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

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Halliwell B, Gutteridge JMC. Oxygen toxicity, Oxygen radicals, transition metals and disease. Biochem J. 1984; 219: 1-14.

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Editorial:

Medical education

Amal Swidan *FICMS*

The importance of medical education need not to be emphasized, it is the long yet joyful journey, challenging study that transfer high school graduate into medical doctors who relive pain, prolong life and improve its quality via their knowledge, skills and attitudes.

Health care is largely medical care especially in Iraq where paramedics' roles are not very much fulfilled.

Making a medical doctor in six years period may not be a simple job particularly nowadays with medical knowledge growing exponentially, information technology pushing medical education as well as medical practice towards contemporary methodologies. Major changes that involved all people without any exception, i.e., students and patients,

Medical students are coming from different social backgrounds with different attitudes. In our country high school graduate in general and those who get admitted into medical schools in particular adopt a spoon feeding type of learning that make many of them highly dependent in their consecutive learning.

Patient expectations are changing and they share their care taker increasingly throughout the management process.

More over contemporary medical education institutes should be accredited by certain eligible bodies after meeting specific agreed upon standards.

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Assistant Professor,Vice Dean

From the above, medical education is a vital process that necessitates continuous evaluation and development. One essential question that may come to mind is what to teach?

The medical knowledge is huge in contrast to the relatively limited time available for teaching. Many teachers still feel that they should teach every detail and prepare distended lectures (sometimes information may be repeated by different teachers from different disciplines). Students spend the whole time of a lecture in dictating from a board or a screen.

The lecture may be described in such case as the transfer of teacher's notes to student's notebooks by passing their brains.

The answer to the "What to teach?" may need to categorize medical knowledge into:

- Core knowledge
- Useful to know
- Interesting to know

The first category is the most important as it involves what should be really mastered by students and to achieve this; core knowledge should get clarified to the best of one knowledge, emphasized in teaching and in assessment. Here one may ask what part(s) is to be considered as core knowledge.

The journey of medical education has just come to a relevant station that should be shared with stakeholders.

We all know that graduates of medical colleges work as resident

doctors in public hospitals directed by ministry of health.

Job description for medical graduate is fundamental in stating "Learning Objectives" of any medical course, and this is primarily the duty of ministry of health (the first market for the product of medical education institutes). Unfortunately this is still lacking in Iraq and achieving it may be a great collaborative multidisciplinary national project.

Learning objectives may be defined as: a statement that describes what the **student** can do, feel at the **end of the course.** Quite clearly, learning objectives refer to students not teachers and timed to the end of the course.

Setting learning objectives will guide those in charge through planning most relevant content, most appropriate teaching methods and assessment, in other words successful curriculum.

For example if conducting normal vaginal delivery is among the duties of resident doctors the curriculum should involve all knowledge, skills and attitudes that enable doctors to perform the task successfully and safely.

Ensuring mastery here may oblige students to perform the act under supervision for certain number of times before getting graduated.
Actual clinical training may merely include observing delivery or even one stage of it.

Another example may be cardio-pulmonary resuscitation, a life-saving measure that should perfectly be mastered by every student before getting graduated.

The second category, useful to know should be taught and included in exams but to lesser extent. The third category, interesting to know may be covered in seminars and tutorials.

- ✓ *How to teach*

A written curriculum is a must for every successful course, as it will be a plan of action for pursuing particular outcome within preset time table through ensuring the completion of every item in the syllabus, passing exams and fulfilling all requirements. Contents and methods are included in a written curriculum.
Whatever teaching methods are agreed upon in any curriculum, a good teacher should bear in mind each of the following:

- **Clarity** of all spoken or written material to every student including those sitting at the end of class room. Voice should be loud enough. In large class rooms amplifiers may be necessary. Translating every new word or term especially when teaching language is not mother language, as in our situation.
- **Making teaching meaningful** by explaining outlines of a lecture or a course in advance, by relating material to students daily lives and/or their future career.
- **Individualization,** as students are not the same they should be treated carefully. Some of them are rapid, dominant, or the other way around. Keep in mind that 95% of people can learn but with different pace and methods.
- **Caring for** students is humanitarian behavior that may foster those young people with the highly demanding medical education n especially in Iraq with its incredible tensions and difficulties.
- Active learning, teaching differs from talking. Active participation of students is fundamental, and can be done by raising questions and appraising responses accordingly.

- Feed back is helpful for students to know their strength and to improve their weakness.

Revising curricula on regular basis may even be another must to maintain the quality of outcome. Planning curriculum need high level of expertise, team work and enough time.

Adopting spiral type of curriculum, and teaching clinical knowledge and skills early in medical colleges may be a suggestion.

Managing curriculum in an architectural method that depend and emphasize the outcome may be a good alternative to the current mechanical type of management where the basic concern is continuity of daily schedule.

In the end of this editorial may I say that all difficulties one may face whether during study or practice of medicine are fundamental parts of the amazing picture of this career. medical challenges, if I can say, is worthy in regard to the status of a successful medical doctor.

Effects of Oral Zinc Sulfate 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.

Aims: to study the effects of zinc sulfate 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 zinc sulfate were compared to distilled water (control), and prednisolone regarding changes in body weight, colon segment weight, and gross and microscopical scores. Plasma zinc and copper concentrations were measured in control and zinc sulfate groups in both models.

Results: In model 1, severe gross and microscopical damage observed in colon. Gross and microscopical scores of zinc sulfate 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.

Zinc sulfate and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control. The gross and microscopical damages were significantly lowered in zinc sulfate and prednisolone groups.

In both models (1 and 2), a significant decrement in post induction mean plasma zinc level was detected ($p < 0.05$); however, such decrement could be corrected by zinc sulfate therapy.

Conclusions: Acetic acid -induced colitis in rabbits (model II) is preferred for testing the anti-inflammatory effectiveness of new therapeutic modalities. Zinc sulfate has a valid prophylactic activity in this model.

Keywords: Inflammatory bowel disease, induced colitis, acetic acid, zinc sulfate, prednisolone.

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

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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 (H_2O_2), nitric oxide (NO), myeloperoxidase

activity (MPO), and tumor necrosis factor (TNF-alpha) [8].

Zinc has both antioxidant and anti-inflammatory actions. Zinc consideration as an antioxidant stems from its presence in Cu/Zn-Superoxide dismutase, an enzyme with a major role as scavenger of free radicals in the cytoplasm of many types of cells and in the extracellular space.

Hence according we try to explore the possible beneficial prophylactic and therapeutic effect of oral zinc sulfate for induced colitis in rabbits.

Materials and Methods

Colitis was induced in male rabbits by rectal administration of 5% acetic acid-30% ethanol (model 1) [9], or 2% acetic acid (model 2) [10]. 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 zinc sulfate (50 mg/kg/ day dissolved in 10 ml distilled water). Each agent (including distilled water) was administered orally two days prior to induction of colitis, the day of induction, and a dose 22 hours 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 [11], 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 (Table 1) [12].

Colonic samples (0.5 cm of length) were taken from the 8 cm segment, fixed in 10% formaldehyde and the routine 5 micrometer sections were prepared. Tissues were routinely stained with haematoxylin and eosin, coded, and evaluated blindly by light microscopy (with 40x high power objective lens) [13]. Each slide was scored according to Christian, et al., [14] 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.

Scores ranged from 0 to 40, four criteria were depended: 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; Percent involvement from 0-4 as 0%1–25%, 26–50%, 51–75%, 76–100%, respectively. The score (total score) represents the sum of the products of each criterion by the score of the percentage involvement [15].

Plasma zinc and copper concentrations were measured in control and zinc sulfate groups in both models before the first dose of treatment and just before killing with the aid of atomic absorption spectrophotometry.

Results are expressed in tables as means $\pm$ standard error of mean (SEM), or drown 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 $0.01 < p$

≤ 0.05 was considered as significant [16].

Results

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

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

Zinc sulfate treatment and prednisolone treatment reduced the loss of body weight of rabbits in comparison to the control group (Table 2).

The mean (±SEM) post-induction rectal temperature for control group (38.78 ± 0.2Cº) showed a statistically insignificant ($p > 0.05$) increment from the mean pre-induction readings (38.55 ± 0.14Cº). On the other hand, post-induction readings for zinc sulfate group (38.73 ± 0.17Cº) and post-induction readings for prednisolone group (37.84 ± 0.38Cº) decreased insignificantly ($p > 0.05$) from mean pre-induction readings (38.94 ± 0.21Cº), and (38.61 ± 0.15Cº) respectively.

When comparing mean post-induction rectal temperature of zinc sulfate group and prednisolone group to the corresponding readings of control group, the differences were insignificant ($p > 0.05$), while when comparing corresponding readings of prednisolone and zinc sulfate group, there was a significant decrease ($0.01 < p < 0.05$) in mean post-induction rectal temperature of prednisolone group, (Figure 5) .

The mean (±SEM) colon segment weight of zinc sulfate group (2.02 ± 0.14 mg) and prednisolone group (1.87 ± 0.16 mg) were insignificantly ($p > 0.05$) more than that of the control group (1.76 ± 0.10 mg), (Figure 6).

Compared to that of prednisolone group, the mean weight of colon segment of zinc sulfate group did not differ significantly ($p > 0.05$).

The means gross histological score significantly lowered in zinc sulfate group and prednisolone group in comparison to the control group ($p < 0.05$) (Table 3) and (Figure 7).

The mean microscopical score was significantly lowered in zinc sulfate group and prednisolone group in comparison to the control group ($p < 0.05$) (Figure 8).

The effects of zinc sulfate in regards to colonic segment weight, gross histological score, and microscopical score were comparable to those of prednisolone ($p > 0.05$).

In both models, (1 and 2), a significant decrement in post induction mean plasma zinc level was detected ($p < 0.05$); however, such decrement could be corrected by zinc sulfate therapy, on the other hand, post induction mean plasma copper concentration obviously increased when compared to the pre-induction levels, (Table 4) and (Table 5) below shows the changes in plasma concentrations of zinc and

copper in response to induction of colitis and zinc sulfate treatment.

Table 1: Gross mucosal inflammation scoring index. (Modified from Brian, et al., 1997)[12]

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 (±SEM) (g)	Mean Post-induction Body Weight (±SEM) (g)
Control	**1225 ± 86.2**	**1170 ± 88.96 *****
Prednisolone	**1228.3 ± 83**	**1183.3 ± 90***
Zinc Sulfate	**1285.7 ± 90.4**	**1238.6 ± 91.2 ****

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

Table 3: Mean (±SEM) 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 ± (SEM)
Control	7	8.86 ± 0.51
Prednisolone	6	7 ± 2.45 *
Zinc Sulfate	7	6.25 ± 1.23 *

**Significant reduction (0.01< p < 0.05) in comparison with the control*

Table 4: Mean plasma zinc and copper concentrations (ppm) of control and zinc sulfate groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

Group	Plasma zinc concentration		Plasma copper concentration	
	Pre-induction	***Post-induction***	***Pre-induction***	***Post-induction***
Control	**1.35 ± 0.18**	**0.31** ± 0.10**	**0.43 ± 0.08**	**0.68* ± 0.11**
Zinc sulfate	**0.93 ± 0.11**	**0.50* ± 0.10**	**0.34 ± 0.09**	**0.50 ± 0.15**

*** Highly significant reduction (p< 0.01) in comparison with its pre induction level*
** Significant difference (p < 0.05) in comparison with its pre induction level*

Table 5: Mean (±SEM) plasma zinc and copper concentrations (ppm) of control and zinc sulfate groups in acetic acid (2 %)-induced colitis

Group	Plasma zinc concentration		Plasma copper concentration	
	Pre-induction	**Post-induction**	**Pre-induction**	**Post-induction**
Control	**1.66 ± 0.10**	**1.32 ± 0.12 ***	**0.71 ± 0.10**	**0.76 ± 0.08**
Zinc sulfate	**1.39 ± 0.11**	**1.56 ± 0.15**	**0.61 ± 0.11**	**0.81 ± 0.17 ***

