Volume 6 (1) 2008 **ISSN 1681-6579**

IRAQI JOURNAL OF MEDICAL SCIENCES

Iraqi Journal of Medical Sciences

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Editorial:

Cervicovaginal smear (Pap Smear)

Hussam H. Ali *MSc , FICMS* **.**

The broadest amd most successful application of cytopathology has been in the diagnosis of invasive carcinoma of the uterine cervix and precursor lesions through the technique , first described by Aureli Babe's , a Rumanian pathologist , and popularized in 1943 by Dr.George Papanicolaou (1883-1962) at Cornell University and universally known as the pap smear.

The availability of the Pap smear has been accountable for a decrease in deaths from cervical cancer of over 60% during the years from 1950-1980. Unfortunately, as many as 14,000 women still die from cervical cancer every year, related primarily to the fact that most of these women have never had a pap smear or are tested only infrequently.

What is a Pap smear?

Exfoliated cells can be obtained from various body sites for the purpose of obtaining clinical useful information. Many cells and tissues of the body are undergoing constant process of mutation /death/regeneration, and cells that die slough off or exfoliate. Proliferation and maturation of epithelial cells leads ultimately to exfoliation of cells. Methods are available to collect exfoliated cells, primarily from epithelial surfaces. It is also possible to mechanically enhance the exfoliation process to obtain more viable cells or small tissue fragments compared to large tissue sections obtained in surgical biopsies.

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Diagnostic use of the Pap smear

The female genital tract is lined by epithelium. The upper vagina has stratified squamous epithelium, the ectocervix stratified squamous epithelium, the endocervix simple columnar (glandular epithelium), and the endometrium simple columnar (glandular epithelium). All of these epithelia are subjected to the cyclical hormonal influences of estrogen and progesterone during the menstrual cycle, which induces proliferation (increase in the number of cells), and differentiation or maturation (the development of functional and morphologic features of mature cells of the parent tissue type) of these epithelia. Differentiation and maturation of cells are reflected by characteristic morphologic features, which staining techniques allow us to identify. As a point of communication between the outside and inside of the body, the uterine cervix is continually being bombarded by a variety of stressors including mechanical, microbiologic, chemical, and hormonal insults.

In many cases the cellular abnormalities related to pathologic entities present in the cervix can be detected and characterized by means of the Pap smear, based on the morphologic alterations of cells created by theses entities, and on the presence of inflammatory cells and/or the actual presence of microbiologic agents.

The cervical/vaginal Pap smear in adequately collected cellular sample derived from exfoliated or mechanically dislodged cells of the

vagina, cervix , and in some cases endometrium , which have been smeared on a glass slide, adequately preserved and stained , and evaluated cytomorphologically for one or more of the following purposes:

*Detection of occult pathologic abnormalities of the uterine cervix in asymptomatic women.

*Detection of recurrence of known pathologic abnormalities of the uterine cervix.

*Evaluation of a suspected hormonal abnormality.

*Monitoring of hormonal therapy.

Obtaining a Pap Smear

Specific collection procedure utilized will depend on the type of information required or specific indication for performing the Pap smear. The goal of the actual collection procedure is to produce an adequate, valuable smear of cellular material from the vagina and/or cervix which can be submitted to the laboratory, along with appropriate clinical information, to be stained and evaluated in accordance with the indication for the test. In order to accomplish this goal, the smear has to have the following characteristics:

*Adequate numbers of squamous epithelial cells present.

*Evidence that the transformation zone was sampled (i.e., the presence of endocervical cells on the smear).

*Spread in a relatively even monolayer.

*Epithelial cells not obscured by blood, inflammatory cells, or foreign material such as lubricant or talk.

*Appropriately preserved.

The collection procedure actually begins with appropriate instructions of the patient regarding the test. A Pap test should be obtained:

*Annually after the age of 18 or after the beginning of sexual activity.

*During the 2nd half of the menstrual cycle, i.e., at least two weeks after the start of one menstrual period and before the start of the next menstrual period.

*Without intercourse during the 24 hours prior to the test.

*Without douching during the 24 hours prior to the test.

The collection procedure continues with the taking of an accurate sexual and health history. Information which should be required on the requisition form sent to the laboratory includes:

*Patient name

*patient age

*Last menstrual period

*Pregnancy history

*History of hormone use

*History of IUD use risk factors

*Previous abnormal Pap smears

*Relevant clinical information e.g., abnormal bleeding, discharge, pelvic pain, etc.

Limitations of Pap Smears

In spite of the best collections, specimen handling, and screening procedures, there will still be a false negative (missed lesion) rate of at least 4%. Up to 2/3 of false negative Pap smear result from factors related to the collection procedure. However, the natural history of cervical dysplasias and carcinomas is such that there is along time interval (years) from dysplasia to invasive carcinoma. If yearly screening is performed, then the chance of a lesion being missed is very low.

THE VALUE OF P53 NUCLEAR PROTIEN EXPRESSION IN PREDICTING RESPONSE TO INTRAVESICAL MITOMYCIN C CHEMOTHERAPY

Ahmad Abdul-Hameed[1] *FICMS*, **Muhannad Muhsin**[2] *PhD*, **Usama Al-Nasiri**[3] *FRCS.*

Abstract

Background: Alterations of p53 gene are the most common mutations in human cancers. In bladder cancer, p53 mutations have been associated with high tumor grades and advanced stages, as well as progression of superficial disease to muscle invasive disease. Moreover, p53 nuclear over expression appears to be an independent predictor of disease progression and decreased survival after cystectomy.

Objective: The objective of the study is to evaluate the P53 expression percentage in patients with transitional cell carcinoma of the bladder and the relation of this expression to superficial cancer, muscle invasive disease, and carcinoma in situ as far as the tumor grade, clinical stage and response to intravesical mitomycin chemotherapy is concerned.

The expression of P 53 in normal bladder mucosa, taken from patients admitted for ureteral endoscopic procedures, was used as a control group

Method: The expression of p53 protein was studied by immunohistochemical analysis in paraffin embedded specimens from 58 patients with transitional cell carcinoma of the bladder and 20 patients with normal urinary bladder (control group), these patients were admitted for insertion or removal of double J ureteric stents. Patients with superficial tumors (stage T1, Ta) were treated with intravesical chemotherapy with mitomycin C (20-40mg). once weekly for 6 weeks and cystoscopy repeated after 3 months , 6 months and 9 months. Patients with superficial muscle invasive disease were subjected to extended transurethral resection followed by intravesical chemotherapy and cystoscopy repeated at 6 weeks, 3 months and 6 months. No treatment other than MMC was given.

Results: P53 over expression was observed in 29 (50%) out of 58 patients with transitional cell carcinoma of the bladder, with no case of p53 over expression observed in the control group.

A statistically significant relation was noticed between p53 over expression and clinical stage, with p53 over expression was more common in muscle invasive tumors. P53 over expression was also more common in high grade tumors; however, no statistically significant relation between p53 expression and tumor grades and response to intravesical chemotherapy with mitomycin C was noticed.

Conclusions: P53 over expression was noticed in half of the patients with transitional cell carcinomas of the bladder, it was much more common in muscle invasive tumors and more frequent in high grade tumors, however, it seems that p53 status did not predict response to intravesical mitomycin C chemotherapy.

Keywords: Bladder Tumor, p53 over expression, response to Mitomycin C

IRAQI J MED SCI, 2008; VOL.6 (1):3-14

Introduction

The P53 gene is tumor suppressor gene that acts as a guardian of the genome(1) .

It's located on chromosome 17p 13.1 and encodes a 53 kilo Dalton nuclear phosphoprotein with DNA binding properties(2). The P53 protein was first discovered in 1979, and it was classified as tumor antigen at that time(3).however, the realization that P53 is a tumor suppressor gene came in 1989(4). Wild type P53 protein normally

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Received: 14th November2005, Accepted: 19th February 2007.

has short half life and lasts only very briefly in the cell nucleus , where as the mutated form often accumulate for longer times and hence are more easily detected by immunohistochemistry (IHC)[5].

Given the pivotal role that P35 plays in DNA repair, cell cycle arrest and apoptosis, it's not surprising that it is the most commonly mutated gene in human tumors, including genitourinary malignancies, close to 50% of all tumors has P53 mutation [6].

Activation of P53 gene:

The main upstream events that activate P53 include: 1. DNA damage. 2. Hypoxia. 3. Low ribonucleoside triphosphate level.

The exact mechanism by which P53 detects DNA damage and subsequent signal transduction pathways involved are not well defined. However, it appears that DNA strand breaks and DNA repair intermediates activate P53 [8].

Functions of P53 gene:

The functions of P53 are diverse and complex. The main cellular responses following activation of P53 include: 1.Cell cycle regulation.2.DNA repair. 3. Apoptosis [1].

P53 in Urologic Malignancies

Mutational inactivation of P53 is a particularly frequent event in bladder carcinoma and has been associated with high-grade muscle-invasive disease [9]. P53 accumulation in the transitional cell carcinoma cell nuclei, as detected by immunohistochemistry, predicts risk of recurrence and death independent of stage, grade, and lymph node status [10].P53 status in primary tumors may predict not only likelihood of recurrence, but also whether patients will respond to chemotherapy [2]. Mutations in prostate specimens range from 10% to 35% in untreated primary tumors and from 40% to 50% in hormone refractory metastatic disease [11].Mutations in P53 occur in renal cell carcinoma, particularly metastatic lesions. Patients with abnormal expression in primary tumors may have an increased risk of developing metastasis [12]. Penile carcinoma has not been conclusively linked to P53 mutations, even in advanced lesions [13].

P53 relevance in the management of bladder cancer.

Loss of P53 function confers genomic instability, impaired apoptosis, and diminished cell cycle restraint. Alteration of P53 is most common mutation in human cancer, roughly half of all human malignancies, including many urological cancers, exhibit P53 mutations. In bladder cancer, P53 mutation has been associated with higher tumor grade, and advanced stage, as well as progression of superficial disease to muscle invasive. Moreover, P53 nuclear over expression appears to be an independent predictor of disease progression and decreased survival after cystectomy [1].

1. *Superficial disease:* In general, P53 mutations and resultant protein over expression seen on immunostaining in superficial bladder cancer. are much less common than in muscle invasive disease, however, when present, P53 mutations in superficial disease has been correlated with increased grade, recurrence, progression and decreased survival[1].

2. *Muscle invasive disease:* The vast majority of the studies have been demonstrated that P53 mutations and nuclear over expression to be more common among patients with muscle invasive bladder cancer compared to those with superficial disease [1].

P53 over expression was associated

with an increased risk of recurrence and decreased overall survival 20.In some study, P53 status was the only independent predictor of survival in case of organ confined disease at cystectomy [(14)].

3. *Carcinoma in situ:* A significant proportion of patients with carcinoma in situ have P53 mutations and nuclear over expression on IHC. Moreover, P53 mutations antedated the development of stage T2 or T3 disease by a mean of 8 months [(1)].

4. P53 status and response to BCG: Definitive conclusions for P53 status predicting response to BCG are not currently available.[1] still; it seems that P53 nuclear over expression after BCG therapy is an ominous harbinger of disease progression.15. P53 status shows promise in the selection of patients after BCG failure for early cystectomy versus repeats intravesical therapy [(16)].

5. *P53 status and response to radiation:* Theoretically P53 could modify tumor response to radiation through regulation of cell cycle kinetics and apoptosis. Loss of P53 function may increase radiosensitivity via impaired P53 dependent DNA repair processes, whereas over expression by up-regulation has been suggested to confer radio resistance. Overall, P53 status does not seem to predict response to radiation reliably [(1)].

6. *P53 status and response to chemotherapy:* P53 function may also influence tumor response to chemotherapy via regulation of the cell cycle and apoptosis, however, data regarding P53 status as a predictor of response of bladder cancer to chemotherapy is contradictory, and appears to depend on the specific mechanism of action of the chemotherapeutic agent as well as tumor type.

Initially P53 was thought to confer chemoresistance in bladder cancer via impaired apoptosis as seen in breast, colon, and hematological malignancies. In contrast, studies by other investigators support the concept that P53 mutations may confer a chemosensetive phenotype [(1)].

Detection of P53 Mutations:

P53 mutations can be detected by: 1.Single strand conformation polymorphism. 2. Direct DNA sequencing. 3. IHC [(6)].

P53 genetic mutations most commonly result in nuclear over expression of the P53 protein and positive immunostaining.

The P53 protein normally has a short half-life within the cell due to degradation via ubiquitin mediated pathways. However, the half-life is markedly prolonged after missense mutation (by far the most common genetic change of P53), although deletions can cause the same effect. Mutations in the DNA binding region (missense) account for 80% of point mutations and subsequently lead to the greatest decrease in P53 function. Missense mutations of P53 lead to accumulation of protein and nuclear over expression as demonstrated by various IHC techniques. It is crucial to understand that "positive" immunostaining implies missense mutation of the P53 gene with prolonged half-life and nuclear accumulation of the mutant P53 protein.

It is also important to appreciate that up to 20% of P53 mutations are deletions or none sense mutations that result in negative immunostaining despite complete absence of the P53 protein. They are also specific instances in which P53 is over expressed in the absence of mutation, resulting in

"positive" immunostaining without genetic alterations.

Nonetheless, P53 genetic mutations most commonly result in nuclear over expression of the P53 protein and positive immunostaining [17].

IHC analysis has many inherent limitations: 1. Variations in technique. 2. Use of diverse antibodies. 3. Tumor heterogeneity. 4. None standardization of values used to define positive staining. All these factors contribute to the unreliability of results based purely on immunostaining. Nevertheless, a strong correlation exists between P53 mutations and positive IHC for the P53 nuclear protein [1] .

A significant advantage of IHC over DNA sequencing is that IHC is commonly used for the assessment of other antigens as tumor markers in many pathology laboratories. Furthermore, identification of P53 nuclear accumulation in the tumor cells in the absence of gene mutation has been noted, indicating alternative disruption of this pathway [18] .

P53 autoantibodies: Tumor specific P53 autoantibodies have been found in the serum of patients with a variety of malignancies on immunoprecipitation, immunofluorescence, western blot test and enzyme-linked immunosorbent assay, however, Even among patients with known P53 mutations only 20% to 40% will have development of P53 autoantibodies. Although further investigation is clearly warranted, it is suggested that P53 autoantibodies may have a predictive role in bladder cancer survival [1].

Patients, Materials, and Method:

This study is a case-control study conducted at AL-KADIMYA teaching hospital from September 2003 to December 2004 (16 months). 58 patients with transitional cell carcinoma (TCC) of the bladder were included, the mean age of the patients was 58 years (range, 23_ 79 years), 43 patients are male and 15 are females (male / female = 2.8 / 1).

Patients:

Patients with bladder tumors were subjected to: 1.Cystoscopy. 2. Transurethral resection of any visible growth. 3. Bimanual examination under anesthesia before and after tumor resection. Tumor specimens obtained at time of resection were divided into two halves, one half subjected to histopathological examinations and the other half spared for IHC studies. In every case, experienced pathologist examined the tumor.

Patients involved in the study met the following eligibility criteria:

1. The primary tumors were histologically confirmed TCC.
2. Tumor specimens were large enough for histopathological examination and IHC studies.
3. Patients with history of any form of previous treatment, were excluded from the study

The patients were properly staged according to the TNM staging system developed by the American joint committee on cancer, 1997.

Patients with superficial tumors (stage T1, Ta) were treated with intravesical chemotherapy with mitomycin C (20-40mg). once weekly for 6 weeks and cystoscopy repeated after 3 months , 6 months and 9 months. Patients with superficial muscle invasive disease were subjected to extended transurethral resection followed by intravesical chemotherapy and cystoscopy repeated at 6 weeks, 3 months and 6 months.

Control

In the current study, P53 alterations had been studied also in 20 healthy

young adults admitted to cystoscopy for ureteric stent removal or insertion. All these patients were healthy young adults with no history of malignancy, chemotherapy or radiotherapy. The procedure had been explained to these patients and a proper informed consent obtained. Multiple biopsies had been taken from healthy looking bladder mucosa from the posterior wall of the bladder.

Out of 58 patients, 34 had superficial tumors and 24 had muscle invasive tumors. In the former group, the male/female ratio was 27/7, while in the later group it was 16/8. The histological grade showed G1.G2 and G3. 11patients showed G1, 14 patient's shows G2 and10patients showed G3 in the superficial group, while in the muscle invasive group it was 1, 10 and 12 respectively. The clinical staging showed 10 cases with Ta and 24 cases inT2 in the superficial tumor group, while in the muscle invasive group it showed 14 cases with T2,6 cases with T3 and 4 cases withT4 respectively. Twenty six patients received chemotherapy, 8 patients (31%) had recurrence and 18(69%) showed response to chemotherapy.

Table 1: Patients Characteristics.

	Superficial Tumors	Muscle invasive Tumors	Total
No.	34	24	58
Male/ female	27/ 7	16/8	43/15
Histological grade			
G1	11	1	12
G2	14	10	24
G3	10	12	22
Clinical stage			
	Ta 10	T2 14	-
	T1 24	T3 6	-
		T4 4	-
Patients receiving chemotherapy	26	_	26
Recurrence after chemotherapy	8(31%)	_	_
Response to chemotherapy	18(69%)	_	_

Materials:

Antigens in the sections are detected by two stages process, the binding of the primary antibody to a specific antigen, and the subsequent detection of this binding by a colorimetric reaction. The section incubated with: 1.Primary antibody: anti P53 (JC70A mouse IgG1, Kappa, Dako, Denmark). 2. Biotinylated secondary antibody. 3. Diaminobenzidine (DAB) chromogen. 4. Streptovidin horse redishes peroxidase.

Method:

Immunohistochemical analysis:

A standard labeled streptavidin-biotin technique was used for IHC staining.

Four um sections were cut and deparaffinized. Antigenicity were retrieved by microwave treatment in citrate buffer ph = 6.0. The sections were incubated overnight at 4c with 1/100 dilution of anti p53 monoclonal antibody. The incubation was followed by sequential 10-min. incubation with biotinylated-linked antibody and peroxidase-labeled streptovidin. The final incubation used diaminobenzidine (DAB) as the chromogen. Both positive and negative control was included for each run of IHC: a-The negative control was obtained by replacing the primary antibody with a buffer, phosphate buffered saline (PBS). b-A known positive section was used as a positive control.

Evaluation of immunostaining:

The staining results were evaluated in a blinded manner without knowledge of the clinical data by two independent researchers. The cutoff point for P53 positivity was established at > 5% cells with nuclear staining. The nuclear intensity of P53 was classified as negative and positive, positive results included weak, moderate and strong staining.

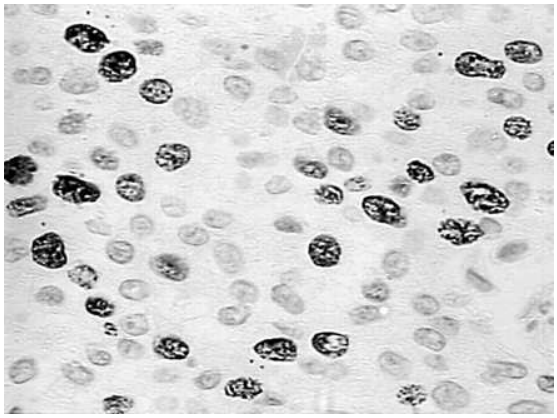

Figure1: P53 Immunohistochemical staining x 400

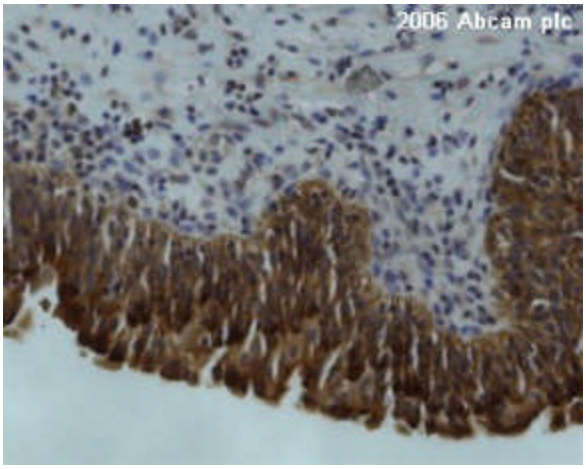

Figure 2: Staining of human urothelium (bladder cancer) tissue sections by IHC (Formalin-fixed paraffin-embedded sections).

Statistical analysis

Statistical analyses were performed using Chi-square test (X2). All P-values less than 0.05 reflected statistically significant differences. Statistics could not be applied in table 8 because one of the values was zero.

Results

The immunohistochemical expression of P53 was observed in 29 (50%) out of 58 patients with TCC of the bladder, however, no case of P53 over expression observed in the IHC of the normal looking bladder mucosa (control group).

A statistically significant difference was noticed between P53 expression and clinical stage, $P < 0.025$ (Table 2), P53 positivity was particularly observed in stage T2-4 invasive TCC of the bladder.

Table 2: P53 Immunoreactivity in Relation to Tumor Types.

Types of Tumor	No. Patients	No.+ve P53 Cases	Percentage
Superficial Tumors	34	11	32.3%
Muscle Invasive Tumors	24	18	75%

$X^2 = 8.6$, $P < 0.005$

P53 over expression was more frequent in grade 3 (68%) than in grade 2 (41%) and in grade 1 (25%), however, no statistically significant difference was noticed between P53 over expression and tumor grades, $P > 0.05$ (Table 3).

Table 3: P53 Immunoreactivity in Relation to Tumor Grades.

Grade	No. Patients	No.P53 + ve	Percentage
I	12	4	25%
II	24	10	41%
III	22	15	68%
Total No.	58	29	50%

$X^2 = 4.91$, $P > 0.05$.

Patients with superficial tumors who received intravesical mitomycin C chemotherapy, 31% showed recurrence within 9 months, while 69% showed no recurrence (Table 1).

Among those who were P53 positive, 44% developed recurrence, compared to 24% in those with p53 negative expression (Table 4), and no statistically significant relation between intravesical mitomycin C chemotherapy and P53 expression was noticed, $P > 0.05$ (Table 4).

Table 4: Patients Outcome Following Intravesical Chemotherapy

Clinical Stage	P53 + ve (No. = 9)		P53 – ve (No. = 17)		Total
	Response	Recurrence	Response	Recurrence	
Ta	1	1	3	1	6
T1	4	3	10	3	20
Total	5	4	13	4	26

$X^2 = 0.923$, $P > 0.05$

In the current study, eight Patients with T2a disease had been treated with extended transurethral resection, intravesical mitomycin C chemotherapy, and close follow up. Among p53 negative patients, two of three respond to this mode of therapy, while all patients (five) with p53 positive expression failed treatment and showed recurrence.(Table 5),and two of them progressed to stage T2b.

Table 5. Outcome of Intravesical Chemotherapy in Patients with T2a Disease

Stage	P53 + ve (No. = 5)		P53 – ve (No. = 3)	
	Response	Recurrence	Response	Recurrence
T2a (no. = 8)	_	5	2	1

Statistics cannot be applied.

Discussion

Different studies have indicated that alterations in the cell cycle regulation are a key event in determining the biological behavior of bladder cancer.[3] an extensive body of literature regarding P53 had accumulated during the last two decades [(1)].

Incidence of P53 over expression in TCC of the bladder:

In the current study, 50% of patients with TCC showed P53 over expression. Although the sample size is small, similar results had been observed in other studies, Toyoaki et al, 2001, retrospectively studied 119 patients with TCC and showed that P53 was over expressed in 61 % .[(19)]In Iraq, Al-Qaysi, 2002, showed that P53 over expressed in 23(57.5%) out of 40 bladder cancer patients[(20)] .

P53 over expression and clinical stage:

In the current study, P53 over expression showed strong statistical relation with tumor stage, P53 over

expression was seen in 25% of superficial tumors (T1, Ta) and 75% of muscle invasive tumors (T2-T4). (Table 2, 6).

Toyoaki et al, 2001, showed statistically significant difference between P53 over expression and tumor stage (P=0.0209), P53 positivity was particularly observed in stage T2-T4 invasive type TCC of the bladder (P=0.0089). [19] Investigators from the international study initiative on bladder cancer analyzed data sets from 25 different centers in an attempt to examine more clearly and definitely the association of P53 mutations in superficial versus muscle invasive disease. Their preliminary report on 1706 patients used a cut off value of 23%, only 25% of patients with superficial tumors had positive P53 immunostaining compared to 48% of those with muscle invasive tumors [21].

It seems that the incidence of p53 over expression in muscle invasive tumors was higher in this study compared to other studies; this difference may be due to 1. Different cut off values used for P53 over expression, for example, in Toyoaki et al study, the cut off value was 10%, while in the current study, it was 5%.2. Variation in intensity scoring, for example, in our study tumors specimens showing weak intensity staining were considered positive while in Toyoaki et al study was considered negative.3.Non uniform methodology for p53 staining, ranging from use of different antibodies to application of dissimilar technique for enhancing epitope expression.

Table 6: P53 Immunoreactivity in Relation to Clinical Stage.

Stage	No. Patients	No. P53+ve	Percentage
Ta	10	2	20%
T1	24	9	37.5%
T2	14	10	71.4%
T3	6	5	83.3%
T4	4	3	75%
Total No.	58	29	50%

$X^2 = 11.34$, $p < 0.025$

P53 status and tumor grade:

In the current study, P53 over expression was more frequent in high grade tumors, being 68% in grade 3, and only 25% in grade 1 tumors and 41% in grade 2 tumors. However, no statistically significant relation between P53 over expression and tumor grade was obtained (Table 3).

These results were in agreement with Toyoaki et al study which showed no statistically significant relation between grades and P53 over expression, with more frequent expression of P53 in grade 3 tumors (72%) compared to 54% in grade 2 tumors and 58% in grade 1 tumors [19]. However AL-Qaysi, 2002, showed significant correlation ($p<0.05$) between P53 over expression and tumor grade [20] .

P53 status and response to intravesical mitomycin C chemotherapy:

In the current study, 26 patients with stage T1and Ta disease treated with intravesical mitomycin C chemotherapy,

among P53 positive cases ,4 of 9 patients failed chemotherapy(44%), compared to 4 of 17 patients in p53 negative group(24%),and no statistically significant relation obtained between p53 expression and intravesical mitomycin C chemotherapy ,P > 0.05.(Table 4).

To the best of our knowledge, no study has reported on the P53 over expression and intravesical chemotherapy. Kawasaki et al showed in vitro abrogation of apoptosis induced by mitomycin C or cisplatin in bladder cell lines with P53 mutations, thus P53 mutations may confer resistance to chemotherapy via impaired apoptosis .[2] On the other hand, Kielb et al suggested that paclitaxel may be more effective against cells with P53 mutations as the mechanism of action requires functionally mutated P53 to induce cell death in human bladder cells.[23] So, data regarding P53 status as a predictor of response of bladder cancer to chemotherapy is contradictory [(1)].

Caliskan et al followed 30 patients with stage Ta and T1 disease subsequently treated with BCG, among P53 positive cases, 5 of 6 failed BCG and progressed to muscle invasion or metastasis, compared to only 6 of 24 that were P53 negative.[24] Pages et al evaluated 43 patients with stage T1 tumors and found that P53 status before treatment with BCG could not define a group of BCG non responders.[25] So definitive conclusions for P53 status predicting response to BCG are not currently available[(1)] .

P53 status and T2a disease:

In the current study, eight patients with T2a disease had been treated with transurethral resection, intravesical mitomycin C chemotherapy, and close follow up. All p53 positive cases (five cases) failed this mode of therapy and two of these cases progressed to T2b disease, while among p53 negative cases, two out of three patients respond and showed no recurrence.(Table 5).This may suggest that patients with T2a disease and negative p53 expression may respond to bladder sparing approach ,however two points should be taken in considerations 1.The short time of the follow up (only 6 months),so patients who had respond to this modality may develop recurrence in the future.2.The small number of the patients(only eight patients).

Herr et al suggested that patients with stage T2 bladder cancer and negative P53 status may be watched if responsive to methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) therapy, while those with stage T3 disease or T2 tumors with positive P53 immunostaining should proceed directly to cystectomy. In their study, P53 status was the only independent predictor of survival in cases of organ confined disease at cystectomy. Patients with T3b or greater pathological stage carried a poor prognosis regardless of P53 status.[(24)] In contrast; Tiguert et al were unable to correlate P53 mutations in patients with muscle invasive bladder cancer treated with cystectomy to cancer specific survival. Furthermore, P53 status has not been shown to predict disease free survival in patients with nodes positive disease, [26] Cote and Chatterjee reported that the only patients with bladder cancer to benefit from adjuvant chemotherapy were those with P53 altered tumors [(28)]. On the other hand, Qureshi et al found that P53 status did not predict response to cisplatin based chemotherapy and failed to predict overall cancer specific survival in their series of patients with

stage T2-4 disease[27]. so data regarding p53 status as a predictor of response of bladder cancer to chemotherapy is contradictory and appears to depend on the specific mechanism of action of the chemotherapeutic agent as well as tumor type. The final conclusion of this work showed that Close to 50% of TCC of the bladder showed P53 over expression which was more common among patients with muscle invasive bladder TCC compared to those with superficial disease, and it was more frequent in high grade tumors (G3), but no statistical relationship between P53 expression and tumor grade had been noticed. P53 status did not predict response to intravesical mitomycin C chemotherapy. Patients with muscle invasive disease and positive p53 immunostianing may need more aggressive intervention than those with negative p53 expression.

Furthers studies with a larger size sample are suggested taking the following points into consideration:

1. Using a uniform methodology for p53 staining, using similar antibodies and similar technique for enhancing antigen expression.
2. Adherence to a generally accepted cut- off value and staining intensity.
3. Study the effect of p53 over expression on response to different chemotherapeutic agents and to other modalities of therapy like radiotherapy and cystectomy.
4. Extending the time of the study for longer period.
5. Study the impact of p53 over expression on bladder sparing procedure in muscle invasive T2 disease.
6. Study the correlation between p53 expression and other types of bladder tumors like seqamous cell carcinomas and adenocarcinomas.

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Effects of Turmeric, and Black Cumin on Induced Colitis in Rabbits

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Abstract

Back ground: The failure of current treatment strategies to control many cases of IBD makes a strong stimulus to find out new modalities of treatment.

Objective: to study the effects of oral curcumin, and black cumin on induced colitis in rabbits.

Materials and Methods: Colitis was induced in rabbits by rectal acetic acid-ethanol (model 1), or acetic acid (model 2). The effects of tested agents (curcumin, and black cumin) were compared to distilled water (control), and prednisolone regarding changes in body weight, colon segment weight, and gross and microscopical scores.

Result: In model 1, severe gross and microscopical damage observed in colon. Gross and microscopical scores of curcumin group were not significantly different from that of control and of prednisolone groups.

In model 2, a less severe inflammation occurred; yet, an evident gross and microscopical damage were observed.

Black cumin and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control. The gross and microscopical damages were apparently lowered when black cumin, curcumin and prednisolone were used, but these changes were significant for prednisolone, and black cumin (grossly), and for prednisolone (microscopically).

The gross and microscopical effects of curcumin, and black cumin were comparable to those of prednisolone.

Conclusion: Acetic acid-induced colitis in rabbits (model I) is preferred for testing the anti-inflammatory effectiveness of new therapeutic modalities.

Black cumin oil and curcumin have an anti-inflammatory activity in this model.

Keyword: Inflammatory bowel disease, free oxygen radicals, induced colitis, acetic acid, curcumin, black cumin.

IRAQI J MED SCI, 2008; VOL.6 (1):15-27

Introduction

Idiopathic inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic or relapsing immune activation and inflammation within the gastrointestinal tract [(1)]. Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of idiopathic IBD [(2)].

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Received: 16th August 2007, Accepted:10th March 2008.

Ulcerative colitis and CD pursue a protracted, relapsing and remitting course, usually extending over years [(3)].

Recent studies pointed to the important role of free oxygen radicals in the pathogenesis of IBD both in animal models of induced colitis and in human beings.

One of the more commonly used models of Induced Colitis in Rabbits is acetic acid induced colitis [(4)].This experimentally induced colitis is similar to the human condition in certain aspects {e.g., acute inflammation with neutrophil infiltration [(5)], increased concentrations of LT B4, and PG E2 [(6)], superoxide dismutase [(7,8)]. and increased

production of inflammatory mediators, such as hydrogen peroxide (H2O2), nitric oxide (NO), myeloperoxidase activity (MPO), and tumor necrosis factor (TNF-alpha)} [8].

Black cumin contains about 0.5-1.5% volatile oils including Nigellone and thymoquinone, which are responsible for Black cumin's anti-histamine, anti-oxidant, and anti-infective effect. Both are effective in their own standards, however, black cumin oil is more concentrated than the herb itself [9]. Antioxidant activity of Nigella sativa essential oil is documented in many studies [10].

Many studies showed that curcumin, exhibits antiinflammatory، antitumor, and antioxidative properties [11, 12]. and it has been found to have an acceptable safety in human. A phase 1 human trial with 25 subjects using up to 8000 mg of curcumin per day for 3 months found no toxicity from curcumin. Five other human trials using 1125-2500 mg of curcumin per day have also found it to be safe. These human studies have found some evidence of anti-inflammatory activity of curcumin [13].

Materials and Methods

Colitis was induced in male rabbits by rectal administration of %٥acetic acid-30% ethanol (model 1) [14], or 2% acetic acid (model 2)[15]. The animals were allowed to have free access to food and water all over the period of study except for 24 hours before induction of colitis (fasting). Animals in different groups were orally administered 10 ml of distilled water (control), prednisolone (2 mg/kg/day dissolved in 10 ml distilled water), or curcumin (50 mg/kg/day dissolved in 10 ml of distilled water) (model I), black cumin oil (0.2 ml/kg/day, each dose was followed by 5 ml of distilled water orally in addition to distilled water, prednisolone, and curcumin (model II).

Each agent (including distilled water) was administered orally two days prior to induction of colitis, the day of induction, and a dose ٢٢hours post-induction (i.e., 2 hours prior to killing of the animal). Twenty-four hours after induction, the animals were sacrificed and the abdomen was opened longitudinally, and a segment of colon 8 cm [16]. proximal to anus was removed for assessment of colonic inflammation.

The effects were observed as changes in body weight, colon segment weight and gross histological score (using a dissecting microscope) (Table-1) [17].

Colonic samples (0.5 cm of length) were taken from the 8 cm segment, fixed in 10% formaldehyde and the routine 5 micron paraffin embedded sections were prepared. Tissues were routinely stained with haematoxylin and eosin, coded, and evaluated blindly by light microscopy with low and high power (40 xs) objective lenses [18]. Each slide was scored according to Christian, et al., [19]. to assess the extent of colonic inflammation. The score ranges from 0 to 40 (total score), which represents the sum of the products of each criterion by the score of the percentage involvement. All evaluations were performed by observers unaware of the treatment groups.

Criteria of scores divided as follows:

- Inflammation severity scored from 0-3 as None, Mild, Moderate، Severe respectively,
- Inflammation extent from 0-3 as None, Mucosa، Submucosa, Transmural, respectively,
- Crypt damage from 0-4 as None, Basal 1/3 damage, Basal 2/3 damage, Crypt lost; surface epithelium present, Crypt and surface epithelium lost respectively,

- Per cent involvement from 0-4 as 1-25%, 26-50%, 51-75%, 76-100% respectively,

The score (total score) represents the sum of the products of each of the first three criteria by the score of the percentage involvement [20].

Statistical analysis

Results are expressed in tables as means ± standard error of the mean (SE), or drowns as bar charts. Paired student's T test was applied for data from the same group, while unpaired student's T test was used for data of different groups. When P value was < 0.01, it was considered as highly significant, while $p < 0.05$ was considered as significant [21].

Results

In model one, 5% acetic acid- 30% ethanol induced a severe gross and microscopical damage in colon with marked increments in weight of colonic segment. Gross score and colon segment weight of curcumin group were not significantly different from those of the control and of the prednisolone groups ($p > 0.05$), (Fig. 1, Fig. 2, and Fig. 3).

Microscopical score of curcumin group was also not significantly different from that of the control group ($p > 0.05$), but, it was significantly lower than prednisolone group ($p < 0.05$) (Fig. 4).

In model two, 2% acetic acid induced a less severe form of inflammation in colon; yet, it had a marked effect in reducing the body weight of rabbits and with evident gross and microscopical damage in colon.

Curcumin, Black cumin and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control group (Table 2).

The mean (±SE) post-induction rectal temperature for control group (38.78 ± 0.2Cº), curcumin group (38.81 ± 0.2Cº), and black cumin group (38.88 ± 0.09Cº) showed a statistically insignificant ($p < 0.05$) increment from the mean pre-induction readings (38.55± 0.14Cº), (38.56 ± 0.25Cº), and (38.74 ± 0.21Cº) respectively. While post-induction readings for prednisolone group (37.84 ± 0.38Cº) decreased insignificantly ($p > 0.05$) from mean pre-induction reading (38.61 ± 0.15Cº).

When comparing mean post-induction rectal temperature of different treatment groups to the corresponding readings of control group, the differences were insignificant ($p > 0.05$), while when comparing corresponding readings of prednisolone and other treatment groups (curcumin, and black cumin groups) there was a significant decrease ($p < 0.05$) in mean post-induction rectal temperature of prednisolone group, (Fig. 5).

The mean colon segment weight of curcumin group and prednisolone group were insignificantly ($p > 0.05$) more than that of the control group. While that of black cumin group was insignificantly ($p > 0.05$) less than that of the control group, (Fig.6).

As shown in (Table 3), there was an obvious reduction of the mean gross histological score of all treatment groups from that of the control group and this reduction was significant ($p < 0.05$) for prednisolone group and black cumin group, while it was not significant ($p > 0.05$) for curcumin group (Fig. 7).

There was an obvious reduction of the mean microscopical histological score of all treatment groups compared to the control group and this reduction was significant ($p < 0.05$) for prednisolone group only, but not significant for black cumin and curcumin groups, (Fig. 8).

The effects of curcumin and black cumin in regards to colonic segment

weight, gross histological score, and microscopical score were comparable to those of prednisolone ($p > 0.05$).

