higher educational level follow dietary recommendation and adopt other risk avoidance behaviors.

Smokers' risk of developing CVD is 2-4 times than that of non smokers [(11)], this finding goes in parallel with the result of this study. Smoking narrows the blood vessels causing an increase in blood pressure and heart rate as well as leads to reduced blood flow in the arteries; this reduced flow can lead to a heart attack [(12)].

The risk of CCU admission was 2.4 times higher among alcoholic than in non alcoholic, this result is comparable with previous studies, which showed direct association between alcohol drinking and risk of heart attack [(9,13)].

The current study showed statistical significant association between hypertension and CCU admission, and this in agreement with previous studies [(5, 9)], and this could be due to the fact that the main effects of hypertension on the heart are a direct result of excess pressure or resistance against which the heart must eject. Hypertension can also cause deleterious

changes in the coronary arteries adversely, affecting blood flow to the heart [14].

The study reported that patients with diabetes were 1.5 times more liable for cardiovascular disease than non diabetic , this finding is due to that people with diabetes are more likely than others to develop additional heart risk factors such as (high blood pressure, obesity, and high cholesterol) so that instead of one heart – disease risk , they have a collection [15].

Obesity significantly associated with cardiovascular disease occurrence [16], the result in this study demonstrated statistical significant association between BMI and CCU admission, this result supported by other studies [9, 17].

Family history is an important risk factor for ischemic heart diseases, the present study found statistical significant association between CCU admission and positive family history of (hypertension, diabetes, ischemic heart disease and death due to heart disease) in the first degree relatives of the cases. This finding might be due to certain environmental risk factors like nutritional habits and common pathophysiologic pathway [9].

Conclusion

The admission to CCU was more common in the age group **(**60-69) years and the females were more likely than males need CCU Admission, patients with the following risk factors were in more need to CCU admission than others: smoking, elevated body mass index (BMI), Hypertension, diabetes, and positive family history of various cardiovascular diseases among first degree relatives of the cases.

Recommendation

To raise the awareness of the people about the risk factors of cardiovascular diseases and how to avoid them through effective health and nutrition education programs and encourages the people to adopt healthy life style.

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Correspondence to: Dr. Najlaa F Jamil,
E-mail: basil.jassar@hotmail.com
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The Role of Matrix Metalloproteinase-2 and -9 *in situ* Hybridization in Bladder Cancer Progression

Areej A Hussein[1] *PhD*, **Jasim M Karhoot**[2] *PhD*, **Alaa Gh Hussain**[3] *FICMS (Path.)*

[1]Dept. of Microbiology, College of Medicine, Diyala University, [2]Dept. of Microbiology, College of Medicine, Baghdad University, [3]Dept. of Pathology & Forensic Medicine, College of medicine, Al-Nahrain University

Abstract

Background Transitional cell carcinomas (TCC) of the bladder are a major health problem and can be a leading cause of death. There are several proteolytic enzymes which are responsible for the degradation of the extra cellular components and have an essential role in tumor invasion and metastasis. MMP-2 and MMP-9 are the most important class of these enzymes.

Objective To assess the In situ hybridization expression of MMP-2 and MMP-9 in TCC of the bladder.

Methods Fifty formalin fixed, paraffin embedded of TCC of the bladder tissue blocks from Specialized Surgical Hospital in Baghdad, were included in this study. In addition ten apparently normal bladder autopsies were collected from the Forensic Medicine Institute Archives used as control group. Tissue blocks were sectioned on charged slides to be used for In situ hybridization, for the detection of MMP-2 and MMP-9.

Results The expression of MMP-2 and MMP-9 in TCC of the bladder tissues in the present study was 64 % for both and strong relationship between expression of MMP-2 and MMP-9 and TCC of the bladder was detected.

Conclusion MMP-2 and MMP-9 play an important role in progression of transitional cell carcinoma of the bladder.

Key words Bladder cancer, Matrix Metalloproteinases, invasion, metastasis, carcinogenesis.

Introduction

Urinary bladder cancer is one of the most common cancers worlds wide; with the highest incidence in industrialized countries [(1)].It's occurrence is strongly associated with cigarette smoking and the use of certain chemicals [(2)].

More than 90% of bladder cancers begin in the lining of the bladder wall and are known as transitional cell carcinomas (TCC). About 5% of bladder cancers are squamous cell carcinomas (SCC). There are also uncommon bladder cancers, such as adenocarcinoma and small cell carcinoma, which are responsible for less than 2% of all bladder cancers [(3)].

There are many markers associating with the progression of bladder carcinoma, such as depth of invasion, stage, grade and multiplicity. Unfortunately they are inaccurate, that is why more clinical prognostic markers are needed. Matrix metalloproteinases are a family of endopeptidases that are capable of degrading most components of the extracellular matrix (ECM) [(4)]. Of them, gelatinase-A (MMP-2) and gelatinase-B (MMP-9) are able to degrade extracellular matrix protein, including type IV collagen. Gelatinases have been linked to cell invasion and the process of metastasis [(5)].

Several studies that zymographical analysis of the levels of MMP-9 and active MMP-2 showed a significant increase with tumor grade and invasiveness, however, the correlation between the levels of both gelatinases with recurrence in superficial tumors or progression in invasive tumors was not significant [6].

To our knowledge there is no Iraqi study had focused on the possible role of MMP-2, MMP-9 during bladder cancer progression in order that this study will try to take the first step in detection of these markers and study the correlation with different parameters such as age, gender, grade and pattern of growth and presence or absence of muscle invasion in transitional cell carcinoma of the bladder.

Methods

Patients and tissue samples

Fifty patients with bladder carcinoma, 35 males and 15 female with an age ranged from 25 to 70 years, were included in this retrospective study, The patients' samples were collected from the archives of histopathology laboratories of Specialized Surgical Hospital in Baghdad From February 2009 till June 2009. The diagnosis of these tissue blocks were primarily based on the obtained histopathological records of bladder biopsy samples that had been accompanied in the hospital laboratory. Confirmatory histopathological re-evaluation of each obtained tissue blocks was done by specialist pathologist. In addition ten apparently normal bladder autopsies were collected from the Forensic Medicine Institute Archives. They were 5 males and 5 female and the range of the age was the same as patients group. Formalin-fixed, paraffin embedded blocks tissue were sectioned (4µm) thickness, from each tissue block, one section was stained with Haematoxylin and Eosin, and 2 sections were mounted on charged slides to be used for *In situ* hybridization for the detection of MMP-2 and MMP-9**.**

In situ hybridization procedure

Serial tissue sections were cut 4µm thick and were positioned on positive charged slides. The slides were placed in 60˚C oven over night. The tissue sections were deparaffinized, the slides were dehydrated by graded alcohol concentration (100%, 95%, 70%) and distal water. The slides were treated with proteinase K solution and dehydrated. One drop of the biotinylated long cDNA probe for human MMP-2 and MMP-9(Maxim Biotech Cat. No.: IH-60025 and IH-60028). Hybridization/ detection kit were used purchased from Maxim Biotech/USA Cat. Number IH-6001(IHD-0050) was placed on the tissue section in oven at 70˚C for 8-10 minutes. After that the slides were placed in a humid chamber and incubated over night at 37°C to allow hybridization of the probe with the target nucleic acid. The slides were soaked in 1X detergent wash at 37˚C until the cover slips fall, and then treated with RNase A solution and streptavidin-AP-conjugate. One to two drops of 5-bromo-4-chloro-3-indolyl phosphartel/nitro blue tetrazolium substrate-chromogen solution (BCIP/NBT) conjugate were placed on tissue section at room temperature for about 30 minutes; the latter was monitored by viewing the slides under the microscope. A blue colored precipitate will from at the site of the probe in positive cells. Slides were then counterstained using nuclear fast red and sections were mounted with a permanent-mounting medium (DPX). Finally the examination and scoring were done under light microscope by a pathologist at power 400 according to the scoring system of [7].

Statistical analysis was done using Chi-Square test for tables with frequencies percentages, range, mean and standard deviation. Values were considered statistically significant when $p<0.05$.

Results

Histopathpological classification

Fifty formalin-fixed, paraffin embedded blocks were collected from bladder carcinoma patients and histopathologial re-examination with hematoxylin and eosin stain was done. The specimens were graded according to world health organization classification [8]. As follow: Grade I: well differentiated transitional cell carcinoma of the bladder (n=4) (8%), Grade II: moderately differentiated transitional cell carcinoma of the bladder (n=31) (62%) and Grade III: poorly differentiated transitional cell carcinoma of the bladder (n=15) (30%).

Each carcinoma was also distributed according to the pattern of growth, as follows: papillary type (n=28) (56%) and solid type (n=22) (44%). More ever, muscle invasion was seen in 26 cases (52%) while non-invasion was seen in 24 cases (48%).

Results of in situ hybridization detection of MMP-2 and MMP-9

The results showed in table 1 and figure 1, 2 which were demonstrated that 32 cases (64%) of bladder carcinoma cases were positive for both of them, while 18 cases (36%) cases were negative for both of them. On the other hand statistical analysis were demonstrated a highly significant differences in MMP-2 and MMP-9 expression among patients with transitional cell carcinoma of the bladder when compared with healthy control group.

Table 1. The expression of MMP-2 and MMP-9 in patients with transitional cell carcinoma of the bladder.

Result of MMP expression			MMP-2 Expression	MMP-9 Expression	Comparison of Significance	
Patients	Positive	Low Intermediate High Total	10 17 5 32 (64 %)	19 10 3 32 (64 %)	p- value 0.01	Sig. Highly Sig. P<0.01
	Negative	N %	18 (36%)	18 (36%)		
	Total	N %	50 (100%)	50 (100%)		
Controls	Positive Negative	N % N %	0 10 (100%)	0 10 (100%)		

Table (2): Correlation of MMP-2 scores and related with different parameters.

Parameters		MMP-2 scores			Comparison of Significance	
		Low	Intermediate	High	p-value	Sig.
Age	25-39 40-54 55-70	1 (10%) 1 (10%) 8 (80%)	0 3 (17.6%) 14 (82.4%)	1 (20%) 1 (20%) 3 (60%)	0.48	Non Sig. (P>0.05)
Gender	Male Female	8 (80%) 2 (20%)	11 (64.7%) 6 (35.3%)	2 (40%) 3 (60%)	0.33	Non Sig. (P>0.05)
Tumor grade	I II III	0 7 (70%) 3 (30%)	1 (5.6%) 11 (64.7%) 5 (29.4%)	0 1 (20%) 4 (80%)	0.13	Non Sig. (P>0.05)
Pattern of growth	Papillary Solid Invasive	5 (50%) 5 (50%) 4 (40%)	8 (47.1%) 9 (52.9%) 10 (58.8%)	1(20%) 4 (80%) 4 (80%)	0.07	Non Sig. (P>0.05)
Muscle invasion	Non invasive	6(60%)	7 (41.2%)	1 (20%)		

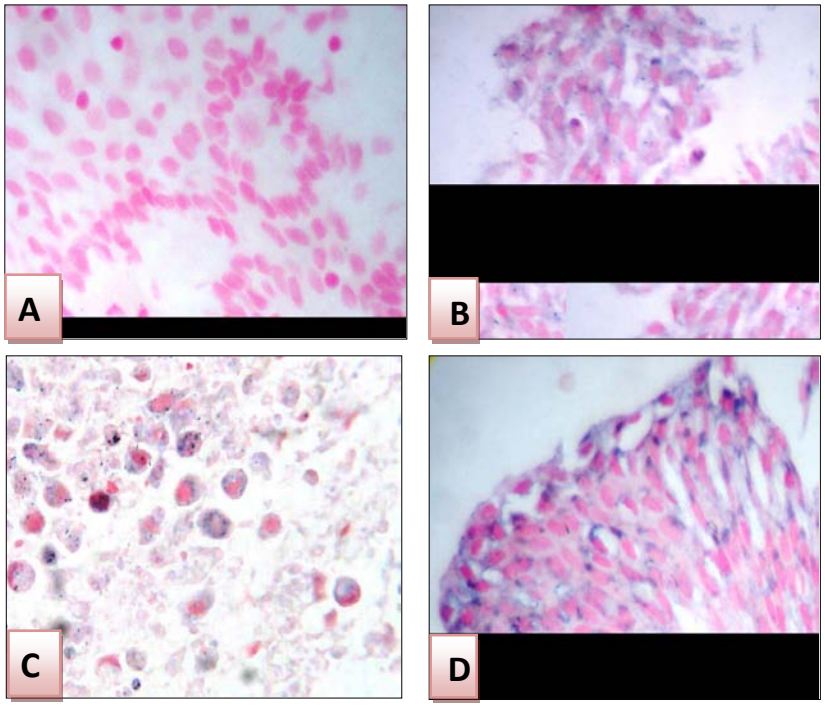

Figure 1. *In situ* hybridization for MMP-2 of patient with TCC of the bladder, stained by BCIP/NBT-Chromogen and counter stained with nuclear fast red (NFR), magnification power, 400. A-Negative expression, B-low MMP-2 positive expression, C-intermediate MMP-2 positive expression, D-High MMP-2 positive expression.

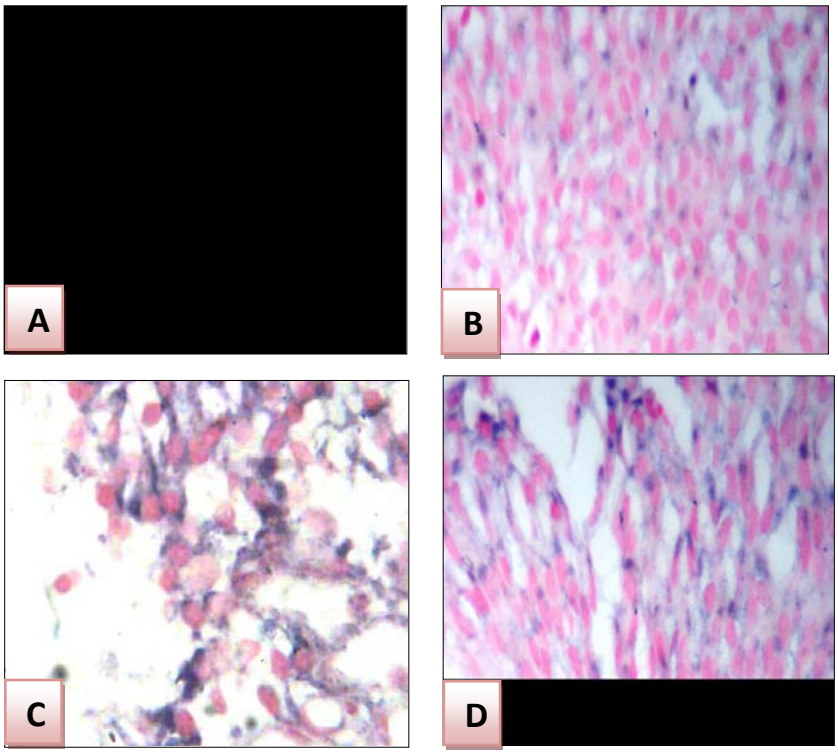

Figure 2. *In situ* hybridization for MMP-9 of patient with TCC of the bladder, stained by BCIP/NBT-Chromogen and counter stained with nuclear fast red (NFR), (Magnification power, 400). A-Negative expression, B-low MMP-9 positive expression, C-intermediate MMP-9 positive expression, D-High MMP-9 positive expression.

muscle invasive. The results of frequency distribution of positive and negative MMP-2 and -9 showed significant correlations between MMP-2 expression and pattern of growth while no correlation occur with MMP-9 based on Chi-square test of analysis (Table 4 and 5).