** Significant difference (p < 0.05) in comparison with the pre-induction levels*

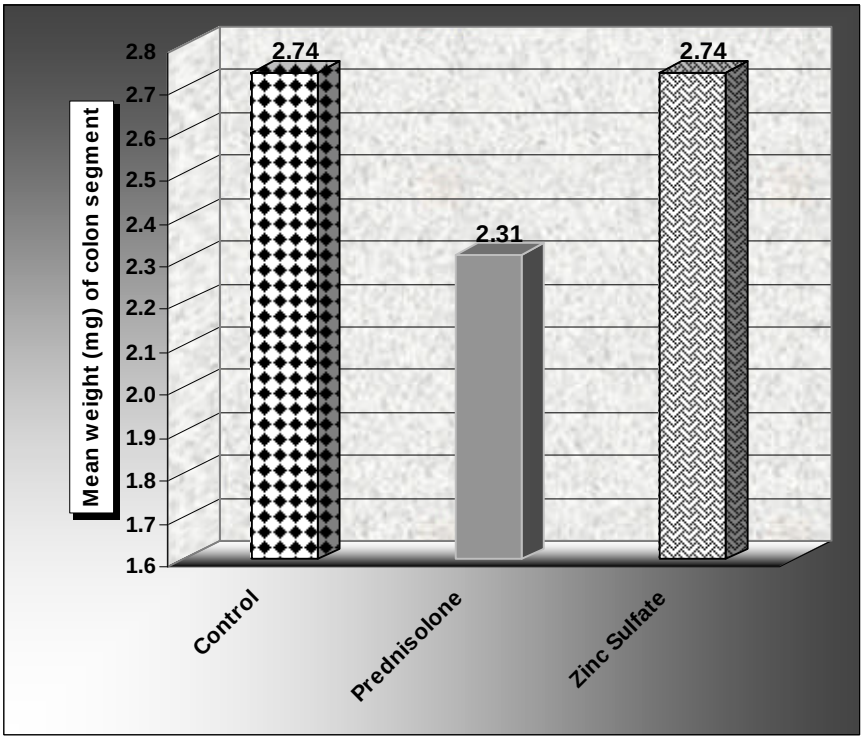


Figure 1: Mean weight of colon segment (mg) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

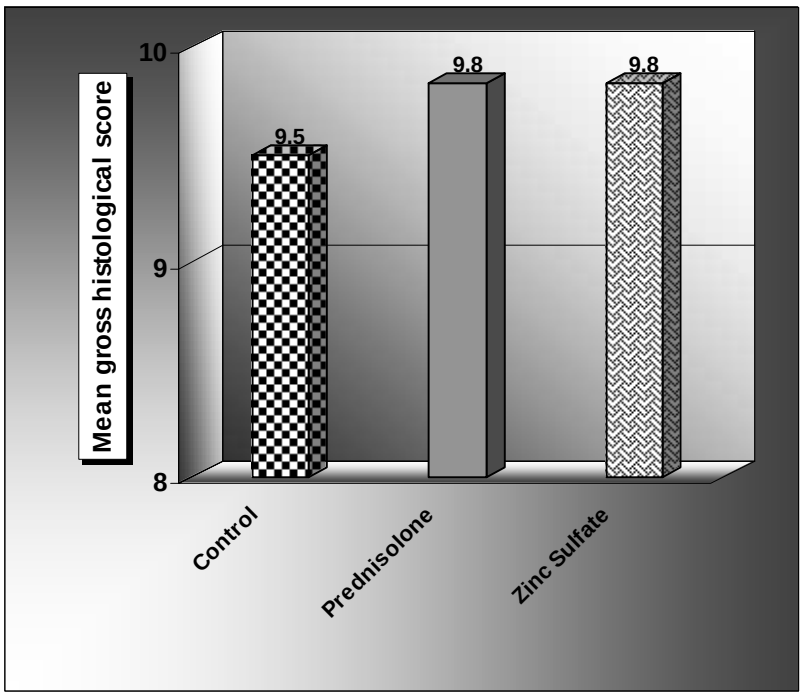
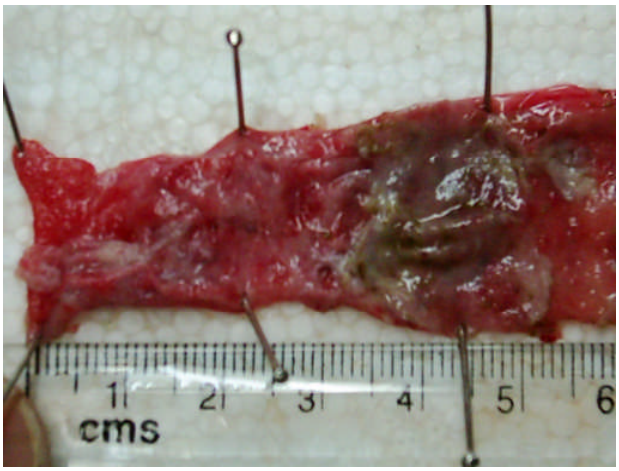


Figure 2: Mean gross histological score (0-10) of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis.



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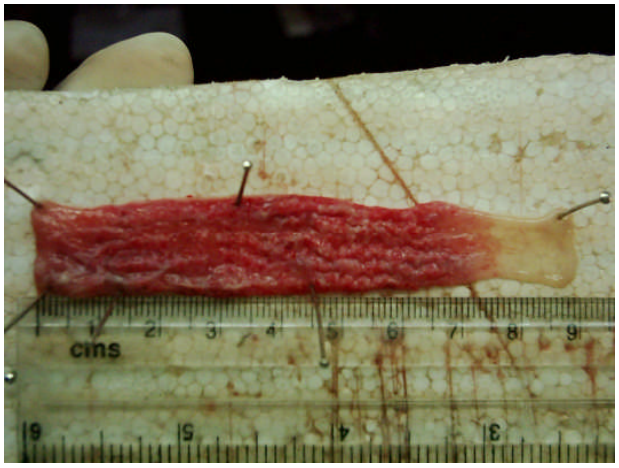

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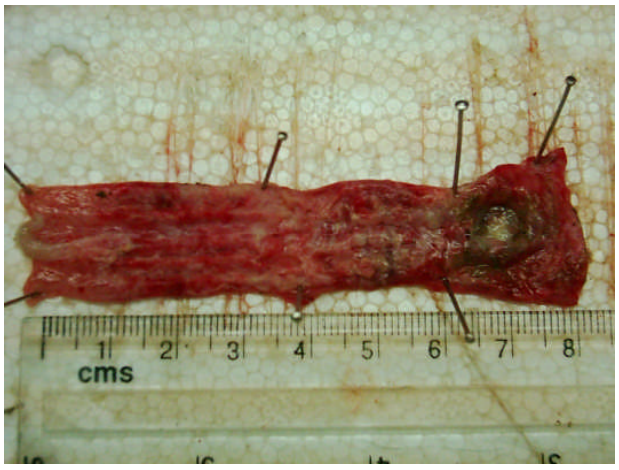

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Figure 3**: The gross appearance of colon segments of control and treatment groups in acetic acid (5 %) - ethanol (30%)-induced colitis, A: control, B: prednisolone, C: zinc sulfate.**

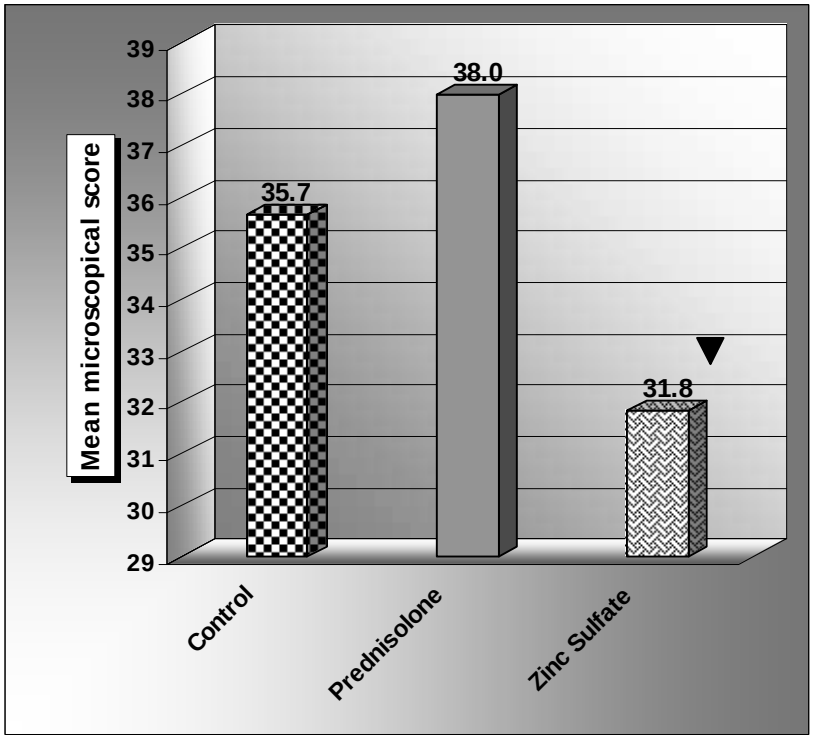


Figure (4): Mean microscopical histological score of the colon (0-40), of control and treatment groups in Acetic acid (5 %) - Ethanol (30%)-induced colitis

▼ ***Significant difference (0.01< p < 0.05) in comparison with prednisolone group***

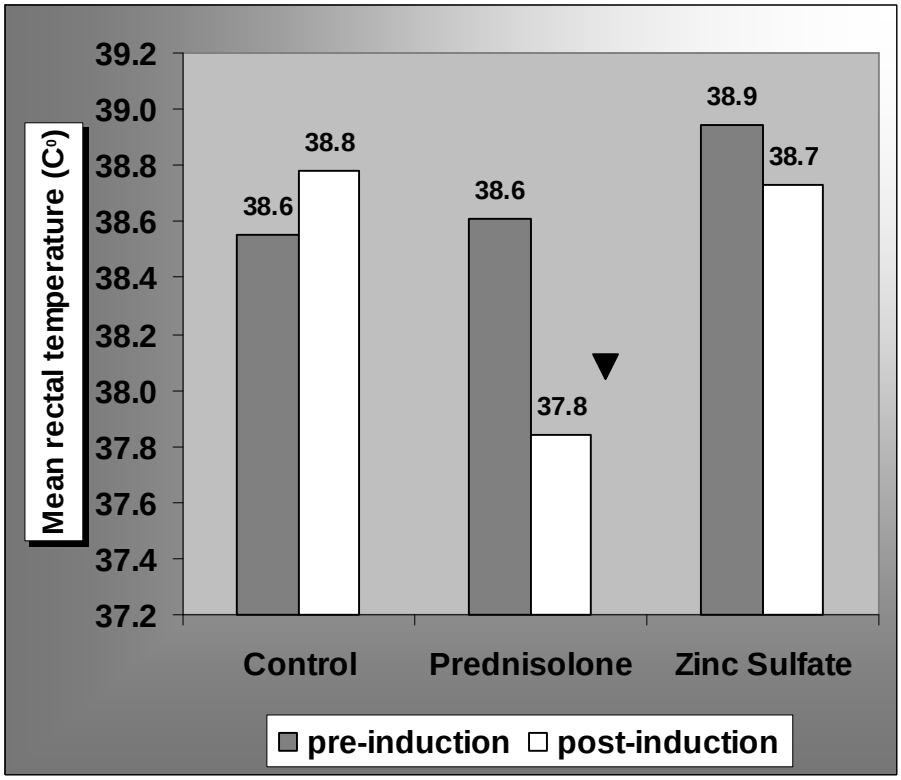


Figure (5): The mean pre-induction and post-induction rectal temperature of rabbits in control and treatment groups in acetic acid (2%) - induced colitis
▼*Significant reduction ($p < 0.05$) in comparison with pos-induction rectal temperature of zinc sulfate group*

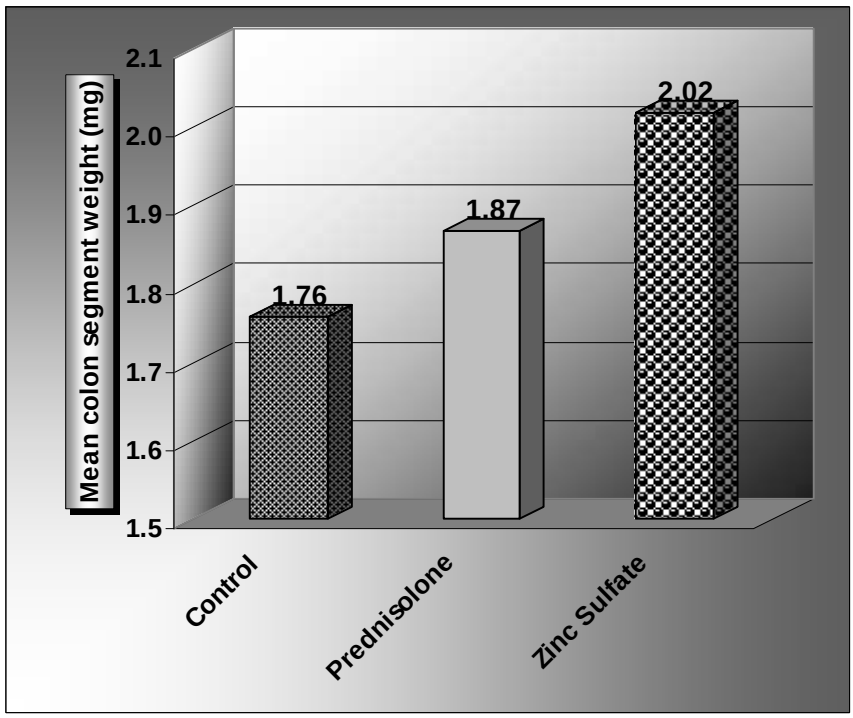
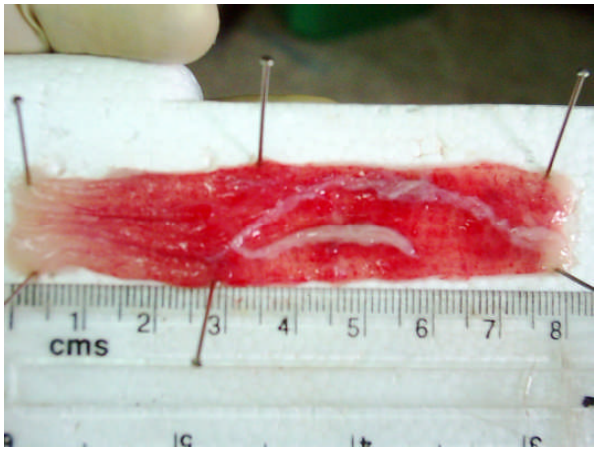