Table 1: Gross mucosal inflammation scoring index. (Modified from Brian, et al., 1997)[17]

Score	Macroscopic Appearance
0	Normal
1	No ulcer; mild petechia/hypervascularity
2	No ulcer; moderate petechia/hypervascularity
3	Ulcer <1 cm with petechia/hypervascularity
4	Same as above at 2 or more sites
5	Ulcer ≥ 1 cm with petechia/hypervascularity
6	Ulcer ≥ 2 cm with petechia/hypervascularity
7	Ulcer ≥ 3 cm with petechia/hypervascularity
8	Ulcer ≥ 4 cm with petechia/hypervascularity
9	Ulcer ≥ 5 cm with petechia/hypervascularity
10	Ulcer ≥ 6 cm with petechia/hypervascularity

Table 2: Mean Initial and Post-induction Body Weight (g) of control and treatment groups in Acetic acid (2 %)-induced colitis

Groups	Mean Initial Body Weight (±SE) (g)	Mean Post-induction Body Weight (±SE) (g)
Control	1225 ± 86.2	1170 ± 88.96 ***
Prednisolone	1228.3 ± 83	1183.3 ± 90*
Curcumin	1168.3 ± 55	1046 ± 29**
Black cumin	1266.67 ± 117.7	1216.67 ± 107.3*

**** Highly significant ($p< 0.005$) in comparison with the initial B.Wt*
*** Highly significant ($p < 0.01$) in comparison with the initial B.Wt*
** Significant $p < 0.05$ in comparison with the initial B.Wt*

Table 3: Mean (±SE) gross histological score (0-10) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

Groups	No. of Rabbits	Mean gross score ± (SE)
Control	7	8.86 ± 0.51
Prednisolone	6	7 ± 2.45 *
Curcumin	6	7.17 ± 1.49
Black cumin	6	5 ± 1.67*

**Significant reduction ($p < 0.05$) in comparison with the control*

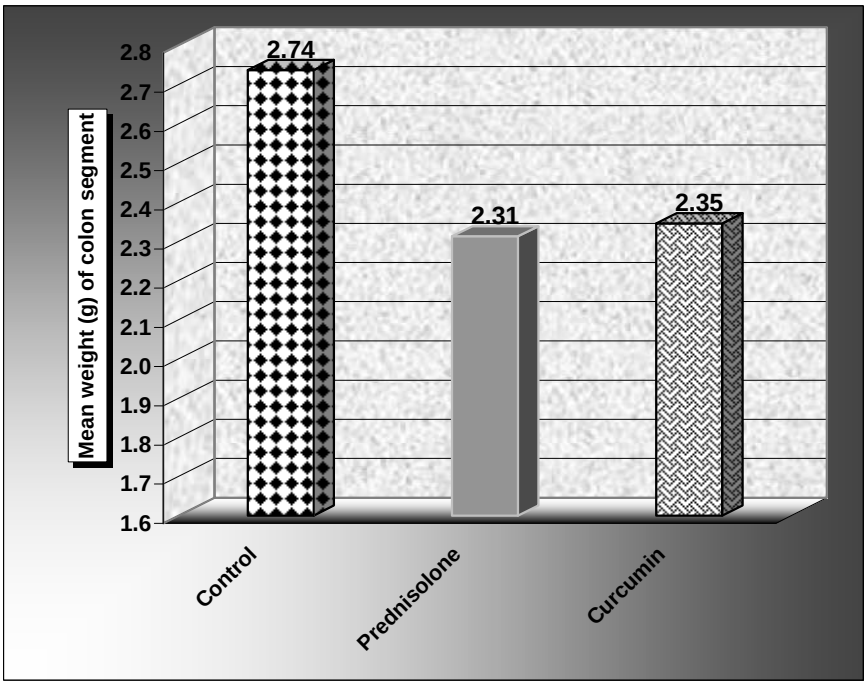


Figure 1: Mean weight of colon segment (g) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

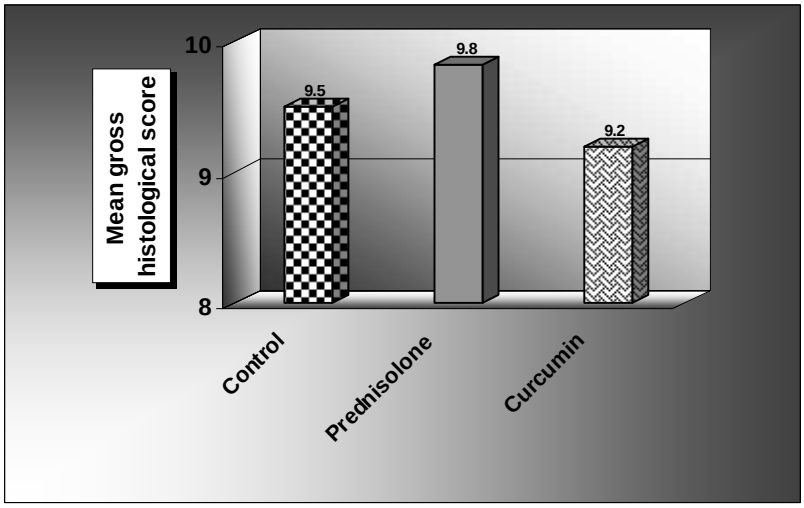
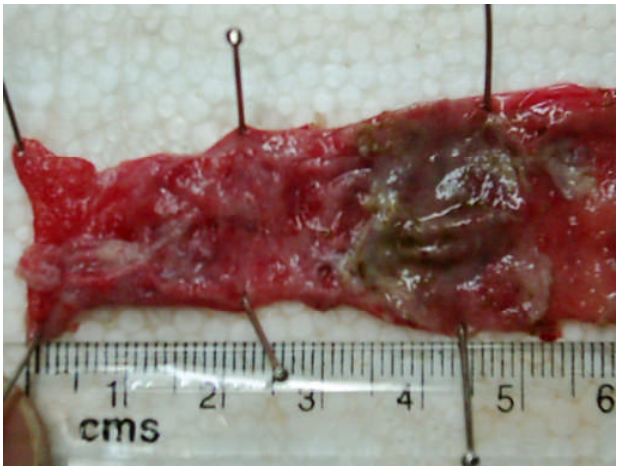


Figure 2: Mean gross histological score (0-10) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis



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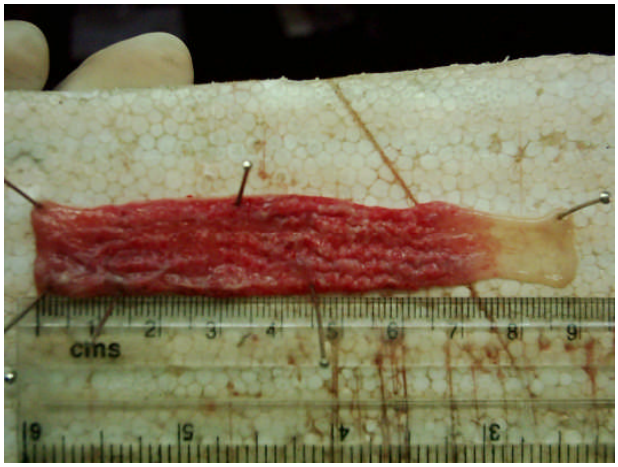

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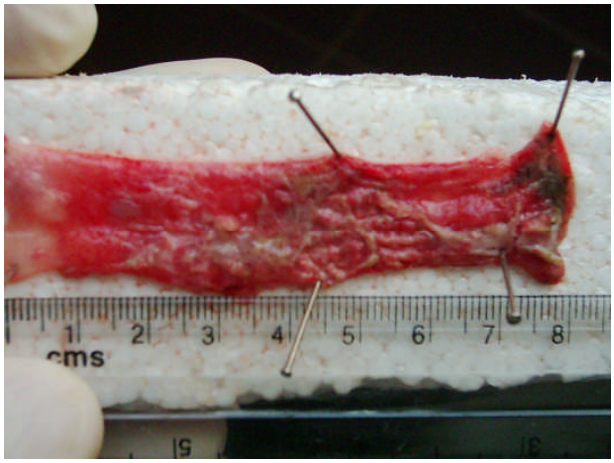

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Figure 3: the gross appearance of colon segments of control and treatment groups in acetic acid (5 %) - ethanol (30%)-induced colitis, A: control, B: prednisolone, C: curcumin.

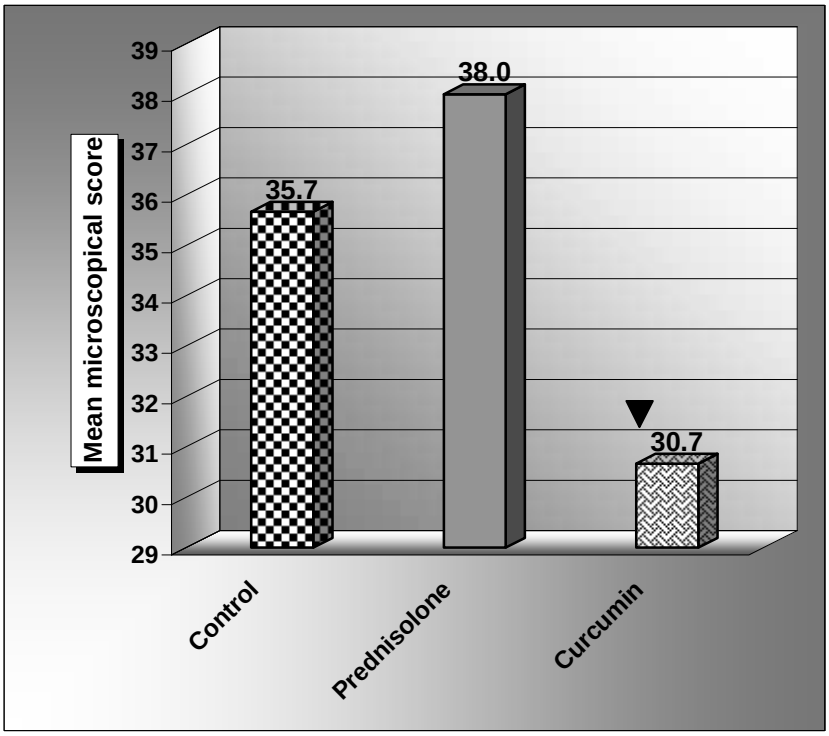

Figure 4: Mean microscopical histological score of the colon (0-40), of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis
▼ ***Significant difference (p < 0.05) in comparison with prednisolone group***

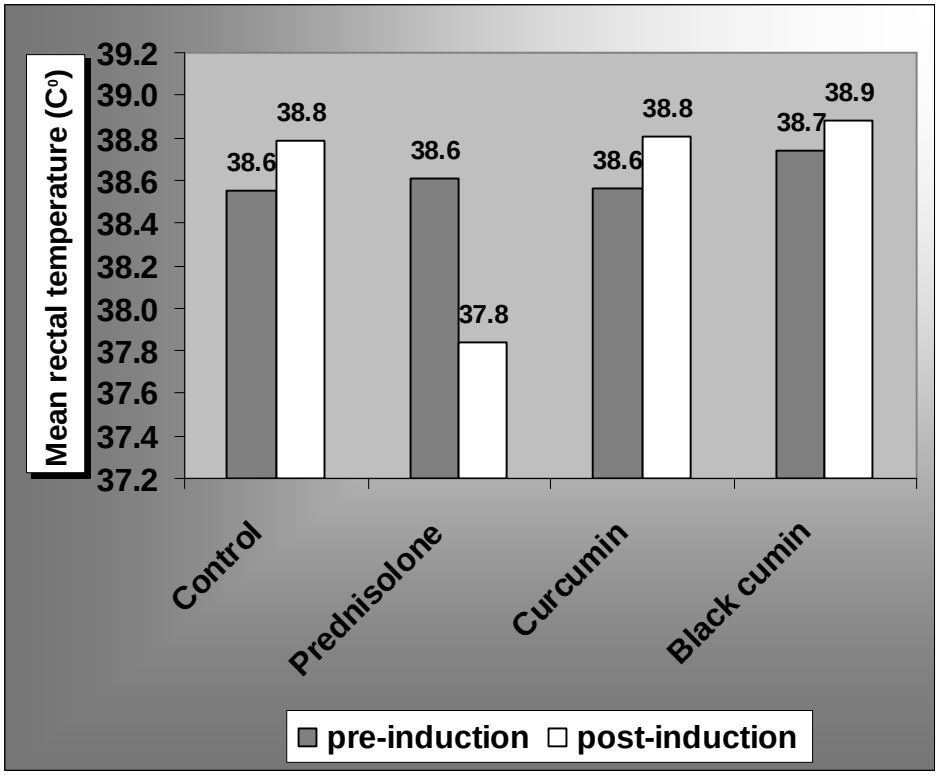

Figure 5: The mean pre-induction and post-induction rectal temperature of rabbits in control and treatment groups in acetic acid (2%) - induced colitis
▼*Significant reduction (p < 0.05) in comparison with post-induction rectal temperature of curcumin and black cumin groups*

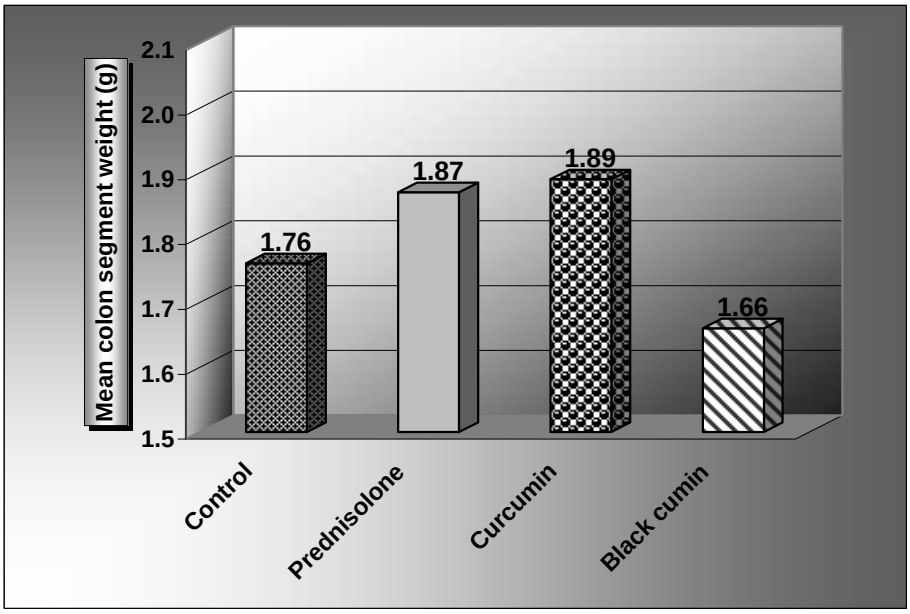

Figure 6: Mean colon segment weight (g) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

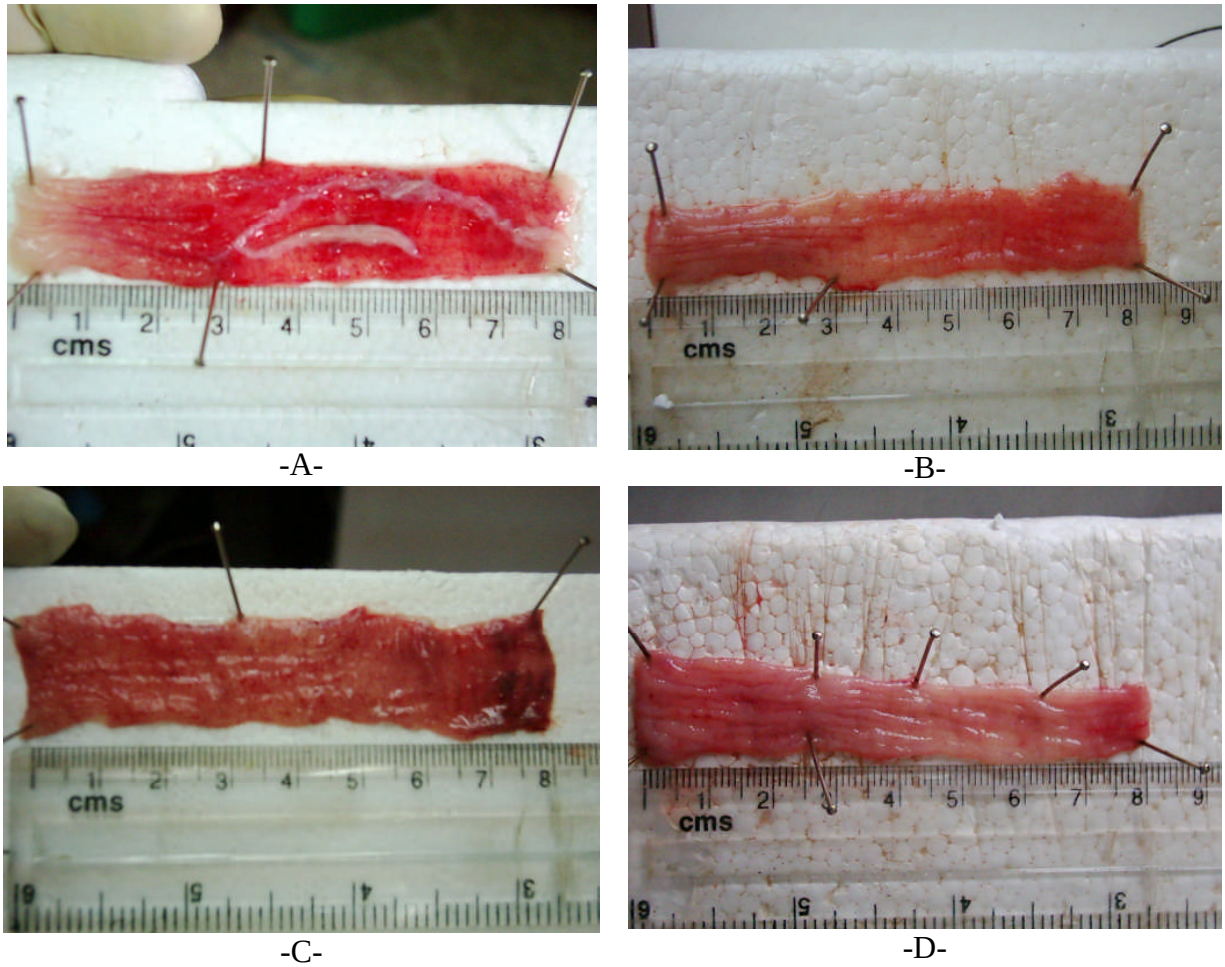

Figure 7: the gross appearance of colon segments of control and treatment groups in acetic acid (2%)-induced colitis, A: control, B: prednisolone, C: curcumin, D: black cumin

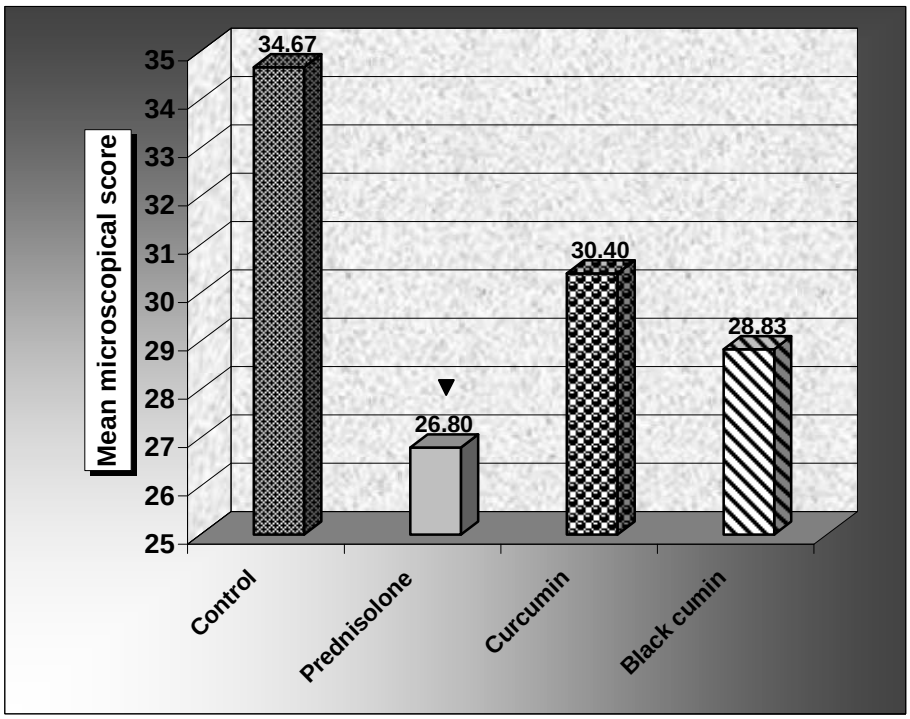

Figure (8): Mean microscopical histological score (0-40) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

▼ ***Significant reduction ($p < 0.05$) in comparison with the control group***

Discussion

Various animal models have provided a foundation for future investigation into the mechanisms responsible for IBD, which will hopefully result in the development and testing of novel therapeutic regimens [(22)].

Acetic acid-induced colitis is used widely because of its reproducibility (with lesions occurring in 100% of animals). In addition, it provides an inexpensive model useful in comparing the effectiveness of novel therapeutic agents. [(23)]. Its similarity with human IBD in many aspects make researchers still use it as one of the models of induced colitis.

Pilot studies done prior to the present work governed the selection of the two models of colitis induction particularly the second model, i.e., acetic acid (2%) that was used in rabbits for the first time.

In the first model of the present study, ethanol was used in combination with acetic acid in order to decrease the mucosal barrier [(24)]. so that the damaging effect of the acid was found to be more severe and deeper than that induced by acetic acid alone.

Finding an orally effective anti-inflammatory agent is of a major importance since the advantages of oral route are well known.

Moreover, selection of such route of administration in the present in vivo study could give a chance for the tested agent to act systemically and / or locally at the colon.

Prednisolone, the oral corticosteroid used commonly as a standard therapy to control acute attacks of IBD [(3)]. was used in the present study as what is called a positive control [(25, 26)].

It was found that non-steroidal antiinflammatory drugs exacerbate experimental colitis in rats [27]. For that, these agents were not used in this study.

Turmeric (the dried rhizome of the perennial herb *Curmcua Loga;* a major constituent of which is curcumin) and black cumin oil are relatively safe and used orally as food additives, and are available and relatively cheep; besides, they are known to have anti-inflammatory and antioxidant properties، which may be the starting point for an effective drug therapy in IBD.

The schedule of therapy (2 days before and 1 day after induction of colitis) was dependent in this study to evaluate mainly the possible prophylactic role of the tested agents in addition to their effectiveness in initial therapy for acute attacks of colitis, which is the major problem of the relapsing and remitting IBD.

In model 1 in this study, the insignificant difference in the means of weight of colonic segment of curcumin, and prednisolone groups from that of the control group may be due to the severe form of inflammation and edema induced by the 5% Acetic acid-30% Ethanol in all groups. For the same reason there was no significant correlation between the gross histological score and the segment weight in this model correlation coefficient (r) = 0.185, $p > 0.05$.

In comparison with model 1, although there were no significant differences ($p > 0.05$) in model 2 in colon segment weight, but there was a positive correlation between the gross histological scores and the corresponding segment weight ($r = 0.37$, $p < 0.05$). Rachmilewitz، et al., (1995) [28]. showed that the weight of colon segment involved by inflammation is increased and could be decreased by an inhibitor of nitric oxide synthase.

Regarding the mean gross histological score in model 1, its insignificant difference for all treatment groups from that for the control group, could be explained by severity of inflammation induced by acetic acid (5%)–ethanol (30%) that even could abolish the expected prednisolone effect (mean gross score = 9.8 ± 0.16). In model 2, the obvious reductions in mean gross histological score for all treatment groups pointed to the effectiveness of the tested agents، particularly prednisolone, and black cumin oil, to reduce inflammatory process in acetic acid (2%) model.

Moreover, compared to effect of prednisolone on mean gross histological score, curcumin (models 1 and 2), and black cumin oil (model 2) had comparable effects; this could indicate their possible potent initial anti-inflammatory effects.

In model 1, the insignificant differences (obtained by all tested agents) in mean microscopical histological scores which simulated what was found in regard to mean gross score (see above), enforced the idea of unsuitability of acetic acid (5%) –ethanol (30%) model to evaluate the effectiveness of tested agents in initial treatment of induced colitis in rabbits.

In model 2, the reductions in microscopical scores induced by curcumin and black cumin oil emphasized the effective anti-inflammatory role of these agents particularly when these findings conjugated with their anti-inflammatory effects detected grossly (see above). However, for black cumin group, p value = 0.056 (i.e., so near to the significance level).

In model 2, compared to control group، prednisolone and black cumin oil exerted a better apparent protective

role than curcumin regarding the induced reduction in mean body weight.

The significant reduction of mean body temperature of prednisolone group from the corresponding readings for the other treatment groups may point to its effect on the body temperature in this model (unknown mechanism). Although , all of the means of body temperature were within the normal range (37.8. - 39.4. C).

Results of the present study revealed that curcumin in a daily oral dose of 50 mg/kg had a comparable effect with that of prednisolone (given orally in a dose of 2 mg/kg/day) against acetic acid –induced colitis in rabbits.

Salh B., et al., (2003) [29]. concluded that curcumin was able to attenuate experimental colitis through a mechanism correlated with the inhibition of the activation of NFkappaB. Other studies showed that intestinal mucosal CD4 (+) T cells and B cells increase in animals treated with curcumin, suggesting that curcumin modulates lymphocyte- mediated immune functions [30].

In the present study, results of black cumin oil administered orally in a daily dose of 0.2 ml/kg/day revealed its potential efficacy in attenuation of experimentally-induced colitis in rabbits; such efficacy was comparable to that of prednisolone given orally in a dose of 2 mg/kg/day.

The anti-inflammatory and analgesic effects of black cumin are documented by Al-Ghamdi (2001) [31]. Mansour, et al., (2001) [32] and Badary, et al., (2003) [33]. found that thymoquinone (a volatile oil of black cumin) to be a potent superoxide anion scavenger of different free radicals thus may play an important role as antioxidant. El- Abhar, et al., (2003) [34]. found that black cumin oil or thymoquinone administered orally have a protective activity against gastric mucosal injury in rats which were subjected to ischemia/reperfusion protocols. Thus, the present study supports the protective role of black cumin oil administered orally against induced injury in gastrointestinal mucosa.

Conclusions

This study showed that:

1. Acetic acid (2%) administered rectally is preferred in induction of colitis in rabbits for testing the anti-inflammatory effectiveness of new therapeutic modalities.

2. Oral black cumin oil (in dose of 0.2 ml/kg/day) had a potential efficacy in attenuation of acetic acid -induced colitis in rabbits.

3. Oral curcumin (in dose of 50 mg/kg/day) had an accepted intiinflammatory activity against acetic acid –induced colitis in rabbits.

Recommendations

- Further in vivo studies are required to elaborate the preferred doses, safety, and exact mechanism of action of black cumin oil، and curcumin in prophylaxis and treatment of experimentally induced colitis.
- Subsequently, clinical studies are recommended to explore the potential anti-inflammatory effect and safety of black cumin oil either as monotherapy or as adjunct to the routinely used treatment of patients with inflammatory bowel diseases.

Acknowledgment

We wish to acknowledge the kind co-operation of the staff of Department of Pharmacology and Department of Pathology, College of Medicine, Al-Nahrain University; for providing great help during the whole period of the study.

Finally, we are grateful to all those who provided us with help، whatever it were.

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A STUDY ON HUMAN PAPILLOMAVIRUS USING IN SITU HYBRIDIZATION TECHNIQUE AND ITS ROLE IN CERVICAL NEOPLASIA

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Abstract

Background: clinical epidemiological studies have shown that human papillomaviruses play major role in the development of different types of cervical lesions, are therefore considered as the major infectious etiological agents of genital lesions and cancer.

***Objective*:** to determine the prevalence of HPV DNA by using in situ hybridization technique among archival tissue specimen of the uterine cervical lesions and normal cervical postmortem tissue biopsies.

***Material & Methods*:** Eighty cervical tissue samples were included in this study. 70 archival tissue biopsy samples comprised a risk group for HPV infection and / or cervical neoplasia; these were selected for the years from 1998 to 2005 from histopathology files of Al-kadhimiya teaching hospital, Al-Ilwiya teaching hospital, Al-Yarmouk hospital, Medical city department of teaching laboratories, and from four private laboratories. The patients mean age was 43.1 years with a range of 20 to85 years. The remaining 10 normal cervical postmortem tissue biopsies were obtained from the institute of forensic medicine and considered as control group. These autopsies were taken from virgin female cervices, their mean age 23.1 years with a range of 18-30 years. In Situ Hybridization was performed for the detection of HPV on cervical tissue.

Results: All normal control cases showed no specific signals for HPV DNA. 6 (30%) of 20 cases of cervical tissue with codylomatous changes, 1 (11.11%) of 9 cases of CIN I.3 (21.43%) of 14 cases of CIN II/III, and 9 (33.33%) of 27 cases of ISCC were shown to be positive for HPV 6/11/16/18/31/33 DNA.

Conclusion: The In situ hybridization enabling direct visualization of viral tissue distribution and better substantiate HPV as a causal agent in cervical neoplasia.A significant association ($p < 0.05$) was found between Insitu Hybridization signal pattern and the histological type of cervical noeplasia

Keywords: Human papilloma virus, cervical intra epithelial neoplasia, cervical carcinoma, in situ hybridization technique.

IRAQI J MED SCI, 2008; VOL.6 (1): 28-37

Introduction

Various epidemiologic and studies have established a strong correlation between genital infection with human papilloma virus (HPV) and development of uterine cervix cancer (1).

This type of neoplasm represents approximately 6% of all human cancer and is the second most common form found in women (2).

The application in recent years of molecular biological techniques has resulted in the exponential growth of knowledge related to the biology and pathogenicity of HPV. To date, 78 different viral types causing human infection in the skin and mucosa are known (3,4). It has been found that 80 to 100 % of advanced neoplasm and

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Received: 18th September 2006, Accepted: 17th December 2007.

invasive cervical carcinomas are caused by only few types of mucosotropic HPV. From the clinical point of view these have been classified as high {types 16 and 18}, intermediate {types 31, 33 and 35} or low {types6 and11} risk, and it is the type 16 that is the most prevalent in invasive carcinomas (30-60%). it has therefore been suggested that patients follow up and therapy should directed against the specific type of viral infection (5).

Since its introduction in 1969 by Gall and Pardue, in situ hybridization techniques have become important tools to detect nucleic acid target sequence(6).

This method has been widely used for detection of HPV nucleic acid .It easy to handle, reliable method for HPV detection and typing, working on both Pap smear and paraffin-embedded sections (7, 8).

The type of signal (cofluent, punctuate) may reflect either episomal or integrated forms of viral target DNA or the intensity of the signal HPV copy number. The main advantage of the in situ format is the ability to correlate DNA probe results with cellular morphology (9). Its main problem until now was its low sensitivity, with a detection limit of10 to50 copies per cell in formalin-fixed samples 7 therefore several attempts have been undertaken to enhance the sensitivity of ISH. Signal-amplified in situ technique have been developed to detect a small number of HPV nucleic sequences with high sensitivity by using a biotinylated probe for immunohistochemical detection of HPV in formalin-fixed, paraffin-embedded biopsy tissue sections. This assay is able to detect as few as 1 to 2 copies of target sequence per nucleus (10).

In premalignant lesions, the HPV genome is typically maintained in its episomal form. However, the majority of invasive cervical carcinomas contain HPV DNA that has integrated in to the host cell genome. Thus, the integration event is temporarily associated with the acquisition of malignant phenotype (11, 12, and 13). Integration has been shown in over 80% of HPV-16 positive SCCs and in 100% of HPV-18 positive SCCs (14).

Materials &Methods

Tissue samples: A total of 80-tissue sample from the uterine cervix were included in this study. 70 of 80 sample comprised a risk group in our study were obtained from archival paraffin embedded bocks selected for the years from 1998 to 2005 from histopathology files of Al- Kadhimya Teaching Hospital, Al-Ilwiya Hospital, Al-Yramouk Hospital, Medical city department of teaching laboratories, and from four private labarotories, These patients either had hysterectomy or punch biopsy, their mean age was 43.1 years with a range of 20 to85 years.

The remaining 10 samples (autopsies), which comprised a normal control group, were obtained from the institute of Forensic Medicine. These autopsies were taken from virgin female cervices; their mean age was 23.1 years with a range of 18-30 years, based on the fact that HPV infection is practically non-existent in celibate population (16).

Ethical approval for use of all specimens was obtained and the histopathological diagnosis was confirmed by review of freshly prepared hematoxylin and eosin-stained slides by certified pathologist and classified according to criteria outlined by the Word Health Organization (WHO).

Histopathologicaly, there were 10 cases of normal cervix, 20 cases of condylomatous changes (8 cases of mild,8 cases of moderate and 4 cases of sever), 23 cases of cervical intraepithelial neoplasia (9 cases CIN 1, 7 cases of CIN 2 ,and 7 of CIN 3) ,and 27 of squamous cell carcinoma of either the keratinizing or non keratinizing large-cell type (6 cases of well differentiated ,16 moderately differentiated, and 5 of poorly differentiated.

In Situ Hybridization for detection of HPV 6/11/16/18/31/33 DNA in Paraffin Embedded Sections:

Principle of the test: A biotinylated probe hybridized to the target sequence (HPV DNA), and then an anti-biotin antibody (Linker 1) binds to the biotin on the hybridized probe. In order to re introduce biotin into the system, an immunoglobulin (Linker 2) that is coupled with substantially higher numbers of biotin moieties than the probe binds to linker 1 antibody. These additional layers initiate amplification of the signal. To visualize the antibody/probe complex, streptavidin – alkaline phosphatase conjugate is added. Upon addition of the substrate BCIP/NBT (Bromo-chloro-indolyl-phosphate/Nitroblue Tetrazolium) solution, an intense blue signal appears at the specific site of the hybridized probe.

DNA probe Hybridization/Detection System In Situ Kit (Maxim Biotech, USA) was used.

The paraffin-embedded sections were cut into 4-6µm thicknesses, placed on Fisher brand positively charged slides and left over night to dry at room temperature.

The procedure of in situ hybridization was carried out in accordance with manufacturer's instructions. The process include a prehybridization steps for preparation of tissue slides, hybridization steps by placing 10-20µl of the ready use DNA probe/hybridization solution onto the tissue sections and the sections were covered with cover slips, then the slides were placed in the oven at 95Ċ for 10 minutes to denature the DNA. After that, the slides were put in the humid chamber and incubated at 37 Ċ overnight to allow hybridization of the probe with target nucleic acid. In the next morning, there are post hybridization steps for preparation of slides.

Slides were examine by light microscope and evaluated for integration status of HPV.A dot-like signal evenly distributed over most cell nuclei was interpreted as integrated HPV, and a patchy signal unevenly distributed over cell nuclei; was interpreted as episomal. Mixture of the two-signal pattern was interpreted as both integrated and episomal (mixed) [17].

Quality control:

Cervical tissue sections previously tested positive for HPV by PCR technique were used as positive control tissue.

Cervical tissue sections from virgin female autopsy were employed as negative control tissue.

Statistical Analysis

ANOVA analysis program was used to calculate the Mean, Standard deviation and difference in the mean of age among different histological types.

Statistical analysis by Chi-square – test was done to look for the relationships between the presence of HPV infection and the extent of histological

abnormality. All analysis was performed using SPSS program. A p value of less than 0.05 was considered statistically significant.

Results

Study population:

The study group consisted of 80 samples their age ranges from 18 to 85 years, with a mean of (40.61±13.668 S.D Years). The histological diagnosis was normal in 10 cases (12.5%), condylomatous in 20 cases (25.0%), CIN I in 9 cases (11.3%), CINII/III in 14 cases (17.5%) (Seven CIN II and seven CINIII) and invasive cancer (squamous cell carcinoma) in 27 cases (33.8%). Details of the subjects and their numbers, mean and the range of age are shown in (table 1).

There was highly significant difference in the mean of age between groups (p value< 0.01), and as shown in (table 2)

The mean age of control group (23.10±3.814 years) was significantly lower than the risk groups for HPV infection and /or cervical neoplasia (p<0.01).

Within the risk groups, the mean age of patients with condylomatous changes was significantly lower than ISCC (33.25±11.125 years Vs 49.67±10.126 years) (p <0.01). Whereas there was no significant difference between the mean age of patients with CIN I and the mean age of patients with CIN II/III or ISCC (42.00±9.605 years Vs 45.29±12.964 and 49.67±10.126 years, respectively) (p >0.05).Also there was no significant difference between the mean age of patients with CIN II/III and the mean age of patients with ISCC (45.29±12.964 Vs 49.67±10.126) (p>0.05).

Detection of HPV 6/11/16/18/31/33 DNAs in cervical lesions:

Detection of HPV6/11/16/18/31/33 DNAs was carried out on all specimens, and as shown in (table 3).

The In situ hybridization was considered satisfactory when (a) the endogenous positive control probe gave dark, even signal over almost every nucleus, indicating adequate digestion and availability of the target DNA, (b) the negative control probe gave no signal, (c) control section gave the appropriate reaction.

In (table 3), the Chi-square showed no significant association (p>0.05) between HPV infection and the extent of histological abnormalities. Although these results may give a rough idea about the frequency of HPV presence in different histological type of cervical lesions, they did not reach statistical significance because of limited number of cases tested and the using of probe that detected not only high risk but also detected high risk and intermediate risk HPV types (6/11/16/18/31/33, collectively).

Insitu hybridization signals in the nuclei showed various patterns but could be morphologically classified in to diffuse, dot, and mixed patterns. All normal cases showed no specific signals for HPV DNA. Of the condylomatous changes 6 of 20 cases (30%), displayed ISH signals for HPV DNA, and of these 6 cases, 5 showed diffuse patterns and only one case showed mixed pattern. In CIN I, only one of 9 cases (11.11%) was positive for HPV DNA and showed diffuse pattern. In CIN II/III, 3 of 14(21.43%) were positive for HPV DNA and showed diffuse, dot and mixed pattern respectively. Diffuse pattern was usually seen in the cells located from the middle to the superficial layer of the dysplastic epithelium, especially in the cells showing koilocytosis or marked

nuclear pleomorphism. While dot pattern tended to extend deep in to the parabasal and basal cells of the dysplastic epithelium. In ISCC, 9 of 27(33.33%) were positive for HPV DNA, and of these 9 cases, 3 showed dot signals and the remaining 6 cases showed mixed patterns. In general, diffuse Insitu hybridization signals were heterogeneous and focally distributed in both CIN and ISCC, whereas dot Insitu hybridization signals were entirely and evenly scattered on all of the cell nuclei as shown in (table 4).

It was evident from these data that dot or mixed Insitu hybridization signal were highest in the HPV positive CIN II/III and ISCC cases compared to condylomatous changes and CIN I .The Chi-square test showed that there was a significant association ($p<0.05$) between the Insitu hybridization signal pattern and the histologic types, and as shown in (table 5). The comparison between the observed and the expected values within CINII/III and the ISCC revealed a significantly higher integrated HPV (mixed or dot Insitu hybridization signals) compared to episomal HPV (diffuse Insitu hybridization signals).

Table 1: Age of the patients involved in the study

Lesions	Category	NO. (%)	Mean of age±S.D.*	Minus	Maximum
Normal	control	10(12.5)	23.1±3.814	18	30
Condylomatous changes	Case group1	20(25.0)	33.25±11.125	20	57
CIN I	Case group2	9(11.3)	42.00±9.605	26	60
CINII/CINIII	Case group3	14(17.5)	45.29±12.964	22	67
ISCC	Case group4	27(33.8)	49.67±10.126	35	85
Total		80(100)	40.61±13.668	18	85

* S.D.: standard deviation

Table 2: The difference in the mean of age among different histological types.

Histological type	Condylomatous changes	CIN I	CINII/III	ISCC
Normal	P < 0.05*	P <0.01**	P<0.01**	P <0.01**
Condylomatous changes		P >0.05	P >0.05	P <0.01**
CIN I			P >0.05	P >0.05
CIN II/CINIII				P>0.05
ISCC				

*Significant ** highly significant (ANOVA test).

Table 3: Number of HPV- positive and HPV- negative cases among different histological types of cervical tissue.*

Type	NO.	HPV Status			
		HPV-Positive		HPV-negative	
		NO.	%	NO.	%
Normal	10	0	0	10	100
Condylomatous changes	20	6	30	14	70
CIN I	9	1	11.11	8	88.89
CIN II/III	14	3	21.43	11	78.57
ISCC	27 Histological	9	33.33	18	66.67

*X^2 =5.751, P >0.05.

Table 4: Frequency and signals patterns of HPV 6/11/16/18/31/33 DNAs in relation to histological type of the cases examined

Histological type (NO.)	NO.OF HPV 6/11/16/18/31/33 DNAs positive (%)	Signal pattern		
		diffuse	dot	mixed
Normal(10)	0/10(0)	0	0	0
Condylomatous changes(20)	6/20(30)	5	0	1
CIN I(9)	1 /9 (11.11)	1	0	0
CIN II/III (14)	3/14 (21.43)	1	1	1
ISCC(27)	9 /27 (33.33)	0	3	6

Table 5: Association between HPV signal pattern and different histological types*

HPV6/11/16/18 /31/33 signal pattern	Observed/ Expected value	Histological type (NO.)					Total
		Normal	Condylomatous changes	CIN I	CIN II/III	ISCC	
Negative	O value	10	14	8	11	18	61
	E value	7.5	15.3	6.9	10.7	20.6	
Diffuse	O value	0	5	1	1	0	7
	E value	0.9	1.8	0.8	1.2	2.4	
Dot	O value	0	0	0	1	3	4
	E value	0.5	1.0	0.5	0.7	1.4	
Mixed	O value	0	1	0	1	6	8
	E value	1.0	2.0	0.9	1.4	2.7	
Total		10	20	9	14	27	80

*X^2 =21.381, P<0.05.

O= observed value, E= expected value

Discussion

In our retrospective study, it was not possible to evaluate the association between cervical lesions and different risk factors due to lack of clinical information. However, an obvious difference was noticed in the mean age of normal groups (23.10±3.814 years) was significantly lower than the risk groups (p, 0.01). This difference in age is likely attributed to the fact that the

females in the group with normal cervix were virgin and therefore were more likely to be young. While the mean age of patients with condylomatous changes was significantly lower than ISCC (33.25±11.125 years Vs 49.68±10.126 years) ($p<0.01$) and this follows the same epidemiological trend of the disease in developing countries that is a woman may become infected with HPV when she become sexually active and because persistent infection with high risk HPV is necessary for the development of CIN , the mean time between the initial infection and manifestation of invasive cervical cancer is estimated at about 15 years. Assessment of the minimal interval between HPV infection and manifestation of cervical cancer is currently the subject of many studies. This long development period suggest that, in addition to persistent infection with high risk HPV and immunological factors, the development of malignancy requires changes in the cellular genome of the HPV- infected cells 18.

There was no significant difference between the mean age of patients with CIN I and the mean age of patients with CIN II/III and ISCC (42.00±9.605 years Vs 45.29±12.964 and 49.67±10.126 years, respectively) ($P> 0.05$). In addition, there was no significant difference between the mean age of patients of patients with CINII/III and the mean age of patients with ISCC (45.29±12.964 years Vs 49.67±10,126 years) ($P >0.05$) and this consistent with a study suggest that approximately 15% of low grade, CIN I progress to high grade cervical lesion (CINII/III) within two years. Another study found that about one third of high-grade lesions (CINII/III) progress to cancer within ten years 8.

However, the peak incidence of cervical cancer in women more than 40 years old may be explained by the long duration of infection in older women infected with high-risk HPV genotypes. Moreover, persistent high-risk HPV infection in turn may increase the risk for the development and persistence of SILs 4. These facts should stress the need for early measures to detect these cervical lesions by cytological screening and hopefully prevent the development of cervical cancer 15.

Detection of HPV 6/11/16/18/31/33 DNAs in cervical lesions

In situ hybridization techniques are effective methods to detect and localize HPV DNA within the affected tissues. It is a reproducible, sensitive, specific, and precise method for localizing nucleic acid sequences in clinical tissue sections with exquisite preservation of tissue morphology. As an adjunct to conventional histopathology, Insitu hybridization may contribute to the understanding, diagnosis, and prognosis of disease 19.

The HPV DNA was detected by ISH with nonisotopic-labeled probe. The available probes for HPV detection consisted of a mixture of HPV probes 6/11/16/18/31/33. ISH for HPV DNAs was carried out on all 80 specimens. The results are summarized in table 3. All normal cases showed no specific signals for HPV DNA. Six (30%) of cases of cervical tissue with condylomatous changes, 1(11.11%) of nine cases of CIN I, 3 (21.43 %) of 14 cases of CIN II/III and 9 (33.33 %) of 27 cases of ISCC showed to be positive for HPV 6/11/16/18/31/33 DNA.