Table 2 and 3 demonstrated the correlation between expression of MMP-2 score and MMP-9score, with different variables. The results showed that there were no significant differences between *in situ* hybridization expression of both MMP-2 and MMP-9 with age, gender, grade, pattern of growth, and

Table 3. Correlation of MMP-9 scores and related with different parameters.

Parameters		MMP-2 scores			Comparison of Significance	
		Low	Intermediate	High	p-value	Sig.
Age	25-39 40-54 55-70	0 4 (21.1%) 15 (78.9%)	1 (10%) 1 (10%) 8 (80%)	1 (33.3%) 1 (33.3%) 1 (33.3%)	0.37	Non Sig. (P>0.05)
Gender	Male Female	14 (73.7%) 5 (26.3%)	9 (90%) 1 (10%)	2 (66.7%) 1 (33.3%)	0.28	Non Sig. (P>0.05)
Tumor grade	I II III	3 (15.8%) 12 (63.3%) 4 (21.1%)	0 6 (60%) 4 (40%)	0 1 (33.3%) 2 (66.7%)	0.50	Non Sig. (P>0.05)
Pattern of growth	Papillary Solid Invasive	11 (57.9%) 8 (42.1%) 10 (52.6%)	5 (50%) 5 (50%) 3 (30%)	1 (33.3%) 2 (66.7%) 2 (66.7%)	0.80	Non Sig. (P>0.05)
Muscle invasion	Non invasive	9 (47.4%)	7 (70%)	1 (33.3%)		

Table 4. *In situ* hybridization expression of positive and negative MMP-2 and related with clinicpathological profile of patients with TCC.

Variables		MMP-2		Comparison of Significance	
		positive	negative	p-value	Sig.
Age	25-39 40-54 55-70	2 (6.3%) 5 (15.6%) 25 (78.1%)	2 (11.1%) 6 (33.3%) 10 (55.6%)	0.24	Non Sig. (P>0.05)
Gender	Male Female	21 (65.6%) 11 (34.4%)	14 (77.8) 4 (22.2%)	0.36	Non Sig. (P>0.05)
Tumor grade	I II III	1 (3.1%) 19 (59.4%) 12 (37.55%)	3 (16.7%) 12 (66.7%) 3 (16.7%)	0.10	Non Sig. (P>0.05)
Pattern of growth	Papillary Solid	14 (43.8%) 18 (56.2%)	14 (77.8%) 4 (22.2%)	0.02	Sig. (P<0.05)
Muscle invasion	Invasive Non invasive	18 (56.2%) 14 (43.8%)	6 (33.3%) 12 (66.7%)		

Table 5. *In situ* hybridization expression of positive and negative MMP-9 and related with clinicpathological profile of patients with TCC.

Variables		MMP-2		Comparison of Significance	
		positive	negative	p-value	Sig.
Age	25-39 40-54 55-70	2 (6.2%) 6 (18.8%) 24 (75%)	2 (11.1%) 5 (27.8%) 11 (61.1%)	0.58	Non Sig. (P>0.05)
Gender	Male Female	25 (78.1%) 7 (71.4%)	10 (55.6%) 8 (44.4%)	0.95	Non Sig. (P>0.05)
Tumor grade	I II III	3 (9.4%) 19 (59.4%) 10 (31.3%)	1 (5.6%) 12 (66.7%) 5 (27.8%)	0.83	Non Sig. (P>0.05)
Pattern of growth	Papillary Solid	17 (53.1%) 15 (46.9%)	11 (61.1%) 7 (38.9%)	0.58	Non Sig. (P>0.05)
Muscle invasion	Invasive Non invasive	15 (56.9%) 17 (53.1%)	9 (50%) 9 (50%)	0.83	Non Sig. (P>0.05)

Discussion

Matrix metalloproteinases (MMPs) are involved in cellular proliferation, migration, invasion and metastasis [(9)]. MMP-2 and MMP-9 are thought to play a central role in these processes, in view of their ability to degrade many Extracellular Matrix (ECM) components and other substrates [(10)].

In general, the role of MMPs in carcinogenesis seems to be very complex, sometimes even controversial, according to some preclinical findings [(11)].

In vitro studies had indicated that MMP-2 and MMP-9 activity may determine the invasion capacity of the bladder carcinoma cell line [(12)].

The current study had demonstrated that MMP-2 and MMP-9 were over expressed in transitional cell carcinoma of the bladder. These results might possibly reflect the association between cellular expression of MMP-2 and MMP-9 and bladder tumor genesis. This was in agreement with the findings of other authors [(6,13,14)] since they found over expression of this enzyme in transitional cell carcinoma of the bladder. In comparison with other studies both enzyme are increased in malignant tissues compared to their benign counterparts [(15).] This raises the question why MMPs expression was rare in benign tumor, we know that benign tumor have no metastasis and no invasion, so that there is no need for additional degradation of ECM, and finally no need for exaggerated MMPs expression. In fact, analysis of both primary and metastatic tumors demonstrated increased MMPs at the metastatic site had pointed out their role in tumor migration and spread [(16)].

In this study, the results showed no correlation between MMP-2 expression and age, gender, also any correlation with tumor grade and muscle invasion not observed. In the present study the results were in agreement with Grignon et al [(13)] who did not found any association between the expression of MMP-2 immunoreaction protein in bladder cancer tissue or the grade or stage in TCC of the bladder. However, this result was also in agreement with the results of Kanayama et al [(17)] who reported that MMP-2 contributes to the invasive properties of bladder carcinoma. Moreover, in some study where gelatine zymography was used, the expression of activated MMP-2 was higher in invasive tumor tissue [(18)]. Other study has also shown a correlation to grade and stage [(19)].

Concerning with MMP-9 expression, positivity did not correlate to the age, gender of the patients, pattern of growth, grade of the tumor. This result was in agreement with the findings of Ozdemir et al [20] who pointed out that no correlation between MMP-9 over expression and tumor grade was recorded. Mohammed et al [21] measured the level of MMP-9 in serum by western blot technique and revealed that serum level of MMP-9 showed highly significant elevation compared to healthy normal subjects but this elevation did not correlate with age, gender or even grade of the disease. Durkan and co workers [22] found no correlation to grade but instead, the MMP-9 levels measured by enzyme-linked immunoassay (ELISA) correlated to stage when measuring MMP-9 protein in urine samples of bladder cancer patients. It had been found that patients with no relapse had a higher urine MMP-9 protein level than patients with relapses, the difference being statistically significant [23].

MMP-9 is quite well examined in bladder cancer whether using tissue samples, serum or urine detection, it seems that high or elevated expression of MMP-9 enzyme correlates to clinical stage or histological grade of the tumor [24,25].

The results are consistent with result of previous studies suggesting that MMP-2 and MMP-9 may play an important role in TCC of the bladder or could facilitate its progression.

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Correspondence to: Dr. Areej A Hussein
E-mail: Areej.2002@yahoo.com
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Neutrophil Activation Through the Expression of CD11a, CD11b and CD11c and Its Role With Complement C3 And C4 Levels in Patients With Pre-eclampcia

Thura J Kadhum[1] *MBChB,* **Nidhal AM Mohammed**[1] *PhD,* **Malka S Al-Saadi**[2]

[1]Dept. of Medical Microbiology, College of Medicine, Al-Nahrain University, [2]Dept. of Gynecology & Obstetrics, College of Medicine, Al-Nahrain University.

Abstract

Background The leukocyte integrin that plays a major role in neutrophil activities is CD11b. In addition to mediating neutrophil adherence to endothelial cells, CD11b binds to the complement component iC3b and directs phagocytosis and intracellular lysis of microorganisms.

Objective To determine whether neutrophil activation, through the increased surface expression of the cell surface markers CD11a, CD11b, CD11c have a correlation with the values of complement components C3 and C4 in pregnant women with pre-eclampsia.

Methods This study was conducted at the AlKadhemiya Teaching Hospital. Patients were 60 pregnant women in labour subdivided into three groups:
Group A: 20 pregnant women with severe pre-eclampsia.
Group B: 20 pregnant women with mild-moderate pre-eclampsia.
Group C: 20 normotensive pregnant women (control group).
We performed the following laboratory measurements for all groups: total white blood cell WBC count, cell surface expression of CD11a, CD11b, CD11c by direct immunofluorescent technique, and serum complement C3 and C4 levels by radioimmunodiffusion method (RID).

Results There was a significant difference in neutrophil activation as detected by the cell surface expression of CD11a, CD11b, CD11c being higher in the pre-eclamptic group than the control group. The incidence of neutrophil activation was significantly higher among patients in group A compared to the other groups. There was a significant difference in the serum level of C3 and C4 in the pre-eclamptic group being higher in group A and B than group C.

Conclusions No correlation was found between the markers of neutrophil activation (CD11a, CD11b, CD11c) and the serum levels of (C3, C4) in the pre-eclamptic group. The incidence of adverse maternal and neonatal outcome is significantly higher among patients in group A compared to the other two groups.

Key words Preeclampsia, complement serum level, CD11a, CD11b, CD11C, neutrophil.

Introduction

Women with chronic hypertension and pregnancy-induced hypertension are at substantial risk for developing pre-eclampsia/eclampsia, a disease with high fetomaternal morbidity and mortality. However, the etiology of this disease is still unknown [(1)].

Preeclampsia complicates 3-5% of first pregnancies and 1% of subsequent pregnancies with around 5-10% of cases being severe [(2)].

Hypertension with proteinuria or oedema or both induced by pregnancy after the 20th week of gestation [3].

The presentation is very variable and although hypertension and proteinuria are the two signs most easily detected they are not consistently present, 20% of women with preeclampsia are normotensive and 30% have no premonitory proteinuria [3].

The increased incidence of pregnancy induced hypertension noted among patients over the age of 35 years probably reflects undiagnosed chronic hypertension with superimposed pregnancy induced hypertension [4]. During an inflammatory response, neutrophil activation leads to neutrophil binding and transmigration through endothelial cells. This occurs through an interaction between endothelial cell adhesion molecules and surface receptors on polymorphonuclear neutrophils. Activation of leukocyte integrins mediates firm adherence of activated neutrophils to the endothelium, with subsequent release of substances mediating vascular damage [5].

Surface expression of L-selectin (CD62L), which is involved in the initial adhesion process, is downregulated as adherent neutrophils emigrate through vascular endothelium [6].

Several lines of evidence have documented neutrophil activation in pre-eclampsia as follows:

1. Neutrophil degranulation products, including elastase and lactoferrin, are present in higher concentrations in the serum of women with pregnancy-induced hypertension [7].
2. The production of leukotrienes and superoxides by leucocytes are also increased [8].
3. Neutrophil integrin expression is altered [9].

The leucocyte integrins represent a subclass of a large family of proteins that mediate interactions between cells and their extracellular environment [6].

The leucocyte integrins-known as CD11a/CD18, CD11b/CD18 and CD11c/CD18 (CD=cluster of differentiation)-mediate the interactions of leucocytes with each other, with extracellular particles and with endothelial cells [5]. Integrins have found to play a role in platelet aggregation, immune functions, and tissue repair and tumor invasion [10].

The leucocyte integrin that plays a major role in neutrophil activities is CD11b. On stimulation, CD11b is translocated to the neutrophil cell surface. In addition to mediating neutrophil adherence to endothelial cells, CD11b binds to the complement component iC3b and directs phagocytosis and intracellular lysis of microorganisms. CD11b also recognizes fibrin and fibrinogen and can mediate neutrophil adherence to the extracellular matrix [11].

CD11a, which is crucial to lymphocyte adherence, is also present on the neutrophil surface and plays a role in antibody-dependent killing.

CD11c is also found on neutrophils, also binds to complement component iC_3b and appears to play a role in neutrophil-endothelial interactions [12]. So we intended in this study to focus on the role of these molecules in patients with pre-eclampsia.

Methods

This study was conducted on 60 pregnant women presented with labor pain attending Alkadhemiya Teaching Hospital starting from the 1st of October, 2001 to the end of April, 2002. Forty pre-eclamptic women were studied and 20 apparently healthy pregnant women as a control. The following inclusion criteria were followed; Pre-eclampsia was diagnosed by the occurrence of hypertension (upper limit of normal blood pressure value was 139/89 mmHg) in combination with proteinuria and/or edema, developing after 20 weeks gestation in a previously normotensive nonproteinuric patient. Age range from 18-40 years, less than 6 weeks

gestational, singleton pregnancy. The exclusion criteria were:

Multiple pregnancy, history of essential hypertension, diabetes mellitus, renal disease, hepatic disease, blood disorders, epilepsy or other medical diseases, history of drug intake other than supplements.

Women included in this study were subdivided into three groups:

Group A: Twenty pregnant women in labor with severe pre-eclampsia which was evidenced by a systolic blood pressure of >160 mmHg and diastolic blood pressure of >110 mmHg with proteinuria >5 gm/24 hours and/or edema.

Group B: Twenty pregnant women in labor with mild-moderate pre-eclampsia which was evidenced by a systolic blood pressure of 140-159 mmHg and a diastolic blood pressure of 90-109 mmHg with proteinuria and/or edema.

Group C: Twenty apparently healthy pregnant women in labor of comparable age, parity and stage of pregnancy as a control group.

Blood pressure was recorded in the sitting position with a cuff that is large enough for the subjects arm on at least two occasions 6 hours apart.

Korotkoff phase 5 (K5) disappearance of the sound was used to detect the diastolic blood pressure.

Proteinuria was diagnosed by collecting clean catch midstream urine sample in a clean dry container, and then urine protein was determined using the reagent strips (Albustix).

A reading of 2+ (1 gm/L) or more or equal to 1+ (0.3 gm/L) if the specific gravity is less than 1030 was considered to be positive result for proteinuria (significant proteinuria).

After medical, surgical and obstetric history was taken for all women who were subjected to full physical and obstetric examination and they were followed during their labor, delivery, postnatal period and peurperium with follow up of their newborn during their postnatal period.