Figure (6): Mean colon segment weight (mg) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis



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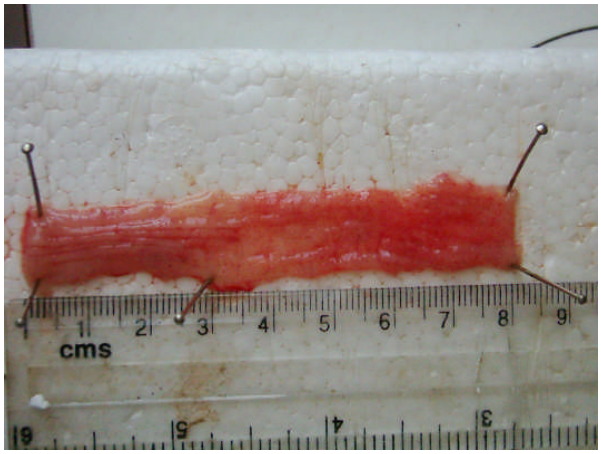

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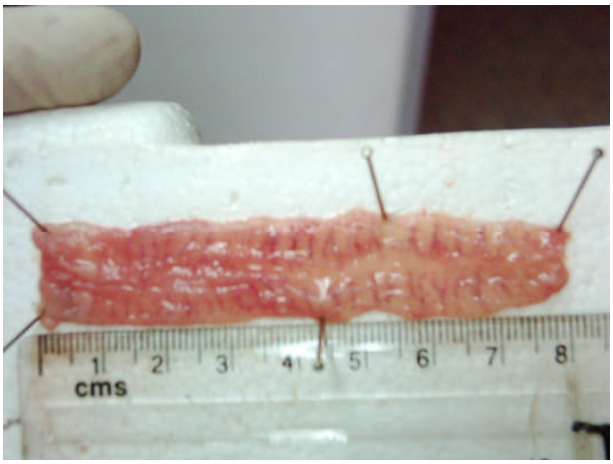

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Figure (7) **the gross appearance of colon segments of control and treatment groups in acetic acid (2%)-induced colitis, A: control, B: prednisolone, C: zinc sulfate.**

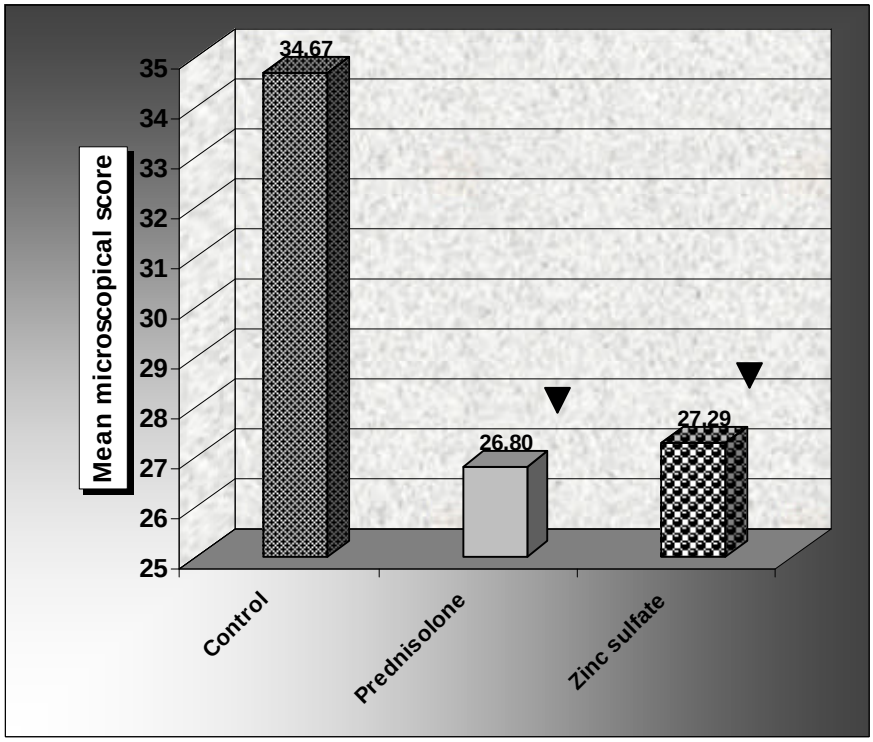

Figure (8): Mean microscopical histological score (0-40) of rabbits in control and treatment groups in acetic acid (2%) - induced colitis

▼ ***Significant reduction (0.01< 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 [17].

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 [18].

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%) which 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 [19] 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 [20, 21].

It was found that non-steroidal antiinflammatory drugs exacerbate experimental colitis in rats [22]. For that these agents were not used in this study.

Zinc sulfate is relatively safe and used orally as a drug, and it is available and relatively cheep, besides, it is 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 zinc sulfate, 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.

There were no significant differences ($p > 0.05$) in model 2 in colon segment weight. Rachmilewitz, et al., [23] showed that 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, (prednisolone and zinc sulfate), to reduce inflammatory process in acetic acid (2%) model.

Moreover, compared to effect of prednisolone on mean gross histological score, zinc sulfate (models 1 and 2) had comparable effect; this could indicate its possible potent initial anti-inflammatory effects.

In model 1, the insignificant differences induced by both 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 new tested agents in initial treatment of induced colitis in rabbits.

In model 2, prednisolone-induced and zinc sulfate-induced significant micro-scopical improvements emphasized the effective anti-inflammatory role of these agents particularly when these findings conjugated with their anti-inflammatory effects detected grossly (see above).

In model 2 in this study, compared to control group, prednisolone exerted a better apparent protective role than zinc sulfate regarding the induced reduction in mean body weight.

Although there was a significant reduction of mean body temperature of prednisolone group from the corresponding readings for the other groups, the means of body temperature were all within the normal range (37.8° - 39.4° C).

In model 1, the highly significant reduction in the mean

post-induction plasma zinc concentrations for rabbits in the control group from its mean pre-induction level and the similar significant reduction in mean post-induction zinc level in model 2 showed that acetic acid induced colitis model is associated with zinc decrement, and it simulates the results obtained in human IBD [(24)].

In the present study, zinc sulfate therapy markedly corrected the plasma zinc values in both models; this result showed that zinc sulfate (in a daily dose of 50 mg/kg taken orally for 3 successive days) was sufficiently absorbed and did not cause toxicity. Zinc homeostatic mechanisms probably could not cope with the well known redistribution of plasma zinc induced initially by inflammatory processes without an external zinc supplementation [(25)].

Plasma copper measurement was used to identify whether such a high dose of zinc sulfate therapy 50 mg/kg/day caused secondary copper deficiency; the latter causes copper deficiency associated anemia in human [(26)].

In model 1, mean plasma copper concentration of the control group was significantly increased post-induction from that of the pre-induction level, $P< 0.05$. While for the zinc sulfate group there is an insignificant increase in plasma copper caused by zinc sulfate therapy.

In model 2, the similar increment in mean post-induction plasma copper from the pre-induction level is expected because of the associated zinc deficiency, but this increment was statistically not significant which may be because of the less severe zinc decrement in comparison to that in model 1.

The significant increment in mean post-induction plasma copper from mean pre-induction level is unexplainable yet.

In the present study, oral zinc sulfate (50 mg/kg/day) appeared to have a considerable prophylactic activity that was comparable to that of oral prednisolone (2 mg/kg/day) against acetic acid –induced colitis in rabbits.

Duggan, et al., [(27)] emphasized that zinc deficiency may predispose the intestinal tract to damage by free radicals and increased NO activity. Furthermore, Mulder, et al., [(28)] documented the decrease in intestinal copper/zinc containing proteins that have antioxidant function in inflammatory bowel disease.

Di-Leo, et al., [(29)] showed that administration of zinc sulfate in a dose of 30 mg/kg/day had a little effect on the short-term course of experimental colitis; this probably in contradict to the encouraged results obtained in the present study. This probably due to difference in the applied dose of zinc sulfate in the present study (50 mg/kg/day) which was administered 2 days prior and further 1 day post induction of colitis. On the other hand, Luk, et al., [(30)] demonstrated the protective effect of zinc sulfate on dinitrobenzene-induced colitis when administered rectally; this could point to the possibility of a potential cumulative effect of zinc sulfate given in a sufficient dose when used concomitantly via both oral and rectal routes of administration.

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Serum Lipid in Early Rheumatoid Arthritis

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Abstract

Background: Several investigators reported an excess of cardiovascular morbidity and mortality among RA patients. The majority of cardiovascular deaths results from accelerated atherosclerosis.

Elevated plasma total cholesterol (TC) and low –density lipoprotein cholesterol (LDL-C), decreased high – density lipoprotein cholesterol (HDL-C) are strong factors for atherosclerotic events.

Objectives: This study was done to:

1- Show the changes of serum lipid profile in patients with ERA.

2- Explain the pathological role of changing lipid profile in ERA and to demonstrate the changes of atherogenic ratio in ERA patients.

3- Presenting the correlation between lipid profile and different inflammatory markers especially ESR and CRP.

Method: Twenty five patients with ERA who met the American college of rheumatology (ACR) 1987 criteria for rheumatoid arthritis (RA) had early disease with disease duration of less than one year without prior use of disease modifying antirheumatic drugs (DMARDS) and or systemic steroids were examined for their lipid profile level and the relation of the atheroganic ratio to their disease were investigated during the period between March –December 2006 in the department of rheumatology at Al –Kadymia teaching hospital .Lipid profil (TC,LDL-C,HDL –C and TG)ESR and C-reactive protein were determined for both the patients and control groups.

Results: The results of the study revealed that ERA patients exhibited higher serum levels of total cholesterol (TC) low- density lipoprotein cholesterol (LDL) and triglycerides (TG) .Where as their serum high –density lipoprotein cholesterol (HDL –C) levels were significantly lower compared to control .As a consequence the atherogenic ratio of TC /HDL –C as well as that of LDL-C/HDL –C was significantly higher in ERA patients compared to controls and these changes were correlated with laboratory changes especially GRP and ESR .

Conclusion: ERA patients are characterized by an atherogenic profile in comparision with the healthy control subject .Recognition and treatment of early rheumatoid arthritis and reduction of these and other cardiac risk factor has greater impact on the course of the diseases.

Keywords: Lipid profile – rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disorder of unknown etiology that primarily involves joints. Extra – articular features of RA including anemia fatigue subcutaneous (rheumatoid) nodules pleuropericarditis neuropathy sclerities splenomegaly sjogrens syndrome vasculitis and renal disease may occur during the course of the disease [(1)].

There is an increased risk of premature death due to coronary artery disease in patients with RA and there may be an increase risk of heart failure [(2)]. The risk for decreased life expectancy and early cardiovascular mortality in particular, among people with rheumatoid arthritis is increasingly recognized. The increased

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risk of coronary heart disease may precede the onset and diagnosis of RA [3].

The risk in RA for cardiovascular death, thought to be increased more than tow folds over the general population, appears to be independent of the known cardiac risk factors. Risk factors for atherosclerotic events and cardiovascular disease include male sex, increased age, elevated plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and decreased high – density lipoprotein cholesterol (HDL-C), high blood pressure, smoking and diabetes mellitus[4].

The risk of sudden death of myocardial infraction appears to be increase in patients with RA. Athermatous plaques in the carotid artery and greater intima – media thickness are two markers that suggest the presences of generalized atherosclerosis.

By these measures, patients with RA have a greater burden of atherosclerotic disease than control .The prevalence of carotid plaques as detected ultrasonographically in patients with RA is correlated with the duration of disease.

- Changes in the endothelium due to circulating immune complexes, cytokines or C-reactive protein [5].
- Ahypocoagulable state due to increased plasma levels of fibrinogen ,von willebrand factor ,plasminogen activator inhibitor -1, and/or other acute phase reactants that correlate with the ESR[6].
- Direct vascular injury due to the dyslipidemia associated with glucocorticoid therapy or rheumatoid vasculitis [7].
- Depletion of circulating endothelial cell progenators [8].

Continued active inflammation as provided by repeated elevation of the erythrocyte sedimentation rate (ESR) to 60mm/hr may be an independent risk factor for cardiovascular death in patients with RA [9, 10].