Our results were consistent with a molecular biology studies carried by Mohammed Ali (2002) in Iraq 20, who

found that 83.3 %, 33.3% and 24.24 % of archival tissues from patients with condylomata accumenata, preinvasive cervical neoplasia and ISCC, respectively were found to be HPV-DNA-PCR positive. Another study carried by Niakan et al (2003) in Iran 21 , showed that HPV DNA was detected in the nuclei of ISCC tumor cells in 13 (65%) of 20 cases using Insitu hybridization techniques.

Many of the studies were performed in non-Moslem western populations. The finding of these studies are often showed higher prevalence of HPV DNA in cervical lesions ranges from 25 to 90 %.HPV-16 accounts for the highest proportion, followed by HPV-18, HPV -45, and HPV- 31 , but their incidence varies depending on the country (22).

Our study was limited to the realistic possibility of determining significant clinical association between the extent of histological abnormality and the detection of HPV-DNA. From the overview of cervical cancer in Iraq we found that cancers of the cervix uteri constitute 1.4 % of the total number of cancers with annual number of 113 new cases of cervical cancer reported in 1995, 1996 and1997, respectively (Iraqi cancer board, 1999).

In this study, a significant association ($p < 0.05$) was found between Insitu hybridization signal pattern and the histological types, and as shown in (table 4) and (table 5) .Dot and mixed signal were detected among tissues with carcinoma and high grade CIN lesions but were rare among tissues with codylomatous changes (only one case showed mixed signal) and CIN I lesions, which characterized by diffuse signals occupying entire nuclei detectable in the mid/superficial layers, especially in the koilocytes, which are morphologically characteristics of HPV infection and probably indicate site of viral DNA replication in the superficial layers of the epithelium . This is consistent with the concept that papillomavirus replicates and undergo gene expression as a function of epithelial maturation (23).

In our study 1 of 3 HPV-positive high grade CIN showed increased from dot nuclear staining in the parabasal and intermediate layers to homogenous, strong nuclear staining in the superficial layers, confirming an increase in virus copy number, as has been reported previously by Stevenson et al. (2000) 24 . But HPV was not detected by the assay in basal/parabasal cells of CIN I lesions and cervical tissue with condylomatous changes.

The employment of in situ hybridization technique appears to be useful in detecting HPV infection within a histological test, and can be used as an indicator of HPV status. Our study showed that no significant association between the extent of histological abnormality and HPV infection. But there was a significant association between the Insitu hybridization signal pattern and the extent of histological abnormality. In all ISCC positive for HPV DNA, Insitu hybridization revealed a basic dot or mixed signal pattern in the nuclei, suggesting that HPV DNA integrated into tumor cell DNA. On the other hand, almost all codylomatous and CIN I displayed a diffuse, mainly epifocal, signal pattern.

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The Physiological Effect Of Chewing Khat Leaves On Human Spermatogenesis

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Abstract

Background: It was estimated that several million people are frequent users of khat, living in countries between South East Africa and Arabian Peninsula.

Khat (Catha Edulis Forsk) is described as an evergreen shrub of the species plant family celastraceae.

Chewing of khat leaves and ingesting the juices that contain the psychoactive substance, Cathinone, produces sympathomimetic and CNS stimulation.

It has deleterious effects on the rate of spermatogenesis, decreasing the sperm count; percentage of sperm motility and increase the number of abnormal sperm forms.

Objective: To prove that chewing khat affects the human spermatogenesis and sexual behavior; and to emphasize the cathinone effect on the human spermatogenesis and to explore the sexual behavioral changes due to khat use.

Materials and methods: A fifty healthy Yemeni khat chewers aged between 20 to 50 years were randomly selected. A questionnaire survey method was used to investigate their sexual behavior and a routine semen analysis was done.

Result: The study showed that 94% of the khat chewers have sperm counts below the normal range while 6% above the normal range; about 72% of the khat chewers have less than 60% active motile sperms and abnormal sperm forms while 28% of the khat chewers have more than 60% active motile sperm and abnormal sperm forms.

A correlation was found between the period of khat consumption and an initial decrease in sperm count and the percentage of active motile sperm with subsequent increase in the numbers of abnormal sperms.

A strong association was found in chewer's using high quality of khat and a decrease in sperm counts and the percentage of active motile sperms with increase the numbers of abnormal sperms when compared with low quality of khat.

***Conclusion*:** In Yemen, the habit of chewing khat affects human spermatogenesis. Khat chewing affects elderly people more than younger ones and the high quality of khat affects spermatogenesis more than low quality of khat variety due to its higher content of cathinone.

Keywords: Khat, Catha Edulis, Cathinone, spermatogenesis.

IRAQI J MED SCI, 2008; VOL.6 (1): 38-44

Introduction

It was estimated that several million people are frequent users of khat, living in countries between South-East Africa and South-West Arabia. Nowadays, due to the development of road networks and the availability of air transport, habitual use of khat spread to remote regions and countries for example US, and UK [1, 2] .

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Received: 15th March 2007, Accepted: 4th March 2008.

There are a variety of different names for khat in different countries. In Yemen more than 40 different khat types are known [3].

Khat is plant of an evergreen shrub, family (celastraceae), genus (Catha), Species (Edulis), and parts used (leaves) [4].

Khat grows best at 3000 - 6000 feet above sea level. The height of the khat tree varies from small to large; it may reach 20 feet in height. Khat tree is a seedless plant; this may explain its limitation to Yemen and nearby Ethiopia and eastein Africa. Its leaves and twigs can be harvested many times throughout the year [5].

Traditionally, khat leaves are chewed in the company of others as a social and cultural activity. During the chewing of khat leaves, the juice is swallowed with the saliva. The residue is not spit out but gathered in the cheek, usually for the whole period of chewing. During khat chewing, considerable amounts of liquids as tea and soft drinks, are also ingested.

The swallowed juices contain the psychoactive substance *cathinone*, which produces sympathomimetic activity and CNS stimulation analogous to those produced by amphetamine [6].

Historically, the original source of khat was first observed in Ethiopia and from there it was transferred to Yemen in the thirteenth century.

The earliest scientific report on khat presented to western literature was written in the eighteenth century when the botanist *Peter Forskal* identified the plant in Yemen (*Catha edulis*) [7].

Several investigations and research works have been done in attempt to expose the harmful effects of khat chewing on the health of the consumers.

Khat was found to contain many chemicals, among which are alkaloids including (cathinone, cathine, ephedrine), tannic acids, amino acids, choline, minerals, and vitamins [8].

In view of this finding arises a question concerning the extent to which each of the different substances present in khat contributed to the effects observed after khat chewing.

Some reports said that khat was used historically for medical purposes; other reports associate khat to socio-medical problems [9], and even other reports mention psychiatric disorders secondary to khat- chewing [10].

Several authors report that the cultivation of khat in Yemen has increased considerably over the years. Because its net return per unit area is greater than that of coffee, khat is currently being grown in many areas previously used to grow coffee.

It was recently reported in Yemen that khat represents about 12.5% of the gross domestic product and that the consumer spends about 19% of his income on khat.

In Yemen, the law does not prohibit the use of khat. However, and over the last few years, khat consumption by the public task force is being advised against [11].

The chemical aspects of Khat are Cathinone, the alkaloid present in khat leaves, is quite similar to structure to amphetamine. The only structural difference between the two is that the methylene group found in the a position in amphetamine is replaced by a carbonyl group in cathinone. However, cathinone has a shorter duration of action in comparison to amphetamine. Cathinone is estimated to be one third as potent as amphetamine and ten times as potent as cathine [12].

It was found that during the decomposition of the plant through drying and storage, cathinone contained within the plant is enzymatically converted to cathine.

Cathinone was placed into *Schedule I* of the controlled substances; Cathine is ruled as Schedule IV substance.

The amount of khat chewed is variable. It depends on the consumer and the duration of the party. The average amount per person is one bundle of khat.

Pharmacological aspect of Khat; the most common route of use of khat is chewing.

However it can be smoked or drunk like tea. The khat-chewer prefers fresh leaves and shrubs filling his/her mouth to capacity, chewing intermittently to release the active component, cathinone, which is rapidly absorbed after oral administration, in contrast to cathine [13].

Cathinone is lipophilic; it penetrates into CNS with more ease than cathine.

The peripheral effect induced by khat can thus be considered to be predominantly due to
cathine while the central effects are due to cathinone.

It was found that the maximal effects of cathinone are observed 30 min after initiation of chewing, whereas the maximal effects of cathine are seen after 3 hrs [(12)].

Cathinone is metabolized rapidly in the liver into norephedrine and is almost entirely excreted in this form. Only 2% of cathinone appears in the unchanged form in urine.

The results of various studies in vitro and in vivo experiments suggest that the effects of khat alkaloids are due to amphetamine-like effect at the cellular level and that cathinone is the main psychoactive constituent of khat.

Khat has various effects on each of the following systems: CNS, alimentary system, liver, CVS, respiratory system, eyes, pregnancy and lactation, genitourinary system, metabolism and endocrine system, sexual potency [(13, 14, 15, 16, and 17)].

In 1973 the WHO expert committee on drug dependence included khat type *« Catha Edulis Frosk »* in their group of *dependence–producing drugs*.

The categories of drug dependence they produced were:

1. Morphine type.
2. Barbiturate - alcohol type
3. Cocaine type
4. Cannabis type
5. Amphetamine type.
6. Khat type.
7. Hallucinogen type (18)

Material and methods

Fifty Yemeni volunteers, who aged twenty to fifty years, were selected randomly from khat chewers. Before signing the application form, the volunteers were informed about the aims of the research.

First, the volunteers have to give over their semen, each participant was asked to perform masturbation in the bathroom of the laboratory and give over his semen sample in a clean container that was labeled using numbers by the laboratory technician.

A routine semen analysis to investigate three basic parameters (sperm count, percentages of active motile sperms, and abnormal formed sperms) was done immediately after of liquefaction of the semen, the results of the three basic semen parameters were documented. These three parameters were chosen because of their importance in the evaluation of spermatogenesis. The results of semen parameters that were obtained in the present study were compared with normal values stated by the most recent textbooks of physiology. ***Second,*** the volunteers were asked to answer to certain questions concerning their sexual behaviors in private

Results

The study showed that 94% of the khat chewers have sperm counts below the normal range while 6% above the normal range; about 72% of the khat chewers have less than 60% active motile sperms and abnormal sperm forms while 28% of the khat chewers have more than 60% active motile sperm and abnormal sperm forms. Show in (Table 1, 2, 3)

A correlation between the period of khat consumption and an initial decrease in sperm count and the percentage of active motile sperm with subsequent increase in the numbers of abnormal sperms. Show in (Table 4,5,6,7)

A strong association was found in chewer's using high quality of khat and an decrease in sperm counts and the percentage of active motile sperms with increase the numbers of abnormal sperms when compared with low quality of khat (Table 8).

Table 1: The variation in the average sperm count between normal people and khat chewers.

Normal averaged sperm count	120 million / ml	
Average sperm count in khat chewers	40 million /ml	33 %
Chewers with less than 120 million / ml	47	94%
Chewers with more than 120 million / ml	3	6%

Table 2: The variation in the average percentage of active motile sperm between normal people and khat chewers.

Normal average percentage of active motile sperm.	More than 60 %	
Average percentage of active motile sperm in khat chewers.	35 %	
Chewers with less than 60 %	36	72%
Chewers with more than 60 %	14	28%

Table 3: The variation in the average percentage of abnormal sperm forms between normal people and khat chewers.

Normal average percentage of abnormal sperm form.	10 % or less	
Average percentage of abnormal sperm form in khat chewers.	26 %	
Chewers with more than 10%	36	72%
Chewers with 10% or less	14	28%

Table 4: Relation between khat consumption periods and chewer's sperm count and chewer's percentage of active motile sperm.and chewer's percentage of abnormal sperm forms.

Years	0-5			6-11			12-17			19-23		
Basic semen parameters *	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Number	18	18	18	19	19	19	7	7	7	6	6	6
Average	49.84	44.72	24.16	36.72	30.20	25.00	38.32	33.57	25.71	20.50	25.00	35.0
Standard deviation	43.10	26.64	9.11	36.30	23.44	10.27	32.17	19.30	10.96	17.25	14.14	4.47

* Basic semen parameters (A= sperm count, B= percentage of active motile sperm, C= abnormal sperm forms)

Table 5: Relation between khat chewing weekly frequency and chewers sperm count and chewer's percentage of active motile sperm, and chewer's percentage of abnormal sperm forms.

Hours	2 – 3			4-5			6-7		
Basic semen parameters	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Number	8	8	8	27	27	27	15	15	15
Average	73.02	55.00	18.12	38.08	32.40	26.29	26.09	30.66	31.00
Standard deviation	56.45	29.76	8.42	30.34	20.44	9.66	29.94	23.21	6.86

Table 6: Relation between khat chewing daily frequency and chewers' sperm count and chewer's percentage of active motile sperm, and chewer's percentage of abnormal sperm forms

Days	2 – 3			4 – 5			6 – 7		
Basic semen parameters	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Average	59.72	48.00	19.00	41.50	38.75	24.06	30.68	28.12	30.20
Standard deviation	55.27	32.93	11.25	33.91	22.84	9.34	27.02	22.18	8.53
Number	10	10	10	16	16	16	24	24	24

Table 7: Relation of sperm count between Khat chewer's age groups. and chewer's percentage of active motile sperm and chewer's percentage of abnormal sperm forms

Years	20 - 25			26 - 31			32 - 37			38 - 43			>43		
Basic semen parameters	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Number	16	16	16	15	15	15	10	10	10	7	7	7	2	2	2
Average	50.12	45.6	21.9	41.0	33.7	25.0	34.3	33.5	27.0	25.2	25.0	30.7	14.5	20.0	37.5
Standard deviation	46.78	27.9	9.8	33.3	23.4	7.3	27.6	22.9	8.2	23.8	15.5	13.0	16.3	7.1	3.5

Table 8: Relation ship between khat quality and chewers sperm count, percentage of active motile sperm and percentage of abnormal sperm forms

.

Basic semen parameters	Sperm count		Abnormal sperm motile		Abnormal sperm forms	
	High quality	Low quality	High quality	Low quality	High quality	Low quality
Number	22	28	22	28	22	28
Average	11.54	61.31	18.40	48.92	31.13	22.67
Standard deviation	9.80	35.49	11.89	22.62	7.54	9.37

Discussion

Our discussion on khat-chewing effects on the human spermatogenesis is based on the following:

To prove that the habit of chewing khat leaves affects the three basic semen parameters. The total average sperm count and total percentages of active motile sperms were found to be significantly decreased, while the total percentage of abnormal form sperms was found to be significantly increased in the volunteers' spermogram indicators.

To emphasize the results of the research we classified all khat-chewers into different groups according to their consumption duration and frequency of khat chewing. A significant negative correlation was found between these factors and all the three basic semen parameters. The effects of khat desired by the chewers are those associated with stimulation of the CNS. Cathinone, the principle active substance from khat leaves, was found in much higher levels in the high quality of khat than in the low quality of khat.

For this reason, we grouped the khat chewers according to the quality of khat. The group including the high quality of khat chewers showed more worsening in the average values of the three basic semen parameters as compared with the group including the low quality of khat chewers.

We observed a high percentage of the chewers were complaining of:

- Increased sexual desire (libido) (90%).
- Spermatorrhea (70%)
- Decrease sexual potency (56%).

- Wet dreams (emission) (36%).
- Testicular pain (26%).

Recommendation

1- People education through mass media about the adverse effect of khat on male reproductive system.

2- Prohibit the use of khat among our people though law legislation.

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Effect of Laser light on lymphocyte Apoptosis

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Abstract

Background: Apoptosis is a physiological type of cell death characterized by certain morphological nuclear and biochemical changes. This process deletes the unwanted cells by a clean mechanism that does not evoke any inflammating changes.

Objective: To show the effect of laser light on the lymphocyte apoptosis.

Subject & Methods: The study was conducted on lymphocyte apoptosis. The taken number of apoptotic lymphocyte was estimated before and after exposure.

Results: Results showed that there was a significant increase in the percentage of lymphocyte apoptosis after exposure to 630nm laser light, which was further increased by increasing the time of exposure.

Conclusion: Lymphocyte apoptosis can be induced by low dose of laser and increasing the exposure time can increase this.

Keywords: He-Ne Laser light, lymphocyte apoptosis

IRAQI J MED SCI, 2008; VOL.6 (1):45-51

Introduction

Apoptosis is a genetically controlled process that's why it's called programmed cell death; it plays an important role in tissue homeostasis; differentiation and development like in the separation of fingers during limb development [(1)].

Apoptosis can be triggered by different stimuli which can be intracellular or extracellular; some known extracellular signals include receptor ligand such as TNF-α, Fas-ligand as well as signaling proteins that cross the membrane such as Granzyme- B and non-protein activators as calcium and radiation [(2)]. The intracellular apoptotic signals as reactive oxygen species or laser therapy trigger apoptosis at the mitochondrial membrane, this cause opening of mitochondrial membrane transition pore, which lead to release of cytochrome C- and execution of apoptotic process. [(3)], both, intracellular and extracellular, passes in two phases: an initial commitment phase (when the cell responds to signal that commit the cell to undergo self destruction) and an executional phase (when cell death can not be stopped) [(4)], which results in typical morphological changes of apoptotic cell death; as plasma membrane blebbing, shrinkage of the cell, nuclear chromatin condensation, lastly fragmentation into apoptotic bodies that will be phagocytosed by neighboring cells like macrophages [(5)].

Surface changes are probably due to the cleavage of cytoskeletal protein, such as fodrin, gelsolin, pectin, actin and cytokeratin [(6)].

Materials& Methods

Fresh blood samples were obtained from the anti cubital vein of twenty six healthy subjects with sterile EDTA tube, the sample diluted 1:1 with isotonic phosphate buffered saline (PH 7.4). Two milliliters of this mixture were carefully layered on the top of

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Received: 20th March 2007, Accepted: 12th November 2007.

four milliliters of ficoll, in a ten milliliters siliconized glass centrifuge tube, lymphocyte separation done according to method of Boyum and Scan., 1968 [7]. The above compound was centrifuged in cold centrifuge at (3100 rpm), for 30minutes at 24°C. After centrifugation, lymphocyte forms a white buffy coat at the interface of the blood plasma which was aspirated by Pasteur pipette and transferred into a ten ml siliconized tube, washed three times by PBS for 20min until a pellet was formed, the lymphocyte pellet re-suspended in 0.5 ml of PBS.

The lymphocyte then was exposed to laser beam (633nm) for 15 minutes and for 30 minutes and both conditions were studied as follows:

A. Cell count and viability:

The number of lymphocytes were counted using Heamocytometer counting chamber, the results were expressed as cell/mm^3 [8]. Trypan blue exclusion test was done to assess cell viability. A known volume of lymphocyte suspension (100µl) was mixed with an equal volume of trypan blue dye (the concentration of trypan blue 0.2%) and examined immediately under light microscope.

One ml of the rest volume of the lymphocyte suspension drawn to a Westergren tube fixed by holding it directly in front of the laser beam so that the beam passes directly through the opening end of the tube and thus the whole suspension was exposed to the light for (15minutes and 30minutes.), then after each of the different exposure time, cell count, viability were estimated.

Morphology of lymphocyte was studied after staining of lymphocytes by acridine orange according to procedure of Vacca (1985) [9]. Slides were examined by fluorescent microscope.

Mitochondria membrane permeability changes were studied using mitolight (mitochondrial permeabilization detection kit (chemicon).

In healthy cells, the dye accumulates and aggregates in mitochondria giving off a bright red fluorescence
(λem = 585-590 nm), while in apoptotic cell the mitochondrial membrane brakes down and so the dye remains in the cytosol in its monomeric form giving off a green fluorescence (λem = 527-530 nm).

B. Cellular morphology:

The morphological characteristics of lymphocyte apoptosis were assessed according to the method of Willingham (1999) [10]. The morphological changes related to apoptotic process as stated by Collins [11], these changes include: Membrane blebbing, cell shrinkage, chromatin condensation and fragmentation of nuclear material, DNA fragmentation, and lastly formation of apoptotic bodies. Cellular morphology was assessed after staining the cells by acridine orange (DNA binding specific dye) [8], and examined freshly by fluorescent microscope.

Results

T-test was used for the analysis of the results. The results of the lymphocyte apoptosis was assessed before and after irradiation with 630nm laser light, included, cellular changes related to the staining cell by trypan blue and examined by light microscope, (figure 1). The percentage of lymphocyte has apoptosis increased significantly ($P< 0.0005$) after irradiation as shown in (figure 2).

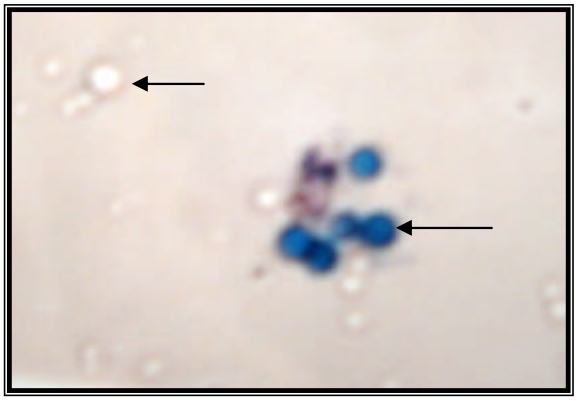


Figure 1: Trypan blue keeping out test white cell normal, dark cell (apoptotic) stained blue. Light microscope (10X)

The morphological changes included: membrane bleb formation, nuclear changes like condensation of nuclear material, kidney-shaped nucleus, fragmentation of nuclei, the percentage of these cells increased significantly (P< 0.005) after irradiation, (figure 3). After increasing the exposure time of lymphocyte to laser (30 minutes), the percentage of lymphocyte apoptosis significantly increased (P< 0.0005), (figure 4).

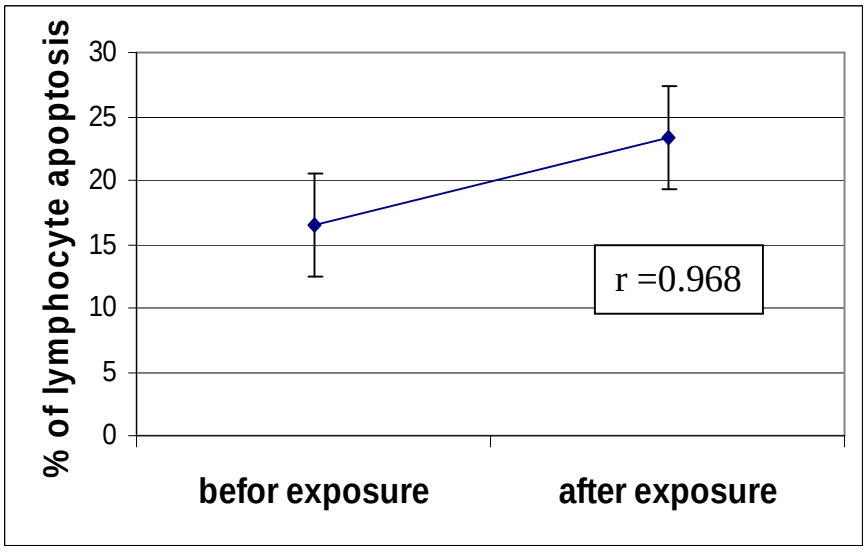


Figure 2: Comparison of the percentage of lymphocyte apoptosis before and after the exposure to laser by Trypan blue exclusion test using light microscope

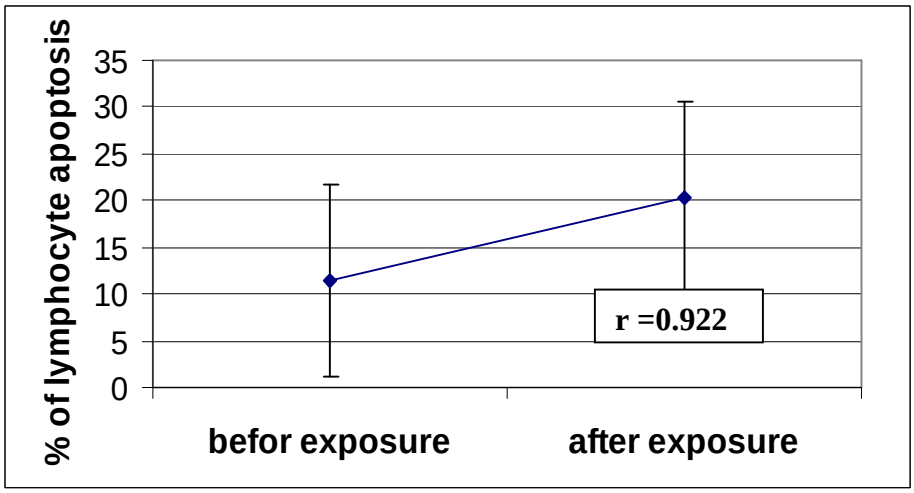


Figure 3: Comparison of the percentage of the lymphocyte apoptosis stained by acridine orange before and after the exposure to laser by using fluorescent microscope.

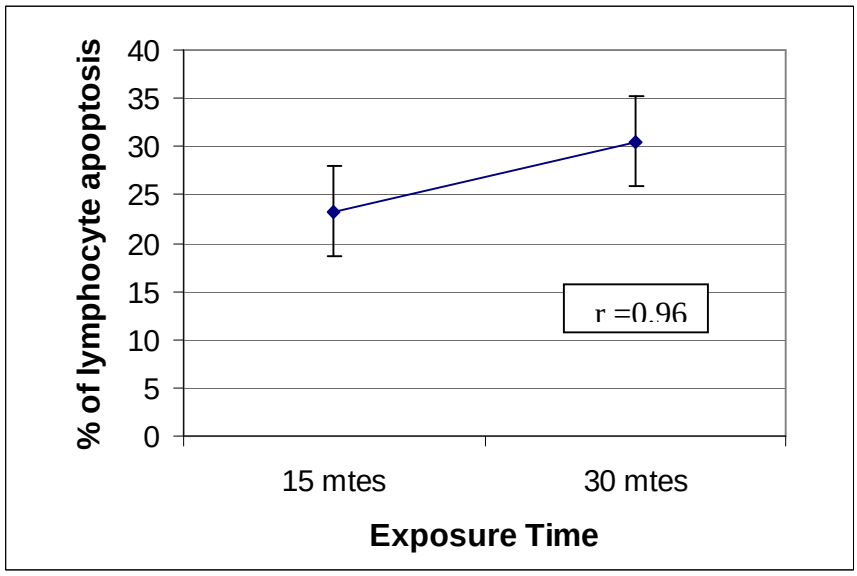
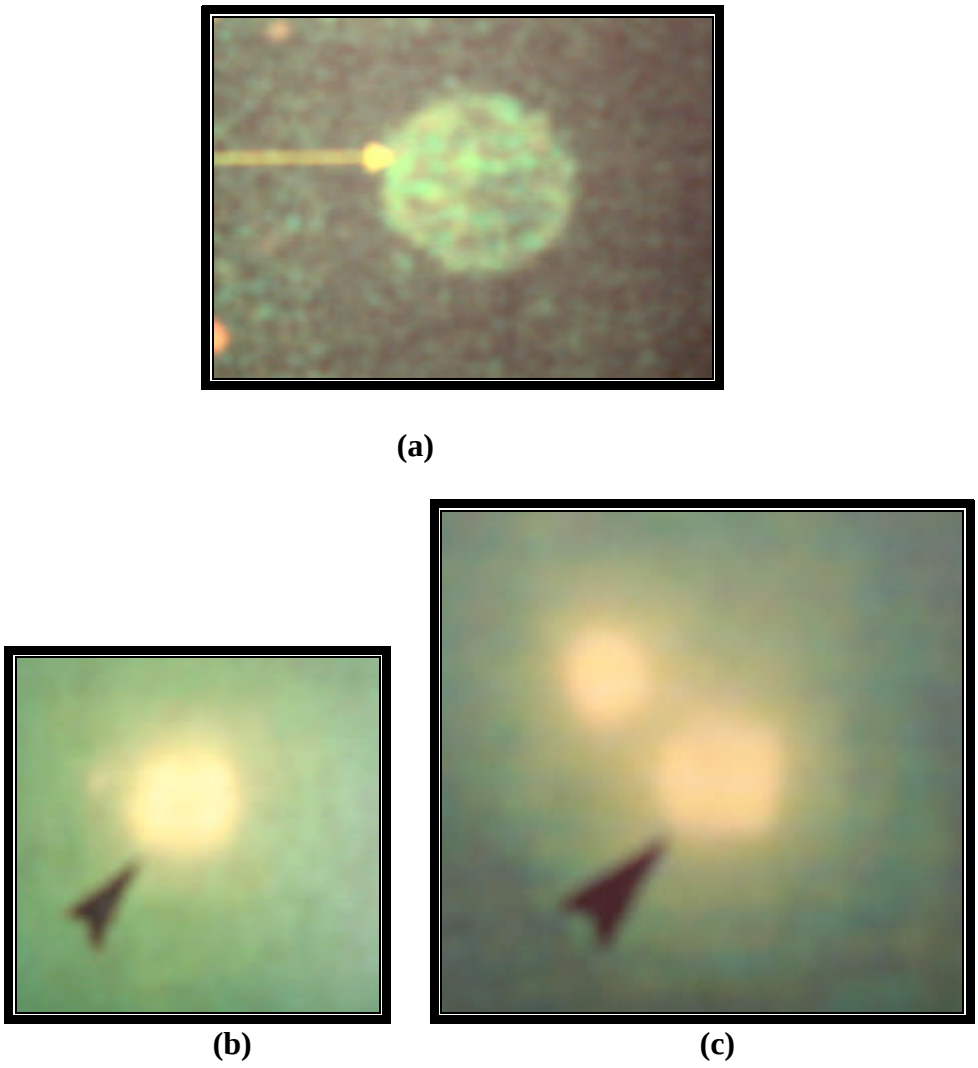


Figure 4: Effect of increasing the exposure time on lymphocyte apoptosis by morphological changes.

A morphological change was observed for lymphocyte having apoptosis, which stained by achridine orange and examined by fluorescent microscope (figure 5).

(Figure 5) shows lymphocyte stained by DNA specific dye (Acridin Orange): (5.a) the normal cell shows intact rounded cell with yellow fluorescence. While in figure (5.b) shows apoptotic cell with membrane bleb formation then separation of cell into two fragments as shown in figure (5.c).

The morphological changes related to mitochondria are shown in (figure 6). In normal lymphocyte the dye accumulate in mitochondria giving off a bright red fluorescence (6.a) while in apoptotic cell the mitochondrial membrane disturbed and the dye will gives off a green fluorescence (6.b).



(a)

(b) **(c)**

Figure 5: Lymphocyte stained by acridin orange (DNA binding specific dye) shown by fluorescent microscope 40X.
a. Normal lymphocyte cell.
b. Membrane bleb formation.
c. Fragmentation of nucleas into two parts.

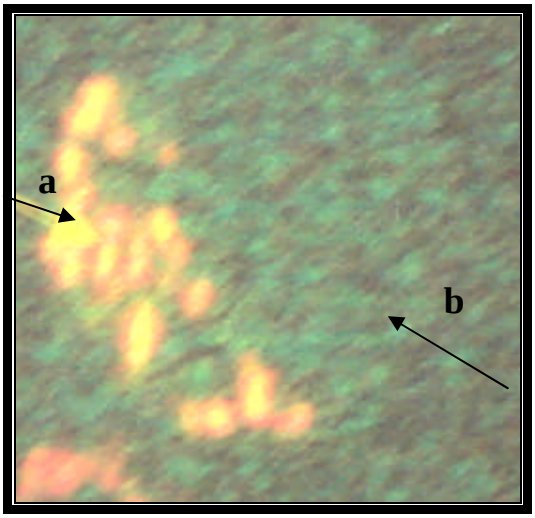


Figure 6: Mitochondria (mitolight detection kit)
a. Normal cell mitochondria (red).
b. Arrow apoptotic cell show green
fluorescence of mitochondria shown by fluorescent microscope (40X).

Discussion

Lymphocyte apoptosis can be induced by many factors some acts on cell membrane and can trigger lymphocyte apoptosis by external pathway like fas ligand and granzyme-B, or withdrawal of growth factors like IL-2. All these can stimulate lymphocyte apoptosis by external pathway that lead to activation of caspases 8, 9, 7 and other down stream caspases [12].

Other factors can stimulate lymphocyte apoptosis after penetrating cell membrane and act directly on internal apoptosis pathway or mitochondrial pathway, this trigger like laser.

Results in figures 2, 3 and 4 shows that there is an increase in lymphocyte apoptosis caused by the exposure to laser light, which increased further by increasing time of exposure. This could be interpreted by: the mechanism of photon energy conversion in laser medicine is heating. A photobiological reaction involves the absorption of a specific wavelength of light by the functioning photoreceptor (photoaccceptor) molecule (molecules capable of absorbing the wavelength used for irradiation resulting in a photobiological response), after absorbing the light of the wavelength used for irradiation this molecule assumes an electronically excited state [13]. So the supplied heating due to irradiation with laser light 633 nm will penetrate lymphocyte membrane and cause disruption of mitochondrial membrane potential caused changes in mitochondrial optical properties [14] which may lead to release of cytochrom-C that stimulate down stream caspases 3 and 6 and lead to lymphocyte apoptosis [15]. This was proved by mitolight detection kit in this study, which showed changes in color upon changes in mitochondrial membrane potential which was induced by laser therapy

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Effect of tamoxifen on antioxidant vitamins, uric acid and gamma-glutamyl transpeptidase in breast cancer patients

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Abstract

Background: Many research works clearly indicate that free radical and reactive oxygen species play a major role in the etiology and development of breast cancer in postmenopausal women. Available literatures suggest that tamoxifen is a potent suppressor of lipid peroxidation in experimental animals**.**

Objectives: The objective of this study is to understand the antioxidant status and oxidative stress in breast malignancy of postmenopausal women before and after treatment with tamoxifen.

Methods: Eight to nine months' tamoxifen therapy (10 mg twice daily) in 19 postmenopausal women was conducted. Serum levels of vitamin A, E & C were determined; also uric acid& GGT were determined. The results were correlated with serum MDA levels. The results were compared with those in patients with breast benign tumors (N=21) and control group (N=23).

Results: A highly significant decrease in antioxidant vitamins levels in breast cancer patients were noticed (P<0.001) compared with those of benign tumor. Also a significant increase in uric acid, GGT, and MDA levels was observed in cancer patients (P<0.01). There was a significant increase in antioxidant vitamins (P<0.01) and significant decrease in uric acid, GGT and MDA levels in cancer patients after treatment with tamoxifen.

Conclusion: The results suggest that tamoxifen exerts a significant effect on the rate of lipid peroxidation and a major improvement in antioxidant status

Keywords: Breast cancer, Tamoxifen, Antioxidant Vitamins, GGT

IRAQI J MED SCI, 2008; VOL.6 (1):52-59

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Introduction

Breast cancer is a complex and important malignancy among women in modern societies. In the year 2000 more than 500,000 death cases were attributed to worldwide breast cancer [(1)]. In Iraq this disease showed a multiple increases during the last decade according to the Ministry of Health [(2)]. Despite the importance of the disease, its etiology and pathogeneses have not been elucidated [(3)].

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury [(4, 5)]. The cellular oxidative damage is caused primarily by free radicals and reactive oxygen species. Free radicals have the ability to bind most normal cellular compounds; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids [(4)]. A disturbance of the balance between formation of active oxygen metabolites and the rate at which they are scavenged by different types of antioxidants is referred to as oxidative stress [(5)]. Prime targets of reactive oxygen species are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation, which may lead to damage of the cell structure and function [(6)]. Additionally, decomposition of lipid hydroperoxides yields a wide variety of

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Received: 15th April 2007, Accepted: 17th December 2007.

end products, including malondialdehyde (MDA) [6]. MDA was considered as a marker of oxidative stress by many investigators [7].

Growing evidence has indicated the role of antioxidant vitamins in cancer prevention and treatment [8]. Antioxidant vitamins were known to prevent the cell damage caused by reactive oxygen species (ROS) and keep the immune system intact against the diverse effect of free radicals [9].

Tamoxifen, a nonsteroidal antiestrogenic drug is widely used in breast cancer cases. It induces tumor regression in women with advanced metastatic breast cancer [10]. The anti-tumor activity of tamoxifen is still uncertain. However, anti tumor activity of tamoxifen is largely believed to be due to occupying the intracellular estrogen receptor sites in target tissue thus, it's blocking the action of biologically active estrogen and estradiol. Recent reports demonstrate that tamoxifen exerts antiproliferative effects on estrogen receptor- positive breast cancer cells [11]. Additional anti proliferative effects of tamoxifen may be related to its inhibition of protein kinase [12] and it's binding to calmodulin, a protein that plays a role in DNA synthesis. Therefore, this drug acts as a suppressor of breast cancer not only through acting as a competitor to estrogen but also through mechanism. No previous studies had elucidated the relationship between antioxidant status and oxidative stress in breast cancer patients treated with tamoxifen.

In this study, the changes in serum levels of vitamin A, E & C, uric acid& GGT were determined in postmenopausal women before and after

treatment with tamoxifen. The results were correlated with those of MDA in the sera of the corresponding patients.

Patients and methods

Forty postmenopausal women inflicted with infiltrative ductal carcinoma were involved in this study. The patients were referred to Baghdad Teaching Hospital and the Hospital of Radiotherapy and Nuclear Medicine, Baghdad, Iraq, for the period starting from June 2004 to the end of July 2005. All cases were diagnosed by histopathological and radiological procedures provided in those medical centers. The mean age of the patients was 48±12 years with a range of 38-72 years. Body mass index of the corresponding patients was 21.9±7.5 Kg/m^2. Nineteen subjects of those cancer patients were treated with tamoxifen (10 mg twice daily) for eight to nine months by the same medical center.

Twenty-three patients with benign tumor (fibroadenoma) were used as pathological control. Healthy control group consisted of 21 postmenopausal women with comparable age and body mass index.

Ten milliliters samples of venous blood were taken from all groups. Those samples were taken in malignant cases before tamoxifen treatment (MBT group) and after treatment with the drug (MAT group). Blood samples were left for 20 minutes at room temperature. After blood coagulation, the sera were separated by centrifugation at 3000-x g for 15 minutes. Hemolyzed samples were discarded.

The concentration of vitamin A in the collected serum samples was determined according to the modified method of Neeld and Pearson [13]. Vitamin E was determined according to the method of Hashim & Schuttriger [14], while vitamin C was determined according to the method of Toronto [15]. Standard curves were established for each of the above determination using authentic samples of those vitamins. Serum uric acid was measured by enzymatic colorimetric assay using test kit supplied by uric acid Giesse diagnostics, Italy. Gamma glutamyl transpeptidase (GGT) activity was also

determined by the test kit of the above company. The method depends on the use of gamma glutamyl para anilide as substrate [16]. Malondialdehyde (MDA) levels were determined according to the method of Buege and Auts [17].

All results were expressed as mean ± SD. Descriptive and inferential statistic were used to describe the results and their interrelationship.

Results

Vitamin A shows a high significant decrease in cancer patients compared with that of patients with benign tumors as shown in table (2): the results show also a highly significant increase in the levels of this vitamin in cancer patients after treatment (MAT). A similar trend of significance was noticed in the serum level of vitamins E&C in different groups.

A significant decrease in serum GGT activity occurs in MAT group compared with MBT group. Similar changes were observed in the levels of serum uric acid of the corresponding groups. A gradual and significant decrease in serum MDA levels in MAT group compared with MBT group was seen.

(Table 3) shows ANOVA analysis and the results of correlation between oxidative stress index (represented by MDA level) and concentration of antioxidant vitamins, uric acid and GGT in MBT group and MAT group of breast cancer patients.

Highly significant correlations were noticed between MDA& vitamin A ($P<0.001$). Also a highly significant correlation was observed between MDA& vitamin C ($P<0.01$) and MDA& uric acid ($P<0.001$) while there is a significant correlation between MDA& vitamin E ($P<0.05$).

Table 1: Serum levels of antioxidant vitaminA,E &C, uric acid, gamma- glutamyl transpeptidase (GGT), and malondialdehyde (MDA) in different cases of breast tumors.

Gtoup type (No.of studies)	Component	Mean	SD	SE
(MBT)(N=40)	Vit.A (mg/dl) $x10^{-3}$	16.06	8.99	1.42
(MAT)(N=19)		48.31	17.60	4.01
(B)(N=23)		39.24	15.69	3.27
(C)(N=21)		51.49	21.74	4.74
MBT	Vit.E (mg/dl) $x10^{-2}$	36.68	18.67	2.90
MAT		77.70	20.75	4.64
B		82.93	22.78	4.75
C		102.74	27.96	6.01
MBT	Vit.C (mg/dl)	0.387	0.141	0.031
MAT		1.110	0.349	0.078
B		1.067	0.321	0.067
C		1.621	0.527	0.115
MBT	GGT (IU/L)	103.98	18.88	4.22
MAT		41.16	9.46	2.15
B		39.13	7.83	1.63
C		20.72	8.64	1.88

MBT	Uric Acid (mmol/L)	539.2	41.3	9.24
MAT		380.5	63.5	14.20
B		346.3	114.3	23.80
C		237.5	108.6	23.10
MBT	MDA (µmol/L)	2.211	0.312	0.061
MAT		0.988	0.323	0.072
B		0.949	0.315	0.065
C		0.701	0.179	0.039

MBT: Malignant before treatment
MAT: Malignant after treatment
B: Benign tumor group
C: healthy control

(Table 1) reveals the mean values of the sera levels of vitamin A, vitamin E, vitamin C, GGT activity, uric acid and MDA in women with breast malignancy before treatment (MBT group) and for those after treatment with tamoxifen (MAT group). The table shows also the mean values of the up-mentioned components in postmenopausal women with benign tumors (group B) and those for healthy women as control group (group C).

