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Iraqi Journal of Medical Sciences

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Issued Quarterly

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The Association among Diet, Prebiotic and Probiotic

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The human gastrointestinal tract that typically refers to stomach and intestine is colonized by an intricate community of microorganisms. The stomach is a home of typically 10^3 colony forming units (CFU)/g content [1]. The large intestine is the main colonization site of more than 500 indigenous microbial species which can reach up to 10^{12} CFU/g lumen contents [1,2]

A wide range of compounds that have both positive and negative effects on gut physiology is produced through fermentation process by predominantly strict anaerobe gut microflora. For instance, short-chain fatty acids (SCFA), mainly butyrate supplies energy metabolism for the large gut mucosa and colonic cell growth. This SCFA is the end fermentation products of complex carbohydrate and protein that usually present in human diet. In contrast, H_2S produced by sulfate-reducing bacteria is highly toxic and may induce ulcerative colitis [1].

From the host's perspective, the key function of gut microflora is to prevent colonization by potentially harmful microorganisms. The imbalanced gut microflora has been linked to the development of certain disorders such as gastroenteritis, colon cancer and inflammatory bowel disease [3]. The composition of gut microflora is considered to be fairly stable over long periods. However, numerous factors such as competition for nutrients, metabolic interaction among bacteria, various host condition and individual dietary preferences may influence alteration of the pattern [4]. Therefore, it is of the foremost interest to manipulate the gut microflora composition toward an increased number of beneficial bacteria that provide health promising properties to the gut.

The groups of beneficial bacteria that help maintain health and treat disease is broadly known as probiotic. Several definitions of probiotic have been suggested for over the years. Fuller [5] defined probiotic as a live microbial food supplements which have beneficial effects on the host by improving its intestinal microbial balance.

A probiotic bacterium should fulfill certain criteria to be described as useful. These include acid and bile stability, adherence to intestinal cells, persistence for some time in the gut, ability to produce antimicrobial substances, antagonism against pathogenic bacteria, ability to modulate the immune response, being of human origin and having generally regarded as safe (GRAS) status [6].

In human, probiotic has been associated with lactobacilli (e.g. *Lactobacillus acidophilus*, *L. delbruekii* and *L. casei*) and bifidobacteria (e.g. *Bifidobacterium bifidum*, *B. adolescentis*, *B. infantis* and *B. longum*). Other known bacteria

include streptococci (e.g. *Streptococcus lactis* and *S. salivarius* ss. thermophilus), nonpathogenic *E. coli* and *Saccharomyces boulardii* [7].

A practical approach in increasing the number and activities of probiotic is through dietary supplementation, particularly with intake of the so called prebiotic. Gibson and Roberfroid [7] defined a prebiotic as 'a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'.

They revealed that food constituents can be categorized as prebiotic if meet the following requirements: 1) Resistant to hydrolysis and absorption in the upper part of gastrointestinal tract; 2) Act as selective substrate for one or a limited number of beneficial bacteria commensal to the colon; 3) Able to alter the colonic flora in favor to healthier composition; and 4) Induce luminal or systemic properties that are beneficial to the host health. Fructooligosaccharides (FOS), inulin, lactulose and galactooligosaccharides are commercially available prebiotic of proven efficacy.

Inulin and FOS can be found in human breast milk and in food such as banana, asparagus, leeks, onion, garlic, wheat, chicory and tomatoes [8]. Galactooligosaccharides (GOS), a mixture of oligosaccharides derived from lactose is frequently used as supplement in food and infant formula milk [8,9]. In their in vitro study, Wang and Gibson [9] demonstrated that FOS and inulin are selectively fermented by most strains of bifidobacteria.

The prebiotic effects of inulin and oligofructose in vivo have also been shown in some studies [10,11]. Moreover, the ability of these oligosaccharides in increasing the numbers of gut probiotic, particularly bifidobacteria has been shown in many human feeding studies. Breast milk is rich in human oligosaccharides and therefore the number of bifidobacteria in the gut microflora of breast-fed infants is higher than that in formula-fed infants [7].

The predominance of bifidobacteria in breast-fed infants is usually associated with lower risk of intestinal infection. However, Moro et al [12] reported that after 28 days of feeding, the number of fecal bifidobacteria and lactobacilli in infant fed with a cow milk supplemented with FOS and GOS were significantly increased compared to the placebo group.

The link between prebiotic and probiotic has been pronounced to enhance the efficacy of the both agents in maintaining the health of intestine. Synbiotics have been defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal track, by selectively stimulating the growth and/or by activating the metabolism of a limited number of health promoting bacteria, and thus improving host welfare' [7].

There are only few studies carried out to investigate the efficacy of synbiotics in human. Bouhnik et al [13] investigated the effect of symbiotic containing *Bifidobacterium* spp. and inulin fermented milk in healthy people. The authors reported that intake of *Bifidobacterium* spp. significantly increased fecal bifidobacteria, but no extra numbers of that particular probiotic was observed merely due to the addition of inulin. However, 2 weeks after trials, the volunteers who received symbiotic product had significantly higher number of *Bifidobacterium* spp. compared to those receiving probiotic alone. In addition, it was found that the trend whereby *Bifidobacterium* spp population decreases in the gut microflora of eldery may be reversed by the consumption of inulin [12].

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Antibiotic Susceptibilities of Gram Negative Aerobic Bacteria Isolated from Urinary Tract Infections in Community

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Abstract

Background Urinary tract infection (UTI) caused by bacteria that can also live in the digestive tract, in vagina, or around the urethra most often these bacteria, enter the urethra and travel to the bladder and kidneys and prostate (in men).

Objective To determine the most common gram negative aerobic bacteria caused UTI in both sex and different ages, and to study the antibiotic susceptibility in order to determine the most effective antibiotics that can cure UTI.

Methods Prospective study of 311 samples of urine has been collected from out patients complaining signs and symptoms of UTI. Isolation and Identification of causative bacteria was concluded, antibiotic susceptibility test has been done, and statistical analysis chi square had done.

Results 125 urine samples obtained from 25 male and 100 female show growths of Gram negative aerobic bacilli. No bacterial growth was defined in the rest of urine samples. Single bacterium was identified in 120 samples, while 5 samples identified as a mixed infection with two kinds of bacteria. In 55 (44%) of cases, *Echerishia Coli* was isolated; in 41 (32.8%) *Klebsiella Pneumoniae*; in 17 (13.6%) *Proteus mirabilis* plus *P. Vulgaris*; and 12 (9.6%) *Pseudomonas aeruginosa*. The percentage of resistance for *E. Coli* varies from 73% to 86%, to Ceftzidime, Ceftriaxone, and Trimethoprim Sulfamethaxozol; for *K. pneumoniae* it ranges from 71% to 100% for Amoxicillin, Pipracillin, Trimethoprim Sulfamethaxozole, and Colistin; for *P. mirabilis* plus *P. vulgaris* ranging from 66% to 100% for Cetazidime, Trimethoprim Sulfamethaxozol, and Cefotaxime; and for *P. aeruginose* ranges from 66% to 100% for Cefazidime, Colistin, Nafcillin, Gentamycin, Trimethoprim Sulfamethaxozol.

Conclusion *E. Coli* caused UTI in female higher than men in the community, and the gram negative–rods had multi antibiotic resistant.

Keyword UTI, Enterobacteriacae, Antibiotic susceptibility

Introduction

Urinary tract Infection (UTI) caused by bacteria that can also live in digestive tract, in vagina, or around the urethra, most often these bacteria, enter the urethra and ascend to the bladder and kidneys and prostate (in men), usually our body removes the bacteria and we have no symptoms [(1)]. Urinary bladder infection has become the most common urinary problem among children, according to conservative estimate, 3% of girls and 1% of boys have had a detected urinary tract infection (UTI) by the age of 11 [(2)].

Women are more likely to get UTIs than men, UTIs can be dangerous especially for older people and pregnant women, as well as for those with diabetes and those who have difficulty urination [(1,3)].

Many substances, such as soap, bubbles bath, stool or clothing can cause soreness of urethra, which makes it easier for bacteria to invade and get into the bladder and multiply [(2,4)].

Anatomic abnormality Increases the risk of bladder infections [3]. Large amount of stool sitting in colon can press up against the bladder and urethra, thus making it more difficult for the bladder to drain completely this allows bacteria to grow [4].

Microorganisms Causing UTI

The Urethra hosts a resident micro flora that colonizes its transitional epithelium, consisting of coagulase negative Staphylococci, Virdans and non hemolytic Streptococci, Lactobacilli, *Diphtheroids (Corynebacterium species)*, nonpatho*genic Neisseria* species, transient gram-negative aerobic bacilli (including Entero bacteriaceae), anaerobic cocci, *Propionibacterium* spp. anaerobic gram-negative cocci and bacilli, commensal *Mycobacterium* spp. commensal *Mycoplasma* species and occasionally Yeasts [3].

Members of the family enterobacteriaceae are among the most important human pathogens. They comprise approximately 80% of gram-negative bacteria and 50% of all isolates identified in hospital laboratories in the United States.

Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis Entrobacter Spp. and *Serratia marcescens* account for the majority of Enterobacteriaceae isolated from clinical specimens [4].

Chlamydia and mycoplasma may also cause UTIs in both men and women and may be sexually transmitted; that is why infections require treatment of both partners [5]. HIV virus may cause the UTI infection [6].

UTI are treated with antibacterial drugs. The sensitivity test is especially useful in helping the doctor to select the most effective drug. The drug most often used to treat routine uncomplicated UTI are listed below [7]:

1. Trimethoprim (Trimpex)
2. Trimethoprim sulfamethoxazole (Bactrim, Septra, Cotrim)
3. Amoxicillin (Amoxil, Trimox, Wymox)
4. Nitrofurantoin (Macrodantin, Furadantin)
5. Ampicillin (Omnipen, Polycillin, Principen, Totacillin)

A class of drugs called Quinolones includes four drugs approved in recent years four treating UTI and they include Ofloxacin (Floxin), Norfloxacin (Noroxin), Ciprofloxacin (Cipro), and trovafloxin (Trovan) [1].

The natural alternative to antibiotics is D-Mannos which is a simple sugar and close cousin of glucose can cure more than 90% of all bladder infections with 1 to 2 days and has no adverse side effects of any kind [2].

Nitric oxide (NO) has been found to possess microbicidal effects against a number of pathogens including DNA and RNA virus families [7].Oral therapy can begin from as early as two months of age and Clavulanate, Cephlexin is recommended for cystitis [15].

Methods

This study was carried out in Al-Karkh Surgery Hospital in Baghdad city, during the period 29th April 2007 to 30th June 2008. Three hundred and eleven urine samples were collected from patients who were presented to the outpatient with signs and symptoms of UTI.

Midstream urine samples were collected in sterile containers, and divided in two portions; the first portion is used for general urine examination and second portion for culture on nutrient agar, blood agar and McConkey agar plates, using sterile standard loop (1ml) then incubated at 37±2°C for 20-24 hours.

Positive urine analysis findings include leukocytes, erythrocytes, bacteria, and squamousal epithelial cells [9] chi-square analysis was done to show significant difference between groups.

Antibiotic susceptibility test done on clinically significant bacterial isolates according to standard modified "Kirby-Baur method" [9,10].

Result

The urine samples were due to patients who were in the age range of 10 to 70 years; and the patient group comprises 224 females and 87 males (Table 1).

Table 1. Age Stages of UTI patients and Bacterial isolates.

Age group	Sex	Bacterial isolates								No Bacterial growth
		Escherichia coli		*Klebsiella pneumoniae*		*P. mirabilis* + *P.vulgars*		*Pseudomonas aeruginose*		
		No.	%	No.	%	No.	%	No.	%	No.
10-20	Male	0	0	1	1.25	0	0	1	1.25	7
	Female	15	18.5	7	8.75	2	2.5	1	1.25	25
21-30	Male	0	0	1	1.25	2	2.5	0	0	9
	Female	10	12.5	7	8.75	5	6.25	2	2.5	30
31-40	Male	0	0	4	5	0	0	1	1.25	10
	Female	8	10	4	5	4	5	3	3.75	20
41-50	Male	2	2.5	2	2.5	1	1.25	1	1.25	10
	Female	10	12.5	6	7.5	1	1.25	1	1.25	20
51-60	Male	1	1.25	3	3.75	0	0	1	1.25	14
	Female	4	5	2	2.5	0	0	0	0	19
61-70	Male	2	2.5	1	1.25	0	0	1	1.25	12
	Female	3	3.75	3	3.75	2	2.5	0	0	10
Total										
Males	25	5	6.25	12	15	3	3.75	5	6.25	62
Females	100	50	62.5	29	36.25	14	17.5	7	8.75	124
all	125	55	68.75	41	51.25	17	21.25	12	15	186
%			44		32.8		13.6		9.6	

Test statistics

	E. coli	*K. pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*
Chi-square [a,b]	151.675	187.328	218.521	334.583
Df	11	11	11	11
Asymp.sig	000	000	000	000

a-o cells (0%) have expected frequencies less than 5 minimum cell frequency is 25.9.
b-o cells (0%) have expected frequencies less than 5 minimum cell frequency is 28.3.
c-o cells (0%) have expected frequencies less than 5 minimum cell frequency is 24.0.
one degree of freedom 0.05, schedule value of chi square
for 11= 19.68
for 10=18.31

	E. coli	*K. pneumoniae*	*Proteus spp.*	*Pseudomonas aeruginosa*
Chi-square [a,b]	194.196	263.084	349.592	382.080
Df	3	3	3	3
Asymp.sig	000	000	000	000

a-o cells (0%) have expected frequencies less than 5. The expected minimum cell frequency is 77.8
Schedule value of chi-square 7.81

A significant growth of aerobic Gram negative bacilli as a single infection was observed in 125 sample (40%) (Table 1) and as mixed infection in 5 sample (3.2%) (Table 2); while there was

no bacterial growth in 170 (60%) of urine samples (Table 1). There were 55 (44%) infections of *E. coli*, 41 (32.8%) infection by *klebsiella pneumoniae* 17(13.6%) infection by *Proteas spp.* and 12(9.6%) infection by P*seudomonas aeruginosa.*

Table 2. Type of mixed pathogens isolated from UTI patients.

Bacteria	Frequency	% Σ single + mixed Bacterial isolates.
Pseudomonas aeruginosa+Klebsiella pneumonae	4	% 2.9
Escherichia Coli+Proteus spp.	2	% 1.48
Klebsiella Pneumoniae+Pseudomonos aeruginosa	2	% 1.48
Pseudomonas aeruginosa+Escherichia coli	1	% 0.74
Escherichia coli+Klebsiella Pneumoniae	1	% 0.74
	Σ = 10	

Statistically, there was a significant relationship between age, gender and type of infections.

The antimicrobial susceptibility test had been done, by using 16 antibiotic produced by Al-Razi Center, and BD BB™ Sensi-Disc™ Antimicrobial. The results were illustrated in table 3.

The resistant percentage to 16 antibiotics was reported as follow: for *E. Coli* was to Ceftazidine 86% then 80% to ceftriaxone, %73 to Trimethoprim sulfamethaxozol, 55% Colistin, 53% Cefotaxime, 45% Nalidixic acid, 42.8% Gentamicin, Tobramycin, 30% Nafcillin, 25% Amoxicillin, 17% Chloramphenicol, 11% Amikacin, 10% Pipracillin, 8% Nitrofurantoin, and still Kanemycin effective against all the *E. coli* strains.

Antibiotic resistant of *K. pneumoniae* was 100% Amoxicillin, 100% pipracillin, 85% Trimethoprime sulfamethaxozol, 71% Colistin, 60% Tetracyclin, 40% Ceftriaxone, Nitrofurantoin, Nalidixic acid, 33% Chloramphenical, 25% Cefotaxime and *K.pneumoniae* was sensitive 100% for kanamycin, Amikacin Nafcillin, Tobramycin.

Antibiotic resistant of P*roteus spp.* 100% Ceftazidime, 80% Trimethoprim Sulfamethoxazol, 66% Cefotaxime, 60% Nitrofurantoin, 58% Nalidixic acid, 50% Colistin, Cefriaxone Chloromphenicol 33% and *proteus spp.* still sensitive to Amikacin, Gentamicin, Nitrofurantoin, Tobramycin.

Pseudomones aeruginosa resistant to antibiotic was 100% Ceftazidime, Colistin Nafeillin, 66% Gentamycin; Trimethoprim Sulfamethaxozol, 50% Tobramycin; Chloramphenicol, Ceftazidime, 33% Nalidixic acid.

Table 3. Antibiotic Susceptibility

Antibiotic	*E. coli*			*K. Pneumoniae*			*Proteus spp.*			*Pseudomonas aeruginosa*		
	R	R%	S	R	R%	S	R	R%	S	R	R%	S
Amikacin	2	11%	16	0	0%	4	0	0	2	0	0	5
Amoxicillin	9	25%	27	10	100%	0	0	0	0	0	0	0
Coftriaxone	8	80%	2	4	40%	6	5	50%	5	0	0	5
Ceftazidime	13	86%	2	0	0	0	4	100%	0	5	100%	0
Cefotaxime (Claforan)	8	53%	7	1	25%	4	2	66%	1	0	0	0
Chloramphenicol	4	17%	19	5	33%	10	3	33%	6	1	50%	1
Colistin	16	55%	13	10	71%	4	3	50%	3	3	100%	0
Gentamicin	3	42.2%	4	0	0	2	0	0	2	2	66%	1
Nalidixic acid (Nigram)	25	45%	30	10	40%	15	7	58%	5	3	33%	6
Nafcillin	4	30%	9	0	0	6	0	0	0	3	100%	0
Nitrofurantion	2	8%	23	6	40%	9	0	0	3	1	0	0
Kanamycin	0	0	10	0	0	5	0	0	0	0	0	0
Pipracillin	10	10%	0	5	100%	0						
Tobramycin	6	42.8%	8	0	0	2	0	0	3	1	50%	1
Trimethoprim sulfamethaxozol (TMP-SMX)	19	73%	7	17	85%	3	4	80%	1	2	66%	1

R=Resistance, S=Sensitive

Discussion

The results of present study showed that the urine cultures reveal no growth of bacteria even in the presence of signs and symptoms of UTI this may be due to other pathogens rather than gram positive bacteria, like fungi, viruses [6].

This result is agreement with [11] except some deviation because of the hospitalized in patients in urological surgery wards, where the nosocomial infections exactly caused by *K. pneumniae* and *P. aeruginosa* [11,12].

P. aeruginosa is important cause of urinary tract infections in patients with urinary catheters. This organism is able to colonize the surface of catheter, forming abiofilm that interferes with activities of antimicrobial agents and host defense mechanism [13]. This result agrees slightly with another study on Urinary tract infections in patients with renal stones [14] which were, the highest percentage of pathogens to *E. coli*, then *Proteus spp., Klebsiella pneumoniae, Pseudomonas aeruginosa*, respectively [14].

Also this result is in agreement with another study had done in France about the UTI in community [15] which showed that the most recovered pathogens were *E. coli* (71%), *Proteus mirabilis* 9%, *Klebsiella pneumonia* 6%, *Pseudomonas aerugenosa* 2%, [15].

And this results goes slightly with a study on community acquired UT1 which was *E. coli* 73% *K. pneumoniae* 6.7% ; *P.aeruginosa* 2.2%, *Proteus spp*. 2.2% [16].

Resistance to antibiotic is most often observed in the hospital setting. Unfortunately, there has been a major worldwide increase within the community in *E. coli* resistance to standard antibiotics, the (Eco. Sens. Project) has been designed to investigate resistant UTI bacteria in 17 European countries [12].

In our study we found that 38% of *E. coli* was resistant to the same antibiotic used in the European study and the difference may due to Ecology and Community circumstances.

The survey of sensitivity to antibiotic agent in Japan, clearly indicated trend for increasing resistance to fluoroqinolones among

enterococci and *P. aeruginosa* isolated from UTI [18] and this agreement with our study.In a European study [12] founded that *E. coli* Resistance to Tmp-smx was 14.1%; Nitrofurantoin and gentamycin 3% and this did not agree with our study.

E. coli is the most common bacteria in UTI. The rates of resistance to common UTI antibiotic vary; however, depending on regions and institution with the highest rate of resistance are those in which antibiotics are heavily prescribed.In European study, for example, the resistance rates were highest in Portugal and Spain and lowest in the Nordic Countries [12]. UTI was higher in females than males and *E. coli* was the commonest pathogen while *K. pneumoiae* infect hospitalized males more than females.

Multi antibiotic resistance transfer between aerobic rods enteric bacteria in community rather than hospital. Multiple organisms are often seen in UTI causes mixed infections which have multi antibiotic resistance.

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Role of Alcoholic Turmeric (*Curcuma longa*) Extract in Outcome of *in vitro* Sperm Activation for Infertile Patients

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Abstract

Background Semen samples are prepared for assisted reproduction by selecting a population of highly motile, morphologically normal sperm and removing the seminal plasma, leukocytes and bacteria. Culture media provide the spermatozoa with needs that maintain optimal function of spermatozoa to give rise excellent results during semen preparation.

Objective The aim of this study was to investigate the effect of alcoholic extraction of turmeric (AET) on sperm parameters during *in vitro* sperm activation (ISA) for asthenozoospermic (AZ) and oligoasthenozoospermic (OAZ) patients.

Methods Seventy four infertile patients were included, and classified into two groups according to their sperm parameters. Semen sample was divided into 3 aliquots. One mL of Earl's medium either alone (control group) or supplied with one concentration of AET (5 µg/mL or 10 µg/mL) was over layered the pellet, and the three tubes were incubated at 37 ^{o}C for 30 min in air incubator. Sperm concentration, motility, grades activity, progressive motility, normal morphology and agglutination were assessed pre- and post-activation *in vitro*.

Results Results revealed an enhancement of most sperm parameters for control and both treated groups post-activation as compared to pre-activation. Post-ISA, sperm parameters for both treated groups were better than the control group. However, best results for improvement of sperm parameters were assessed within treated group (5 µg/mL of AET).

Conclusions The lower concentration of alcoholic turmeric extraction enhanced human sperm parameters during ISA without any harmful effects on sperm physiology. The results are also useful as a guide for further standardization of turmeric extracts used for pharmaceutical purposes in the techniques of assisted reproduction.

Key words Male infertility, Sperm activation, Turmeric.

Introduction

Thousands of chemical structures have been identified in plant foods that have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of metabolism and antimicrobial effects [1]. Turmeric is a spice that comes from the root *Curcuma longa*, a member of the ginger family, Zingaberaceae [2,3]. Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%) [4]. Therefore spice turmeric, an important ingredient in the food preparation,

blocks the formation of hazardous Maillard reaction products and its mutagenic activity [5].
Turmeric has been used for its medicinal properties for various indications and through different routes of administration, including topical, oral, and inhalation [2]. Moreover, curcumin, the active principle isolated from turmeric, exhibits antimutagenic and anticarcinogenic activity [5]. The active principles of turmeric have been evidenced in several animal studies to exert hypolipidemic and antioxidant properties [6]. However, curcumin have been reported to interfere with inflammatory processes [1].
The dietary turmeric is an effective anti-mutagen and it may be useful in chemoprevention against cancers of different organs [7]. The potential efficacy of fresh turmeric paste to heal wounds was tested in a preclinical study in an animal model [8]. Some research suggests that as an antioxidant, turmeric may help in the prevention of conditions such as cancer and heart disease. It was reported that the turmeric may lower levels of low-density lipoprotein cholesterol ("bad cholesterol") and total cholesterol in the blood [9]. Also, turmeric may lower blood sugar and may have additive effects with diabetes medications, and increased sperm count and motility. In addition, turmeric may stimulate contractions of the uterus and may alter menstrual periods [10,11].
The development of assisted reproductive techniques (ARTs) as treatment modalities for infertility during the last two decades has led to the development of a wide range of different sperm preparation methods [12] and culture media [13]. Initially, different media were generated, and contained the same components, yet each medium was characterized by having a different component at a high concentration [14]. However, sperm isolation procedures involve the minimum of physical trauma because the shearing forces associated with centrifugation stimulate reactive oxygen species (ROS) generation in human semen samples [15]. Spermatozoa are very vulnerable to oxidative stress by virtue of their high cellular content of unsaturated fatty acids and limited protection by cytoplasmic antioxidant enzymes [16]. Therefore, the aim of the present study was to investigate the effect of alcoholic turmeric extraction (AET) on human sperm parameters during *in vitro* activation for asthenozoospermic and oligoasthenozoospermic patients.

Methods

1. Infertile patients:
This study was conducted at Institute of Embryo Research and Infertility Treatment, Al-Nahrain University, Baghdad, IRAQ. In the present study, seventy four infertile patients were involved, and classified into two major groups according to parameters of spermatozoa involving asthenozoospermic (AZ, no. 28) and oligoasthenozoospermic (OAZ, no. 46) patients. Complete history and physical examination for infertile patients were achieved before starting this study.

2. Seminal fluid analysis (SFA):
The semen sample was collected after 3-5 days of abstinence directly into a clean, sterile, wide open Petri dish by masturbation in a room near to the laboratory. The containers were labeled with name and age of patients, abstinence period and time of collection. According to manual of WHO [17], SFA was done and involved macroscopic and microscopic examinations as mentioned in details by Fakhrildin [18].

3. Preparation of alcoholic turmeric extract:
High quality of local Turmeric was purchased. Pieces of turmeric were further cleaned from dusts and crushed into turmeric powder using electrical mixer. 10 g of turmeric powder were added to 100 mL of 90% ethanol in a clean glass beaker, and the mixture was stirred for 4 hours period, then the mixture was filtered using Whattman filter paper to collect alcoholic extraction of turmeric (AET) in another clean glass beaker [6,19]. AET was divided into 5 parts in wide open glass Petri dishes for evaporation

of ethanol throughout one night in the laboratory, and using evaporator for complete evaporation. Finally, the residual of AET, as dried material, was weighted and stored in dark universal glass tube for further use. Then, 0.5 mg and 1 mg of dried AET were dissolved in 100 mL of Earl's medium to prepare 5 µg/mL and 10 µg/mL of AET, respectively.

4. *In vitro* sperm activation (ISA):
One mL of liquefied semen sample was placed inside Falcone conical tube and mixed with 0.5 mL of Earl's medium, then; suspension was centrifuged at 2250 rpm for 6 minutes at room temperature. The supernatant was discarded, and the pellet was overlayered with 1 mL of Earl's medium either alone (control group) or enriched with two concentrations (T1; 5 µg/mL and T2; 10 µg/mL) of alcoholic turmeric extract. During *in vitro* sperm activation, three tubes were incubated at 37^{o}C for 30 minutes. Thereafter, sperm parameters were examined in the three groups.

5. Experimental design:
This study was designed to investigate the effects of alcoholic turmeric extraction on outcome of ISA for asthenozoospermic and oligoasthenozoospermic semen samples. Therefore, examinations of SFA as pre-activation group at start, and other three groups of control and two treated (T1; 5 µg/mL and T2; 10 µg/mL) groups as post-activation groups. Examination of sperm parameters involves sperm concentration, motility (%), grades activity (%), normal morphology (%) and agglutination (%).

6. Statistical analysis:
All values were expressed as Mean $\pm$ S.E.M. Statistical Package for Social Studies (SPSS; version 14) was used for statistical analysis. Depending on experimental design and groups of this study, paired *t*-test and two way ANOVA test were applied to compare between groups of pre- and post-activation, and to compare among control and post-activation groups. P value at < 0.05 was considered as statistically significant.

Results

Results of the present study for asthenozoospermic patients showed that the percentages of sperm motility and progressive sperm motility were decreased as compared to standard criteria of WHO (1999). Meanwhile, percentage of sperm agglutination was higher when compared to normal WHO criteria 1999 (Table 1).
Sperm concentration, non-progressive sperm motility (%), immotile sperm (%) and sperm agglutination (%) for control and treated groups were significantly decreased ($P<0.01$) in the post-activation as compared to the pre-activation (Table 1).
In contrast, percentages of sperm motility, progressive motile sperm and normal sperm morphology for three groups were increased significantly ($P<0.01$) in the post-activation when compared to pre-activation (Table 1).
Among control and both treated groups, non significant differences ($P>0.05$) were observed in the sperm concentration, non-progressive motile sperm (%), normal sperm morphology (%) and sperm agglutination (%) as presented in table 1.
From the same table, percentages of sperm motility and progressive motile sperm for T1 group were significantly increased ($P<0.05$) as compared to the control and T2 group. However, significant reduction ($P<0.05$) in the percentage of immotile sperm for T1 group when compared to the control and T2 groups (Table 1).

Table 1. Parameters of *in vitro* pre- and post-activated sperm using Earl's medium enriched with two concentrations of alcoholic turmeric extract for asthenozoospermic patients

Sperm parameters		Asthenozoospermic semen samples No = 28				WHO, 1999 criteria
		Pre-activation	Post-activation			
			Control	T1; 5 mg/mL	T2; 10 mg/mL	
Sperm concentration		54.68±2.73	15.72±1.98	20.84±1.83	18.08±1.94	20X 10^6 sperm/mL
Sperm Motility (%)		38.76±2.42	78.66±2.81	88.29±2.63†‡	80.21±2.17	≥ 50 %
Sperm grade activity (%)	PMS	29.55±1.74	75.61±1.89	86.26±2.48†‡	77.78±2.67	≥ 50 % of grades (A+B)
	NPMS	9.24±1.46	2.04±0.83	2.09±0.64	2.41±0.72	
	IS	61.23±1.38	21.35±1.13	11.68±0.81†‡	19.80±0.87	
Normal sperm morphology (%)		46.27±1.75	83.56±2.37	85.12±2.71	84.29±2.79	≥ 30 %
Sperm agglutination (%)		12.43±1.23	-	-	-	< 10 %

PMS = Progressive motile sperm, MPMS = Non-progressive motile sperm, IS = immotile sperm, * = P<0.01 as compared to three groups of post-activation, † = P<0.01 as compared to the control group of post-activation, ‡ = P<0.05 as compared to another treated group of post-activation.

Table (2) shows sperm parameters for oligoasthenozoospermic patients complaining from reduction in the sperm concentration and motility (%) when compared to normal criteria of WHO (1999). As compared to pre-activation *in vitro*, there were significant reductions (P<0.01) in sperm concentration, immotile sperm (%) and sperm agglutination (%) in the post-activation of three groups. Conversely, percentages of sperm motility, progressive motile sperm and normal sperm morphology for control and both treated groups were significantly increased (P<0.01) in the post-activation as compared to pre-activation (Table 2).