A concentration of C reactive protein (CRP) 5mg/L may also be an independent risk factor for cardiovascular death [11, 12].

In general, and with some variations between different studies, the lipid profile of patient with active or untreated RA is primarily characterized by a decrease in serum levels of HDL-C where as contrasting result have been published on the serum levels of TC and LDL-C importantly, the reduction in HDL-C has as a consequence the increase in the TC/HDL-C ratio [13, 14]. This ratio represents an atherogenic index which is an important prognostic marker for cardiovascular disease indeed the risk of myocardial infarction increases considerably when this ratio is higher than five and it should ideally be four or less. The serum TC and HDL-C levels in RA are inversely correlated with disease activity suggesting potential role for inflammation in the atherogenic profile and the higher atherosclerotic risk observed in RA [15, 16].

Use of methotrexate and anti-tumor necroses factor (anti-TNF) agents may have a beneficial effect upon cardiovascular morbidity and mortality [17]. Because an increased prevalence of coronary atherosclerois may contribute to the elevated mortality rates of patients with RA the combination of lipid –lowering and anti-inflammatory would be a compelling rationale for the use of statins [18].

Methods

Inclusion criteria: Twenty five patients who met the American college of rheumatology (ACR) 1987 criteria for rheumatoid arthritis (RA) had early disease with duration of less than one year without prior use of disease

modifying antirheumatic drugs (DMARDs) and or systemic steroids were investigated during the period between March –December 2006 in the department of rheumatology at Al Kadhimiya Teaching Hospital.

Smokers or patients suffering from conditions that affect the lipid profile such as diabitus mellitus ,hypothyrodisim liver or kidney disease ,Cushing's syndrome ,obesity (body mass index >30)and a history of familial dyslipidemia ,were excluded . In addition patients receiving medication affecting lipid metabolism such as lipid –lowering drugs,beta blockers ,oral contraceptives estrogen ,progesterone ,thyroxin and vitamine E were excluded from the study . Twenty five apparently healthy, non smoking subjects also participated in the study and were considered as a control group. These subjects fulfilled the same exclusion criteria reported for the patient group. None of the subjects participating in the control group had a history of coronery heart disease. The control group was proportionally matched for age and sex to the patient group.

Overnight fasting blood samples were obtained from both ERA patients and the control groups. Serum lipids were determined within six hours of blood sampling .TC-, triglycerides and HDL –C were determined with enzymatic colorimetric method using shimadzu micro –flow meter CL-720 .LDL-C was estimated using the friedewald formula.

Friedewald formula=serum TC [serum HDL+Serum TG/5] [22]**.**

IgM rheumatoid factor was measured by EELIZA method. ESR was measured by the modified Westergen method. In addition complete blood count with differential, as well as serum glucose, liver and kidney function tests, were performed for all patients.

All data were analyzed by excel programme using the independent t-test of unequal variances considering ($p<0.05$) as significant difference.

Results

During the selection period (March –December 2006),twenty –five patients were included in the study .There were 22 women and 3 men with a mean age of 54.2=\9.6 years and mean disease duration of 0.5+-0.3 years ,The clinical characteristics and lipid profiles of patients and control are described in (Table 1).

1-Serum Total Cholesterol LDL-C and TG

The results of the patients had shown a high mean serum level of cholesterol in comparison to control group with a significant difference ($p<0.05$) as shown in (Table 2).

The mean serum level of LDL-C in patients was higher than the mean serum level of LDL-C in control group .But a non –significant difference ($p>0.05$) as shown in (Table 3).

On the other hand the mean serum TG level in patients was higher than the mean serum TG level in control group. There was a non-significant difference between serum cholesterol level of patients in comparison to that in control group ($p>o.o5$) as shown in (Table 4).

2- Serum HDL-C

The mean serum level of HDL-C in patients with ERA was lower than the mean serum level of control group. With a significant difference ($p<0.05$) as shown in (Table 5) .

3-The Atherogenic ratio

As a consequence of the above mentioned results regarding total cholesterol LDL-C , HDL-C and TG the mean atherogenic ratio of TC HDL-C was higher in patients with ERA than in control group with

significant difference ($p<0.05$) m as shown in (Table 6) .

The LDL-C, HDL-C was also higher in patients with ERA than the control group with a significant difference ($p<0.05$) as shown in (Table 7).

4- Correlation of TC , CRP , HDL-C , ESR

There was a significant direct correlation between serum TC and CRP in patients with ERA ($p<0.05$).

Serum HDL-C had shown a significant correlation in relation to CRP in patients with ERA ($p<0.05$), with an inverse correlation.

Serum TC had a significant correlation to ESR in patients with ERA ($p<0.05$), with a direct correlation as shown in (Figure 3).

Serum HDL-C had a significant correlation with ESR in patients with ERA ($p<0.05$), with an inverse correlation.

Table 1: Comparison between mean values ± standard deviation of patients with ERA and control group

Parameter	Control values ± S.D.	ERA patients values ± S.D.
Sex (male / female)	5/20	3/22
Age (year)	5504 ± 10.4	54.2 ± 9.6
Body mass index (BMI) (Kg/m^2)	25.3 ± 1.8	25.2 ± 2.4
IgM Rheumatoid factor (+/-)	0/0	21/4
C – reactive protein	2.1 ± 0.7	24.4 ± 10.8
ESR (mm/hr)	501 ± 1.7	49.9 ± 11.2
TC (mg/dl)	192.2 ± 18.5	217.9 ± 37.5
LDL (mg/dl)	125.7 ± 14.7	141.2 ± 24.2
HDL (mg/dl)	52.0 ± 5.5	40.5 ± 7.9
TG (mg/dl)	98.3 ± 13.4	133.4 ± 27.5
TC/HDL	3.74 ± 0.7	5.69 ± 1.37
LDL/HDL	205 ± 0.57	4.04 ± 0.9

Table 2: The relationship between Serum Total Cholesterol level in ERA patients and control group

Groups	Serum level of total cholesterol (mean ± S.D.) mg/dl	P value
Patients with ERA	217.9 ± 37.5	0.004
Control group	192.2 ± 18.5	

Table 3: The relationship between Serum LDL level in ERA patients and control group

Groups	Serum level of LDL (mean ± S.D.) mg/dl	P value
Patients with ERA	141.2 ± 24.2	0.07
Control group	125.7 ± 14.7	

Table 4: The relationship between Serum TG level in ERA patients and control group

Groups	Serum level of TG (mean ± S.D.) mg/dl	P value
Patients with ERA	133.4 ± 27.5	1.72
Control group	98.3 ± 13.4	

Table 5: The relation ship between Serum HDL-C level in ERA patients and control group

Groups	Serum level of HDL (mean ± S.D.) mg/dl	P value
Patients with ERA	40.5 ± 7.9	0.009
Control group	52.0 ± 5.5	

Table 6: Atherogenic ratio in ERA patients and control group

Groups	TC/ HDL (mean ± S.D.)	P value
Patients with ERA	5.6 ± 1.3	0.004
Control group	3.7 ± 0.7	

Table 7: LDL/HDL in ERA patients and control group

Groups	LDL/ HDL (mean ± S.D.)	P value
Patients with ERA	4.04 ± 0.9	0.004
Control group	2.5 ± 0.5	

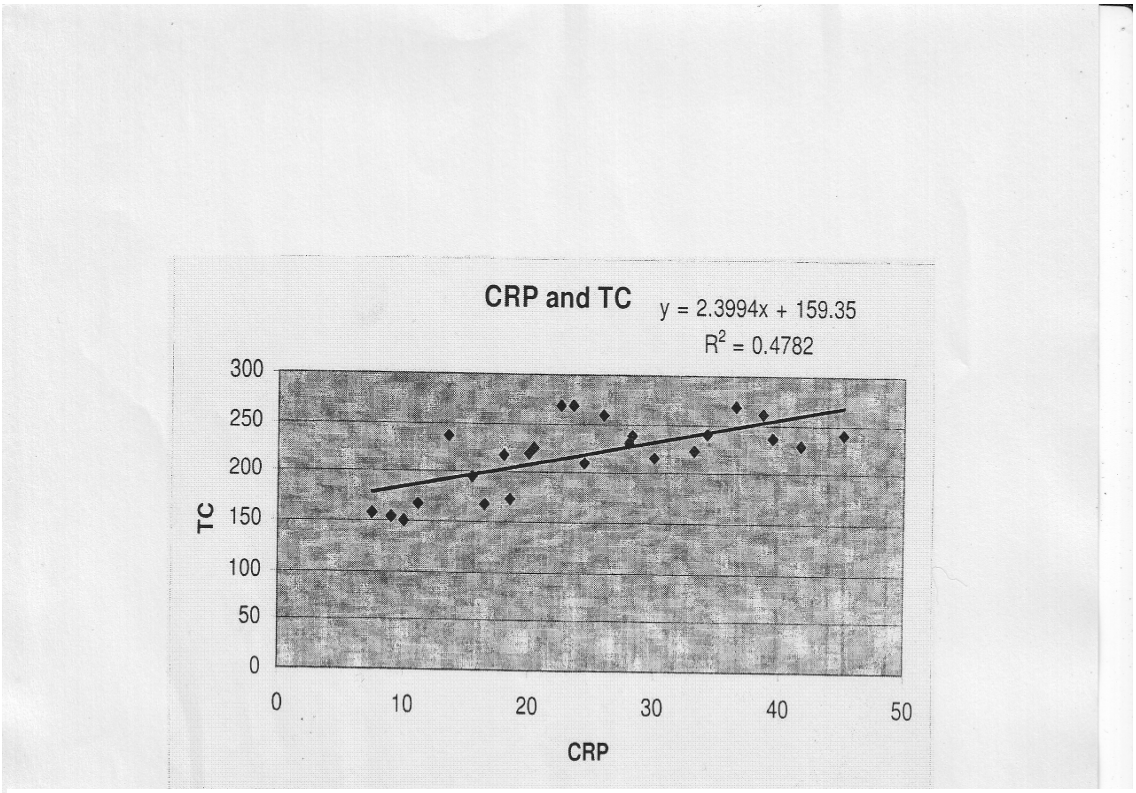

Figure 1: The correlation between CRP and TC in ERA patients.

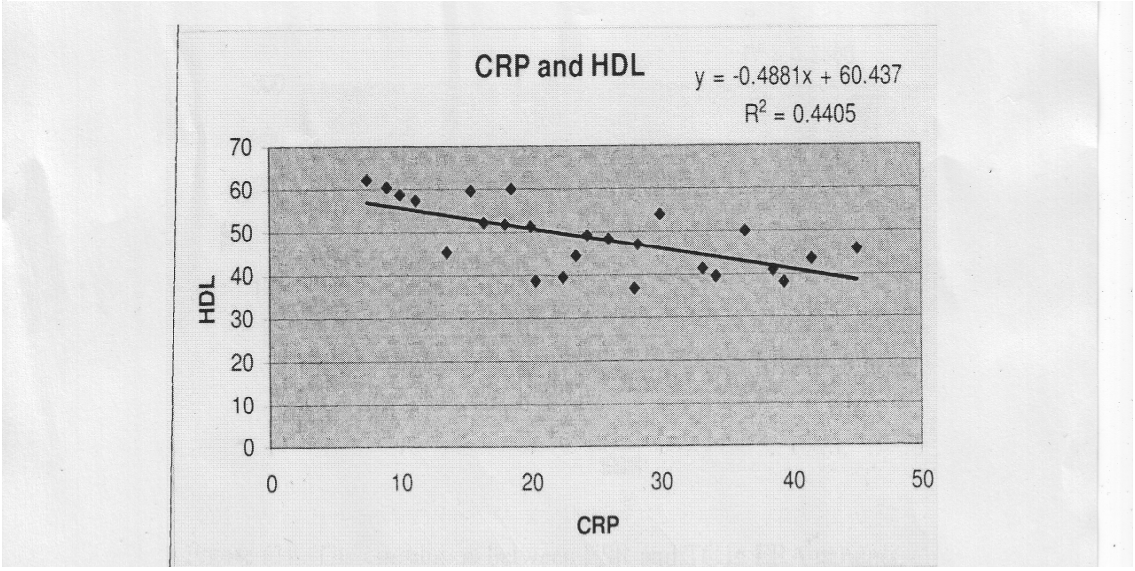

Figure 2: The correlation between CRP and HDL in ERA patients.