In addition to the routine laboratory tests done, we performed the following laboratory measurements for all groups:

- Total white blood cell WBC count.
- Determination of cell surface adhesion molecule (CD11a, CD11b, CD11c) by direct immunofluorescent technique.The technique described by Eggleton et al (1989) [13] was used for neutrophil separation.

Preparation of slides:

10 µl of cell suspension per well on teflon coated slides, left to dry, then fixed by fixator (prepared by mixing 90 parts of 95% ethanol, 5 parts methanol, 5 parts of 100% isopropanol).

Slides dipped in fixator for 30 minutes and allowed to dry at room temperature, then slides checked with microscope for even spread of cells, then stored at -20 C till assayed.

Direct immunofluorescent test (DIFT):

The teflon slides precoated with neutrophil suspension were removed from freezer and allowed to reach room temperature, then washed with PBS, then slides were layed flat in humidity chamber then 10 µl of fluorochrome conjugated monoclonal antibodies at 1/10 dilution were added to each well. Cover chamber and slides were left undisturbed in incubator at 37 C. Slides then transferred to staining jar filled with PBS at room temperature, then we placed a drop of mounting media on each well of slides (9 parts glycerol to 1 part carbonate buffer PH=9) to enhance fluorescence and retard fading on exposure to UV light, then cover slides were lowered into place slowly to avoid bubbles.

Examination of the slides: slides were examined with fluorescence microscope at 490nm. In each spot 200 neutrophils were counted, the positive labeled cells were identified by their bright green color. The percentages of positive cells were found using the following formula: (labeled cells/200)×100

Measurement of serum complements C3 and C4 levels:

The serum level of C3 and C4 for each patient was estimated using the Radioimmunodiffusion method (RID), whichwas done according to manufactuers instruction.

Statistical analysis:

The statistical analysis of the data was performed using the following tests: Chi square test, Student t-test and Correlation coefficient test.

Results

Figure 1 shows the mean WBC count for all groups. There was no significant difference in the mean total WBC count for all groups ($p > 0.05$).

Figure 2 shows the mean percentage positive cells for CD11a for all groups. We found a higher percentage among patients of group A (49.4% ± 2.63) compared to those in group B (30.75% ± 1.67) and group C (16.65% ± 1.30) which was statistically significant ($p < 0.0001$).

Figure 3 shows the mean percentage of positive cells for CD11b. We found a statistically significant higher percentage among patients of group A (41.9% ± 1.57) compared to group B (24.15 % ± 1.11) and group C (16.95% ± 1.33) ($p < 0.001$).

Figure 4 shows the mean percentage of positive cells for CD11c. We found a higher percentage among patients of group A and B (33.1% ± 1.29, 18.05% ± 1.58 respectively) compared to group C (15.15 % ± 1.13) being statistically significant for A and C, B and C ($p < 0.0001$) but statistically not significant for A and B ($p > 0.05$).

Figure 5 shows the mean serum concentration of C3 and C4 for all groups. We found a statistically higher concentration among the pre-eclamptic group as compared to the control group ($p < 0.0001$) but there was no correlation with disease severity with p value for A and B ($p > 0.05$).

To evaluate the relationship between the percentage of positive cells for CD11a, CD11b, CD11c and the serum complement C3 and C4 levels in the pre-eclamptic group, the correlation coefficient was calculated and no correlation was found between the two parameters.

Table 1 shows the relation between the mean percentage positive cells for CD11a, CD11b, CD11c and the mean serum complement C3 and C4 levels for the pre-eclamptic group.

Table 1: The relationship between CD11a, CD11b, CD11c and C3, C4 among the pre-eclamptic group

Group A		C3 (mg/dl)	C4 (mg/dl)
CD11a (% positive cells)	Correlation Coefficient	-0.129	-0.077
	Significance	0.589	0.748
CD11b (% positive cells)	Correlation Coefficient	-0.50	0.378
	Significance	0.835	0.10
CD11c (% positive cells)	Correlation Coefficient	0.121	-0.34
	Significance	0.587	0.887
Group B		**C3 (mg/dl)**	**C4 (mg/dl)**
CD11a (% positive cells)	Correlation Coefficient	-0.254	-0.005
	Significance	0.280	0.985
CD11b (% positive cells)	Correlation Coefficient	0.078	0.118
	Significance	0.747	0.621
CD11c (% positive cells)	Correlation Coefficient	-0.320	0.04
	Significance	0.169	0.868

Discussion

Pre-eclampsia is characterized by platelet activation, vasoconstriction and vascular damage [14], all changes which strongly imply disordered endothelial cell function. Neutrophils have been implicated in the pathogenesis of atherosclerosis [15] and through their ability to produce reactive oxygen species, have been presumed to play a role in the vascular damage of pre-eclampsia.

The expression of neutrophil cell adhesion molecules have been described as a useful marker of in vivo activation in various pathologic situations, including chronic renal failure [16], endotoxemia [17], trauma and sepsis [18].

Neutrophil priming and activation can best be identified by a transformation in cellular adhesion molecule expression — most notably by a rise in the integrins, predominantly CD11b and to a lesser degree CD11a, CD11c and a fall in the level of the selectin CD62L. These are constitutively expressed on human neutrophils and are upregulated on the cell surface [19].

The total WBC count does not change during pregnancy [20]. In our study we found that the total WBC count remained within the normal range in the three groups with mean value of 9202.5 cell/mm^3 $\pm$ 577.63 in group A, 8059 cell/mm^3 $\pm$ 619.72 in group B, 7650 cell/mm^3 $\pm$ 482.5 in group C.

The present study showed that polymorphonuclear neutrophils are activated in pre-eclamptic women presented with labour pain through the changes of several markers. The neutrophil response was evaluated by quantitating the cell surface expression of the integrins CD11a, CD11b, CD11c.

The present study showed a significant increase in cell surface expression of CD11a, CD11b, CD11c in group A (49.4%, 41.9%, 33.1% respectively) and group B (30.75%, 24.15%, and 18.05% respectively) as compared to group C (16.65%, 16.95%, and 15.15% respectively).

This result is consistent with that of Sabatier et al. 2000 who found that neutrophils were activated in pre-eclamptic women and in patients with isolated intrauterine growth restriction through the increased cell surface expression of Cd11b and CD62L [21].

In our study we measured the serum complement levels, C3 and C4 and it was found that a significant increase in the levels of C3 in group A and B (210.4 mg/dl, 192.7 mg/dl respectively) as compared to group C (135.6 mg/dl) and increased levels of C4 in group A and B (52.42 mg/dl, 48.75 mg/dl respectively) as compared to group C (37.2 mg/dl) but with no correlation with disease severity as there was no statistical difference between group A and B ($p > 0.05$).

While Mellenbakken et al, 2001 [22] found only a decrease in the level of C4 but no changes in the level of C3 between normotensive and pre-eclamptic subjects.

de-Messias et al, 2000 [23] in their study on Brazilian pre-eclamptic women showed a significantly higher level of C3 and C4 in the pre-eclamptic group when compared to the normal pregnancies, but none of the studied variables showed statistically significant differences regarding the severity of pre-eclampsia.

These results confirm the activation of complement in pre-eclampsia, suggesting that this activation is related to the disease manifestations.

In our study we could not find a positive correlation between the markers of neutrophil activation and the markers of complement activation in the pre-eclamptic women and it may be that other factors of the complement cascade operate on the neutrophil or that the effect of complement on neutrophil is local and cannot be detected systemically, this is in agreement with the study of Mellenbakken et al, who found no correlation between neutrophil activation and systemic complement activation in patients with pre-eclampsia[22].

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Correspondence to: Dr. Nidhal AM Mohammed.
E- mail: dr.nidhalmohammed@yahoo.com
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Pathological Changes in Genital Organs of Female Albino Mice After Treatment with Pentoxifylline

Rajiha A AL-Naimi [1] *MSc*, **Salema L Hassan**[1] *MSc*, **Saad S AL-Dujaily**[2] *PhD*

[1]Dept. of Poultry Diseases, College of Veterinary Medicine, Baghdad University,[2] Institute of Embryo Research & Infertility Treatment, Al-Nahrain University.

Abstract

Background Little to our knowledge has been attempting to show the effect of pentoxifylline (PTX) on ovulation or oogenesis. In a few studies of man with asthenospermia oral treatment with PTX produced significant increase in sperm concentration and motility.

Objective Study the effect of PTX on ovulation and oogenesis in albino mice.

Methods Sixty albino mice randomly divided into 6 equal groups: Group 1 received tap water and considered as a control group. Group 2 treated with 16 mg of PTX/Kg/BW/daily for 2 weeks. Group 3, 4, 5, and 6 treated with same dose for 4, 6, 8, 10 weeks respectively. Post-mortem examination done according to the time of treatment and the reproductive systems were excised and processed for light microscopic examination.

Result PTX administration causes an increase in the diameter of the ovary and in the total number of ovarian follicles and their diameters especially Graafian and secondary follicles. In addition, highly significant increase in the numbers of corpus lutea especially in those groups treated for longer time was noticed. Moreover, an increase in the thickness of uterine and oviduct epithelial lining due to hyperplasia and an increase in the diameter of endometrial glands and oviducts was demonstrated.

Conclusions We concluded that administration of PTX has a significant effect on the female genital organs especially if given in small doses for 10 weeks. This will definitely influence reproduction and litter size manifested after mating with untreated males.

Key words Pentoxifylline, ovary, uterus, fallopian tube

Introduction

Reproductive efficiency is the major factor that affects profitability in many live stock production systems. Inefficient reproduction may be caused by numerous factors; including increased genetic selections for meat or milk production traits, early embryonic and fetal loss, failure to reach puberty at an optimum age or an inability of young females to conceive early in the breeding seasons, environmental stress such as temperature extremes or changes in photoperiod (day and night cycle), production of sperms with a low potential for fertilization, and limited sex drive [(1,2).]

Pentoxifylline (PTX) is a dimethyl xanthine derivative belongs to a group of vasoactive drugs used in humans for the treatment of peripheral and cerebral vascular diseases caused by impairment of the microcirculation [(3)]. PTX is used also in the treatment of male infertility in human by enhancing sperm motility both *in vivo* [(4)] and *in vitro* [(5)], in cases of normozoospermia and asthenozoospermia [(6)]. It has an inhibitory effect on

Phosphodiesterase that enhances sperm motility by increasing intracellular cAMP [7].

In veterinary practice, it is used to improve microcirculation and enhance healing of skin lesions in dogs, and for the treatment of endotoxemia, laminitis, navicular disease, and improve motility of the cryopreserved spermatozoa in stallions [8].

Studies on reproductive performance in rats, mice, and rabbits revealed no evidence of impaired fertility or harm to the fetus due to PTX [9]. In addition, females with endometriosis-associated infertility may get benefit from the use of PTX without significantly affecting embryo development [10].

To the best of our knowledge little studies has been attempted to show the effect of PTX on ovulation or oogenesis. Therefore, the aim of this study is to accomplish this task and study its effect on some parts of the genital organs like ovary, oviduct, uterus, and vagina.

Methods

1- Experimental design:

Sixty mice were divided into 6 equal groups, after labeling them (at ear or tail) and weighing them using mechanical balance as follows:

G_1: treated orally with tap water, which was regarded as a control. G_2: treated orally with 16 mg/B.W/daily for 2 weeks. G_3: treated orally with 16 mg/B.W/daily for 4 weeks. G_4: treated orally with 16 mg /B.W/daily for 6 weeks. G_5: treated orally with 16 mg/B.W/daily for 8 weeks. G_6: treated orally with 16 mg/B.W/daily for 10 weeks.

2- Treatment:

PTX is presented in the form of coated tablets containing 400mg (Aventis USA).

160 mg of the coated tablets were dissolved in 100 ml of tap water to obtain a stock solution from which 0.1 ml was given orally for each 10 gm of the living body weight of the experimental mice. This amount of the solution will provide a dose of 16 mg/kg Bw/day of the drug. The dose was individually adjusted according to the weight of each animal and was given via a fine plastic stomach tube given to G2, G3, G4, G5 and G6. Control group (G1) was given tap water only.

3- Parameters:

Clinical signs and symptoms and macroscopic and microscopic findings.

A- Clinical signs and symptoms:

Clinical signs were closely observed and continuously recorded along the period of experiment which is 10 weeks, also any change in activity or behavior was noted.

B-Macroscopic and microscopic examination:

At the end of each experimental group, the animals were anesthetized, then the abdomen of each animal was opened and the organs wanted were excised. Then the genital organs were examined for any changes in size, and color then small pieces were kept in 10% neutral buffer formalin for fixation processed routinely in histokinette, cut at 5µm thickness by microtome and stained with haematoxylin and Eosin stain then examine under light microscope [11].

Parameters used in studying the tissue section are: [12]

A. Ovaries:

Diameter of the ovary, number of all follicles, number and diameter of Graafian (antral) follicles (GF), number and diameter of submature follicles, and number of corpus luteum (CL).

B. Oviducts:

Diameter of oviduct (ampulla) and thickness of epithelial cell layer.

C. Uterus:

Diameter of endometrial glands and thickness of epithelial layer.

Data from treated and control groups were expressed as Mean± Standard error of Mean (S.E.M) and analyzed using student's t-test Differences between values were considered significant at $p < 0.05$ and highly significant at $p < 0.01$[13].

Results

1- Clinical Signs and Symptoms:-

All treated animals were active end showed an increase appetite for food consumption during

the study period, while the control group showed normal food consumption.

2- Macroscopic findings:-

There were no clear macroscopic changes, except that there were differences in dimensions of the genital organs. The morphological changes in the diameter of ovary were highly significant ($p < 0.01$) increased in G3 and G6 (1386±4.15, 1412.7±4.23) µm respectively with a significant increase ($p < 0.05$) in G2, G4 and G5 (1306.8±6.53, 1159.3±4.60, 1158±4.603) µm respectively compared with that of the control group (1009.8±0.56) µm (Table 5). Gross examination showed an enlargement of the genital organs (Figure 1).

3- Histopathological examination

1- Ovaries:

There were no any inflammatory changes in all examined genital organs. The microscopic examination showed highly significant increase in the diameter of the ovaries ($p < 0.01$) in G3 and G6 (1386±4.15, 1412.7±4.23) µm, and a significant increase ($p < 0.05$) in G2, G4 and G5 (1306.8±6.53, 1159.3±4.60, 1158±4.63) µm respectively compared with that of control (1009.8±8.56) µm (Table 5). The diameter of the primary follicles showed a significant increase ($p < 0.05$) in G2, G3 and G4 (118.56±3.31, 71.63±3.58, 74.1±3.70) µm compared with that of the control group (93.86±0.89) µm. The diameter of secondary follicles showed highly significant increase ($p < 0.05$) in G5 and G6 (407.55±4.07, 427.31±3.84) µm with significant increase ($p < 0.01$) in G2, G3 and G4 (271.7±3.53, 333.45±3.01, 365.65±2.19) µm. The diameter of Graaffian follicle was significant in G3, G4, G5 and G6 (363.09±2.54, 382.85±2.67, 382.85±3.06) µm (Table 1 and Figures 2-4).