Table 2: The comparison of serum components in the different groups of breast tumors and healthy control.

	P-Value			
Component	**M vs. C**	**M vs. B**	**M vs. C**	**MAT vs. MBT**
Vit. A	0.001	0.001	0.01	0.001
Vit. E	0.01	0.01	0.01	0.001
Vit. C	0.01	0.02	0.04	0.001
GGT	0.01	0.01	0.05	0.01
Uric Acid	0.001	0.01	0.01	0.001
MDA	0.01	0.02	0.05	0.01

GGT: Gamma- glutamyltranspeptidase
MDA: Malondialdehyde

Table 3: Correlation coefficients and the significance levels of different serum chemical components in patients with breast tumors.

Component vs MDA	MBT					MAT				
	Slope	**Intercept**	**R^2**	**r**	**P**	**Slope**	**Intercept**	**R^2**	**r**	**P**
Vit. A	-67.4	2.28	0.372	0.160	<0.05	-13.86	1.66	0.659	0.754	<0.001
Vit. E	-2.25	2.75	0.432	0.658	<0.02	-0.73	1.55	0.220	0.466	<0.05
Vit, C	-0.002	0.98	0.151	0.386	<0.1	-0.003	0.32	0.46	0.676	<0.001
GGT	0.01	1.12	0.783	0.884	<0.001	0.02	0.12	0.380	0.616	<0.001
Uric acid	0.002	0.98	0.150	0.386	<0.1	0.003	0.03	0.460	0.676	<0.001

GGT: Gamma- glutamyltranspeptidase
MDA: Malondialdehyde

Discussion

There is a growing evidence for the role of free radicals and reactive oxygen species in the initiation and promotion of different kinds of malignant tumors [7]. Many investigators attributed such increased incidence of cancer with advancing age to the increasing level of free radicals reaction with age and to decreased ability of the immune system to detoxify those free radicals [8, 9]. A variety of pathological events such as diabetes, atherosclerosis and aging were also attributed to the enzymatic and non-enzymatic oxidation of biological molecules [7].

Many vitamins, enzymes, organic molecules and trace elements play a major role in scavenging those free radicals generated from food oxidation and many pollutants.

The results in (table 1) reveal a decrease in the levels of vitamins A, E&C in cancer patients. Such decreases may play a great role in the development of malignancy, since that antioxidant vitamins reveal great actions in the physical and chemical quenching of oxygen and superoxide radicals generated from oxidation processes inside the human cell. Vitamin A was shown to be involved in the stimulation of the immune system and cancer suppressor genes as well as deregulate of oncogenes and block tumorgenesis [18, 19]. Vitamin E was reported to be an important factor in the induction of apoptosis of cancer cells beside its action in quenching of free radicals and increase of the capability of the immune system [20]. Vitamin C is considered the most powerful natural antioxidant, which protects indispensable macromolecule in the human body from damage by free radicals.

The decrease in serum levels of antioxidant vitamins was greater in Iraqi women of breast cancer in comparison with western population. This can be attributed to nutritional differences among different societies and to the

difference in concentration of air, water and food pollutants among those populations [7].

The increase in serum uric acid levels in cancer patients can be referred to tissue hypoxia, which may lead to a concomitant increase in xanthine oxidase activity as reported by Moison et al. [21]. Uric acid is a well-known antioxidant in the human body and this phenomenon must lead to a decrease in its serum level with the development of malignancy. Such net increase in the concentration of uric acid in cancer patients can be attributed to the exceeding effect of increased xanthine oxidase activity (due to tissue hypoxia). This effect combined to increase cell damage, which is a recognized characterstic of cancer tissue. Our results are cosistent with those reported by Kolonel et al [22] and Hameed et al [23]. The significant increase in GGT activity in cancer patients can be attributed to the continuous release of this enzyme from the surface of cancer cells and its direct leakage to the blood circulation. GGT is a membrane-bound enzyme, which is affected significantly in cancer cell more than the normal cell due to changes of cell membrane accompanying carcinogenesis. It is noteworthy to consider this enzyme as tumor progression marker and can be used for monitoring of tumor regression also. Our results are more significant in comparison with those reported by Seth et al [24] and Mishra etal [25].

A highly significant change in MDA levels in cancer patients compared with other groups can be attributed to increase in the byproducts of lipid peroxidation due to transition to malignancy. (Table 3) shows a good negative correlation of this component with antioxidant vitamins while there was a significant positive correlation between MDA and each of uric acid &GGT.

The results of this study reveal the effect of tamoxifen treatment of antioxidant status and oxidative index (represented by serum MDA level) in the respective patients. The highly significant increase in the serum levels of antioxidant vitamins and the highly significant decrease in MDA&GGT after treatment reinforce the importance of using this drug for the recovery of oxidative status in breast cancer patients.

In fact the decrease in lipid peroxidation in the treated women is unexpected finding, because tamoxifen action was thought entirely to be due to its interference with biologically active estrogen and estradiol. Studies about estrogen and its metabolites show that estrogen is a natural antioxidant of membrane lipid peroxidation [26]. The inhibitory action of estrogen on lipid peroxidation remains obscure. However estrogens may donate hydrogen atoms from their phenolic hydroxyl groups to lipid peroxy radicals for the termination stage, moreover, the examination of the chemical structure of tamoxifen shows no apparent reason for why it acts as chain-breaking inhibitor of peroxidation, because there is no group bearing an easily donatable hydrogen atom. However antioxidant activity of tamoxifen may be explained in the following manner: during tamoxifen medication, tamoxifen is hydroxylated and demethylated in the liver under influnce of the enzyme cytochrome P-450 resulting in the formation of 4-hydroxy tamoxifen and N-desmethyl tamoxifen [27]. Like estrogen, 4-hydroxy tamoxifen has a more powerful inhibitory effect on lipid peroxidation than its parent compound. The phenolic hydroxyl group confers this chain breaking antioxidant property and the putative effects on membrane structure [11]. The clinical antiestrogenic activity of tamoxifen is likely because of the effect of the parent compound and its 4-hydroxy metabolite and its relative affinity for estrogen receptor sites [27].

The antioxidant vitamins in breast cancer patients before treatment (MBT group) were significantly lower than that of benign cases and control group as shown in (table 1). This may be due to the fact that in the cancerous stress condition, the requirements for vitamins increased progressively. Beside this, the increased levels of oxygen radicals in untreated patients themselves may reduce the availability of those vitamins in the blood of cancer patients. Both effects tend to reduce antioxidant vitamins level in the sera of cancer patients. In eight to nine months of treatment, the corresponding patients showed markedly elevated levels of antioxidant vitamins and concomitant decrease in uric acid and GGT levels. Reduction of serum MDA levels was noticed. These changes can be explained according to Jordan et al [28] observation who reported that tamoxifen tends to retard cell proliferation rather than kills such cells. The retarded cell proliferation leads to decrease in the utilization of antioxidant vitamins. This explanation can be reflected on the decrease in uric acid, GGT and MDA levels in the sera of MAT group in comparison with MBT group of breast cancer patients.

Finally, from all the above observations it can be concluded that tamoxifen inhibits the effects of free radicals and initiates the regeneration and recovery of antioxidant vitamins in women.

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Alteration in the phenotype of peripheral blood T lymphocyte in patients with idiopathic preterm labour

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Abstract

Objective: The current study aimed to detect some changes occurred on the surface of T lymphocytes manifested by CD3, CD4 and CD8 molecules that may have a role in patients with idiopathic preterm labour.

Setting: Al-Kadimyia Teaching Hospital / Department of Obstetrics & Gynecology for a period of one year (March 2002-March 2003).

Study design: Thirty patients with idiopathic preterm labour were enrolled in this study in addition to thirty healthy pregnant women as a control group. Blood samples were taken from both groups, lymphocytes were separated and immunofluorescent labeled by monoclonal antibodies to CD3, CD4 and CD8 surface markers.

Results: Patients have a significant low percentage of these surface markers in comparison with control subject.

Conclusion: The above findings confirm the suppression of cellular immunity in patients with idiopathic preterm labour.

Keywords: Preterm labour, Lymphocytes, CD3+, CD4+ and CD8+ cells

IRAQI J MED SCI, 2008; VOL.6 (1):60-64

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Introduction

Preterm labour is the major cause of perinatal mortality and morbidity [(1)]. It is one of the most serious problems facing obstetrician and other perinatal health care [(2,3)]The basic function of immune system is to combat the numerous pathogens that are present in environment and the lymphocytes considered the key constituent of it. Only mature functional lymphocytes express a number of characteristic surface proteins and these CD-proteins are associated with T-cells receptor through non-covalent attachment.

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Received: 2nd May 2007, Accepted: 25th June 2007.

They serve to transmit signals from these receptors into cytoplasm and must be present for the receptor to be transported out of the cell surface. Almost exclusively T-lineage cells express the CD3 proteins and their presence is commonly used to identify T-cells in extrathymic tissues. The CD8 antigen is expressed on cells that have cytotoxic activity, these are extremely important in the defense against viral infection. The CD4 expressed on T-helper cells which promote proliferation, maturation and immune reaction of all other types of lymphocytes [(4)]. Mucosa of female genital tract is an immune barrier containing IgA secreting plasma cells, dendritic cells, and CD4+ and CD8+ T lymphocytes [(5)]. The uterus is not immunologically privileged site, it is well vascularized with good lymphatic drainage and can reject foreign tissue. Lymphatics are found in the uterus and cervix, some of which contain IgA, IgG, which is found to be increased in localized infection and unexplained

infertility, also CD4+ T lymphocytes and macrophage, are increased in cervical secretion in HIV infection [6]. High percentage of the decidual cells (75%) are composed of immune cells namely macrophage (20%), lymphocyte (10%), natural killer cells NK (45%) while the remaining 25% are stromal cells [7].

This study was conducted to see whether immunologic changes are present in patients with idiopathic preterm labour manifested by changing the percentage of certain surface molecules like CD3, CD4 and CD8.

Patients and methods

Cases were studied in Al-kadimyia Teaching Hospital and were divided into two groups, the patient's group (group A) includes thirty pregnant women with idiopathic preterm labour (PL) between 26 weeks and less than completed 37 weeks of pregnancy and was chosen depending on definition criteria. Other thirty healthy pregnant women with comparable gestational age were included as a control group (group B). The exclusion criteria includes patients with rupture membrane, chorioamnionitis, dead or compromised fetus, gross fetal congenital anomalies, multiple pregnancy, women with suggestive history of cervical incompetence and polyhydramnios. The gestational age was calculated from the first day of the last menstrual cycle and early ultrasonogtraphy. Patients enrolled in this study underwent general and obstetrical examination, routine investigations were done and they were observed and managed appropriately during labour. All newborns received by pediatrician and were admitted to the neonatal care unit for follow up. White blood cell (WBC) and differential count including lymphocyte percentage were calculated.

Sampling: five ml. of blood was collected from all women groups. Blood was collected with heparin. Lymphocytes separation was conducted according to Boyum; 1968[8] and Galun et al; 1994[9].

Regarding statistical analysis the results of CD markers were reported as percentage, mean, SD and SE. The significance was conducted using Student t-test and chi-square test was used in some comparisons.

Results

Total number of lymphocytes in peripheral blood for both groups showed no significant difference with P value = 0.761 as shown in (table 1).

Table 1: Total lymphocyte count in group A compared to group B

	No. Of patients	Mean	SD	SE
Group A	30	1727.03	832.9897	152.0824
Group B	30	1669.56	808.6800	147.6441

There was a significant decrease in the mean percentage of CD3+ cells in group A compared with group B with a P value < 0.00006 as shown in (table 2).

Table 2: CD3+ cells in group A compared to group B.

	No. Of patients	Mean	SD	SE
Group A	30	56.33	6.0591	1.1062
Group B	30	74.93	4.4947	0.8206

When we checked the percentage of CD4+ and CD8+ cells, results showed that these subset of T lymphocytes also significantly decreased in group A in comparison with group B with a P value ≤ 0.0000215 and ≤ 0.0000316 respectively as shown in (table 3) and (table 4).

Table 3: CD4+ cells in group A compared to group B

	No. Of patients	Mean	SD	SE
Group A	30	26.33	7.0238	1.2524
Group B	30	43.76	3.3496	0.6115

Table 4: CD8+ cells in group A compared to group B

	No. Of patients	Mean	SD	SE
Group A	30	5.73	2.5180	0.4597
Group B	30	17.10	1.6887	0.3083

(Table 5) shows a difference in the ratio of CD4+/CD8+ in both groups. The ratio was statistically significant with P value ≤ 0.0054.

Table 5: Ratio between CD4+ and CD8+ cells in group A compared to group B

	No. Of patients	Mean	SD	SE
Group A	30	4.59	2.7834	0.2750
Group B	30	2.55	1.98353	0.19834

Discussion

In 60 -70% of preterm labour, no identified cause was definite; we tried to determine if there is a role for immunologic changes in causing this state. The success of virtually all immune response depends on the remarkable ability of T cells, which accounts for about 75% of total peripheral lymphocytes [(10)]. T lymphocytes represented by CD3 positive marker found to be with a mean value of 74.9% in group B and a range of 70-80%. This result lie within normal value of T-cell which was consistent with what Branch-Dw who found that T cells is not appreciably altered during normal pregnancy [(11)], knowing that normal range of CD3+ in non pregnant individual is $0.9\text{-}2.8x10^9$/L which corresponds to 80% of total lymphocyte[(7)]. In contrast, CD3+ percentage was lower in group A with a mean value of 56.3% Which was statistically significant. This was in agreement with Oleszezuk et al[(12)]who found that CD3+ percentage decreases significantly in patients with idiopathic PL. Roughly 70% of T-cells in peripheral blood are CD4+CD8- and 20% are CD4-CD8+, while 5% are double positive CD4+CD8+. During normal pregnancy there is slight decrease in CD4+ (43.7% of total lymphocyte), which correspond to 63% of T-cells. In this study group A shows statistically significant lower level of CD4+ percentage with a mean value of 26.3% (equivalent to 46.7% of T-cells). This result was similar to what's found by Brandt et al [(13)], who suggest suppression in cell mediated immunity occurred in patients with preterm labour. CD8+cells constitute 20-25% of T cells, which constitute 18% of total lymphocyte [(14)]. In this study CD8+ found to be with a mean value of 5.73% in group A and a mean value of 17.1% in group B, this difference is significant with a P value of < 0.000316 and this is in agreement with (15&16) suggesting a suppressed cellular immunity in patients with idiopathic PL. The higher ratio of CD4+/CD8+ in group A compared with group B, this result reflects a suppression of the main two subsets of cellular immunity (helper & cytotoxic) in preterm labour patients.

From this study we concluded that an idiopathic preterm labour could be mediated through an alteration in immune system, which is represented by suppression of cellular immunity.

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Distribution of HLA class I and class II Antigens in T1DM Children and their Siblings

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Abstract

Background: Genomic studies have confirmed that the main locus defining the genetic susceptibility to T1DM is encoded within the Major Histocompatibility Complex- HLA (Human Leukocyte Antigen) region on human chromosome 6.

Objective: To investigate the role of HLA-class I and class II antigens in the etiology of type 1 diabetes mellitus (T1DM) and in prediction of this disease in siblings.

Patients & methods: Sixty T1DM children who were newly onset of the disease (diagnosed less than five months) were selected. Their age ranged from 3-17 years. Another 50 healthy siblings were available for investigation of HLA-typing, their ages range from 3-16 years. Eighty apparently healthy control subjects, matched for age (4-17) years, sex and ethnic backgrounds (Iraqi Arabs), underwent the HLA-typing. Serological typing of HLA antigens was done by microlymphocytotoxicity assay.

Results & recommendations: At HLA-class I region, T1DM patients showed a significant increased frequency of antigens A9 (40.0 vs. 18.75%) and B8 (28.33 vs. 8.75%) as compared to control subjects, while at HLA-class II region, DR3 and DR4 were significantly increased in patients (53.33 vs. 26.25% and 50.0 vs. 12.5% respectively) as compared to controls. In addition to that, T1DM was significantly associated with DQ2 (33.33 vs. 15%) and DQ3 (40.0 vs. 20%) antigens as compared to controls, suggesting that these antigens had a role in disease susceptibility, while the frequency of DR2 and DQ1 antigens were significantly lowered in patients compared to controls (6.66 vs. 25% and 6.66 vs. 22.5% respectively). These molecules might have protective effect. In siblings a significant increase frequency of DR4 antigen (34.0 vs. 12.5%) was observed in comparison to controls, suggesting that it might be much useful for predicting T1DM in affected families. It is potentially valuable to predict TIDM in siblings by screening for HLA risky alleles in correlation with autoantibodies.

Keywords: T1DM patients, Siblings, HLA.

IRAQI J MED SCI, 2008; VOL.6 (1):65-73

Introduction

Type1Diabetes Mellitus (T1DM) is one of the most common chronic childhood diseases.

Its incidence seems to be increasing in countries around the world and is predicted to be about 40% higher in 2010 than 1997 [(1)]. It is known to be polygenic disease that appears from interaction of mutation of multiple genes [(2)]. Genomic studies have confirmed that the main locus defining the genetic susceptibility to T1DM is encoded within the Major Histocompatibility Complex- HLA (Human Leukocyte Antigen) region on human chromosome

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Received:23th May 2007, Accepted: 16th December 2007.

6 [3]. The MHC consists of three major regions, A, B, and C that encode class I genes, and the D region which encodes class II genes. The class I molecules are highly polymorphic and present peptide fragments of foreign antigens to cytotoxic T- Lymphocytes. The class II molecules present foreign processed antigen to helper T- Lymphocytes and thus are involved in initiating the immune responses [4]. There are two major classes of class II genes, the DR and DQ genes. It has been estimated that 60% of the genetic susceptibility to T1DM is conferred by the HLA [5].

The role of HLA alleles in T1DM was first indicated by the association with HLA-B8, -B15, and -B18 [6], and then with HLA- DR3 and DR4 encoded by the DRB1 locus and susceptibility with the DQB1 and DQA1 genes, which are in linkage disequilibrium with DR3 and DR4 [7, 8, 9]. DQB1 *0201 and DQB1 *0302 present a high risk for disease occurrence [10]. A study conducted by Mezal, showed that A1, B8, and DR3 were the high risk antigens [11], while others found that A24, B8, B15, DR3, DR4, DQ2, and DQ3 were highly associated with T1DM among Iraqi patients [12]. Strong natural protection against T1DM is also conferred by the DR2.DQ6 haplotypes [13], which occurs in approximately 20% of the healthy white population but it is rarely found among patients with diabetes. DR2, DQ1, DQ4, and B35 were found among the protective alleles in Iraqi patients [11, 12].

In the present study, we investigate the occurrence of T1DM in relation to genetic predisposition in children and their siblings through the HLA polymorphism.

Subjects, Materials and Methods

Subjects:

Sixty Iraqi Type 1 diabetic patients (28 males and 32 females) were subjected to this study. The patients attended the National Diabetes Center at Al-Mustansiriya University/ College of Medicine during the period May 2004 - October 2005. Their ages ranged from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). All the patients were treated with daily replacement doses of insulin at the time of blood sampling. Fifty healthy siblings (25 males and 25 females) of type 1 diabetes patients were available for investigation of the HLA-typing. Their ages ranged from 3 – 16 years. For the purpose of comparisons, 80 healthy control subjects matched for age (4-17 years old), sex and ethnic back ground (Iraqi Arabs) were selected who have no history or clinical evidence of type, 1 diabetes or any chronic diseases and obvious abnormalities as a control group for HLA typing.

Serological Typing of HLA Antigens:

Ten milliliter of venous blood was drawn from each subject (patients, siblings, and controls). The collected blood was displaced into glass universal tubes containing heparin (10 IU /ml) as anticoagulant. Lymphocytes were separated from the whole blood using Ficoll- Isopaque density centrifugation (Flow-Laboratories, UK) as described by [14]. The test was carried out in histocompatibility laboratory in Al-Karama hospital. The collected cells were suspended in washing medium (RPMI-1640 free serum cultured media) (Euroclone, UK) and centrifuged three

times, then the lymphocytes were resuspended in 2 ml warm RPMI-1640 supplemented with 10% heat inactivated human type AB serum. The lymphocyte population were separated by nylon wool method to T- cells that were used in the phenotyping of HLA- class I (A, B, and C) antigens and B- cells that were used for phenotyping of class II (DR and DQ) antigens [15]. Cells were counted and their viability was determined. The viability of cells was 95% and above. Microlymphocytotoxicity assay was used for both HLA- class I and class II typing [16].

Statistical analysis

Regarding of HLA and disease association the frequency distribution for selected variables was done first. The strength of disease association with particular HLA antigen was determined by calculating the relative risk (RR) and etiological fraction (EF), and if the association is negative, therefore the preventive fraction (PF) was calculated [17]. The significance of such association was assessed by Fisher exact probability.

Results

The frequencies of HLA antigens (-A; -B; -C; -DR; and -DQ) were compared between T1DM patients and controls, siblings and controls and between T1DM patients and siblings.

HLA association with T1DM:

The distribution of HLA-A; -B; -C; -DR; and -DQ antigens with their frequencies in T1DM patients and controls are presented in (table 1), while antigens showing significant variations between patients and controls are given in (table 2).

At HLA-A locus, the antigen A9 was significantly increased (P= 0.004) in the patients and such differences were associated with RR value of 2.88 and EF value of 0.261. This positive association remained significant after correction (PC= 0.032).

At HLA-B locus three antigens were significantly increased. They were B8(28.33 vs. 8.75%), B12(11.66 vs. 2.50%), and B15(11.66 vs. 2.00%) in the T1DM patients (P=0.002, 0.032, and 0.018 respectively) in comparison to controls. Such differences were associated with RR values of 4.122, 5.150, and 9.113 respectively, and EF values of 0.214, 0.093, and 0.103 respectively. However one positive association remained significant after correction (PC= 0.032) and this was with B8. In contrast, B35 and B51 antigens were significantly decreased in the patients compared to controls (3.33 vs. 13.75% and 15.0 vs. 28.75% respectively), but such negative associations failed to remain significant after correction.

At HLA-C locus, Cw7 antigen was significantly increased in T1DM patients (31.66 v. 16.25%, P=0.026, RR=2.388, and EF= 0.183). In the other hand, the Cw4 antigen was significantly decreased in the patients compared to controls (6.66 vs. 18.75%, P=0.031). However both associations failed to reach a significant level after correction.

At HLA-class II region (DR-loci), three antigens showed different frequencies in patients and controls, and these were DR2, DR3, and DR4. Increased frequencies of DR3 (53.33 vs. 26.25%) and of DR4 (50.0 vs. 12.5%) were observed in patients. The two positive associations were associated with RR values of 3.210 and 7.00 respectively and EF values of 0.366 and 0.428 respectively. Such positive associations were highly significant ($P=9.7x10^{-3}$ and $1x10^{-5}$ respectively) even after correction (PC=0.008 and

$9x10^{-5}$ respectively). In contrast DR2 antigen was significantly decreased in the patients (6.66 vs. 25.0%). Such negative association was significant before (P=0.003) and after correction (PC=0.027).

At HLA-DQ loci, two antigens DQ2 and DQ3 were significantly increased in the patients compared with controls. DQ2: (33.33 vs. 15.0%, P=0.009, RR=2.833, EF=0.215; DQ3: 40.0 vs. 20.0%, P=0.008, RR=2.666 and EF=0.249). The two positive associations remained significant after correction (PC=0.027and 0.024 respectively). The antigen DQ1 was significantly decreased in T1DM patients (6.66 vs. 22.5%) compared to controls, and such negative association (P=0.008) remained significant after correction (PC=0.024).

HLA Association with T1DM in Siblings:

The distribution of HLA-A; -B; -C; -DR and -DQ antigens in siblings and controls are given in (table 1), while antigens showing significant variations between siblings and controls are listed in (table 2).

At HLA-A locus, the antigen A2 showed a decreased frequency in siblings of T1DM patients (22.0 vs. 37.5%). This negative association was significant (P=0.047) before correction, but not after.

At HLA-B locus, the antigen B12 showed a significant (P=0.036), increased frequency (12.0 vs. 2.5%) with RR value of 5.318 and EF value of 0.097. Correcting the probability of this antigen failed to reach a significant level. In contrast, the B51 antigen showed negative association with the disease in the siblings. The antigen had a frequency of 12.0% in the siblings, while in the controls, the frequency was 28.75%. Although the association was significant (P=0.019), the corrected probability failed again to attain a significant level (PC=0.304).

At HLA-class II region (DR loci), an increased frequency of antigen DR4 (34.0 vs. 12.5%, P=0.003) was observed in the siblings. The RR value of such positive association was 2.428, and the EF value was 0.176. This association was significant (P=0.003) before correction, and after correction (PC=0.027). On the other hand, DR5 antigen showed a decreased frequency in the siblings as compared to controls (2.0 vs. 11.25% respectively). Such negative association was significant before correction (P=0.049) but not after (PC=0.441).

T1DM Patients vs. Siblings

As listed in (table 2), both the T1DM patients and their siblings shared the A2 and DQ1, may be considered as protective antigens, while A9, B8, DR3 and DR4 were susceptible ones. No other antigen in the present study was found to be common between the patients and their siblings. Such associations were significant before correction but not after.

Table 1: HLA antigen frequencies in control, T1DM patients and Siblings groups.

HLA-antigens	Control (Number = 80)		TIDM patients (Number =60)		Siblings (Number = 50)	
HLA-A locus	**No.**	**%**	**No.**	**%**	**No.**	**%**
A1	15	18.75	12	20.00	5	10.0
A2	30	37.50	26	43.33	11	22.0

A3	7	8.75	5	8.33	2	4.00
A9	15	18.75	24	40.00	10	20.0
A10	10	12.50	12	20.00	5	10.0
A11	16	20.00	0	ND	0	ND
A19	32	40.00	16	26.66	11	22.0
A28	8	10.00	7	11.66	3	6.00
HLA-B locus						
B7	6	7.50	6	10.00	7	14.00
B8	7	8.75	17	28.33	5	10.00
B12	2	2.50	7	11.66	6	12.00
B13	2	2.50	2	3.33	1	2.00
B14	4	5.00	0	ND	0	ND
B15	1	2.00	7	11.66	1	2.00
B16	2	2.50	6	10.00	1	2.00
B17	1	1.25	0	ND	1	2.00
B18	4	5.00	2	3.33	5	10.00
B27	5	6.25	2	3.33	2	4.00
B35	11	13.75	2	3.33	2	4.00
B37	4	5.00	7	11.66	1	2.00
B40	2	2.50	3	5.00	3	6.00
B41	8	10.00	5	8.33	2	4.00
B51	23	28.75	9	15.00	6	12.00
B73	2	2.50	2	3.33	4	8.00
HLA-C_W locus						
Cw**2**	3	3.75	3	5.00	3	6.00
Cw4	15	18.75	4	6.66	8	16.00
Cw5	2	2.50	2	3.33	0	ND
Cw7	13	16.25	19	31.66	10.	20.00
HLA-DR locus						
DR1	18	22.50	21	35.00	12	24.00
DR2	20	25.00	4	6.66	7	14.00
DR3	21	26.25	32	53.33	18	36.00
DR4	10	12.50	30	50.00	17.0	34.00
DR5	1	11.25	2	3.33	1	2.00
DR6	3	3.75	6	10.00	3	6.00
DR7	12	15.00	14	23.33	10	20.00
DR8	8	10.00	11	18.33	6	12.00
DR10	0	ND	4	6.66	6	12.00
HLA-DQ locus						
DQ1	18	22.5	4	6.66	11	22.00
DQ2	12	15.00	20	33.33	11	22.00
DQ3	16	20.00	24	40.00	15	30.00

Table 2: Antigens of HLA-class I and class II regions showing significant variations between T1DM patients, siblings and controls.

HLA	T1DM vs. control					Siblings vs. control					TIDM vs siblings	
	RR	EF	PF	P	PC	RR	EF	PF	P	PC	P	PC
A2	–	–	–	–	–	0.470	–	0.198	0.047	NS	0.014	NS
A9	2.88	0.261		0.004	0.032	–	–	–	–	–	0.019	NS
B8	4.122	0.214	–	0.002	0.032	–	–	–	–	–	0.014	NS
B12	5.150	0.093	–	0.032	NS	5.318	0.097	–	0.036	NS	–	–
B15	9.113	0.103	–	0.018	NS	–	–	–	–	–	–	–
B35	0.216	–	0.107	0.031	NS	–	–	–	–	–	–	–
B51	0.437	–	0.162	0.041	NS	0.337	–	0.191	0.019	NS	–	–
Cw4	0.309	–	0.128	0.031	NS	–	–	–	–	–	–	–
Cw7	2.388	0.183	–	0.026	NS	–	–	–	–	–	–	–
DR2	0.214	–	0.195	0.003	0.027	–	–	–	–	–	–	–
DR3	3.210	0.366	–	$9.7x10^{-3}$	0.008	–	–	–	–	–	0.051	NS
DR4	7.00	0.428	–	$1x10^{-5}$	$9x10^{-5}$	2.428	0.176	–	0.003	0.027	0.026	NS
DR5	–	–	–	–	–	0.160	–	0.095	0.049	NS	–	–
DQ1	0.246	–	0.168	0.008	0.024	–	–	–	–	–	0.019	NS
DQ2	2.833	0.215	–	0.009	0.027	–	–	–	–	–	–	–
DQ3	2.666	0.249	–	0.008	0.024	–	–	–	–	–	–	–

RR: relative risk; EF: Etiological fraction; PF: Preventive fraction; P: Fisher exact probability; PC: Corrected probability

Discussion

The present study demonstrated that immunogenetic predisposition may be considered as an important factor for the development of T1DM in association with the HLA antigens in which markers of human HLA showed different distributions in patients, siblings and controls.

At HLA class I region, significant increased frequencies of antigen A9 and B8 were observed in the patients. Other HLA T1DM association studies carried out in other world populations revealed an association with other HLA-class I antigens; B8 and B15 in Finnish population [18], in addition to A1, A2, B56, B62, Cw3 and Cw7 [19], A24 in Japanese [20]. Such differences can be explained in the ground of racial differences, especially if we consider that HLA antigens show different frequencies in different populations including Iraqis. HLA-A1 and B8 were found to be associated with T1DM in Basrah population (11) while Al-Samarria, found a very high significant association between HLA-A24, B8 and B15 and T1DM in her study that was conducted in Baghdad [12]**.** The EF value can range from 0 (no association) to 1 (maximum association). That means a value of 1 for an antigen is interpreted that this antigen is fully responsible for the development of the disease other wise if the value is in between 0 and 1, it indicates that this marker is partially involved in the disease development [17],

and other factors like environment factors can be involved. The EF value of A9 (0.261) and B8 (0.214) support the previous hypothesis and so other factors in association with these antigens contribute the rest percentage required in the development of T1DM**.** At HLA-class II region, further antigens had positive associations with T1DM. These were DR3, DR4, DQ2 and DQ3. The polymorphism of HLA-class II loci has gained much interest in the HLA disease association studies. However, multiple studies have reported association between HLA-DR and DQ phenotypes and T1DM. DQ2.DR3 and DQ3.DR4 haplotypes reported as high risk alleles in Caucasians [21], DR4, DQ4 but not DR3 were found to be dominant in Japanese [22], while DR3, DR4, DR9 and DQ2 were the only alleles positively associated with T1DM in Koreans [23]. In Finland, DQ2/DQ3 genotype was found to be associated with genetic susceptibility and was more frequent in children diagnosed <5 years of age [24], and in diabetes-associated autoantibodies emerged in children with predisposing HLA-DQ alleles after 3 months of age [25]. In Lebanese, 77% and 40% of T1DM patients were positive for DQ2 and DQ3 respectively (10). Others reported high significant association of HLA-DR3, DR4, DQ2 and DQ3 with T1DM in Iraqi patients (12). Studies of HLA genes at the molecular levels showed that this association with HLA-DR is secondary to a stronger link with certain HLA-DQ variants.

The critical factor is the amino acid at position 57 in the HLA-DQB chain. Genetic variants of DQB which encode the amino acid aspartate at this position seem to confer protection against T1DM, whereas variants encoding other amino acids increase the risk. Hence the HLA-DR3 and DR4 association arises because these DR-alleles are linked to DQB alleles which do not encode aspartate [4]. It is worthy to note that amino acid 57 in HLA-DQB lies in the "antigen binding groove". It was reported that class I HLA-24 gene promotes pancreatic β-cells destruction in an additive manner in the patients with T1DM susceptible HLA-class II genes [26]. Antigens B35, B51, Cw4, DR2 and DQ1 showed a negative association with the disease, but after correction only the DR2 and DQ1 antigens remained significant. These antigens may have protective effect especially if we consider PF values to be 0.195 for DR2 and 0.163 for DQ1 antigens.

In siblings, a significant increased frequency of antigens B12 and DR4 was observed in comparison with control subjects, but this positive association remained significant only for DR4 antigen after correction with RR value of 2.428 and EF value of 0.176. Concerning other world population studies, HLA-DR4 was found to be associated with the presence of ICA (7%) in siblings of T1DM Mexican-American patients [27]. This locus is known to be associated with T1DM risk particularly with in type 1 diabetes families [28]. Thus it may be much more useful for predicting T1DM in affected families than in population. A highly diabetogenic subset of DR4 haplotypes was detected among T1DM patient's sibling, suggesting that DR typing is 6-10 times less powerful as predictor of T1DM in the population than among patients siblings [7].

Clearly, the structural differences seen between the predisposing and protective HLA molecules will affect

their ability to bind or interact with diabetogenic antigens and the T- cell receptor (TCR) of autoreactive β-cell specific T-cells [29]. Several mechanisms have been proposed to explain how this might influence the risk of developing autoimmune T1DM. The protective HLA molecules may form stable complexes with self antigens in the thymus, leading to efficient deletion of potentially autoreactive T cells (negative selection). In contrast, the less stable complexes formed by the predisposing HLA-molecules may result in inefficient T-cell removal and the release of autoreactive T cells into the periphery [30]. The predisposing and protective HLA molecules may interact differently with the TCRs of autoreactive T cells, affecting the phenotype of the T cells (proinflammatory versus regulatory) [29] or their activation status (Proliferative versus anergised) [31]. This immunomodulatory hypothesis is supported by the observation that DQ1 can protect against the development of diabetes, even after the onset of β-cell autoimmunity [32].

The HLA- class I (-A9 and -B8) and class II (-DR3, -DR4, -DQ2, -DQ3) antigens were significantly increased in T1DM patients and they may played an important role in the etiology of the disease, while -DR2 and -DQ1 antigens were significantly decreased in those patients. In siblings a significant increase was observed in HLA-DR4 antigen compared to controls.

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Histological modulation of adult rat's thyroid in response to anti-oxidant factor

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Abstract

Background: The pineal neurohormone namely melatonin regulates the bodily endocrine glands including thyroid gland, by controlling the function of pituitary gland.

Objective: To study the effects of different doses of dietary melatonin (as antioxidant) on male rat's thyroid, *"histologically*".

Methods: Melatonin was supplied to adult Wister albino rats, for successive 14 days. Rats were divided into 6 groups. Group I was the control. Group II, III, IV, V and VI were given a daily dose of 125, 250, 500, 750 and 1000 µg / kg body weight, respectively. After last day of treatment, animals were killed under effect of anesthesia and thyroid gland was taken for histological study.

Results: The results showed no significant effects on thyroid with the regarded as normal therapeutic dosages, whereas significant damaging effects were seen with the higher doses.

Conclusion: Dietary melatonin has no bad effect on adult rat's thyroid within therapeutic doses, but it had highly damaging changes in large doses.

Keywords: Thyroid, melatonin, anti-oxidant, and endocrine.

IRAQI J MED SCI, 2008; VOL.6 (1):74-80

Introduction

Melatonin is the principal pineal neuro-hormone [(1)]. It regulates every bodily hormone by regulating the function of pituitary in a rhythmic manner [(2)]. The free radical theory of damaging events concluded that any stress process is caused by free radical oxidative agents, and this fact has been well investigated within the context of oxidant/antioxidant balance [(3)]. Melatonin is at the top of many dietary antioxidants; such as vitamin E, glutathione and strawberry extract. Antioxidants from food sources appear to be promising for prevention of most stressful events [(4)].

Thyroid gland is an important member in the endocrine system, and its diseases can lead to serious consequences for human health [(5 & 6)], hence the study of protective role of anti-oxidative agent (such as melatonin) is continuous and could provide a new strategies for the management of many important thyroid related diseases, which might be extremely useful in the context of endocrine-therapy and pharmacology planning in favor of human wellness [(7)].

Materials and methods

Adult male Wister albino rats, 48 in number, were used in this work. They were kept in an animal room, with a temperature ranging between 20 -24 C°, the light - dark cycle was 12:12. They fed a control pellet diet with free access to food, except for one and half hour prior to melatonin containing meal. Dietary melatonin was provided as a single daily dose, 2 hours prior to sundown. Water was offered *ad libitum*.

Animals were divided into 6 groups, each consisting of 8 rats. Group

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Received: 21st June 2007, Accepted: 29th October 2007.

I was the control: rats were provided with the same type of drug containing meal, but no drug was added (placebo), though, they were also deprived from food one and half hour prior to the time of treatment as other groups. Group II, III, IV, V and VI were given dietary melatonin as a single daily dose of 125, 250, 500, 750 and 1000 µg/kg body weight, in sequence, mixed with their food, for fourteen successive days. In this study the doses of drug was regarded to be given in a low or high dose according to previous studies [8, 9, 10 & 11].

After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole thyroid gland was removed, separated from the surrounding connective tissues under a dissecting microscope, weighed by an electric sensitive balance and used for paraffin section, using Bouin's solution for fixation and (Haematoxylin & Eosin) for staining [12], then 5 serial sections of 3 µm thickness from the mid- part of the organ were studied by light microscopy.

Histological study was done both as descriptive and morphometric. The morphometric analysis was estimated by using Zeiss Integrating Micrometer – disk Turret I of 25 point - system, used on a light microscope: which measures the relative surface area by counting the points superimposed through a disk put on the microscopic eye piece during slide examination, so the number of these points positively related to the relative measurement of the surface area [13 & 14], the total points falling on each thyroid follicle's wall, as well as lumen, were calculated. From each section 5 fields were taken randomly examined at 150X magnification. All the values were taken as mean ± SD of 8 rats. The significance of difference between each of treated groups and its control was evaluated by student – t – test (15).

Results

Descriptive and morphometric studies for all groups were done, as follows:

Body weight: There was no significant effect on the over all body weight ($P>0.05$) as shown in (table 1).

Thyroid weight was not affected significantly in group II, III, and IV whereas it was significantly affected (increased) in group V and VI (table 2).

Morphometric results:

(1) The number of points (superimposed through the Micrometer – disk Turret) overlying the epithelial wall of the acini (follicles), was significantly unaffected till the dose of 500µg/kg, then it was significantly decreased at the dose of 750µg/kg, and a great increase was clear at group received 1000 µg/kg (Table3).

(2) The number of points superimposed on the lumen of the acini, followed an opposite manner to that of the wall (Table3).

The descriptive histological result:

Cells of the epithelial wall of thyroid acini, in the groups treated with 125, 250, and 500µg/kg were almost very similar to those of the control group; so each acinus was bound by a single layer of specialized thyroid epithelium which rest on a thin basement membrane and enclosed a lumen filled with thyroid colloid (a pink staining homogenous material). Some acini had sequamous epithelium; other acini had cuboidal or low columnar epithelium in the same given area (Fig.1).

In groups received 750 µg/kg dose, cells were seen commonly as flattened sequamous epithelium lined the acini which were filled by pink thyroid colloid with no vacuoles at the peripheries of their lumens between the colloid and epithelial cells (Fig.2).

The group treated with 1000 µg / kg dose: The thyroid acini were viewed with thickened basement membrane.

There was abundance in the number of thyroid epithelial cells and they appeared to be taller (Fig.3). The acini were looked smaller with less amount of colloid and the edges of the colloid were scalloped (Fig.4).