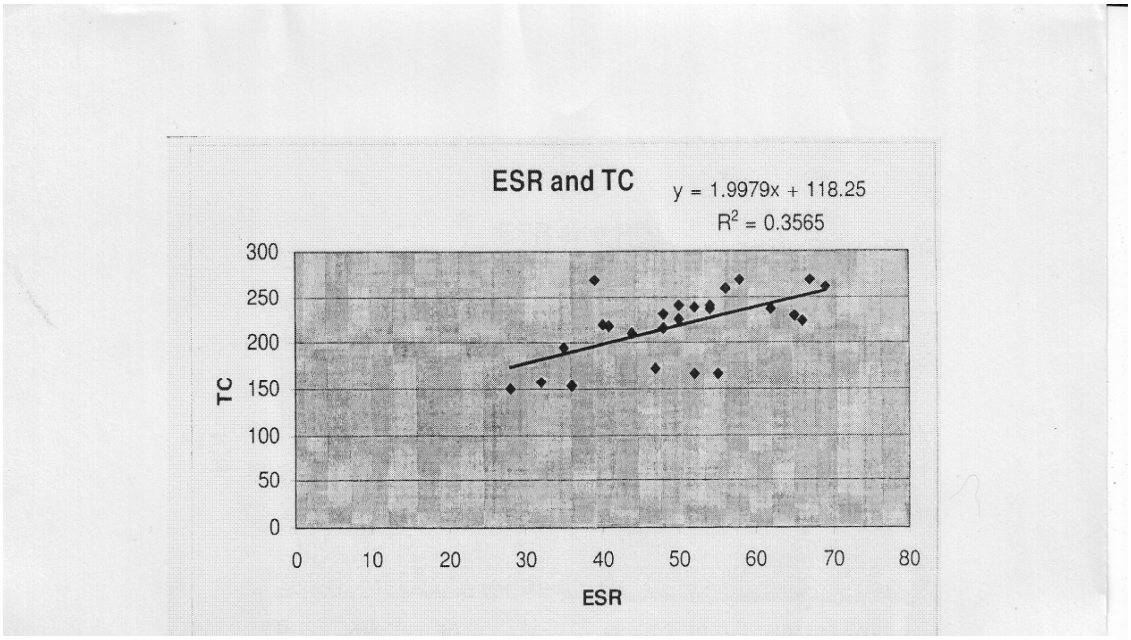

Figure 3: The correlation between ESR and TC in ERA patients.

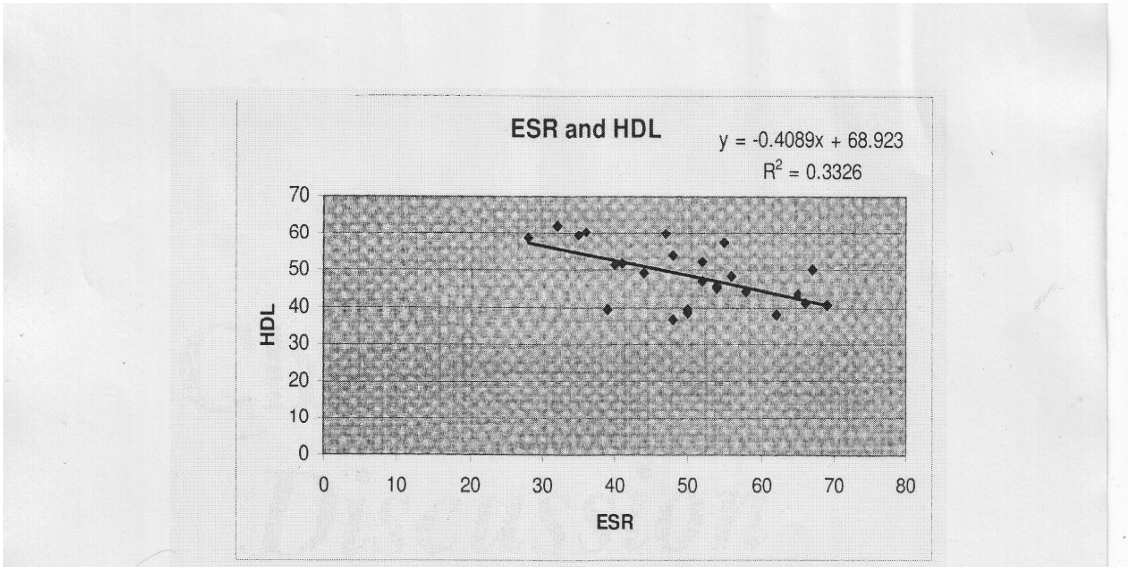

Figure 4: The correlation between HDL and ESR in ERA patients.

Discussion

The objective of this study was to determine the lipid profile of ERA patients had to investigate whether this could be influenced by disease activity in the early stages of rheumatoid arthritis. According to the results, patients with ERA exhibited an atherogenic lipid profile characterized by a significantly reduced serum level of HDL-C and as a sequence an increase in the atheroginic ratio of TC/HDL-C or LDL-C/HDL-C was observed in ERA patients, suggesting that these patients are possibly exposed to a higher risk of atherosclerosis.

The lipid profile of patients with ERA has been evaluated in several studies. Some of these studies have reported lower levels of HDL-C and TC, higher TC/HDL-C and LDL-C/HDL-C ratios in active and/or untreated disease than in general population [19, 20]. On the other hand other studies did not show significantly different lipid levels from those observed in the healthy population [21, 22], while others referred to an overall reduction in all lipid sub-fractions in cases of active disease [23].

These contrasting results may be attributed to the size of the sample, the type of the study (prospective or cross-sectional) and difference in the disease type (established or early) or to difference in disease activity. Patients in remission or with controlled disease show an increase in HDL-C levels and a reduction in the atherogenic index compared to patients with active disease.

Systemic inflammation may also play a role in the development of atherosclerosis [24]. In fact the increase in acute phase reactants in cardiovascular events has already been documented [25]. It has even been suggested that RA and atherosclerosis may share a common predisposing factor [26, 27].

CRP is the common denominator for both diseases CRP which increase in active disease may contribute to atherosclerosis because it stimulates macrophages to produce tissue factor, a procoagulant that is found in atherosclerosis plaques [28, 29]. The presence of CRP in atheromatic lesions also suggests a (cause and effect) relationship between this acute phase reactant and coronary events [30].

An important observation is that ERA patients exhibit low HDL serum levels. The decrement in HDL-C was inversely correlated with the increment of either CRP levels or ESR values. This suggests that inflammation is an important determinant for the reduced HDL-C levels observed in ERA patients. It is possible that RA patients may have some classic risk factors for atherosclerosis development [33, 34]. However it is not correct to attribute the increase prevalence of atherosclerosis observe in RA patients to those factors. In our study, we tried to exclude patients with classic risk factors for atherosclerosis and we found that ERA patients with high disease activity showed an adverse lipid profile before the commencement of therapy.

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Histological effects of melatonin on male rat's alveolar macrophages

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Abstract

Background: Melatonin is a neuro-hormone of the pineal gland. It increases and enhances immunity, whether in animals or human. The mononuclear – phagocyte system; is a single functional immune unit. The pulmonary alveolar macrophages are one of the most important members included within this immune unit.

Objective: This work tried to study the effect of different doses of dietary melatonin on adult rat's pulmonary alveolar macrophages.

Methods: Melatonin was supplied to adult 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 a daily dose of melatonin as 125, 250, 500, 750 and 1000 µg / kg body weight, respectively. After the last day of treatment, the left lung of the rat was removed under anesthesia for histological study.

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

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

Key words: Melatonin, immunity, and alveolar macrophages.

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Introduction

Melatonin is a neuro-hormone of the pineal gland, secreted mostly at night time [(1)]. It increases and enhances both cellular and humoral immunity, whether in animals or human [(2, 3)]. The mononuclear – phagocyte system; is a single functional unit of immunological system, consists of bone marrow precursors (monoblasts and promonocytes), circulating monocytes and tissue macrophages, both free and fixed (histiocytes).

Thus as one member of the immune system; macrophages are affected and activated by melatonin effect [(1, 2, 3)] .The pulmonary alveolar macrophages are one of the most important members included within this immune unit [(4, 5)]. It would be of great interest to study the effect of dietary melatonin on these alveolar macrophages.

Materials and methods

Forty eight Adult male Wister albino rats were used in this work. They were kept in an animal room, with a temperature of 22±2C°, 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

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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 drug used in this work was N-Acetyl-5-methoxytryptamine (melatonin) tablets, from Nature's Bounty INC, Bohemia NY 11716, and USA. After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole left lung was removed, and processed for paraffin section, using Bouin's solution for fixation and (Haematoxylin & Eosin) for staining. Then 5 serial sections of 5 µm thickness were studied [6].

Histological study was done both as descriptive and morphometric by light microscope. The morphometric data were estimated by using objective micrometer used on a light microscope; by which a distance of 10µm could be calculated, so the average widest diameter of pulmomary alveolar macrophages, as well as the diameter of their nuclei, was 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 [7].

Results

The morphometric results:

In all of the treated groups; there were significant increase ($p<0.05$) both in the average widest diameter of the macrophages and the average diameter of their nuclei (Table1).

The descriptive results:

Unusual types of cells were appeared; among which was the large type of macrophages seen mostly in group II and III. They were large polygonal, with darkly stained cytoplasm containing many vesicles, vacuoles and particles. Their nucleus was pale basophilic and eccentric. They were frequently located near the blood vessels in comparison with the control (Figure1 and 2).

The second type of cells seen was the epithelioid cells; they were large type of macrophages with voluminous pink-stained cytoplasm and pale basophilic, eccentric nucleus. They were viewed mostly in the group IV, V and VI (Figure 3).

Multinucleated giant cells were regarded to represent the third type of unusual cells. They were noticed only in animals received doses of 750 and 1000µg/kg.Those cells were very large, irregular in shape, with pale acidophilic cytoplasm filled with granules, vesicles and debris (Figure 4).

The vascularity of the pulmonary tissues as a whole was positively increased with the stepping up doses of melatonin, till the last dose 1000µg/kg; where areas of hemorrhages appeared (Figure5).

Table1: Average widest pulmonary alveolar Macrophage's diameter and their nuclear diameter in µm, of adult rats; treated with dietary melatonin.

Daily dose of melatonin in µg/kg body wt	***Average alveolar Macrophage's diameter (in µm)***	***Average nuclear diameter of alveolar Macrophage's (in µm)***
Control	16.3±1.6	9.7±1.4
125	19.1±1.8*	11.2±1.8†
250	29.7±2.9**	15.1±2.7**
500	44.3±5.1**	18.9±4.2**
750	91.4±11.4**	(multiple) 21.3±7.1††
1000	168.5±21.6**	(multiple) 24.1±9.1‡

-Data were expressed as mean ± SD.
(* P<0.004; **P<0.0001; † P<0.03; †† P<0.008; ‡ P<0.006).

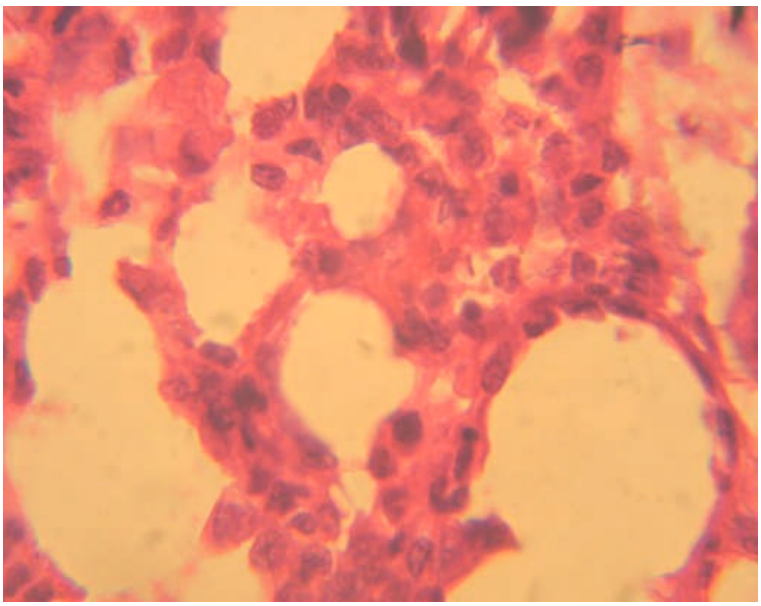

Figure 1: Lung tissue in the control adult rat. H&E, X400

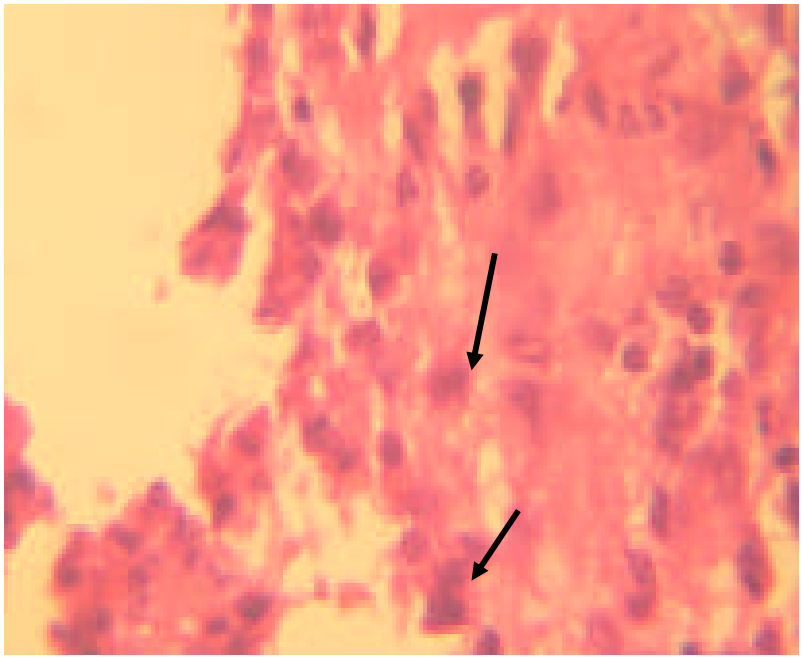

Figure 2: Lung tissue in adult rat treated with 250 µg/kg doses; macrophage (arrows). H&E, X400.