Concerning the total number of follicles, there is a highly significant increase ($p < 0.01$) in G6 (25.0±0.5) and a significant increase ($p < 0.05$) in G2, G3, G4 and G5 (20.0±0.6, 21.0±0.63, 23.0±0.46, 23.0±0.69) respectively compared to the control (18±0.36). The numbers of primary follicles showed a high significant increase ($p < 0.01$) in G2 (12.0±0.36) and a significant increase in G3, G4, G5, and G6 (8.0±0.24, 6.0±0.24, 3.0±0.21 and 8.0±0.56) respectively compared with that of the control (3.0±0.12). The numbers of secondary follicles showed a significant increase ($p < 0.05$) in G_3, G_4, G_5 and G_6 (5±0.15, 4±0.12, 4±0.12, 4±0.16) respectively compared with that of control (3.0±0.12). The numbers of Graaffian follicles showed significant increase ($p < 0.05$) among all treated groups (3.0±0.18, 5.0±0.15, 5.0±0.1, 6.0±0.18, 6.0±0.12) respectively compared with that of the control (4.0±0.16). The numbers of corpus luteum were highly significant increase ($p < 0.01$) in G5 and G6 (8.0±0.24, 12.0±0.24) and the increment was higher than that of the control group. G2 and G3 showed significant increase ($p < 0.05$) (3.0±0.18, 3.0±0.21) compared with that of the control (Table 2 and Figure 5).

2- Uteri and oviducts changes:

All treated groups showed proliferation of the lining epithelium due to hyperplasia leading to increase in the thickness of the epithelial lining, with presence of mitotic figures. The increase changes are highly significant increased ($p < 0.01$) in G3, G4, G5 and G6 (37.05±0.79, 49.4±0.82, 61.75±0.92, 86.45±2.43) respectively, while G2 showed significant increase ($p < 0.05$) in the thickness compared to that of the control (12.35±4.03) µm. The endometrial glands became larger and appear with more numerous colloids in their lumen. The endometrial glands diameters showed highly significant increase ($p < 0.01$) in G3, G4, G5 and G6 (54.34±0.62) µm for each groups, while G2 showed significant increase ($p < 0.05$) (43.225±0.74) µm compared to that of the control (33.11±2.07). (Table 3 and Figures 6-9).

Similar changes to uteri were detected in the mucosa of oviducts, the thickness of epithelial lining cells was highly significant increased ($p < 0.01$) in G4, G5 and G6 (123.5±10.8, 138.32±4.22 and 163.02±2.99) µm respectively, while G2 and G3 showed a significant increase ($p < 0.05$) (86.45±2.44) µm for each as compared with that of the control (59.28±2.81)

µm, also the results showed an increase in the diameters of oviducts (ampulla region) with highly significant increase ($p < 0.01$) in G_3, G_4, G_5, and G_6 (326.7±15.2, 346.5±15.1, 485.1±18.6 and 514.8±21.2) µm respectively, while G_2 showed a significant increase ($p < 0.05$) (277.2±4.66) µm as compared with that of the control one (247.5±3.33) µm (Table 3 and figure 10).

Other pathological changes were an increased mucus secretion of the cervix, which projects as fine strands into the lumen (Figure 11), and hyperkeratosis of the vagina (Figure 12) especially in the animals treated for 10 wks (G6).

Table 1. Morphological changes in the diameter of the ovary after oral administration of pentoxifylline to mature female mice

Diameter (µm)	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
Diameter of ovary	1009.8± 0.56	*1306.8± 6.53	**1386± 4.15	1159.3± 4.60	*1158± 4.63	**1412.7± 4.23
Diameter of G.F	345.8± 0.89	358.15± 4.65	*363.09± 2.54	*382.85± 2.67	*382.85± 3.44	*382.85± 3.06
Diameter of secondary F.	222.3± 3.99	*271.7± 3.53	*333.45± 3.01	*365.65± 2.19	**407.55± 4.07	**427.31± 3.84
Diameter of primary F.	93.86± 0.89	*118.56± 3.31	*71.63± 3.58	*74.1± 3.70	93.86± 2.81	93.86± 2.81

Values are mean ± standard error (SEM), (n=6 animals/groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$); G.F: Graafian follicle.

Table 2. Morphological changes in the number of ovarian follicles after oral administration of pentoxifylline to mature female mice

Follicles number	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
No. of all follicles	18±0.36	*20±0.6	*21±0.63	*23±0.46	*23±0.69	**25±0.5
No. of G.F	4±0.16	*3±0.18	*5±0.15	*5±0.1	*6±0.18	*6±0.12
No. of secondary F	3±0.12	3±0.18	*5±0.15	*4±0.12	*4±0.12	*4±0.16
No. of primary F	5±0.1	**12±0.36	*8±0.24	*6±0.24	*3±0.21	*8±0.56
No. of C.L	2±0.14	*3±0.18	2±0.14	*3±0.21	**8±0.24	**12±0.24

Values are mean ± standard error (SEM), (n=6 animals/groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$); G.F: Graafian follicle, C.L: Corpus luteum.

Table 3. Morphological changes in the uteri and oviducts after treatment with pentoxifylline to mature female mice

(µm)/Diameter	Control group	2 weeks treatment	4 weeks treatment	6 weeks treatment	8 weeks treatment	10 weeks treatment
	G1	G2	G3	G4	G5	G6
Ut.Epi.	12.35±4.03	*24.7±0.52	**37.05±0.79	**49.4±0.82	**61.75±0.92	**86.45±2.43
E.G.	33.11±2.07	*43.225±0.74	**54.34±0.62	**54.34±0.62	**54.34±0.62	**54.34±0.62
OD.Diam	247.5±3.33	*277.2±4.66	**326.7±15.2	**346.5±15.1	**485.1±18.6	**514.8±21.2
OD.epith	59.28±2.81	*86.45±2.44	*86.45±2.43	**123.5±10.8	**138.32±4.22	**163.02±2.99

Values are mean ± standard error (SEM), (n=6 animals/ groups), *Significant changes ($p < 0.05$), ** Highly significant changes ($p < 0.01$), Ut.Epi.: Uterine epithelia., E.G.: Endometrial gland, OD. Diam: Oviduct diameter, OD.epith: Oviduct epithelia.

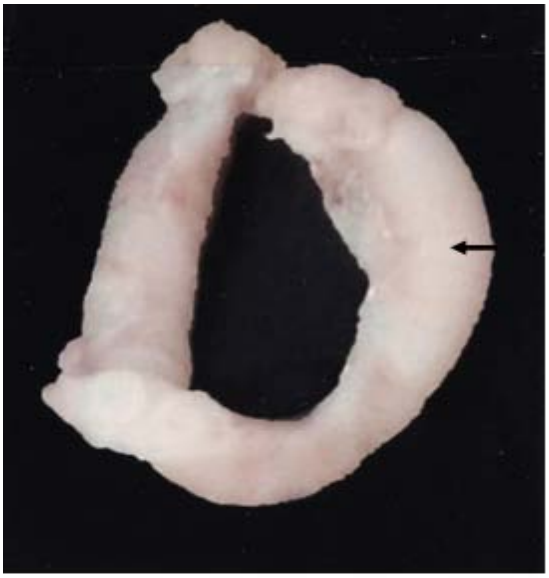

Figure 1: Genital system of one animal treated with (16 mg/kg bw/day for 6 wks) shows the enlargement of the uterus (⟶) with thickening of the uterine wall.

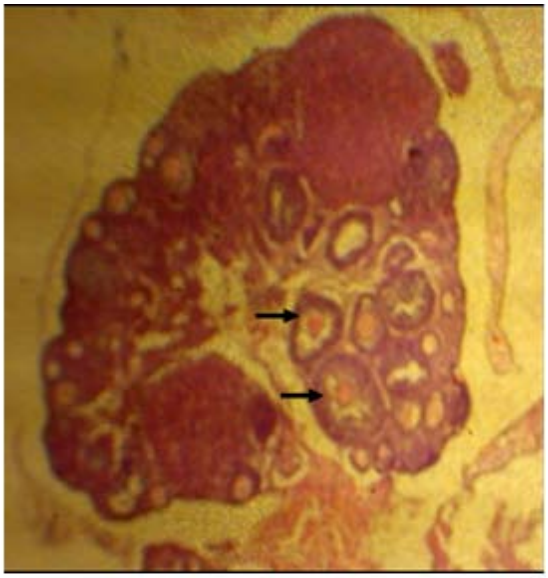

Figure 2: Histopathological section of the ovary from one animal treated with (16 mg/kg bw/day for 2 wks) showed increase in the diameter of the ovary and follicles (⟶) (H&E X 100).

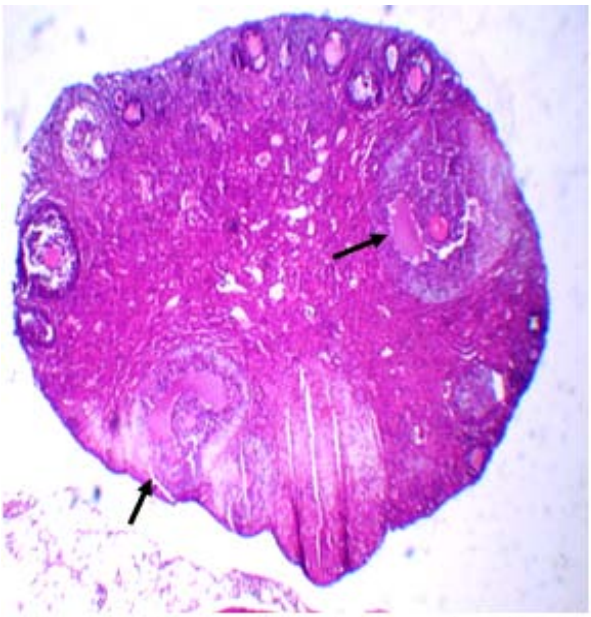

Figure 3: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 4 wks) showed increase in the diameter of the ovary and follicles (⟶) (H&E X 100).

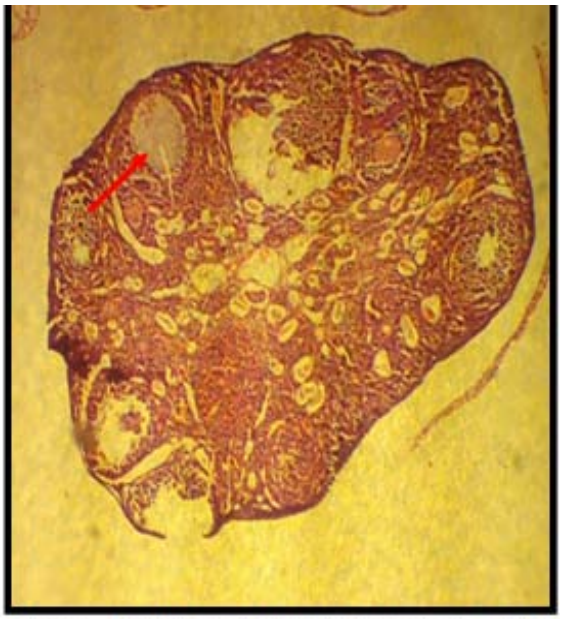

Figure 4: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) showed large number of follicles in different stages with congestion of blood vessels (⟶) (H&E X 100).

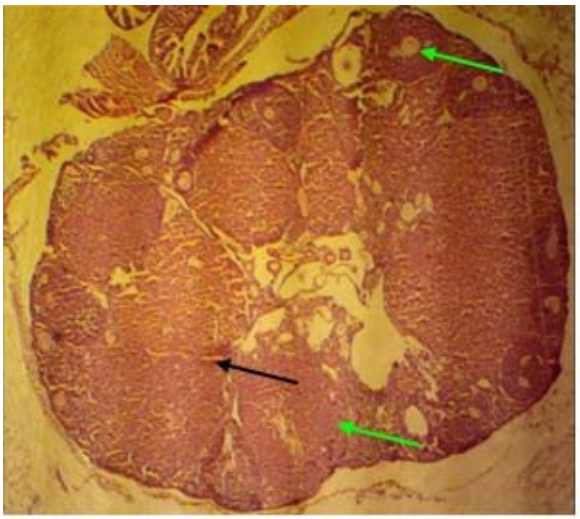

Figure 5: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) shows the presence of large number of corpus lutea (→), and the increase in the diameter of the ovary and the number of follicles (→) (H&E X 100).

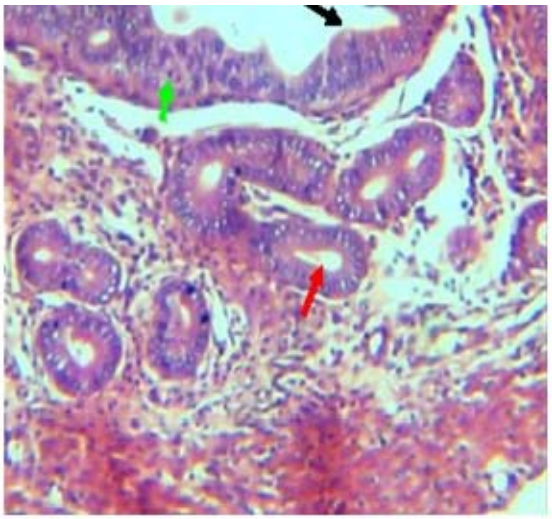

Figure 6: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 6 wks) shows the hyperplastic endometrial lining cells (→) with presence of mitotic figure (→) and the dilated endometrial glands (→) (H &E, X 100).

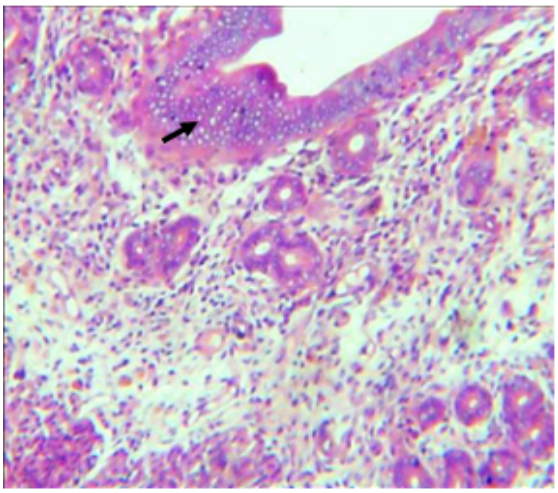

Figure 7: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 8 wks) shows marked thickness of epithelial lining cells of the endometrium also increase number of stromal cells (→) (H&E, X 100).