From the same table, non significant differences (P>0.05) were noticed in the sperm concentration, normal sperm morphology (%) and sperm agglutination (%) in the post-activation against the control and both treated groups. However, percentages of sperm motility and progressive motile sperm for T1 group were significantly increased (P<0.05) as compared to the control group. In contrast, significant reduction (P<0.01) was assessed in the immotile sperm (%) for T1 group when compared to the control group. Post-activation *in vitro* caused a significant reduction (P<0.05) in non-progressive motile sperm (%) of control and T1 groups as compared to T2 group.

Table 2. Parameters of *in vitro* pre- and post-activated sperm using Earl's medium enriched with two concentrations of alcoholic turmeric extract for oligoasthenozoospermic patients

Sperm parameters		Asthenozoospermic semen samples No = 28				WHO, 1999 criteria
		Pre-activation	Post-activation			
			Control	T1; 5 mg/mL	T2; 10 mg/mL	
Sperm concentration		17.26±1.39	6.82±0.82	8.68±1.07	8.13±1.16	20X 10^6 perm/mL
Sperm Motility (%)		35.92±1.72	76.79±2.28	87.23±2.17†	83.72±2.03	≥ 50 %
Sperm grade activity (%)	PMS	26.58±1.52	72.18±1.84	79.37±1.81†	74.50±1.93	≥ 50 % of grades (A+B)
	NPMS	9.32±0.73	4.61±0.47	5.86±0.64	9.22±0.81†‡	
	IS	64.09±1.52	23.22±1.17	12.76±0.45†	16.29±0.73	
Normal sperm morphology (%)		36.81±1.31	81.46±1.73	83.87±1.79	83.27±1.58	≥ 30 %
Sperm agglutination (%)		6.67±0.74	-	-	-	< 10 %

PMS = Progressive motile sperm, MPMS = Non-progressive motile sperm, IS = immotile sperm, * = P<0.01 as compared to three groups of post-activation, † = P<0.01 as compared to the control group of post-activation, ‡ = P<0.05 as compared to another treated group of post-activation.

Discussion

After *in vitro* sperm activation (ISA), all sperm parameters for asthenozoospermic (AZ) and oligoasthenozoospermic (OAZ) patients were enhanced in the control and both treated groups, except for sperm concentration. The reduction in the sperm concentration results from swim-up of only active motile sperm during ISA [20]. However, most sperm motility parameters and sperm progressive motility (%) especially were improved in both AET-treated groups better than the control group. In this study, we used one centrifugation step because the original sperm parameters for both groups of infertile patients were not severe cases. However, strategies involving repeated high speed centrifugation are also occasionally used in an effort to harvest as many cells as possible from the ejaculates of severely OAZ patients [21]. In addition, it has been demonstrated that the method employed for preparing spermatozoa influences ROS production by human sperm suspensions and this inversely correlates with the fertilizing potential of the spermatozoa *in vitro* [22].

Numerous studies have indicated that the AET improves Ca^{2+}-transport to correct the defective Ca^{2+} homeostasis [4], and reduces cholesterol content [23,24]. It was reported that the reduction of cholesterol is combined with an increase of α-tocopherol level in rat plasma, suggesting *in vivo* interaction between curcumin and α-tocopherol that may increase the bioavailability of vitamin E and decrease cholesterol levels [25]. Turmeric has many active components, but curcumin is the most potent ingredient. It is a powerful anti-inflammatory and anti-oxidant and has greater effects in preventing free radical damage, compared with vitamins C, E and superoxide dismutase [26]. However, modulation in Ca^{2+} and reduction of cholesterol are important prerequisites for sperm hyperactivation, capacitation, acrosomal reaction and fertilization [27,28].

Under *in vivo* conditions, potentially fertile spermatozoa are separated from immotile spermatozoa, debris and seminal plasma in the female genital tract by active migration through the cervical mucus. During this process, only progressively motile spermatozoa are selected [20]. Researchers were able to show that morphologically abnormal sperm with low motility produced the highest levels of ROS [29]. Also, the results of experiments suggest that abnormal sperm with low motility produce high levels of ROS which can then subsequently damage and affect the motility of otherwise normal sperm within the semen population [30]. Actually, as soon as seminal plasma is removed, the spermatozoa become vulnerable to free radical attack by contaminating leukocytes and both sperm function and DNA integrity can be compromised [21]. In addition, harmful effect of centrifugation process during sperm preparation [31]. For this reason, AET was used.

Also, AET lowers reactive oxygen species (ROS) generated by centrifugation during ISA through antioxidant activity [32,33]. Spermatozoa are rich in polyunsaturated fatty acids and more liable for lipid peroxidation by ROS [23]. ROS and their derivatives can damage various biomolecules including lipids, proteins and DNA. There is evidence suggesting that free radicals and ROS play a significant role in many cases of male infertility [30]. Curcumin, the active ingredient of the turmeric, is a strong antioxidant, and reportedly several times more potent than vitamin E as a free radical scavenger [1]. *In vitro*, curcumin can significantly inhibit the generation of ROS like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages [34]. This is brought about by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase [35]. Curcumin binds to lipoxygenase in a noncompetitive manner [36].

In contrast, it was reported that curcumin inhibits human sperm motility and has the potential for the development of intravaginal contraception [36]. These harmful effects of curcumin on sperm motility may be indirectly through physiological and biochemical effects

on vagina and its lining epithelium by increasing NO which has negative activity on sperm motility [23].

From the results of the present study, it can be concluded that the low concentrations of alcoholic turmeric extract enhanced human sperm parameters during ISA without any harmful effects on sperm physiology. The results are also useful as a guide for further standardization of turmeric extracts used for pharmaceutical purposes in the techniques of assisted reproduction.

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Anthropometric Study of Pubic Tubercle and Its Clinical Implications

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Abstract

Background Abdominal wall is the site of opposing physical forces that may eventually result in the appearance of the hernias. The external abdominal hernias are the most common forms, the inguinal hernia being the commonly encountered type [75% of the abdominal hernia]. Many factors are responsible for the formation of the inguinal hernia but, what makes a few people more susceptible to this situation is still clearly not proved. Few of the previous studies have concluded that the low lying pubic tubercle is associated with the development of the inguinal hernia.

Objectives This study was designed to investigate the distance between the anterior superior iliac spines and the perpendicular distance of the pubic tubercle from the interspinal line.

Methods 50 males with inguinal hernia compared with the control group comprising of 60 adult healthy males.

Results This study revealed that both parameters (interspinal SS distance and the pubic tubercle height ST) in the study group were significantly greater than that in the control group. The distance from mid inguinal point to the superficial inguinal ring was also measured in both the study and control groups and the results show that the distance is shorter in the individuals with the inguinal hernia. Identification of the structural characteristics of inguinal region enables the surgeon to perform the surgical technique appropriately.

Conclusion The low pubic tubercle group of cases has more tendencies for herniation. The unusual origin of internal oblique muscle in group II with low lying tubercle is far away, from the external half of the inguinal ligament, leaving the internal ring unprotected during abdominal muscle contraction, which is another causation of hernia development.

Key words Inguinal hernia, pubic tubercle, inguinal canal, anterior superior iliac spine

Introduction

Sir Astley Paston Cooper in 1804 has said that "No disease of human body, belonging to the province of the surgeons, requires in its treatment a better combination of accurate anatomical knowledge with surgical skill than hernia in all its variety [(1)]. Among all spontaneous external abdominal hernias, inguinal hernia is the most commonly encountered type. The statistics show that the indirect inguinal hernia is the most common of all forms of the hernia, affecting the males seven times more than the females[(2)]. There are various defensive mechanisms of the inguinal canal to prevent the formation of hernia which are based on anatomical factors. Anatomic variations of different structures facilitating herniation have been assessed by many authors. The origin of the internal oblique muscle from the inguinal ligament far away from the pubic tubercle and its lower fibers not covering the internal ring has been implicated in the indirect inguinal hernia [(3)]. The various degree of incompleteness of the internal oblique muscle in the inguinal region lead to the essential predisposition to direct inguinal hernia [(4)]. Other factors are an increase in the size of Hessert's triangle [(5)]. One

important factor that determines the probability of an individual to suffer from an inguinal hernia is the location of the pubic tubercle. Many authors have concluded that persons with low lying pubic tubercle are at a higher risk and more prone to hernia [6,7]. The aim of the present study is to measure the distance between the two anterior superior iliac spines and the perpendicular distance of the pubic tubercle from the interspinal line, in individuals with inguinal hernia and to compare them with normal healthy individuals. Also a comparative study of the length of the inguinal canal is done.

Methods

The study group comprised of 50 males who were diagnosed with inguinal hernia and the control group consisted of 60 healthy male individuals. The age of individuals in both the groups was between 25 and 40years. A detailed history was taken to rule out any fracture or anomaly and such persons were excluded from the study. The subjects were asked to lie down in relaxed supine position on a hard bed, keeping their lower limb straight so that both the anterior superior iliac spines were at the same level. The line joining the two anterior superior iliac spines was measured and was named as SS line. Then the pubic tubercle was marked and the vertical distance of the pubic tubercle from the SS line was measured and named as ST line (Figure 1). The mid point between the anterior superior iliac spine and the pubic symphysis was marked as the midinguinal point and the distance from it to the centre of the superficial inguinal ring was measured, the inguinal ligament length was measured as well. All these measurement thus obtained were tabulated and analyzed using Chi-square test and students't' test.

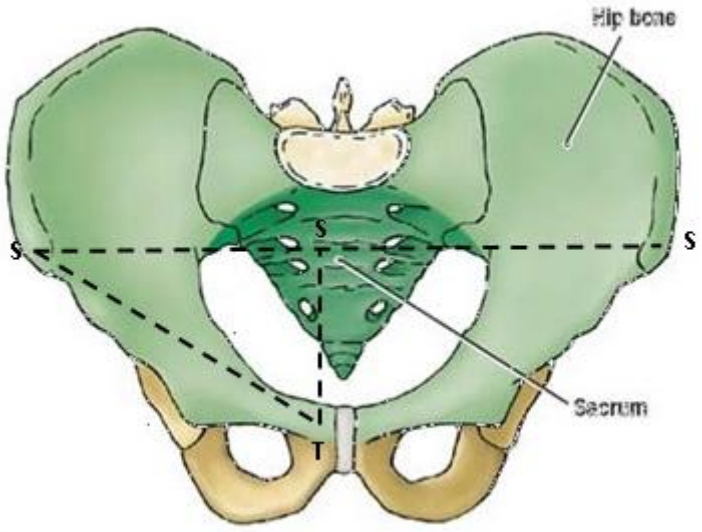

Figure 1. Graphic illustration for the measurement, SS, interspinal distance. ST, the pubic tubercle height

Results

The following tables depict the various results [Table 1 and Table 2].

Table 1. Comparison of the SS line and ST line in the study and control group

Group		N	Mean(cm)± SD	' t' value
SS line	Study	50	23.3040±1.28176	2.04400
	Control	60	22.6462±1.00568	p= .046
ST line	Study	50	7.8115±0.82526	5.56400
	Control	60	6.5440±0.80056	p= .001

Table 2. Comparison of the distance from the mid inguinal point to the superficial inguinal ring

Group	N	Mean± SD	' t' Value
Study	50	5.640±0.635	1.41000
Control	60	6.089±0.607	P=0.173

Table 3. Position of the pubic tubercle in the two groups.

Group	<7.5 Count (%) Group I	>7.5 Count (%) Group II
Study	13 (26%)	37 (74%)
Control	55 (91.5%)	5 (8.3%)

Discussion

The causation of inguinal hernia is multifactorial with evolutionary, congenital, environmental, genetic factors and also the general state of health all contributing to its development. The low lying pubic tubercle predisposes to the development of inguinal hernia. Africans have much higher incidence of inguinal hernia as compared to Europeans since the Africans has comparatively more oblique pelvis(low lying pubic tubercle) than the Europeans [8].

Sehgal et al (2000) in their study have classified the subjects as(Group I) "High lying pubic tubercle" i.e. those with ST line less than or equal to 7.5 cm and (Group II) "Low lying pubic tubercle" i.e. those with ST line more than 7.5 cm. They observed that in 73.6 % of cases and only 16% of controls belonged to Group II and concluded that the low lying pubic tubercle was a predisposing factor for inguinal hernia [6].

The change in posture from pronograde to upright has caused reduction in efficiency of shutter mechanism of inguinal canal leading to the development of inguinal [9]

In the present study 74% of cases belonged to the Group II whereas 91.5% of controls belonged to Group I [Table 3]. The mean value of ST line in our study group is 7.8115+0.82526 which is significantly greater (p=0.001) than the controls the mean value being 6.5440+0.80056. Lopez- Cano et al (2005) have stated that the low pubic arch group showed a significantly longer inguinal ligament and a greater angle made by the superior border of the suprainguinal space and inguinal ligament at its medial insertion. The lower the pubic tubercles are located, the more often morphological alterations are found in the external oblique, internal oblique, transversus, cremastric muscles and the fascia transversalis [7, 10].

The shutter-like mechanism at the internal inguinal ring is provided by contraction of the arching fibers of the internal oblique muscle, which, when shortened, approximate themselves to the inguinal ligament and compress the spermatic cord [11].

The unusual origin and insertion of internal oblique and transverses abdominis muscle, results in an ineffective shutter mechanism of the inguinal canal [10].

The low pubic tubercle group showed a significantly longer inguinal ligament than the high pubic tubercle group. The greater length of inguinal ligament and a larger suprainguinal angle may account for a greater area of suprainguinal space which may account for a deficient function of the shutter mechanism [11]. Harris and White associated a greater length of inguinal ligament with a higher tendency to develop inguinal hernia [13].

Ajmani and Ajmani (1983) have noticed that in the inguinal hernia patients, the origin of internal oblique from the inguinal ligament was away from the pubic tubercle and its lower fibers did not cover the deep inguinal ring leaving it unprotected, allowing the hernial sac to push out when the intra-abdominal pressure is raised [3].

In addition to above mentioned pathophsiological factors, the inguinal canal in that study group with low lying pubic tubercle being more longer and more oblique so the hernia sac will push out easier through the canal as the more gravitational effect than when the canal is more or less horizontal or oblique in normal group. So we can state that the functional significance of the inguinal region is modified by bony, ligament and muscular variations and therefore the identification of the structural characteristics enables the surgeon to perform the surgical technique appropriately, be it classical hernia repair or laparoscopic approach for prosthetic mesh implantation.

This anthropometric study of pelvis will enable the surgeons to categorize people with low lying pubic tubercle as liable for hernia development so they should be precautious in doing their daily activities.

On the other hand those patients with low lying pubic tubercle developed inguinal hernia preferably to make hernioraphy for the posterior wall and do reinforcement for the deep ring by mesh for example since they have unprotected deep ring and weak shutter mechanism.

Conclusion

The low pubic tubercle group of cases has more tendencies for herniation. The longer the inguinal ligament, the larger the suprainguinal region and the larger Hessert's triangle. Which leads to less efficient shutter mechanism.

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The Proliferative Profile of the Rhombencephalicdemilune in the Developing Rat Cerebellum: A quantitative HistochemicalStudy

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Abstract

Background Studies have shown that the cerebellum develops within the dorsal metencephalon creating a rhombencephalicdemilune (RD) which represents the formation site of the cerebellum granular cells progenitors. These studies used different histological techniques but all have provided qualitative information regarding the biosynthesis and cell mitosis at the RD.

Objective Quantifying the proliferative activity of the cellsat the RD during the embryonic period.

Methods Six age groups from day 16 to day 21 albino rat embryos Rattusrattusnorvegicus were investigated with Ag-NOR staining technique to quantify cell proliferation.

Results There wasa statistically significant difference ($p<0.01$) between cellular activity at different age groups with a surge during embryonic day 18.

Conclusions Correlation with other studies revealed that Ag-NOR staining technique, which reflects protein biosynthesis and nuclear mitotic activity, provided a valuable quantitative measure of cellular proliferation in the developing rat cerebellum.

Keywords Rhombencephalicdemilune, Rat, Developing Cerebellum, Ag-NOR, Quantitative, Histochemistry

Introduction

The cerebellar primordium was described as a crescent shaped anlage or cluster of cells that appear at embryonic day 13. This primordium is divided into three parts: lateral, subisthmal and postisthmalprimordia, which were believed to be the sources of different components of the developing cerebellum [(1)]. Later studies gave a morphological delineation of the developing cerebellar primordium as being a dorsal metencephalic anlage since the metencephalon has floor and roof plates, and the cerebellum develops within the roof plate or the "dorsal metencephalon"[(2)]. Thus, the rhombencephalicdemilune(RD) is the crescent-shape area surrounding the dorsal metencephalic anlage caudally and laterally, forming the caudal part of the roof plate of the forth ventricle in the developing embryo; it is the zone where the progenerators of the cerebellar external germinal layer(EGL) cells are located. These cells spread radially from caudal to rostral direction during the embryonic day 17,which signals the transformation of the cerebellar primordium into the primitive cerebellum [(2,3)].

These events weredemonstrated by different histochemical methods includingshort-term and long-term survival thymidine autoradiograms [(4)], lectins [(5)], immune histochemistry[(6)], and enzyme histochemistry [(7)].Such methodsuse different markers for migration, histological differentiation and cell process dynamics, in particular during complex embryonic development at the dorsal metencephalic anlage[(1,8,9)]. All these methods only provide subjective information regarding the proliferative profile of the RDthat has

specific spatiotemporal variation during prenatal development; it appears at embryonic day 17 and disappears at day 21 [2,10,11].

In the proliferating cells, nucleolar organizer regions (NORs) are loops of DNA which contain ribosomal RNA genes important for the synthesis of proteins. These NORsare stained with silver colloid technique, and the resultis known as Ag-NOR dots [12].

The number and area of Ag-NORs are an accurate index of activity and cell proliferation in terms of protein synthesis[13]. Hence, Ag-NORstain is used to measure the biosynthetic profileand cell mitotic activityby demonstrating the amount of rRNA that increases during cell replication [14]. It can be used as an indicator related to the proliferative capacity of normal and neoplastic cells [15].This work aims at assigning a quantitative proliferation index for the cells of the RDduring their embryonic development by the application of Ag-NOR staining technique.

Methods

A sample of twelve albino rats *Rattusrattusnorvegicus* was divided into six age groups from embryonic day 16 to embryonic day 21 and brain tissue specimens were obtained by decapitation.Tissue blocks were immersed in Bouin's fixative for 16 hours at room temperature (25°C) and parasagittal paraffin sections of 6 micrometer thickness were prepared for embryonic age day 16 through day 21.

Sections were stained according to the method of Ploton [16]. Dewaxingin xylene was done for 3-5 minutes then pre-incubation in glycine solution (made by dissolving glycine powder (AnalaR) in 99% ethanol alcohol) for 10-20 minutes followed by rehydration in descending concentrations of ethanol alcohol (100%, 90%, 80% and 70%) each for 3 minutes.

Colloidal developer solution was made by dissolving 2 g of gelatin powder (Agar LTD) in 100 ml of double deionized distilled water (2% w/v). This was added to 1% aqueous formic acid.Developer solution was mixed 1:2 volumes with 50 g/dl aqueous freshly prepared double deionized silver nitrate (M & B) solution filtered through mini-pore filter paper under dark room conditions.

Histological sections were left in silver colloid solution for 45 minutes at 37°C in an air incubator. Background stain was reduced through holding the slides perpendicularly in Coplin's jars where the precipitate remains at the bottom.Sections were washed in running double deionized distilled water for 10-15 minutes then treated with 10% nitric acid solution (Fluka) for 30 seconds, washed well with flowing double deionized distilled water and immersed in 5% sodium thiosulphate (AnalaR) (w/v) solution for 5 minutes to provide a permanent preparation.

Finally, dehydration was achieved by ascending concentrations of ethanol alcohol (70%, 80%, 90% and 100%), each for 3 minutes, then clearing with xylene and mounting with Eukitta mounting medium.Examination of sections was done under light microscope (1250X oil immersion) using a systemic random selection of 5 fields per section of each age group.Fifty cells with Ag-NOR stained nuclei were identified in each field and the average number of staining dots per each cell was obtained [14].

Results

An orientation view is seen in figure 1 that shows a compact cellular layer at the RD region with extensions towards the EGL and the neuroepithelial layer. Purkinje cells are observed as a less packed stratum deep to the EGL, while fronds of cellular projections from the medullary velum mark the development of the choroid plexus at the roof of the forth ventricle.In figure 2, the cells of the RD are magnified to reveal the Ag-NOR dots within the nuclei.The averages of Ag-NORs per cell nucleus in different age groups of rat embryo RD are shown in table 1.

With the aim of analyzing the differences between the various age groups, a single factor ANOVA was applied regarding the Ag-NOR parameter evaluated: mean Ag-NOR number

per cell.The results show a statistically significant difference between the various age groups studied (P<0.01), as shown in table 2.

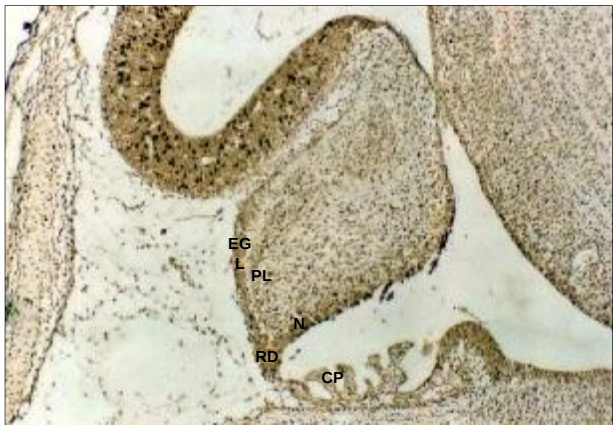
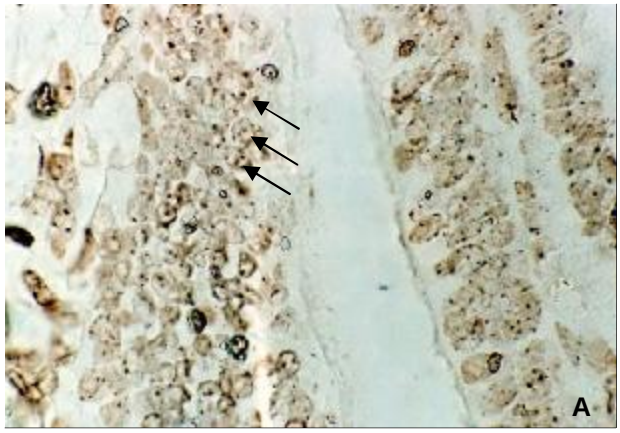
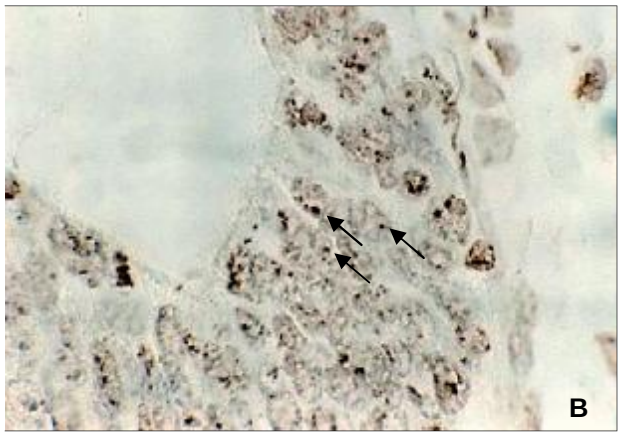


Figure 1. Parasagittal section of the developing cerebellum of a18 days aged rat embryo showing the rhombencephalicdemilune (RD) with the appearance of the cerebellar external germinal layer (EGL), Purkinje layer (PL) and neuroepithelium (N). The choroid plexus (CP) is seen at the roof of the 4th ventricle. Ag-NOR stain. 100X.





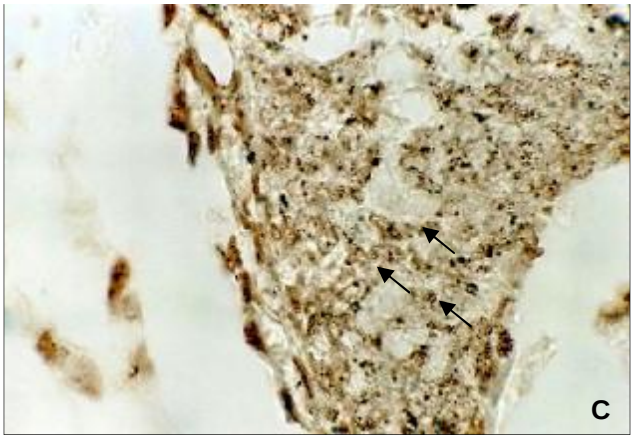


Figure 2.Cells of the rhombencephalicdemilune have increasing mitotic activity from day 16 (A), day 17 (B) with maximum at day 18 (C) reflected by the increasing number of Ag-NOR "dots" (arrows) in the proliferating cells. Ag-NOR stain. 1250X Oil immersion

Table 1. Average numbers of Ag-NOR per cell nucleus identified within five fields of each section in different age groups of rat embryo rhombencephalicdemilune

Section	Age (days)					
	16	17	18	19	20	21
1	1.64	2.74	7.74	1.56	1.40	1.32
2	1.44	2.40	6.92	1.60	1.36	1.06
3	1.62	2.34	6.86	1.64	1.32	1.28
4	1.58	2.88	6.72	1.62	1.38	1.40
5	1.38	2.62	7.02	1.66	1.41	1.24
Mean ±S.D	1.53± 0.12	2.60± 0.23	7.05± 0.40	1.62± 0.04	1.37± 0.04	1.26± 0.13

Table 2. ANOVA Single Factor

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	126.1153	5	25.22306	622.0748	1.46E-24	3.89507
Within Groups	0.97312	24	0.040547			
Total	127.0884	29				

Discussion

Several works designate the RD as the birth place of the EGL of the cerebellum; this region undergoes cellular changes in correlation with the spatiotemporal variations that results in the spread of the EGLto cover the outer surface of the cerebellum during development[1,2].

The proliferative activity of the RD studied previously gave a clue that the caudal part of the dorsal metencephalic anlage is the formation site of the granular cells progenitors in the EGL [2,3]. But whether designated as a superior [11] or upper rhombic lip [17], the observations made on this formation site were based on qualitative measures performed to characterize the proliferating cells of the region.

In order to investigate the possibility of establishing a quantitative method for the estimation of cellular activity at the RD during embryonic development, the Ag-NOR staining technique was employed in this work because it is considered as a rapidand easy way to obtain an estimation of protein synthesis rate [13,18];the number of Ag-NOR dots is an accurate index of activity and cell proliferation in terms of protein synthesis [19,20].

Although studies performed on various regions of the CNS, such as the hypothalamus and the hippocampus, did not analyze the proliferation pattern seen during the various developmental stages, the Ag-NOR technique alone or in combination with other histological stains have revealed quantitative associations among cell proliferation and different aspects of functions in animals and in humans [21-23].

Our results showed a significant "surge" in cell proliferative activity in terms of Ag-NOR dots per cell nucleus during the embryonic day 18 (Table 1). Such results conform to observations made by other studies when the nuclear transitory zone starts to appear at the embryonic day 14 and the cortical transitory zone at the embryonic day 15 [1].Further dynamic re-arrangement and translocation of the cells at these zones during the embryonic day 16 and thereafter is in the direction of the EGL dispersion, as noticed in figure 1 which demarcatesthe extension of RD cells towards the newly formed EGL. This dispersion, beginning at the embryonic day 17, signals the transformation of the cerebellar primordiuminto the primitive cerebellum, and it is coupled with the progressive maturation of the cerebellar cortex and the deep nuclei [8,9]. Consequently, this study revealed that the proliferative activity of the cells of the RD reaches its peak one day after the appearance of granular cells progenitors when cell "migration" is at maximum [24].

In conclusion, the Ag-NOR staining technique was successfully used in this study to yield a quantitative index of cell proliferation at the RDin concordance with previous qualitative works. This technique is recommended for the investigation of protein biosynthesis and cellular activity in the developing CNS.

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Renal and Myopathy Lesions of Dirofilaria immitis in Natural Infected Dogs

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Abstract

Background Dirofilaria immitis has been increasingly recognized worldwide as inadvertent human pathogens. The epidemiological survey usual hosts of theses nematodes are domestic and wild carnivores. The disease is regarded as one of the most dangerous threat for the dog health. The adult worms take up residence in the heart, lungs and surrounding blood vessels.

Objective This study attempts to shed the light on relationship between glomerular lesions and heart filarial (Dirofilaria immitis) infection.

Methods Dirofilaria immitis was isolated from 98 dogs out of 457 dogs that were autopsied for the time between April 2008 and May 2010, in Al-Hindya area, Karbala province. Parameters concerning parasitological and pathological changes are used in this study to determine the significance of the results.

Results Grossly many adult heartworms were found in the right ventricle of infected hearts and the cross section revealed body cavity, thick cuticle with coelomyarian and polymyarin muscle type, accompanied by infiltration of inflammatory cells mainly eosinophils and lymphocytes between muscle fibers. The microscopical changes in the kidney were necrotic and sloughing of epithelial cells, cystic dilation of collecting tubules of medulla containing hyaline casts, with glomeruli showing membranous nephropathy.

Conclusions This is the first histological report of canine filariasis in Iraq particularly in Karbala province. It is important to keep in mind that pathologic changes in heartworm disease may be well advanced before the appearance of clinical signs of the disease. In heartworm disease the circulatory system is not the only system affected. The renal, hepatic & pulmonary systems can be secondarily affected. In our work the observations support the previous hypotheses of immature and possibly adult worms, contribute to the glomerulonephropathy.

Key wards Canine heartworm, interstitial nephritis, membranous nephropathy.

Introduction

Dirofilaria immitis is a filarial nematode whose primary host is the dog. It is known as canine heartworm, can infect humans and cause a pulmonary lesion (human pulmonary dirofilariasis) that can easily be mistaken for a malignant tumor on imaging studies [(1)]. The larvae injected by infected mosquito into the skin migrate through the muscles to the pulmonary blood vessels reaching the pulmonary arteries, where they continue to mature. Adult worms are found primarily in the pulmonary arteries, right side of the heart and in severe infections adult worm was found in the abdominal cavity of the dog during spaying [(2)].

Chronic dirofilaria disease results from progressive proliferative endarteritis and thromboembolism of the pulmonary artery caused mostly by adult worms and not by juvenile migrating worms [3].

Histopathologically studies strongly suggest that filariasis may be of importance in relation with canine interstitial nephritis (IN) [4]. Some severely infected interstitial nephritis cases were shown to have necrosis with hemosiderin deposition and tubular degeneration also might be related with heart filariasis [5].