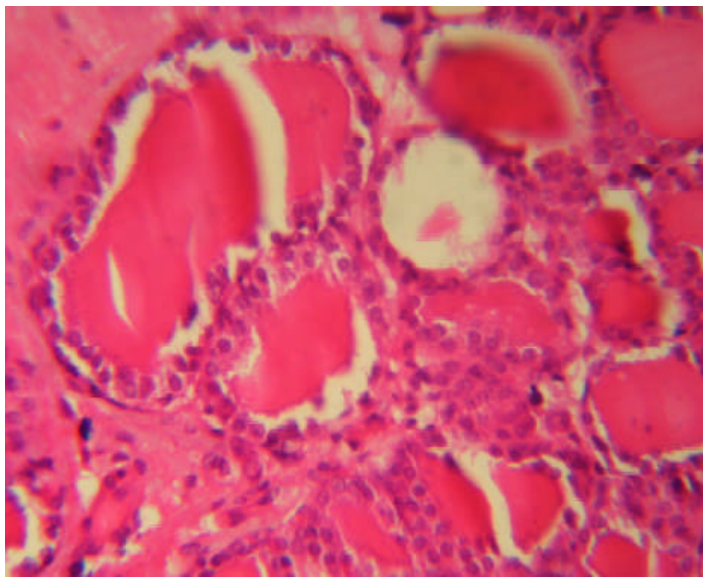

Figure 1: Thyroid tissues in control group of male rat H&E stain X100

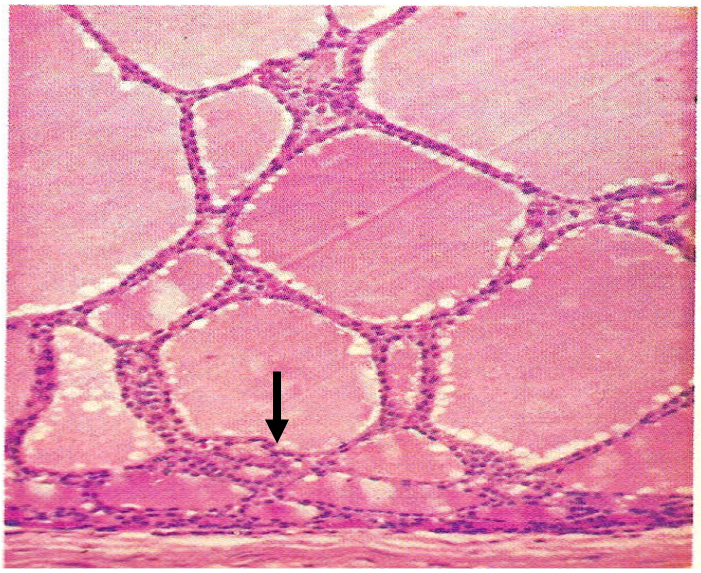

Figure 2: Thyroid acinus in male rat treated with 750µg/kg melatonin. The epithelial acinar cells are thin and almost sequamous (arrow), H&E stain X100

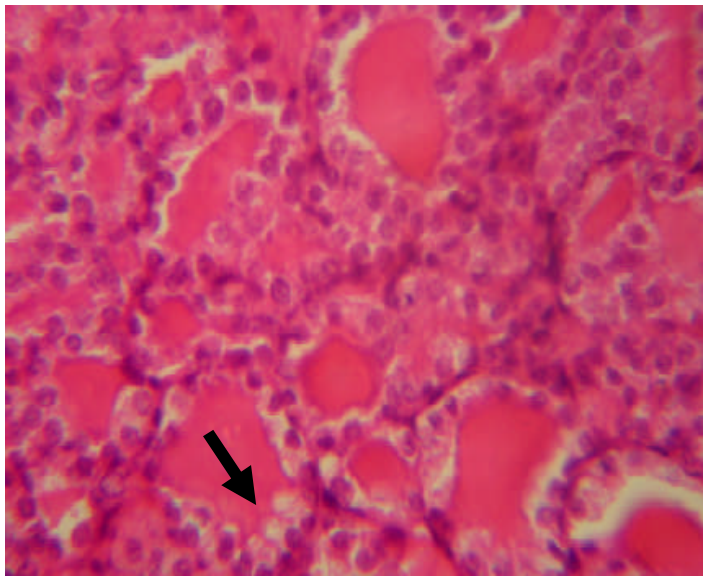

Figure 3: Thyroid tissues in male rat treated with 1000µg/kg melatonin: edges of colloid are scalloped (arrow), H&E stain X100

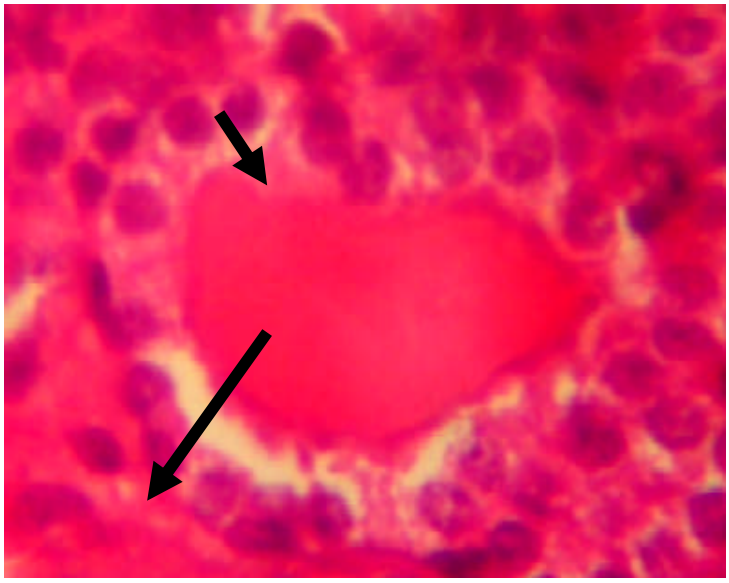

Figure 4: Thyroid acinus in male rat treated with 1000µg/kg melatonin: the columnar epithelium rest on thick basement membrane (arrow), H&E stain X400

Table 1: The effect of dietary melatonin on body weight (Grams) of 8 wk old male rats.

dose of Daily melatonin (µ/kg) body weight	*Body wt of rats at 1st day of experiment*	*Body wt of rats at last day of experiment*
Control	343.00±2.9	423.71±5.1
125	345.00±3.9	416.11±4.6 **NS**
250	343.25±2.9	425.24±5.1 **NS**
500	346.00±2.8	413.06±4.9 **NS**
750	345.25±4.6	418.25±9.7 **NS**
1000	345.50±5.1	416.57±8.2 **NS**

-Results were expressed in mean ± SD of 8 rats.
-All differences were statistically not significant =NS,
(P>0.05) when compared with its control.

Table2: Effect of melatonin on thyroid weight of adult male rats.

Daily dose of melatonin (µg/kg body weight)	*Thyroid weight at autopsy (mg)*
Control	19.14±2.33
125	20.17±3.17 **NS**
250	21.09±3.09 **NS**
500	19.18±2.98 **NS**
750	24.13±4.46*****
1000	25.16±5.13******

-Results were expressed in mean± SD of 8 rats
-The difference of each dose group was statistically significant when compared with its control (* P<0.0003; ** P<0.0001; NS not significant).

Table3: Number of points (seen through the Micrometer – disk Turret) overlying the thyroid acini wall and lumen of adult rats treated with dietary melatonin (in unit area of 0.0025mm^2).

Daily dose of melatonin in µg/kg body wt	*Points on thyroid acini wall*	*Points on thyroid acini lumen*
Control	14.18±1.14	8.86±1.22
125	14.91±1.41 **NS**	9.41±1.32 **NS**
250	14.26±1.21 **NS**	9.35±1.24 **NS**
500	14.67±1.12 **NS**	9.21±1.33 **NS**
750	11.27±1.22*	12.24±1.24***
1000	21.11±1.13**	6.12±1.14***

-Data were expressed as mean ± SD of 8 rats.
-When any dose-group was compared with its control, the differences were statistically significant (* P<0.00004;** P<0.00001; *** P<0.001
NS= non significant).

Discussion

There were no changes in the body weight of rats in all groups, this might be explained by the fact that exogenous melatonin causes no effect on the overall body weight in rats [16 & 17]. This is probably because the food intake in rats does not affected by melatonin administration [18]. The effect of melatonin on thyroid tissues could be explained by the fact that melatonin acts through specific receptors in all body tissues [19]. Once melatonin reaches any bodily tissue it exerts its action immediately, and melatonin has a dose – dependent physiologic action [20] so this is why in the low doses 125, 250 and 500µg/kg, no significant effect was noticed, while with higher doses 750 and 1000 µg/kg there was an obvious histological modification which leaded certainly to a physiological disturbance, because the function of thyroid acini, is determined from its size, height of the lining epithelium and the amount of its colloid [1 & 5]. Those results could be explained by the fact that melatonin has damaging effects only when it is administered in high doses [20 & 21].

The thickening of the basement membrane could be resulted from the increase in production of fibrocollagenous tissues, since melatonin hormone has special effect on fibroblasts [22 & 23], which are the active collagen – secreting cells and the basic forming cells of the connective tissues [1].

The group treated with the dose of 750µg/kg has the histological figure very similar to that of the hypothyriodism; i.e. large acini lined by a layer of almost flattened sequamous glandular cells, together with abundance of colloid which are known histolopathlogical features of hypothyroidism, and a decrease in the thyroid secretion level [1, 5 & 6]. The cause for that hypothyroidism might be the vast stimulation of thyroid gland by that high dose of melatonin which stimulated the hypothalamic- pituitary-thyroid axis, thence a large quantity of thyroid hormone especially thyroxin was secreted and accumulated within the thyroid acini that gave a view quite similar to thyroid hypofunction status [5 & 6]. In group V and VI (table 2): in both groups the gland weight was increased although they showed different histological picture, which could be explained by the fact that any thyroid disorder may induces its enlargement whether there is hypothyroidism or hyperthyroidism [1 & 6].

The shrink and regression in the amount of thyroid colloid was so clear at dose of 1000µg/kg, that might be discussed by the fact that melatonin over dosage may stimulate the anterior pituitary to produce thyroid stimulating hormone (TSH) which leads to massive stimulation of thyroid gland to secrete its hormones, mainly thyroxin (T4) and tri-iodothyronine (T3), so this would give the histological appearance very similar to thyroid tissues of group VI (8). The acini were looked smaller with less amount of colloid and the edges of the colloid were scalloped (Fig.4) indicating an active reuptake of thyroid secretion (5). All the destructive and damaging effects could be due to sustained time of treatment, because the effect of melatonin may differ with duration of administrated course [24]. In this study the doses of 125, 250 and 500µg/kg were regarded as therapeutic doses since no destructive effects were highlighted with those doses, and it is well documented that any drug would be considered to be given within therapeutic dose if that dose has no bad effect [25 & 26]. In this study the doses of drug was regarded to be given in a low or high dose according to previous studies [8, 9, 10, & 11].

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Histological effect of melatonin hormone on adult rat's prostate

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Abstract

Background: The prostate is the largest of the accessory glands of the male reproductive tract. Its secretion serves as a diluent and vehicle for transport of sperms from male to female, so its function is very important for the normal fertility. Melatonin is the basic neuro-hormone of the pineal gland, regulates the sexual and reproductive activities in all mammals including man.

Objective: This work aimed to study the effect of different doses of dietary melatonin on adult rat's prostate, *"histologically*".

Methods: Melatonin was supplied to adult Wister albino rats, for successive 30 days. Rats were divided into 6 groups. Group I was the control. Group II, III, IV, V and VI were given (mixed with their diet) a daily dose of 125, 250, 500, 750 and 1000 µg / kg body weight, respectively. The dietary melatonin was supplied to rats mixed with their food. After the last day of treatment, animals were killed under effect of anesthesia; prostate was removed for histological study.

Results: The results showed significant beneficial effects on prostate by normal therapeutic dosages, whereas significant damaging effects were seen with further stepping up doses.

Conclusion: Dietary melatonin has good effects on the rat's prostate within therapeutic doses, whereas it had highly damaging changes in overabundance.

Keywords: Prostate, melatonin, and fertility.

IRAQI J MED SCI, 2008;VOL.6(1):81-88

Introduction

The prostate is the largest of the accessory glands of the male reproductive tract. Its secretion serves as a diluent and vehicle for transport of sperms from male to female, so its function is very important for the normal fertility [(1)]. Melatonin is the basic neuro-hormone of the pineal gland. This hormone evidently plays an important regulatory role in the sexual and reproductive activities in all mammals including man [(2 & 3)].

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Received: 21st June 2007, Accepted: 29th October 2007

Melatonin limits human prostate cancer cell growth by a mechanism which involves the regulation of androgen receptor function but it is not clear whether other mechanisms may also be involved [(4)], hence, it would be of great interest to study the relationship between melatonin and prostate function.

Materials and methods

Adult male Wister albino rats (8 weeks old), 48 in number, were used in this work. They were kept in an animal room, with a temperature of 22±2Cº, the light - dark cycle was 12:12. Water was offered *ad libitum*. They fed a control diet with free access to food, except for one and half hour prior to melatonin containing meal. Dietary melatonin was provided as a single daily dose, 2 hours prior to sunset.

Animals were divided into 6 groups, each consisting of 8 rats. Group I was the control: rats were provided with the same type of drug containing meal, but no drug was added (placebo), though, they were also deprived from food one and half hour prior to the time of treatment as other groups. Group II, III, IV, V and VI were given dietary melatonin as a daily dose of 125, 250, 500, 750 and 1000 µg/kg body weight, in sequence, for 30 successive days. The dietary melatonin was supplied to rats by mixing it with their food, in only one meal, 2 hours prior to sun set. After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole prostate was removed, separated from the surrounding connective tissues under a dissecting microscope, weighed by an electric sensitive balance and used for paraffin section, using Bouin's solution for fixation and (Haematoxylin & Eosin) for staining [(5)], then 5 serial sections of 5 µm thickness from the left lobe were studied.

Histological study was done both as descriptive and morphometric by light microscope. The morphometric data were estimated by using Zeiss Integrating Micrometer – disk Turret I of 25 point system (which measures the relative surface area by counting the points superimposed through a disk put on the microscopic eye piece during slide examination, so the number of these points positively related with the relative measurement of the surface area), the total points falling on the fibromuscular stroma were calculated. From each section 5 fields were taken randomly examined at 150X magnification. Also by using objective micrometer used on a light microscope; by which a distance of 10µm could be calculated, the average height of the glandular cells, as well as the diameter of their nuclei, were estimated. All the values were taken as mean ± SD of 8 rats. The significance of difference between each of treated groups and its control was evaluated by student – t – test [(6)].

Results

Descriptive and morphometric studies for all groups were done, as follows:

Prostate weight was affected significantly in all groups; it was enlarged with the increasing doses of melatonin till the dose 500µg/kg, and then regressed gradually with increasing doses (table 1).

In all of the treated groups; the cells kept their arrangement to form the prostatic mucosal glands, showing the general architecture of a typical prostatic gland with its papillary pattern of ingrowths (Fig.2). The glandular epithelial lining was appeared to get taller with the increment of melatonin dose (Fig.2, 3 & 4); till the dose of 500µg/kg, after which, it seemed to be regressed (Table2). The nuclei of the glandular epithelial cells; gradually got lighter in color and larger, with the increase in melatonin doses till the dose of 500µg/kg, afterwards it gradually regressed and became darker, in the same manner as their cells did (Table2).

The smooth muscular and fibrocollagenous stroma was increasingly enlarged with the increase in the dose of melatonin (because the number of counted points seen through the eye piece- disk was increased) as shown in table 3. There was an increase in the vascularity in all of the treated groups, proportionate positively with the given doses of melatonin.

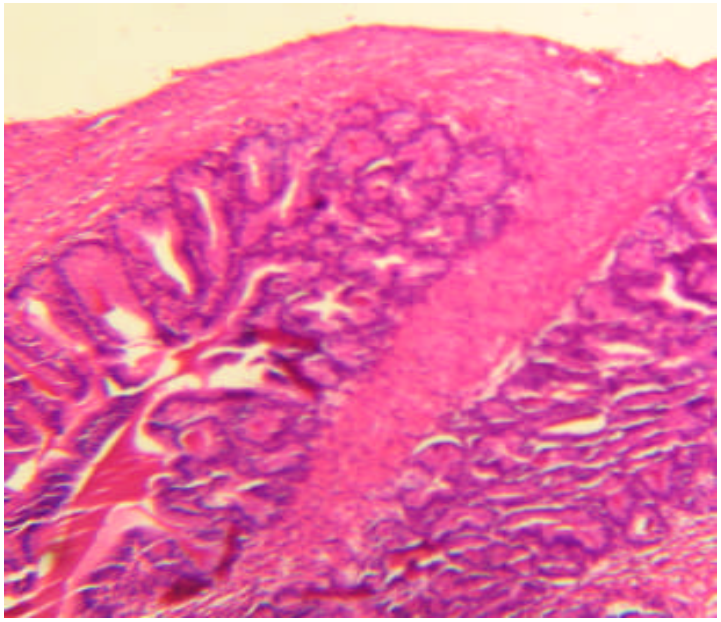

Figure 1: Prostate of control adult rat, X 200, H & E.

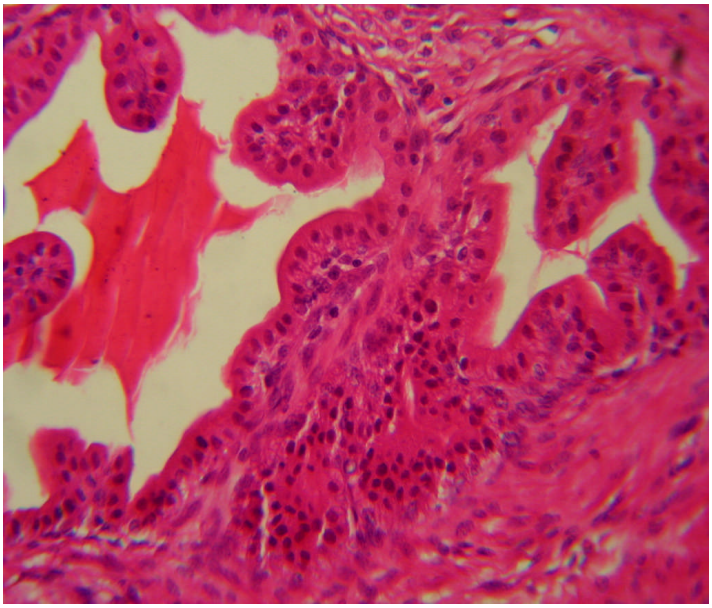

Figure 2: Prostate of adult rat treated with 500µg/kg dose, X 200, H & E.

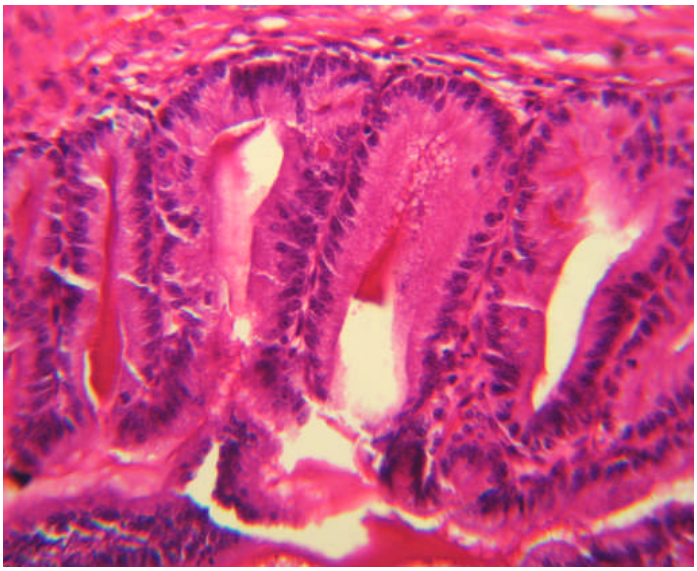

Figure 3: Prostate of adult rat treated with 500µg/kg dose, X 200, H & E.

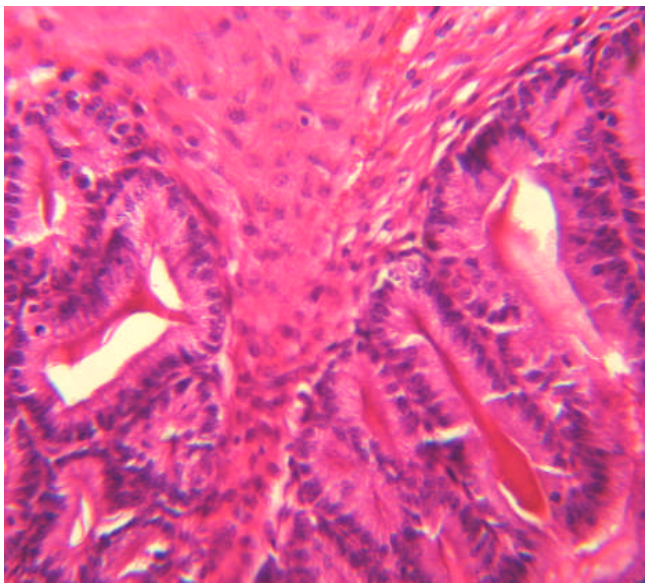

Figure 4: Prostate of adult rat treated with 1000µg/kg dose, X 200, H & E.

Table1: the effect of melatonin on prostatic weight of adult male rats.

Daily dose of melatonin in µg/kg body weight	***Prostatic weight at autopsy(mg)***
Control	74.2±3.3
125	78.9±2.1*
250	85.2±2.2**
500	91.9±3.3**
750	69.6±1.7**
1000	61.2±1.3**

-Results were expressed in mean± SD of 8 rats.
-The difference of each dose-group was statistically significant when compared with its control:
(* P<0.0002; ** P<0.0001).

Table2: Average cell height and nuclear diameter in µm, in the prostate of adult rats treated with dietary melatonin.

Daily dose of melatonin in µg/kg body wt	***Average cell height of prostatic epithelium (in µm)***	***Average nucleus diameter of prostatic epithelium (in µm)***
Control	39.2±1.4	21.4±1.3
125	41.2±1.5*	22.6±1.1†
250	42.8±0.9**	22.9±1.6††
500	44.1±1.6***	23.5±0.7‡
750	37.1±0.8**	20.2±1.1†
1000	32.3±1.4***	19.1±0.8‡

-Data were expressed as mean ± SD of 8 rats.
-When any dose-group was compared with its control, the difference was statistically significant:
(* P<0.003; **P<0.0003; *** P<0.0001;
† P<0.04; †† P<0.02; ‡ P<0.005).

Table3: Number of points overlying the fibromuscular stroma in prostate of adult rats treated with dietary melatonin (in unit area of 0.0025mm^2).

Daily dose of melatonin in µg/kg body wt	***Points on prostatic stroma***
Control	15.1±1.3
125	15.6±0.9 **NS**
250	16.1±1.1 **NS**
500	17.2±1.2*
750	18.0±1.4**
1000	18.7±1.5**

-Data were expressed as mean ± SD of 8 rats.
-When any dose-group was compared with its control, the difference was statistically significant:
(* P<0.001; ** P<0.0009; NS= non significant).

Discussion

The prostatic weight was significantly affected by melatonin in the instant work. The explanation for this might be highlighted by the fact that prostatic weight principally follows its function status [7 & 8]. The physiological condition of the prostate determined basically by its glandular epithelial histological appearance; so it is considered to be actively functioning whenever its epithelial cells are tall columnar with pale large nuclei, whereas it is said to be insufficient in case its epithelia are atrophied, low cuboidal or squamous with dark relatively small nuclei [1, 7 & 8]. The glandular cells height and diameter of their nuclei showed a clear positive effect of melatonin on those parameters; i.e., they were steadily increased with the stepping up the doses up to the level of 500 µg/kg, then after decreased gradually. This could be due to the concept that melatonin is well designed to exert its physiologic action in a dose – dependent manner, being stimulating at normal therapeutic level and harmful at its overabundance [9 & 10]. The glandular cells significantly got taller with more and more larger nuclei observed in those groups treated with 125, 250 and 500 µg/kg dose, then regressed at 750 and 1000 µg/kg dose, these findings might indicate the inhancement in the function of epithelial glandular cells, as a consequence of exogenous melatonin on those cells, affecting them directly through melatonin receptors found in all tissues and cells [11], and/or indirectly through the hypothalamic-hypophysial-gonadal axis stimulating the secretion of FSH thereby promotes other sexual hormones secretion [12]. Nevertheless, there could be probably an induction of Sertoli cells by melatonin supplement to secret surplus amount of androgen binding

protein (Abp), which binds testosterone and hydroxytesteron produced out side the genital ducts, high concentration of these hormones are required within the genital epithelium and lumen for normal function [13 & 14]. The cell height and size of nuclei diminished with 750 µg/kg doses and a great regression noticed at 1000 µg/kg dose. The suggestion for those finding could be through suppression of hormone inhibin, which is secreted by Sertoil cells normally, inhibiting the secretion of FSH by the pituitary under control of hypothalamus and plays an important feed back role in controlling the secretion of sex hormones, which could be the cause of that regression consequently [1&14]. The significant effect on average diameter of nuclei in all of treated groups; may lead to the impression that melatonin could affect most of the cell activities, since the nucleus is the archive of the cell [13].

The buildup in prostatic weight might be the consequence of the raise in the cell height and nuclear bulk. The number of points overlying the stroma was increasing incrementally in all treated groups, which also could contribute to that enlargement of prostate. This large increment in septal thickness perhaps, due to the allowance in production of fibrocollagenous tissue; once there are any damaging events to any organ [7 & 14]. Moreover Melatonin hormone has special effect on fibroblasts, which are the active collagen – secreting cells and the basic forming cells of the connective tissues [1 & 15]. The other contributor to the prostate enlargement could be the dilated blood vessels, because melatonin has a well-known vasodilator action [16]. Those results could be explained by the fact that melatonin has damaging effects only when it is administered in excess [9 & 10].

The results of the instant work went with the concept that melatonin administration within a therapeutic dose might be helpful in the amelioration of the fertility state [3 & 17].

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Immunocytochemical detection of some apoptosis regulating proteins (P53 and Bcl-2) in Peripheral Blood Lymphocytes of Rheumatoid arthritis patients

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Abstract

Background: There are several regulatory proteins involved in the control of lymphocyte apoptosis. Their impairment may play a role in the pathogenesis of several autoimmune diseases. Recent studies have reported impairment in the apoptosis of peripheral blood lymphocytes (PBLs) in patients with rheumatoid arthritis (RA).
Objective: The aim of this study is to evaluate the cellular expression of P53 and BcI-2 proteins in the PBLs and their roles in the apoptotic process, and correlate their cellular expressions with the percent of peripheral T cell population.
Methods: This study involved forty-six RA patients were examined and compared with 17 healthy control individuals of similar ages. Lymphocytes were separated from peripheral blood samples, the assessment of their cellular expression of CD3 and regulatory proteins p53 and Bcl-2 by immunocytochemistry staining method.
Results: The results showed abundant accumulation of CD3 T lymphocytes in the peripheral circulation of RA patients in comparison with controls. A highly significant increased percentage of Bcl-2 protein expression in RA PBLs, compared to healthy control ($p<0.001$) while there was no such statistical difference regarding P53 expression in PBLs from both groups ($p=0.278$). The results of linear regression showed a significant correlation between the increased peripheral blood T lymphocytes and cellular percentage of Bcl-2 protein expression ($p<0.001$), while there was no such correlation with the percentage of P53 expression ($p=0.587$).
Conclusion: in conclusion of these results, we found an increase in the peripheral blood T lymphocytes from patients with RA that could be resulted from the noticed up-regulation of cellular expression of Bcl-2 protein, rather than with changes in cellular expression of P53 protein.
Keyword: Rheumatoid arthritis, apoptosis, P53, Bcl-2, immunocytochemistry.

IRAQI J MED SCI ,2008;VOL.6(1):89-98

Introduction

Apoptosis or programmed cell death is a physiologic, genetically encoded program that results in cell death. It plays an important role in the elimination of unwanted cells during development and also as a balancing factor in maintaining tissue homeostasis, including that of the immune system [(1)].

Apoptosis also has anti-autoimmune mechanism that deletes potentially pathogenic autoreactive lymphocytes, and limits tissue damage in autoimmune diseases, including Rheumatoid Arthritis (RA) [(2)].

The etiopathogenesis of RA is not fully understood. However, it has become increasingly clear that T cells play a crucial role in the induction and maintenance of RA lesions [(3)]. Meanwhile, it has been recently postulated that the increased number with abnormal differentiation pattern observed in peripheral lymphocytes in RA patients might be related to an abnormality in the apoptotic pathway [(4)]. This suggested being due to lymphocyte expression of apoptosis-related molecules leading to

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Received: 2nd September 2007, Accepted: 17th December 2007.

suppression of the apoptotic process; while suppression of apoptosis of T lymphocytes leads to their survival which relates to the chronic and relapsing characters of RA [5, 4].

P53 and Bcl-2 proteins are known to play a central role in the regulation of apoptosis. P53 is a tumor-suppressor gene that controls cellular proliferation. In its natural form (wild-type) P53 can bind to DNA and prevent cells from entering the S (synthetic) phase of the cell cycle so as to allow time for DNA repair. Alternatively, P53 dependent events can eliminate the cells by sending them down to an irreversible apoptotic pathway. Thus P53 allows the DNA either to be repaired or ultimately destroyed before replication renders the damage perminanent. On the other hand, the mitochondrial-mediated apoptosis is partially controlled by the family of Bcl-2 proteins, one of the biologically most relevant classes of apoptosis regulators [6]. The Bcl-2 protein was originally identified as the primary cause of some B-cell lymphoma (hence the designation Bcl), but was subsequently found to have strong anti-apoptotic activity in a variety of cell types, including lymphocytes [7]. Upregulation of Bcl-2 proteins may cause systemic autoimmune disease through accumulation of activated T lymphocytes [8, 9].

The present study aims to evaluate P53 and Bcl-2 expression in PBLs isolated from RA patients regarding their role in the apoptotic process.

Subjects and methods

Patients and controls:

The study groups consisted of forty six Iraqi patients with RA fulfilled the American College of Rheumatology (ACR) classification criteria [10], were recruited from the out-patient clinic at the Department of Rheumatology and Rehabilitation, Al-Kadhumyia Teaching Hospital in Baghdad. Also 17 age-and sex-matched healthy controls were enrolled in the study. These controls were healthy blood donors.

The scoring system of present disease activity was done according to modified DAS28-3, that combines of both clinical and laboratory parameters. The clinical examination of joint swelling and tenderness was performed for 28 joints (include the same joints: shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and the knees [11]. While the general immunolaboratory assessments included erythrocyte sedimentation rate, C-reactive protein, and RF. Clinical and laboratory characteristics of the patients included in the study are summarized in (Table 1).

Blood samples and slides preparation:

A blood sample (Five ml venous blood) was aspirated from a suitable vein from all patients and unaffected controls. Blood was collected in pyrogen-free silicone-coated tubes with heparin. The blood samples were used for lymphocyte separation according to Isopaque-ficoll technique (originally described by Boyum in 1968) [12].

Heparinised peripheral blood was diluted 1/1 with phosphate buffered saline (PBS), and mononuclear cells were isolated by ficoll density gradient centrifugation at 2000 rpm for 20 minutes. Mononuclear cells were washed three times with PBS for 5 minutes, resuspended at 1 x 10^6 cells/ml, and fixed on poly-L-lysine-coated glass slides, wrapped, and kept at -20°C until assayed.

Measurements of T-cell population and apoptosis regulating proteins:

The percentage of PBLs reactivity was semi quantified by immunocytochemistry staining method.

Briefly, these precoated charged slides were removed from freezer, allowed to reach room temperature, unwrapped and then dipping the slides into PBS-filled jar for about 5 minutes and slides were placed on a flat level surface, then endogenous peroxidase was quenched by initial incubation of the smears by enough drops

of Peroxidase block for 5 minutes at room temperature then rinse with PBS from a wash bottle, slides then placed in PBS wash bath for 2 minutes and excess buffer were taped and wiped around smears. Then, enough power block reagent (1/10 diluted in PBS) were applied for 5 minutes and excess blocking reagent were taped but not washed to ovoid non-specific binding of antibodies. Then, the coated lymphocytes were covered by 20 µl of 1/30 diluted mouse monoclonal Ab (primary Ab) specific for human CD3, bcl2 and p53. Slides then incubated at 37°C for 1hr, then unreacted monoclonal Ab was removed by three cycle of washing with PBS each two minutes, then slides were washed wiped around the smear. After that enough solution of biotinylated secondary antibody (anti-mouse Ab) were applied to cover each smear, distributed evenly over the precoated slides then placed in humid chamber for 1 hour at 37°C and washed in buffer and bathed in PBS for 5 minutes then wiped around smear. Enough solution of streptavidin conjugated peroxidase were applied to cover the smear and slides were placed in humid chamber for 1 hour at 37°C then washed in buffer and bathed in PBS for 5 minutes then wiped around the wells. Then enough drops of freshly prepared DAB working solution were applied to cover the section at room temperature for 10 minutes or until the color is observed then the reaction terminated by rinsing gently with distilled water from a washing bottle. Slides then placed in bath of hematoxyline for 30 seconds at room temperature. Slides were rinsed gently with distilled water from a wash bottle then rinsed under gently running tap water for 5 minutes. A drop of mounting medium (DPX) was placed onto the wet smear and the spot quickly covered with a cover slip. Slides were let to dry.

Slides were examined under 400X-magnification power of light microscope (ZEISS). The dark brown (homogenous or membranous) staining identified positive labeled cells see (figure 1).

Statistical analysis

The percentage of each of the tested marker expression on PBLs was calculated by a simple calibration of percentage of reactivity as following formula: Percentage of expression= (No. of positive cells/ total No. of cells) ×100%.

Statistical differences were analyzed using Independent sample-test. P-values <0.01 were considered statistically significant. Simple linear regression was used to assess the relationship between studied variables.

Results

The study included forty-six RA patients (four men and forty two women), mean age (47.67 years) ranged in age from (25-66 year) with mean disease duration (6.5 years). Our patients were classified according to DAS into two main group the majority of them, 37 patients (80.4%) presented with high disease activity and the remainder were minimum disease group consist of 9 patients (19.6%).

Immunocytochemical examination:

The percentage of peripheral blood T cell population was indicated by CD3 reactivity. In our study we found significantly elevated percentage of CD3 positive cells in RA patients when compared with healthy controls ($p \leq 0.001$). Also, there was highly significant overexpression of Bcl-2 in RA patient than those from control group ($p \leq 0.001$), and no significant difference in the P53 expression between RA patients and control groups (p=0.278).

In RA patients and according to disease activity groups, we compare the percentage of expression of studied markers between and found that no significant statistical differences in CD3, Bcl-2 and P53 expressions (p=0.686, p=0.130 and p=0.823) respectively.

***Relation ship between CD3 with Bcl-2 and P53 PBL expression*:**

Our study reported that there was an increased T-cell population in the peripheral circulation, we try to investigate wether this increase related to anti apoptotic effect of Bcl-2 overexpression or due to P53 reduced expression. The results of linear regression found that there was highly significant linear relation between increased peripheral T cell population and the increased Bcl-2 expression ($p \leq 0.001$) (figure 4.A), but there was no relation between increased peripheral T cell population and P53 expression ($p=0.587$) (figure 4.B).

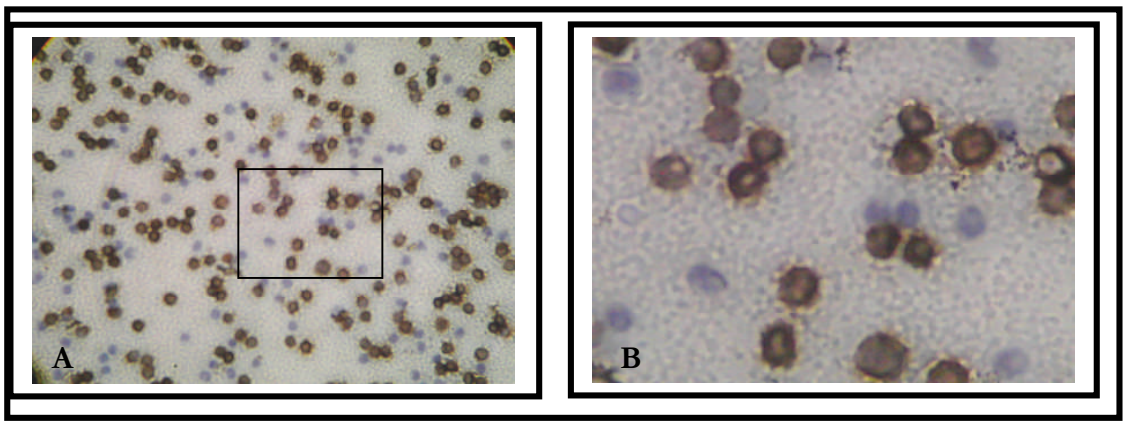

Figure 1. Immunocytochemical staining of PBL from RA patients stained with anti human Bcl-2 mAb, visualized by peroxidase/DAB (brown) and counter stained with hematoxylin. A: Low power magnifications of 400X. B: High power magnifications of 1000X.

Table 1. patients and control characterstics. data are presented as means (SD).

	Controls	RA patients	RA patients	
			High disease activity group	Minimum disease activity group
Women/men	**15/2**	**42/4**	**34/3**	**8/1**
Age	**48.6 (10)**	**47.67(12.09)**	**48.06(11.96)**	**46.45(12.97)**
Disease duration (months)	**----**	**88.61(72.88)**	**92.34(68.28)**	**76.73(92.67)**
ESR (mm/1st h	**12.50(3.31)**	**67.43(20.26)**	**70.94(19.54)**	**53(17.33)**
CRP (mg/l)	**10.20(15.24)**	**43.956(55.078)**	**49.78(59.53)**	**20(17.75)**
Tender joints	**-----**	**10.58(5.42)**	**12.54(4.62)**	**4.77(3.19)**
Swollen joints	**-----**	**7.35(4.52)**	**8.63(4.36)**	**3.66(1.80)**
DAS-28 (3)	**-----**	**5.77(0.83)**	**6.11(0.63)**	**4.844(0.24)**
RF sero-positive (No. (%))	**3(21.4%)**	**34 (73.9%)**	**27(72.9%)**	**6(63.54%)**
Duration of morning stiffness (minutes)	**-----**	**76.41(41.30)**	**84(41.72)**	**52.27(30.28)**

ESR=erythrocytes sedimentation rate, CRP= C reactive protein, DAS= disease activity score, RF=rheumatoid factor.

Table 2. Mean (standard deviation) of PBL cellular expression of CD3 and apoptosis regulating proteins (Bcl-2 and P53) in RA patients and control group.

	Controls	RA patients	RA patients	
			High disease activity group	Minimum disease activity group
CD3	72.92(0.44)	79.21(1.42)	79.26(1.33)	79.04(1.8)
Bcl-2	20.5(3.55)	34.17(6.5)	35(5.48)	31.55(9.1)
P53	2.17(0.68)	3.26(0.32)	3.2(3.1)	3.45(3.78)

Table 3. Results of comparison among different study groups using independent sample t-test.

	RA patients *vs.* Controls	High disease activity group *vs.* Minimum disease activity group
CD3	p=0.000**	P=0.686
Bcl-2	P=0.000**	P=0.130
P53	P=0.278	P=0.823

(*) significant($p \leq 0.05$),
(**) highly significant ($p \leq 0.005$)

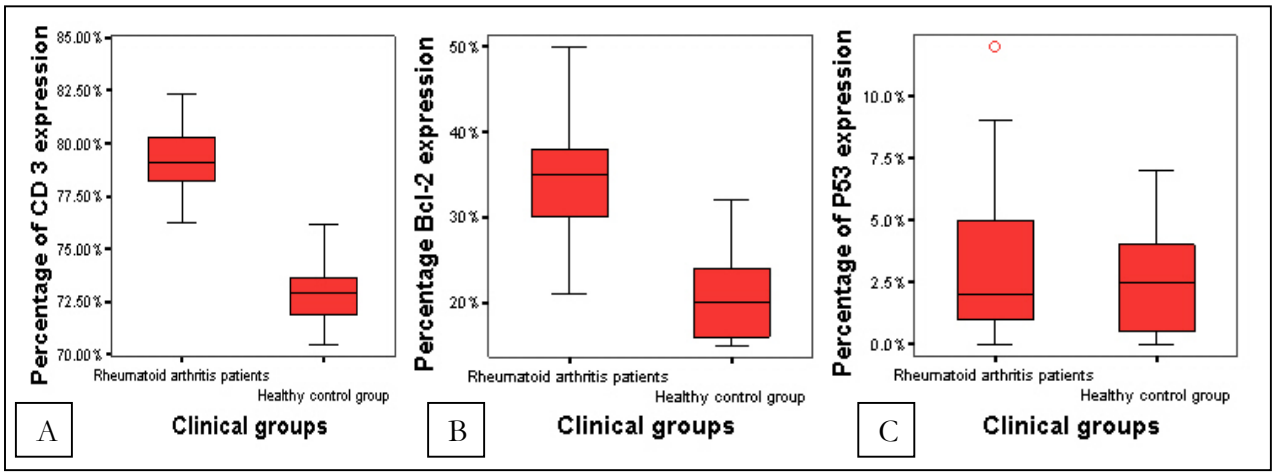

Figure 2. Cellular expression of CD3 (A), Bcl-2 (B) and P53 (C) in peripheral blood lymphocytes from RA patients and control group measured by immunoytochemistry method. Box plots represent median (line), 25th and 75th centiles (box), and whiskers indicate the 10th and 90th centiles.