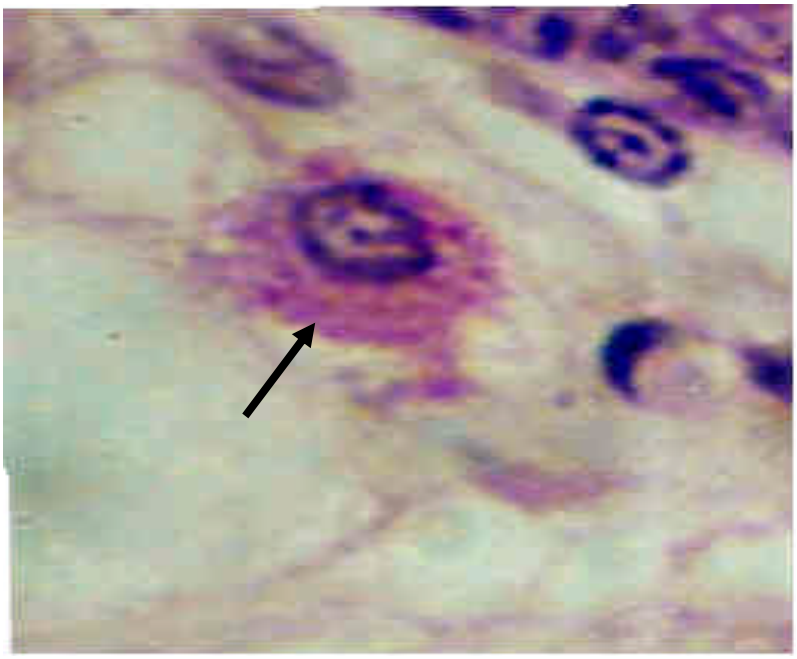

Figure 3: Epithelioid cell (arrow). H&E, X 800.

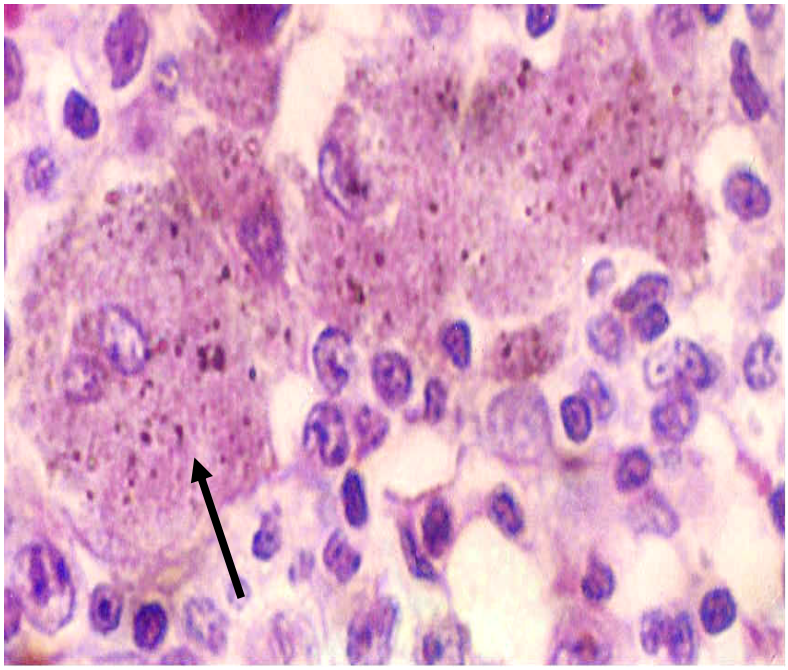

Figure 4: Multinucleated giant cell (arrow).H&E, X450

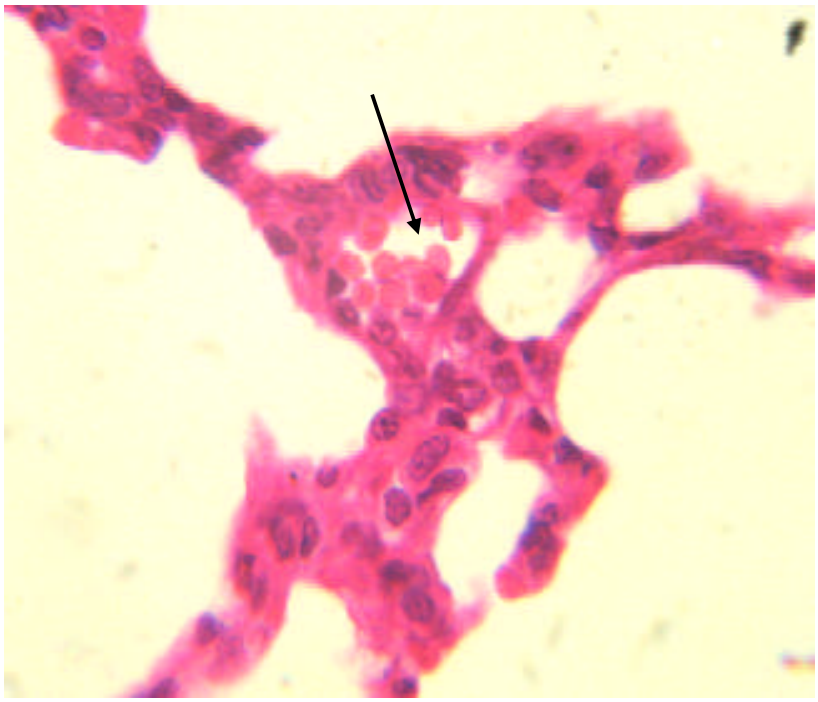

Figure 5: Dilated blood vessel (arrow) in lung tissues in rat given a dose of 500µg/kg. H&E, X400

Discussion

The average widest diameter of pulmomary alveolar macrophages, as well as the diameter of their nuclei was significantly increased by melatonin in the instant work. The explanation for this might be highlighted by the fact that those parameters principally follow the macrophage function status [8, 9]. The physiological condition of the pulmomary alveolar macrophages is determined basically by their histological appearance; so they are considered to be actively functioning whenever their size are larger with paler and larger nuclei, whereas they are said to be insufficient in case they are being smaller with darker relatively smaller nuclei [4, 5, 10]. Hence in the groups treated with 125 and 250µg/kg dose; the unusually large macrophages reflected an actively functioning cells, whereas, the epithelioid cells might represent the over stimulating view, since they are well documented to be seen only in hyper stimulating situations [2, 3, 5, 10].

The other interestingly existed cells in the ongoing study were the multinucleated giant cells; which appeared to highlight a very toxic condition, because those exceptionally rare cells are formed when epithelioid cells coalesce to make huge multinucleated masses termed the foreign body giant cells; that regarded as a characteristic finding consequently seen only in pathological and/or toxic cases. This could be due to the concept that melatonin is a well designed to exert its physiologic action in a dose – dependent manner, being stimulating at normal therapeutic level and harmful at its overabundance [11, 12]. Those findings might indicate the enhancement in the function of macrophages, as a consequence of exogenous melatonin on those cells, affecting them directly through melatonin receptors found in all tissues and cells [13, 14], and/or indirectly through the well known cytokines, namely; granulocyte – macrophage colony stimulating factor (GM – CSF), which is secreted by the macrophages, endothelium and T lymphocytes; activating these cells, and/or through macrophage colony stimulating factor (M- CSF), since the melatonin is the hub director of all types of immunity[2, 3, 15, 16].

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 [4, 5].

The dilated blood vessels watched in the forgoing study; could be due to the fact, that melatonin has a well known vasodilator action [17]. Those results could be explained by the fact; that melatonin has damaging effects only when it is administered in excess [11, 12].

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 immunity status [18, 19, 20].

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Effect of melatonin on histology of the epididymidis of adult rat.

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Abstract

Background: The spermatozoa are provided with the needed capacity for normal motility, in the epididymidis, so the function of epididymidis 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: To study the effect of different doses of dietary melatonin on the histology of adult rat's epididymidis.

Methods: Melatonin was supplied to adult Wister albino rats with their diet, for successive 30 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 the last day of treatment, animals were killed under effect of anesthesia; epididymidis was removed, fixed in Bouin's solution and processed routinely for histological study.

Results: The results showed significant positive effects on epididymidis, since it increased the epididymal wall thickness, epididymal, as well as spermatozoal clump within epididymal tubules, with normal therapeutic dosages, whereas significant damaging effects were seen with raising dosages.

Conclusion: Dietary melatonin has clear positive effects on the rat's epididymidis within therapeutic doses, since it increased the epididymal wall thickness, epididymal, as well as spermatozoal clump within epididymal tubules, whereas it had highly damaging changes in surplus doses.

Keywords: Epididymidis, melatonin, and infertility.

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Introduction

The epididymidis is the site of accumulation, storage and physiological maturation of spermatozoa; hence spermatozoa get their capacity for normal motility [1, 2], so the function of this part of male genital system is very vital for the normal fertility [3, 4].

Melatonin is the basic neuro-hormone of the pineal gland [5, 6].

This hormone evidently plays an important regulatory role in the sexual and reproductive activities in all mammals including man [7, 8, 9], hence, it would be of great interest to study the relationship between melatonin and epididymidis structural and so functional status. Histological morphometric study could be 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 each epididymidis wall, lumen, and

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spermatozoa clump within the lumen, could give the idea about the structural and hence the functional status of epidiymidis.

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 of 22±2C°, 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 sundown.

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. After the last day of treatment, all animals were killed by dissection under effect of diethyl ether. The whole epididymidis was removed, separated from the surrounding connective tissues under a dissecting microscope, weighed by an electric sensitive balance. Fixed in Bouin's solution, embedded in paraffin, and processed routinely for histological study. Then 5 serial sections of 5 µm thickness from the mid- part (body) of the left organ were stained with Haematoxylin & Eosin and selected for study [10, 11]. epididymidis was removed, under a dissecting microscope, weighed by an electric sensitive balance.

Histological study was done both as descriptive and morphometric by a 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 each epididymidis wall, lumen, and spermatozoa clump within the 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 [12].

Results

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

Epididymidis weight was unaffected significantly in all groups (Table 1).

Morphometric results:

(1) The number of points overlying the epididymal epithelial wall, was raised till the dose of 500µg/kg, then it was significantly decreased at the dose of 750µg/kg, and a great decrease was clear at group received 1000 µg/kg (Table2).

(2) The number of points superimposed on the lumen of the epididymidis, followed an opposite manner to that of the wall (Table2).

(3) The number of points superimposed on the spermatozoa clump within lumen of the epididymidis, followed an opposite manner to that of the wall (Table2).

The descriptive histological result:

Cells of the epididymal epithelial wall, in the groups treated with 125, and 250µg/kg were almost thicker than that of the control group (Figure 1); so each epididymal duct was bound by a single layer of specialized epithelium which rest on a thick basement membrane and enclosed a lumen filled with clumps of spermatozoa which are more abundant than that of the control. The epididymal duct had tall columnar epithelium bearing numerous very long microvilli, and basal nuclei (Figure 2).In the group treated with 250µg/kg, apoptotic and pyknotic cells seen frequently.

In groups received 500 µg/kg dose, cells of epididymal duct were seen commonly tall columnar epithelium, having basal nuclei which were appeared as more crowded at the periphery of the duct, with unusually long micovilli, lined narrow lumina which were noticed to have less population of spermatozoa (Figure 3).

The group treated with 1000 µg / kg dose: The epididymal duct was viewed with thickened basement membrane, some areas showed fibrosis & necrotic changes. There was abundance in the number of spermatozoa , epithelial cells were looked more or less regressed in their height, their nuclei were viewed as less abundant at periphery of the duct, the micrivili also seemed to be shorter, and the lumina were appeared larger (Figure 4).