The most glomerular and interstitial lesions are usually observed in dogs with high microfilaria counts and long infection periods due to prolonged release of antigenic material into the blood stream by inducing in situ formation or trapping of performed complex [6]. Microscopical evaluation of kidney revealed a diffuse hypercellularity and thickening of glomerular basement membrane. While transmission electron microscopy revealed deposits in the mesangium, subendothelium and sub-epithelium. These lesions are compatible with membranoproliferative glomerulonephritis type III in humans [8].

Due to concern over the potential public safety and health risk, the present work carried out to describe the nephropathy of dirofilariasis in natural infected dog; also give the general histological description of the parasite. To the author's knowledge this is the first histopathological study of heartworm in dogs in Iraq particularly in Al-Hindya part of Karbala province.

Methods

From April 2008 to May 2010, four hundred and fifty seven stray dogs were shot by an authorized policeman and investigation for the presence of heart worms (D. immitis). These dogs were from different village of Al-Hindya area, Karbala province. No attempt was made to select these dogs on the basis of sex, breed, color, or type of coat. Approximately 5 ml of blood was drawn from the femoral vein and direct from the heart of each dog, were tested by the Knott method [9] for the presence of microfilaria (larval stage) of the parasite. After shooting of these dogs, they were brought to the veterinary clinic in the province for the purpose of conducting postmortem and further investigations. Each organ like liver, spleen, lung, heart, and kidney were carefully examined. Tissue samples from kidney were fixed in 10% formalin. Paraffin sections were made by a routine procedure and stained with Hematoxcylin and Eosin [10]. D. immitis found in the right ventricle and pulmonary arteries were removed from 98 natural infected dogs (Figure 1).

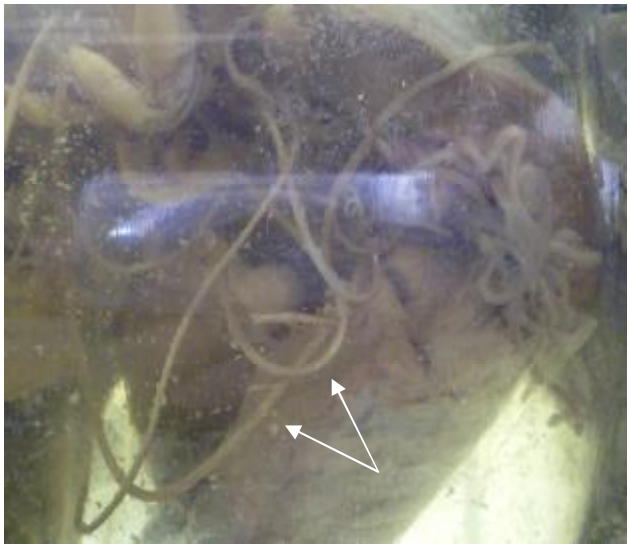

Figure 1. Canine filariasis: Many adult *D. immitis* in the heart of a dog

Result

Light microscopic study of kidney section showed several lesions of varying degrees of severity. The histopathological finding in cross-section of parasite showed presence of body cavity thick cuticle and coelomyarian and polymyarin muscle type with intestine tract (Figure 2). While the myocardial findings revealed infiltration of inflammatory cells mainly eosinophils and

lymphocytes between degenerated muscle fibers (Figures 3 and 4), with fragmentation and separation of some muscle bundle by intramuscular edema (Figure 5) as well as present of PMNCs in the lumen of some blood vessels.

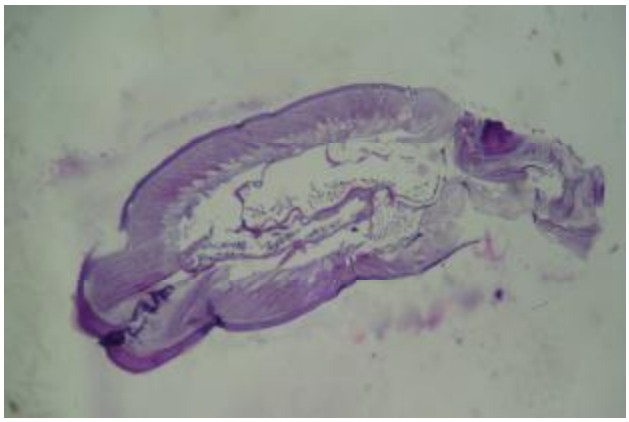

Figure 2. Cross section of D. *immitis* shows thick cuticle, coelomyarian and polymyarian muscle. H & E. (10X)

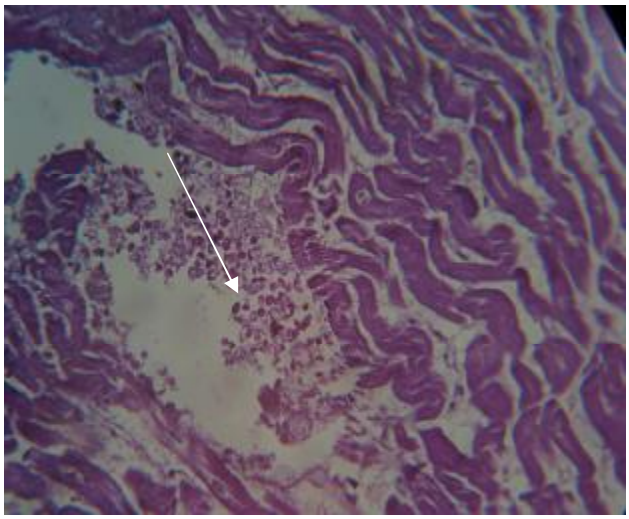

Figure 3. Heart muscle of infected dog shows mild infiltration of eosinophils between muscle fibers. H & E (10x)

Some glomeruli were collapsed associated with atrophy of tuft and dilatation of urinary space (Figure 6) with periglomerular fibrosis. Some glomeruli showed features with membranous nephropathy characterized by diffuse thickening of the basement membrane; also the change is diffuse affecting all capillaries in glomerular tuft (Figure 7).

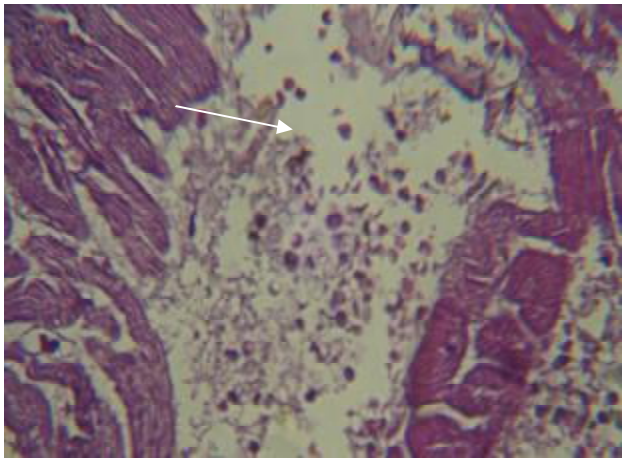

Figure 4. Heart muscle of infected dog shows infiltration of interstitial with MNCs inflammatory edema. H&E 20x

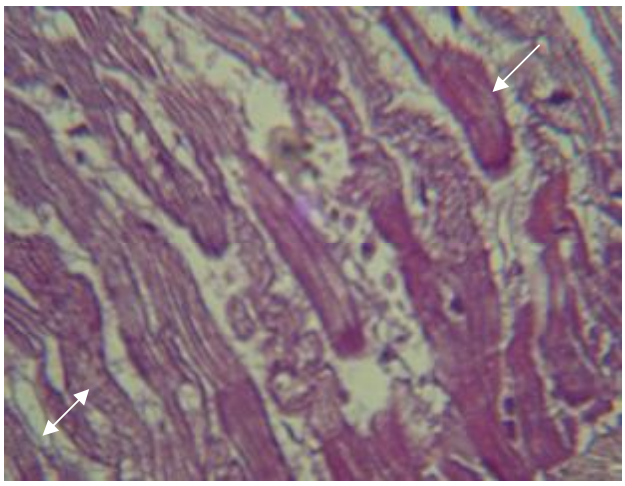

Figure 5. Heart muscle of infected dog shows the hyalinized degenerated muscle fiber (↙) and separated by cellular infiltration and cellular edema (↗↙). H & E (20X)

The epithelial lining of proximal and distal convoluted tubules showed varies degree degenerative changes range from mild to severe. Some of the tubules were necrotic with sloughing of their epithelial lining cells (Figure 8).

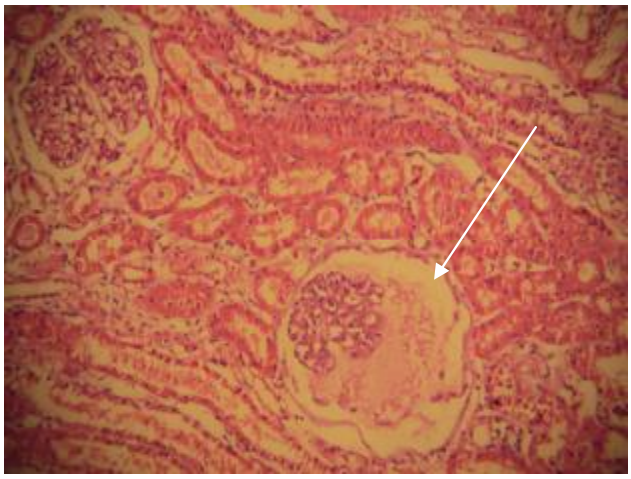

Figure 6. Kidney of infected dog shows one glomerular collapsed with atrophy of tuft, dilatation of urinary space & hyper-cellular of the other one. H&E (10x).

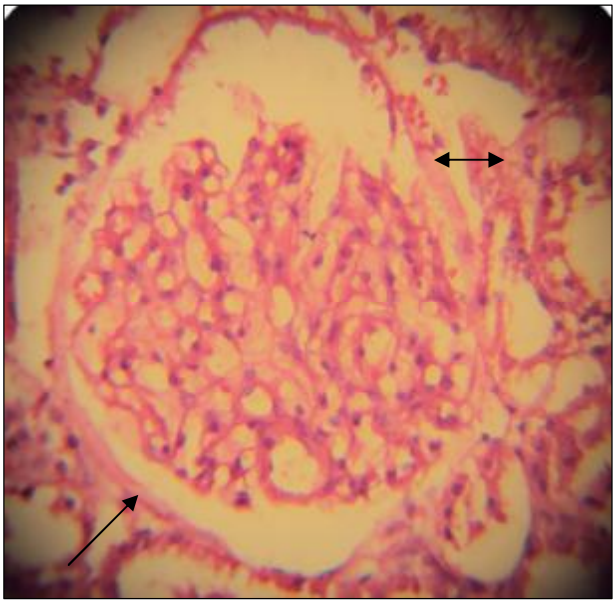

Figure 7. Kidney of infected dog shows diffuse thickening of the basement membrane (↔) and capillaries of glomerular tuft (↗). H&E (40x).

Cystic dilatation of collecting tubules of medulla containing hyaline casts in the lumen were observed (Figure 9). There was focal infiltration of lymphocyte and plasma cells in the interstitial tissue (Figures 10a and 10b) accompanied with fibrous thickening of renal capsule (Figure 11).

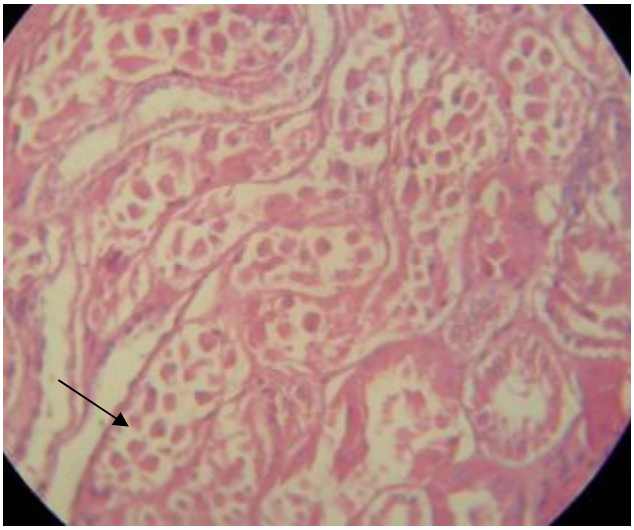

Figure 8. Kidney of infected dog shows most of epithelial lining the tubules has died & sloughed into the lumen. H&E (10x).

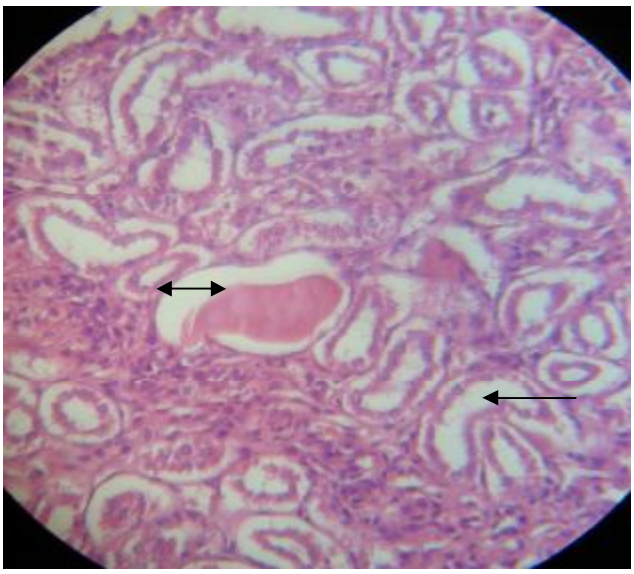

Figure 9. Kidney of infected dog shows cystic dilatation of collecting tubules (←) of medulla containing hyaline casts (↔). H & E 20x

The blood vessels were congested and thickened (Figures 12 and 13) with perivascular lymphocytic cuffing. In addition, some sections

of interstitial reaction have various degrees of fibrosis.

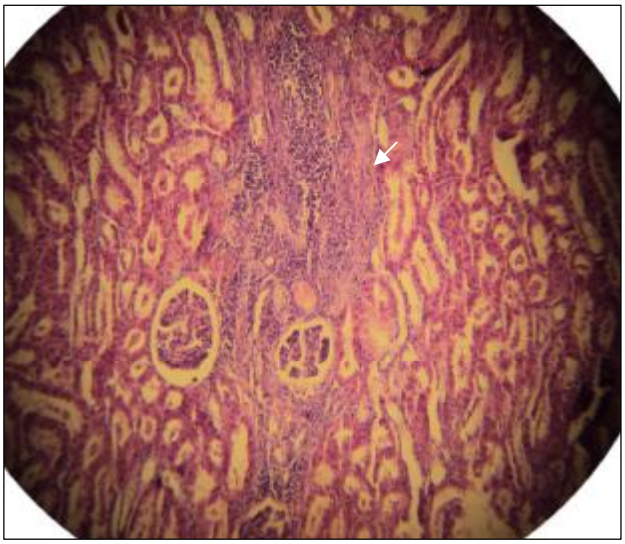

Figure (10a) kidney of infected dog shows focal infiltration of lymphocyte and plasma cells with tubular dilatation. H & E. 10x

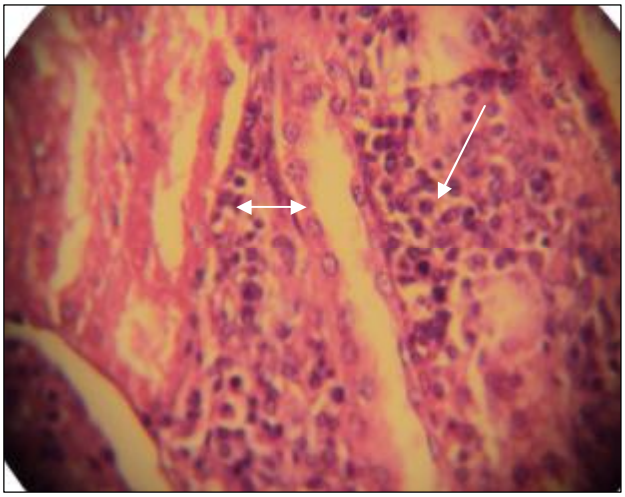

Figure 10b. Kidney of infected dog shows infiltration of lymphocyte and plasma cells in the interstitial tissue (↙) with cystic tubular dilatation (⟷). H&E10x

Discussion

In the present study canine filariosis was detected for the first time in Al-Hindya part, Karbala province, Iraq. Its prevalence and epidemiological aspects of D. immitis in dogs were mentioned in previous reported [11].

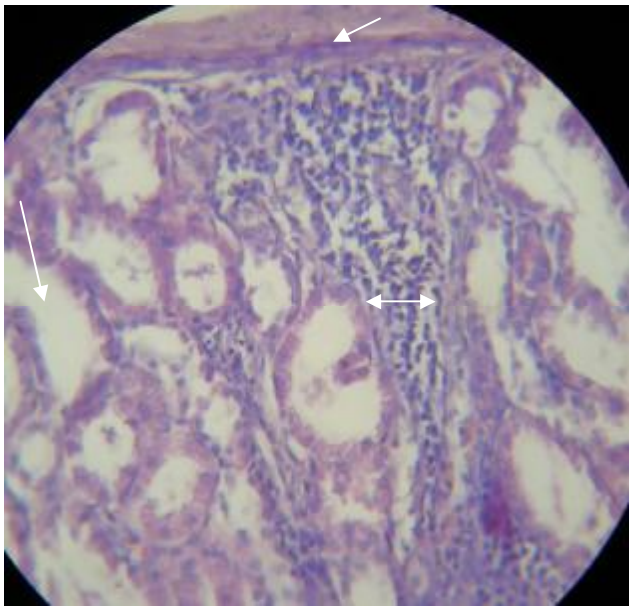

Figure 11. Kidney of infected dog shows fibrous thickening of renal capsule (↙) with focal, sub cortical infiltration of lymphocyte and plasma cells (⟷) and cystic tubular dilatation (↓).
H & E 20x

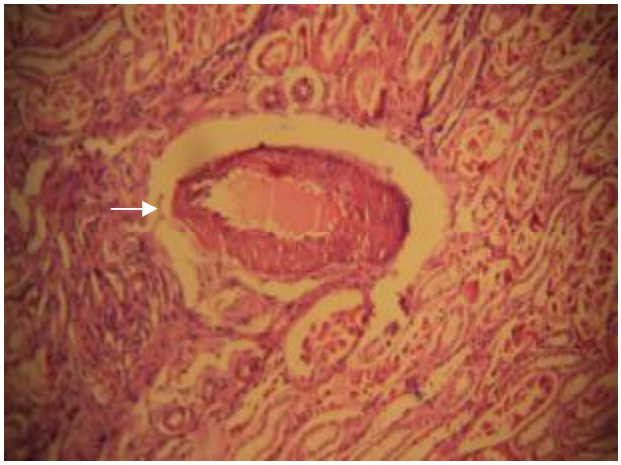

Figure 12. Kidney of infected dog shows thickened blood vessel with congestion. H & E. 10x

The Severity of pathology of heartworm in dogs is determined by worm numbers, duration of

infection, host activity level, and induction of host immune response in the lungs and kidneys (immune complex glomerulonephritis) [12].

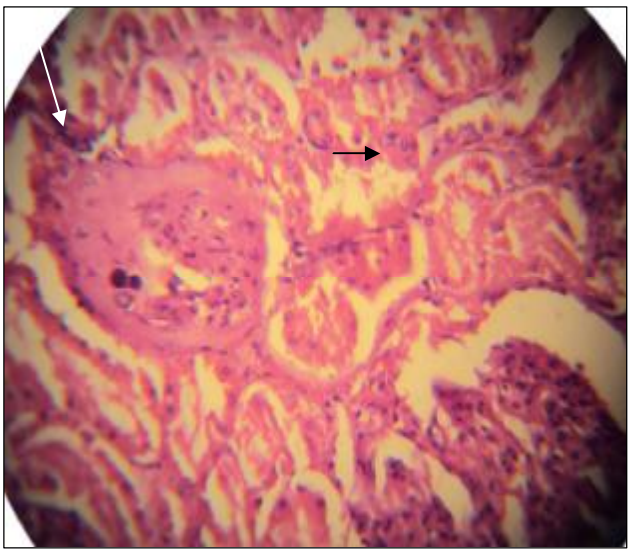

Figure 13. Kidney of infected dog shows severe tubular swelling (⟶) and the wall of small artery is fibrosed with luminal reduction (↘). H&E 20X

In the present study it was observed that muscle layer of the polymyarin and coelomyarian muscle type was well developed, particularly at the level of the lateral cords as described in [13,14].

Our results were in agreement with the results of [15] who found that some sections of the helminthes were surrounded by a cellular reaction inside the vessel, while others were free in the lumen of the artery.

In this study the histological examination of renal biopsy specimens revealed that the lesions were seemed difficult to consider that glomerular lesions resulted from only the primary interstitial affection in focal lesion type as well as diffusety, these results agree with the results of [16]. Feride et al in 2007 who observed interstitial nephritis in the kidneys and thickening of the glomerular capillaries in the glomerulus, and this observation agreed with our data.

In this study the grades of interstitial mononuclear infiltration and fibrosis varied between individual cases. This is attributable to either stages of the disease at autopsy or difference in pathogenesis [4].

Monlux (1953) Described focal mononuclear cell infiltration in the inner medulla of the kidney in filarial- infected dogs. To the present work we found infiltration of inflammatory cells mainly eosinophils and lymphocytes between degenerated muscle fibers, also Grauer (1987) and Naruse (1976) reported a general leukocytosis was seen in dogs with *D. immitis,* and they added that, although eosinophilia and neutrophilia could be seen, the percent of distribution of leukocyte showed individual differences.

Tubular changes in some severely infected cases also might be related with heart filariasis [20]. However, the result of the present study revealed that canine interstitial nephritis cases without any glomerular changes were about 22.5% and that most interstitial lesions were associated with glomerular changes as previously described by [21,22]. Also the thickening of the glomerular basement membrane has occurred independently [12] and this observation agreed with our data.

The pathogenesis of glomerulonephritis due to *D. immitis* has been widely discussed and considered to be the most frequent type of membranoproliferative glomerulonephritis in dogs [23,24]. The most sever lesions are usually observed in dogs with high Mf counts and long infection periods due to prolonged release of antigenic material into the blood stream by inducing in situ formation or trapping of performed complexes [25].

Abramowsky et al (1981) Considered glomerular lesions are another complication of filariasis. Another fact is that the observed lesions in this study may represent a natural form of an immunopathogenic mechanism of glomerular damage in which filarial antigen becomes

uniformly localized in the glamor and elicits an autologour antibody response.

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The Influence of Maternal Ketonuria on Biophysical Fetal Test Results in the Evaluation of Postterm Pregnancy

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Abstract

Background The perinatal morbidity and mortality increase significantly as pregnancy progresses beyond term. The ketone production as a result of dehydration becomes significant in the latter portion of pregnancy.

Objective To determine whether ketonuria, a commonly assessed urinary marker of maternal starvation and dehydration, is associated with abnormal fetal test results in the setting of postterm pregnancy.

Methods During a one-year period (March 2007-March 2008), a total of 180 visits of 100 patients of postterm pregnancies (≥ 41 weeks' gestation) occurred at Baghdad Teaching Hospital. Maternal assessment included vital signs and urinalysis. The presence and degree of maternal ketonuria was correlated against abnormal results of fetal heart rate tests, non stress tests, amniotic fluid index measurements (the biophysical profile scores) performed on the same day.

Results There were 180 evaluations suitable for inclusion in the study. Clinically detectable ketonuria occurred in 13.9% of the patients studied. Patients with clinically detectable ketonuria were at increased risk relative to patients without ketonuria for abnormal outcomes during postterm testing, including the presence of oligohydramnios (28% vs. 9.7%; p <0.018), non reactive non stress tests (12% vs. 2.6%; p <0.03), and variable, late fetal heart rate decelerations (20% vs. 8.3%; p <0.05).

Conclusions Maternal ketonuria among patients with postterm pregnancy was associated with a >2 fold increase in the occurrence of oligohydramnios, a 3-fold increases in non reactive non stress tests, and a significant increase in fetal heart rate decelerations.

Key words maternal ketonuria; biophysical fetal test; postterm pregnancy

Introduction

Postterm pregnancy: A pregnancy which has gone beyond 42 weeks or 294 days from the first day of the last menstrual period (LMP) [1].

The incidence of postterm pregnancy varies from 3% to 10 %[2,3]. The retrospective studies indicate that there is an increased risk of perinatal mortality after 42 weeks [4]. The presumed pathogenesis of the complications associated with post date pregnancy relates to progressive uteroplacental insufficiency. As gestational age advances, uteroplacental insufficiency leads to fetal asphyxia, oligohydramnios, meconium aspiration, and in severe cases fetal central nervous system damage and death [5,6].

The mainstay of antenatal management lies first in the diagnosis of postterm pregnancy. Once diagnosed the pregnancy can be terminated by induction of labor or managed conservatively until spontaneous onset of labor. Whilst awaiting spontaneous onset of labor, fetal well being should be monitored by appropriate available tests as non stress test, biological profile test, Amniotic Fluid Index [A.F.I].

Ketonuria: The increased excretion of ketone bodies in the urine higher than 1mg/24h. known as ketonuria which resulted from the

excessive formation of ketone bodies that results in increased blood levels (ketonemia).The overall condition is called ketosis [7,8].

The metabolism in normal pregnancy is characterized by more exaggerated excursions in maternal nutrient fuels and rapid development of ketonemia during fasting, a pattern described as "accelerated starvation"[9, 10].

The changes in accelerated starvation, a more rapidly evolving decline in glucose and increases in free fatty acids and β-hydroxybutyrate during fasting than occurs outside of pregnancy, are thought to be mediated by the increased nutrient flux to the fetus and by the secretion of hormones that exert lipolytic and anti insulin action, such as human placental lactogen and prolactin [11].

Methods

A prospective study was carried out at Baghdad Teaching Hospital, (Medical City Hospital) for the period from 1st March 2007 to 1st of March 2008.

During the study period, 180pregnancy evaluations were performed on 100postterm pregnant women ≥ 41 weeks' gestation according to last menstrual period and early ultrasonography. They were selected from antenatal clinics and they were followed up to the time of the delivery.

Eight patients were excluded from further analysis, one patient had gestational diabetes, and three patients had other high risk pregnancy complications, such as hypertension or renal disease. Three patients were with late registration for antenatal care or with an uncertain gestational age.

The protocol used for expectant management for those postterm pregnant women has been published elsewhere. After the initial visit antenatal assessment was continued in these cases on a twice weekly basis.

A complete obstetrical and medical history was taken from each patient with special attention for the last menstrual period.

The Expected date of delivery (EDD) that was calculated from the menstrual history was confirmed by an ultrasound examination performed between 12 and 20 weeks of gestation. The maternal assessment also consisted of vital signs, blood pressure, temperature, pulse rate, body weight, height, and urinalysis.

Measurement of ketonuria:

Urinalysis was performed with a Combi-Urine test strips (Panreac. QuimicaA, Company Head Office: Barcelona) that simultaneously evaluated urinary glucose, urobilinogen, ketones, pH, protein, and specific gravity. Assessment of ketonuria with this product used the nitroprusside reaction technique and yielded a semi-quantitative reading of urinary acetoacetic acid concentration. The nitroprusside reaction is a sensitive assay for ketone bodies in urine. Values of ketonuria, as assessed with Combi-Urine test ranged from negative to large. Each patient thus had 1 of the following 4 possible values recorded for ketonuria: negative, 0 mg/dl; small, 1 to 30 mg/dl; moderate, 30 to 40 mg/dl; and large, >40 mg/dl.

Procedure:

The urine used for the analysis was fresh, uncentrifuged and collected in clean containers, free of detergents. The test strip was immersed in the urine for approximately 2 seconds. The reagent areas on the strip were compared with corresponding color chart on the container about 60 seconds after immersion.

Fetal assessment included non-stress test (NST), ultrasonographic determination of the amniotic fluid index (AFI) and biophysical profile (BPP).

- Non-stress test: NST result was considered abnormal in the presence of recurrent fetal heart rate (FHR) variable decelerations, late decelerations or 120 min. of non reactivity.
- Amniotic Fluid Index: AFI was quantified by means of the four-quadrant method described by Phelps et al, (1999). Oligohydramnios was defined as AFI<5.1 cm.

- Biophysical profile: (BPP) was considered normal if the score was 8 or more, including normal amount of liquor. In the presence of the oligohydramnios, an abnormal FHR tracing, patients were referred for labor induction. Patients with normal fetal test results were assessed twice weekly until abnormal results were obtained or spontaneous labor occurred.

Statistical analysis

Data were collected, arranged and tabulated in a number, percentage for discrete variables and mean±SD for continuous variables. Data were checked and transferred to a personal computer using SPSS 7.5 (Statistical Package for Social Science) and format for statistical analysis association between different variable were measured by using student t-test, fisher exact probability test. *p* value of <0.05 was considered to be significant.

Results

During the study period, 180 urinalyses were performed on one hundred patients, their age ranged between (19-42) years and parity (0-6). A mean of 1.8 evaluations per patient was performed (range, 1-3 evaluations per patient). Twenty-five evaluations showed some degree of ketonuria and one hundred fifty-five showed no ketonuria, for an overall incidence of ketonuria of 13.9% in this population. Most patients with ketonuria had relatively minor levels of ketone bodies in the urine (Table 1).

Table 1. The distribution of ketonuria in the study

Parameter	Degree of ketonuria				Total
	No ketone	Small ketones	Moderate ketones	Large ketones	
No. (%)of women	155(86.1%)	13(7.2%)	7(3.9%)	5(2.8%)	180(100%)
Total	155(86.1%)	25(13.9%)			180(100%)

Demographically, the mean age of patients with ketonuria±S.D was 26.28±6.47 years and in patients with no-ketonuria was 27.08±5.45 years. There were no statistically significant differences regarding their mean age (Table 2).

Table 2. Maternal demographic parameters

Parameter	Ketonuria Group (n=25)	No ketonuria Group (n=155)	Statistical Significance
Mean maternal age ±S.D	26.28±6.47	27.08±5.45	*P*=0.506
Nulliparous no. (%)	12(48%)	73(47.1%)	*P*=0.155
Mean Parity ±S.D	1.24±1.65	1.2±1.7	*P*=0.078
Mean height (in) ±S.D	63.67±1.55	63.38±5.06	*P*=0.779
Mean weight (kg) ±S.D	86.20±8.22	82.52±12.04	*P*=0.142

Our bedside assessments for evidence of maternal dehydration, a potential contributor to maternal ketonuria, reveal a significant difference in mean urine specific gravity between patients with and without ketonuria, where the mean urine specific gravity was 1.18 ±0.80 in patients with ketonuria and 1.01±0.007 in patients without ketonuria. The *p* value was less than 0.011 (Table 3).