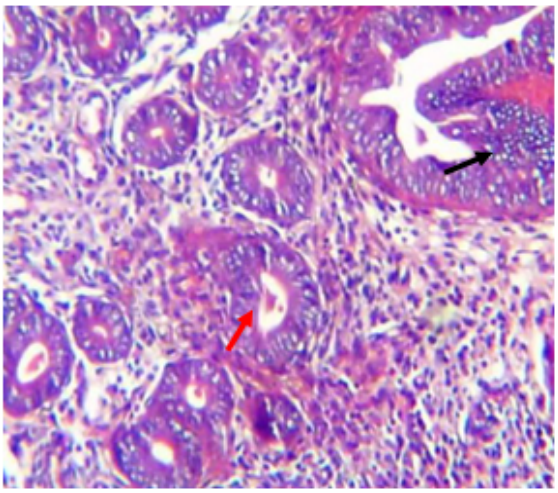

Figure 8: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows marked hyperplasia of epithelial of endometrium with formation of papillary projections toward the lumen of the uterus with marked proliferation of stromal cells (H &E, X 100).

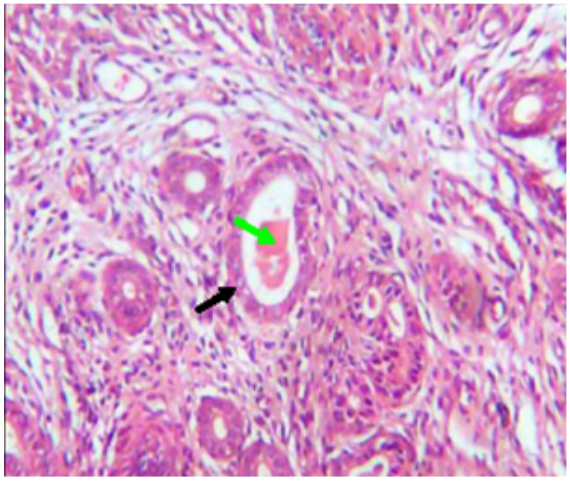

Figure 9: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows proliferation of fibrous connective tissue around the endometrial glands leading to dilatation, many of these glands contain colloids (H &E, X 100).

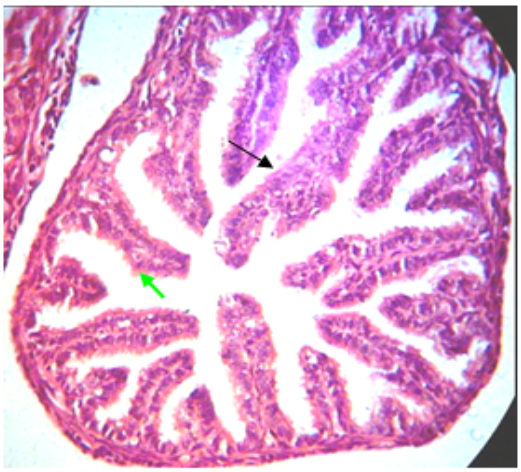

Figure 10: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows marked thickness of epithelial lining mucosa with proliferation of epithelial lining cells forming the branching papillary projections towards the lumen (⟶) with increase in the number of cilia (⟶) (H & E. X 40).

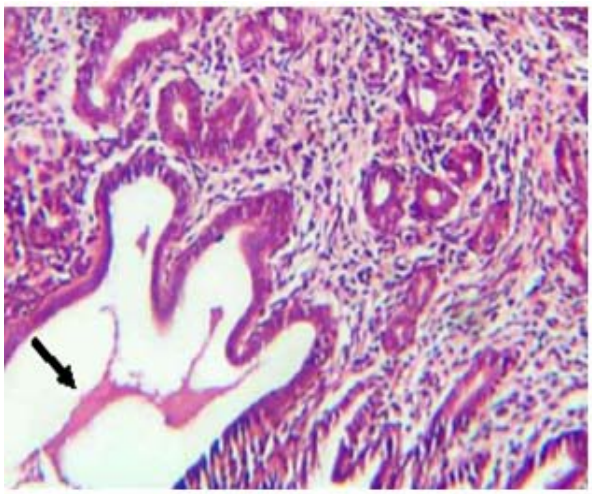

Figure 11: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows the increase in the secretion of mucus seen as fine strands in the lumen (⟶) (H&E, X 100).

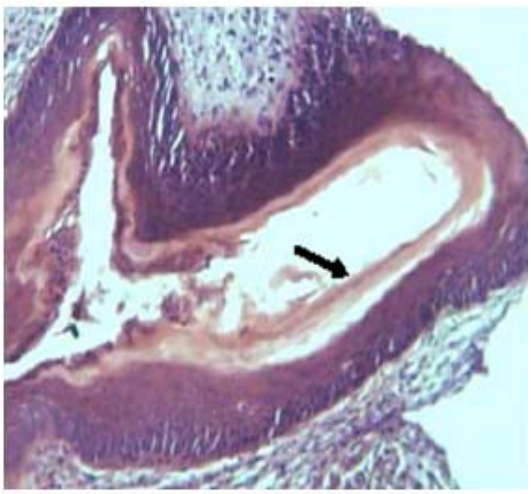

Figure 12: Histopathological section of the ovary of one animal treated with (16 mg/kg bw/day for 10 wks) shows the increase amount of keratin (⟶) (H&E, X 100).

Discussion

The microscopic findings in ovaries revealed a clear increase in the mean of ovarian diameters and an increase in the total number of follicles and in their diameters with acquisition of more number of corpus luteum (C.L) in the treated groups as compared with that of the control group which showed formation of few (C.L) especially in those groups that were treated for longer duration. These findings are due to increasing levels of estradiol (E2), follicular stimulating hormone (FSH) and luteinizing hormone LH [14]. Follicular growth and development depends on the production of E2 [15]. E2 acts within the follicle as autocrine or

paracrine manner, it promotes the proliferation of granulosa cells and increases their response to FSH, also stimulate the proliferation of theca interna cells, and these events give the follicle progressively greater capacity to produce E2 and makes it increasingly sensitive to FSH as it matures. By these actions, E2 increases its own production, simultaneously, E2 and FSH induce granulosal cells to creates receptors for LH, in preantral follicles granulosal cells have few receptors for LH which are not responsive to LH which in contrast, the granulosal cells of preovulatory follicles have abundant LH receptors and consequently have acquired sensitivity to LH [16].

The positive results of uteri due to treatment with PTX consisting of hyperplasia of uterine epithelial lining cells leading to increase in the thickness of uterine epithelial lining cells, and hypertrophy of endometrial glands reflected as an increase in the diameter of endometrial glands. These results reflects the importance of oral treatment of the drug which lead to the elevation of E2 [14]. Since it is well known that estrogen causes marked cells proliferation in the mucosal cell lining and greatly increases development of the endometrial glands which later aids in providing nutrition to the implanted ovum [17]. Although, the increase in the thickness of endometrium improves the pregnancy rate in females having a thin endometrium before treatment [18]. The only risk factor in treatment for more than 10 wks is that hyperplasia may leads to neoplasia [19].

Estrogen has important effects on mucus secretion of the cervix, it increase the secretion of mucus, which become abundant, clear, and non-viscous. All these characteristics are most pronounced at ovulation and allow sperms, which are deposited in the vagina, to move easily through the mucus on their way to the uterus and uterine tubes [20].

Oviducts observations can be attributed to the increase in E2 level [14] .The influence of the steroidal hormones on the fallopian tubes appear to be quite significant, since their effects on the mucosal lining of fallopian tubes are similar to those seen in the uterine endometrium.

They cause the glandular tissues to proliferate and, they cause an increase in the number of ciliated epithelial cells of the fallopian tubes also, the activity of the cilia is considerably enhanced, these cilia helps to propel the fertilized ovum in the direction of the cavity [16,21], and this is exactly seen in the treated groups compared with that of control group.

The experiment showed the treated groups enter subsequent estrus cycles because of the elevation in the reproductive hormones, which might lead to improvement in folliculogenesis. Estrogens causes the vaginal epithelium to proliferate and to show an increased cornification [22,23]. This was clear in both vaginal smears and tissue sections in addition to the elevation in the LH, which is the physiological signal for ovulation [16].

All previous results revealed to the effect of PTX on different parts of the genital organs, which might reflect itself on reproductions and the number of new generations.

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Correspondence to: Dr. Rajiha A. AL-Naimi,
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Some Observations on the Occurrence of β-Thalassemia in Mosul

Mohaisen H Adaay[1] *PhD*, Moayed M Al-Anzy[2] *MSc*, Abdul-Monaim H Al-Samarrai[3] *PhD*, Khudair A Al-Tikriti[3] *PhD*, Firas A Al-Samarrai[4] *MBChB*.

[1]Institute of Embryo Research & Infertility Treatment, Al-Nahrain University, [2]College of Pharmacy, Tikrit University, [3]College of Medicine, Tikrit University, [4] Dept. of Internal Medicine, Jordanian University Hospital.

Abstract

Background β-thalassemia is found in Mediterranean, Saudia Arabia, Jordan, Egypt and Yemen. Survey in Iraq showed that β-thalassemia trait is carried by 4.5-5% of the population.

Objective To determine factors that is associated with thalassemia in Mosul district.

Methods 105 thalassemia blood transfusion dependent children with the age of 2.5–18 years attending Ibn Al-Atheer Teaching Hospital in Mosul city during 2005 were included in this study, 45 healthy subjects served as control. Blood groups, Hb, and PCV were evaluated. SI, TIBC, and TS levels were also determined.

Results The occurrence of homozygous β-thalassemia is significantly higher in the offspring of first cousin marriages."O" blood group represents a highest percentage (42.8%) among the thalassemic patients. Hb, PCV and TIBC were below the control measurements. Whereas SI and TS were above that of the controls.

Conclusions Consanguinity seems to be increasing the size of the disease due to the limited health education of the parents about the disease.

Key words Thalassemia, Hb, PCV, SI, TIBC, TS

Introduction

Thalassemia has been classified by the world health organization as a major public health problem [(1)]. It occurs throughout the world and regarded as one of the major health problems in endemic regions as the Mediterranean countries, Middle East, North Africa and Asia [(2-4)]. The thalassemia trait is characterized by a reduction in or absence of synthesis of one or more globin chains in the hemoglobin (Hb) molecule [(5)]. B-thalassemia is found in Arabic countries especially those which are located on the Mediterranean, Saudia Arabia, Jordan, Syria and Yemen [(6)]. Survey in Iraq showed that β-thalassemia trait is carried by 4.5-5% of the population [(7)]. The disease remains incurable with complications result from iron overload as result of blood transfusion and increased intestinal absorption of iron[(8)].

The aim of the present study is to determine some factors that are associated with thalassemia in Mosul district such as age and gender distribution of the disease, the relation between thalassemia and consanguinity of the parents, to measure Hb and packed cell volume (PCV) before blood transfusion and to investigate the serum levels of serum iron (SI), total iron binding capacity (TIBC) and transferrin saturation (TS).

Methods

One hundred and five thalassemia blood transfusion dependent children with the age of 2.5-18 years (62 males and 43 females) attending Ibn Al-Atheer Teaching Hospital in Mosul city during 2005 were included in this study. The local ethical committee approved the study. Fifty four healthy children with the

age of 4-17 years served as a control group. Five mls of venous blood were drawn from the cubital vein using disposable needles and syringes without using tourniquet. Blood groups were determined according to the methods of Rowley and Milkins [9]. PCV and Hb were estimated using the methods of Bain et al, [10]. SI and TIBC conducted according to Varely et al, [11].The mean, standard deviation, correlation coefficient and t-test were used for statistical analysis. The differences were considered significant when $p \leq 0.05$.

Results

Age and gender distribution of patients with homozygous β-thalassemia is shown in Table 1. A mean age of 8.91 years was recorded with a significantly higher ($p < 0.001$) incidence among males. The effect of consanguinity on the occurrence of homozygous β-thalassemia is shown in Table 2. The percent of thalassemia is significantly higher ($p < 0.001$) in the offspring of first and second cousin marriages in comparison with unrelated marriages.

As concerning the hematological parameters, Table 3 shows the blood group distribution in homozygous β-thalassemia. O^+ blood group was the highest (42.86%) among the thalassemic patients.

Hb and PCV levels were significantly ($p < 0.05$ and $p < 0.001$) below normal respectively in all of the 105 thalassemic patients Table 4. The mean Hb value was 8.04±1.1 g/dl while the mean PCV % was 25.6±3.5 in comparison with 13.5±0.9 and 39.7±2.8 in the control group respectively.

Referring to the iron status, Table 5 shows the iron levels in thalassemic patients and control groups. In the thalassemic patients, the mean SI was 183.4±44.3 µg/dl, the mean TS was 79.1±21.4 and the mean TIBC was 239.2±46.2 µg/dl, in comparison with 101.5±21.1 µg/dl, 32.7± 8.1 and 302 ± 45.1 µg/dl in the control group respectively. The mean SI and TS in the thalassemic patients were significantly ($p < 0.001$) higher than in the control group; whereas the meanTIBC was significantly ($p < 0.001$) lower than in the controls.

Table 1. Age and gender distribution of patients with homozygous β-thalassemia

Gender		Age(year)				Total
		< 2-6	> 6-10	> 10-14	> 14-18	
Male	No.	22	16	19	5	62
	%	20.9	15.2	18.1	4.7	59.1*
Female	No.	13	11	10	9	43
	%	12.3	10.4	9.5	8.5	40.9
Total	No.	35	27	29	14	105
	%	33.3	25.7	27.6	13.3	100

*$p < 0.001$ in comparison with female.