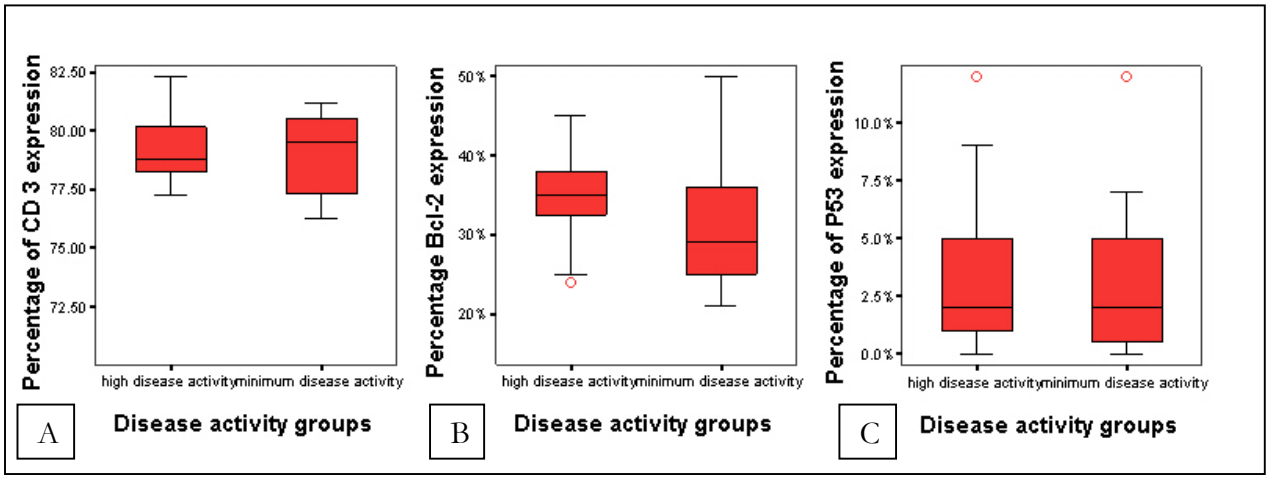

Figure 3. Cellular expression of CD3 (A), Bcl-2 (B) and P53 (C) in peripheral blood lymphocytes from RA patients suffering from high disease activity and minimum disease activity measured by immunoytochemistry method. Box plots represent median (line), 25th and 75th centiles (box), and whiskers indicates the 10th and 90th centiles

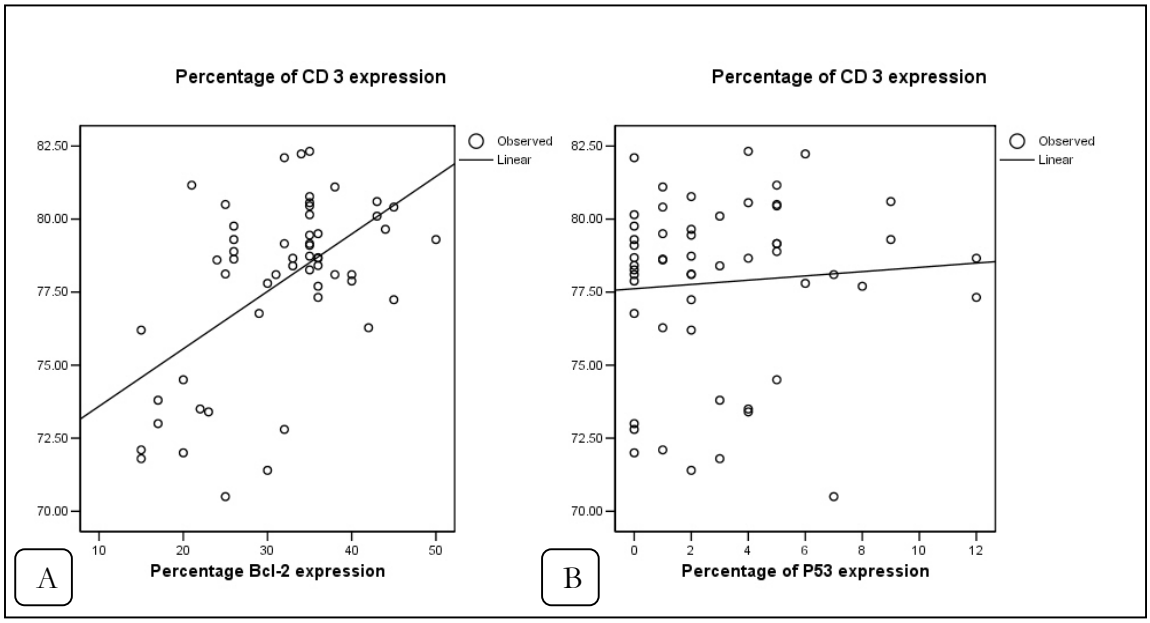

Figure 4. Relation between PBL expression of CD3 and Bcl-2 (A), and P53 (B) from RA patients. Points represent individual peripheral blood samples. Data show line of linear regression ($p \leq 0.001$) and ($p = 0.587$) for (A) and (B) respectively.

Discussion

Rheumatoid arthritis (RA) is a common autoimmune disease in which patients suffer from chronic inflammatory synovitis that is dominated by the presence of macrophages, neutrophils, lymphocytes and synovial fibroblasts. Synovial macrophages are highly resistant to apoptotic stimuli [13].

Several lines of evidence have shown the importance of T lymphocytes in the pathogenesis of rheumatoid arthritis using animal models of arthritis [16–21]. The data also suggest that both regulatory and pathogenic T lymphocytes might be involved [22–26]. In human rheumatoid arthritis (RA), clonal expansion of T cells in both peripheral blood and local synovium has been described repeatedly in the past 10 years. The clonally expanded T lymphocytes appear to be autoreactive [27, 28].

We recorded abundant accumulation of CD3+ve T-cells in the peripheral circulation with highly statistical significant difference in the mean percentage of expression than those of controls. In RA, workers proposed that thymic output is prematurely compromised in RA patients and a compensatory expansion of peripheral T cells results in a contracted and distorted repertoire. However, a shift of a contracted repertoire towards autoreactivity may result in increasing the size of individual clones and probably increase the risk of self-recognition. This could amplify their potential to cause ongoing autoimmune disease [14].

Lymphocytes are critical player in the pathogenesis of RA, in which apoptosis may play divergent roles. The study of lymphocyte development and survival, programmed cell death and the genes regulating this process has become a focus of interest and defects in lymphocyte apoptosis are hypothesized to contribute to development of autoimmunity in RA [15].

These studies suggest that pathogenic T lymphocytes accumulate *in vivo* for unknown reasons and cause autoimmune arthritis in RA patients. The mechanisms for the accumulation of those pathogenic T lymphocytes are still unknown, while a failure of apoptosis has been proposed to be an important mechanism [29-38].

However, the mechanisms of accumulation and expansion of autoreactive T lymphocytes in RA patients are still unknown. There may be three possibilities for accumulation and expansion of autoreactive lymphocytes in RA patients: one is a continual input of autoreactive lymphocytes into the peripheral lymphocyte pool, certain genetic backgrounds may predispose an individual to accumulate autoreactive T cells *in vivo*; [39, 40] the second is a failure to suppress autoreactive lymphocytes via anergy; [41, 42, 43] the third possibility is a failure to remove autoreactive lymphocytes from the peripheral lymphocyte pool by apoptosis [44]. While limited data are available regarding functional relationships among different T lymphocytes in RA, the positive effect of inducing T-cell tolerance in treating RA in both animal models and human clinical trials implicates that breakdown of peripheral tolerance plays an important role in the pathogenesis of RA [45, 46].

Activation-induced cell death (AICD) is an important mechanism by which the immune system can eliminate peripherally activated lymphocytes, maintain homeostasis and maintain peripheral tolerance in the immune system [31–33]. Previous data support that a failure of T-lymphocyte apoptosis is involved in the pathogenesis of RA [32, 33]. Most available studies were performed in RA synovium [34]. The data suggests that T-cell apoptosis is suppressed [35]. It could be due to activated CD14+ cells that secrete a soluble survival signal (CD14 cocktail) that protects activated lymphocytes from undergoing AICD [45].

Apoptosis is controlled genetically where P53 and Bcl-2 play a central role in its regulation [46, 47]. Actively proliferating

cells typically express Bcl-2 that protects them against apoptotic stimuli while terminally differentiated cells lose Bcl-2 expression, found that Bcl-2 protein in PBLs overexpressed and proposed a defect in the mechanism of deletion of over-produced lymphocytes that probably play role in the pathogenesis of RA [34].

Our results clearly demonstrate that there is uniform underexpression of p53 in PBLs from patients with RA. Other investigations have addressed the role of p53 in RA. The majority of these studies have focused on the synovium. It has been proposed that high levels of oxidative stress in rheumatoid synovium may cause somatic mutations in the TP53 gene [48]. Mutations in synovial p53 may allow pathologic proliferation of synovial cells that may lead to joint destruction and other clinical manifestations of RA. Alternatively, it has been proposed that the cytokine, macrophage migratory inhibitory factor (MIF), may cause decreased cellular p53 levels [49, 50]. Elevated MIF levels may contribute to the underexpression of p53 in PBMCs from RA patients. However, our results clearly show that T lymphocytes, representing 80% of our PBMC preparations, are defective in p53-mediated apoptosis, and T lymphocytes are not known to respond to MIF. There is also evidence that p53 maintains tolerance in lymphocytes by regulating cell cycle progression. Human T lymphocytes from peripheral blood or intestinal lamina propria show an inverse relationship between p53 levels and the rate of progression through the cell cycle [51]. Cell cycle delays mediated by elevated levels of p53 in lamina propria T lymphocytes may be a mechanism that maintains tolerance against environmental antigens. Preliminary studies by Leech et al, using an antigen-induced arthritis model on a p53_/_ background, revealed that T lymphocytes proliferate more readily and produce more IFN_ in the absence of p53 [52]. However, our results agreed by Mass et al. when found that P53 protein was reduced in lymphocytes of patients with RA, making the cells less likely to under go apoptosis [53]. Similar results in models of collagen-induced arthritis [48] suggest that inflammatory responses may be exacerbated in the absence of p53.

These data show that the highly differentiated and apparently unstable state PBLs in RA may result in part from active inhibition of T cell apoptosis by environmental factors associated with the inflammation itself.

In conclusion defective lymphocyte apoptosis is playing an important role in RA inflammation. It could most probably mediate through other mechanism rather than the P53 pathway (P53 independent). Mean while, overe-xpression of Bcl-2 protein by PBLs could protect them from apoptosis, leading to their persistence and chronic characters of the disease.

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Evaluation of stroke risk factors among hospitalized patients with ischemic stroke in Baghdad

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Abstract

Introduction: Identification of stroke risk factors is of great importance to prevent the disease and its sequels.

Objective: Is to analyze the known stroke risk factors and to study the education level, housing condition and economic status as possible relevant stroke risk factors.

Patients and methods: 510 patients with ischemic stroke of different types and severity who were admitted to 3 hospitals in Baghdad. The patients were examined and investigated thoroughly. Special emphasis was concentrated on education level, housing condition and economic status.

Results: 510 patients were studied, 215 females and 295 males. The study showed 16 % higher male prevalence. Hypertension is commonest risk factor in the present study (75.5%) followed by other risk factors. the study showed a higher number of stroke patients [44.7%] was illiterate, in comparison to [9%] of the patients with the higher education. The study showed more prevalence of the stroke patients [90.39%] live in crowded small houses. The study showed [36%] of stroke patients belongs to families with below 100 USD monthly incomes.

Discussion: Higher prevalence of diabetes mellitus, hypertension, smoking and also poor health awareness in patients with low educational levels, poor economic state and poor housing condition.

Conclusion: Stroke is increased in low educational levels, poor economic state and poor housing condition. . There is a high percentage of hypertension not previously diagnosed despite stigmata of chronic hypertension.

Key words: stroke, risk factors

IRAQI J MED SCI ,2008;VOL.6(1):99-107

Introduction

Stroke is a sudden focal neurological syndrome due to cerebro-vascular disease[(1)].it is the commonest neurological disorder admitted in the general medical wards[(2)] it forms 50 % of the neurological wards admission [(2,3)] .Stroke is the second leading cause of death world wide [4], stroke is the commonest cause of morbidity world wide [(1,2,3,4)].

High mortality and morbidity burden of stroke impacts a high economic and social burden on the families and society [(5)].

Because of all these impacts added to low frequency of successful acute stroke treatment [(2, 3, 5)], makes identification and prevention of stroke risk factors is of utmost importance to minimize the whole impacts of stroke.

Stroke risk factors are classified into modifiable factors, which can be identified and treated; fortunately they are more frequent than non – modifiable risk factors. Efforts of researchers are directed to identify more risk factors in their societies and to analyze the difference between these societies.

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Received: 16th September2007, Accepted:23th March 2008.

The aim of this paper is to re evaluate the known stroke risk factors in hospitalized patients in Baghdad and to study socio- economic status and education level as a risk factor in relation to stroke incidence.

Patients and methods

510 patients with stroke were admitted in the neurological wards at Alkindi teaching hospital, Al-yarmok teaching hospital and Medical City teaching hospital; during the period between April /2004 to September/ 2006.

All the patients were asked about their detailed present illness, past medical history, drug history, gynecological history for females and social history; special concern was emphasized on monthly income of the patient's family; we divided families into:

- Those with below 100 USD/month incomes.
- Those with 101—200 USD/month incomes.
- Those with 201--300 USD/month incomes.
- Those with 301--400 USD/month incomes.
- Those with 401--500 USD/month incomes.
- Those with more than 500 USD/month incomes.

Than the patients were divided according to their educational level into;

- Illiterate group
- Can read and write group [primary school]
- Secondary school level group
- Higher education level group

Also we asked about house condition whether it is crowded with three members or more of the family living in one room or not crowded with two or less members living in one room.

Detailed medical and neurological clinical examinations were done by neurologist.

All the patients had Brain CT scanning, complete blood picture, serum calcium, serum potassium, serum sodium, fasting blood sugar, fasting total serum cholesterol, blood urea, serum creatinine, pro-thrombin time, partial thrombo- plastine time, ECG, trans -thoracic echocardiography and Doppler of the Carotids.

Anti-cardiolipin antibodies, ANF, Rheumatoid factor, Pethrgy test were done for those below 40 years. Patient diagnosed as Antiphospholipidantibody disease, other connective tissue disease or Behcets disease were excluded from the study because of the direct relation of stroke in those patients to one cause rather than a risk factor.

The patients considered diabetic when he was known to be diabetic on anti-diabetic treatment or more than [7 mmol/L] 120 mg/dl fasting blood sugar test and random more than 2 hours postprandial blood sugar more than [11.1mmol/L] 200 mg/dl [this is according to WHO criteria of diabetes mellitus] [7], and considered hypertensive when he had tow weeks apart 2 blood pressure readings of systolic BP 160mmhg and /or diastolic BP 95 mmhg. [As in Framingham's study][6], or if there is history of hypertension and the patient on antihypertensive.

Heart diseases included in this study are disorder of heart rate, ischemic heart disease, valvular heart disease, cardiomyopathy and left sided heart failure.

Total Cholesterol level above 220 mg /dl is considered as a hyper-cholestrolemia [as in Qisilbash metanalysis of 10 studies] [8]. PCV above 55% and above 48% in male and female respectively, was considered as polycythemia. Carotid

stenosis of any extent above 50% stenosis by Doppler study is reported in the present study. Obesity was considered if the weight is 20% more than the standard [length in cm -100] [12]. The patient was considered as having migraine when he has history of typical recurrent attacks of migraine of more than four hours unilateral or bilateral throbbing headache, that was associated with nausea, vomiting and either photophobia or phonophopia [1].

Results

510 patients were studied, 215 females and 295 males, the males are affected more than female by 16% see (Table 1).

As seen from (Table 1) the stroke percentage is higher in male than female by 16 % in patients between 40 and 70 years age ; exception to this is the approximately no gender rates of difference below age of 40 years and above age of 70 years.

The study showed increase the number of patients with older age, especially after age of 50 years there is doubling of the incidence until there is decline of the incidence after the age of 80 years (Table 1).

The maximum stroke estimate is between 71-80 years [34.9%] and the minimum estimate is below age of 40[3.33%] see (Table 1).The present study reported hypertension in 75.5% of the patients, smoking in 61.5%, other factors prevalence was shown in (Table 1).

The present study showed 28% [110 out of 381 patients] of the hypertensive patients not known to be hypertensive previously but have stigmata of chronic hypertension [retinal changes and ECG strain pattern of hypertension]

The study showed [36%] [184/510] of stroke patients belongs to families with below 100 USD monthly income, 22.4% of the patients belongs to families with monthly income between 101- 200 USD, and 15.2% between 201-300 USD, 10.7% between 301-400USD, 9.4% between 401-500 USD and 6.3% of the patients families with more than 500 USD monthly income see (figure 1).

There is a higher incidence [44.7%] of illiterate patients, in comparison to [9%] of the patients with the higher education. See (figure 2).

There are [461] patients [90.39%] live in crowded small houses and the rest [49] patients [9.6%] live in non-crowded houses.

There are 473 patients [90.39%] having WBCS counts between 4000-11000 cells/mm2; 35 patients [6.9%] having more than 11000cells/mm2 and only 2 patients [0.39%] having below 4000 cells/mm2.

Table 1: age/ gender distribution

Age /years	male	female	total
----40	10 [59%]	7 [41%]	17 [3.33%]
41-50	18 [62%]	11 [38%]	29 [5.7%]
51-60	51 [61%]	32 [39%]	83 [16.27%]
61-70	102 [67%]	50 [33%]	152 [29.8%]
71-80	91 [51%]	87 [49%]	178 [34.9%]
80---	23 [46%]	28 [54%]	51 [10%]
Total	295 [58%]	215 [42%]	510

X2= 12.702 DF = 5, P-Value = 0.026

Table 2: Risk Factors in the Present Study

				age/year					
		-40	**41-50**	**51-60**	**61-70**	**71-80**	**80-**	total	%
hypertension		15	17	61	115	130	42	380	74.5%
SMOKING		9	23	55	98	113	16	314	61.5%
heart diseases		5	11	45	85	97	40	283	55%
diabetes mellitus		2	5	50	90	95	25	267	52%
TIA		3	16	35	69	81	25	229	45%
hyper cholestrolemia		14	8	15	45	75	15	172	33.7%
carotid stenosis/dopplar		1	2	23	40	61	15	142	28%
polycythemia		2	7	12	33	24	8	86	17%
carotid bruit		0	1	19	22	25	8	75	15%
obesity		2	15	12	25	17	3	74	14.5%
migraine		8	12	6	0	0	0	26	5%
Alcoholism		3	5	5	2	2	1	18	3.5%
Pill		2	1	0	0	0	0	3	0.58%

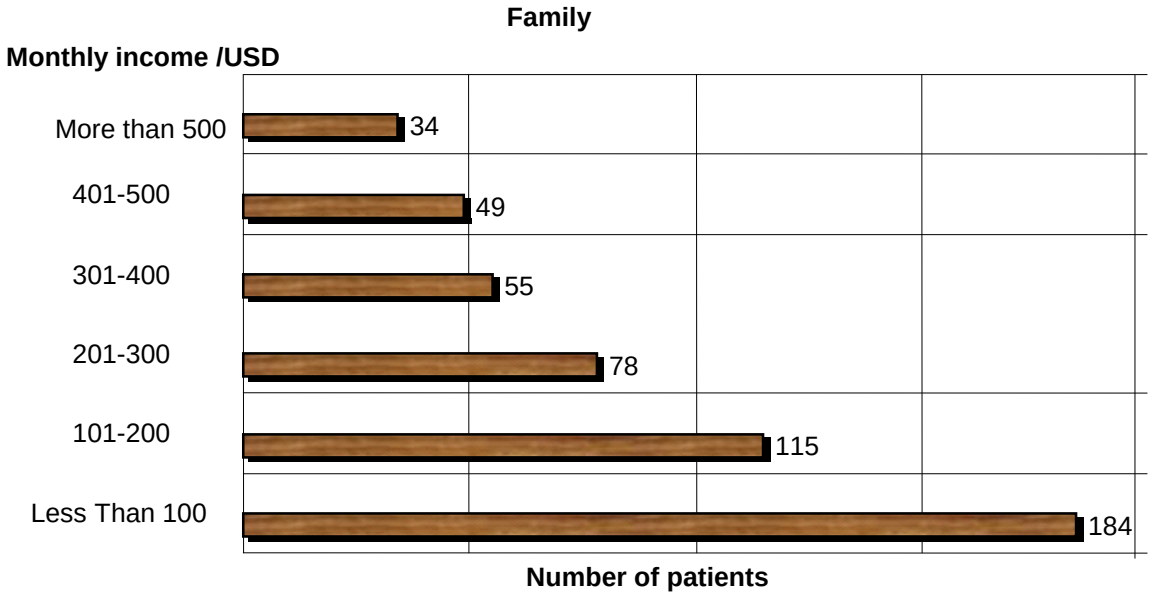

Figure 1: Relation of stroke incidence to the monthly income of the patients

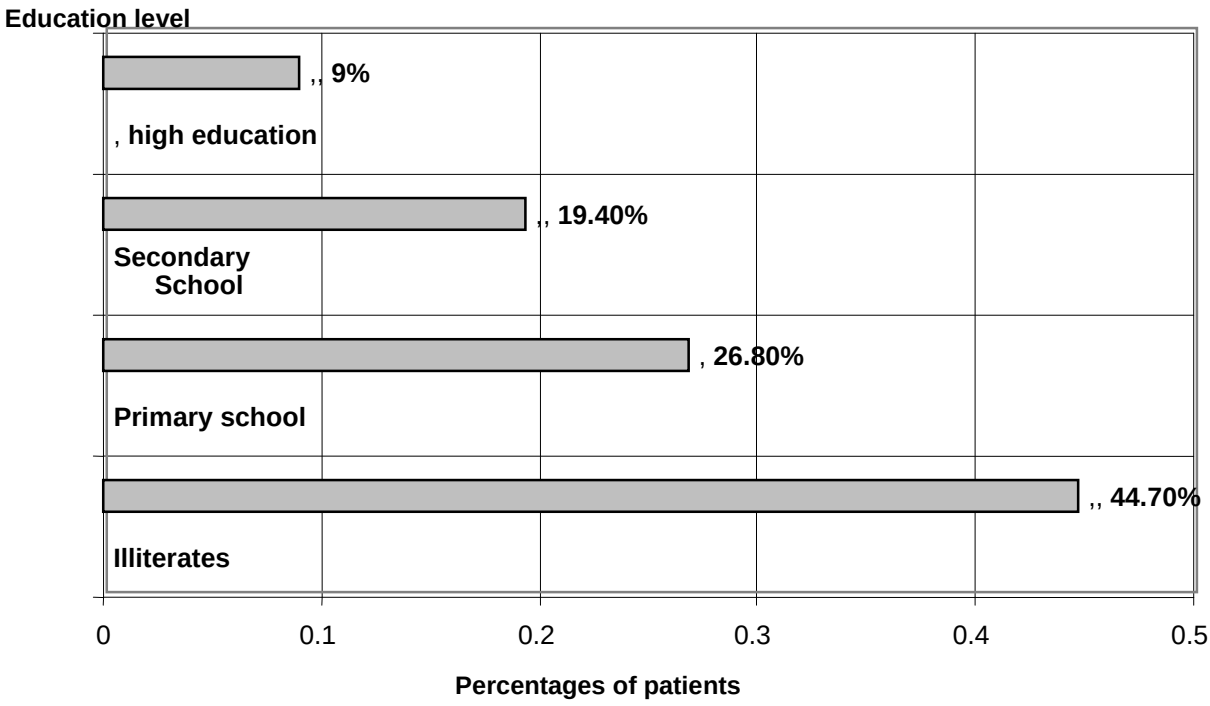

Figure 2: Relation of stroke incidence to Level of education of the patients

Discussion

Stroke is the most common preventable neurological disease [(9)].

The low frequency of treatment options in comparison to the grave high disease burden on the families and public health [(9)]; all these makes most of the efforts to be directed toward recognition of modifiable risk factors ,in order to prevent and to reduce the disease occurrence to minimal incidence rates .

Scientist and neurologist all over the world try to find new risk factors; most of the researchers based their work on the Framingham study[10].

Age is the most powerful predictor of stroke [(11)]; The present study showed that below age of 80 years there is an exponential increase of stroke percentage with the raise of age (Table 1), this increase with increasing age is in agreement to Suleiman, Khalil and Almehedawi study [12], Abu-nayla-Salman study[13], North Manhattan stroke study [15] and Framingham study [14] .

The study shows a decline of stroke percentage after the age of 80 years. And this is contrasting Suleiman, Khalil and Almehedawi study [12], Abu-nayla and-Selman study [13], North Manhattan stroke study [15] and Framingham study [14] in which there are doubling of stroke rates after the age of 80 higher than the younger ages. The more frequent age affected by stroke in the present study is between 71-80 years; and this is in approximate to white American age result in North Manhattan study which reveals the mean age of stroke in white American is [80 ± 9 years] population and [67 ± 12 years] Hispanic American.

The present study showed [16%] higher male affection than female between 40-70 years, and this result is agreeing the results of Suleiman, Khalil and Almehedawi study [12] , Abu-nayla-Salman study[13] .According to

American heart association, men's incidence of stroke exceeds women's by 19% [16]; this figure is in approximation to 16% difference in the present study. Sacco and Chong report 24 -30% greater male incidences; which is higher than the gender disparity of the present study. The higher male rate in the present study is contrasting the results of the Framingham study, which showed non-significant gender difference [14]. The present study shows a non significant gender difference at the extremes of age, as seen from (figure 1) there is nearly equal gender affection below age of 40 years and above age of 70 years; this non significant gender difference above 80 years in the present study is in agreement to result of American heart association [16] but contrast results of Suleiman, Khalil and Almehedawi study [12] and, Abu-nayla-Selman study [13]. Hormonal changes in the elderly female with decrease and loss of the protective effect of progesterone may lead to nearly equal rates of stroke in both genders.

Elevation of systolic blood pressure above 160 mmhg and /or elevation of diastolic blood pressure above 95mmhg are the commonest risk factors we have seen in the present study; it is found in 74.5% of the patients in this study. The result of hypertension frequency in the present study is similar to the result of north Manhattan stroke study, which reveals 63% among white American stroke patients and 79% among Hispanic American stroke patients [18]. The result of hypertension in the present study is higher than that of Suleiman, Khalil and Almehedawi study [12] , Abu-nayla-Selman study [13] and Framingham study in which the attributable stroke risk for hypertension ranged from 35-50% [4]. This higher rate of hypertension in comparison to the above studies is explained on the increased frequency and prevalence of hypertension in our society in the later years.

The present study showed 28% [110out of 381 patients] of the hypertensive patients not known to be hypertensive previously but have stigmata of chronic hypertension [retinal changes and ECG strain pattern of hypertension]; in fact this is very high rate of undetected hypertensive patients indicate either or both poor health awareness of the general population or poor health care service; and support the name of silent killer for the hypertension [19]. There is a lot of studies prove the significant relationship between control of blood pressure [even with in normal range] and the reduction of the incidence of stroke [20]; the joint national committee on prevention, detection, evaluation and treatment created a new category of pre hypertension may be at high risk for stroke.

The present study showed a significant number of patients having a WBC count more than 11000cells /mm2; and we raise the role of WBCS as risk factor for stroke or as a factor predicting the stroke progress; we think this need further analysis of the role of WBCS in relation to stroke and stroke out come.

Smoking is the second most common risk factor in the present study, [61.5%] of the patients is smoker. The present study smoking rate is higher than that of North Manhattan study [18%], abu-nayla and Selman study [23%][13] and in Suleiman, Khalil and A-lmehdawi study [29%] [12] Framingham study [20-40%] [14]. The effect of smoking is same in the different age groups in the present study and this contrasting the diminished smoking as a risk factor of stroke with increasing age proved by Shinton and Beever metaanalysis [21].

In a metaanalysis of 32 studies there is relative pooled risk for stroke in the smoker of 1.5 also there is dose – response relationship.

We think that removal of restriction on cigarette import resulting in its lower price added to higher monthly income of wide part of Iraqi population in the later years; all these lead to expansion of the smoking problem as seen from present study.

Heart diseases were reported in 55% of the present study, heart diseases rate was approximately similar to the result of 61% tare of cardiac diseases in White Americans in the North Manhattans study and higher than 32% of the stroke patients in Hispanic American by North Manhattans study of white American [22]. Also heart disease rate in the present study is higher than that of [25%] in Suleiman, Khalil and Almehedawi study[12] and the [28%] in Abu-nayla-Salman study [13] and Framingham study [14].

The present study shows [52%] of the stroke patients were diabetic type 1 or 11; this rate is in agreement to Abu-nayla-Selman study which showed [50%] of the patients are diabetic [13] the estimate of diabetes mellitus in the present study is higher than [32.3%] percentage of patients having diabetes mellitus in Suleiman, Khalil and Almehedawi study [12]. North Manhattan stroke study showed diabetes mellitus frequency of 41% among Hispanic American and 26% in white American.

Lindegurd and Hillbom have suggested that diabetic women had a greater risk of stroke than diabetic men [23].

More stressful life event in our country makes diabetes and hypertension was at higher prevalence than other countries.

TIA preceding stroke was present in [45%]; this result is in agreement with Herman-Lyman study but higher than Suleiman, Khalil and Almehedawi study [35.5%][12], Abu-nayla-Selman study [6.5%] [13]. Bigger sample of the present study than Suleiman, Khalil and Almehedawi study [only 61 patients][12], Abu-nayla-Selman study [only 250 patients][13]; explains the higher TIA rate in the present study, which is in approximation to Fisher CM who found 42% of stroke patients had preceding TIA[24].

Hypercholesterolemia was seen in [33.7%] of the present study stroke patients; this result is higher than Suleiman, Khalil and Almehedawi study [9.7%][12]; this result is nearly equal to 29% of White American having Hypercholesterolemia and 35% of Hispanic American. The result of hypercholesterolemia in the present study is statistically highly significant. The relationship between high cholesterol and stroke is less clear than the relationship with ischemic heart disease in other studies; in large metaanalysis including over 450000people found no association between cholesterol and stroke. Clinical trials using statins showed a substantial decrease in stroke incidence [24].

Carotid stenosis by Doppler study was seen in 28% of the present study patients. Carotid bruit was seen in 15% of the patients, all of them had stenosis by Doppler more than 60% of the carotid artery lumen; these results are higher than Suleiman, Khalil and Almehedawi study [6%][12].

Polycythemia was seen in [17%] of the patients in the present study; this figure is higher than Suleiman, Khalil and Almehedawi study [5%] [12], Abu-nayla-Salman study [2.4%] [13]. . All these high PCV were attributed to smoking and its chronic obstructive pulmonary disease sequel.

Obesity was a feature of [14.5%] of the present study; this is similar to 15% in Suleiman, Khalil and Almehedawi study .a case control study from North Manhattan found that men with waist-hip ratio greater than 0.93 and women greater

than 0.86 were at higher risk of stroke, and this ratio had a greater risk at younger age group [24]. Obesity as measured by body mass index also considered as a risk factor for stroke [25]. Some investigators considered obesity as secondary stroke risk factors because obesity was associated with higher incidence of hypertension and diabetes mellitus [26].

Migraine rate in the present study is [5%] of the patients, other study done in Iraq not searching for migraine as a risk factor for stroke as in Suleiman, Khalil and Almehedawi study [12] , Abu-nayla-Salman study[13].

Alcoholism was seen in [3.5%] of the present study patients, this is lesser than Suleiman, Khalil and Almehedawi study which reports 19.4% of the patients were alcoholics [12], and lesser than [7.6%] in Abu-nayla-Salman study[13]. Among patients 39 years and older who were hospitalized for acute stroke, found [37%] and [23%] of Hispanic American and white American respectively were alcoholics; and this low rate of alcoholism in our patients in compares to American patients is related to religious prohibition of alcohol in our society. Frequency of patients taking pills [0.58%] is in agreement to that seen in Suleiman, Khalil and Almehedawi study [0. 25%] [12].

The present study showed a highly significant correlation with low income, there is inverse relationship between stroke estimate and the monthly income. And this is related to non-healthy dietary habit, poor health awareness and non-compliance with treatment of other medical disease.

The present study shows a significant association of stroke with crowded house; this association is related to poverty, stressful life and lack of exercises.

There was a highly significant increase of stroke rate with low education level especially the illiterate patients; this association is attributed to poor health awareness and poor health access.

Conclusion

1.There is grave expansion of the stroke risk factors estimate in our community later years in comparison to previous studies in the previous years.

2.There is strong relationship of the increased stroke frequency with the low income, crowded houses and low educational background.

3.There is large percentage of the hypertensive patients was undetected; either because of lack of access to health care or due to inefficient health care.

Recommendation

1. We need a wide national health program to educate about the risk factors of stroke and its primary and secondary prevention.

2. We need advanced centers specialized in the treatment and researches of stroke to improve our understanding of the real dimensions of the problem in our country and than to ease its control.

3. We need to improve our primary health centers to increase the detection of risk factors; as this will lead to reduction of the stroke as well as ischemic heart diseases and definitely this will reduce so much the heavy burdens of these diseases.

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Possible association of HLA class I and II molecules with Ulcerative colitis in Iraqi patients

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Abstract

Background: The etiology and pathogenesis of ulcerative colitis has not yet been elucidated. However, attention has been brought to the belief that a genetic factor plays a crucial role in the development of the disease.

Objectives: This study was established to shed light on the possible association of HLA class I and class II antigen with ulcerative colitis.

Methods: The study included 105 subjects .It comprised of 35 ulcerative colitis patients and 70 apparently healthy controls. Lymphocytotoxicity assay has been used to assess HLA- typing.

***Results*:** Comparison between ulcerative colitis patients and healthy control showed positive significant associations of HLA –B14, B27 and DR52 antigens with patients, however, only B27 maintained a significant P value after adjustment. Meanwhile the frequencies of HLA -B35, B41 and Cw6 were decreased in patients when compared with control group.

Conclusions: These findings demonstrated that HLA –B14, B27 and DR52 might play a role in ulcerative colitis susceptibility, while HLA-B35, B41 and Cw6 may confer protective effects against ulcerative colitis.

Keywords: Ulcerative colitis, Genetic factors, HLA.

IRAQI J MED SCI, 2008;VOL.6 (1): 108-113

Introduction

Ulcerative Colitis (UC) and Crohn's Disease (CD) are classified as the chronic idiopathic forms of Inflammatory Bowel Disease (IBD). Ulcerative Colitis is an inflammatory disorder that affects the rectum and colon [1, 2]. All races and ethnic groups in the world may have UC; however, there is a clear higher incidence in developed countries compared with the less-developed one. Although the etiology and pathogenesis of this disorder has not yet been elucidated, there has been a significant progress in recent years regarding the potential genetic and environmental factors that contribute to the pathogenesis of UC in animal models and human disease [3].

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Received: 21st October 2007, Accepted: 28th October 2007.

The important clue has come from genetic-epidemiological studies, which have suggested a role of genetic factors in the etiology of the disease. Human Leukocyte Antigen (HLA) genes are a good candidate for such role, Stasangi *et al* [4] reported that genes of major histocompatibility complex are implicated as important inherited determinants of susceptibility to UC and may also influence the pattern of disease. Another study done by Trachtenberg *et al* [5] demonstrated an association between UC and HLA class II genes, and mentioned that the interaction between DR and DQ may determine the extent of a disease risk, on the other hand, Shorter and associates [6] observed a limited association between UC and the expression of HLA –B5.

The large majority of reported association studies in UC have denoted a positive association with HLA –DR2 and a negative association with HLA-DR4. The most significant association between this disease and HLA–DR2

have been observed in Japanese population, moreover, it has been observed in pooled Caucasian population studies [7,8]. The present study was established to shed light on the possible association of HLA class I and class II antigen with ulcerative colitis.

Subjects and Methods

Subjects:

The present study included 35 Iraqi UC patients (16 females and 19 males; mean age was 41.6±15.7 years (ranged between 18-83 years). They were attending the Gastroenterology and Hepatology Teaching Hospital". The result of them was compared with the results of 70 healthy (age and gender matched) control group.

HLA-Typing

Microlymphocytotoxicity assay was applied for HLA-typing as described by Terasaki and MeClelland [9] and modified by Dick *et al*, and Bender [10, 11]. The isolation of lymphocytes from the whole blood by a single step centrifuged technique (density gradient centrifugation). T and B-lymphocytes were separated by a nylon wool column before testing for HLA DR and DQ antigens.

Statistical analysis

Univariate analysis was applied for the data depending on logistic regression and the results were reported as odds ratio (ORs). The results were presented as percentage frequencies, and the analysis included estimating the EF and PF values. An estimate was considered statistically significant if its P value was less than an α level of significance of 0.05. Adjustment for the hypothetically proposed increase in the alpha level of significance in case of multiple comparisons of different HLA antigens was done by multiplying the P value by the number of comparisons in each HLA locus (Emery, 1976 and Sorlie, 1995).

Results

A total of 35 patients were typed for HLA class I and II antigens. The frequency distribution of various class I and II antigens for studied groups are presented in table (1). Comparison between UC patients and healthy controls showed several antigens deviations in their frequencies. HLA-B14, B27 and DR52 were observed with increased frequencies in the patients (25.7, 34.3 and 17.1%, respectively) versus healthy controls (5.7, 4.3 and 4.3% respectively), with P-values of 0.007, <0.001 and 0.039 respectively, however, only B27 maintained a significant P value after adjustment. In contrast HLA-B35, B41 and Cw6 were observed with decreased frequencies in patients when compared with controls.

Table1:Antigens frequency of the HLA class I (A,B and C)and class II(DR and DQ)of the U C patients and healthy controls

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	Ulacerative colitis (n=35)		Healthy controls (n=70)		OR	Inverse OR	Chi	P	Adjusted P	EF	PF
	N	%	N	%							
HLA-A locus											
1	10	28.6	16	22.9	1.4	**	0.41	0.52*[NS]*	**	0.074	**
2	9	25.7	25	35.7	0.6	1.6	1.06	0.3*[NS]*	**	**	0.135
3	4	11.4	14	20.0	0.5	1.9	1.18	0.28*[NS]*	**	**	0.097

9	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
10	4	11.4	1	1.4	8.9	**	3.69	0.05[NS]	**	0.101	**
11	7	20.0	8	11.4	1.9	**	1.37	0.24[NS]	**	0.097	**
23	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
24	12	34.3	13	18.6	2.3	**	3.09	0.08[NS]	**	0.193	**
25	2	5.7	1	1.4	4.2	**	1.33	0.25[NS]	**	0.043	**
26	2	5.7	7	10.0	0.5	1.8	0.53	0.47[NS]	**	**	0.045
28	5	14.3	9	12.9	1.1	**	0.04	0.84[NS]	**	0.016	**
29	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
30	3	8.6	12	17.1	0.5	2.2	1.35	0.25[NS]	**	**	0.094
31	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
32	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
33	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
34	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
36	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	7		15								
HLA-B locus											
5	1	2.9	3	4.3	0.7	1.5	0.13	0.72[NS]	**	**	0.015
7	3	8.6	7	10.0	0.8	1.2	0.06	0.81[NS]	**	**	0.016
8	2	5.7	7	10.0	0.5	1.8	0.53	0.47[NS]	**	**	0.045
13	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
14	9	25.7	4	5.7	5.7	**	7.32	0.007	0.22[NS]	0.212	**
15	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
17	2	5.7	4	5.7	1.0	**	0.00	1[NS]	**	**	**
18	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
21	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
22	1	2.9	0	0.0	6.1	**	2.13	0.14[NS]	**	0.024	**
27	12	34.3	3	4.3	11.7	**	12.69	0.000	0.012	0.313	**
35	0	0.0	12	17.1	0.1	15.2	6.59	0.010	0.33[NS]	**	**
37	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
38	3	8.6	5	7.1	1.2	**	0.07	0.8[NS]	**	0.015	**
39	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
40	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
41	0	0.0	9	12.9	0.1	11.0	5.01	0.025	0.8[NS]	**	**
44	5	14.3	6	8.6	1.8	**	0.80	0.37[NS]	**	0.063	**
45	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
47	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
49	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
50	2	5.7	8	11.4	0.5	2.1	0.85	0.36[NS]	**	**	0.061
51	3	8.6	14	20.0	0.4	2.7	2.12	0.15[NS]	**	**	0.125
52	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
53	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
56	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
57	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
62	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
63	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**

70	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	22		23								
HLA-Cw locus											
1	4	11.4	2	2.9	4.4	**	2.74	0.1[NS]	**	0.088	**
2	3	8.6	5	7.1	1.2	**	0.07	0.8[NS]	**	0.015	**
3	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
4	3	8.6	16	22.9	0.3	3.2	2.97	0.08[NS]	**	**	0.156
5	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
6	0	0.0	9	12.9	0.1	11.0	5.01	0.025	0.2[NS]	**	**
7	4	11.4	13	18.6	0.6	1.8	0.86	0.35[NS]	**	**	0.081
8	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	55		88								
HLA-DR locus											
1	1	2.9	8	11.4	0.2	4.4	1.87	0.17[NS]	**	**	0.088
2	12	34.3	17	24.3	1.6	**	1.16	0.28[NS]	**	0.132	**
3	8	22.9	16	22.9	1.0	**	0.00	1[NS]	**	**	**
4	10	28.6	16	22.9	1.4	**	0.41	0.52[NS]	**	0.074	**
5	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
6	0	0.0	4	5.7	0.2	4.8	1.98	0.16[NS]	**	**	**
7	10	28.6	15	21.4	1.5	**	0.65	0.42[NS]	**	0.091	**
8	1	2.9	8	11.4	0.2	4.4	1.87	0.17[NS]	**	**	0.088
9	0	0.0	6	8.6	0.1	7.2	3.26	0.07[NS]	**	**	**
10	5	14.3	8	11.4	1.3	**	0.17	0.68[NS]	**	0.032	**
11	0	0.0	6	8.6	0.1	7.2	3.26	0.07[NS]	**	**	**
12	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
13	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
14	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
15	2	5.7	11	15.7	0.3	3.1	1.98	0.16[NS]	**	**	0.106
52	6	17.1	3	4.3	4.6	**	4.26	0.039	0.66[NS]	0.134	**
53	4	11.4	3	4.3	2.9	**	1.78	0.18[NS]	**	0.075	**
Blank	6		6								
HLA-Dq locus											
1	10	28.6	26	37.1	0.7	1.5	0.76	0.38[NS]	**	**	0.120
2	10	28.6	18	25.7	1.2	**	0.10	0.76[NS]	**	0.038	**
3	7	20.0	25	35.7	0.5	2.2	2.65	0.1[NS]	**	**	0.196
4	8	22.9	16	22.9	1.0	**	0.00	1[NS]	**	**	**
Blank	35	100.0	55	78.6							

Discussion

Susceptibility to IBD is partially genetically determined and the HLA genes are candidates for a role in genetic susceptibility to IBD, because their products play a central role in immune response (12).