Table 3. Comparison in urine specific gravity between patients with and without ketonuria

Parameter	Ketonuria group (n=25)	No ketonuria group (n=155)	Statistical significance
Mean urine specific gravity ±S.D	1.18±0.80	1.01±0.007	*p* <0.011

The mean amniotic fluid index ±S.D (8.69±4.47 cm) was statistically lower in patients with ketonuria than in patients without ketonuria (10.50±4.24 cm; Table 4). This observation of an association of diminished amniotic fluid volume with ketonuria remained significant after patients were stratified according to degree of ketonuria. The *p* value was less than 0.05 (Figure 1).

Table 4. Antenatal testing outcomes

Parameter	Ketonuria group(n=25)	No ketonuria group(n=155)	Statistical significance
Mean AFI±S.D	8.69±4.47	10.50±4.24	*p*<0.05
Oligohydramnios no. (%)	7(28.0%)	15(9.7%)	*p*<0.018
Spontaneous FHR deceleration no. (%)	5(20%)	13(8.3%)	*p*<0.05
Non reactive NST no. (%)	3(12.0%)	4(2.6%)	*p*<0.03
Mean BPP ±S.D	8.80±1.15	9.40±7.46	*p*<0.69

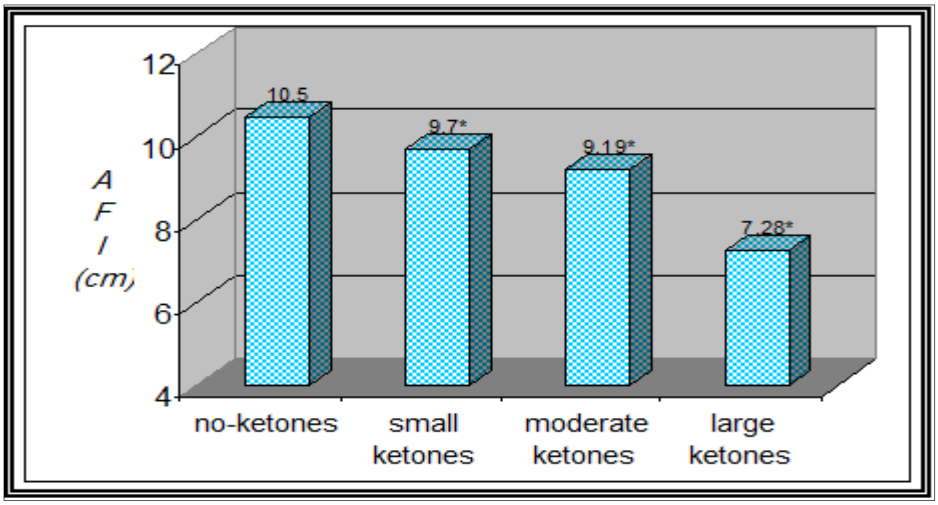

Figure 1. Average amniotic fluid index (AFI) according to degree of ketonuria. Asterisk, *p*<0.05, versus no ketones

To determine the clinical significance of the effect of ketonuria on amniotic fluid volume, we also compared the frequencies of oligohydramnios between patients with and without ketonuria. Our results indicate a >2 fold increase in the incidence of oligohydramnios among patients with ketonuria (28%) relative to those without ketonuria (9.7%). The *p* value was less than 0.01 (Table 4). Furthermore; the incidence of oligohydramnios was directly related to the degree of ketonuria. The highest incidence of oligohydramnios was (60%) occurred among patients with large ketonuria, whereas the lowest incidence was (9.7%) occurred among patients without ketonuria, which was statistically significant (*p* <0.004; Figure 2).

The incidence of oligohydramnios in patients with moderate ketonuria was (28.6%) and in patients with small ketonuria was (15.4%), both were significantly different when compared with patients without ketonuria (p <0.01; Figure 2).

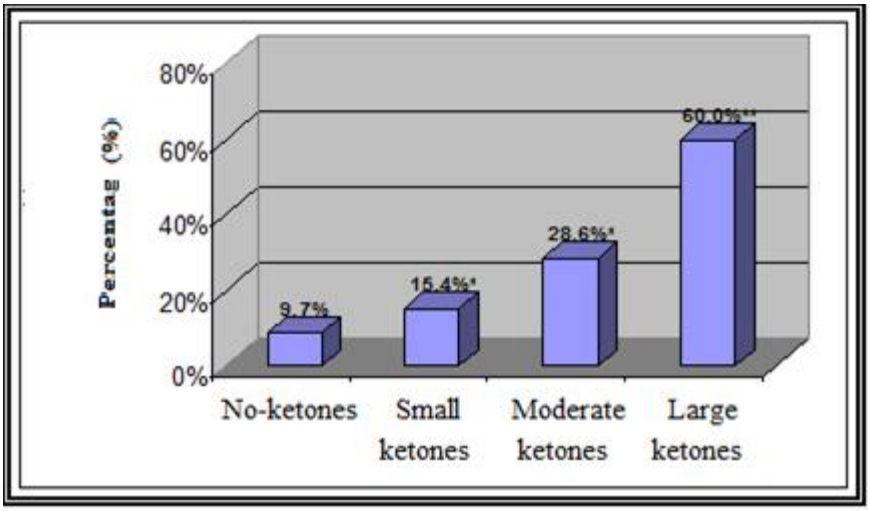

Figure 2. Percentages of patients with oligohydramnios according to degree of ketonuria. Asterisk, p <0.01, versus no ketones; 2asterisks, p <0.004, versus no ketones

When patients were stratified according to amniotic fluid index, it was found that; the highest incidence of ketonuria was (31.8%) occurred among patients with oligohydramnios which was more than twice as likely to have ketonuria as were patients with any other level of amniotic fluid volume (Figure3).

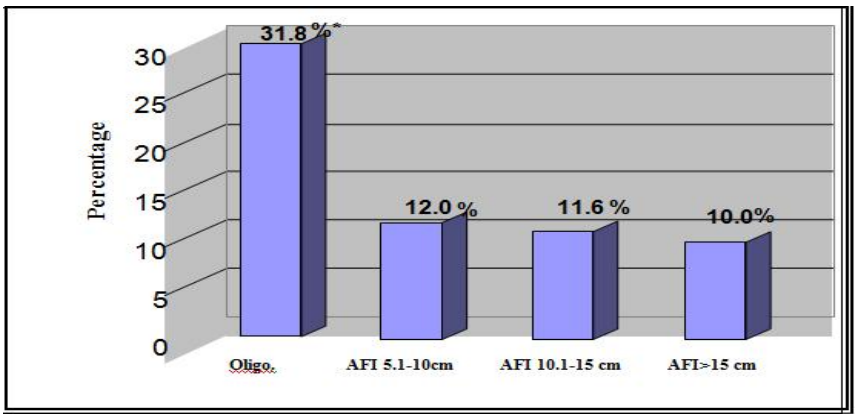

Figure 3.Percentages of patients with ketonuria according to AFI categories. Asterisk, p <0.05, versus any other group.Oligo, oligohydramnios

Spontaneous Fetal Heart Rate(FHR) decelerations were also more common among patients with ketonuria(20%) than among patients without ketonuria (8.3%), which was statistically significant (p<0.05; Table 4).No statistical association was found between the degree of ketonuria and the presence of FHR decelerations(Table 5).

Table 5. Percentages of patients with FHR decelerations and non reactive Non-stress test $(NST)_s$ according to degree of ketonuria (small, moderate, large ketones; *p* value, versus no ketones)

Parameter	Degree of ketonuria								*p* value
	No ketones (n=155)		Small ketones (n=13)		Moderate ketones (n=7)		Large ketones (n=5)		
	No.	%	No.	%	No.	%	No.	%	
Spontaneous FHR decelerations	13	8.3	3	23.0	1	14.3	1	20.0	>0.05
Non reactive NST_s	4	2.6	1	7.7	1	14.3	1	20.0	<0.05

Patients with ketonuria were statistically more likely to have non reactive NST_s [(12.0%) vs. (2.6%); *p* <0.03, table 4]. Patients with large ketonuria were almost 7 times more likely to have non reactive NST_s than were those without ketonuria (20% vs. 2.6%; *p* <0.03, Figure4).

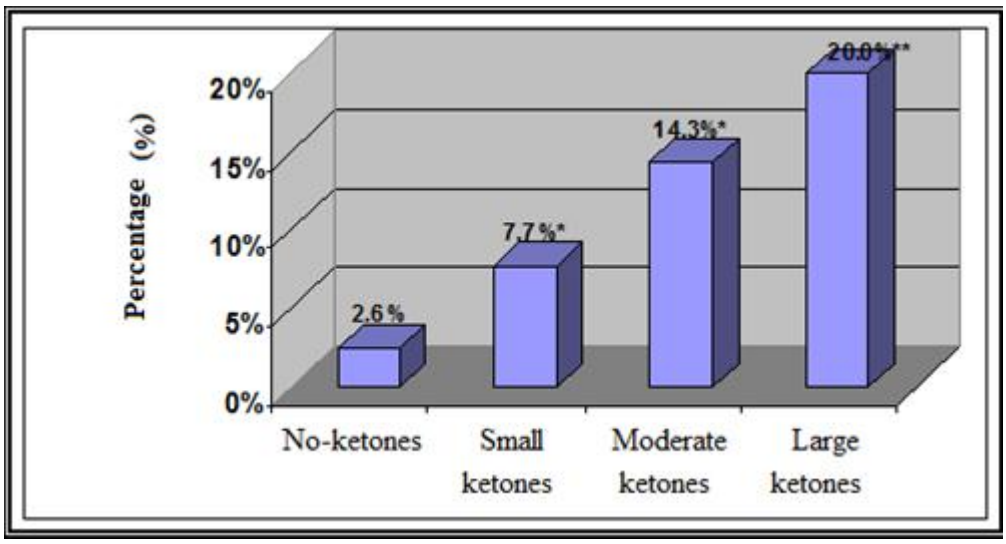

Figure 4. Percentages of patients with non-reactive NST_saccording to degree of ketonuria. Asterisk, *p* <0.05, versus no ketones; 2 asterisks, *p* <0.03 versus no ketones

Discussion

Perinatal morbidity and mortality increase significantly as pregnancy progresses beyond term [12]. Approximately 18.5% of all pregnancies continue to >41 weeks' gestation and 4% to 10% continue to >42 weeks [13]. Although the risks associated with prolonged pregnancy have been chronicled, the management of this common condition remains controversial. The process of enhanced ketone production as a result of accelerated starvation or dehydration becomes significant in the latter portion of pregnancy [14].

Decreased Amniotic Fluid Index In our study the mean amniotic fluid index±SD (8.69±4.47 cm) was lower in patients with ketonuria than in patients without ketonuria (10.5 ±4.24 cm). This association of diminished amniotic fluid volume with ketonuria remained significant after patients were stratified according to degree of ketonuria (Figure1), and it was also found that, the highest incidence of ketonuria (31.8%) occurred among patients with

oligohydramnios which was more than twice as likely to have ketonuria as were patients with any other level of AFI (Figure 3).

It was found that the maternal ketonemia resulting in ketonuria, is a hyperosmolar condition [15]. The maternal condition involving hyperosmolarity, such as maternal dehydration, result in a diminution in fetal amniotic fluid volume from decreased fetal urination [16].These studies provide preliminary evidence to support the hypothesis that ketone bodies passed from the mother to the fetus may elicit alterations in amniotic fluid volume and FHR reactivity.

Our results could be also explained by other studies on the relationship between the maternal fluid volume and the amniotic fluid volume, which demonstrated an increase in the amniotic fluid volume in response to maternal hydration [17-19].

It was found that maternal oral hydration increased the AFI by approximately 16% where as fluid restriction decreased the AFI by 8% in women with normal amniotic fluid. These findings support previous data that maternal hydration increased the AFI by 31% in women with decreased amniotic fluid and suggest that maternal fluid volume or osmolality may have a role in maintaining the amniotic fluid volume [17].

Abnormal FHR tracing: As shown in the result of our study spontaneous FHR decelerations were more common among patients with ketonuria (20%) than among patients without ketonuria (8.3%), and it was more likely to have non reactive NST_s (12%) than were those without ketonuria (2.6%). The association of ketonuria and non reactive NST_s increased directly with each increment of increasing ketonuria (Table 5, Figure 4) patients with large ketonuria were 7 times more likely to have non-reactive NST_s than were those without ketonuria. This could be explained by one of the followings:

- One of the most commonly documented fetal physiologic alterations resulting from exposure to ketone bodies involves the neurologic system [20-24]. Bhasin et al (1982), found that synthesis of pyrimidines in the fetal brain decreases significantly in the presence of ketone bodies. This is seen as an attempt by the fetus to reduce its' metabolic demands and conserve energy in the face of possible maternal nutritional deprivation [20].
- It was hypothesized that the presence of a non reactive NST is an early manifestation of these neurologic alterations in some fetuses exposed to maternal ketone bodies. Support for this hypothesis is provided by the fact that the response (FHR non-reactivity) appears to be dose related to the degree of ketonuria (Figure 4). Other support for this concept is provided by previous studies that have documented FHR abnormalities in the presence of extreme quantities of ketone bodies such as those seen in diabetic ketoacidosis[25,26]. Alternatively, it is possible that maternal ketonuria is a marker for maternal dehydration and hypovolemia. Subsequent fetal hypovolemia could explain both FHR changes and decreased amniotic fluid volume.

It was a small study, the relatively small number of patients in the groups with moderate (n=7) and large (n=5) ketonuria may have reduced our ability to generalize with respect to the frequency of specific outcomes in these groups. It should be noted, however, that statistically significant relationship between fetal test results and degree of ketonuria existed regardless of the exact cutoff level used to define the severity of ketonuria. Although the maternal ketonuria is a reversible condition [25], it is impossible from this study to determine whether the oligohydramnios and FHR abnormalities found in patients with ketonuria represented reversible alterations in fetal test results or additional evidence in support of the need for immediate delivery.

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Polypropylene Mesh in Stress Urinary Incontinence

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Abstract

Background The pubovaginal sling (PVS) is a safe and durable surgical procedure for stress urinary incontinence (SUI) of all types. Among numerous modifications of the procedure is using synthetic sling material to decrease surgical morbidity and increase long-term success.

Objective To present the results of pubovaginal sling with a polypropylene mesh in women with SUI.

Methods We studied 12 consecutive patients who underwent PVS procedure using polypropylene mesh for SUI between January 2008 and April 2009. Stress urinary incontinence was demonstrated by positive cough test, filling cystometry. Urethral hyper mobility was demonstrated with straining cotton swab (≥ 30), with different grades of vaginal wall prolapse. Urodynamic study was not performed. All these patients were treated with pubovaginal sling (PVS) with a low-cost polypropylene mesh confectioned by the surgeon. The sling was placed at the level of the proximal half of the urethra and tied with adequate tension, but not obstructing the bladder outlet. Postoperatively, the patients were evaluated at 6-month with a symptom questionnaire, physical examination, and postvoid residual volume determination. Demographic criteria, complications during surgery and post operative period, and subjective cure rate at three months were assessed.

Results Twelve patients with mean age of 55.5 years and median parity of 4 years underwent bladder neck sling surgery using polypropylene mesh. Body weight range was 45-68 kg. No intraoperative or major postoperative complications were reported. Mean duration of surgery was 65.5 minutes (60-120 minutes). Concomitant procedures were performed, including cystocele repair (n= 10) rectocele repair (n=11). Mean duration of hospital stay was 2 days (1-5 days). Ten patients had complete cure of SUI, one patient had significant decrease in the severity of stress urinary incontinence. One patient had persistent SUI.

Conclusions The construction of a pubovaginal sling using a low-cost polypropylene mesh is a safe and effective technique for the relief of SUI. It should be considered an alternative, especially in patients with weak rectus fascia.

Key words stress urinary incontinence; pubovaginal slings; polypropylene

Introduction

Stress urinary incontinence (SUI) is a disorder commonly affecting females of all age groups compromising their quality of life. The bothersome symptoms of SUI adversely affect the social relationships and activities, restrict physical pursuits, impair personal hygiene and lead to avoidance of sexual relationship [1]. Several risk factors have been implicated in causation of SUI: weak collagen, age, childbearing, obesity, constipation, advanced pelvic organ prolapse and chronic obstructive airway disease [2]. SUI is thought to occur as a result of bladder neck/urethral hypermobility and/or neuromuscular defects [3]. Neuromuscular defects lead to the intrinsic sphincter deficiency. Pubovaginal slings (PVS) have become standard modality of treatment in last decade after work of Delancy et al who had shown that the anterior vaginal wall acts as a hammock for the vesical neck and urethra [4]. Over last few years many procedures using

autologous material (rectus sheath, fascia lata) or synthetic material (polypropylene, mersilene) have been reported in literature [5,6]. Mersilene was the first synthetic material to be used as pubovaginal sling [7], while polypropylene has been recently described. The main advantage of the use of synthetic material is avoidance of morbidity of harvesting autologous material and avoids the risk of transmission of an infective disease of cadaveric tissue. Furthermore, they are not biodegradable and the tensile strength does not decrease with passage of time [8]. The main disadvantage of the synthetic material is the risk of erosion of the sling to the vaginal mucosa or urethra and infection [6,9].

The main indication to use polypropylene mesh is in patients with previous pelvic surgery in which there will be difficulty in preparing flaps from the rectus fascia and also in patients with weak rectus fascia. The aim of the present study was to evaluate the surgical results, intraoperative and postoperative complications in patients with stress urinary incontinence undergoing a pubovaginal sling procedure usinga low-cost polypropylene mesh.

Methods

Between January 2008 and April 2009, a total of 12 consecutive women with stress urinary incontinence (SUI) underwent pubovaginal sling procedure with polypropylene mesh in Al-KadhimiyaTeaching Hospital.

Inclusion criteria were primary treatment of stress urinary incontinence (SUI) and showing SUI on filling cystometry without detrussor over activity. Straining Cotton swab ≥ 30 test was used indicating urethral hyper mobility. Urodynamic study was not performed.

Preoperative assessment consisted of recording patient's demographic details, detailed urinary history, and physical examination, vaginal examination to assess for bladder neck mobility, prolapse and obvious incontinence. Neurological examinations and urinalysis were also performed.

Exclusion criteria were recurrent and difficult-to-treat urinary tract infections, significant symptoms of urge urinary incontinence, a history of, or detrusor over activity detected at cystometry, post voiding residual >150 mL, bladder capacity <200 mL, or physical/mental impairment.

The following complications were recorded: excessive blood loss, bladder perforation, urethral lesion, and other intraoperative complications. Postoperative complications that were considered were the need for catheterization >24 hours, postoperative bleeding,retropubic hematoma, wound infection, difficulty in bladder emptying, urinary obstruction, suprapubic pain,mesh erosion, and dysparunia .

All patients were asked to restrict any lifting after surgery and abstinence from sexual intercourse for 12 weeks.

Follow-up:

All patients were asked to come in for a follow-up at the outpatient department 1 week after being discharged.Postoperative outcome variables were assessed at each office visit included SUI symptoms, de novo or worsening urge incontinence, and urinary retention.

Surgical outcome in the continence status was defined at six or more months during follow-up after surgery using a questionnaire assessment reported by patients themselves when patients were interviewed. A patient was classified as cured if she was dry and without urinary complaints. If the patient still suffers from some degree of stress incontinence, she is classified as improved, and failure is registered if urinary incontinence was unchanged or worse. They were also asked about their voiding condition.

The pelvis was examined thoroughly for any vaginal erosion of the sling and a stress test undertaken when the patients had a full bladder.

Operative technique:

The patients were placed in the dorsal lithotomy position allowing free access to the perineum and lower abdomen.Eighteen Fr Foleys was placed in urinary bladder and balloon was palpated at bladder neck. Two lower abdominal transverse incisions 2cm in length on either side of the midline to the abdominal apponeurosis above the upper border of the symphysis pubis were done. Two parts of polypropylene mesh of 10 x 1.5 cm in size were preparedand soaked in gentamicin solution.Saline was infiltrated into the anterior vaginal wall to facilitate dissection.

A midline vertical incision is made in the anterior vaginal wall. This was deep enough to cut through the vaginal skin and pubocervical fascia. Separation of pubocervical fascia from the vaginal skin was done.

A long curved forceps (Robert forceps) was introduced through the sub pubic fossa to bring the tip of the mesh to the vaginal wound. The same procedure is repeated on the other side and the straps of polypropylene mesh were drawn down in to the vagina. Cystoscopy was performed after that to check for any injury in the bladder. The two flaps crossed over each other under the urethrovesical junction and suturing of the mesh to the pubocervical fascia was done. The tension on the sling is avoided by placing a hemostat between the bladder neck and sling .Cutting the excess of mesh and suturing its tip to the rectus fascia was done.

The excess vaginal skin was removed and the vaginal skin was closed by a series of interrupted no. 1 polyglycolic acid. Vaginal pack was inserted. The suprapubic incision was closed with a running 3-0 monofilament suture. Vaginal pack was removed 24hr. after the operation.

Foley catheter was removed morning after the operation and a voiding trial was initiated 4 hours after that and measurement of voided urine volume and catheterization then was performed to assess the post voiding residual urine volume,If it was less than 50 ml the catheter was removed.

Results

A total of 12 consecutive patients, with a mean age of 55.5 years (range 33-60) and a median parity of 4(range 1–6) were included in this study. One patient had a history of prior three cesarean sections and another patient had prior one cesarean section. No patient had undergone prior anti-incontinence surgery. The body weight range was 45–68 kg. Two (33.3%) patients were menopausal.The Clinical characteristics of patients are shown in Table 1.

Table 1 Patient's Characteristics

Characteristics	
mean Age (range) years median	55.5 (33-60)
Parity (range)	4 (1-6)
median Vaginal deliveries(range)	2 (0–4)
Body weight (kg)	45-68
menopausal state	2 (33.3%)
Prior cesarean section	2 (33.3%)

Mean operating time was 65.5 min (60-120 minutes).Concomitant surgery was anterior colporrhaphy and colpoperineorrhaphy.The operative data are shown in table 2.There was no bladder or urethral injury. Only one patient have blood loss of about 500 cc from dissection of the retropubic space the haemostasis was ensured with suturing of the bleeding areas.

Table 2. Operative Data

Characteristics	Median	Range
Operation time (min)	65.5	60-120
Hemoglobin change (g/dL)	1.8	0.4-2.7
Hospital stay (days)	2	1-5
Concomitant surgery		
Anterior colporrhaphy (No.)		10
Colpoperineorrhaphy (No.)		11

Overall SUI was cured in 10 (83.3%) and improved in 1 (8.3%). One patient had persistence of SUI with at least 6 months follow

up. Overall patient's satisfaction rate was 91.6 % as shown in table 3.

One patient developed difficulty in the initiation and maintenance of voiding following surgery. Post voiding residual urine volume was 50 ml.

Before pubovaginal sling surgery, 3 patients (25%) had urgency, of which urgency resolved in 2 and persisted in 1 after surgery. De novo urgency appeared in one patient (8.3%). Short and long term complications and overall patient's satisfactions are shown in table 3.

Table 3.Complications

COMPLICATIONS	N	%
Bladder perforation	0	0
De novo urgency (transient)	1	8.3
Retropubic hematoma	0	0
Difficulty emptying	1	8.3
Long-term complications	N	%
Urinary obstruction	0	0
Suprapubic pain	0	0
Mesh erosion	0	0
Dyspareunia	0	0
Patients' satisfaction	11	91.6

Discussion

Stress urinary incontinence is a common condition affecting females of all ages. Numerous surgical procedures have been described in literatures for the treatment of SUI, but in last decade there has been increased inclination of urologists towards use of pubovaginal slings. Numerous materials are available for use in PVS - synthetic and autologous [5,6]. The use of these graft substitutes have flourished in recent years. It has been shown that the females suffering from SUI have higher plasma proteolytic activity in comparison to age and sex matched controls, thus use of autologous material to treat SUI becomes questionable [10].

Choosing an artificial sling simplifies the operative procedure, in that the graft is readily available and does not require harvesting from a second operative site. The readiness and ease of preparation decreases the operative time, patient discomfort and potential postoperative complications. Synthetic materials also bypass the potential problems of inadequate length and strength associated with autologous grafts. Furthermore the synthetic sling is non degradable, tensile strength does not decrease with passage of time and allows tissue ingrowth between the interstices of the mesh [8].

Studies confirm that the choice of a tension free vaginal tape(TVT) polypropylene mesh allow high success rates and the TVT simplified the SUI therapy, becoming one of most common options for the treatment of this disease [6,11]. Thus, the industry has offered different kits to make the slings, but most of the time the costs are prohibitive for public health systems with few financial resources.Fransberet al and Jung Hun Lee et al also used pubovaginal sling with low cost polypropylene mesh for correction of stress urinary incontinence [12,13].

In this study, the results for PVS procedure, using polypropylene mesh slings in the treatment of female SUI, are comparable with what has been previously reported in the literature [14]. Our results also uphold previous findings that most concomitant urge symptoms can be resolved or improved after a successful sling operation [14]. Morgan et al [15] reported a 74% cure rate of preoperative urge incontinence using the PVS procedure. However, they did not find any preoperative variable that predicted the resolution of urge incontinence postoperatively except for concomitant anterior colporrhaphy, which correlated most closely with the resolution of urge incontinence.

Use of polypropylene mesh is yielding encouraging results but major concern remains erosion into the urinary tract. Tying the sutures at the end of the mesh loosely can minimize the erosion. Creation of adequate vaginal mucosal flaps prevents ischemic necrosis of the flaps. We did not encounter any such instance of mesh erosion in any of the patient till last

follow up, while a recent study has shown that the erosion rate with use of the polypropylene mesh is less than 5% [16,17]. These findings correlate well with decreased incidence of infection or urinary retention and our study correlates well with these findings. Also the urethral erosion can be minimized by loosely anchoring the sutures at the rectus sheath level. It also helps in decreasing the incidence of postoperative urinary retention and urgency. Careful dissection of the vaginal epithelium from the endopelvic fascia is important because a dissection which extends too deep will compromise the thickness of the endopelvic fascia. While applying the sling beneath the urethra, the endopelvic fascia acts as a buffer between the sling and the urethra. An adequate thickness of fascial buffer can prevent direct compression of the sling on the urethra, avoiding the eventual development of urethral obstruction or erosion.

There were few steps taken to reduce the infection like removing vaginal pack within 24 hours, soaking the mesh with antibiotic saline and intra-operatively wound was repeatedly washed with antibiotic saline.

Assessment of the outcome of the sling procedure basically depends upon the patient subjective assessment. Our preoperative work up of the patient did not include routine urodynamic testing. Urodynamic assessment of the patient was done in cases were there was history suggestive of detrusor over activity. Our assessment regarding outcome of the procedure was subjective. In immediate postoperative period subjective cure rates was 91%. Jarvis et al had reported a subjective cure rate of 82.4% in their study on use of synthetic slings in treatment of SUI [18]. Synthetic slings have been shown to produce durable results both objective and subjective 81.63% and 81.2% respectively in patients with SUI [19].

The present results suggest that polypropylene mesh can be a good sling material for treating female SUI, although the conclusion is tentative, as possible complications of sling infection and erosion of the synthetic sling from the vaginal epithelium may occur in the long term. Careful dissection of the vaginal epithelium and providing a thick endopelvic fascia buffer between the sling and urethra might prevent these serious complications.

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Expression of b-HCG in Breast Tumors

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Abstract

Background: Human chorionic gonadotropin (HCG) is a glycoprotein hormone, which consists of two polypeptide subunits (alpha and beta), produced by syncytial trophoblast cells of the placenta during pregnancy. Ectopic HCG production occurs in many tumors including breast tumor.

Objectives: The objective of the study is to investigate the expression of β–HCG in breast tumor and its correlation with pathological prognostic factors (age, tumor type, site, size, histological grade, lymphocytic infiltration, vascular invasion and lymph node involvement).

Methods: A total of 44 breast tumors were selected, consisting of eight benign lesions (Fibrocystic disease in four cases and fibroadenoma in the rest) and 36 malignant breast tumors (invasive ductal carcinoma (21 cases) all of not-otherwise-specified type (NOS), in situ ductal carcinoma (6 cases), invasive lobular carcinoma (6 cases) and in situ lobular carcinoma (3 cases).

Results: β-HCG expression was found in 6 of 36 malignant breast tumors (16.7%). None of the benign breast lesions showed β-HCG expression. High expression of β-HCG is seen more frequently in infiltrative ductal carcinoma with higher-grade tumor and in old age group (≥50 years).

Conclusion: It was concluded that high expression of β-HCG is seen more frequently in infiltrative ductal carcinoma with higher grade. There was a high β-HCG expression in tumors more than or equal to 5-cm diameter.

Key words: Breast tumors, β-HCG

Introduction

Breast cancer is the most common cancer among women world wide, and is the second most common after lung cancer for both genders, according to the World Health Organization [(1)].

Human Chorionic Gonadotropin (HCG) is a glycoprotein hormone, which consists of two polypeptide subunits (alpha and beta), produced by syncytial trophoblast cells of the placenta during pregnancy [(2,3)]. The β-subunit of glycoprotein hormone is unique, giving the biological and immunological specificity of the HCG hormone [(4)]. Ectopic HCG production occurs in many tumors including breast [(5)]. Several reports have shown that the production of this hormone by a neoplasm is associated with a more aggressive behavior [(6)].

A number of studies using peripheral blood of breast cancer patients, showed a wide variety in the frequency of raised levels of β-HCG [(7,8)].

The ectopic production of HCG by nontrophoblastic tumors is well documented. Adenocarcinoma arising in the mammary gland

has been shown to stain positively for the Beta subunit of HCG [5].

All tumors found by radioimmunoassay to contain β-HCG were also found to be immunohistochemically positive in formalin fixed tissue whereas those not containing measurable HCG did not stain significantly [9].

The presence of increased serum levels of HCG and its metabolites is generally agreed to be a sign of poor prognosis [10,11].

Methods

This is a retrospective study of forty-four cases of surgical lesions (total mastectomy and excisional biopsies). The cases were consist of eight benign lesions (Fibrocystic disease (four cases) and fibroadenoma (four cases)) and 36 malignant breast tumors (invasive ductal carcinoma (21 cases) all of not-otherwise-specified type (NOS), in situ ductal carcinoma (six cases), invasive lobular carcinoma (six cases) and in situ lobular carcinoma (three cases)). These cases were retrieved from Al-Kadhemia Teaching Hospital Laboratory and Medical City Hospital Laboratory for the period June, 2003 to June, 2005. For each case representative sections were stained with haematoxylin and eosin (H & E) and others were stained immunohistochemically for β-HCG from the available formalin fixed paraffin embedded tissues.

Haematoxylin and eosin stained sections were examined for the type of tumor, histological grading (WHO and Bloom-Rechardson grading system), also for identifying vascular invasion and lymphocytic infiltration.