Table 2. Consanguinity and β-thalassemia major

Consanguinity	No.	%
Offspring of first cousin marriage	58	55.2
Offspring of second cousin marriage	17	16.2
Offspring of far relative marriage	13	12.4
Offspring of unrelated marriage	17	16.2
Total	105	100

Table 3. Blood group distribution in homozygous β-thalassemia

Blood group	Patient		Mosul Study [20] n=3177	Iraq Study n=24063
	No.	%	%	%
A^+	28	26.67	28.8	30.2
B^+	18	17.14*	23.9	26.7
O^+	45	42.86*	34.7	34.7
AB^+	9	8.57	12.6	8.4
A^-	3	2.86		
B^-	1	0.95		
O^-	1	0.95		
Total	105	100	100	100

**p* < 0.05 in comparison with Mosul and Iraq study

Table 4. Hematological data in patients and control group according to gender

Group	Gender	No.	Hb (g/dl)	PCV%
			Mean ±SD	Mean ±SD
Patients	M	62	8.2±1.05	26.1±3.2
	F	43	7.6±1.2	24.9±4.8
	Total	105	8.04±1.1*	25.6±3.5**
Control	M	28	13.9±0.8	40.6±2.6
	F	24	12.9±0.8	38.7±2.6
	Total	52	13.5±0.9	39.7±2.8

**p* < 0.05 in comparison with the control, **p* < 0.01 in comparison with the control.

Table 5. Iron levels in homozygous β- thalassaemia and normal subjects

Group	Gender	SI (µg/dl)	TIBC (µg/dl)	TS (%)
		Mean ±SD	Mean ±SD	Mean ±SD
Patients	M	192.1±45.8	237.4±44.80	82.9±20.04
	F	169.8±38.4	241±48.8	73.2±22.5
	Total	183.4±44.3*	239.2±46.2*	79.1±21.4*
Control	M	113.4±21.4	296.8±48.3	37.7±9.1
	F	91.8±20.8	310.1±42.1	29.6±7.1
	Total	101.5±21.2	302±45.1	32.7±8.1

**p* < 0.01 in comparison with the control.

Discussion

β-thalassemia is a common genetic disorder in the Mediterranean countries and the Middle East [2,12]. The present study focused on the occurrence of this disease in "Mosul" due to the appreciable number of patients with thalassemia major encountered in Mosul pediatric hospitals all around the year, the known endemicity of malaria in the area to which the prevalence of the disease probably related since there is a possibility of an association between beta thalassemia and malaria [13] and the high rate of consanguineous marriage among the rural.

A mean age of 8.91 years of the patients was found to be on regular blood transfusion which is higher than that found in other study "7.71 years" [6]. AS concerning the gender distribution of the disease, the study showed a higher

occurrence among the males (59.04%) than in the females (40.96%) which is comparable to previous studies [6,14,15].

Consanguinity seems to play an essential role in increasing the size of the problem in Mosul since 71.4% of the patients studied were the product of marriages between first and second cousins. Al-Haj (15) found a higher results (88%) whereas a lower result (41.6%) was reported by Awad[6].

Hematological tests showed low Hb and PCV levels with a means of 8.04 g/dl and 25.6% respectively compared with 13.5 g/dl and 39.7% in the control group. These results of low Hb among patients can be explained by the limited health education of the parents about the disease ,so that , blood transfusion was used only when the patient showed clinical symptoms caused by severe anemia or simply just to sustain life [16-17] . Whereas reports from other countries focused on a supertransfusion program (maintaining Hb level above 12 g/dl) or hypertransfusion program (where the Hb level never allowed dropping below 9 g/dl) [18-19].

As compared to normal blood group distribution in both Mosul [20] and Iraqi [21] population, the present study showed that the frequency of patients with blood group "O" is higher than in the normal population; on the other hand, the frequency of patients with blood group "B" is lower than the control. No significant difference was found in blood group "A" and "AB". This result is comparable to that reported by Al-Haj Ahmed [14], but disagrees with the study conducted by Awad [6] on Mosul district.

Generalized iron loading of the organs has been a recognized complication of thalassemia major [8]. SI and TS values were found to be higher (183.4 µg/dl, and 79.1%) than in the control group (101.5 µg/dl and 32.7%). These findings are in agreement with other studies [22-24] which refer to an increase in SI and TS in addition to other parameters.In patients with thalassemia, the excess iron results not only from blood transfusion but also from increased iron absorption secondary to the ineffective erythropoiesis, which is associated with an increased plasma iron turnover [25-26].

It is concluded from this study that β-thalassemia patients were prevalent in Mosul city compared with other regions of Iraq, consanguinity is clearly obvious especially among first cousin marriages and that all patients have shown anemia which is more common in females than males.

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Correspondence to: Dr. Mohaisen H Adaay,
E-mail: dr_mohsin2004@yahoo.com
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Intravesical Mitomycin C Instillation to Delay Recurrence of Superficial Bladder Cancer (Long-Term *versus* Short-Term Protocols)

Ahmed A Al-Azzawi[1] *FIBMS*, **Mohammed Sh Al-Zaidy**[1] *FIBMS*, **Ahmed H Ismael**[2] *PhD*

[1]Dept. of Medicine, [2]Dept. Pharmacology, College of Medicine, Al-Nahrain University

Abstract

Background Majority of patients with bladder cancer present with superficial tumor, which is of three types: papillary carcinoma confined to the bladder epithelium, tumor invading but confined to the lamina propria, and carcinoma in situ (cis). Transurethral resection represents the effective management of superficial tumors but with high recurrence rate (75%) that can be minimized to 50% by using cytotoxic drug or immunotherapy.

Objective To compare two method lines of treatment in respect to long - term versus short - term therapy of Mitomycin C (MMC)Intravesical instillation after transurethral resection of superficial transitional carcinoma of urinary bladder to detect the duration of disease free interval, recurrence rate and adverse effects.

Methods A prospective, randomized, two parallel and compared lines of treatment study involved 50 patients who were evaluated after transurethral resection of superficial bladder cancer with median follow-up of 30 months. They were informed about the study and their approval to participate in the study was taken. Group A (25 patients) received intravesical MMC 30 mg weekly for 6 weeks, while group B (25 patients) continue to receive further 12 monthly instillations. Statistical analysis was performed by using Kaplan - Meier methods and chi square test.

Results After follow-up of 30 months, 28% of patients who received short course of intravesical MMC were disease free in comparison to those with long – term treatment in group B which shows significantly higher rate of disease free (56%). Regarding the adverse effects, no significant differences in incidence were noted between two groups.

Conclusion The data from the present study confirm both efficacy and safety of using intensive, prolong regimen of intravesical MMC instillation for superficial bladder cancer.

Key words Mitomycin C, superficial transitional carcinoma, and transurethral resection.

Introduction

Bladder cancer is the third most prevalent disease among male patients, the tenth among female patients in United States [(1)]. The majority of patients (75% - 80%) with bladder cancer initially present with superficial tumor [(2)], which comprising three different types: stage Ta- papillary carcinoma confined to the bladder epithelium, stage T1-tumor invading but confined to the lamina propria, and hot, non papillary carcinoma in situ (cis) [(1-3)].

Transurethral resection (TUR) can effectively control the primary tumor and confirm the superficial transitional cellular nature of the disease. Because of high recurrence rate and / or progression of the tumor after TUR (75%), there is increasing interest in intravesical instillation of Cytotoxic drug or immunotherapy - Bacillus Calmette-Guerin (BCG) and become common practice after TUR in order to prevent recurrence in more than 50% of transitional cell carcinoma (cis, Ta, T1) [(4-6)].

Intravesical trials are divided into 2 major categories:

1- Therapeutic trial designed to treat established disease.

2- Prophylactic / adjuvant, designed to prevent recurrence.

Seventy–six clinical studies had been reported on short-term intravesical therapy within the last 20 years, their result rate of the net benefit was: Thiotepa (8-27%),

Doxorubicin (12-23%), Mitomycin C (13-35%) [6,7].

Japanese studies have shown that Mitomycin C is most effective chemotherapeutic agent in influencing superficial bladder carcinoma, when given intravesically[8]. A clinical study has shown that complete disappearance of bladder tumor can be achieved in 44-49 % of patients [7,8].

A prospective follow-up study was designed to compare long term versus short term therapy of Mitomycin C intravesical instillation after transurethral resection of superficial transitional carcinoma of urinary bladder with respect to duration of disease free interval, recurrence rate and adverse effects.

Methods

The study involved 50 patients with superficial transitional cell carcinoma of the bladder (grade I-III, staging as cis, Ta, T1) diagnosed after TUR. They were collected in the period from January 2007 to November 2009 in AL-Kadhimyia Teaching Hospital and were informed about the study. Patients of the study were free of other diseases like diabetes mellitus, hypertension and ischemic heart diseases.

After 15 days (TUR) all patient under went either short (group A) or long (group B) cores of MMC therapy regiment after taking their approval to participate in the study.

All of the 50 patients (group A and B) started their therapy with intravesical installation of 30 mg of MMC in 50 ml of normal saline to be returned for at least one hour, this procedure was repeated weekly for 6 weeks for those patients in group A (short term therapy, while patients in group B (long term therapy) continued to receive the same dose of MMC monthly up to 12 months.

Regarding the follow up and response assessment as well as adverse (toxic) effect. All patients of the study were subjected to cytological urine analysis as well as cytoscopic examination. These procedures started 3 months following the start of protocol and to be repeated every 3 months for the first year and then every 6 months up to the end of the study period (30 months).

The complete response was defined as no residual carcinoma cystoscopically and negative urine cytology for the malignant cells.

Statistical analysis was done for recurrence free rate (disease free rate), performed by Kaplan-Meier method for both groups and were tested with long rank test and chi square test. Excluding criteria were: the presence of another cancer, previous local or systemic chemotherapy or radiotherapy and renal failure.

Results

From January 2007 to November 2009, 50 patients were treated and their response to MMC intravesical therapy was evaluated. They were of age ranging from 23 to 78 years (median is 56 years). The majority of patients were males (84%). Regarding clinicopathological features, 66% of the patients had solitary tumor and 70% were having Ta bladder cancer (Table 1).

Table 1. Clinicopathological profile of 50 patients with superficial transitional cell cancer of the bladder.

Criteria of the study group		Regimen Therapy				Total	
		Group A		Group B			
		GMA		GMB		No.	%
		No.	(%)	No.	(%)		
Sex	Men	20	80	22	84	42	84
	Women	5	23	3	16	8	16
Age	≤ 30	3	12	0	0	3	6
	≤ 50	6	24	6	24	12	24
	≥ 50	16	64	19	76	35	7
Stage	Ta	16	64	19	76	35	70
	T1	6	24	4	16	10	20
	cis	3	12	2	8	5	10
Multicentricity	Solitary	18	72	15	60	33	66
	Multiple	4	16	7	28	11	22
	Unkown	3	12	3	12	6	12

In assessment of the response to treatment, our study revealed that significantly higher percentage rate (56%) in group B in comparison to that of group A (28%) were free of cancer after the end of the study period (30 months) as in table 2 and figures 1 and 2.

Table 2. Crude results of end points after long term follow-up

Results	Group A		Group B		Total		X^2	*P* value
	No.	%	No.	%	No.	%		
* Recurrence of tumor	18/25	72	11/25	44	29	58	4.11	< 0.05
** Stage								
Ta	12/16	48	8/19	32	20/35	40		
T_1	4/6	16	2/4	8	6/10	12	0.2	< 0.05
cis	2/3	8	½	4	3/5	6		
*** Grade								
GI	12/18	48	6/15	24	18/33	36		
GII	3/4	12	3/7	12	6/11	12	1.66	< 0.05
GIII	3/3	12	2/3	8	5/6	10		

*$P < 0.05$ Significant, ** $X^2 = 0.2$ $P > 0.05$ Not Significant, *** $X^2 = 1.66$ $P > 0.05$ Not Significant

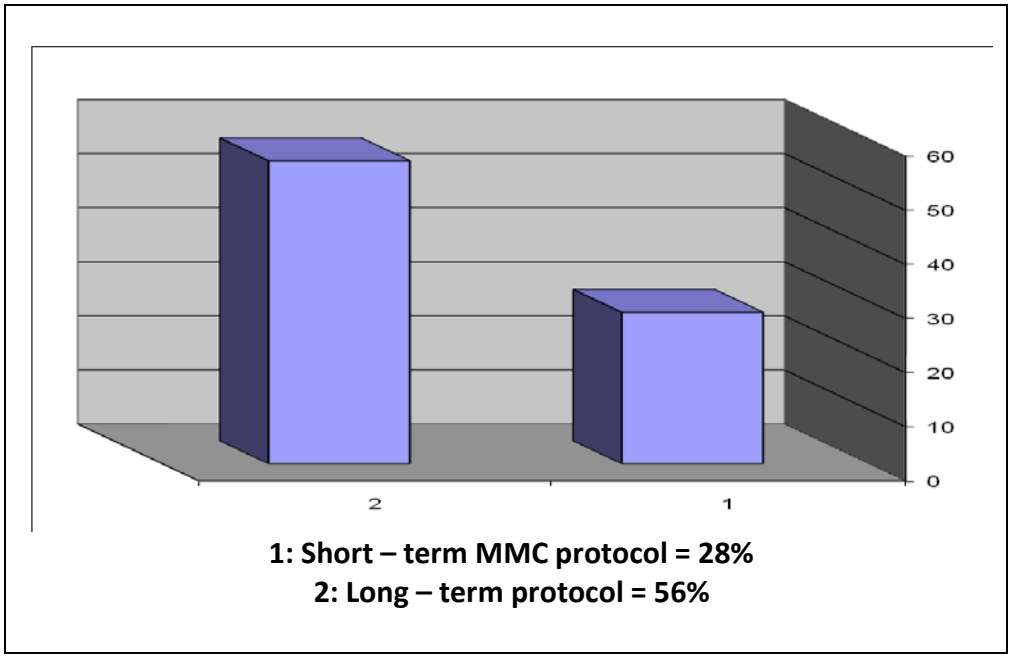

Figure 1. Percentage of disease free patients in short & long – term MMC protocols in both groups of the study

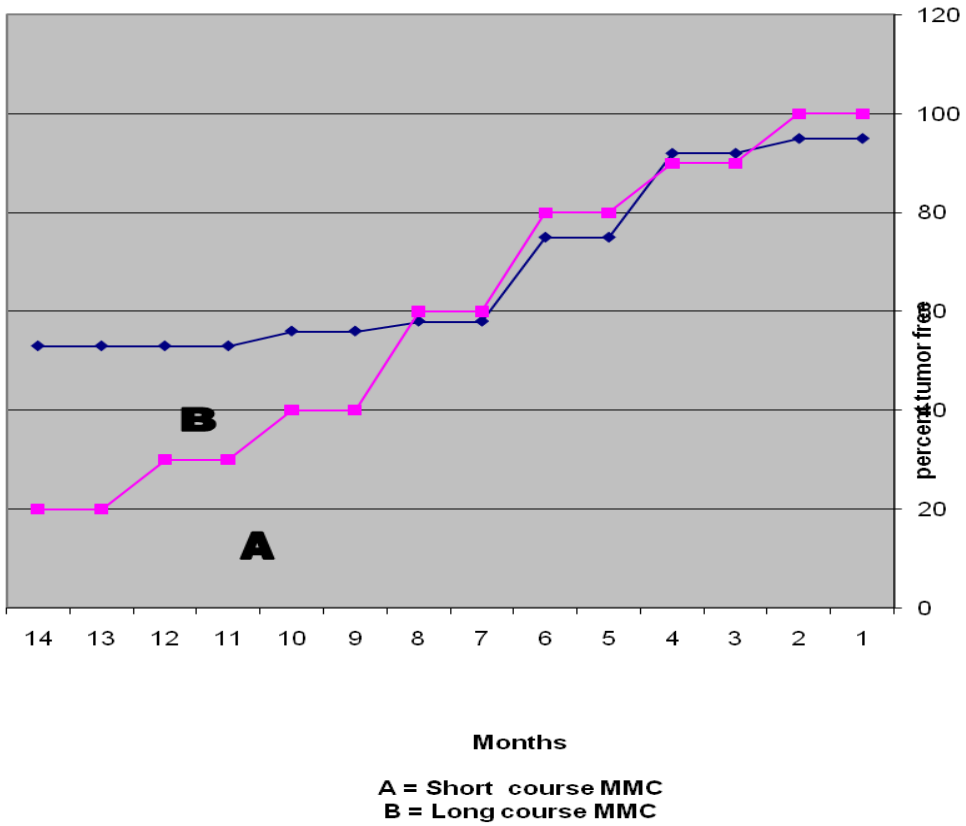

Figure 2. Kaplan- Mieir plot of percentage of patients with papilary tumors cis, Ta, & T1 grades 1 to 3 transitional cell cancer free of tumor after TUR & short MMC protocol versus long term. Long rank test, *p = 0.04.*

In addition to free cancer response assessment, the adverse effects of MMC therapy in both groups were evaluated. Higher percentage of patients in group B affected with bladder irritation (36%) and urinary tract infection (52%). On the contrary, 52% of patients in group A demonstrated positive hematuria. However all these differences were of no statistical significance (x^2=1.07 and *p* value > 0.005) as in table 3.