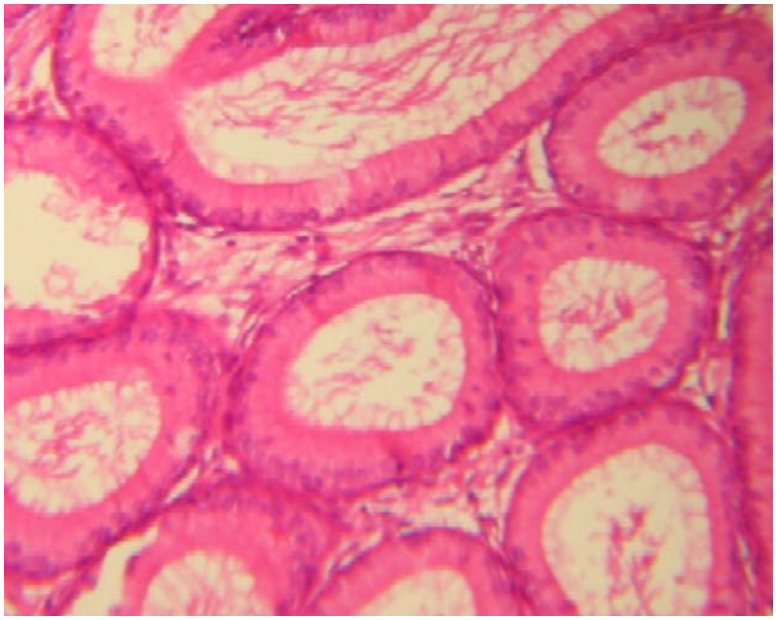

Figure 1: Epididymidis in control adult rat, X 200, H&E.

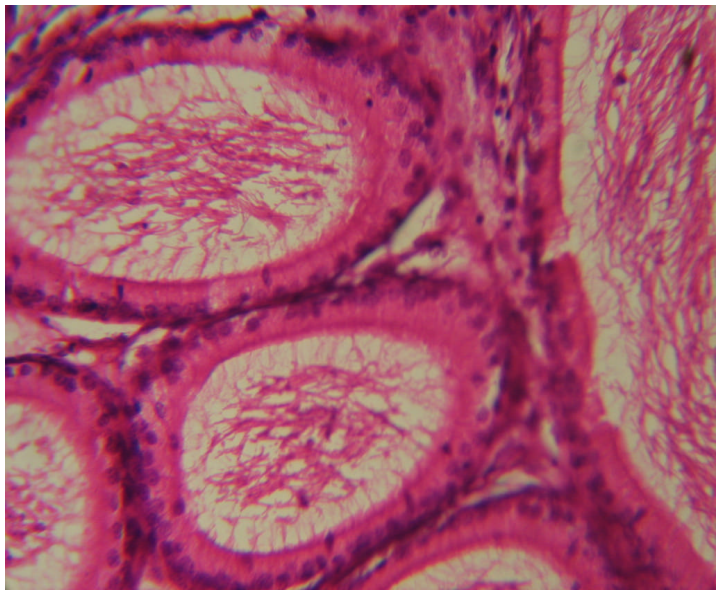

Figure 2: Epididymidis of adult rat, treatedwith 250µg/kg dose, epididymal wall thickness increased, lumen dilated and spermatozoal Clumps became abundant than that of control, X 200, H&E.

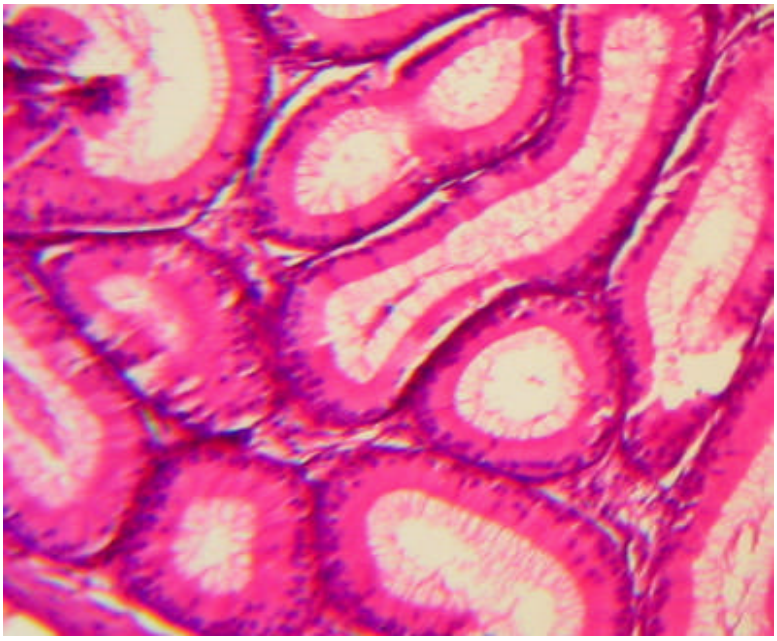

Figure 3: Epididymidis of adult rat treated with 500µg/kg dose, epididymal wall thickness increased, lumen became smaller and spermatozoal clumps diminished than that of control, X 200, H&E.

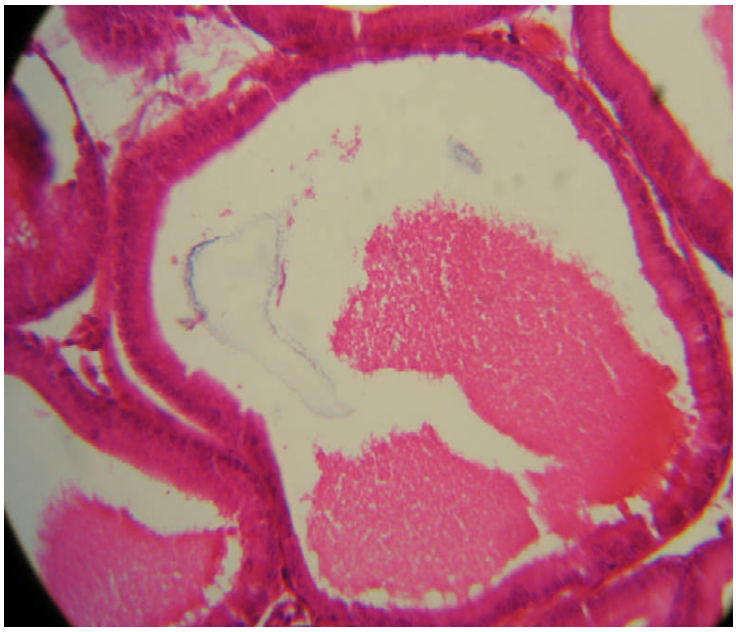

Figure 4: Epididymidis of adult rat treated with1000µg/kg dose, epididymal wall thickness decreased, lumen dilated hugely and spermatozoal clumps became much abundant than that of control, X 200, H & E.

Table 1: The Effect of Melatonin on Epididymal Weight of Adult Male Rats.

Daily dose of melatonin in µg/kg body weight	***Epididymal weight at autopsy (in mg) of adult rats***
Control	4.3±64.3
125	4.9±28.4 **NS**
250	5.1±23.2 **NS**
500	5.9±26.3 **NS**
750	6.6±31.1 **NS**
1000	9.2±61.3 **NS**

-Results were expressed in mean± SD of 8 rats.
-The difference of each dose group was statistically insignificant when compared with its control:
(* P>0.05; NS= not significant).

Table 2: Number of Points Overlying the Wall and Lumen, as well as the Spermatozoal Clump, in Epididymidis 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 epididymal wall***	***Points on epididymal lumen***	***Points on epididymal Spermatozoal clump***
Control	16.17±1.93	31.96±1.34	7.33±2.15
125	17.16±0.09 **NS**	41.41±1.21*	10.15±0.11‡
250	17.98±1.01†	40.35±1.02*	11.06±1.02*
500	29.87±1.62*	10.26±1.31*	5.09±1.42**
750	21.26±1.07 **NS**	19.24±1.28*	6.92±1.91**NS**
1000	11.13±1.05*	51.92±1.97*	24.43±2.16*

-Data were expressed as mean ± SD of 8 rats.
-When any dose-group was compared with its control, the difference was statistically significant:
(* P<0.00001; ** P<0.004 † P<0.008; ‡ P<0.02; NS= non significant).

Discussion

The epididymal weight was significantly unaffected by melatonin in the instant work (Table 1). The explanation for this might be highlighted by the fact that epididymal weight principally dose not follow its function status [13]. Changes in epididymal wall thickness, lumen diameters, and spermatozoal clumps showed a clear positive effect of melatonin on those parameters (Table 2); i.e., they were steadily increased with the increase in amount of doses up to the level of 500 µg/kg dose, then after decreased with 750 µg/kg dose and they were noticed to increased again at 1000 µg/kg dose. This could be due to the concept that melatonin is well designed to exert its physiologic action in dose – dependent manner, being stimulating at normal therapeutic level and harmful at higher doses [14, 15].

The epididymal tubule wall was significantly thicker with more frequent existence of nuclei observed in those groups treated with 125 , 250 and 500 µg/kg dose, and much less in group of 750 µg/kg ,then regressed at 1000 µg/kg dose, these findings might indicate the increase in number of epidiymal epithelial cells, which could be the consequence of exogenous melatonin on the those cells, and affecting their function directly through melatonin receptors found in all tissues and cells [16], or indirectly through the pituitary gland affecting its secretion of FSH there by promotes other sexual

hormones secretion [17]. Nevertheless, direct and/or indirect role, also there could be probably an induction of Sertoli cells to secret increasing 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[18, 19]. The epididymal wall thickness was decreased with 750 µg/kg doses and a great regression noticed at 1000 µg/kg dose. This could be due to the concept that melatonin is stimulating at normal level and harmful at higher doses [14, 15].

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 therefore plays an important feed back role in controlling the suppression of inhibin, which could be the cause of that regression consequently [4, 13].

The number of points overlying the spermatozoa clump within the duct was increasing incrementally in the groups treated with the dose of 125 and 250 µg/kg, then at the dose of 500 µg/kg, it was adversely proportionate with those points on the wall & lumen of the tubules, this may be due to the effect of melatonin either directly on the main cells of spermatogenic lineage, through melatonin receptors proposed to be present in all body tissues and cells [16], and / or indirectly by melatonin effect on hypothalamic-hypophysial axis suppresses the secretion of FSH, hence decreases cells of spermatogenic lineage activity and number [20]. The other proposal explanation could be through over stimulation of these Leyding cells by melatonin inducing over secretion of androgen; which acts by its negative feed back mechnism on hypothalamus leading to suppression of FSH secretion also [1, 4] .

The increase in frequency of apoptotic and pyknotic cells seen in groups treated with 250 µg/kg, might be caused by the effect of melatonin on Sertoli cells to control the large number of spermatogenic cells competing for survival in a so called programmed cell death (apoptosis) which is very different from that which occurs as a direct result of deleterious events to the cells, termed necrosis [1, 4, 13].

In the group treated with 1000 µg/kg dose, some areas showed fibrosis & necrotic changes, this picture offers the extent of the highly damaging effect exerted by that dose of melatonin, since in any damaging events to any given tissue, a similar histological view will be sighted [1, 13, 17, 21]. Those results could be explained by the fact that melatonin has damaging effects only when it is administered in high doses [14, 15]. 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], which are the active collagen – secreting cells and the basic forming cells of the connective tissues [1, 4, 23]. The increase in spermatozoa clump might be the consequence of decrease in motility of the spermatozoa so accumulated inside the widened lunina [13, 17].

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Cardiac arrhythmias in chronic obstructive pulmonary disease

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Abstract

Background: As a result of hypoxia, acidosis and effect of drugs used in chronic obstructive pulmonary disease arrhythmia can develop in many patients. Improvement in pulmonary function will result in decreasing the incidence of arrhythmia

Aim of study: To describe the frequency of cardiac arrhythmia in patient with COPD recorded by 24 hour holter monitoring and their relationship to clinical and homodynamic factors.

Patients and methods: Fifty patient with COPD and fifty patients with normal people monitored by pulmonary function test, 12 lead standards ECG and 24 hour holter monitor.

Result: Different types of arrhythmia were seen in patients with COPD and there is increase incidence of arrhythmia with the development of cor-pulmonal

Key words: ECG (electrocardiograph), COPD (chronic obstructive pulmonary disease), arrhythmia, ectopic.

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Introduction

The initial evaluation of patient suspected of having a cardiac arrhythmia begins with careful history, addressing specific questions regarding the presence of palpitation, syncope, spells of lightheadedness, chest pain or symptoms of congestive heart failure[1].

Palpitation may results from irregularities in cardiac rate or rhythm or a change in contractility of the heart [2]. The physician should inquired about circumstances that can trigger the arrhythmia, such as emotionally up setting event, ingestion of caffeine-containing beverages, cigarette smoking, exercise, excessive alcohol intake, or gastrointestinal problems[1,2].

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A careful diet and drug history can be useful, for example, in revealing that palpitations develop only after the use of nasal decongestant that contains sympathomimetic vasoconstrictor or in revealing that the patient has been exposed to street drugs such as cocaine. Clinical States that predict the genesis of arrhythmias should be considered, such as thyrotoxicosis, pericarditis, mitral valve prolapse [3, 4].

Variety of familial disorders can result in arrhythmias including myotonic dystrophy, Duchene muscular dystrophy, dilated cardiomyopathy and congenital conduction disorders can result in sudden death duo to arrhythmias [5, 6, 7].