Statistical analysis of the data was performed using Chi square test (contingency table). The results were considered statistically significant when the alfa level of significance (p) was equal or less than 0.05.

Results

The expression of β-HCG was positive in 6 of 36 malignant breast lesions (16.7%). None of the benign or insitu breast lesions were positive for β-HCG. Five out of 21 invasive ductal carcinoma (23.8%) were β-HCG positive (Figure 1).

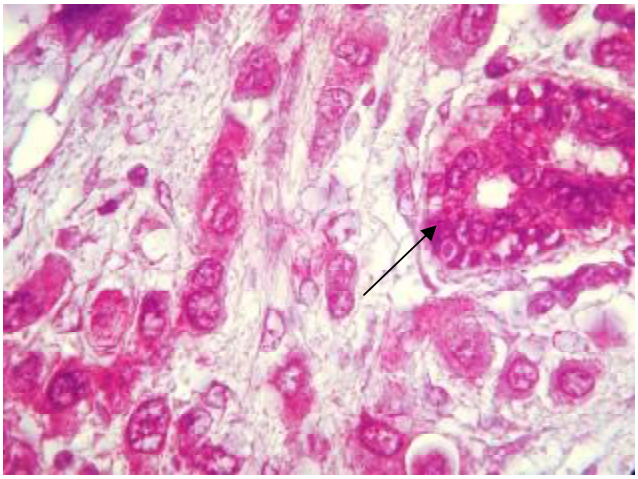

Figure 1. Invasive ductal carcinoma shows positive reactivity for **β**-HCG. Grade **III**. The cytoplasm stains with red color. (X 1000) (Immunohistochemical staining, alkaline phosphatase method).

One out of 6 invasive lobular carcinoma (16.7%) were β-HCG positive (Figure 2).

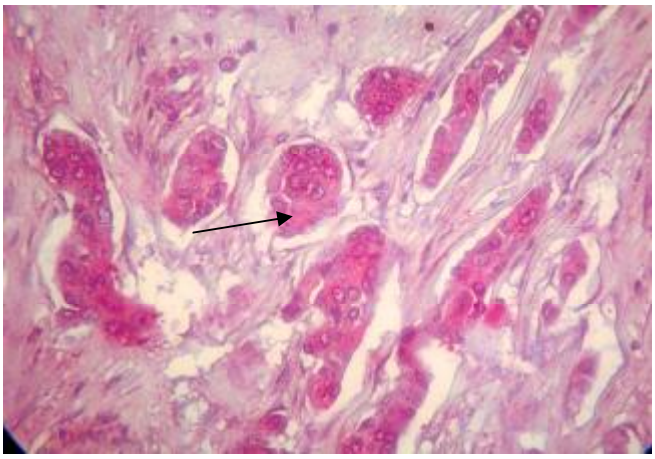

Figure 2. Invasive lobular carcinoma shows positive reactivity for **β**-HCG Grade **II**. The cytoplasm stain with red color (x 400). (Immunohistochemical staining, alkaline phosphatase method).

No statistical difference for both ductal and lobular carcinoma (P= 0.146) and (P=0.427), respectively. In invasive ductal carcinoma 3 out of 5 cases (60%) were focally positive β-HCG. In invasive lobular carcinoma 1 of 1 case (100%) was Focal positive β-HCG. Statistical analysis revealed no significant difference (P=0.836) (Table 1).

Table 1. β-HCG expression in breast carcinoma according to Pattern of staining

Type of malignancy	Pattern of staining		Total
	Focal	Diffuse	
Invasive ductal carcinoma	3 (60%)	2 (40%)	5
Invasive lobular carcinoma	1(100%)	0	1
Total	4	2	6

P = 0.836 (not significant)

Two of the six β-HCG positive cases were for women of less than 50 years old group and the other four cases were for more than 50 years old. Statistical analysis revealed a significant difference (P=0.039) (Table 2).

Table 2. β-HCG expression in breast carcinoma relation to age groups

Age group (years)	b-HCG expression		Total
	Positive	Negative	
≤ 35 years	0	9 (100%)	9
36-49 years	2 (8.3%)	22 (91.7%)	24
≥ 50 years	4 (36.4%)	7 (63.6%)	11
Total	6	38	44

P = 0.039 (significant difference)

The six β-HCG positive cases were distributed equally between left and right-breast. Statistically there was no significant difference between 2 sites (P =0.821). Two out of 14 cases (14.3%) of tumor size (2-5 cm) were positive β-HCG.

Four out of 20 cases (20%) of tumor size (> 5 cm) showed β-HCG expressions, although statistically there was no significant difference (P=0.511) (Table 3).

Table 3. β-HCG expression in breast carcinoma in relation to size

Tumor size	b-HCG expression		Total
	Positive	Negative	
≤ 2 cm	0	2 (100%)	2
2-5 cm	2 (14.3 %)	12 (85.7%)	14
> 5 cm	4 (20%)	16 (80%)	20
Total	6	30	36

P = 0.511 (not significant).

According to WHO grading system, β-HCG expressions were found in 2 of 8 (25%) of moderate differentiated ductal carcinoma and in 3 of 12 cases (25%) of poorly differentiated ductal carcinoma. Statistically there was no significant difference (P=0.795). However, according to modified Bloom- Richardson grading system of 21 invasive ductal carcinoma, 3 out of 12 cases (25%) of grade II expressed β-HCG, 2 out of 6 cases (33.3%) of grade III showed positive β-HCG expression and none of the grade I express β-HCG, Statistically there was no significant difference (P=0.704). While one out of 3 invasive lobular carcinoma of grade II (33.3%) showed positive β-HCG. Statistically there was no significant difference (P=0.691) (Table 4).

Table 4. β-HCG expression in breast carcinoma relation to grading system

Tumor grade		β-HCG expression		Total
		Positive	Negative	
1. Invasive ductal carcinoma				
1. WHO grading system*	Well differentiated	0	1 (100%)	1
	Moderate differentiated	2 (25%)	6 (75%)	8
	Poor differentiated	3 (25%)	9 (25%)	12
	Total	5	16	21
2. modified Bloom-Richardson** Grading System	Grade I	0	3 (100%)	3
	Grade II	3 (25%)	9 (75%)	12
	Grade III	2 (33.3%)	4 (66.7%)	6
	Total	5	16	21
2. Invasive lobular carcinoma				
1. modified Bloom-Richardson*** Grading system	Grade I	0	2 (100%)	2
	Grade II	1 (33.3%)	2 (66.7%)	3
	Grade III	0	1 (100%)	1
	Total	1	5	6

* P = 0.795 (not significant), ** P = 0.704 (not significant), *** P = 0.691 (not significant).

Discussions

Many studies had varying results, which led to marked discrepancies in the frequency of β-HCG detection in tumor specimens.

In this study β-HCG frequency considerably less than that of Agnantis et al (1992) [5] and higher than that of Nishiyama et al (1980) [12].

There are several explanations for the discrepant results, first sample size, second is the heterogencity of the tumor cells, and the HCG containing cells may be quite rare and sparsely scattered throughout large areas of tumor or in few clusters (Focal). In this study 60% were focally distributed for ductal carcinoma and 100% for lobular carcinoma which is similar to that reported by Nishiyams et al (1980) [12] and Bellet et al (1980) [13]. Thus the more sections are examined for each tumor the higher percentage of positive results would be expected.

A third factor involves differences in the staining methods used. Another factor that may account for some of the discrepancies involves the method of tissue preparation most of the studies have used formalin fixed, paraffin-embedded specimens including the present study while in McManus et al (1976) [14] who found highest rate

of β-HCG detection in tissue specimen studied fresh frozen sections.

Finally, the discordant results among the various studies may have been due, in part to different specificities of the antibodies used to detect β-HCG.

There is a continuing need to derive markers in breast tumors or in the sera of breast cancer patients, which may give a guide as to future prognosis as well as to, monitor the course of the disease.

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Seroconversion Rate of Hepatitis C Virus Infection among Haemodialysis Patients in AL-Kadhimiya Teaching Hospital (Dialysis Unit)

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Abstract

Background Hepatitis C virus (HCV) infection is a serious public health problem throughout the world. Chronic haemodialysis patients are at higher risk for acquiring hepatitis C virus (HCV) infection.

Objective To assess the rate of seroconversion of hepatitis C virus (HCV) infection every month for one year duration, to evaluate the possible associated risk factors and the relationship of hepatitis C virus infection with blood transfusion and duration of hemodialysis.

Methods Fifty seven patients, 37 males (65%) and 20 females (35%), who were on regular haemodialysis in Al-Kadhymia Teaching Hospital were studied during the period between January 2009 and December 2009. Patients were analyzed monthly with anti –HCV antibodies using a commercial enzyme-linked immuno-sorbant assay BioKit (bioelisa HCV 4.0) and serum Alanin aminotransferase measurements.

Results Twenty three patients (40.3%) were HCV positive of whom, 13 were males (56.5%) and 10 were females (43.5%). History of blood transfusion, number of blood transfusion and duration of haemodialysis, had significant correlation in acquiring HCV infection.

Conclusion Seroconversion of HCV infection was of high rate incidence (40.3%). Duration of HD, history of blood transfusion and number of blood transfusion(s) are factors affect the rate of seroconversion of HCV infection in patients on regular HD. The higher rate of seroconversion of HCV infection needs further research to identify the causes and to establish a well organized prophylactic program by using more sophisticated and accurate investigation.

Key words Hepatitis C virus infection, Hemodialysis, Chronic renal failure

Introduction

Hepatitis C virus (HCV) infection is a serious public health problem throughout the world, patients with chronic renal failure on haemodialysis are at higher risk to hepatitis C virus infection, with prevalence varies among different countries and haemodialysis centers [(1,2)].

Haemodialysis patients are more vulnerable to HCV infection than others because of history of blood transfusion, frequent injection and partial immunosuppression. The duration of haemodialysis treatment and nosocomial HCV transmission have also been suggested as contributing factors [(3-5)].

The typical test for anti-HCV is an enzyme immunoassay, which can occasionally yield a false-positive result, a recombinant Immunoblot assay can be used to confirm anti-HCV reactivity. The diagnosis of hepatitis C is confirmed more aptly by a qualitative sensitive assay for HCV RNA in serum such as reverse transcriptase (Polymerase Chain Reaction) [(6.7)].

Several commercial assays are available to quantify HCV RNA levels in serum (e.g. bDNA, RTPCR) but these tests have been difficult to

standardize, most patients with chronic hepatitis C have 10^5 to 10^7 IU of HCV RNA in serum, and levels are usually stable over time [8-11].

Dose reduction of Ribavirin in patients with renal failure undergoing dialysis might helpful, neither the optimal regimen nor the efficacy of therapy is established in this patients [1,2,6].

Aim of study

To assess the rate of seroconversion of hepatitis C virus (HCV) infection every month for one year duration and to evaluate the possible associated risk factors and the relationship of hepatitis C virus infection with duration of hemodialysis.

Methods

Patient Selection

This study was carried out in dialysis unit Al-Kadhimiya Teaching Hospital, in Baghdad between 1st of January and 31st of December 2009. Baseline data about patients was obtained from routine history and clinical examination this includes age, sex, duration of haemodialysis, previous blood transfusion, number of blood transfusion, drug intake (erythropoietin) and intravenous drug abuse, occupation and history of previous illness.

Laboratory Data

Blood samples were collected from all patients monthly and sera were screened by standard techniques using a commercial enzyme-linked immuno-sorbant assay Bio Kit (bioelisa HCV 4.0) for the presence of anti-HCV antibodies. Serum ALT was determine in the study group and it performed each month (normal value <20 U/L), cut-off value of serum ALT in our laboratories was 94 U/L.

Statistical Analysis

Data were analyzed by spss-17. Quantitative data presented using the Mean ±SD. Comparison of qualitative data was done by Chi-square, t-test and p value of <0.05 was considered as significant. Correlation coefficient between variables was applied.

Results

The study involved 57 patients with end stage renal disease treated with HD; they were 37 males (65%) and 20 were females (35%). The mean age of the study population was 41.28±14.37 years range from 18-71 years. HCV infection was detected in 23 patients (40.3%); they included 13 males patients (56.5%) and 10 females (43.5%) as seen in table 1.

Table 1. Demographic Features of the study population

Group	No	%
Total number	57	100
HCV positive male patients	13	22.9
HCV negative male patients	24	42.1
HCV positive female patients	10	17.5
HCV negative female patients	10	17.5
Mean age ±SD	41.28±14.37years	
Age range	18-71 years	

The over-all seroconversion of HCV infection in male patients was 35.1% while it was 50% in females, but the relationship between sex and seropositivity was not significant (p >0.05). The mean age of HCV positive and HCV negative patients were 40.2±13.1 and 42±15.3 years, respectively (Table 2).

Table 2. Comparison of HCV Positive and Negative Patients on Haemodialysis

Parameter		HCV Positive Patients n=23	HCV Negative Patients n=34	P Value
Age		40.2±13.1	42±15.3	0.6
Gender	Male	13 (56.5%)	24 (70.5%)	0.2
	Female	10 (43.5%)	10 (29.5%)	

Regarding the duration on haemodialysis it is found that (40.3%) of the study populations were being dialyzed for one year the frequency of HD was2-3 times each week (mean 2.1). The seroconversion of HCV infection among patients on haemodialysis more than six months was (51.4%) as compared to (22.7%) among patients on dialysis for less than six months (Table 3).

Table 3. Relationship between Duration and Frequency of HD (No. of dialysis/time period) and Risk of Acquire Infection

Duration of haemodialysis	Number of patients	HCV +ve	HCV –ve	P Value
More than 6 months	35 (61.4%)	18 (51.4%)	17 (48.6%)	0.03
Less than 6 months	22 (38.6%)	5 (22.7%)	17 (77.3%)	

During one year of haemodialysis, 23(40.3%) new cases were identified with seroconversion of HCV infection. The frequency of seroconversion of HCV infection among chronic haemodialysis patients are significantly increasing with duration of haemodialysis (Table 4).

Table 4. Rate of Seroconversion of HCV Infection Monthly in Al-Kadhymia Teaching Hospital

Time of Haemodialysis	Number of Patients	HCV +ve	
		Number	%
January	38	2	5.2
February	38	2	5.2
March	39	3	7.7
April	40	9	12.5
May	40	10	22.5
June	42	11	23.8
July	45	11	24.4
August	47	16	23.4
September	48	16	33.3
October	50	19	32
November	54	23	35.1
December	57		40.3
Total number	57	23	40.3*

*Correlation Coefficient is significant

The twenty three new cases were identified with seroconversion of HCV infection, twenty out of twenty three of study patients investigated monthly serum ALT. Blood screening showed variable ALT levels preceding the anti-HCV seroconversion (Table 5).

Table 5. Serum ALT level 4 month before the Seroconversion of HCV Infection

Case number	ALT levels 4months before seroconversion					ALT/Anti HCV seroconversion	
	Month 1	Month 2	Month 3	Month 4	Mean ± SD	value	Month
04	10	28	86	>94	54.50 ± 41	>94	April
05	27	80	>94	13	53.50 ± 39.50	13	April
06	16	21	76	>94	51.75 ± 39	15	May
07	26	34	26	63	37.25 ± 17.50	>94	May
08	12	11	57	>94	43.50 ± 39.90	56	May
09	14	13	17	22	16.50 ± 4	19	May
10	24	19	34	33	27.50 ± 7.20	63	June
11	22	52	73	>94	60.25 ± 30.70	>94	July
12	18	35	85	>94	58 ± 37.20	26	August
13	7	25	18	78	32 ± 31.54	>94	September
14	15	18	40	46	29.75 ± 15.54	76	September
15	25	14	>94	>94	56.75 ± 43.20	>94	September
16	10	16	15	>94	33.75 ± 40.25	65	September
17	15	26	13	>94	37 ± 38.42	31	November
18	11	14	>94	13	33 ± 40.68	>94	November
19	61	69	89	60	69.75 ± 13.40	90	November
20	25	37	30	93	46.25 ± 31.55	>94	December
21	26	39	>94	90	62.25 ± 34.70	54	December
22	12	51	28	>94	46.25 ± 35.63	18	December
23	14	69	50	>94	56.75 ± 33.70	94	December
Total	Mean ± SD = 43.96±13.74					(63. 9±32.33)*	

*statistically significant p< 0.05 using t-test

A total 40 study patients (70.1%) were received blood transfusion; out of the total, 23 were HCV positive (52.5%) compared to the rest 17 patients (29.9%) who were not transfused, 2 were HCV positive (11.8%). 24 patients were transfused with more than five units of blood; among them, 16 were HCV positive (66.6%) as compared to 16 patients were received less than five units of blood; among them,5 were HCV positive (31.25%) as seen in table 6.

Table 6. Relationship between History of Blood Transfusion/Number of Blood Transfusion and Seroconversion of HCV Infection Hospital

Parameter	No.	HCV+ve Patients	HCV-ve Patients	P Value
+ve history of blood transfusion	Yes (n=40) No (n=17)	21(52.5%) 2 (11.8%)	19 (47.5%) 15 (88.2%)	0.004
No. of blood transfusion	5units (n=24) 5 units (n=16)	16(66.6%) 5(31.25%)	8(33.4%) 11(68.75%)	0.027

Thirteen males and ten female participants were seropositive, but the relationship between sex and seropositivity was not significant ($p > 0.05$). The relationship between duration of haemodialysis, history of blood transfusion and number of transfused units and seropositivity were statistically significant ($p < 0.05$).

Discussion

The seroconversion and seroprevalence of HCV infection among dialysis patients is generally much higher than healthy blood donors, it ranges from 1 to >80% in different series [12-15], this wide difference may reflect the demographic variations among the general population in these countries, however, the dialysis process itself and the level of hygiene standards influence the prevalence of HCV infection [16-19].

The results of the current study indicate that the cumulative rate of seroconversion during one year of HCV infection is (40.3%). But the seroprevalence of HCV infection in HD unit in AL-Kadhimiya Teaching Hospital (36.8%) nearly same as other centers of HD in Baghdad (39.5%) but higher than the results of studies conducted in Nineveh (15.3%) and Basra (7.5%) [20-22].

Also the present study shows higher rate of seroconversion than European countries (12-17.7%) and nearly same results were found in regional countries (32%) and lower than the results found in Egypt and Pakistan (44,56%) respectively [23-26].

The duration of the present study already lasted for 12 months and showed a high seroconversion rate of HCV infection over a short certain period, characterizing an outbreak of HCV infection in this period (epidimicity). It seems that the HD environment play a role in the transmission of HCV, the possible routes of transmission may be through contact of patients with contaminated environmental surfaces and sharing equipments (i.e. gloves, Clamps, sphygmomanometer, dressing and needles).

There is no statistical difference between male (56.5%) and female patients (43.5) in the assessment of the rate of seroconversion of HCV infection ($p=0.2$).

There is significant correlation between the rate of seroconversion and the duration of hemodialysis which has been noted in the current study which is compatible with the results of regional countries such as Iran and Jordan [27,28].

During the investigation from (January 2009 to December 2009), ALT was evaluated in only 23 patients 4 months prior to the appearance of HCV antibodies, the level of ALT varied at the initial stages of infection , reaching up to the level of >94 U/L (the cutoff reading level in the adopted laboratory) in the period preceding the 4 months of the appearance of anti-HCV antibodies in the serum the study results were compatible with the study done by Engel et al which showed variable s ALT levels preceding the appearance of anti-HCV antibodies [29]. It should be noted that the appearance of antibodies in haemodialysis patients delayed in comparison with non-haemodialysis patients and seroconversion may depend on each patient response and most of uremic patients reaching end stage renal disease are immunocompromised [30].

Many patients with end stage renal disease need blood transfusion(s) for correction of anemia [31,32]. In this study the data showed that HCV infection was detected in (52.5%) of patients who had received blood transfusion(s); versus (11.8%) of HD patients who did not have history of receiving blood transfusion(s) were HCV positive, this was statistically significant different ($p=0.004$). The irregular intake of erythropoietin throughout the year (the drugs most of time was unavailable which necessitate frequent blood transfusions might explain the increase rate of seroconversion of HCV infection). Furthermore, it has been noted that the number of transfusions was directly proportional to the seroconversion of HCV infection in the study group. The results were compatible with study

by Shaheen FA. Study in whom analyzed the blood transfusion(s), both independently and in combination with other risk factors for acquiring HCV infection, HCV infection was detected in 34% of patients who had received blood transfusion(s) versus 16% of HD patients who did not have history of receiving blood transfusion were HCV positive [33].

Conclusion

There is higher rate of seroconversion of HCV infection among patients undergoing HD therapy. Duration of HD, history and number of blood transfusion(s) affect the rate of seroconversion of HCV infection in patients on regular HD. Laboratory test results showed variable ALT preceding seroconversion of HCV infection.

It has been suggested that, the patients on HD need strict adherence to infection control measures in dialysis unit. Measures which should be considered include prevention of patient-to-patient contamination and separate haemodialysis systems for HCV seropositive patients. The data also reinforce the importance of serological screening at the onset of dialysis treatment and at regular intervals thereafter to identify all HCV-infected patients. Further detailed research on the role of blood transfusions in acquiring HCV infection is required.

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A Comparative Study between the Side Effects of Copper Intrauterine Device in Women with Non-scarred and Scarred Uterus

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Abstract

Background So many women think that the use of intrauterine devices in a scarred uterus carries high complications like increased uterine bleeding and pain and these side-effects may result in early removal of the device.

Objective The study was conducted to show the difference in the side effects of Copper intrauterine device (CuT380A) in women after vaginal deliveries and those with previous scar of cesarean section.

Methods The study group consisted of 411women who were using CuT380A device for contraception which has been fitted for more than 3months (240 of them had vaginal deliveries and 171 had one or more cesarean section scar). Complications of the CuT380A device were compared in both groups in regard to heavy vaginal bleeding (menorrhagia), painful menstrual cycles (dysmenorrhea), cycle irregularity, other types of pain (pelvic pain and backache) and infection. Both groups were further studied and complications were compared according to the duration of intrauterine device insertion.

Results The most common side-effects related to CuT380A were bleeding and pain. Menorrhagia was recorded in 35.42% and 29.24% while dysmenorrhea in 27.08% and 34.50% women of the non-scarred and scarred uterus groups respectively. These side effects were not statistically different between the two groups and they decreased significantly with time; menorrhagia decreased from 40.54% to 27.17% (P-value=0.035) and from 36.27% to 18.84% (P-value=0.014) while dysmenorrhea decreased from 31.76% to 19.57% (P-value=0.039) and from 41.18% to 24.64% (P-value=0.026) in non-scarred and scarred uterus groups.

Conclusion The study revealed that the side-effects of CuT380A device did not differ between non-scarred and scarred uterus and that menorrhagia and dysmenorrhea significantly decreased with time.

Key words CuT380A, non-scarred uterus, scarred uterus.

Introduction

The intrauterine device (IUD) is one of the most convenient methods of birth control because, once inserted, it requires no daily attention from the woman and does not interfere with sexual activity[1].There are a variety of modern IUD in many shapes and sizes available to women in developing countries. To date, the most effective and longest lasting IUD is the Copper T380A (CuT380A), which is being used in 70 countries around the world[2,3], with about 127 million current users [4]. The method is safe, rapidly reversible, inexpensive, highly effective, long-acting and non-hormonal; these attributes make it unique and desirable for many users [2,5].

The copper intrauterine device (IUD) can cause side effectsin some women[5], women are more likely to have increased menstrual loss and

dysmenorrhea but the usually have regular menstrual cycle[6]. It is also possible for women to bleed small amounts daily in the first three to five months of use[6].

The effect of IUD–particularly the effect on local prostaglandins-on the endometrium tends to cause increased menstrual bleeding and dysmenorrhea[7]. Bleeding can be both heavier and more prolonged particularly during the first three to 6 months of use[7].

Anecdotal information accumulated from clinicians and some published information suggests thatside effects from the copper IUD decrease over time [8,9].

IUDs side effects like increased bleeding and pain cause removal of the device within the first year in up to 15% of users[10]; still higher percentages tolerate some level of theseside effects, yet retain use of the method[5].

The study is conducted to highlight the difference in the side effects of CuT380A in women with previous vaginal deliveries and those with previous cesarean section scar.

Methods

This prospective cross-sectional study was conducted during the period from the 1st of Jan.2008 to the 1st of Jan.2010 in a private clinic. Informed consent was taken from each eligible woman.

A total of four hundred & eleven women having CuT380A (Copper T, Leiras OY, Finland) for more than 3months were included in the study, some of these women had the intrauterine device fitted before the study period and others during it.

Two hundred and forty of them had vaginal deliveries (non-scarred uterus group), while the remaining 171 women had one or more cesarean section scar (up to three scars) in their reproductive history (scarred uterus group). All IUDs were inserted by the authorwithin the first 5 days of the menstrual cycle.

Each woman had at least three visits where thorough history and clinical examination including pelvic examination was done. Women with history of operations in the uterus (i.e., myomectomy, hysterotomy and metroplasty) were excluded from the study. All were healthy with regular menstrual cycles before insertion of the intrauterine device and no history of significant gynecological or medical disorders.

The participants were subjected to a questionnaire using special form that include the age, parity, number of cesarean sections, duration of IUDs insertion, days of bleeding, cycle regularity, cyclic pain and amount of bleeding before and after IUDs insertion and other complaints (backache, chronic pelvic pain and infection) and IUDs removal.

Dysmenorrhea was considered when the patient complained from cyclic pain which was not experienced by the patient before IUDs insertion which necessitated medical treatment (i.e. NSAID). Menorrhagia was considered when more number of pads where changed with passage of clots. Recurrent genital infection was considered when the patient gave history of repeated attacks of infection with clinical and bacteriological evidence of genital infection after IUDs insertion. Other gynecological complaints (backache, chronic pelvic pain and inter-menstrual spotting) were also recorded.

The data collected and arranged in tables and classified according to the groups of patients and duration of insertion. We compared first the side effects of intrauterine device between the two groups of women, then they were further divided into subgroups according to the duration of intrauterine device insertion; since most side-effects decrease over time especially within the first12-24months, we chose to compare these effects before and after 12months.

The two groups were also compared for rate of intrauterine device removal rate according to the cause of removal.

All these groups were subjected to comparison and statistical analysis using descriptive statistics (table, frequency and percentage) and inferential statistics (chi-square test) to find

any association between variable data. P-value less than 0.05 was considered significant.

Results

The age of the participants range from 18 to 47 years (mean of 31.2, SD ±7.26) for the non-scarred uterus group and (31.6, SD ±6.57) for the scarred uterus group. No statistical difference is seen between the two groups (P = 0.597).

Regarding the parity: women with 0-2 children; 88(36.67%), 62(36.26%) non-scarred and scarred uterus groups, women with 3-4 children; 93(38.75%), 80(46.78%) respectively and women with more than 5 children: 59(24.58%), 29(16.96%) respectively. No statistical difference present between these groupsrespectively (P = 0.120).

Duration of the menstrual flow after IUDs insertion is not different between the two groups, with a mean of 5.28(SD ±1.54) days for the non-scarred uterus group and 5.45(SD ±1.36) days for the scarred uterus group and the P is 0.246.

Table 1 compares the side-effects of CuT380A device between the two groups; menorrhagia is more prevalent in the non-scarred uterus group: 85women (35.42%) compared to 50(29.24%) in the scarred-uterus group with a P-value of 0.189, while dysmenorrhea is more in those with history of previous scar: [59(34.50%) vs. 65(27.08%) women in the non-scarred uterus group, P=0.106].

Regular cycles are observed more in the non-scarred uterus group; only 19.58%of women have irregular cycles compared to 18.71%suffer from inter-menstrual spotting and irregular cycles in the scarred uterus group. No statistical difference is found between them (P = 0.825).

The women who suffered from backache and pelvic pain are more in the scarred uterus group (as seen in the table).

Incidence of genital infection is comparable in both groups; 12(5.00%) and 8(4.68%) women respectively with a P = 0.881.

The duration of IUD insertion is "less than 12 months" in 148(61.67%) women with non-scarred uterus compared to 102(59.65%) with scarred uterus, and the duration is "equals-more than 12months" in 92(38.33%) and 69(40.35%) in both groups respectively. No statistical differences are detected (P = 0.680).

Table 1. Comparison between the side-effects of copper IUD in non-scarred and scarred uterus

Complications	NVD (n=240)		C/S (n=171)		P-value
	No.	(%)	No.	(%)	
Menorrhagia	85	35.42	50	29.24	0.189
Dysmenorrhea	65	27.08	59	34.50	0.106
Irregular cycle	47	19.58	32	18.71	0.825
Backache	21	8.75	18	10.53	0.545
Pelvic pain	19	7.92	17	9.94	0.474
Genital infection	12	5.00	8	4.68	0.881

NVD = Normal vaginal delivery, C/S = caesarian section

The complications according to the duration of IUD insertion are studied in both groups (table 2 and 3). Some parameters like menorrhagia and dysmenorrhea shows statistical significant decline after 12months of insertion in both groups: menorrhagia [40.54%, 27.17%, (P=0.035) vs. 36.27%, 18.84%, (P=0.014) for non-scarred and scarred uterus groups], dysmenorrhea [31.76%, 19.57%, (P = 0.039) vs. 41.18%, 24.64%, (P=0.026) respectively] while other parameters shows non-significant changes, but when we compare these effects according to duration after insertion between non-scarred and scarred uterus (Table 4), no

significant statistical differences are detected between all parameters.The study shows also that 80(33.3%) women and 48(28.07%) women are symptom-free in non-scarred and scarred uterus groups.

Table 2. Complications according to the duration of copperIUD insertion after vaginal delivery (non-scarred group)

Complications	Duration after vaginal delivery				P-value
	<12months		≥12months		
	No.	%	No.	%	
Menorrhagia	60	40.54	25	27.17	0.035
Dysmenorrhea	47	31.76	18	19.57	0.039
Irregular cycle	27	18.24	20	21.74	0.507
Backache	14	9.46	7	7.61	0.622
Pelvic pain	12	8.11	7	7.61	0.889
Genital infection	8	5.41	4	4.35	0.715
Total	148	61.67%	92	38.33%	

Table 3.Complications according to the duration of copper IUD insertion after cesarean section (scarred group)

Complications	Duration after scar				P-value
	<12months		≥12months		
	No.	%	No.	%	
Menorrhagia	37	36.27	13	18.84	0.014
Dysmenorrhea	42	41.18	17	24.64	0.026
Irregular cycle	20	19.61	12	17.39	0.715
Backache	10	9.80	8	11.59	0.708
Pelvic pain	9	8.82	8	11.59	0.552
Genital infection	5	4.90	3	4.35	0.866
Total	102	59.65%	69	40.35%	

Table 4.Comparison between the side-effects of copper IUD in non-scarred and scarred uterus according to the duration after insertion

Complication	Normal Vaginal Delivery		Caesarian Section		P-value
	<12months	≥12months	<12months	≥12months	
Menorrhagia	60	25	37	13	0.670
Dysmenorrhea	47	18	42	17	0.890
Irregular cycle	27	20	20	12	0.653
Backache	14	7	10	8	0.477
Pelvic pain	12	7	9	8	0.535
Genital infection	8	4	5	3	0.848

As shown in table 5, a total of 58(24.17%) and 33(19.30%) women in non-scarred and scarred uterus groups removed the intrauterine device for different reasons. Menorrhagia and different types of pain resulted in 15.51%, 12.12% IUD removal in both groups

representing the majority of medical cause for removal, while 44(75.86%), 26(78.79%) women respectively removed the IUD for other reasons (desire for pregnancy, fear from side-effects like possibility of perforation, fear from future infertility, coital problems, religious causes…).