Table 3. Adverse effects of the short and long term intravesical MMC therapy.

Toxicity		Group A		Group B	
		No.	%	No.	%
Bladder irritation	Mild	5	20	4	16
	Moderate	2	8	3	12
	Severe	1	4	2	8
	Total	8	32	9	36
Microscopical hematuria		13	52	10	40
Urinary tract infection		9	36	13	52
Systemic allergy		1	4	0	0

Discussion

Numerous studies have demonstrated the efficacy of post-TUR intravesical administration of MMC in lowering recurrence rate, at least for 2-3 years of superficial transitional cell carcinoma of the urinary bladder after first observation [8-10].

The results of previous clinical studies showed that the MMC is beneficial in prevention of tumor recurrence in 13-35% of patients in comparison to other chemotherapeutic agents like Thiotepa (8-27%) and Doxorubicin (12-23%) [10,11].

Soloway and Ford reported that recurrence rate was 10 times as great in patients failed to respond to MMC than those showing complete response [12].

The data obtained from our study give an evidence that significantly improve the disease - free survival after the continuation of intravesical MMC for 12 monthly instillations [10] a trend in favor of MMC protocol in group B and Kaplan- Meier curve clear that tendency. Further, the majority of patients tolerate long – term intravesical MMC therapy well [9,12].

Further efforts to select patient were separated according to the presence or absence of ABH (blood group type A, B, and H) antigen on the tumor: the more aggressive ABH- negative tumor were found to recur less frequently than ABH positive tumor [8,10]. Moreover many questions still remain unanswered in relation to scheme of installation, when the ideal time to start treatment? What is the optimal duration of treatment? What is the ideal interval between two installations? All those questions have to be answered in longer duration study.

As a conclusion, the data from the study confirm both efficacy and safety of using intensive; prolong courses of intravesical MMC installation.

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Correspondence to Dr. Ahmed H Ismael
E-mail: ahmed_haqi2008@yahoo.com

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Serum Levels of Interleukin-1 Alpha and Interleukin-6 in Acute Coronary Syndrome Patients

Mohammed O Hamzah[1] *MSc*, **Kismat M Turki**[2] *PhD*

[1]Dept. of Chemistry and Biochemistry, College of medicine, Al-Nahrain University, [2]Dept. of Biochemistry, College of Medical College, Baghdad University

Abstract

Background Cytokines are responsible for the modulation of immunological and inflammatory processes and play a significant role in the pathogenesis of acute coronary syndrome patients (ACS).

Objective This study aims to investigate the serum levels of IL-1α and IL-6 in ACS patients.

Methods The study covered 140 subjects. It comprised a total of 101 patients with ACS patients [62 with acute myocardial infarction (AMI) and 39 with unstable angina (UA)], compared with 39 healthy individuals with no history of cardiac disease. Serum IL-1α and IL-6 analysis was performed by ELISA.

Results The present results revealed that there were significant elevation in mean serum levels of IL-1α and IL-6 in patients with ACS (AMI and UA) as compared to healthy control (P<0.001). Moreover, the levels of these cytokines were significantly higher in AMI patients when compared to UA patients (P<0.001).

Conclusion These finding suggest that IL-1α and IL-6 play an important role in pathogenesis of ACS.

Keywords Acute coronary syndrome, IL-1α and IL-6.

Introduction

Atherosclerosis is a complex multifactorial process resulting from an excessive inflammatory response to various forms of injurious stimuli to the arterial wall [(1)]. The transition of a stable coronary atherosclerotic lesion into a ruptured and/or eroded plaque results in the clinical manifestation of ACS [(2)]. The understanding of the factors that induce such events is essential for the prevention and treatment of atherosclerosis. Mechanistically, atherosclerotic plaque instability is the consequence of a complex inflammatory response of the vessel wall ignited by activated macrophages and T-cells leading to proteolytic degradation of connective tissue matrix, excessive pro-inflammatory cytokine production, and apoptosis of vascular wall cells [(3, 4)]. The well known locally generated markers of inflammation are TNF -α and IL-6 [(5)]. Together, TNF and IL-1 stimulate the production of IL-6 by smooth muscle cells [(6)]. IL-6 gene transcripts are expressed in human atheromatous lesions [(7)], and IL-6 is the main hepatic stimulus for C-reactive protein (CRP) production [(8)]. Patients with ACS demonstrate elevated serum levels of IL-1α and IL-6, indicative of a systemic inflammatory response [(9, 10)]. In contrast, serum levels of the potent anti-inflammatory cytokine IL-10 have recently been shown to be decreased in patients with ACS [(11)], thus it may favor plaque instability and the development of ACS. Whereas elevation of systemic markers of inflammation is firmly established to predict an unfavorable outcome in patients [(12)]. The present study was undertaken to assess the serum levels of IL-1α and IL-6 in patients with ACS.

Methods

Subjects

The patients group:

The current study comprised of 101 patients with ACS (37 females and 64 males; mean age 51.89 years, ranged between 21-85years

The patients group was classified into two groups: Group 1, includes 62(61.37%) patients with AMI and Group 2 includes 39 patients (38.63%) with UA.

The clinical examination and diagnosis were performed by physician specialized in in Ibn Al-Nafees Cardiac Specialty Teaching Hospital and AL-KindyTeaching Hospital.

The control group:

A control group included 39 subjects who had no history or clinical evidence of cardiac diseases or any chronic disease.

Estimation of serum of IL-1α and IL-6:

IL-1α and IL-6 were determined in serum using commercially available ELISA Kit. (BioSource, Europe S.A. Company, Belgium).

Statistical analysis:

Comparisons between groups were performed using ANOVA and student's *t* tests and P values less than 0.05 was considered statistically significant.

Results:

It is shown in Table 1, there were a significant elevation in the serum mean levels of IL-1α among AMI and UA patients (255.11 pg/ml and 64.58 pg/ml respectively) in comparison to that of healthy control (10.82 pg/ml) P<0.001. Significant differences were observed in the mean levels of IL-1α in patients with AMI as compared to those with UA (P<0.001).

Regarding the concentration of serum IL-6, Table 2 revealed significant increase in AMI and UA patients (266.11 pg/ml &91.77 pg/ml respectively) in comparison to that of healthy control (5.71 pg/ml) (P<0.01). Moreover, significant increase was noticed in serum levels of IL-6 in AMI when compared to UA patients (P<0.05).

Table 1: IL-1α level in patients with myocardial infarction and unstable angina compared to the healthy controls

Serum IL-1α	Acute Myocardial infarction	unstable angina	healthy controls	P (ANOVA-TEST)
Minimum	110.61	46.77	3.06	
Maximum	131	166.55	28.83	
Mean±SEM	255.11±7.40	64.58±3.70	10.82±0.70	<0.001
Number	62	39	39	
P (T-TEST)				
AMI versus UA: p<0.001				
ACS versus HC: p<0.001				

Table 2: IL-6 level in patients with myocardial infarction and unstable angina compared to the healthy controls

Serum IL-6	myocardial infarction	unstable angina	healthy controls	P (ANOVA-TEST
Minimum	98.65	57.83	3.06	
Maximum	389.98	185.65	10.89	
Mean±SEM	266.11±7.16	91.77±4.97	5.711±0.37	<0.001
No.	62	39	39	
P (T-TEST)				
AMI versus UA p<0.001				
ACS versus HC p<0.001				

Discussion

Thrombus formation over vulnerable disrupted atherosclerotic plaques has been implicated as an important mechanism in the development of the acute ischemic syndromes of unstable angina, AMI and sudden death [13]. Inflammatory and immunologic mediators may play crucial roles in plaque rupture. Macrophages and T-cells are known to be important components of atherosclerotic lesions, which can generate and release cytokines that play important roles in ACS. Various inflammatory markers and cytokines are associated with atherosclerosis and its progression to clinical syndromes. A number of pro-inflammatory cytokines, including IL-1, IL-6, IL-12 and interferon- are expressed in human atherosclerotic plaques. These cytokines alone or in combination contribute to the local inflammatory response, and may have great impact on plaque formation and progression [14].

High level of IL-1α and IL-6 in ACS patients observed in this study was comparable with other studies [15-17] who reported similar increase of these cytokines. IL-1α has wide range of target cells including cardiomyocytes and vascular smooth muscle cells. IL-1α induces postanoid dependent hypotension in rabbits *in vivo* and stimulates human smooth muscle cells to secrete IL-6. In chronically ischemic myocardium where focal necrosis was documented, enhanced levels of IL-1 mRNAs were found indicating a role of this cytokine in myocardial inflammation [18]. In contrast with present results Heinisch et al, did not find elevation in IL-1α levels, but they noticed elevation in IL-β in AMI patients [19].

Human vascular smooth muscle cells express and secrete IL-6 after IL-1 stimulation or during proliferation. Furthermore, hypoxic cardiomyocytes have been shown to produce IL-6 which could contribute to ventricular dysfunction as observed after myocardial ischemia and reperfusion [18]. Hirano *et al*. reported that IL-6 was expressed in cardiac myxoma cells at much higher levels than inactivated lymphocytes. There is now evidence that in patients suffering from acute myocardial infarction, IL-6 may affect the progression and the healing process of this illness, because IL-6 serum levels seem to be elevated in these patients [20].

In agreement with current results, Luo and associates reported that serum levels of IL-6 was significantly higher in ACS patients (including AMI and UA) than in healthy control and stable angina patients [21]. Similarly Sen and Sharman studied 100 patients with ACS and they found that serum IL-6 was elevated in ACs patients as compared to healthy control, furthermore, they observed that levels of IL-6 was significantly higher in AMI patients than in UA patients . So they concluded that IL-6 with other pro-inflammatory cytokines may be used for the identification patients with AMI /UA [22].

In conclusion the findings suggest that IL-1α and IL-6 play an important role in pathogenesis of ACS. Serum levels of IL-1α and IL-6 may have some diagnostic value for ACS, and can be useful marker reflecting disease stability.

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Correspondence to: Mohammed O Hamzah
E-mail: moh_alsafi75@yhoo.com
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The Effect of EDTA with Single or Combination of Antibiotics on Pseudomonas aeruginosa Isolates in Vitro

Abdul-Kareem H Abd[1] *PhD*, **Ahmed R Abu-Raghif**[1] *PhD*, **Ahmed NF Al-Azzawi**[2] *MSc*

[1]Dept. of pharmacology, College of Medicine, Al-Nahrain University, [2]Dept. of Biotechnology, College of Science, Baghdad University.

Abstract

Back ground P. aeruginosa is one of the most common causes of infection in burns and wounds and it is the major cause of death in burn patients. This organism is frequently feared because it causes severe hospital-acquired infections, especially in immunocompromised hosts, and is often antibiotic resistant; complicating the choice of therapy. Thus, there is continuous need for enhancing the antibacterial efficacy of antibiotics against P aeroginosa.

Objective This study was conducted to determine the MIC of antibiotics used in combination for resistant isolates of Pseudomonas aeruginosa and measure the effect of EDTA in increasing the inhibition effect of these antibiotics.

Methods P. aeruginosa was identified microscopically and biochemically .The swap samples from burns and wounds were collected from patients of AL-YarmooK, Baghdad and Al-Kadhumia Teaching Hospitals. Minimum inhibitory concentration (MIC) was used to evaluate antibiotics effectiveness, while fractional inhibitory concentration (FIC) was used to evaluate the effect of antibiotics combination on pathogenic bacteria (P.aeruginosa). Disk diffusion assay were used to determine the inhibition zone of antibiotic disk (with and without EDTA) against P. aeruginosa.

Results Ten isolates were selected according to their pattern of resistance as those showing multi-drug resistance and tested to specify their minimum inhibitory concentration for (amikacin, gentamicin, ceftazidime, and ciprofloxacin). Amikacin had the lowest MIC compared with others. Among combinations, the combination of ß-lactam antibiotics with amikacin was found to be the most effective combination. Results showed that EDTA increases the effect of antibiotic against P. aeruginosa isolates especially when it was combined with aminoglycoside antibiotics.

Conclusion Amikacin is the most effective agent against Pseudomonas aeruginosa especially when combined with ceftazidime, more over; EDTA increases the activity of antibiotic against pseudomonas aeruginosa especially when combined with aminoglycoside antibiotics.

Keywords P aeruginosa, minimum inhibitory concentration (MIC), Fractional inhibitory concentration (FIC), Ethylenediaminetetraactic acid (EDTA).

Introduction

Pseudomonas aeruginosa is an important nosocomial pathogen due to its ubiquitous presence wherever there is water. The devastating effect of infection on patients is usually the result of an excessive immune response, and the organism's high resistance to both host defenses and antibacterial agents [(1)].

P. aeruginosa constitute a large group of the normal aerobic intestinal flora. Within the intestine they generally not causing a disease and may even contribute to normal function and nutrition, but these organisms become pathogenic only when they reach tissue outside the intestinal tract, particularly the urinary tract, biliary tract, meninges, lung, eye,

kidney, ear, intestine and damage or burn skin, causing inflammation at these sites only when normal host defense are inadequate, particularly in early infancy, old age, and in terminal stages of other diseases [2, 3].

P. aeruginosa is one of the most common causes of infection in burns and wounds. It could cause burn sepsis through bacterial colonization of the burn site, destruction of the mechanical barrier to tissue invasion and multiple systemic immunological defects related to serious burns. P. aeruginosa is a major cause of death in burn patients [4].