In the present study, there was a positive significant association of

HLA- B 14, B 2 7 and DR52 in patients with UC as compared with healthy group, however; only B27 maintained a significant P value after adjustment. This result is in agreement with other studies [5, 13, 14], while at variance with the result of Perri and colleagues [15], who demonstrated that patients with UC had a significantly increased frequency of DQ6 and D Q 7 and a decreased frequency of DQ5 and DQ8 when compared with ethnically matched healthy controls, as well as no significant differences in HLA class 1 and HLA-DR antigens was observed. Also present study showed decreased frequencies of HLA-B35, B41 and Cw6 in patients when compared with controls, so, might confer protective effects against ulcerative colitis.

The discrepancies emerged in this study compared with other studies could be, in part, probably due to the highly modified techniques they used for the same purposes, e.g, PCR, however, if the environmental and genetic factors excluded, which yet to be elucidated, the small number of our study groups may be a considerable contributor to these discrepancies.

HLA-B27 showed a significant high frequency in patient group as compared with healthy control. The appearance of HLA-B27 antigen among UC patient who suffering from extraintestinal manifestation (arthralgia), can support involvement of autoimmunologic mechanism in joint inflammation, that B27 antigen was found to be correlated with arthralgia in the UC patients [13]. This result which mimicked what reported by many authors; Tiwana and colleagues [16] referred to a significant HLA associations identified with specific extraintestinal manifestations of IBD, with the best characterized one was the association of Ankylosing Spondylitis (AS) with HLA-B27. Ankylosing spondylitis has been observed to be associated with UC, an association, which may contribute, to differences in endogenous peptide presentation by HLA-B27 subtypes and be relevant in the disease pathogenesis. HLA associations here possibly involve arthritic peptides and molecular mimicry; molecular analysis has shown that Klebsiella microbes possess antigens, which cross-react with self-antigens, such as HLA-B27 and spinal collagens [17]. It is thought that perhaps AS starts when the defenses of the intestine start breaking down and bacteria from the intestine pass into the blood stream directly into the region where the sacroiliac joints are located.

In conclusion, these findings demonstrated that HLA-B14, B27 and DR52 might play a role in UC susceptibility, suggesting HLA-based different etiopathogenesis, in addition some of these molecules might play a role in development of some extraintestinal manifestations observed in UC patients. On the other hand HLA-B35, B41 and Cw6 might confer protective effects against UC.

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Maternal placental vasculopathy and infection in patients with preterm delivery

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Abstract

Background: Premature delivery remains the most important cause of neonatal mortality and there is considerable amount of information in the literature indicating a strong association between maternal placental vasculopathy and chorioamnionitis with preterm delivery.

Objective:.To outline the association between maternal placental vasculopathy and chorioamnionitis with preterm labor and premature rupture of membranes.

Patients & Methods: We performed a case control study conducted on 54 patients who were delivered preterm 37 patients because of preterm labor and 17 because of premature rupture of membranes, and 54 patients who were delivered at term after uncomplicated pregnancy for the period from January 2004 to July 2005. We studied the clinical information's obtained include demographic data, gestational age, obstetric history, route of delivery, infants birth weight and placental histopathological features. All the patients were seen at Gynecology &Obstetrics department at al kadhimyah teaching hospital in Baghdad.

Results: Maternal placental vascular lesions were present in 13 (35.1%) patients with preterm labor, and six (35.3%) patients, with premature rupture of membranes while only 6 (11.1) of control patients. Histopathological features suggestive of Infection of the placenta were found in 14 (37.8%) patients with preterm delivery and 6 (35.3%) patients with premature rupture of membranes and eight (14.8%) of control patients. ***Conclusion:*** It is possible to identify two subgroups of patients among those who are delivered preterm because of preterm labor or premature rupture of membrane, one with infection of the product of conception and another with maternal placental vasculopathy.

Keywords: premature rupture of membranes: placental disorders, decidaul vasculopathy, infection, preterm labor, premature membranes.

IRAQI J MED SCI ,2008;VOL.6(1):114-120

Introduction

Preterm labor is the leading cause of perinatal morbidity and mortality all over the world. It usually results in preterm birth, a complication that affects 8 to 10 percent of births.

Strategies to prevent preterm delivery have focused on early diagnosis of preterm labor and on clinical markers such as cervical change, uterine contractions, bleeding and changes in fetal behavioral states.

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Received: 5th November 2007, Accepted:23th March 2008.

Diagnosing early preterm labor is difficult and has a high false-positive rate. False diagnoses of preterm labor have resulted in unnecessary and potentially hazardous treatment for thousands of women. Improved methods of early diagnosis would be a significant advance in the treatment of women at risk for preterm labor [(1)].

The development and wide spread use of tocolytic agents over the past 2 decades has not appeared to substantially affect the overall incidence of preterm delivery [(2)].

The human placenta is an under examined organ. The clinical indications for placental examination have no gold standards. The histopathological examination and diagnosis of the placenta may

crucial information.

It is possible to highlight treatable maternal conditions and identify placental or fetal conditions that can be recurrent or inherited Preterm delivery therefore remains one of the most poorly understood mechanisms of perinatal morbidity and mortality [3].

Not all patients with preterm labor or premature rupture of membranes have infection; therefore, conditions other than infection should have an important role in producing these problems. The purpose of this research is to study the contribution of placental pathology to the understanding of the structural and functional abnormalities that may precede the preterm delivery.

Patients &Methods

The patient's population was composed of 108 patients with singleton pregnancies. 37 were delivered preterm after spontaneous premature uterine contractions, 17 were delivered preterm after premature rupture of membranes. The gestational age of preterm group was between 24 and 34 weeks gestation, and 54 were delivered at term after uncomplicated pregnancies.

From January 2004 to July 2005, we studied the clinical information's obtained include demographic data, gestational age, obstetric history, route of delivery, infants birth weight and placental histopathological features. All patients were seen at gynecology & obstetrics department at Al Kadhimyah teaching hospital, in Baghdad.

Delivered placentas were placed in clean plastic bag, taken to the laboratory where processing and histologic studies performed. The placentas of all deliveries were processed and interpreted by senior consultant pathologist according to standardized protocol [4,5,6, and 7].

Histopathological Examination of Placenta

Each placenta was studied grossly on a clean surface, and then histopatholgical examination of the placenta including a piece from membranes stripped, portion of umbilical cord, and several blocks of the placenta were dissected from maternal side.

Histological chorioamnionitis was characterized by the presence of polymorphonuclear leukocytes with or without associated necrosis in the fetal membranes and subchorionic fibrin plate. The degree of severity was graded subjectively into three grading system based on the number and the extent of infiltration of neutrophils (figure 1).

Maternal placental vasculopathy was diagnosed when segment of spiral arteries attached to maternal surface of the placenta failed to show the presence of adaptive changes and remained as small muscular vessels with well defined wall, containing recent or old organized thrombi, (figure 2), additional histopathological criteria were the presence of uneven accelerated abnormally small fibrotic chorionic villi with abnormally thin syncytiotrophoblastic knots, and multiple placental infarcts (figure 3).

Statistical analysis

The significance of differences observed between control group and preterm labor and premature rupture of membranes groups were determined with student t test.

Results

The descriptive variables for patients in the three groups (preterm labor, premature rupture of membranes, control) are shown in table 1.As expected there were significant differences between control group and study groups (premature labor and premature rupture of membranes) with respect to gestational age at time of delivery and birth weight. The incidence of primigravid patients in the three groups was not statistically different (Table 1).

Table 2 shows the classification of

patients in to five groups: infection, maternal placental vasculopathy, mixed lesion, abruption placentae, and normal findings. The prevalence of infection and maternal placental vasculopathy was significantly higher in patients with preterm labor than control women who were delivered at term (P<0.05).

Abruptio placentae occurred significantly more frequently in patients with preterm labor than in control(Table 2).

Table 3 shows the classification of patients with premature of membranes in to five different groups according to histopathological examination. The prevalence of infection and maternal placental vasculopathy was significantly higher in patients with premature rupture of membranes than in control women who were delivered at term. (Table 3)

The number of patients with normal findings was significantly larger in the control group than in the preterm labor or premature rupture of membranes (P<0.05).

Of the six patients with premature rupture of membranes classified as having infection four had grade III chorioamnionitis.Sever histologic amnionitis (grade III) was significantly more frequent in patients with premature rupture of membranes and preterm labor than in the control group (Table 4).

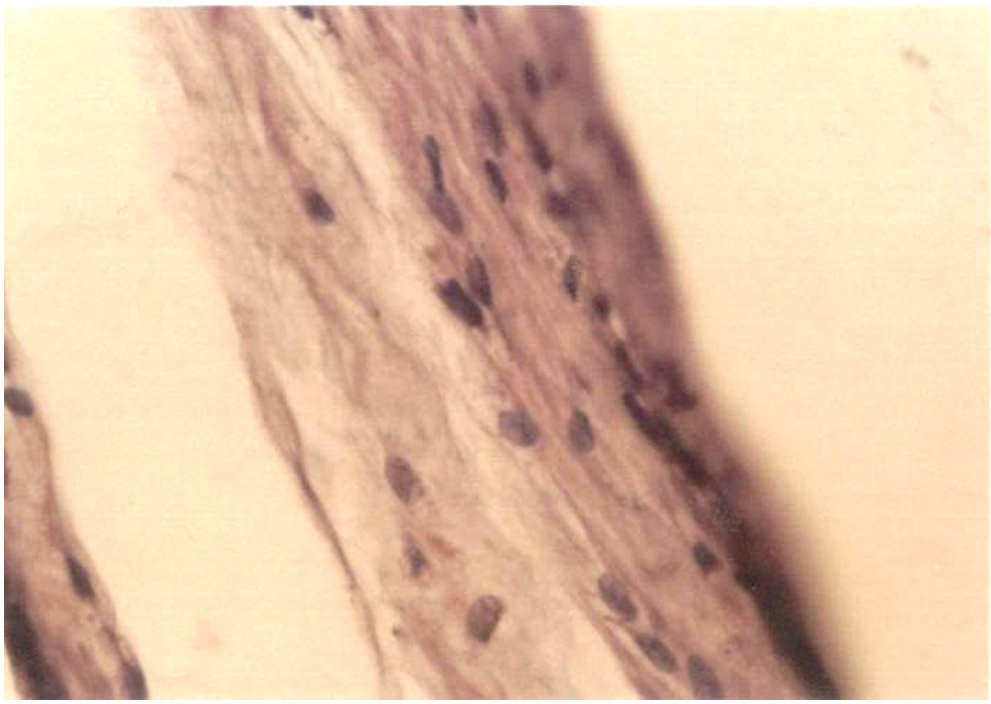

Figure 1: mild degree of chorioamnionitis with acute inflammtory cells appearing as dark blue spots. H&E(X 400).

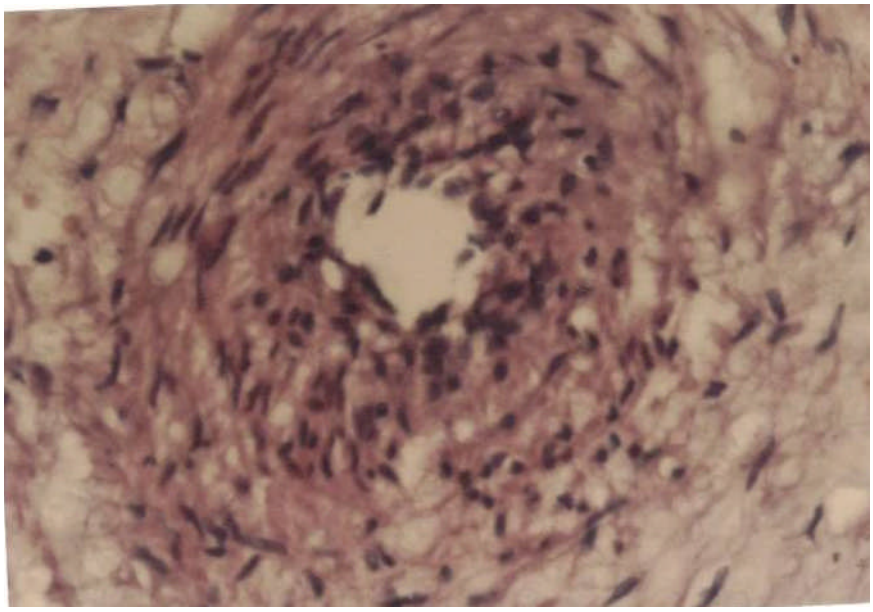

Figure 2: Features of maternal placental vasculopathy. H&E. (X400).

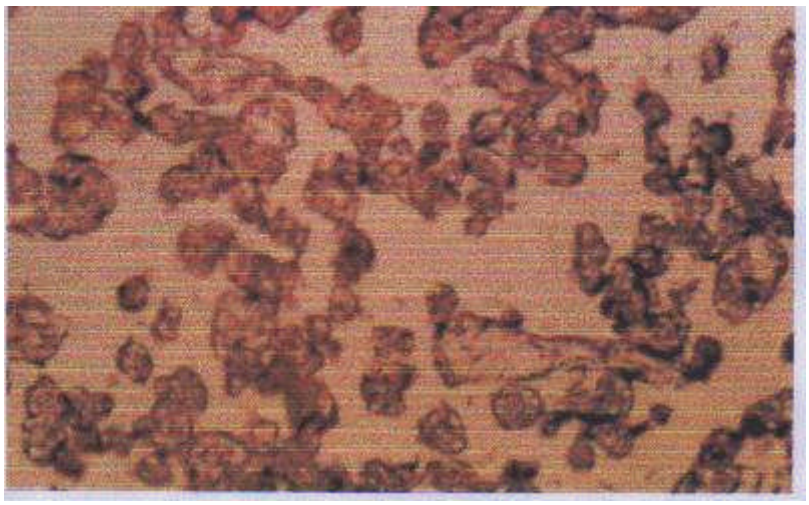

Figure 3: Small and even abnormal fibrotic chorionic villi

Table 1:Characteristics of patients

characteristics	Preterm labor(no.=37)(mean ±SE)	significance	Control(no.54) (mean±SE)	Significance	*PROM(no.=17) (mean±SE)
Maternal Age	24±6	NS	25±5.2	NS	26±5
Gravidity	3±1.4	NS	3.3±1.3	NS	32±1.5
parity	0.6±0.9	NS	0.9±1.1	NS	0.9±1.0
Gestational age(wk)	31.4±3.3	P<0.05	39.0±1.2	P<0.05	31.8±3.0
Cesarean delivery	6(16.2)	NS	8(14.8%)	NS	3(17.6%)
Birth weight	1760±330	P<0.05	3352±450	P<0.05	1795±321

NS= non significance
P= P value
*Premature rupture of membranes

Table 2:Classification of patients with preterm labor according to placental histopathology

Findings	Preterm labor (no. 37)	Control (no.54)	Significance
Infection	14(37.8%)	8(14.8%)	P<0.05
Maternal placental vascular lesion	13(35.1%)	6(11.1%)	P<0.05
Mixed lesion	3(8.1%)	2(3.7%)	NS
Abruptio placentae	3(8.1%)	0(0%)	P<0.05
Negative findings	4(10.8%)	38(70.3%)	P<0.05

NS= non significance
P= P value

Table 3: classification of patients with premature rupture of membranes (PROM) according to placental histopathology.

Findings	PROM (no.17)	Control (no.54)	Significance
Chorioamnionitis	6(35.3%)	8(14.8%)	P<0.05
Maternal placental vascular lesion	6(35.3%)	6(11.1%)	P<0.05
Mixed lesion	1(5.9%)	2(3.7%)	NS
Abruptio placentae	1(5.9%)	0(0%)	NS
Negative findings	2(11.8%)	38(70.3%)	P<0.05

NS= non significance
P= P value

Table 4: severity of histologic amnionitis

Grade	Preterm labor(no.=14)	Significance	Control(no.=8)	Significance	PROM(no.=6)
I	2(14.3%)	P<0.05	6(75%)	P<0.05	1(16.6%)
II	3(21.4%)	NS	2(25%)	NS	1(16.6%)
III	9(64.3%)	P<0.05	0(0%)	P<0.05	4(66.6%)

NS= non significance
P= P value

Discussion

Our data suggest the presence of two well defined subgroups among patients with preterm labor and premature rupture of membranes one of them is characterized by the presence of infection of the product of conception and the other by the presence of maternal placental vascular abnormalities consisting of lack of adaptive changes in the decidual portion of the spiral arterioles and the presence of uneven accelerated maturation of the villi, multiple syncitial knots and placental infarcts.

Placental inflammation is a common finding in preterm gestations. It is most often not associated with clinical evidence of infection, so the diagnosis is usually made at the time of histologic placental examination. Silent chorioamnionitis is a significant cause of "uncomplicated" preterm labor refractory to conventional methods of tocolysis [8]

The finding of an association between chorioamnionitis and premature labor and PROM is not surprising and has been clearly and widely studied by many authors [5,9,10,11,12,13,14]. Our study showed that histopathological evidence of infection was found in 37.8 % of cases of preterm labor and 35.3% of patients with premature rupture of membranes, which is similar to that found by Fernando Arias [15].

This association is found so frequently that the possibility of a cause effect relationship between infection and preterm labor and PROM is widely accepted among experts in this field. [14, 16, 17, 18] ,which proved in our study.

Less popular is the idea of an association between maternal placental vasculopathy and preterm labor and premature rupture of membranes. The changes in maternal vascular compartment of the placenta in patients with preterm labor and premature rupture of membranes are similar to those found in patients with preeclampsia, in those with fetal growth retardation and in some patients with repetitive second- trimester fetal death. [19].

Maternal placental vasculopathy was found in 35.1% of patients with preterm labor and 35.3% of patients with premature rupture of membranes, which is similar to that found by fernando arias [15].

Ultimately a logarithms that combine sociodemographic factors, clinical and ultrasonographic findings, biochemical markers and a reasonable understanding of the pathophysiology of the mechanisms of preterm labor will be required if any progress is to be made in improving the rate of preterm delivery and consequent perinatal morbidity and mortality.

Until the pathophysiology of prematurity is, better understood effective methods of prevention or appropriate intervention will continue to elude clincians.In cases of vascular

complications, diagnostic and therapeutic research should be directed at subclinical manifestations of the underlying pathologic mechanism to prevent reaching a critical threshold at which labor is initiated. Care must continue to be taken however not to prolong pregnancies in which the uterine environment is no longer able to support the appropriate growth and/or survival of the fetus.

It was estimated that at least of 1/4th of preterm deliveries in the present study and others were associated with chorioamnionitis and maternal placental vascular changes, occurring either alone or with PROM, I recommended for further studies in that field including studying different lines of microbiological, immunological and further pathological studies.

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Detection of IL-8, IL-10 and IFN- γ mRNA in trophoblast tissues of recurrent spontaneous abortion using in situ hyperdization

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Abstract

Background: Th1-type cytokines secretion such as IFN-γ, and Th2 cytokines such as IL-10, have been shown to exert deleterious effects on pregnancy, inhibiting fetal growth and development .

Aim: Measurement of the locally concentrations of selected Th1 and Th2 cytokines in women with a history of recurrent spontaneous abortion (RSA) at the time of abortion using *in situ* hyperdization technique.

Methods: A total of one hundred and nineteen women, ranged from the mean age (23.9 − 28.5)years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5 + 0.68);Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4 ± 0.85)and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9 ± 0.88). From each patient and control, placental tissues were collected. Trophoblasts tissues (an image for the local microenvironment) were screened to determine their in situ levels of IL-10 and IFN-γ based on cDNA probes (for in-situ hybridization, ISH).

Results: There was a significant increase in the level of IL-10 within trophoblast tissues biopsies exclusively from women with successful pregnancies (group C) ($p < 0.001$). On the other hand, IFN-γ was found predominantly expressed in trophoblast tissue biopsies of patients with RSA whether IHC or ISH were conducted ($p< 0.05$). Accordingly, only trophoblast tissues biopsies from patients with RSA revealed a significant increase in the ratio of IFN-γ/IL-10 levels expressed as determined by in situ hybridization in comparison to the same ration calculated from trophoblasts tissues of women with successful pregnancies (group C) ($p< 0.001$). as marker for Th2 immune response, during successful pregnancies. Furthermore, the current study failed to demonstrate a significant difference in the tissue levels of IL-8 between RSA and control group ($p> 0.05$) and no significant different between non-RSA and control ($p>0.05$) , (always $p < 0.05$).

Conclusion: These outcomes may further support the possible exisance of an immune response that orchestrates abortive phenomena and the possible protective role of IL-10.

***Keywords*:** Recurrent spontaneous abortion (RSA), in-situ hybridization, ISH, cytokine,IL-8,IL-10,IFN γ.

IRAQI J MED SCI, 2008;VOL.6(1):121-127

Introduction

Recurrent spontaneous abortion is one of the important complications in pregnancy, its incidence is 0.5–1%, and the etiology of RSA is varied, and includes maternal or paternal chromosomal aberrations, uterine anatomic abnormalities, endocrine disorders, infections, and reproductive autoimmune defects.

However, the etiology is undetermined in 40–60% of women with recurrent abortion [1, 2].

Successful human pregnancy appears to be an immunological paradox, in that the fetus represents a semi-allograft developing in the potentially hostile environment of the maternal immune system [3, 4]. One important mechanism involves the down-regulation of the cellular immune response, which has been shown to be dependent upon the suppression of T-helper (Th)1 and T-cytotoxic (Tc)1cells,which produce interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, and the up- regulation of Th2 and Tc2 cells,

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Received: 8th January 2008, Accepted 7th April 2008.

which produce IL-4, IL-6, IL-10 and IL-13 [5-8].

Previous investigations of Th1/Th2 immune responses during pregnancy were able to show that a distinct shift towards Th2-type reactions occurs, especially at the feto-maternal interface [9-12].

On the other hand, Th1-type cytokine secretion such as IFN-γ and Thl/Th2 ratio has been shown to exert deleterious effects on pregnancy, inhibiting fetal growth and development [13].

In addition, IL-8 may be indirectly stimulated via endotoxin-induced inflammatory cytokine, such as IFN-γ, TNF-α and IL-1-α, these cytokines are known to up regulate IL-8 expression in hemopoietic cells [14]. IL-8 displays both inflamm-atory and growth –regulating properties [15], but is notable for its selective chemotaxis , degranulation, and activation of neutrophils [16]. During pregnancy IL-8 induced activation of neutrophils, collagenase and elastase activity in intrauterine environment has been imp-licated in mechanisms of rupture of fetal membranes [17] and cervical ripening [18, 19].

Subjects, materials and methods

One hundred and nineteen women attending the Obstetrics and Gynecology department of Al-Kadhimyia Teaching Hospital in Baghdad between December 2004 and August 2005 were the subject of this study. Included recurrent spontaneous abortion (RSA); non-RSA(first and second abortion) and successful pregnancy (full term) as a control groups.

The gestational age was calculated for each patient from data of the last menstrual period.

These one hundred and nineteen women were grouped into three groups:

Group A: the study group included 62 pregnant ladies all of whom gave a history of previous 3-6 consecutive abortions. History was taken from the patients taking into consideration their hospital records in addition to their previous medical reports (all of them had no family history of genetic disease).

Group B: included 34 pregnant ladies with incomplete abortion for the first time or second time.

Group C: included 23 pregnant ladies had at least two previous normal pregnancies taken as comparison group. All this was done under the supervision of a senior gynecologist.

Trophoblastic tissue was collected from the evacuation of retained pieces during the procedure of curettage and placed in 10% formaldehyde. Two to three paraffin embedded blocks were prepared for each patient. Staining with haematoxyline and eosin was carried out to decide which block can be used in the study (only sections that contained trophoplastic tissue were included in this study). These cases were subjected for in situ hybridization protocols with the different markers included in this study.

In situ hybridization (ISH) using DNA Probe Hybridization/Detection System In situ kit (Maxim Biotech, Inc., USA). For the detection of IL-8, IFN-γ and IL-10, the biotinylated DNA probe hybridize to the target sequence (IL-8 or IFN-γ or IL-10 mRNA sequence), then a streptavidin-AP (streptavidin-alkaline phosphatase) conjugate is applied followed by addition of the substrate bromo-chloro-indolyl-phosphate/nitro-blue tetrazqlium (BCIP/NBT) , which yields an intense blue-black signal appears at the specific site of the hybridized probe. This directly streptavidin-AP conjugate linked to the biotinylated probe provides a rapid and highly sensitive detection method.The procedure included the following steps: Paraffin embedded sections were cut into 5 µm thickness, placed on

Fisherbrand positively charged slides and left overnight to dry at room temperature. In each ISH run negative control slides were included which were obtained by omitting the probe and using hybridization solution only, this was undertaken under identical test conditions (on the same slide). Another negative control slides were obtained by using RNase pretreatment, which abolished the hybridization signals, this was performed by placing 1-2 drops of RNase A onto the tissue sections and incubating the slides at 37°C for 2hr according to [20,21]. Poor tissue quality or target RNA degradation may give false negative results or poor signal. This could be verified by using a probe to an abundant RNA target like the probe of a housekeeping gene which is a sequence or gene product that is constitutively expressed in most tissue types such as actin or tubulin. In each ISH run the biotinylated housekeeping gene probe in a dilution of 1:3 by the hybridization solution, was used. reactive lymphocytes within the tissue were considered as internal positive control for IFN-γ detection . Placental tissue obtained from the women who had had elective pregnancy termination, was considered as a positive control tissue for IL-10 detection using ISH. Placental tissue obtained from the women with normal vaginal delivery, was considered as a positive control tissue for IL-8 detection using ISH . The expression of IL-8, IFN-γ and IL-10 mRNA was measured by the same scoring system, by counting the number of positive trophoblastic cells, which gave a blue-black (BCIP/NBT) nuclear staining under the light microscope, counting of positive cells was done with the assistance of histopathologist. The number of villi cells that were positively and negatively stained was expressed as a ratio. The extent of the ISH signal in the villi was determined in 10 fields (XI00 magnification). In each field the total number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotro-phoblast in a given villous was graded as 3, (75-100%); 2, (25-75%); or 1, (<25%). The total staining score was divided by the number of whole villi per field in 10 fields. These scores (between 1 and 3) were added for each field, and a score between 10 and 30 was gained for each sample. The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers, the percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields.

Results

In (Table1), Chi-square test of significant was conducted to examine the association between IL-8 ;IL-10 and IFN-γ mRNA expression in trophoblasts tissue in the three groups of investigated women ,it was found that highly significant association (p<0.001) between them in the three scoring levels. The results showed that percentages of IL-8 and IFN-γ were elevated in 94.3% (33/35) and 82.9%(29/35) ,respectively in women with RSA (group A). It was found that 90% (9/10) of women in control group showed highest level of IL-10 and 62.9%(22/35) of women with RSA have moderated level (score 2).

Table 1: Comparison of the prevalence of IL-8, IL-10 and IFN-γ mRNA (ISH assay) in trophoblasts depend on the scoring level.

	score[a]	Groups			Total (n=61)	Chi-Square P value
		A(n=35) No (%)	B(n=16) No (%)	C(n=10) No (%)		
IL-8	**2**	2 (5.7)	16 (100)	5 (50)	**23**	0.000**
	3	33 (94.3)	0	5 (50)	**38**	
IL-10	**1**	13 (37.1)	0	0	**13**	0.000**
	2	22 (62.9)	16 (100)	1 (10)	**39**	
	3	0	0	9 (90)	**9**	
IFN-γ	**1**	0	0	5 (50)	**5**	0.000**
	2	6 (17.1)	16 (100)	5 (50)	**27**	
	3	29 (82.9)	0	0	**29**	

Score[a]: 1<25%; 2(25-74) %; 3(75-100) %

ANOVA test analysis in table (2) showed that the expression of IL-10 by trophoblasts tissue ,was significantly higher in successful pregnancy (group C) than in recurrent spontaneous abortion (group A) (p<0.001) the mean ± SE (88.5±2.5), mean versus (25.5±0.9); whereas (group A) showed a high value with a highly significant difference (p>0.001)of IFN-γ in comparison with control (group C), the mean ± SE (84.3±1.7; 25.7±1.9 ,respectively). In women with RSA group it was found that IL-8 was highly significant different from that in non-RSA groups (85.2±1.3 versus 63.0±2.1) and there was no significant difference (p>0.05) between RSA group's patients and controls group (67.3±7.5).

Table 2: **Comparison between the mean percent of the expression of IL-8, IL-10 and IFN-γ (ISH assay) in the trophoblasts of studied groups.**

Variable	Group	n=61	Mean± SE	F test *p* value	Sig. between groups	
					groups	*P* value
IL-8	**A**	35	85.2 ± 1.3	<0.01	**A –B**	0.000**
	B	16	63.0 ±2.1		**A –C**	0.122
	C	10	67.3 ±7.5		**B – C**	٠.٩٣٣
IL-10	**A**	35	25.5 ±0.9	<0.01	**A –B**	0.000**
	B	16	51.9 ±2.1		**A –C**	0.000**
	C	10	88.5 ±2.5		**B – C**	0.000**
IFN-γ	**A**	35	84.3 ±1.7	<0.01	**A –B**	0.000**
	B	16	52.1±2.03		**A –C**	0.000**
	C	10	±1.9٢٥.٧		**B – C**	0.000**

*=significant difference(p<0.05) ; **= highly significant difference(p<0.01)

Discussion

In the current study, we evaluated the expression of IL-10, IFN-γ and IL-8 in human placental tissues (trophoblastic tissue), from all three groups: RSA, non-RSA and Control (successful pregnancy).

Much of the work on spontaneous abortions in humans has focused on the

analyses of maternal responses and local changes that occur following abortion. Evidence from studies on murine and human pregnancy points to a strong association between maternal Th2-type (IL-4 ,IL-6,IL-10) immunity and successful pregnancy on the one hand and between Th1-type (IL-2 and IFN-γ) immune reactivity and pregnancy loss on the other[22].Moreover, during pregnancy, IL-8 is produced by a variety of cells, mainly monocytes/ macrophages [23]. IL-8 induced activation of neutrophils and elastase activity in the intrauterine environment has been implicated in the mechanisms of rupture of fetal membrane and cervical ripening [24].

The pro-inflammatory cytokine, IFN-γ was targeted as a reflective for type 1 immune response in this study, because of its Th1 polarizing effect due to its potential role in generating Th1 cells, mediating their effects functions and regulating Thl/Th2 balance [25]. On the other hand, 1L-10 was targeted in this study as a reflective for Type 2 immune response because it is an important anti-inflammatory cytokine contributing to the outcome of pregnancy due to its important modulatory effects against the pro-inflammatory cytokines [26-28].

The current study demonstrated that 29/35 (82.9%) of the cases in RSA (group A) showed high level of IFN-γ *in situ* expression, with a highly significant difference (p <0.001) from those with non-RSA (group B)in whom the expression of IFN-γ was in moderated level ,16/16(100 %) of cases and from those with successful pregnancy (group C) the expression of IFN-γ was 5/10 (50%) in moderated level and 5/10(50%) in lowest level, no cases found to express high level. A part from the causes of this significant increase in the expression of IFN-γ in women with recurrent abortion, revision was made for the previous studies that examined the association between Thl type cytokines and recurrent abortion. First studies in Hill's laboratory [29] had shown that peripheral blood mononuclear cells (PBMC) of women with a history of RSA when stimulated with a trophoblast antigen extract produced significantly higher concentrations of the Thl cytokines, IFN-γ and TNF-α , as compared with normal pregnancy[30,31].This results in agreement with the similar local study that showed, highly significantly increased (p<0.001) expression of IFNγ in women with RSA compared with first abortion or elective pregnancy termination [32] and when compared with successful pregnancy[33,34] because of that IFN-γ, have been hypothesized to play a role in pathogenesis of recurrent abortion.

Highly significantly increase (p<0.001) in situ expression of IL-10 was found in women with successful pregnancy (group C). It was found that 9/10 (90%) of women in groups C in highest level of IL-10 compared with RSA (group A) which was no cases found in high level but 22/35(62.9%) was in moderate level and was found 16/16(100%) of women with non – RSA was in moderate level. This result is consistence with similar local study by that showed, highly significantly increased (p<0.001) expression of IL-10 in women with first abortion or elective pregnancy termination compared with RSA [32]; This significantly lower IL-10 expression could be attributed to defect in Th2 and Tc2 cells at the feto-maternal interface or to the accumulation failure of Th2 cells at the implantation site in women with RSA [35]. Several lines of evidence suggest that IL-10 may play a major role in influencing the activity of the placental trophoblast, which has been proposed as a key cell type in regulating the fetal immunoprotection [36-38].

Since it is directly involved in down-regulating Th1-type activity by inhibiting IFN-γ production, IL-10 has been proposed to play an important immunoregulatory role in pregnancy by maintaining a bias away from the detrimental Th1-type of reactivity [39, 28].

There are many confounding studies held the notion on the balance of Thl and Th2 cells at the circulation and implantation site, expressing them as a ratio of Thl/Th2 cytokines, so that, another dimension was added to the results of this study when it examined the ratio of IFN-γ/IL-10 in situ expression in women with RSA which was significantly higher (p<0.001) in trophoblasts tissue, and about (19.1 times) than that of successful pregnancy (group C). This significantly high IFN- γ/IL-10 ratio lends further support to the findings in this study as it was in consistence with the previous studies (41-34).

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Interferon Alpha -2b in plantar fasciitis

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Abstract

Background: Plantar fasciitis is the most common cause of heel pain but treatment remains empirical.

Objective: To investigate the effect of interferon alpha 2b on pain, morning stiffness and tenderness of heels in patient with plantar fasciitis.

Methods: Three hundred seventy six patients with plantar fasciitis enrolled in this study. The patients divided into two groups: Group one received interferon alpha 2b 3MIU\evry 3days and group two treated with diclofenac. 50 mg two times daily with heel pads. The study conducted for 12 week.

Results: 92% of group one patients who received interferon alpha 2b became completely asymptomatic at week 6. At week 12 96.8% became completely free of symptoms.

Conclusion: Treatment with interferon alpha 2b in patients who had plantar fasciitis seems to be effective bringing complete cure

Key words: interferon alpha 2b, planter fasciitis

IRAQI J MED SCI ,2008;VOL.6(1):128-131

Introduction

Plantar fasciitis is the most common cause of heel pain .It is the consequence of biochemical faults that cause tension of the intrinsic muscles and of the plantar fascia at its insertion to the calcaneus. Classically the patient complains of heel pain on first arising and after a period of rest symptoms diminish with walking [1,2].

Pain and tenderness are maximal at the point of insertion of fascia into the medial tubercle just anterior to the weight- bearing area of the heel or extend distally along the fascia as it courses to the toes; the foot usually appears to be normal[3]. Over time and with repetitive stress micro tears can occur in the origin of the plantar fascia generating inflammatory response consisting of collagen necrosis hyperplasia and matrix calcification. Most patients have calcaneal spurs but some do not. Plantar fasciitis refers specifically to the clinical syndrome of pain, inflammation and fibrosis of the plantar fascia and its calcaneal insertion. Stress along the plantar fascia is increased with obesity, over use and inappropriate footwear[3,4].

Diagnosis of plantar fasciitis is based on history, examination and imaging technique. Pain and morning stiffness, involving the heel and plantar surface of the foot. Examination shows local tenderness over the anteromedial portion of the plantar surface of the calcaneous with worsening of pain on passive dorsiflexion of the toes[5].

Radiography is of little value in the diagnosis but is important to rule out other disorders[6].

The presence of infracalcaneal heel spurs on radiographs correlates poorly with symptoms. The plantar fascia can be seen on a satisfactory conventional lateral radiograph[7].

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Received 20th May 2007: Accepted 7th January 2007

MRI is of great value in defining soft tissue abnormalities showing thickening of plantar fascia or calcaneouse [8, 9].

Histopathology of plantar fsciitis is characterized by collagen degeneration angiofibroblastic hyperplasia chondroid metaplasia and calcification of degenerated matrix. Therapy consists of resting, unloading and stabilizing foot. Nonsteroidal anti-inflammatory drugs diminish the pain and stiffness but are not curative. Splinting, casting and orthotics can be considered[10].

Heel pad or cushion application of local heat and local injection of corticosteroids at the insertion of the plantar fascia may be used. Surgical approaches such as fasciotomy and spur excision are infrequently used[11,12].

Methods

This study was conducted in Alkadymia Teaching Hospital in Baghdad during the period from 18th September 2006 to17th November 2006, 376 patients enrolled in this study .352 women (93.6%) and 24 men (6.4%) Their age rang {21-74} years mean 43 years. Disease duration was (6+_2months) with heel pain diagnosed according to the history, examination and radiographic findings.

Subjects who enrolled in the study underwent routine biochemical blood analysis in order to exclude other diseases. All diabetic patients had been excluded from the study. X-ray of the feet were obtained in all patients

Evaluation of subjects includes physical examination, which has particular focus on the pattern of feet involvement. Patients were divided into two groups: Group one composed of 188 patients treated with interferon alpha 2b 3MIU {3milion international units MIU} every 3days and 50mg diclofenac two times daily.

Group two treated with diclofenac 50m twice daily with heel pads. Statistical analysis was done application of student's t test

Patients were included irrespective of past or present treatment {nonsteroidal anti-inflammatory drugs, NSAID's or disease modifying anti-rheumatic drugs DMARD's}.

We examined all heel -x-rays reports that stated either normal or a calcaneal spure. All patients were assessed carefully. At the first visit the patient is asked about the duration of symptoms. The heels are examined for the presence of injury, soft tissue swelling and tenderness.

Inclusion criteria were:

1- Pain and morning stiffness involving the heel and plantar surface of the foot

2- Local tenderness of the plantar surface of the calcaneous

3- Worsening of calcaneal pain with passive dorsiflextion of the toes

4-Other diseases like fractures or diabetes mellitus…. Etc…should be excluded.

Written consent was obtained from each subject prior to treatment.

Clinical examination of the patients was carried out at intervals of 2weeks.

The outcome and satisfaction with therapy i.e. relief of symptoms and signs of the disease was taken as a pointer to the efficacy of therapy.

Results

After a week 12 patients left the study because of the unability to attend the follow up clinic or because of the side effect of interferon {fever, rigor, myalgia}. Following treatment with interferon alpha 2b improvement was seen in 92% {162 patiens} in 2weeks and 4 weeks follow up. However 85% {150 patients} were completely

Asymptomatic at week 6. 16 patients showed improvement in pain severity but continued to have mild pain and stiffness .4.8% {9 patients} who had partial improvement they became

completely free of symptoms at week 12 raising the number to 96.8%. No major adverse effects has been observed due to interferon alpha 2b except certain symptoms developed in 18 patients which are {fever , myalgia and rigor} treated successfully with one gram acetamenophen .In group two {Diclofenace group} they did not show any improvement in their symptoms. The pain and morning stiffness continued and the local tenderness of the heel was the same.

Discussion

The severity of symptoms before and after therapy is very important. There was a substantial decline in severity in group one {interferon group} in comparison with group two who received diclofenac only. The results observed in the present study demonstrate that complete disappearance of symptoms and signs of plantar fasciitis can be achieved with interferon alpha 2b. It is believed that direct anti-proliferative action, inhibition of virus replication and modulation of the host immune response play important role s in its function(13).

Interferon alpha 2b has been shown to posses many of the activities of natural human alpha- interferon preparations(14).

The inteferons are family of naturally occurring small proteins and glcyoproteins produced and secreted by cells in response to biological inducers. They exert their cellular activities by binding to specific membrane receptors on the cell surface(15).

Once bound to the cell membrane interferons initiate a complex sequence of intracellular events these include the induction of modulating activities such as enhancement of the phagocytic activity of macrophages and augmentation of the specific cytotoxisty of lymphocytes for target cells .Any of these activities might contribute to interferon alpha 2btheraputic effects(16,17).

Interferon alpha 2b is very effective drug in the treatment of plantar fasciitis.