Table 5.IUDs removal according to the cause of removal

Cause of removal	Non-scarred group		Scarred group		P-value
	No.	%	No.	%	
Menorrhagia	6	10.34	2	6.06	0.488
Dysmenorrhea and other types of pain	3	5.17	2	6.06	0.858
All other side-effects (inter-menstrual bleeding, infection)	5	8.62	3	9.09	0.939
Other causes (desire for pregnancy, fear from side-effects…)	44	75.86	26	78.79	0.750
Total	58	100	33	100	

Discussion

Many studies published on IUDs have consistently reported findings in favor of IUD use. Notable among these findings are: IUDs are not abortifacients; newly developed IUDs are highly effective and the efficacy is long-lasting, with lower removal rates attributable to bleeding and/or pain [(11)].These complaints are not uncommon among IUD users in the first months after insertion [(1)]. Complications and complaints reported during the study period were mostly related to menstrual disturbances. The most frequently reported menstrual complaints among both groups were menorrhagia anddysmenorrhea.

Different studies showed menorrhagia as one of the important menstrual disturbance after loop insertion:

Daniel andMishell[(12)] found that Copper IUDs were associated with increased menstrual blood loss by about 50%, particularly during the first few post-insertion cycles.This amount of blood loss does not usually cause anemia [(13)].Other studies gave different values for menorrhagia ranged from 6.2%, 35.4% and 56.3%[(1,5,15)].Comparable ranges of values are obtained in our study (35.42% for non-scarred uterus group and 29.24% for the scarred uterus group).

The incidence of excessive bleeding was found to be the highest at the 12th-month follow-up by V. Parikh and Gandhi [(14)] and de Araujo, et al [(16)] showed a decline in menorrhagia from 10.6% in the first 3months compared to 4.3% after one year while Hubacher et al [(5)] showed 56.3% at 9-19weeks and 53.9% at 19-39 weeks. In our study, incidence of menorrhagia was reported to be significantly lower in women with "equals-more than 12 months" fitted loopfor bothnon-scarred and scarred uterus groups [from 40.54% to 27.17% for NVD cases (P=0.035) and from 36.27% to 18.84% (P=0.014) for C/S cases].

Days of menstrual cycle in women after TCu380A reached 5.9days [(5)], we had 5.28(SD ±1.54) days for the non-scarred uterus group and 5.45(SD ±1.36) days for the scarred uterus group and a P-value of 0.246.

In their study of Hubacher et al[(5)], inter-menstrual spotting was present in 20% at 9-19weeks increasing to 24.9% at 19–39 weeks; our values ranged from19.58% for NVD cases and 18.71% for C/S cases. With time, inter-menstrual spotting was increasing in NVD cases (from 18.24% to 21.74%) and decreasing in C/S cases (from 19.61% to 17.39%) although both were non-significant changes. In addition, 30.6 % of the TCu380A IUD users reported dysmenorrhea, while Reinprayoon et al [(1)] reported 59.1%. Our study showed 65 (27.08%) vs.59 (34.50%) in non-scarred, scarred uterus groups respectively.

A large study of 2700 Copper IUD users in India found that complaints of pain and bleeding decreased over a 24-month period [17]. Moreover, Hubacher and coworkers reported menstrual pain of 38.3% at 9weeks, 30.6 at 9-19weeks and 32.6% at19-39weeks. Menstrual pain was decreasing significantly with time in both groups of the study.

The relatively high number of reports of menstrual complaints did not result in a large number of IUD removals because of bleeding and pain. The 12-month gross cumulative life table rates for removal because of bleeding and pain were only 3.79 per 100 women for the TCu380A[1]. The one-year continuation rate among parous women using the Copper T 380A is 92.1%. This rate suggests that this IUD is very well tolerated [12], in our study bleeding and pain resulted in 15.51%, 12.12% IUD removal in non-scarred and scarred uterus groups.

D. Hubacher, P. Chen and S. Park [5] reported different types of inter-menstrual pain as 18.7%, our study shows backache as 8.75% and 10.53%, pelvic pain 7.92% and 9.94% in scarred and non-scarred uterus groups.

de Araujo et al [16] reported 7.7% pain of different types at 3months, decreasing to 6.4% pain at 1year and 4.6% at 2years, a decline that is seen in the non-scarred uterus group only in the current study (although not significant).

Vaginitis and cervicitis were the most commonly reported types of genital infection in a study of D. Reinprayoon[1] with a range of 5%–7%, our study results are also comparable (12(5.00%), 8(4.68%) respectively).

Our study reveals that menstrual complaints significantly decrease with time but no difference between the two groups while inter-menstrual complaints not significantly change with time and also no difference between the two study groups.

Women's overall satisfaction with TCu380A remains high, as evidenced by the average first-year continuation rate of 78% [18]. Although side effects from TCu380A decreased over time, the product still causes problems that often lead to premature removal[19].But sincesexually active women need protection from pregnancy, the copper T380A with its low rates of failure during the initial 5–10 years of use plus the long, highly effective life, the absence of systemic effects such as those associated with steroid contraception and the economic benefits of using a supply-free reversible contraceptive for up to 20 years permits women as young as those in their mid to late 20s to use a single device until they experience menopause. Continuation of contraception with this device poses, after observation, no known additional risks to IUD users, who remain at liberty to change their choice of contraception at any time before menopause [20].

Conclusion

The study revealed that the side-effects and rate of IUD removal did not differ between the non-scarred and scarred uterus groups and that even though some of those side-effects decreased with time (menorrhagia and dysmenorrhea) but they were not different between the two groups.

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A study on Heavy Metals and Antibiotic Resistance of *Staphylococcus aureus* Isolated from Clinical Specimens

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Abstract

Background The trace heavy metals such as Cobalt, Zinc, Copper and Nickel play important roles in bacteria; they regulate a wide array of metabolic function as coenzyme or cofactors. However, some metals like arsenic, mercury and cadmium, are not essential for growth and extremely toxic. Understanding of metal resistance in Staphylococci in association with antibiotics resistance has progressed rapidly in the last years with well-established cadmium, mercury, antimony and arsenic resistance system encoded by plasmids.

Objective To evaluate antibiotic and heavy metal resistance in *Staphylococcus aureus* isolates.

Methods Thirty *S. aureus* isolates were collected from different clinical specimens. The minimum inhibitory concentration of thirty *S. aureus* isolates was determined for four types of antibiotics, which were tetracycline, gentamicin, cefotaxime and penicillin-G. Resistance of *S. aureus* isolates to heavy metals ions (Cobalt, Zinc, Mercury and Cadmium) were tested. Ethidium bromide was used as a curing agent with freshly growing *S. aureus* to study resistance features link with antibiotic and heavy metal resistance.

Results The minimum inhibitory concentration of thirty *S. aureus* 83.3% of the isolates were resisting tetracycline 80% of the isolates were resisting gentamicin 93.3% of the isolates were resisting cefotaxime, and 80% of the isolates were resisting penicillin-G. While, 93.3% of isolates found to be resistant for Cobalt ions, 86.6% resisted Zinc ions, 86.6% resisted Mercury ions. While, 83.3% of isolates resisted Cadmium ions. Using Ethidium bromide as a curing agent showed two groups of cured colonies.

Conclusions There is strong relationship between multiple antibiotic resistances and multiple heavy metal resistance In addition; there may be two to three types of plasmids depending on results obtained from curing experiment.

Key wards Heavy metals, *S aureus*, antibiotics, resistance.

Introduction

Staphylococcus aureus was responsible for a wide range of infections, from mild skin infections to wound infections and bacteraemia. Although the introduction of antibiotics over the last 50 yr has lowered the mortality rate from *S. aureus* infections, the bacteria have developed resistance mechanisms to all antimicrobial agents that have been produced [(1).]

Some strains of *S. aureus* express many potential virulence factors that are lack in other strains. *Staphylococcus aureus* infections can be treated with commonly used antibiotics [(2)]. In recent years some strains of *S. aureus* have become resistant to some antibiotics

which means that it is not killed by antibiotics that's take great attention [3].

Heavy metals consist of a group of about 40 elements. Many are essential for growth of both prokaryotic and eukaryotic organisms, and therefore are required at low concentration. However, some metals like arsenic, mercury and cadmium, are not essential for growth and extremely toxic even at low concentration [4]. The trace heavy metals such as Cobalt, Zinc, Copper and Nickel play important roles in bacteria; they regulate a wide array of metabolic function as coenzyme or cofactors, as catalysts or acid in the enzymes and as structural stabilizer of enzymes and DNA binding protein [5].

Understanding of metal resistance in Staphylococci has progressed rapidly in the last years with well-established cadmium, mercury, antimony and arsenic resistance system encoded by plasmids [6]. Little is known about transport of the resistance to zinc and cobalt (chromosomal encoded) ions in *S. aureus* [7].

The aim of this study was to evaluate antibiotic resistance and tolerance to some heavy metals linked with antibiotic resistance in *S. aureus* and making a curing experiment to demonstrate the relationship of antibiotic resistance and heavy metal tolerance with any cured plasmid could harboring such traits.

Methods

Collection of Isolates: One hundred thirty isolates of *s. aureus* were obtained from different clinical specimens were collected from Al-Yarmouk and Al-Kadhimiya hospitals from 74 female and 56 male. Of these, 30 isolates were identified as *S. aureus* (17 isolates from female and 13 isolates from male). On the basis of their colony morphology, Gram's stain and positive results in coagulase, DNase, catalase, mannitol fermentation, and for the further confirmation the isolates were identified by API staph system.

Antibiotic and heavy metals solution: Tetracycline stock solution prepared at concentration 25000 µg/ml, cefotaxime stock solution prepared at concentration 10000 µg/ml, gentamicin stock solution prepared at concentration 8000 µg/ml and penicillin-G stock solution prepared at concentration 80000µg/ml, then the stock solutions were sterilized by filtration and kept at 4°C, until used [9]. Heavy metals used were Zn $(CH_3COO)_2.2H_2O$, Co $(CH_3COO)_2.4H_2O$, $CdCl_2$ and $HgCl_2$ prepared as stock solution and sterilized by filtration and kept at 4°C until used.

Ethidium Bromide solution 10 mg/ml (Curing solution) was prepared by dissolving 0.2gm of ethidium bromide in 20 ml distilled water and stirred on magnetic stirrer for few hours to ensure that the ethidium bromide has dissolved then it was sterilized by filtration, and stored in a dark bottle at 4°C [8].

Minimum Inhibitory Concentration (MIC) tests. Inocula of selected isolates were grown in 5ml nutrient broth, then 0.1ml of each culture were inoculated in series of 5ml fresh nutrient broth containing various concentrations of antibiotics or heavy metals solutions (8, 16, 32, 64, 128, 256, 512 and 1024 µg/ml for antibiotics) and (5, 10, 20, 40, 80, 160, 320, 640 and 1280 µg/ml for heavy metals) for each isolates of *S. aureus,* then all tubes were incubated at 37°C for 24 hours. 100 µl from each tube were spread on brain heart infusion agar plates and all plates were incubated at 37°C for 24 hours. The lowest concentration of the antibiotics or heavy metals solutions that inhibited the growth of bacterial isolates was considered as the minimum inhibitory concentration (MIC) [9].

Plasmid DNA curing. Cells of the selected isolate were grown in 5ml of nutrient broth. 0.1ml samples of each culture were inoculated in series of 5ml fresh nutrient broth tubes containing various concentrations of ethidium bromide (50, 100, 200, 400, 600, 800 and 1000 µg/ml). All tubes were incubated at 37°C for 24-48 hours. The growth density of the deferent tubes was measured visually and compared with the control to determine the

effect of each concentration of curing agent on bacterial growth [10]. The lowest concentration of the curing agent that inhibits the growth of bacterial isolate was considered as the minimum inhibitory concentration (MIC).

Selection of Cured Cells. After treatment of bacterial isolate with standard curing agent and the isolation of survivors on nutrient agar, survivors were analyzed for the presence or absence of drug resistance as a result of elimination of the plasmid by selecting 100 colonies of bacterial isolates from each treatment. These colonies were replica plated (using toothpick) on nutrient agar plate (master plates) and on nutrient agar plates containing an antibiotics and other nutrient agar plate containing a heavy metals to which the original isolate is resistant [10]. If a colony was able to grow on the master plate but not on the selective agar containing the appropriate antibiotic or heavy metal, it means that, the cells of this colony are cured cells that lost plasmid responsible for resistance to the antibiotic or heavy metal. The percentage of the cured cells was determined.

Results

Antibiotic sensitivity test of *S. aureus* isolates.Antibiotic sensitivity test performed with twelve types of antibiotics. The percentage of resistance were 93.3%, 83.3%, 83.3%, 80%, 50%, 33.3%, 30%, 30%, 20%, 20% and 3.3% to the following antibiotics cefotaxime, carbenicillin, tetracycline, gentamicin, cephalixin, fusidic acid, chloramphinicol, bacitracin, vancomycin, streptomycin and imipenem. No resistance was found to amikacin.

The minimum inhibitory concentration (MIC) of antibiotics of *S.aureus* isolates *Staphylococcus aureus* isolates showed high percentage of resistance for the four types of antibiotics that were tested against, 93.3% of *S. aureus* isolates showed resistance for cefotaxime, of these 46.6% showed high resistance level at 64 µg/ml, while 13.3% of isolates showed a lower resistance level at 128 µg/ml. Eighty percent of *S. aureus* isolates showed resistance for penicillin-G. Of these, 50% showed high resistance level at 128 µg/ml, While 6.6% of isolates showed a lower resistance level at 512 µg/. *S. aureus* isolates which represented 83.3 % showed resistant for tetracycline. Of these 56.6% showed high resistance level at 128 µg/ml. While 6.6 of isolates showed a lower resistance level at 256 µg/ml. Eighty percent of *S. aureus* isolates showed resistance for gentamicin. Of these, 56.6% of were resisting 32 µg/ml in high resistance level. While 10% of isolates showed a lower resistance level at 16 µg/ml (Table 1).

Table 1. Resistance percentage of *S.aureus* isolates for different concentration of antibiotics.

Antibiotic	% Resistance of *S.aureus* for the following concentrations (µg/ml)								% Sensitive isolates
	8	16	32	64	128	256	512	1024	
TE	-	-	10	10	56.6	6.6	-	-	16.6
CN	-	10	56.6	13.3	-	-	-	-	20
CTX	-	-	-	46.6	13.3	33.3	-	-	6.6
P-G	-	-	-	6.6	50	16.6	6.6	-	20

CN: Gentamicin, P-G: Penicillin-G, CTX: Cefotaxime, TE: Tetracycline

Heavy metal resistance of *S.aureus* isolates:

There were 86.6% of isolates resist Zinc ions. About 40 % of the tested *S.aureus* isolates showed high resistance level (most of bacterial isolates resist it) at concentration 0.64 mg/ml, while 6.6 % of isolates showed lower resistance

level at 2 mg/ml concentration. There were 93.3% of the isolates resist Cobalt ions, 30 % of the tested *S.aureus* isolates showed high resistance level (most of bacterial isolates resist it) at concentration 0.16 mg/ml. While 6.6 % of isolates showed lower resisting level at concentration 0.02 mg/ml.There were 83.3% of isolates resisting Cadmium ions. About 33.3% of the tested *S. aureus* isolates showed high resistance level (most of bacterial isolates resist it) at 0.02 mg/ml. while, 3.3 % of isolates showed lower resistance level at 0.01 mg/ml. There were 86.6 % of isolates resist Mercury ions, 56.6 % of the tested *S.aureus* isolates showed high resistance level at 0.02 mg/ml, while 6.6 % of isolates showed low resistance level at 0.04 mg/ml concentration (Table 2).

Table 2. Resistance percentage of locally isolated *S.aureus* for different concentrations of heavy metals.

H.M	% Resistance of *S.aureus* isolates for the following Concentrations (mg/ml)										% Sensitive isolates
	0.005	0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.0	
Zn	-	-	-	-	-	13.3	16.6	40	10	6.6	13.3
Co	-	-	6.6	20	-	30	16.6	10	10	-	6.6
Cd	-	3.3	33.3	16.6	10	20	-	-	-	-	16.6
Hg	23.3	-	56.6	6.6	-	-	-	-	-	-	13.3

H.M= heavy metals, Zn = Zinc, Co = Cobalt, Cd = Cadmuim, Hg = Mercury

Relationship between heavy metals resistance and antibiotics resistance *S. aureus* isolates which represented 94.4 % (17 from 18-quadruple heavy metal resistance *S. aureus* isolates) were resistant to tetracycline at concentrations ranged between (32 -256 µg/ml). In addition, 94.4 % (17 from 18-quadruple heavy metal resistance *S. aureus* isolates) were resistant to cefotaxime at concentrations ranged between (64-256µg/ml). *S. aureus* isolates which represented 88.8 % (16 from 18-quadruple heavy metal resistance *S. aureus* isolates) was resistant to gentamicin at concentrations ranged between (16-64µg/ml). Also, 88.8% (16 from 18-quadruple heavy metal resistance *S. aureus* isolates) resisted penicillin-G at concentrations ranged between (64-256µg/ml).

Curing of plasmid DNA of *S.aureus* isolate R2 with Ethedium bromide

One isolate had been chosen designated as isolate R2 resistance because it has multi drug and metal resistance and it shows effective growth among the 30 *S.aureus* isolates. Table 3 showed that 100 µg/ml of Ethedium bromide was the less concentration which had noticeable inhibitory effect on bacterial growth for the isolate R2 compared with control growth.

Depending on curing experiment which indicated that may be there were two types of cured colonies; colonies lost resistance for Zinc, Cobalt, Cadmium, Penicillin-G and Tetracycline, colonies lost resistance for Zinc, Cobalt, Cadmium, Penicillin-G, Tetracycline and Cefotaxime this indicated loosing for more than one type of plasmid in the last type of colonies of *S. aureus* isolates. While there were no loss of Genamicin and Mercury resistance, which indicated that these markers are not, located on plasmid DNA (located on chromosome or on mega plasmid). That means that may be there were two to three types of plasmids depending on results obtained from curing experiment as shown in table 3.

Table 3: Number of cured bacterial colonies that lost resistance to antibiotics and heavy metals after treatment with Ethedium bromide.

Resistance phenotype	*Staphylococcus aureus*	
	Wild type	Cured cells
Zn, Co, Cd, p-G, TE, CTX	100 % resistance	3 % sensitive
Zn, Co, Cd, P-G, TE	100 % resistance	97 % sensitive
Hg	100 % resistance	100 % resistance
CN	100 % resistance	100 % resistance

Zn = zinc, Co = cobalt, Cd = cadmium, Hg = mercury, P-G = Penicillin-G, TE = Tetracycline

Discussion

The high percentage of *S.aureus* might be due its role as the main cause of nosocomial infections. It is also one of the most important infectious agents, which can cause an opportunistic infection because it is a part of body normal flora [(11)]. *Staphylococcas aureus* isolates showed high percentage of resistance for the four types of antibiotics tested against .These results are in agreement with that of Booth *et al* (2001) which found that 90% of isolates were resistant to β-lactam antibiotics. Production of β-lactamase is the main cause of high resistance of *S.aureus* to β-lactam antibiotics since the β- lactam ring is the main constituent of β-lactam antibiotics molecules [(12)]. The frequent use of tetracycline to treat wound infections locally may elevate resistance percentage of *S.aureus* for this antibiotic. The mechanism of resistance for tetracycline performed by ribosomal protection, active efflux and decrease uptake [(1)]. Kuroda *et al.* reported that, about 45% of the total isolates of *S.aureus* carried a 35.5 kb plasmid and these isolates always showed resistance to gentamicin, tobramycin, kanamycin, amikacin, astromicin, and arbekacin. The plasmid carried resistance here may be transferred easily and that explain the elevated percentage of resistance to gentamicin [(13)].

The highest zinc resistance among bacterial isolates was also reported by Xiong and Jayaswal (1998) [(7)]. The molecular mechanism of resistance involves a number of proteins. These protein molecules either export the metal ions out of the cell or detoxify or sequester them so that the cells can grow in an environment containing high level of toxic metals [(7)]. The highest Cobalt resistance among bacterial isolates was also reported by Xiong and Jayaswal [(7)]. There were 83.3% of isolates resisting Cadmium ions. While there were 86.6 % of isolates resist Mercury ions. Novick and Roth (1968) reported that certain isolates of *S.aureus* carried resistance factors to some inorganic ions including Mercuric, Cadmium, Arsenate and Lead; also they reported that penicillinase plasmids In *S.aureus* carried resistance factors to some inorganic ions including Arsenate, Lead, Mercuric and Cadmium [(14)].

The results of current study revealed that there is strong relation between resistance to antibiotics and heavy metals.

Genes encoding for metal and antibiotic resistance may be located on the same plasmids and/or transposons, conferring co-resistance [(15)]. Curing and transfer experiments revealed that the 26-kb plasmid encoded resistance to Cadmium, Mercuric chloride, Propamidine isothionate and ethidium bromide [(16)]. However, since bacteria are very likely to be confronted with toxic Mercury concentrations, Mercury resistance determinants are very widespread [(6)]. The mechanism of resistance of *S. aureus* to Mercury considered was referred to the category that the detoxication of noxious substances introduced into bacterial cells by some intracellular mechanisms, which

somehow change them into non-noxious form by reduce Mercury ions to a less toxic oxidation state by the bacterial cell [17]. Plasmids might be capable of encoding resistance to antibiotics specifically related to heavy metals (Silver, Mercury, and Copper) resistance [18].

Depending on curing experiment which indicated that there may be two types of cured colonies; colonies lost resistance for Zinc, Cobalt, Cadmium, penicillin-G and tetracycline, and colonies lost resistance for Zinc, Cobalt, Cadmium, penicillin-G, tetracycline and cefotaxime. This indicated loosing for more than one type of plasmid in the last type of colonies of *S. aureus* isolates. While there were no loss of genamicin and Mercury resistance, which indicated that these markers are not, located on plasmid DNA (located on chromosome or on mega plasmid). Such results indicate the presence of two to three types of plasmids depending on results obtained from curing experiment. The effect of ethedium bromide as interchalating dyes with preferential inhibition of plasmid replication. If the resistance is plasmid mediated, those bacteria with clustered resistance genes are more likely to simultaneously pass on those genes to other bacteria, and those bacteria would then have a better chance at survival. In such a situation, one may suggest an association with antibiotic resistance and metal tolerance [19]. If both or all enes clustered are useful to the organism, it is beneficial to the survival of that organism and its species because those genes are more likely to be transferred together in the event of conjugation. Thus, in an environment with multiple stresses, for example antibiotics and heavy metals, it would be more ecologically favorable, in terms of survival, for a bacterium to acquire resistance to both stresses.

Conclusions

The relationship between multiple antibiotic resistances and multiple heavy metal resistance indicates an environmental biohazard of heavy metal pollution in Iraq. In addition, there may be two to three types of plasmids depending on results obtained from curing experiment. The genes responsible for resistance for some heavy metals and antibiotics may be located on the same plasmid DNA.

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Adherence of Candida Albicans to Uroepithelial Cells

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Abstract

Background Urinary tract infection (UTI) is usually associated with multiplication of microorganisms in urinary tract followed by adhesion of these organisms to the uroepithelial cells which is considered as the first step for colonization and infection. Adhesion of Candida on the epithelium of the urinary tract stands as the first step in the pathogenesis of Candidiasis. The adhesion process is under the impact of many factors.

Objective This study was designed to determine the susceptibility of uroepithelial cells obtained from patients with different urinary tract diseases, for adherence with Candida albicans in vitro.

Methods Forty eight urine samples collected from women attending Medical City Hospital /Baghdad suffering from urinary tract diseases and proved to have Candida growth. Ten urine samples were collected from apparently healthy age matched females and used as a control. The uroepithelial cells of each sample have been collected and incubated with Candida albicans cells and the percentage of uroepithelial cells with an adhered Candida cells (% EC). The mean number of Candida/epithelial cells (C/E) was estimated before and after adherence assay.

Results Klebsiella infection showed the highest percentage of uroepithelial cells with Candida (%EC), next in frequency was samples with high number of uroepithelial cells, then, Streptococcus pyogenes, Escherichia coli, pus cells, and finally samples with high number of RBCs. This study indicates that receptivity of Candida adhesion to the uroepithelial cells of urinary tract have different affinity depending on the type of modulating factor surrounding these uroepithelial cells.

Conclusion From the obtained data it can be concluded that urine findings including bacterial growth might modulate epithelial cell surface and increase their receptivity for Candida adherence.

Key word Adherence, Candida albicans, uroepithelial cells.

Introduction

Urinary tract infections (UTIs) which are not properly treated from their onset can become a real threat in time, finally leading to renal failure. This is partly due to the fact that urinary signs and symptoms are often not reliable in distinguishing upper and lower urinary tract diseases [(1)].

UTI is usually associated with the demonstration of pathogenic organisms in urine; bacteria, fungi or parasite. It is more common among females due to the shortness of urethra [(2)]. Candida play an important role in UTI since Candida albicans and related species are the leading cause of disseminated fungal infection in immuno- compromised diabetics, postoperative patients and in patients with chronic mucocutaneous candidiasis. Candida causes an erosive dermatitis and severe inflammation of vaginal tract [(3)].

An understanding of the mechanism of attachment and a delineation of the adhesive

molecules on the surface of Candida (adhesion) as well as those on cell membranes (receptors) has suggested new approaches to the prevention of serious candidiasis infection. Such adherence enables the organisms to avoid elimination by the cleansing action of mucosal secretions, allowing the yeast to colonize [4].

Adhesion of Candida species on epithelium of the gastrointestinal or urinary tract stands as a critical first step in the pathogenesis of candidal infection [3].

A work on the adherence of some Gram positive bacteria such as Staphylococcus aureus to human epithelial cells was carried out and it was found that fibronectin-binding proteins are the major staphylococcal adhesin responsible to this adherence [5].

The objective of this study is to determine the effect of different urine findings including bacteria on susceptibility of uroepithelial cells obtained from patients with different urinary tract infections, for adherence with Candida species in vitro. It is an attempt to assess the role of urine deposit findings and bacterial growth on modulation of uroepithelial surface receptivity for Candida albicans adhesion.

Methods

Epithelial cells

Uroepithelial cells were collected from freshly voided urine of females with urinary tract disease with or without bacteria. The suspended cells in urine were centrifuged 3000 rpm for 15 minutes. The pellet was washed 2-3 times with phosphate buffer saline (PBS). Using Neubauer chamber, uroepithelial cells were examined and counted for attached bacteria and yeast. Control uroepithelial cells had no evidence of yeast contamination or attachment [6].

Candida growth and isolation

Candida cells were obtained from females urine samples with urinary tract diseases associated with Candida albicans infection by using Sabouraud dextrose agar. Candida isolates were diagnosed by applying appropriate diagnostic tests [7]. The yeast cells were incubated for 48h at 37^{o}C and the cells were suspended in 5 ml PBS with pH 7.2, washed twice with PBS by centrifugation at 3000 rpm for 5minutes. The pellet was collected and resuspended in 5 ml PBS. Yeast cells were diluted with PBS and adjusted to a concentration of 10^8 yeast cells/ ml and kept in deep freeze until used [6].

Urine samples

Urine samples were grouped into; urine from patients with positive bacterial growth, urine from patients with negative bacterial growth and urine samples from controls. The following tests were applied to the above samples:

1. General urine examination: This examination was carried out according to Fischach & Dunning (2004) [8].

It included the following:

-Macroscopic examination e.g. color, reaction, appearance.

-Biochemical tests e.g. sugar, albumin, bile pigment.

-Microscopic examination: urine samples were centrifuged at 3000 rpm for 15 minutes, deposits were examined and the results estimated according to the following scores:

a) Pus cells > 5/HPF considered positive.
b) RBC >3/ HPF considered positive.
c) Casts> 2/HPF considered positive (other than hyaline cast).

Urine culture

Both Gram positive and Gram negative bacteria were isolated on different media. Klebsiella pneumoniae, Escherchia coli and Streptococcus pyogenes were identified in urine samples grouped as samples with bacterial growth. Uroepithelial cells were obtained from urine samples of all groups and incubated with candida albicans.

Adhesion assay

This assay was performed as described by Centeno et al [6]. Uroepithelial cells (0.5 ml from a PBS suspension of 5x10^6 cells per ml) and yeast organisms (0.5 ml from a PBS suspension of 10^8 yeast cells per one ml) were placed in screw top vials (ratio of cells/ yeast 1:20) and incubated at 37^{o}C for 60

minutes in water bath with gentle shaking. After incubation, the suspension was filtered through Nuclepore schisto kit (12 μm pore size) instead of polycarbonate filter Nuclepore Corp., Pleasanton, Calif. unattached yeast cells were removed by rinsing the filter two times with 3-5 ml of pipetted PBS. The filters were labeled and allowed to dry for 24 hours at room temperature. The dried filters were floated, uroepithelial cells down side, on the dye solution (Methylene blue 0.5% in PBS) for 10 to 15 seconds and then removed. Excess dye was removed from filters with three successive rinses by floating filters in PBS – filled Petri plate. After the final rinse, filters were placed, epithelial cells side up, on large glass slides and viewed as wet mounts by light microscopy at 400X. Uroepithelial cells viewed were counted for yeast attachment by preparing 4-6 slides for each sample. Percentage of epithelial cells with attached candida (% EC) and mean number of candida per epithelial cells (C/E) were counted by using direct microscopic examination.

Adherence assay was applied to study Candida adherence to the epithelial cells isolated from patients with urinary tract disease as well as apparently healthy individuals (control).

Statistical analysis

t -Test was used with P value < or = 0.05 considered significant [9].