P. aeruginosa has for long been regarded as an antibiotic-resistant organism, its low permeability outer membrane prevents access of many agents to their sites of action [5].The presence of constitutive and enhanceable efflux mechanisms removing a huge range of antimicrobial agents from the cell is considered as important factor of resistance, especially if coupled with enzymatic mechanisms of resistance [6].

P. aeruginosa is frequently resistant to many commonly used antibiotics (e.g. amikacin, gentamicin, ciprofloxacin, ceftazidime , pipracillin, and tetracyclin) by one or more of the following mechanisms: restricted uptake and efflux; drug inactivation and changes in targets [7].

The MIC indicates the minimal concentration of the antibiotic that must be achieved at the site of infection to inhibit the growth of the microorganism being tested. By knowing the MIC and the theoretical levels of the antibiotics that may be achieved in body fluids such as blood and urine, we can select the appropriate antibiotic, the dosage schedule and the route of administration. Generally a margin of safety of ten times the MIC is desirable to ensure successful treatment of the disease [8].

Most infections in humans with normal host defenses system can be treated with a single antimicrobial agent, but there are indications for the use of combinations of antimicrobials for the treatment of infections. The combinations may provide more broad-spectrum coverage than single antibiotics can provide, decrease the emergence of resistance strains, decrease dose-related toxicity by using reduced dose of both drugs, and to obtain enhanced inhibition of microorganisms by antimicrobial drugs [9].

Salts of Ethylenediaminetetraactic acid (EDTA) have long been used as antimicrobial agents, particularly against bacteria. They have also been used as enhancer of other agents, such as: lysozyme, antibiotics, and irradiation, by increasing permeability of bacterial membrane or by removal or destruction of covalently bound lipid components [10].

Its activity appears to be more when used in combination with antibiotics with activity against gram negative than with gram positive bacteria. This is due to the differences in the cell wall structure of the two groups. Gram positive bacteria contain more phospholipids than peptidoglycans in their cell walls in comparison with Gram negative bacteria [11].

Two modes of action of EDTA has been recognized, first EDTA potentiate the effect of antibiotics by binding to the metal ions which compete with aminoglycosides for cell wall receptor that allow them into bacteria, second EDTA disrupt the lipopolysaccharides structure in the outer membrane of gram negative bacteria, through this disruption the membrane becomes more permeable to other agents such as antibiotics [9].

This study was conducted to determine the MIC of antibiotics to be use in combination against resistant isolates of Pseudomonas aeruginosa and measure the effect of EDTA in increasing the inhibitory effect of these antibiotics.

Methods

Antibiotic powders: were obtained from the manufacturers as follows: Amikacin (AL-Razi center for production of diagnostic kits (Iraq)). Ceftazidim "Gulf pharmaceutical industries (UAE)" Gentamicin (AL-Razi) Ciprofloxacin "BAL-pharma (India)".

Antibiotic Solutions: Gentamicin, amikacin, and ceftazidime were prepared as stock solution of 10 mg/ml of antibiotic powder in distilled water, sterilized by filtration and store at -20°C [(12)]. Ciprofloxacin solution were prepared as stock solution by dissolving 1g of antibiotic powder in 90 ml sterile distilled water, pH adjusted to 5.0 with 1N HCl then volume completed to 100ml, obtaining a final concentration of 10 mg/ml, sterilized by filtration and stored at -20°C [(13)]. Bacterial isolates of P. aeruginosa (ATCC 27583) were obtained from Department of Biology, College of Science, Baghdad University.

EDTA Stock Solution (EDTA solution): is prepared by adding 186.1g of disodium ethylene diamine tetraacetate. $2H_2O$ to 800 ml of D.W., stirring vigorously on a magnetic stirrer, pH was adjusted to 8.0 with NaOH, dispenses into aliquots and sterilized by autoclave giving a final concentration of (5mM) [(14)]. P. aeruginosa was identified microscopically and biochemically [(15)].

Sample Collection: The swap samples from burns and wounds were collected in a sterile tubes containing nutrient broth from patients of AL-YarmooK, Baghdad and Al-Kadhimiya Teaching Hospitals during the period from 1/3/2005 to 15/5/2005.A total of 150 samples were collected and transported to the laboratory within two hrs. of collection.

Bacterial Isolation: Burns and wounds samples were cultured by spreading on MacConkey and blood agar plates. Plates were incubated overnight at 37°C. After the incubation, non fermentative colonies which appeared pale on MacConkey agar were selected and streaked on selective media (Citramid agar and king A agar) and incubated at 37°C for 24 hrs to test the pigmentation related to P. aeruginosa. These colonies were subcultured on brain heart infusion agar to obtain pure culture for further identification [(16)].

Antibiotic Sensitivity by Disk Diffusion Test [(17)]: After incubation, the diameter of inhibition zone was noted and measured in mm, results were determined according to the National Committee for Laboratory Standard [(18)].

Determining the Minimum Inhibitory Concentration for Antibiotic Combination: This test was used to determine the effect of antibiotics combination on pathogenic bacteria (P. aeruginosa)**.** A serial dilution method was used to determine MIC. The result was determined depending on the turbidity of the tube, then the combination whether it's synergistic, additives, antagonistic, or indifferent depending on the fractional inhibitory concentration (FIC), which was determined as follows: (≤0.5) synergism, (0.5 – <1) additive, (1– <2) antagonism, (≥2) indifference, and calculated using the following equation [(19)].

$$\text{FIC} = \frac{\text{MIC for antibiotic in combination}}{\text{MIC for antibiotic alone}}$$

Determining the Effect of EDTA in Combination with Antibiotics: Antibiotic disks were soaked in EDTA solution and disk diffusion assay were used to determine the inhibition zone of antibiotic disk (with and without EDTA) against P. aeruginosa according to the National Committee for Clinical Laboratory Standards and the inhibition zones were measured in (mm)

Results

Isolation and Identification of P. aeruginosa: The identification and characterization of the isolates were carried out according to the cultural, morphological and biochemical tests.

Antibiotic Sensitivity: The results showed that all isolates of P. aeruginosa were sensitive to amikacin, while percentage of resistant isolates for the remaining antibiotics as fallow: (30%) gentamicin, 72% ceftazidime, 26% ciprofloxacin.

The result showed that isolates No. (A1, A2, A5, A9, A10, A11, A18, A19, A20, A23) had the

highest level of resistance so that they were selected to study the effect of antibiotics combination and inhibitory effect of EDTA against P. aeruginosa.

Minimum Inhibitory Concentration (MIC): Ten isolates (which had the highest level of resistance) were tested to determine the MIC of amikacin, gentamicin (which represented the aminoglycoside), Ceftazidime (which represented the β-lactam), and Ciprofloxacin (which represented the flouroquinolons). Amikacin showed the lowest MIC level for P. aeruginosa which rang from (0.12- 4) µg/ml.While other antibiotics in this study had higher range of MIC as follow: ceftazidime (2-4) µg/ml, ciprofloxacin (2-8) µg/ml and gentamicin (0.5-8) µg/ml (Table 1).

Table 1. MIC value for four antibiotics (µg/ml) tested against P. aeruginosa isolates.

P aeurogenosa Isolates	AK	GN	CIP	CAZ
A1	4	8	-	4
A2	0.5	-	8	2
A5	0.5	-	8	-
A9	1	1	2	-
A10	2	8	-	-
A11	4	-	-	-
A18	0.12	0.5	-	-
A19	0.5	-	-	-
A20	2	-	-	4
A23	0.5	0.5	-	-

(AK) Amikacin, (CN) Gentamicin, (CIP) Ciprofloxacin, (CAZ) Ceftazidime

Antibiotics Combination: The combination between antibiotics for each isolate in this study was based on the selection of antibiotics that have lowest MIC to be used for combination.

Table 2 show the MIC value for antibiotics (amikacin, gentamicin, ceftazidime, ciprofloxacin), before and after combination against P. aeruginosa isolates to determine the effect of antibiotics combination on these isolates, MIC values of antibiotics in combination were found to be lower than MIC values of a single antibiotic.

Table 2 showed that (FIC) values for combination of amikacin with ceftazidime in the isolate No. (1, 2, 11, and 20) was very low in comparison with those (FIC) values of combination of amikacin with gentamicin and ciprofloxacin in the isolate No. (5, 9, 11, 18, 19, and 23).

Table 2. MIC of antibiotic combinations.

P. aeruginosa Isolates	Antibiotics combination	MIC of first antibiotic alone (µg/ml)	MIC of first antibiotic in combination (µg/ml)	MIC of second antibiotic alone (µg/ml)	MIC of second antibiotic in combination (µg/ml)	FIC	Results
A1	AK+CAZ	4	0.25	4	0.25	0.125	Syn
A2	AK+CAZ	0.5	0.03	2	0.125	0.12	Syn
A5	AK+CIP	0.5	0.12	8	2	0.49	Syn
A9	AK+GN	1	0.12	1	0.12	0.24	Syn
A10	AK+GN	2	0.25	8	1	0.25	Syn
A18	AK+GN	0.12	0.03	0.5	0.12	0.49	Syn
A20	AK+CAZ	2	0.125	4	0.25	0.125	Syn
A23	AK+GN	0.5	0.06	0.5	0.06	0.24	Syn

FIC: Fractional Inhibitory Concentration; Ak: Amikacin; Caz: Ceftazidime; Cip: Ciprofloxacin; GN: Gentamicin; Syn: Synergism.

The Effect of Combination of EDTA-Antibiotic on P. aeruginosa Table (3) shows synergistic effect of EDTA with other antibiotics of different groups in which the inhibition zone increased after adding the EDTA to the antibiotics. Inhibition zone of isolate No. A10 was found to be increased by (5mm) when EDTA was combined with gentamicin. While inhibition zone increased by (4 mm) when EDTA was combined with ceftazidime for isolate No A2. Adding EDTA to other antibiotics for different isolates show increased inhibition zone by 2-3 mm.

Table 3. Combination effect of EDTA with antibiotics on p. aeruginosa isolates using disk diffusion assay.

Isolateseses	Antibiotics	ZOI[1] without EDTA	ZOI with EDTA mm
A1	AK[2]	22	24
	Caz[3]	19	22
A2	AK	23	25
	Caz	20	24
A5	AK	24	27
	CIP[4]	18	20
A9	AK	21	24
	GN[5]	20	22
A10	AK	22	25
	GN	19	24
A18	AK	20	23
	GN	18	20
A20	AK	20	22
	Caz	20	23
A23	AK	23	25
	GN	18	21

1: Zone of inhibition (mm) 2: Amikacin 3: Ceftazidime 4: Ciprofloxacin 5: Gentamicin

Discussion

Pseudomonas aeruginosa is a notoriously difficult organism to control with antibiotics or disinfectants. The emergence of prevalence of antibiotic resistance strains is considered as a major therapeutic problem that can be explained by several hypotheses such as, the influence of excessive and /or inappropriate antibiotic use [(20)].

The results show that all isolates of P. aeruginosa were sensitive to amikacin (100%); this result may be related to the lower random use of this antibiotic by patients. This result was in agreement with that of Startchounski et al who found in a study in Russia that resistance percentage of the isolate to amikacin was (1%) [(21)]. Resistance of P. aeruginosa isolates to gentamicin was found to be as low as (30%) and this result was in agreement with that of Brumfitt and Hamilton who found that resistant to gentamicin was (32%) [(22)]. The results of this study also shows that the resistance percentage to ceftazidime was 72%, and this may be due to the ability of this isolates to produce β-lactamase enzyme which break the β-lactam ring and this results is in agreement with that found by Rice et al [(23)]. P. aeruginosa isolates were found sensitive to fluroquinolones like ciprofloxacin in which (26%) of the isolates was found resistant to this antibiotic and it was in agreement with that of Shawar et al [(24)] who indicate lower than 63% of isolate susceptible to ciprofloxacin, this may belong to the wide use of ciprofloxacin as

therapeutic agent for treatment of diseases caused by P. aeruginosa leading to a low level of susceptibility percentage for this antibiotic.

Amikacin remains the first choice among the tested antibiotics in the present study with lowest MIC for P. aeruginosa which range from (0.12- 4) µg/ml, therefore it is the more preferred in the therapy than other antibiotics and these result agreed with that of Bonfiglios [25] who found that MIC of amikacin against P. aeruginosa (2 µg/ml).While other antibiotics in this study have a higher MIC and as follow: ceftazidime was (2, 4 and 4) µg/ml, and this result was consistent with that found by Rolston et al [26] while gentamicin had a MIC range from (0.5-8) µg/ml, and this may be due to the ability of P. aeruginosa isolate to produce β-lactamase which break the β-lactam ring in the structure of antibiotic. For ciprofloxacin the MIC was ranged (2-8) µg/ml and this agreed with that of Craig [27].

The combination between antibiotics for each isolate in this study was based on the selection of antibiotics that have lowest MIC to be used for combination. MIC values of antibiotics in combination were found lower than MIC values of single antibiotic, revealing a synergistic effect of these combinations and those results are similar to those shown by Hollander et al [28]. Table 2 showed that (FIC) values for combination of amikacin with ceftazidime in the isolate No. (1, 2, 11, and 20) is very low in comparison with those (FIC) values of combination of amikacin with gentamicin, ciprofloxacin in the isolate No. (5, 9, 11, 18, 19, and 23), and this indicates that combination of aminoglycoside with β-lactam antibiotic was more effective than combination of aminoglycoside with other group of antibiotics, and this because aminoglycoside antibiotics exert their effects on protein synthesis of bacterium while β-lactam antibiotics exert their effect on bacterium cell wall and this lead to complete destruction of bacteria [8].

Ethylenediaminetetraactic acid has a more complex inhibition-concentration profile. Synergism between EDTA and other antimicrobials agents have been widely reported against P. aeruginosa [29]. From results obtained in table 3 it was found that EDTA had a synergistic effect on aminoglycoside antibiotics (amikacin and gentamicin) against P. aeruginosa, and these results are in agreement with that obtained by Spark [30] who found that EDTA enhance the activity of aminoglycoside antibiotics by binding to the metal ions which compete with aminoglycoside antibiotics for cell wall receptor that allow antibiotics to enter the bacterial cell. Gotthelf [31] had shown that EDTA is capable of reducing the MIC of ciprofloxacin against P. aeruginosa and this result was in agreement with the result of the current study, while the effect of EDTA on ceftazidime also gives synergistic effect, and this result was found similar to that obtained by Vaara [32], and this because EDTA cause destruction of the outer membrane of the bacterial cell altering the permeability to antibiotics which enter the bacterial cell and exert their effect.

In conclusion, amikacin is the most effective agent against Pseudomonas aeruginosa especially when combined with ceftazidime, moreover, EDTA increases the activity of antibiotic against Pseudomonas aeruginosa especially when combined with aminoglycoside.

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Correspondences to: Dr Abdul-kareem H Abd,
E-mail: ar_armat1967@yahoo.com
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