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المجلة العراقية للعلوم الطبية
قائمة المحتويات

المقالات

المجلد السادس، العدد الأول، ١٤٢٩ هـ، ٢٠٠٨م

المجلة العراقية للعلوم الطبية

تعنون المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد ١٤٢٢٢ بغداد، العراق. تلفون و فاكس (٥٢٢٤٣٦٨-١-٩٦٤).

رقم الإيداع في دار الكتب و الوثائق ببغداد ٧٠٩ لسنة ٢٠٠٠

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IRAQI JOURNAL OF MEDICAL SCIENCES

A MEDICAL JOURNAL ENCOMPASSING ALL MEDICAL SPECIALIZATIONS

ISSUED QUARTERLY

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احمد عبد الحميد[١] ،مهند محسن[٢]، أسامه الناصري[٣]

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[١]مستشفى السليمانية التعليمي [جامعة السليمانية]
[٢]فرع الأحياء المجهرية [كلية العلوم-جامعة كربلاء]
[٣]فرع الجراحة البولية [كلية الطب –جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٣-١٤

تأثيرات نبات الكركم والحبة السوداء على التهاب القولون المحدث في الارانب

أزهر عبد الحافظ الجمعة[١] ، أديب أحمد الزبيدي[٢] ،علاء غني[٣]

الخلاصة

خلفية الدراسة: إن فشل إستراتيجية العلاج الحالية في السيطرة على الكثير من حالات داء الأمعاء الالتهابي كان دافعاً قوياً للبحث عن نماذج علاجية جديدة.

هدف الدراسة: لدراسة تأثيرات كل من نبات الكركم والحبة السوداء على التهاب القولون المحدث في الأرانب.

طريقة العمل: تم إحداث التهاب القولون في عدد من الأرانب بواسطة إعطاؤها عن طريق المخرج مزيجا من حامض ألخليك - كحول اثيلي (نموذج ١)، أو حامض ألخليك (نموذج٢).

تم مقارنة تأثيرات كل من نبات الكركم والحبة السوداء بتأثيرات كل من الماء المقطر (مجموعة التحكم)، والبردنيزولون من خلال التغير في وزن الحيوان ، و وزن قطعة القولون، و درجة الفحص النسيجي العيني وألمجهري.

النتائــج: في نموذج (1) ، لوحظ ضرراً شديداً في القولون ثبت عينيا ومجهريا. لم تكن درجات الفحص النسيجي العيني و المجهري لمجموعة الكركمين تختلف اختلافا معتداً به عن مجموعة التحكم ومجموعة البردنيزولون.

أما في نموذج (2) فقد حدث التهاباً في القولون اقل وخامةً، و لكن بالرغم من ذلك لوحظت تأثيرات واضحة بالفحص النسيجي العيني والمجهري للقولون.

قلل كلاً من البردنيزولون والحبة السوداء من فقدان وزن الحيوان بالمقارنة مع مجموعة التحكم.

إن الأضرار العينية والمجهرية قلت بشكل ملحوظ بعد استخدام كل من البردنيزولون والحبة السوداء والكركمين. إلا إن هذه الانخفاضات كانت معتداً بها إحصائيا بالنسبة للبردنيزولون وزيت الحبة السوداء بالنسبة للفحص النسيجي العيني، وللبردنيزولون بالنسبة للفحص النسيجي المجهري.

إن تأثيرات كل من الحبة السوداء و الكركمين على وزن قطعة القولون ومعدلات درجات الفحص النسيجي العيني والمجهري كانت مقاربة لتأثيرات البردنيزولون.

الاستنتــاج: إن نموذج التهاب القولون المحدث بواسطة حامض ألخليك في الأرانب نموذج (1) هو المفضل للتحري عن التأثير المضاد للالتهاب لعقاقير جديدة.

كان لكل من الحبة السوداء والكركمين تأثيرا مضاداً للالتهاب في هذا النموذج.

مفتاح الكلمات: داء الامعاء الالتهابي ، جذور الاوكسجين الحرة، التهاب القولون المحدث، حامض الخليك، الكركمين، الحبة السوداء .

[١]فرع الفارم كولوجي [كلية الطب – جامعة النهرين]
[٢]فرع الفارم كولوجي[كلية الصيدلة – جامعة كربلاء]
[٣]فرع الباثولوجي[كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص١٥-٢٧

دراسة حول الفيروس ألحليمي البشري باستعمال تقانة التهجين الموضعي و علاقته بسرطان عنق الرحم

لقاء رياض الخزاعي[١] ، إسماعيل إبراهيم لطيف[٢] ،أروى مجاهد الشويخ[٢]

الخلاصة

خلفية الدراسة: أظهرت الدراسات السريرية و الإحصائية أن الفايروس ألحليمي البشري يلعب دورا كبيرا في تطور مختلف أنواع إصابات عنق الرحم لذلك اعتبر عامل ملوث أساسي مسبب للإصابات السرطانية في عنق الرحم.

هدف الدراسة: لتحديد تفشي الفايروس ألحليمي البشريDNA باستخدام تقانة التهجين الموضعي في نماذج الأنسجة الأرشيفية المطمورة بشمع البارافين المأخوذة من إصابات عنق الرحم و مقارنتها بخزعات ما بعد الوفاة المأخوذة من أعناق رحم طبيعية.

طرق العمل: تضمن البحث ثمانين عينة و كان سبعون منها مأخوذة من الأنسجة الأرشيفية المطمورة في شمع البارافين و التي تمثل مجموعة الخطر المحتوية على دلائل تشير إلى وجود إصابة بالفايروس الحليمي البشري مع أو وجود حالات سوء نمو ظهارية (السرطانية أو غير السرطانية) و قد اختيرت العينات من ملفات مختبرات التحليلات النسيجية للمدة بين ١٩٩٨ و لغاية ٢٠٠٥ من مستشفى الكاظمية التعليمي، مستشفى العلوية،مستشفى اليرموك التعليمي، المختبرات التعليمية في مستشفى مدينة الطب و بعض المختبرات الخاصة.كان متوسط عمر المرضى ٤٣.١ سنة ضمن مدى يتراوح بين ٢٠-٨٥ سنة.أما العشر عينات المتبقية تمثل خزع بعد الوفاة من عنق أرحام نسوة أبكار كانت أعمارهم تتراوح بين ١٨-٣٠ سنة و بمعدل عمر ٢٣.١ كمجموعة سيطرة اثبت الفحص النسيجي عدم وجود عوارض أو إصابات فيها.لقد تم التحري بواسطة تقانة التهجين الموضعي عن DNA هذا الفايروس في أنسجة عنق الرحم.

النتائج: لقد أظهرت الدراسة انتشار موجبية DNA هذا الفيروس في مجموعة الخطرهي٣٠%،١١.١١%،٢١.٤٣%،٣٣.٣٣% في كل العينات التي تحمل في ثناياها دلائل على الإصابة بالفايروس الحليمي البشري،حالات سوء النمو الظهارية البسيطة ،حالات سوء النمو الظهارية المتوسطة و الشديدة و أخيرا حالات سرطان عنق الرحم المحرشف، على التوالي ، في حين لوحظ عدم وجود DNA الفايروس في مجموعة السيطرة.

الاستنتاج: إن تقانة التهجين الموضعي تجسد القيمة الحقيقية لوجود الفايروس كمسبب للمرض ووجد إن هناك ترابط وثيق بين توزيع علامات التهجين الموضعي والنوع النسيجي لسرطان عنق الرحم.

مفتاح الكلمات: الفايروس الحليمي البشري، سرطان عنق الرحم ،تقانة التهجين الموضعي.

[١]مستشفى الكاظمية التعليمي [كلية الطب _ جامعة النهرين]
[٢] فرع الأحياء المجهرية [كلية طب النهرين – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٢٨-٣٧

التأثير الوظيفي للقات على تكوين الحيامن المنوية البشرية

ناجي عبد الوهاب عبد الله

الخلاصة

خلفية الدراسة: ينمو القات بالأصل في اليمن والحبشة وكينيا ومرتفعات جنوب شرق أفريقيا ومدغشقر حيث إن ملايين من الناس في هذه الأقطار تمضغ أوراق هذه الشجرة الدائمة الخضرة والتي تعتبر من عائلة سيلاستراس٠

تشير الدراسات لسكيشتر وآخرون إلى إن مضغ أوراق القات وبلع العصارة التي تحتوي على المادة الفعالة (كاثينون) يؤدي إلى تنبيه عصبي مركزي محاكي للودي (سمبثاوي) مشابه لتأثيرات الامفيتامين . يؤثر القات على عده أجهزة بالجسم منها ضعف القدرة الجنسية للذكور كالعجز الجنسي ونقص إنتاج الحيامن المنوية ٠

هدف الدراسة: يهدف البحث إلى معرفة تأثير مضغ القات على إنتاج وتكوين الحيامن المنوية البشرية ومدى تأثير المادة الفعالة بالقات على القدرة الجنسية ونقص إنتاج الحيامن المنوية عند الذكور٠

طريقة العمل: تم دراسة خمسون حالة لأشخاص أصحاء متناولين القات تراوحت أعمارهم من ٢٠-٥٠ سنة تم اختيارهم بشكل عشوائي ،أخذت عينة للحيامن المنوية إثناء جاسات مضغ القات لفحصها ودراسة نتائجها مختبريا ، أضافه إلى تعبيه استبيان حول تأثير القات جنسيا على شخص نفسه٠

النتائــج: أظهرت الدراسة إن ٩٤% من متناولين القات يعانون من قله عدد الحيامن المنوية بينما ٦% كان عدد الحيامن المنوية أكثر من المعدل الطبيعي كما تبين أيضا إن أكثر من ٧٢% من متناولين القات لديهم اقل من ٦٠% من الحيامن النشطة المتحركة وحيامن منوية الغير طبيعية و ٢٨% من متناولين القات لديهم أكثر من ٦٠% من الحيامن النشطة المتحركة والحيامن المنوية الغير طبيعية٠

تبين من الدراسة وجود علاقة بين طول الفترة الزمنية لتناول القات ونقص عدد الحيامن المنوية ونسبتها المتحركة النشطة وزيادة الحيامن المنوية الغير طبيعية ٠

أظهرت الدراسة وجود علاقة قوية بين نوعية القات ونقص عدد الحيامن المنوية ونسبتها المتحركة النشطة وزيادة الحيامن المنوية الغير طبيعية ٠

الاستنتاج: إن ظاهرة مضغ القات لها تأثير جانبي على الحيامن المنوية البشرية ، ويكون تأثيرها أكثر كلما تقدم العمر مقارنه بالبالغين ، كما إن تأثير المادة الفعالة الموجود في نوعية القات الجيدة تلعب دور هام في التغيرات التي تطرأ في الحيامن المنوية البشرية٠

مفتاح الكلمات : قات ، كاثا ايدوليس ، كاثينون ، إنتاج الحيامن المنوية ٠

فرع الباراكلينك - وحدة الطب الشرعي [كلية الطب - جامعة عدن – اليمن]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٣٨-٤٤

تأثير أشعة أللیزر على استماتة ألخلایا أللمفیة

رويدة عبد الأمير، إسراء فائق

الخلاصة

خلفية الدراسة: الاستماتة هي نوع فسيولوجي من موت الخلية، تمتاز بتغييرات معينة كيميائية ونووية.يتم بهذه الطريقة التخلص من الخلايا الغير مرغوب فيها بطريقة صحيحة بحيث لا تثير أي تغيرات تسبب الالتهاب.

هدف الدراسة: لبيان تأثير أشعة أللیزر على استماتة الخلايا اللمفية.

طريقة العمل: أجريت هذه الدراسة على الخلايا اللمفيةالمستميتة. يتم تقييم عدد الخلايا اللمفية المستميتة قبل وبعد التعرض لأشعة أللیزر.

النتائج: هذه الدراسة بينت أن هناك زيادة ملحوظة بنسبة الخلايا اللمفية المستميتة بعد تعرضها إلى أشعة أللیزر وكان هناك زيادة أيضاً بهذه النسبة عند زيادة زمن التعرض.

الاستنتاج: ان استماتة الخلايا اللمفية يمكن أن يُحث بواسطة جرعة قليلة من أشعة أللیزر وهذا يمكن أن يزداد بزيادة زمن التعرض لهذه ألأشعة.

مفتاح الكلمات: أشعة أللیزر، استماتة الخلايا اللمفية.

قسم الفسلجة [كلية الطب –جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٤٥-٥١

تأثير عقار التاموكسفين على مستوى الفيتامينات المضادة للتأكسد وحامض اليوريك وأنزيم غاما- جلوتاميل ترانسببتيديز (GGT) في مرضى سرطان الثدي

طارق حفظي [١]، سلوى حميد ناصر الربيعي [٢]، هدى سليم الخالدي [٣]

الخلاصة

خلفية الدراسة: أشارت الكثير من الأبحاث إلى الدور الكبير الذي تلعبه الجذور الحرة وأنواع مختلفة من مركبات الأوكسجين في التسبب وتطور سرطان الثدي لدى النساء بعد انقطاع الطمث. اقترحت الادبيات العلمية المتوفرة كذلك عن دور عقار التاموكسفين في تثبيط عملية اكسدة الدهون في الحيوانات المختبرية .

هدف الدراسة: محاولة فهم الحالة المضادة للتأكسد والجهد ألتأكسدي في حالة السرطان للثدي لدى النساء بعد انقطاع الطمث قبل وبعد العلاج بعقار التاموكسفين .

طريقة العمل: تم في هذه الدراسة علاج ١٩ امرأة بعد انقطاع الطمث ومن المصابات بسرطان الثدي بعقار التاموكسفين (١٠ ملغم مرتين في اليوم). تم تعيين مستويات فيتامينات C,E,A وحامض اليوريك وأنزيم (GGT) في النساء المذكورين. تم مقارنة النتائج المستحصلة لمرضى السرطان مع مرضى الأورام الحميدة (٢١ امرأة) ومجموعة السيطرة (٢٣ امرأة) .

النتائـج: أشارت النتائج إلى حصول انخفاض ملحوظ في مستويات الفيتامينات المضادة للتأكسد في مصول مرضى سرطان الثدي قبل العلاج مقارنة بمرضى الأورام الحميدة ($P<0.001$) كذلك حصول زيادة ملحوظة في مستويات حامض اليوريك وأنزيم (GGT) ومركب مالوندايلديهايد (MDA) في سرطان الثدي مقارنة بمرضى الأورام الحميدة ($P<0.001$). لوحظ حصول زيادة
ملحوظة في مستويات الفيتامينات المذكورة في مصل مرضى سرطان الثدي بعد العلاج بعقار التاموكسفين .

الاستنتاج: تقترح النتائج إن للتاموكسفين تأثيرا ملحوظا على نسبة أكسدة الشحوم كما يؤدي العقار المذكور إلى حصول تحسن واضح في الحالة المضادة للتأكسد في مرضى سرطان الثدي .

مفتاح الكلمات: سرطان الثدي ، التاموكسفين ، الفيتامينات المضادة للتأكسد ، إنزيم GGT.

[١] فرع الكيمياء الحياتية [كلية الطب _ جامعة النهرين]
[٢] فرع الكيمياء [كلية العلوم _ الجامعة المستنصرية]
[٣] فرع الكيمياء الحياتية [كلية طب الكندي – جامعة بغداد]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٥٢-٥٩

التغيرات المظهرية على الخلايا اللمفاوية التائية للولادات المبتسرة التلقائية مجهولة الأسباب

مها البياتي[١] ، نضال عبد المهيمن[٢]، ثريا حسام الدين[٣]

الخلاصة

خلفية الدراسة: وجد في الأبحاث الحديثة الخلايا اللمفاوية التي يعبر على سطحها مستضدات مختلفة لها دور أساسي في إحداث الولادات المبكرة.

هدف الدراسة: هدفت الدراسة الحالية التحري عن بعض التغيرات على سطح الخلايا اللمفاوية متمثلة بمجاميع التمايز CD8,CD4,CD3 التي من الممكن أن يكون لها دور في إحداث الولادات المبتسرة مجهولة الأسباب .

المواد و طريقة العمل: لتحقيق هذا الهدف فقد شملت الدراسة (٣٠) سيدة في حالة ولادة مبتسرة تلقائية مجهولة الأسباب بالإضافة إلى (٣٠) سيدة حامل سليمة لفترات حمل مقاربة لتلك الفترة البالغة للمجموعة الأولى ودون أية بينة لولادة مبكرة كمجموعة سيطرة. تم اخذ عينات دم من كل سيدة حيث فصلت الخلايا اللمفاوية من عينات الدم واجري لها حساب دقيق ثم حضرت سلايدات فلورسينية (وميضية) خاصة لتجمعات التمايز الثالثة والرابعة والثامنة ثم اجري الفحص الوميضي المناعي المباشر لهذه الخلايا.

النتـــائج: تمت مقارنة نسبة كل تجمع تمايزي لمفاوي بين المجموعتين فوجدنا نسبة اقل من كل من التجمع التمايزي الثالث والرابع والثامن في المجموعة الأولى مقارنة بمجموعة السيطرة وكان هذا الفرق ذا دلالة إحصائية كبيرة

الأستنتاجات:أكدت النتائج الأثباط المناعي الخلوي لدى السيدات اللاتي لديهن ولادات مبتسرة .

مفتاح الكلمات: الولادة المبكرة ، الخلايا اللمفاوية ، $CD8^+,CD4^+,CD3^+$

[١] فرع النسائية و التوليد [كلية طب النهرين – جامعة النهرين]
[٢] فرع الأحياء المجهرية [كلية طب النهرين – جامعة النهرين]
[٣] فرع النسائية و التوليد [مستشفى الكاظمية التعليمي]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٦٠-٦٤

التوزيع الجينى لمستضدات التطابق النسيجي- الصنف الأول والصنف الثاني في مرضى السكري من النوع الأول وإخوانهم

إيمان مهدي صالح[١] ، نضال عبد المهيمن[٢] ،ماجد حسين الجيلاوي[٣]

الخلاصة

خلفية الدراسة: أثبتت الدراسات الوراثية بأن الموقع الجيني الذي يحدد قابلية الأصابة بمرض السكري من النوع الأول يقع ضمن مستضدات التطابق النسيجي في الإنسان و المحمول على الكروموسوم رقم 6.

هدف الدراسة :صممت هذه الدراسة للتحري عن دور مستضدات التطابق النسيجي – للصنف الأول والصنف الثاني في إحداث المرض وكذلك في التنبؤ لحدوث المرض في أخوة مرضى السكري.

المرضى وطريقة العمل: شملت الدراسة ستون (٦٠) مريضاً حديثي الإصابة بمرض السكري النوع الأول (مشخصين بالإصابة خلال فترة أقل من خمسة أشهر). تراوحت أعمارهم من (٣-١٧) سنة. وتضمنت الدراسة (٥٠) فرد من أخوة المرضى لغرض أجراء فحص مستضدات التطابق النسيجي ، تراوحت أعمارهم من (٣-١٦) سنة. بالإضافة إلى عينة سيطرة مكونة من (٨٠) شخص (يبدون أصحاء) متطابقون من حيث العمر (٤-١٧) سنة ، الجنس ، العامل العرقي (عراقيين – عرب) وذلك لغرض فحص مستضدات التطابق النسيجي .

النتائج والتوصيات: أظهر المرض زيادة معنوية في تكرار مستضد الصنف الأول A9 (40% ضد 18.75%) والمستضد B8 (28.33% ضد 8.75%) عند المقارنة مع مجموعة السيطرة. بينما أظهرت مستضدات التطابق النسيجي الصنف الثاني زيادة معنوية في المستضد DR3 (53.33% ضد 26.25%) والمستضد DR4 (50% ضد 12.5%). بالإضافة إلى هذا فأن مرض السكري من النوع الأول أظهر ارتباط معنوي مع المستضد DQ2 (33.33% ضد 15%) والمستضد DQ3 (40% ضد 20%) بالمقارنة مع مجموعة السيطرة وقد يكون لهذه الانتجينات دوراً مهيأ للإصابة بالمرض. وهناك انخفاض معنوي في المستضد DR2 (6.66% ضد 25%) عند المقارنة مع مجموعة السيطرة وكذلك المستضد DQ1 (6.66% ضد 22.5%) وقد يكون لها تأثير في الحماية من المرض. كما تصاحبت هذه الزيادة المعنوية في أخوة المرضى مع المستضد DR4 (34% ضد 12.5%) عند مقارنتهم مع مجموعة السيطرة . وبالتالي فقد يكون التحري عن هذا المستضد له أهمية في التنبؤ لمرض السكر في العوائل المصابة بالإضافة إلى التحري عن وجود الأجسام المناعية الذاتية.

مفتاح الكلمات: مرضى السكري من النوع الأول، الإخوة، مستضدات التطبق النسيجي

[١] فرع الأحياء المجهرية[كلية طب الكندي – جامعة بغداد]
[٢]فرع الأحياء المجهرية [كلية طب النهرين – جامعة النهرين]
[٣]قسم التقانة الاحيائية [كلية العلوم- جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٦٥-٧٣

التغيرات النسيجية في الغدة الدرقية لذكور الجرذان البالغة عند الاستجابة لعامل مضادالأكسدة

سامية عياس عليوي[١] ، حيدر جواد كاظم[٢] ،علي عبد الستار[٢]

الخلاصة

خلفية الدراسة: إن الهرمون العصبي للغدة الصنوبرية والمسمى الميلاتونين ، ينظم عمل الغدد الصم في الجسم و بضمنها الغدة الدرقية، وذلك عن طريق السيطرة على عمل الغدة النخامية.
هدف الدراسة: لمعرفة تأثير جرعات مختلفة من هرمون الميلاتونين الغذائي (كعامل مضاد الأكسدة) على الغدة الدرقية لذكور الجرذان، من الناحية النسيجية.
طريقة العمل: تم إعطاء الميلاتونين الغذائي للجرذان البيضاء البالغة لمدة١٤يوما متتالية.قسمت الجرذان الى٦مجاميع.المجموعة ١كانت للمقارنة.المجموعة ٢ ، ٣، ٤، ٥ و ٦، أعطيت ١٢٥,٢٥٠,٥٠٠,٧٥٠,١٠٠٠ ميكروغرام/كغم من وزن الجسم على التوالي .بعد آخر يوم من المعالجة قتلت الحيوانات تحت تأثير المخدر وأزيلت الغدة الدرقية وتم أجراء الفحص النسيجي عليها.
النتائــج: أظهرت النتائج عدم وجود تأثير مهم مع الجرعات التي تعتبر اعتيادية (بحسب الدراسات السابقة). أما في الجرعات العالية فأنه يؤثر تأثيرا ضارا بليغا.
الاستنتاج: إن هرمون الميلاتونين لا يحدث ضررا للغدة الدرقية الجرذان البالغة في الجرعات الاعتيادية لكنه يسبب تلفا كبيرا في الجرعات العالية.
مفتاح الكلمات:الغدة الدرقية، الميلاتونين، مضادات الأكسدة، الغدد الصم

[١]فرع التشريح والأنسجة والأجنة [كلية طب – الجامعة المستنصرية]
[٢]فرع التشريح والأنسجة والأجنة [كلية طب النهرين – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٧٤-٨٠

التأثير النسيجي لهرمون الميلاتونين في البروستات للجرذان البالغة

سامية عباس عليوي

الخلاصة

خلفية الدراسة: البروستات الأكبر في الغدد الإضافية للجهاز التكاثري الذكري ، وإفرازها يوفر عامل تخفيف ووسيلة لنقل الحيامن من الذكر إلى الأنثى ، ولها دور مهم في الخصوبة الطبيعية . إن هرمون الميلاتونين هو الهرمون العصبي الأساسي للغدة الصنوبرية وهو ينظم الفعاليات الجنسية والتكاثرية في الثدييات وبضمنها الإنسان.

هدف الدراسة: معرفة تأثير جرعات مختلفة من الميلاتونين الغذائي على نسيج البروستات في الجرذان البالغة.

طريقة العمل: أعطي الميلاتونين الغذائي للجرذان البيضاء البالغة لمدة ٣٠ يوما متتالية. قسمت الجرذان الى ٦ مجاميع. المجموعة ١ كانت للمقارنة. المجموعة ٢ ، ٣، ٤، ٥ و ٦ أعطيت ١٢٥, ٢٥٠, ٥٠٠, ٧٥٠, ١٠٠٠ ميكروغرام/كغم من وزن الجسم، على التوالي .بعد آخر يوم من المعالجة قتلت الحيوانات تحت تأثير المخدر وأزيلت البروستات وتم أجراء الفحص النسيجي عليها.

النتائج: أظهرت وجود تاثير نافع مهم مع الجرعات الاعتيادية.أما في الجرعات العالية فأنه يؤثر تأثيرا ضارا بليغا.

الاستنتاج: هرمون الميلاتونين يحدث تأثيرات نافعة في بروستات الجرذان البالغة في الجرعات الاعتيادية لكنه يسبب ضررا كبيرا في الجرعات العالية.

مفتاح الكلمات: البروستات، الميلاتونين، الخصوبة.

فرع التشريح والأنسجة والأجنة [كلية طب – الجامعة المستنصرية]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٨١-٨٨

كشف مناعي خلوي كيميائي لبعض البروتينات المسيطرة على عملية الذوي (P53 و Bcl-2) في خلايا الدم اللمفيه ألمحيطيه لمرضى التهاب المفاصل الرثوي

حيدر فيصل غازي ،نضال عبد المهيمن ،عبد الرزاق حردان احمد

الخلاصة

خلفية الدراسة: هنالك العديد من بروتينات السيطرة التي تشترك في عملية ذوى الخلايا اللمفيه وان الخلل الحاصل فيها قد يلعب دوراً مهماً في أمراضية العديد من الأمراض ذاتية المناعه. لقد ذكرت الدراسات الحديثة وجود خلل في عملية الذوي في خلايا الدم اللمفيه ألمحيطيه لمرضى التهاب المفاصل الرثوي.

هدف الدراسة: إن الهدف من هذه الدراسة هو قياس نسبة الإظهار الخلوي لبروتين P53 و Bcl-2 في خلايا الدم اللمفيه المحيطية لمرضى التهاب المفاصل الرثوي ودورها في عملية الذوى، كذلك معرفة العلا قه بين نسبة الإظهار الخلوي لهما مع نسبة خلايا الدم اللمفيه التائية المحيطية.

طريقة العمل: شملت هذه الدراسة ستة وأربعين مريض مصاب بالتهاب المفاصل الرثوي وقورنوا بـ١٧ شخص سليم ذوي أعمار متقاربة. حيث فصلت خلايا الدم اللمفيه من عينات الدم المحيطي وتم فحص نسبة الإظهار المناعي الخلوي للمعلم المناعي CD3 و بروتينات السيطرة P53 و Bcl-2 فيها بطريقة التصبيغ المناعي الخلوي.

النتائــج: لقد أظهرت النتائج تجمع خلايا الدم اللمفيه التائية المحيطية في مجرى الدم المحيطي لمرضى التهاب المفاصل الرثوي مقارنة بمجموعة السيطرة. وجد فرقاً معنوياً عالياً في زيادة نسبة إظهار بروتين Bcl-2 في خلايا الدم اللمفيه المحيطية لمرضى التهاب المفاصل الرثوي مقارنة بمجموعة السيطرة ($P<0.001$) بينما لا يوجد فرق معنوي بالنسبة لنسبة الإظهار الخلوي لبروتين P53 بين المجموعتين($P=0.278$). أظهرت نتائج الانحدار الخطي وجود علاقة معنوية بين زيادة الخلايا اللمفيه التائية ونسبة إظهار بروتينBcl-2 ($P<0.001$) بينما لا توجد تلك العلاقة مع نسبة إظهار بروتينP53 ($P=0.587$).

الاستنتاجات: تم الاستنتاج من خلال نتائج هذه الدراسة وجود زيادة ملحوظة في عدد الخلايا اللمفيه التائية المحيطية للمرضى المصابين بالتهاب المفاصل الرثوي والذي قد يكون ناتج عن خلل في عملية الذوي المتمثلة بالزيادة الملحوظة في الإظهار الخلوي لبروتين Bcl-2 بغض النظر عن نسبة الإظهار الخلوي لP53 بروتين.

مفتاح الكلمات: التهاب المفاصل الرثوي، الذوي، P53،Bcl-2 ، فحص مناعي خلوي كيميائي.

فرع الأحياء المجهرية [كلية الطب - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٨٩-٩٨

تقييم المخاطر بين المرضى الراقدين في مستشفيات بغداد لمرض الطارئة الوعائية الدماغية

زكي نوح الموسوي[١] ،حسن عزيز الحمداني[٢] ،حامد فاخر العزاوي[٣]

الخلاصة

خلفية الدراسة: الضربة الدماغية تشكل عبء ثقيل على المريض وعائلته و لسوء الحظ لا بوجد سبب مباشر ولا معالجة شافية للضربةِ الدماغية ؛ يشكل تعريفِ عوامل ا لخطورةِ المسببة للضربةِ الدماغية أهمية كبيرة ليَمْنعُ المرضَ ومضاعفاته .

هدف الدراسة: تُحليّلَ عواملَ ا لخطورة المسببة للضربةِ الدماغية المعروفةِ ولدِراسَة مستوى التعليم والمستوى الاقتصادي كعوامل ذات علاقة محتملة مع حدوث الضربة الدماغية

طريقة العمل: تمت دراسة ٥١٠ مريض بالضربةِ الدماغية الأحتشائية بأنواعِها وشدتهاِ المختلفةِ الذين أُدخلوا إلى ٣ مستشفيات في بغداد. المرضى فُحِصوا وتُحرّوا كليَّاً. التأكيد الخاصّ رُكّزَ على مستوى التعليم والمنزلةِ الاقتصادية.

النّتائِـج: ٥١٠ مريضا دُرسوا، ٢١٥ أنثى و٢٩٥ ذكر. الدراسة وجدتْ ١٦ % نسبة إصابة ذكور أعلى.كذلك وجدت الدراسة ارتفاع ضغط الدم كعاملُ خطرِ معروفِ في الدراسةِ الحاليةِ (٧٥.٥ %) ثم تَلتْ بعواملِ الخطرِ الأخرى. الدراسة وجدت عدد أعلى مِنْ مرضى الضربةِ [٤٤.٧ %] كَانواْ مرضى أُمّيينَ، بالمقارنة مع [٩ %] من المرضى دوي تعليما العالي. وجدتْ الدراسةُ انتشارا أكثرَ مِنْ مرضى الضربة [٩٠.٣٩ %] يَعِيشونُ في البيوتِ الصغيرةِ المزدحمةِ. الدراسة وّجدتْ [٣٦ %] مِنْ مرضى الضربةِ يَعُودُ إلى العائلاتِ ذوي الدخل تحت ١٠٠ دولار شهرياً .

المناقشة: وجد أن المجموعة الاجتماعية السفلى المجموعة و الاقتصادية الدنيا والمجموعة ذات المستويات التعليمية الدنيا. تتميز بقلة الوعي الصحي أيضاً في تلك المجموعات مِنْ المرضى وجد ارتفاع عوامل الخطر التي تؤدي إلى حدوثِ الضربةِ الدماغية . مثل داء السكّري،و ارتفاع ضغط الدم، والتدخين

الاسـ تنتاج: الضـ ربة مـ رضُ تَوَسُّـ ع فـ ي مجتمعِنـ ا.و هـ ي فـ ي تزايـ دُ فـ ي المسـ تويات التعليميـ ةِ المنخفضةِ،و الحالة الاقتصادية المتدنية وظروف سكنية سيّئة. . هناك نسبة عالية مِنْ ارتفاع ضغط الدم لم تُشخّصُ سابقاً على الرغم مِنْ وجود علاماتِ ارتفاع ضغط الدم المُزمن

مفتاح الكلمات: طارئة وعائية دماغية ، المخاطر

[١]فرع الطب الباطني[كلية طب الكندي – جامعة بغداد]
[٢]فرع الطب الباطني[كلية الطب – جامعة النهرين]
[٣]فرع الطب الباطني[كلية الطب – جامعة بغداد]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص٩٩-١٠٧

احتمالية ارتباط مستضدات الخلايا البيضاء البشرية الصنف الأول والثاني مع التهاب القولون التقرحي في المرضى العراقيين

احمد عبد الحسن عباس الحسن

الخلاصة

خلفية الدراسة: إن أسباب و أمراضية التهاب القولون التقرحي غير واضحة لحد ألان وعلى الرغم من ذلك فان الدراسات لفتت الانتباه إلى إن العوامل الوراثية قد تلعب دور جوهري في المرض.

هدف الدراسة: نظمت هذه الدراسة لكي تسلط الضوء على احتمالية وجود مصاحبة بين مستضدات خلايا الدم البيض البشرية الصنف الأول والثاني مع مرض التهاب القولون التقرحي .

طريقة العمل: تضمنت الدراسة ١٠٥ حالة و تكونت من ٣٥ مريض مصاب بالتهاب القولون التقرحي و ٧٠ شخصا سليما ظاهريا كمجموعة ضابطه.استخدم فحص سمية الخلايا اللمفية Lympho cytotoxicity assay لغرض تنميط التطابق النسيجي HLA-typing.

النتائـــج: أظهرت المقارنة بين المرضى المصابين بالتهاب القولون التقرحي ومجموعة السيطرة الأصحاء مصاحبة معنوية موجبة لكل من مستضدات الخلايا البيض البشرية B14, B27 and DR52 مع المرضى . بينما كانت تكرارات مستضدات الخلايا البيض البشرية B35, B41 and Cw6 قليلة في المرضى عند مقارنتها مع مجموعة السيطرة .

الاستنتاجات: أظهرت النتائج إن B14, B27 and DR52 قد يؤدي دور في التعرض للإصابة بمرض التهاب القولون التقرحي، بينما B35, B41 and Cw6 ربما يمنح حماية ضد الإصابة بالمرض.

مفتاح الكلمات: التهاب القولون التقرحي ، مستضدات الخلايا البيض البشرية ،العوامل الوراثية.

فرع المناعه السريرية [كلية الطب- جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص١٠٨-١١٣

الاعتلال الوعائي السخدي الامي والإصابة الجرثومية بين حالات الولادة المبكرة

لقاء رياض الخزاعي

الخلاصة

خلفية الدراسة: تعد الولادة المبكرة من أهم أسباب الوفاة بين الأطفال ألحديثي الولادة و هناك كمية ضخمة من المعلومات في النشرات المطبوعة تشير إلى وجود صلة قوية بين الاعتلال الوعائي السخدي الامي و التلوث وحالات الولادة المبكرة.

هدف الدراسة:إثبات التلازم بين الاعتلال الوعائي السخدي الامي والتهابات الأغشية و المخاض المبكر وتمزق الأغشية المشيمية السلوية المبكر .

طريقة العمل: لقد قمنا بدراسة ٥٤ حالة ولادة مبكرة ناتجة عن ٣٧ حالة مخاض مبكر و ١٧ حالة تمزق الأغشية قبل الأوان و مقارنتها مع ٥٤ حالة ولادة تمت عند اكتمال مدة الحمل بدون مضاعفات في الفترة من كانون الثاني ٢٠٠٤ إلى تموز ٢٠٠٥ .تمت معالجة جميع الحالات في قسم النسائية و التوليد في مستشفى الكاظمية التعليمي في بغداد.

النتائـج: الاعتلال الوعائي السخدي الامي كان موجودا في ١٣ حالة (٣٥.١%) من حالات المخاض المبكر و ٦ حالات (٣٥.٣%) من حالات تمزق الأغشية قبل الأوان و ٦ حالات (١١.١%) من حالات الضبط .تلوث الأغشية كان موجودا في ١٤ حالة (٣٧.٨%) من حالات المخاض المبكر و ٦ حالات(٣٥.٣%) من حالات تمزق الأغشية قبل الأوان و٨ حالات(١٤.٨%) من حالات الضبط..

الاستنتاج: إن من الممكن تمييز مجموعتين من المرضى في حالات الولادة المبكرة الناتجة عن المخاض المبكر و تمزق الأغشية قبل الأوان الأولى هي مجموعة تلوث الأغشية و الثانية هي مجموعة الاعتلال الوعائي السخدي الامي.

مفتاح الكلمات: *تمزق الأغشية المبكر، أمراض المشيمة، الإصابة الجرثومية، الولادة المبكرة*

فرع النسائية و التوليد[كلية الطب _ جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص١١٤-١٢٠

تحديد الحامض النووى الرايبى الناقل لل IL-8, IL-10 and IFN- γ فى انسجة المشيمه للاجهاض التلقائى المتكرر باستخدام تقنية التهجين الموضعى.

نضال عبد المهيمن[١] ، امال حسين[٢]

الخلاصة

خلفية الدراسة: إن إنتاج المدورات المناعية مثل انترفيرون كاما من قبل الخلايا التائية المساعدة نوع ١ و الانترلوكين ١٠ من قبل النوع ٢ من تلك الخلايا قد تبين إن له تأثيرات مضره على المل و نمو و تطور الجنين.

هدف الدراسة: قياس التركيز الموضعي لبعض من المدورات المناعية باستخدام تقنية اختبار التهجين الموضعي والكشف عن المجسات المهجنة (ISH)لمريضات الإجهاض التلقائي المتكرر .

المواد وطرائق العمل: تضمنت الدراسة الحالية مائة وتسع عشْرة امرأة، تراوحت متوسط أعمارهن بين (٢٣.٩– ٢٨.٥)، تم تقسيمهن إلى ثلاثة مجاميع: مجموعة(أ) إجهاض تلقائي متكرّر (RSA) وعددهن ٦٢ امرأة و متوسط أعمارهن بينَ (٢٨.٥ + ٠.٦٨). مجموعة (ب) - إجهاض تلقائي غير متكرّر (non-RSA) وعددهن ٣٤ امرأة و متوسط أعمارهن بينَ (٢٦.٤ ± ٠.٨٥). مجموعة(ج) - سيطرة (حمل ناجح): وعددهن ٢٣ امرأة ومتوسط أعمارهن بينَ (٢٣.٩ ± ٠.٨٨). تم جمع نماذج النسيج المغذي للجذين (التروفوبلاست) من كل المرضى وكذلك مجموعه السيطرة. تم دراسة تأثير IL-10,IFN-γ,IL-8 باستخدام تقنية اختبار التهجين الموضعي والكشف عن المجسات المهجنة (ISH) .

النتائــج: أظهرت النتائج وجود فرق معنوي عالي ($p<0.01$) بالنسبة لمتوسط النسبة المئوية ل IFN-γ في المجموعة(أ) مقارنة بمجموعة السيطرة (ج) .وكذلك وجود فرق معنوي عالي ($p<0.01$) بالنسبة لمتوسط النسبة المئوية ل IL-10 لمجموعة السيطرة (ج) مقارنة بالمجموعة(أ). تم إيجاد فرق معنوي عالي ($p<0.01$) بين المجموعة(أ) والمجموعة(ج) عند مقارنة متوسط النسبة المئوية ل IFN-γ/IL-10 في نسيج التروفوبلاست . إضافة لذلك، أظهرت نتائج فحص IL-8 عدم وجود فرق معنوي ($p>0.05$) بين متوسط النسبة المئوية ل IL-8 عند مقارنة كل من المجموعة (أ) والمجموعة (ب) مع المجموعة (ج) .ولكن أظهرت النتائج إن هناك فرق معنوي ($p>0.05$) بين متوسط النسبة المئوية ل IL-8 عند مقارنة المجموعة (أ) مع المجموعة (ب).

الاستنتاجات: تدعم نتائج هذه الدراسة إمكانية وجود الاستجابة المناعية الالتهابية والتي تتناغم مع ظاهرة الإجهاض التلقائي المتكرر.

مفتاح الكلمات: إجهاض تلقائي متكرّر، تقنية اختبار التهجين الموضعي،المدورات المناعية، الانترفيرون كاما،انترلوكين ١٠.

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المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص١٢١-١٢٧

التهاب اللفافة الاخمصي

عدنان عبد عنوز

الخلاصة

خلفية الدراسة: التهاب لفافة أخمص القدم من أكثر أسباب الآم كعب القدم ولكن العلاج بقي غير وافي وتجريبي.

هدف الدراسة: لغرض فحص تأثير وفائدة العقار انترفيرون الفاتوبي على الآم كعب القدم وتيبس القدم الصباحي لدى مرضى التهاب لفافة اخمص القدم.

طريقة العمل: ٣٧٦ مريضاً يعانون من التهاب لفافة أخمص القدم ادخلوا في هذه الدراسة تم تقسيم المرضى إلى مجموعتين المجموعة الأولى تناولت انترفيرون الفاتوبي ثلاثة ملايين وحدة كل ثلاثة أيام والمجموعة الثانية تناولت دايكلوفيناك ٥٠ ملغم مرتين في اليوم مع استعمال وسادة للكعب الدراسة استمرت لمدة ١٢ أسبوع .

النتائــج: ٩٢% من المجموعة الأولى التي تناولت انترفيرون الفاتوبي اظهروا تحسناً ملموساً وقد اختفت كافة أعراض المرض بعد مرور ٦ أسابيع في نهاية الأسبوع الثاني عشر ٩٦.٨% من مرضى المجموعة الأولى بدون أعراض مرضية.

الاستنتاجات: علاج مرضى التهاب لفافة أخمص القدم بعقار انترفيرون الفاتوبي اظهر جدوى وفعالية عالية في شفاء المرضى واختفاء أعراض المرض .

مفتاح الكلمات: انترفيرون الفا تو بي – التهاب اللفافه الاخمصي.

فرع الطب [كلية طب النهرين – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ١ ص١٢٨-١٣١