Results

A- Urine from patients with bacterial growth:

The percentage of uroepithelial cells with adhered Candida (%E C) was counted before and after Candida adherence assay. Table (1) showed that %EC after adherence assay were significantly higher (P<0.01) compared to that before assay. The highest differences in % EC before and after assay was noticed with uroepithelial cells obtained from urine infected with Klebsiella (35.3%) followed by that with Streptococcus (32%) and then with E.coli (29.2%).

Table 1. Percentage of EC (%EC) obtained from urine with different bacterial infections before and after Candida adherence assay.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
Klebsiella pneumonia	25.3±6.4	60.6±4.5*	35.3
Escherichia coli	35.9±14.8	65.1±13.7*	29.2
Streptococcus pyogenes	34±7.8	66±1.8*	32

EC= Epithelial cells with Candida, *P< 0.01

The mean number of candida per uroepithelial cell (C/E), (Table 2), was found to be higher with uroepithelial cells obtained from urine sample with different bacterial infections after assay compared to that before assay (P<0.01) and the difference between C/E after assay and before assay was the highest with epithelial cells obtained from urine with Klebsiella (49.6%) followed by that with Streptococcus (47%) and then with E.coli (32.2%).

Table 2. Mean number of Candida per epithelial cell (C/E) obtained from urine with different bacterial infections before and after adherence assay.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
Klebsiella pneumonia	37±18.5	86.6±20.8*	49.6
Escherichia coli	55.8±17.9	89±10.5*	33.2

Streptococcus pyogenes	47±15.1	94±18.2*	47

EC= Epithelial cells with Candida, *P< 0.01

B-Urine from patients with negative bacterial growth but with positive urine deposits:

Table (3) showed that %EC was significantly increased (P<0.01) after Candida assay compared to that before assay. %EC difference was found to be the highest with uroepithelial cells having high number of uroepithelial cells (32.7%) compared to the epithelial cells with pus cells (24.5%)and then epithelial cells with red blood corpuscles (RBCs) (21%).

Table 3. Percentage of EC in epithelial cells obtained from urine deposits with different microscopic findings and no bacterial growth.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
>10 epithelial cells/ HPF	37.5±9.1	70.2±8.1*	32.7
> 5 pus cells/ HPF	36.5±20.3	61±13.2*	24.5
> 3 RBCs/ HPF	48.6±11.2	69.6±11.2*	21

* P < 0.01

Table (4) revealed that C/E in the presence of different urine deposit findings were significantly higher (P<0.01) after adherence assay than before. The positive epithelial findings showed the highest difference followed by RBCs and pus cells (31.5, 26.6, and 25.3 respectively).

Table 4. Mean number of C/E obtained from urine with different abnormal urine findings and no bacterial infection.

Epithelial cells From urine with:	% EC before adherence assay ± SD	% EC after adherence assay± SD	The difference
>10 epithelial cells/ HPF	55.7±21.1	87.2±13.8*	31.5
> 5 pus cells/ HPF	53.7±26.1	79±14.8*	25.3
> 3 RBCs/ HPF	68±13.6	94.6±8.7*	26.6

* P < 0.01

It was noticed that urinary tract disease with and without bacterial growth has an enhancing effect on adherence activity compared to control (Table 5). Percentage of EC in uroepithelial cells obtained from urine with bacterial growth was found to be higher than that without bacterial growth. The same is true for mean number C/E.

Table 5. %EC and C/E mean difference between results before and after Candida adherence assay for patients compared to control.

	Mean of difference		
	Uroepithelial cells from urine with (-) growth	Uroepithelial cells from urine with infection	Control
% EC	32.2	26.1	3.5
Mean C/E	43.3	27.9	5

Discussion

C. albicans are commonly found on mucous membranes (e.g. vagina and gastrointestinal tract) and it is an opportunistic pathogen. Vaginal colonization is increased by Diabetes mellitus, pregnancy and the use of oral contraceptive agents [10]. Previously, colonization with bacteria was thought to inhibit fungal infections in situation where the bacterial flora does not adhere to yeast [11]. Later studies suggested that certain pilliated bacterial strains do significantly enhance yeast adherence to host epithelial cells. It was shown by Ahearn et al [12] that C. albicans is frequently found concomitantly with bacterial urinary tract infections. Working on adherence of three species of Candida it was indicated that C. albicans adhered to vaginal and buccal epithelial cells to a significantly greater degree than other species tested [13]. Date extract inhibit germ tube formation of C. albicans which might contribute to the effects on reducing adhesion (14). It can be concluded from the above data that Candida adherence could be under the effect of many factors.

Adherence of Candida species to mucosa and particularly C. albicans is probably an important initial step in the pathogenesis of infection caused by these yeasts [15]. This adhesion occurs as a result of the interaction between yeast and epithelial cells receptors [13]. It was found that the cell wall of C. albicans contains floccular materials which are probably involved in yeast attachment [6]. Candidal adherence to the epithelium and the production of carboxylic acids of short chain as a product of sugar metabolism, produce an acid environment that could affect the pathological process in several ways such as cleavage of secreted IgA, an important factor to prevent Candida adherence [16].

As Candida adheres, their ability to grow and survive contributes to the hydrolytic enzymes, which are extracellularly secreted by the fungus. They may play a central role in the pathogenesis of candidiasis [17].

It is well known that C. albicans adhesion to mucosal cell is enhanced by several factors such as germ tube production, phospholipase, protease, other extracellular enzymatic activities, carbohydrates, pH and temperature [18]. It was suggested that cell wall mannoprotein is an essential component of the C. albicans adhesins [19].

Adherence of K. pneumoniae to mammalian epithelial cells is mediated by the adhesins, Fim H and MrKD which are associated with type 1 and type 3 fimbriae respectively [20]. All the RD6 isolates of K. pneumoniae were found to adhere strongly to mammalian cells in vitro [21]. Regarding E. coli adhesion, it was stated that E. coli has Fim H gene responsible for adherence to epithelial cells [22].

Many studies have shown that bacterial attachment to host cells can be augmented by pilli [6]. The capacity of K. pneumoniae RD6 isolate (genotype) to cause UTI may be mediated by its striking adherence to mammalian cells [19]. The increase in yeast attachment to epithelial cells preincubated with pilliated bacteria is due to an attachment of yeast to mannose-sensitive pilli on bacteria already attached to epithelial cells. This attachment may be an important factor in C. albicans pathogenicity.

In the present study the possible involvement and capabilities of Candida adherence to epithelial cells isolated from urinary tract of patients in the presence or absence of bacterial growth compared to normal was evaluated in vitro. This study revealed that the percentage of epithelial cells with candida (% E C) was higher in the presence of bacterial growth, especially K. pneumoniae after adherence assay where the difference was 35.3 which is similar to findings reported previously by King et al [13]. Candida adhesion to urogenital epithelial cells in the presence of Streptococcus pyogenese was found to be increased. An in vitro study on oral streptococci showed that these bacteria suppressed C. albicans infection which could be due to competition between Streptococcus and Candida for cell receptors

and alteration of the environment by bacterial infection [23]. On the other hand it was found few years later that, group A streptococci is responsible for the attachment of these organisms to mucosal cells [24], which is in agreement with our study. The same result was detected in the presence of E.coli.

Also the mean number of Candida per epithelial (C/ E)after conducting adherence assay was found to be higher in the presence of bacterial growth in particular K. pneumoniae where the difference was (49.6%), whereas for E. coli (33.2%) and then for S. pyogenes it was (47%) with P value < 0.01

Suman et al [25] concluded that increased receptivity of vaginal epithelial cells to pathogens associated with lower local immunity may play role in the pathogenesis of recurrent UTI in females.

In the present study, urine samples without bacterial growth and high number of epithelial cells showed the highest difference in EC% before and after applying adherence assay (Table 3). From these findings it can be concluded that epithelial cells from urine with high number of sloughed epithelial cells are more susceptible to adhesion than others. This may be explained on the same basis of Williams et al [26] findings which demonstrated that increased adherence was associated with increased keratinization of epithelial cells among smokers. It was stated that Candida showed more adherences to superficial keratinize epithelial cells than intermediate cells [27].

Increased number of RBCs in urine which is seen in 40-60% of patients with acute cystitis. Haematuria may be caused by, non-infective pathological conditions or renal mycobacterial infection with or without pyuria. High umber of RBCs or pus cells indicates certain pathological conditions that are not associated with bacterial growth. Such situation was met with this study, which showed an increase in Candida adhesion (EC %) but to a lower extent compared to those with bacterial growth (Tables 1 & 4) at P value<0.01.

In table (4), C/E in the presence of positive epithelial cells showed a significant increase (31.5) compared to positive pus cells (25.6) and positive RBC (26.6) with P value<0.01.

Finally, table (5) revealed that mean% EC and mean C/E in urine obtained from apparently healthy controls were the lowest in comparison to those with bacterial and without bacterial infections before and after applying adherence assay. Also it was noticed that the mean difference in %EC and C/E in a group with bacterial growth is higher than that with only urine findings group and control. Recently an attempt to prepare an anti-candidal IgA in egg yolk (IgY) showed to decrease Candida adhesion which can be used as an adjunct to antifungal drugs [28].

In conclusion, the increase in adhesion of Candida to uroepithelial cells was associated variably with urinary tract diseases with different urine deposits and bacterial growth factors. These changes in Candida adhesion may be due to modulation effect of these factors on uroepithelial surface.

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Neovascularization in prostatic adenocarcinoma as determined by CD34: A retrospective study

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Abstract

Background Microvessel density is one of the variables that are thought to affect the natural history of prostatic carcinoma in as much as its degree influences tumor progression including the ability of invasion and metastasis.

Objectives To assess microvessel density of prostatic adenocarcinoma, and determine its relationship to serum levels of prostatic specific antigen, and carcinoma grade, as determined by Gleason's score.

Methods Thirty patients with prostatic adenocarcinoma were studied. Parameters assessed are patients' age, serum levels of prostatic specific antigen, and the grade of the carcinomas according to (Gleason's scoring system). The values of the serum levels of prostatic specific antigen and the Gleason score were divided into 3 subgroups for statistical purposes. The degree of angiogenesis was evaluated by assessing microvessel density in sections stained immunohistochemically with CD34.

Results The microvessel density ranged from 10 to 35 (average 20.24 ± 5.95). Statistically significant correlation was found between the mean of microvessel density and serum prostatic specific antigen when the cutoff point of was 11 ng/ml, and with Gleason score when the cutoff point was 7.

Conclusion Microvessel density determination can predict the potential biologic behavior of prostatic adenocarcinoma in individual cases. The incorporation of the serum levels of prostatic specific antigen levels and Gleason scores with the former makes such predictions more practical.

Key words Prostatic adenocarcinoma, microvessel density, serum prostate specific antigen, Gleason score.

Introduction

Prostatic adenocarcinoma (PAC) is a significant cause of morbidity and mortality in elderly males. The occult, clinically undetectable forms are even more common. Thus, it is important to understand the changes seen with the early stages of the disease as well as those that are associated with progression. One of these changes is the cancer cell-mediated neovascularization [(1)]. Any increase in the tumor mass must be preceded by a sufficient vascular supply that helps support the growth, as well as augments its ability to invade and metastasize [(2)]. Cancer cells have been found to produce several angiogenic factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Angiogenesis is initiated as proliferation of endothelial cells with subsequent formation of new vessels from the already existing vascular bed [(3)]. This process is normally under a tight regulatory control through a balance between angiogenic stimulators and inhibitors [(4)].

Neovascularization is assessed by microvessel density (MVD). The most common method utilized to quantify MVD is through calculating the number of vessels in specified areas

microscopically [3]. The latter task can be performed more accurately by staining histological sections with antibodies that specifically identify, and thus highlight endothelial cells. CD34 is one of the most commonly used antibodies for this purpose.

The aims of this study are to assess MVD in PAC, to determine whether there is a correlation between serum levels of prostatic specific antigen (PSA) and microvessel density and to correlate the above findings with the Gleasons score of the tumors.

Methods

A retrospective study from August 2004 through August 2008, thirty patients with PAC was studied. Samples were collected from laboratory of the hospital of specialized surgeries. These were represented by thirty specimens of formalin-fixed, paraffin-embedded blocks along with their histo-pathological reports. Parameters assessed included: the patients' age, serum levels of prostatic specific antigen (S-PSA), and the Gleason's score of the tumor. The nature of the surgical specimens submitted for evaluations included 26 samples were transurethral resection (TURP); prostectomies; and 4 samples were needle core biopsies.

The S-PSA levels were divided into 3 subgroups for statistical purposes; levels less than 10 ng/ml, levels between10-29 ng/ml and levels of 30 ng/ml and more.The range of Gleason score was similarly divided into three categories; 4 or less (well differentiated PAC), 5-6 (moderated differentiated PAC), and 7 or higher (poorly differentiated PAC). Two sections, 3 micron each were cut from paraffin blocks. One was stained with H&E stain, and the other with CD34 immunostain (mouse antiendothelial cell marker CD34 class II monoclonal antibody. Clone QBEnd/10. (Dako EnVisionTM code N1632). Both positive (a hemangioma) and negative (replacing the primary antibody with BPS buffer) controls were run together with the every batch of sections. A positive reaction is indicated by a red-brown colored cytoplasmic precipitate. The degree of angiogenesis was evaluated by assessing microvessel density (MVD) [5].

Microvessels were counted without prior knowledge of the grade of the diseases [6]. For the MVD quantitation, the stained slides were screened to identify areas of highest density of neovascularization ("hot spots"). In each section, five hot spots were chosen. The microvessel count (MVC) is the number of microvessels present within a 200X field (equivalent to 0.740 mm^{2}). The average of the MVCs was obtained by dividing the sum of the five values by 5 [7-9]. All the statistical analyses were performed through the SPSS program (version-12) and Excel application. Inferential statistics used included Binomial ANOVA test, and t-test.

Results

This study included 30 cases of PAC. The range of the patients' age was 52 to 85 year (mean 69.18 ± 7.59 years). The Gleason score was calculated for only 26 out of the 30 cases studied; in four cases the material present were insufficient for such determination being represented by needle biopsy samples. The diagnosis of carcinoma in such cases was made primarily on the presence of perineural invasion, vascular and lymphatic invasions. The mean of MVD was from 10 to 35 (average 20.24 ± 5.95). The mean of MVD in specimens collected from patients with a PSA level of less than 10 ng/ml (3 cases) was 13.2 ± 3.6, whereas those with PSA level of 30 ng/ml or more (11 cases) showed a mean MVD of 22.4 ± 5.2. Put it in another way, all of those with a PSA level of less than 10 ng/ml showed a MVD less than 10. Those cases with a PSA levels lying between 10 to 29 ng/dl (16 cases) showed a mean MVD of 19.5+5.43. Statistical analysis showed that there was significant difference in the mean of MVD in those patients with PSA level of 11 ng/ml or more compared with those expressing PSA level of less than 11 ng/ml. (P = 0.005) as seen in figure 1.

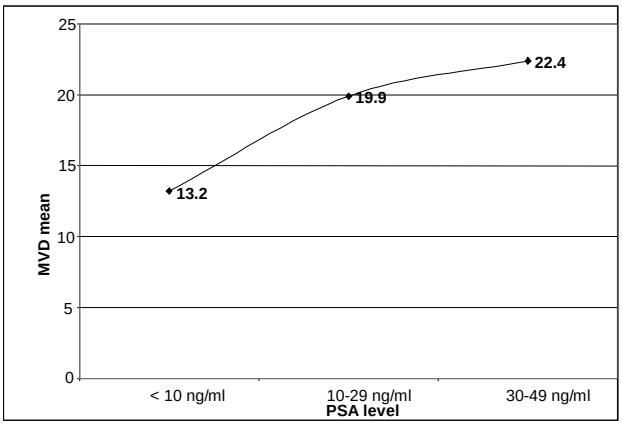

Figure 1. Distribution of the mean MVD in relation to serum PSA level

Regarding the relation between MVD & the grade of PAC, the mean MVD in cases of well differentiated PAC (Gleason score 4 or less) was 16.2 ± 5.7 versus 23.02 ± 4.9 for poorly differentiated PAC cases (Gleason score 7 +) as seen in figure 2.

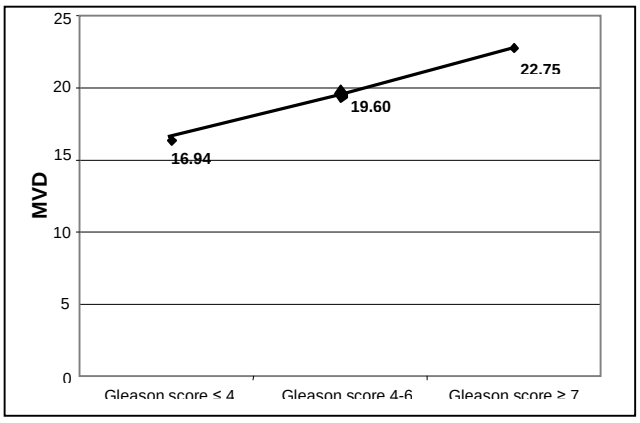

Figure 2. Distribution of the mean MVD in relation to the grade of prostatic cancer (Gleason score)

The majority of the latter group (Ten out of 12 cases; 83.3%) had a MVD count of 20 or more. A statistically significant difference was noted among the means of MVD of those with Gleason score below and those above 7 (p = 0.0036) as seen in (figures 3 and 4).

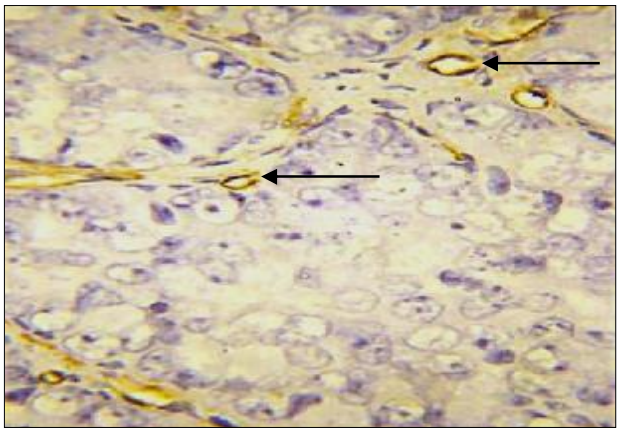

Figure 3. Poorly differentiated adenocarcinoma (Gleason score 9) showing prominent MVD showed the CD34 immunostain as brownish cytoplasmic discoloration highlighting the vascular endothelium. (40x).

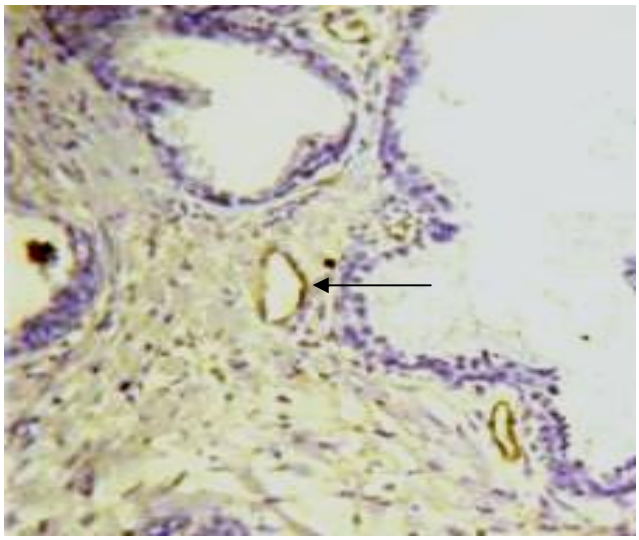

Figure 4. Well differentiated adenocarcinoma (Gleason's score 3) showing low microvessel density. The vessels are highlighted by CD34 immunostaining as brownish cytoplasmic discoloration. (10x).

Discussion

Angiogenesis of malignant tumors is essential for cancer growth, invasion and metastases [10]; PAC is no exception. Several studies have shown that the degree of angiogenesis does indeed influence the natural history of PAC through its correlation with the tumor aggressiveness and metastatic potential [10, 11] A

close correlation has been observed between MVD and patient survival in several malignant tumors including PAC.[7] The correlation between MVD using CD34 marker and such variables as tumor grade (Gleason score), and S-PSA levels were investigated.

This study has substantiated, indirectly, the significance of angiogenesis in relation to the biological behavior of PAC. In this study there was a definite correlation between the MVD of PAC and S-PSA levels; this has reached a statistical significance when the patients were segregated into two groups with a cutoff point of 11 ng/ml. Our results is supported by a similar study carried out by Strohmeyer et al [(12)], despite the fact they used a different marker; a polyclonal antibody against factor VIII. On the other hand, these results were contradicted by Kaygusuz et al [(13)] using CD34, who claimed that no such correlation existed. However, more independent studies on this particular point have to be carried out to clarify the relation between these two variables.

In this study there was a positive correlation between MVD and the grade of PAC as determined by the well-known Gleason score. This correlation also assumed a statistical significance when the cutoff point of the score was 7. These findings were augmented by two other studies; the findings of Bettencourt et al [(14)] and those of Kaygusuz et al [(13)]. In these two studies, like ours, CD34 monoclonal antibody was implemented. As it nearly always the case, the aforementioned conclusion was opposed by Rubin et al [(15)] and Silberman et al[16] through their application of CD31 monclomal antibody (instead of CD34). Although their samples size was larger than ours; but studies utilizing CD34 marker (including ours) are admittedly more reliable since CD34 is acknowledged of being more reliable than CD31 as endothelial cells markers.

Finally, we concluded that the MVD determination can predict the potential biologic behavior of PAC in individual cases. The incorporation of the S-PSA levels and Gleason scores with the former makes such predictions more accurate.

We recommend further studies of similar nature to be carried out with larger samples size, and to investigate in addition other prognostic variables of PAC in relation to MVD. Moreover, to establish a research program to assess the efficacy of angiogenesis therapy alone and in conjugation with radiotherapy and chemotherapy.

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Evaluation of the Occluding Effect of Sensodyne with Strontium Chloride 0n Microleakage through Dentinal Tubules of Endodontically Treated Teeth

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Abstract

Background Dentin hypersensitivity may be defined as pain arising from exposed dentin. The relationship between dentin hypersensitivity and the patency and Microleakage through dentinal tubules has been established in vitro. Currently the most accepted mechanism of nerve activation associated with dentin hypersensitivity appears to be hydrodynamic in nature.

Objectives The concept of tubule occlusion as a method of dentin desensitization is a logical conclusion of the hydrodynamic theory.

Methods Forty two human maxillary anterior teeth were selected, the crowns were cut away at the CEJ, the root canals prepared, cleaned and filled then the coronal access closed with zinc phosphate cement. The specimens were randomly divided into four groups, two of them experimental A and B and two controls C (positive) and D (negative). All the specimens were coated with sticky wax except the ring that had cementum removed while the negative control specimens were entirely coated with sticky wax. All the specimens were stored in human saliva which was changed daily for 21 days with daily brushing of specimens of group A with a Sensodyne tooth paste (that with active ingredient Strontium chloride). At the end of the 21 days, the specimens were immersed in pelikan ink for three days then the sticky wax was removed, after that the teeth were cleared to make them transparent to provide a three dimensional assessment of dye penetration. The teeth were subjected to linear dye penetration measurement and scanning electron microscope analysis to investigate whether Sensodyne with Strontium chloride occlude dentinal tubule orifices.

Results Strontium chloride, the active ingredient of Sensodyne tooth paste has a tubule occluding property which may indicate a therapeutic potential in vivo. Also scanning electron microscopic analysis revealed presence of deposits in and around the tubular orifices.

Conclusion Sensodyne with strontium chloride proved effective (*in vitro*) in occluding the orifices of patent dentinal tubules.

Keywords Dentin hypersensitivity, Dentinal tubules occlusion, Sensodyne with strontium chloride.

Introduction

Dentin hypersensitivity is a common painful dental problem, exposure of cervical dentin and patency of the dentinal tubules may occur through the loss of covering enamel -and / or gingival recession with loss of cementum. Both processes have multifactorial etiology [(1)]. These exposed dentinal tubules transmit various thermal, chemical, and bacterial stimuli from the dentinal surface to the pulp [(2)]. Currently, the hydrodynamic theory [(3)] is the most widely accepted theory for explaining the mechanism of dentin hypersensitivity, this theory explains that fluids move within

dentinal tubules in response to external stimuli and that this movement of fluids stimulate mechanoreceptors in the pulp to cause pain.

Many studies reported that most of the tubules on sensitive dentin are opened and that the occlusion of these tubules reduces the sensitivity by preventing or minimizing the permeability of dentin [4].

This permeability allows the bidirectional movement of materials from the oral cavity to the pulp and vice versa. The pulp irritation associated with Microleakage through dentinal tubules is often dictated by the permeability of dentin [5].

Microleakage may be presumed to occur after exposure of the dentinal tubules to oral fluids, factors affecting this microleakage are:

Patency of the dentinal tubules, number of the dentinal tubules exposed and the exposure time [6].

Natural occlusion of the exposed dentinal tubules can occur through the formation of calculus, deposition of intra tubular crystals from dentinal fluid or saliva, formation of peri tubular dentin or intra tubular deposition of collagen or plasma protein [7].

For certain reasons such as excessive acidic food, traumatic tooth brushing, alteration of the composition of saliva and periodontal diseases and therapy, the external openings of the dentinal tubules may become uncovered and Microleakage may occur [1].

Different findings concerning the occluding effect of desensitizing agents on open dentinal tubules have been reported [1] and the effect of various desensitizing agents to occlude the patent tubules had been reported by a number of studies and various agents had been used either topically or with a dentifrice.

The problem of microleakage through dentinal tubules following the application of a desensitizing agent was not solved and the evidences from previous research works were not clear, for this reason this study was addressed to this problem, the desensitizing agent used was Sensodyne that contains Strontium chloride, the choice was based on the popularity of the product and its relative effectiveness.

The aim of this study was to determine and evaluate the dye microleakage through exposed dentinal tubules at the cervical level of endodontically treated teeth and to evaluate the occluding effect of Sensodyne dentifrice (with the active ingredient Strontium chloride) on the exposed cervical dentinal tubules using the scanning electron microscopy.

Methods

This study was done in Al-Majd General Company for Military Industries in Al-Jadiriya near Al-Furrosiya Club.

Forty two recently extracted human maxillary anterior teeth were randomly collected to be used in this study. No data regarding age, sex, and causes for extractions were recorded.

All the teeth, after extraction were stored in normal saline at room temperature. Any calculus, soft tissue or debris was removed by ultrasonic scaling. Then the crown of each tooth was cut away at the Cementoenamel junction by a diamond disc and conventional handpiece and standard endodontic access preparations were made by carbide round bur No.3. The root canal contents were removed with barbed broachs, patency of the apical foramen was determined using No.1 0 file, and the working length was established 1mm short of the length at which this file exited the foramen.

The canals were chemomechnically prepared to a No. 30 Master Apical File and instrumentation completed by step back flaring to No. 60 file. Copious irrigation with 2.5% solution of sodium hypochlorite was used throughout the canal preparations, and then the canals were dried with paper points.

Master gutta percha cones were fitted to within I mm of the working length in 37 out of 42 teeth, five teeth were not obturated and reserved as positive controls. The canals and gutta-percha cones were coated with zinc -

oxide base sealer and obturation completed using the lateral condensation technique, excess gutta percha was removed with heated instrument to a level apical to the orifices of the canals by two millimeters then the coronal access preparations were closed with zinc phosphate cement. All the teeth were stored in 100% humidity at 37^{o}C for 48 hours to allow the sealer enough time to set. Then in 37 out of the 42 teeth, the dentin was exposed by removing the cementum, in five teeth, the cementum was not removed (dentin not exposed) and reserved as negative controls.

A 3mm wide ring of root surface, 2mm apical to the coronal rim of each specimen was cut by a rotary instrument attached to a special micro -lathe. Each root was fixed by a vise (clamp) horizontally in front of the instrument and this was set to cut only one millimeter all around the root while it was rotating to obtain a collar of 3mm width and 1mm depth around the root in order to remove all the cementum and expose the dentine in this area.

The specimens were then randomly divided into four groups:

1- *Group A:* 16 teeth. In this group, the specimens were treated with the desensitizing agent Sensodyne (with strontium chloride) on the exposed dentin.

2- *Group B:* 16 teeth. In this group, the specimens were not treated with the desensitizing agent on the exposed dentin.

3- *Group* C: (positive control) = 5 teeth, in this group the canals were not obturated but the cementum was removed and dentin exposed

4- *Group D:* (negative control) = 5 teeth, in this group the canals were obturated but the cementum was not removed and the dentin not exposed.

The external surfaces of each specimen of group A, Band C was coated with two layers of sticky wax except for the ring that had cementum removed, while the external surface of specimens in group D was entirely covered with two layers of sticky wax.

The ring cut of the surfaces of specimens of group A, Band C was treated with saturated solution of citric acid pH1 for 30 seconds to remove the smear layer then washed with distilled water for five minutes.

All specimens were stored in whole human saliva collected from the same person and changed daily for 21 days. During this period, the specimens of group A were brushed with Sensodyne dentifrice (including strontium chloride) using a medium brush twice daily for one minute each and then rinsed with distilled water and re-immersed in saliva which was collected daily from my clinic secretary man.

At the end of three weeks, the specimens were removed from the saliva, washed with water and then immersed in Pelikan Ink for72 hours, after that removed and washed with water for 30 minutes and then the sticky wax was removed by a sharp wax knife.

The specimens were decalcified in 5% nitric acid for five days with renewing the acid daily and at the end of five days, the specimens were washed for four hours under running water. After that, the specimens were dehydrated in increasing concentrations of Ethyl alcohol, and then immersed in Methyl Salicylate for 24 hours to make them transparent.

Maximum apical extension of the dye using a Vernier. Two readings were taken for each specimen by two evaluators and the average of the two readings were considered for statistical analysis. The collected data was analyzed by a professional statistician using means, standard deviation, tables and bar charts then the t-test was used to compare between the two experimental groups A and B.

Eight specimens randomly selected, four from each group A and B were examined in a scanning electron microscope at 20 kV after gold platting. Micro- photographs of the exposed dentinal surface were taken at various magnifications.

Many photos were scanned and exposed then we choose the clearest ones. All the specimens were gold – platted and scanned at 20 Kv.

Results

All the experimental teeth of group B (not treated with Sensodyne) showed much more dye penetration than the experimental teeth of group A (treated with Sensodyne), table 1.

Table 1. Level of dye penetration (in mms) for groups A and B.

Specimen No.	Group A treated with Sensodyne	Group B untreated with Sensodyne
1	0.35	1.65
2	0.36	2.10
3	0.54	1.80
4	0.56	2.14
5	0.37	1.57
6	0.58	0.98
7	0.36	1.30
8	0.34	2.19
9	0.50	2.03
10	0.45	1.60
11	0.34	0.99
12	0.51	2.05
13	0.57	1.50
14	0.35	2.14
15	0.58	1.40
16	0.55	1.25
The mean leakage	0.456875	1.668125

The positive control group (group C) demonstrated complete dye penetration, while the negative control group (group D) showed no dye penetration.

Figure 1 shows the comparison between the mean leakages for both groups A and B while table 2 shows the summary of the statistics for both groups A and B (The mean leakage, standard deviation, the maximum and minimum values).

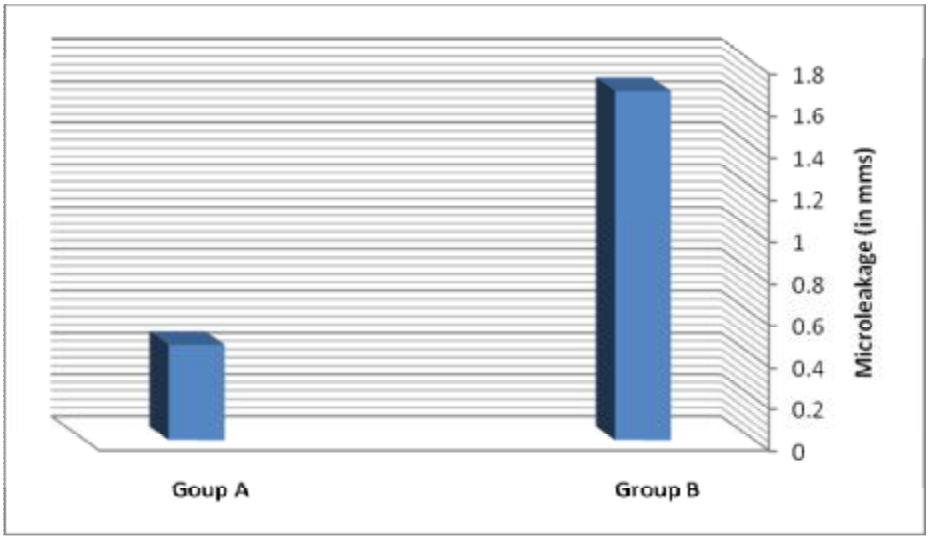

Figure 2. Comparison between mean leakage (in mms) for both groups A and B.

Table 2. Descriptive statistics for experimental groups A and B.

Group	No.	Mean±SD	Maximum Leakage	Minimum Leakage
A	16	0.456375±0.697	0.51	0.14
B	16	1.668125±0.399753	2.19	0.98

Comparative of significance using the t-test showed very highly significant difference ($P<0.0001$) between the two groups through the difference of mean value (leakage).

The scanning electron microscopic analysis showed many deposits on the dentin surface in and around the orifices of the dentinal tubules for specimens of group A (Figure 2).

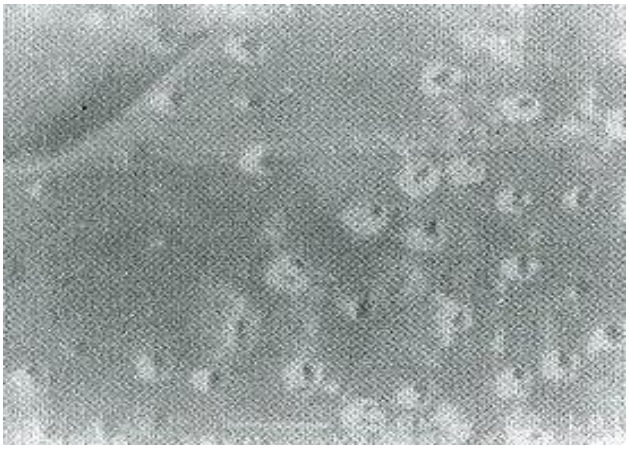

Figure 2. Scanning electron microphotographs showing an area of dentin treated with Sensodyne (Group A).

While the SEM analysis for specimens of group B showed the dentin surface free of any deposits and the dentinal tubules orifices were patent (Figure 3).

Figure 3. Scanning electron microphotographs showing an area of dentin not treated with Sensodyne (Group B).

Discussion

Microleakage is considered as one of the major causes of endodontic failure [8]. Exposure of the root canal space to oral fluids through the loss of temporary seal, recurrent caries, marginal leakage or possibly exposed dentinal tubules may lead to salivary and bacterial contamination [9]. This microleakage may occur between the sealer and the walls of the root canal or between the sealer and root canal filling material.

Microleakage may be presumed to occur after exposure of dentinal tubules to oral cavity fluids, determinant factors are maintenance of patency of dentinal tubules, number of dentinal tubules exposed and the exposure time.

When dentin is exposed due to abrasion, grinding, scaling, caries or cementing a crown, a mat of mineralized material may form within two weeks occluding the dentinal tubules (Natural occlusion) [7], but for many reasons such as excessively acidic food, alteration of the composition of saliva or traumatic tooth brushing, the exposed dentinal tubules remain uncovered and patent [1].

Periodontal diseases and therapy are other important causes of exposure of cervical dentin and dentinal tubules particularly after treatment with citric acid which opens and enlarges the orifices of the tubules.

Studies on the occlusion of dentinal tubules were performed in vivo and in vitro and different findings concerning the occluding effect of the desensitizing agents have been reported. Most of these studies have focused on coronal dentin where important variables such as dentin surface area, thickness and surface characteristics can be controlled.

This study focused on radicular dentin, the permeability of radicular dentin has been observed to be much lower than that of coronal dentin and there is a good correlation between tubule number and diameter and the permeability [10]. In addition to the microleakage through exposed dentinal tubules, the present study investigated the potential tubule - occluding effect of strontium chloride hexahydrate 10% included in Sensodyne toothpaste.

Pelikan Ink was used as a dye and a clearing method was used to study the microleakage which provided us a direct three dimensional measurements.

The cleared positive control teeth (group C) showed complete dye penetration throughout the root canal length while the cleared negative control teeth (group D) showed no dye penetration.

This proved that the dye microleakage in other groups was through the exposed dentin which indicated good experimental design and good coating process.

From the analysis of data of the experimental groups A & B it was found that group B (untreated with Sensodyne) had higher mean of dye penetration than group A (treated with Sensodyne) with high significant difference ($P < 0.0001$) between the two groups. This proved the occluding effect of Sensodyne on the exposed dentinal tubules of specimens of group A. this effect was confirmed by the scanning electron microscopic study of specimens from both groups A & B which showed irregular deposits in and around the orifices of dentinal tubules of specimens of group A . While specimens of group B showed opened orifices of the tubules.

The present study demonstrated that the interface between the canal walls and the root canal filling material could be penetrated by the dye through patent dentinal tubules in the cervical surface of roots exposed to saliva. It could be concluded that the dye penetrated the dentinal tubules and then through the canal walls and sealer which represent the previous leakage of saliva.

This conclusion was comparable to what Berutti [6] found that exposed cervical dentin which was exposed to saliva for various periods showed dye penetration between the canal walls and the sealer to increasing depths proportional to the time of exposure to saliva.

The difference between our study and Berutti [6] was the period of exposure to saliva since in our study it was 21 days while in Berutti [6] study was 20, 40 and 80 days. In addition that Berutti [6] did not include occlusion of exposed dentinal tubules by a desensitizing agent.

Despite the different views about the effect of Sensodyne with strontium chloride in occluding patent dentinal tubules, various clinical trials have verified its desensitizing effect. Many earlier reports have indicated the tubule occluding effect of Sensodyne.

Pawlowska [11] found that strontium chloride binds strongly to dentin and this support our study.

Collins and Perkins [12] found that Sensodyne reduced dentin sensitivity and occluded patent dentinal tubules and this also supports this study.

Jensen and Doering [13] proposed that the mode of action of strontium was by binding to the matrix of the tubules thus reducing its radius; this might explain the results of Minkoff and Axelrod [14] who reported that the effect of strontium chloride emerged during the 2nd week and it significantly reduced dentin hypersensitivity by the end of the twelfth week.

The results of the present study were in agreement with the results of Gillam et al [15] who investigated the potential tubule occluding effect of five selected desensitizing agents: Sensodyne, Butler protects, OxaGel, ABbond 2 and One-Step. They found that all these agents produced some occlusion of the tubules with different levels of coverage.

On the other hand, Topbasi [16] reported that Sensodyne did not have significant effect in

occluding dentinal tubules in vitro but most of the tubules were occluded in vivo. The difference in the results between Topbasi [16] study and ours might be due to the difference in the method of storage since they stored the teeth in distilled water while the teeth in our study were stored in natural human saliva.

The results of the present study disagreed with the results of Clark et al [17], Mostafa et al [18] and Addy and Mostafa [19] who attributed the failure of Sensodyne in occluding dentinal tubules to the silica-based abrasive system in it since it produces characteristic irregular deposits of silica-containing diatomaceous earth on the dentin surface with partial occlusion of the tubules which were washable by the saliva. This difference in the results may be that the present study involved the in vitro application of Sensodyne. In the clinical situation it is doubtful that Sensodyne have such a high concentration, this difference in concentration probably accounted for the differences in the results.

Also, the results of the present study were not comparable with the results of Ling et al [20] who found that Sensodyne toothpaste produced granular-like deposits which were localized on the dentin surface between the orifices and around the periphery of the tubular openings but were largely removed by washing or rinsing. This difference in the results might be due to the differences of techniques and amount of toothpaste applied over time, since Ling et al [20] used two minutes brushing and six hours rotation in saliva then ten seconds rinsing with distilled water.

However, the results of the present study suggested that Sensodyne dentifrice with strontium chloride hexahydrate has a promising effect on the reduction of microleakage through exposed dentinal tubules by its occluding effect on these patent tubules and it performed well in vitro.

Conclusions

With the experimental confines of this in vitro study it was concluded that:

1. It was possible that the interface between the canal wall and the root canal filling material to become recontaminated because of microleakage through patent dentinal tubules in the cervical surface of roots exposed to saliva.
2. The cervical dye penetration was significantly more in group B (untreated with Sensodyne) than in group A (treated with Sensodyne), thus indicating the occluding effect of Sensodyne with strontium chloride on the exposed dentinal tubules.
3. The SEM photomicrographs revealed that Sensodyne with strontium chloride produced precipitates form in the orifices of dentinal tubules resulting in occlusion or reduction of their lumen.
4. Sensodyne with strontium chloride proved effective in vitro, in the in vivo situation it may employ other mechanism of action that cannot be simulated in this in vitro model.

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Carcinoid causing Bowel Obstruction; a Case Report with Literatures Review

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Abstract

In 1907, Siegfried Oberndorfer (1876–1944), a German pathologist at the University of Munich, coined the term karzinoide, or "carcinoma-like," to describe the unique feature of behaving like a benign tumor despite resembling a carcinoma microscopically. Carcinoid tumours are characteristically low grade malignant tumors with neuroendocrine differentiation that have been described in several locations, including the gastrointestinal, respiratory, hepatobiliary, and genitourinary systems. Carcinoid tumors most commonly occur in the gastrointestinal tract (74%) and bronchial system (25%). less than 1% of cases these tumors have been reported in the genitourinary system.

In this report we describe a 55-year-old house wife female who presented with vague gastrointestinal complaints for long period then she developed intestinal obstruction due to small bowel carcinoid. The clinical findings are used to illustrate pathophysiology, classification, management of carcinoid tumours through review of literatures.

Keywords: carcinoids tumours, neuroendocrine tumours, small bowel obstruction.

Introduction

In 1907, Siegfried Oberndorfer (1876–1944), a German pathologist at the University of Munich, coined the term karzinoide, or "carcinoma-like," to describe the unique feature of behaving like a benign tumor despite resembling a carcinoma microscopically [(1)]. Carcinoids of the small bowel arise from the enterochromaffin cells, or Kulchitsky cells, found in the crypts of Lieberkuhn. These cells are also known as argentaffin cells because of their staining by silver compounds. Carcinoids may be classified by embryologic site of origin (foregut, midgut and hindgut) and secretory product (multihormonal and 5-HT, 5-HT and substance P, multihormonal) [(2)]. More recently, the World Health Organization (WHO), in an effort to clarify the classification of carcinoid tumors and to standardize a system that would enable clinicians to compare patients and predict outcomes accurately, proposed a new classification of gastroenteropancreatic NETs; based on their malignant potential [(3)].

Within the gastrointestinal tract, nearly 45% of carcinoids arise in the small intestine, making this the most common location for carcinoid tumors. Likewise, carcinoid tumors account for the highest percentage of small bowel tumors, representing approximately one third of all small intestinal neoplasm's [(4)].

These tumors commonly present in the sixth or seventh decade of life with symptoms of abdominal pain or small bowel obstruction (SBO). Small bowel carcinoids are frequently multiple, exhibiting multicentricity in up to 30% of patients, and often display metastases to the lymph nodes (39%) or the liver (31%). Development of typical carcinoid syndrome is rare, manifesting in approximately 5%-7% of patients; however, younger patients are more

likely to develop carcinoid syndrome and display a worse prognosis [5].

Malignant carcinoid syndrome, the predominant clinical feature of carcinoid tumors, results from excessive secretion of hormone products into the systemic circulation. These hormones (peptides and amines) cause the extreme symptoms of the disease, and a reduction in their circulating blood concentrations through targeted treatment is a therapeutic goal [6].

Carcinoid syndrome does not usually develop until a tumor has metastasized - usually to the liver - and the hormonal products released by the tumor reaches the circulation in substantial concentrations. The likelihood of occurrence and the associated severity of carcinoid syndrome depend on several factors: tumor size, whether its location is in an area draining into the systemic circulation, and the degree of metastasis, Carcinoid syndrome exhibits slow growth with early ill-defined symptoms and is frequently misdiagnosed as "irritable bowel syndrome" or "spastic colon". Surgical removal of the tumors is the primary therapeutic option; chemotherapy is less effective. Octreotide is the primary medical therapy for the management of certain symptoms associated with carcinoid syndrome [7].

After the onset of clinical symptoms, median survival times of 3.5 to 8.5 years have been reported; 5-year survival has ranged from 30% to 67%. The prognosis for poor patient outcome generally correlates inversely with increasing levels of urinary 5-HIAA excretion. Other biochemical indicators of a bad prognosis are high levels in the plasma of neuropeptide K and chromogranin A [8] .

Case report

The patient was a 65-year-old house wife, female who presented with vague gastrointestinal complaints, namely, softer stools, abdominal cramps, and poor appetite for more than 6 months. She developed lower colicky abdominal pain increased in severity and associated with bile stained vomiting of 3 days, the condition not responded to the usual medication and supportive measures so that the patient referred to Al-Karama Teaching Hospital for further evaluation at 25th of June, 2008. On examination an elderly female looks ill, dehydrated, vital signs show tachypnea, tachycardia and hypotension with abdominal distention and sluggish bowel sounds.

Blood investigations show elevated WBC (14000), blood urea (50 mg/ dl) and normal blood sugar and haematocrit. Plain x-ray of abdomen shows dilated small bowel loops with air fluid levels (Figures 1).

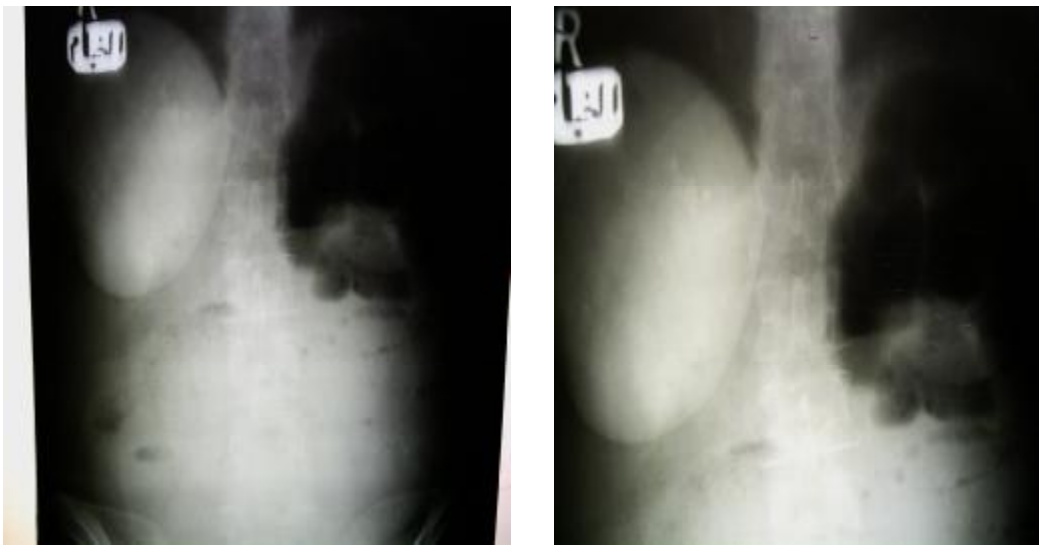

Figure 1. Plain x- ray of abdomen showing dilated segment of bowel with air fluid levels.

The diagnosis of intestinal obstruction confirmed and after adequate resuscitative measures the abdomen explored under general anesthesia through a midline incision where the cause of obstruction identified as a constricting mass in the terminal ileum and right hemicolectomy performed. The patient had uneventful smooth postoperative period while awaiting for the result of histopathology, no further treatment was required a part from follow up using regular ultrasound scanning, CT scan of abdomen and investigation including 5 Hydroxi–Indol Acetic Acid in urine which was negative both in the postoperative period and for 1 year following operation.

Macroscopic findings

Grossly, Segment of bowel, with mesentery and omentum, with the cecum 25 cm in length, with a constricting like mass (Figure 2).

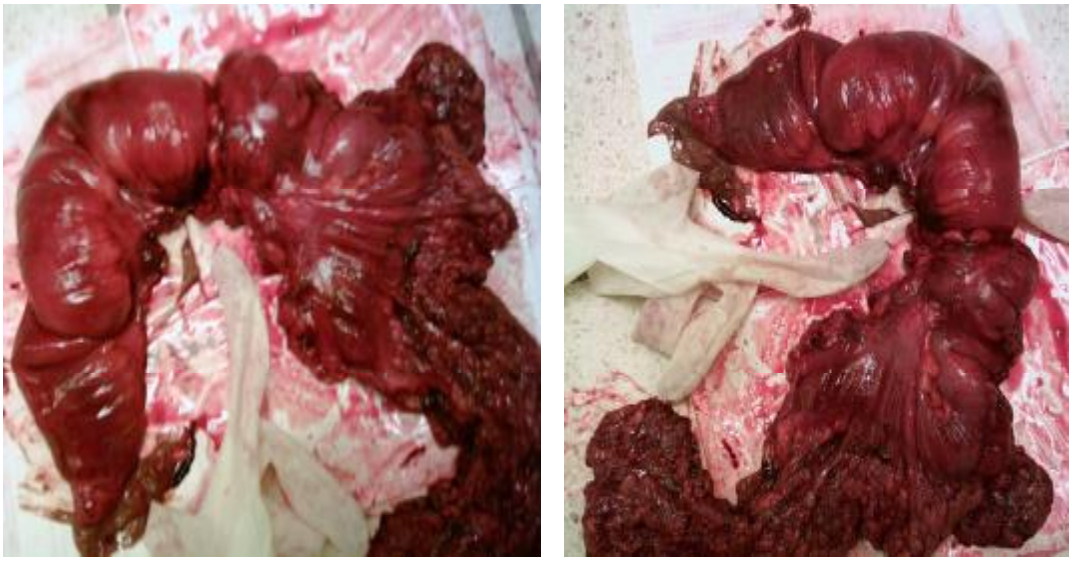

Figure 2. Segment of bowel including the cecum, with mesentery and omentum

Microscopic findings

The histopathological examination revealed solid mass of monotonous appearing cells with small nuclei, inconspicuous cytoplasm with tumor emboli in lymphatic vessels. Picture goes with carcinoid tumour of small intestine; resection margins are free from tumour (Figure 3).

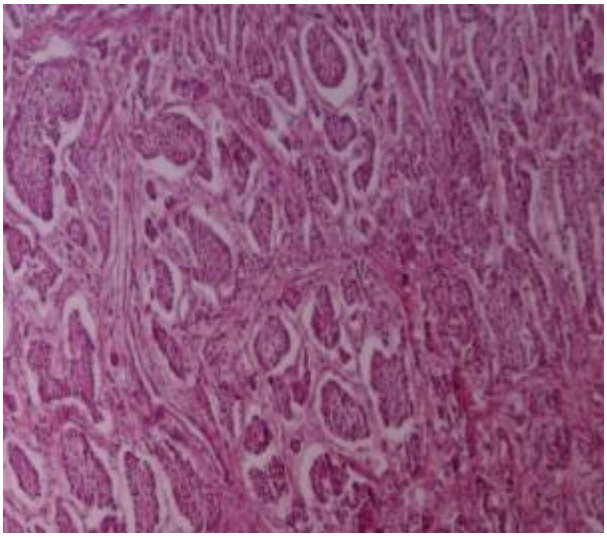

Figure 3. Solid mass of monotonus appearing cells with small nuclei.

Discussion

Carcinoid tumors are characteristically low grade malignant tumors with neuroendocrine differentiation that have been described in several locations, including the gastrointestinal, respiratory, hepatobiliary, and genitourinary systems [9]. The incidence of carcinoid tumours diagnosed during life is rising with gastrointestinal carcinoids making up the majority; earlier estimates were of fewer than 2 per 100 000 per year [10]. More recent studies have found rates approaching 3 per 100 000, the changes in incidence may result more from changes in detection than in the underlying burden of disease [11].

Many clinicians prefer to avoid surgery in patients with carcinoid neoplasia, because of its slow growth and relatively favorable prognosis, nevertheless, the commonest cause of death in patients with carcinoid is advanced metastatic disease, and both clinical and epidemiological data indicate that the more effectively the disease is ablated, the more long-lasting the benefit [12].

Although primary size is correlated with the presence of nodal with or without liver metastases, carcinoid tumours < 1 cm in diameter may be metastatic at presentation, particularly those arising within the small intestine, resection of all sizes of carcinoid with local and regional nodes is preferred to prevent nodal dissemination causing mesenteric ischemia with or without infarction, histopathological assessment helps to determine the need for hemicolectomy [12]. Liver resection has been followed by prolonged 5 year survival in several series and is recommended in appropriate patients to attempt cure or to debulk metastatic disease [13,14].

Chemoembolization may play a role in relieving symptoms and providing sustained tumor control, the aim of hepatic artery chemoembolization is to control hormone-related symptoms, to inhibit growth, and to improve the chances of survival [15]. The technique usually consists of the injection of a mixture of cytotoxic drugs, iodized oil, and Gelfoam (gelatin sponge, Upjohn, Kalamazoo, MI) into the branches of the hepatic artery supplying the tumor, this technique can result in a decrease in 5-HIAA, an improvement of symptoms, and a decrease in size of tumors [15].

Emergency surgery is likely to be indicated in the presence of an acute abdomen, whether carcinoid has previously been identified or not, or is suspected or not – more commonly not. Such surgery should be directed to remove the immediate threat to life, its extent being limited by the condition of the patient. With incipient or established multiorgan failure such circumstances are best managed with limited corrective surgery, reserving until recovery further surgery for tumour clearance or debulking if appropriate. Thus a limited emergency small bowel resection for an obstructing carcinoid tumour might be followed at a later date by elective surgery to remove further small bowel, particularly if by then a second tumour has been identified, and to undertake mesenteric lymphadenectomy [16, 17].

Conclusion

Although carcinoids are rare tumour; they should not be forgotten among causes of intestinal obstruction or patient with vague abdominal symptoms.

Recommendation

A population-based perspective of natural history and of the impact of treatment is essential to formulating an overall approach to tumour management. This is especially true for rarer tumours such as carcinoid.

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Hydatid Cyst of the Rib: A case report

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Abstract

The hydatid cyst is endemic in our country, but bone lesions are less common. The disease often takes the appearance of abscess or malignant lesion. We report a case of a 31-year-old woman with a hydatid cyst of the rib complicated with cutaneous fistula. The surgery allowed both diagnosis and treatment.

Keywords: hydatid cyst, rib, fistula.

Introduction

The hydatid cyst is a parasitic disease caused by *Echinococcus granulosus*, a cestode which remains endemic in some parts of the world.

This study is relevant to Iraq because of the exposure of its rural population to infection due to their proximity to the carnivores, sheep, and bovins. Liver (60%) and lungs (20% to 30%) are the most affected by the disease [(1)]. Osseous hydatidosis is uncommon (0.9% to 4%) especially the ribs. Bone lesions are always primary; secondary lesions are due to recurrence [(2)].

In this case, we present a patient with costal echinococcosis that looks like-as revealed by a cutaneous fistula-an infectious lesion. The course of the disease is usually slow and laboratory tests are often negative. Diagnosis is generally made through the combined assessment of clinical, radiologic, and histopathological test.

Case Report

A 31-year-old woman was admitted to our unit for a skin fistula on the right chest wall. She was not smoker and she had no history of substance abuse. Two years ago, she had suffered from chest pain with swelling of the right chest wall. The swelling was drained two times by general surgeon in private clinic, but still there is recurrence and there is no definite diagnosis.

On admission, physical examination showed a discharging sinus beneath right breast at the right 4th rib's level, with no other abnormality. Blood cell count, hydatid serology, and inflammation markers were normal.

Chest X-rays showed an osteolytic lesion on the 4th right rib (Figure 1). The lung parenchyma and mediastinum were free.

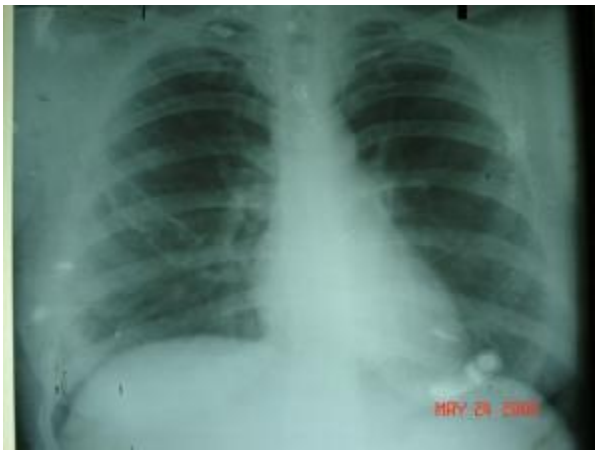

Figure 1: (PA chest x -ray) shows widening 4th rib on right side with osteolytic changes in favor of osteomylitis.

The patient underwent a thoracotomy. An incision was performed along the lateral arch of the 4th rib, where we noticed a hydatid vesicle and bone sequesters. We resect the anterolateral arch of the 4th rib on a 12 cm length. There were no postsurgical complications. Pathological analysis revealed a hydatid cyst of the rib.

Histipathological section shows viable non complicated hydatid cyst with germinal epithelial layer, daughter cyst, laminated membrane H & E 200x (Figure 2).

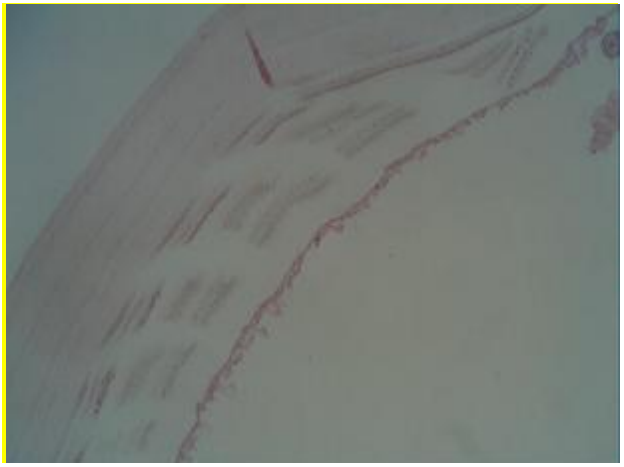

Figure 2. Histopathological section shows eosinophilic laminated membrane in keeping with germinal layer of hydatid cyst. H & E 200x.

Twelve months after this surgery the patient is still in good condition and has no cyst relapse.

Discussion

Hydatidosis, caused by *Echinococcus granulosus*, is still an endemic parasitic disease in the Mediterranean area, South America, North Africa, and Australia. The most common anatomic location of hydatid disease is the liver, followed by the lung. Hydatidosis is located in the bones in 0.5% to 2% of all cases. A hydatid cyst can be seen in any part of the body [3].

Costal hydatid disease is very rare, even in the countries where the disease is endemic. In rib lesions, hydatid cyst destroys the bone matrix and usually infiltrates adjacent tissues which, fortunately, have not happened in our specific case [4]. The disease evolution is generally slow but it is important to note that complications can occur. The diagnosis is usually suspected based on the conditions of life and the radiological aspect. Serological tests might help in the diagnosis, but one has to keep in mind that the best sensitivity barely reaches 82.7% and the best specificity 94.7% [5]. Some authors recommend also CT scan and even ultrasound [6].

Ruptured and infected hydatid cysts are often confused with tumors and/or abscesses [7].

Early diagnosis is important to prevent complications. When an intrathoracic extrapulmonary hydatid cyst lies in the neighborhood of bone structures, it can cause bone destruction. Rupture of a pulmonary hydatid cyst into the pleural space, either spontaneously or during surgery, is the most common cause of pleural hydatidosis or chest wall hydatidosis [4].

In this case, the hydatid cyst is primary; it involved neither the lung nor the liver. The possible mechanism of primary hydatid disease of the chest wall may be as follows: the embryo passes through the duodenal wall into either the portal vein or the periduodenal and perigastric lymphatics. Periduodenal and perigastric lymphatic channels connect with the thoracomediastinal lymphatic and the thoracic duct [8].

The gold standard is to perform surgery in excising the entire rib and to use pre- and postoperative medical treatment [9]. It has been suggested that better results would be achieved by combining surgery and albendazole (10 mg/kg) for presurgery and postoperative prophylaxis, and that large doses over a long period of time would be a good clinical approach and may reduce the incidence of relapse [10]. The preference goes to surgery first followed by 6 months of postoperative prophylaxy by albendazole 400 mg/day, with monthly hepatic balance monitoring, given the risk of hepatotoxicity [9].

Hydatid cyst, especially of the rib, is a very rare disease. However the reported case

demonstrates the importance of this differential diagnosis.

In conclusion, what makes this case special is the remarkably late onset of the disease. Given the patient's history, one might have been led to a wrong diagnosis, especially taking into account that radiology was inconclusive.

CT scan of the chest didn't requested as there is little clinical data that makes hydatid cyst as possible diagnosis.

So we depend on chest X-ray and histopathological report that prove a hydatid cyst and also confirm surgical findings of hydatid cyst membrane inside excised diseased rib.

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