Patients and methods

This is prospective study that was done on one hundred patients who have been admitted to medical ward and out patient clinic of University Hospital of Al-nahrian College of medicine during the period from the first of June to fifteenth of October 2004.These patients were divided into two groups.

Group A, 50 patients age 40—75 years are complaining of cough, exertional dyspnoea, scanty sputum and have history of smoking for at least 30 years.

Group B, 50 patients who are non smoker considered as control

Full history, including drug history and clinical examination was done for both groups. Also investigations were done for both groups include.

1- Chest X rays
2- Pulmonary function test
3- Electrocardiography
4- Serum electrolyte such as serum potassium.
5- Haematological investigation such as measurement of PCV.
6- echocardiographical Study
7- Holter monitoring for 24 hours

Patients who receive bronchodilator and diuretic are included in this study while those who are compiling ischemic heart disease and who receive digoxin or other anti-arrhythmia are excluded from this study.

Result

The result of clinical characteristic and laboratory investigation, pulmonary function test, standard ECG and 24 hour holter monitor were compared between group A (COPD) and the control group are shown in (Table 1 , 2 , 3,and 4) respectively.

Table 1: Characteristic of patients

characteristic	Group A	Group B
age	55±15	55±15
male	80%	80%
female	20%	20%
smoking	98%	0%
PCV	40%-55%	35%--45%
Serum K. µmol/L	3.4—4.7	3.4---5.0
Presence of oedema	20%	0%
History of admission	47%	4%
Pulmonary hypertension by echo.	50%	0%

Table 2: Characteristic of pulmonary function test

Pulmonary function test	Group A	Group B
FEV_1	0.5—2.8	2.6--3.5 L
FVC	0.9—3 L	2.7 L—3.9 L
FEV_1/FVC	33%--68%	90±10%
PEFR	1.4 –5.2 L/S	4---6 L/S
R. volume	2 L—6 L	1—2 L
T.L.capicty	5.2 L—9.8 L	5 L—7 L
Increase R.V.	O.5 L—5.1 L	
Increase T.L.C.	1.3 L—4 L	

FEV=forced expiratory volume, FVC=forced vital capacity, R=Residual, T.L.C=total lung capicty, R.V=residual volume

Table 3: Standard ECG in patients with (COPD) Group A & Group B

Standard ECG	Group A	Group B	P value
No arrhythmia	76%	84%	0.15
Sinus tachycardia	50%	20%	0.0005
P.pulmonal	20%	2%	0.01
Atrial ectopic	18%	8%	0.06
Ventricular ectopic	22%	6%	0.009
Atrial fibrillation	10%	4%	0.12
Atrial flutter	4%	2%	0.28
Run of SVT	2%	0%	o.16

SVT=Supraventricular tachycardia

Table 4: 24 hour holter monitoring both groups & p value

	Group A	Group B	P value
Sinus tachycardia	80%	10%	0.0001
Ventricular ectopic	64%	40%	0.006
Atrial ectopic	72%	50%	0.01
Atrial fibrillation	24%	8%	0.01
Atrial flutter	12%	2%	0.09
Heart block	12%	2%	0.09
S.V tachycardia	20%	4%	0.019
Run of VT	10%	2%	0.05
W.P.W	2%	0%	0.15

VT=ventricular tachycardia, W.P.W=Wolff Parkinson white syndrome

Table 5: Comparison of incidence of arrhythmia detected by the standard ECG and holter monitoring

	Absent%	Present%	Atrial ectopic%	Ventricular ectopic%
Standard ECG	76%	24%	18%	22%
Holter monitoring	16%	84%	72%	64%

Discussion

Many if not most arrhythmias occur intermittently and patients present to their physician having had a previous episode but without an arrhythmia occurring at the time of evaluation. Therefore, the suspicion that an arrhythmic problem exists as well as the necessity and urgency of further evaluation must frequently determined by the history alone [8, 9, 10].

Cardiac arrhythmias are common in patient with COPD. This study supports the fact that both ventricular and Supraventricular premature beat occur frequently in patients with chronic obstructive lung disease. Their frequency in this population is in fact similar to that observed in high risk patient with

coronary heart disease and it is of high frequency when compared to the normal people [11,12].

These study shows, the people with cor-pulmonal manifested by low FEV_1 and low FVC, high residual volume and high total lung capacity and echocardiography finding have high incidence of arrhythmia than control group (B) p. value 0.007.

Thomas and valabhji detect arrhythmia by standard electrocardiogram in 7% of patients with chronic obstructive lung disease [12].

Corzza and pastor examined the frequency of arrhythmia in patients with chronic obstructive lung disease during standard electrocardiograph are 31% of them had arrhythmia [13,]. That compatible with our study which shows frequency of arrhythmias during standard electrocardiography are 24% of patients with chronic obstructive lung disease.

The arrhythmias detected in patients with chronic obstructive pulmonary by 24 hour holter monitoring more frequency than arrhythmias by standard electrocardiography.

Ventricular ectopic, Atrial ectopic, sinus tachycardia, Atrial fibrillation and Supraventricular tachycardia high frequency in patients with chronic obstructive pulmonary disease than other control group as mention in (Table 4).

The mechanism of arrhythmia in chronic pulmonary disease is not known but is probably diverse and multi factorial including hypoxemia, acidosis, bronchodilator therapy and electrolyte imbalance [14].

Conclusion

patients with sever obstructive pulmonary disease have more risky for arrhythmias than other people and most of arrhythmias detected by 24 hours holter monitoring rather than 12 lead ECG. Patients with cor-pulmonal have more risk for arrhythmias. the arrhythmia in COPD include atrial ectopic 72%, ventricular ectopic 64%, atrial fibrillation 24%, atrial flutter 12%, heart block 12%, Supraventricular tachycardia 10%, and W.P.D(Wolff Parkinson white syndrome)2%.

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Thymoma a Clinicopathological Study in Iraqi Patients

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Abstract

Background: Thymoma is a rare tumor, it represent 0.09% of all tumors in Iraq still it's the most frequent anterosuperior mediastinal tumor.

Aim of the study: Study the clinicopathological features of thymoma in Iraqi patients.

Materials and Methods: A retrospective study on Fifty-one cases of thymoma, randomly selected from three specialized cardiothoracic centers in Baghdad for the period from Jan.1991 to Oct.2004. Paraffin blocks were collected, and 4 micrometers sections were stained with Hematoxyline & Eosin stain. Thymoma was classified according to the most recent WHO histopathological classification (1999). And staged according to Masaoka`s staging system (1981), TNM staging system (1991) and GETT staging system (1991.

Results: The fifty one cases included 19 females (37.25%) and 32 males (62.75%) their age ranged from 11-67 years with a mean of (39 +13.6) years. About half of the patients (54.9%) were found to have myasthenia gravis. WHO sub typing revealed that B2 was the most frequent subtype (35.3%). A significant correlation was found between WHO classification system &, Masaoka`s &TNM staging system, and with the sex of the patients.

Conclusion: MG was the only paraneoplastic syndrome diagnosed in this study. A significant correlation was found between WHO classification system & Masaoka`s & TNM staging system, and with the sex of the patients.

Key Words: Thymoma, Myasthenia gravis, Mediastinal tumors.

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Introduction

Thymoma is the neoplasm of thymic epithelial cells, independently of the presence and/or the number of lymphocytes [1, 2].

According to Iraqi cancer registry the frequency of thymoma in IRAQ is 23.5% of all mediastinal tumors, and 0.09% of all cancers in IRAQ [3] In USA the over all incidence of thymoma is 0.15/100,000 persons with slight female predominance. While in Europe the incidence of thymoma is 0.18/100,000 for male and 0.10/100,000 for females [4].

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The most widely used histopathological classification systems were Lattes- Bernatz (L-B) 1962 and Muller-Hermilink (M-H) 1985, at 1999 a system adapted by WHO committee which incorporates between L-B and M-H. The most widely used staging system of thymoma is the Masaoka`s, it depends on encapsulation, degree of invasion and metastasis [5, 6]. Another staging system is adapted the TNM system 1991, in which Masaoka`s system is incorporated with some modifications [7, 8]. A third staging system described by the French study group of thymoma called the GETT system based on level of surgical excision, it tends to down grade the Masaoka`s staging system [9,10] . Prognosis of thymoma depends on several factors as stage of invasion (the single most important factor),

microscopical type (the WHO subtypes are closely correlated to the prognosis and likelihood of invasion as follows: A<AB<B1<B2<B3) [6]. Myasthenia gravis most common paraneoplastic syndrome proved to have no effect on prognosis [9-11].

Materials and Methods

A retrospective study on 51 cases of Thymoma was randomly selected from three cardiothoracic centers in Baghdad (Ibn-Al Nafes Hospital, Special Surgery Hospital, and Al-Kademia Teaching Hospital) from Jan 1991 to Oct.2004. All the specimens considered were completely or subtotally resected mediastinal tumors, tumor other than Thymoma were excluded. Clinical data were taken from patients' case sheets. H& E sections were re-examined and classified according to WHO classification into subtype A (medullary), AB (mixed), B1 (predominantly cortical), B2 (cortical) and B3 (epithelial). Three main staging systems were used Masaoka`s, TNM and GETT staging systems. Staging was accomplished retrospectively using the operative notes and pathological reports. WHO histopathological subtypes and tumor staging systems were correlated with the clinical data.

Results

Forty three cases (84.4%) were malignant Thymoma and 8 (15.6%) were benign. Thirty-two patients (62.7%) were males and 19 (37.3%) were females. Patient's age ranged from 11–67 years (mean of 39 years, SD=13.6) .Major presentation (49%).was at fourth and fifth decades of life. Gender was significantly correlated to Masaoka`s and TNM staging systems (p value <0.05), male patients were presented in advanced stages, while females were in early stages. Forty patients (68.5%) were symptomatic and the most common was myasthenia gravis in 54.9%, followed by chest pain 37.2 %, and dysopnea 25.4%. Myasthenia gravis patients, male to female ratio was (1.5:1), their age ranged from 22–50 years (mean =36 years).

WHO classification shows that most frequent subtype is B2 (35.3%) and least frequent is AB subtype (7.8%). Myasthenia gravis was seen in B2 and B3 subtypes (32%, 29% respectively) A significant correlation was found between WHO subtype and gender (p value <0.05) the most frequent subtype in male was B2 (27.5%) while in female subtypes A & B1 (11.8%) each, (Table 1).

WHO subtypes correlated significantly with Masaoka`s staging system (p value<0.004), (Table 2). WHO individual subtypes correlated significantly with TNM staging systems. (P value <0.004), (Table 3). WHO risk groups did not correlate with GETT staging system neither as an individual subtypes nor as risk groups.

Table 1: Correlation between Gender and WHO (Histopathological) Subtypes of Thymoma.

Sex- Histological type (WHO) correlation.

		Histological type (WHO)					
		A	AB	B1	B2	B3	Total
Male	Number of cases	2	3	4	14	9	32
	Percent of T	3.9%	5.9%	7.8%	27.5%	17.6%	62.7%
Femal	Number of cases	6	1	6	4	2	19
	Percent of T	11.8%	2%	11.8%	7.8%	3.9%	37.3%
Total	Number of cases	8	4	10	18	11	51
	Percent of T	15.7%	7.8%	19.6%	35.3%	21.6%	100%

Table 2:Correlation Between WHO Subtypes of Thymoma and Masaoka`s staging system.

Correlation between Histological type (WHO) and Masaoka's staging system.

			Masaoka's staging system					Total
			I	IIa	IIb	III	IVb	
Histological type (WHO)	Aa	Number of cases	3	3	1	1		8
		Percent of Total	5.9%	5.9%	2%	2%		15.7%
	AB	Number of cases	1	1		1	1	4
		Percent of Total	2%	2%		2%	2%	7.8%
	B1	Number of cases	1	4	1	3	1	10
		Percent of Total	2.0%	7.8%	2%	5.9%	2%	19.6%
	B2	Number of cases	3	2	2	6	5	18
		Percent of Total	5.9%	3.9%	3.9%	11.8%	9.8%	35.3%
	B3	Number of cases		2		4	5	11
		Percent of Total		3.9%		7.8%	9.8%	21.6%
Total		Number of cases	8	12	4	15	12	51
		Percent of Total	15.7%	23.5%	7.8%	29.4%	23.5%	100%

a. A: medullary-spindle cell type; BA: mixed cellularity; B1: predominently cortical; B2: predominently epithelial; B3: well-differentiated, organoid.

Discussion

According to Iraqi cancer registry thymoma is the most frequent mediastinal tumor, it represents (23.5%) of all mediastinal tumors followed by lymphoma (17.6%) and mesothelioma (15.6%), it represents (0.09%) of all tumors [3].

The mean age at time of presentation was (39) years approaching that of Iraqi cancer registry [3]. A significant correlation was seen between the gender and various histopathological subtypes of thymoma were male patients seen frequently with B2,B3 subtypes (27.5%,17.6% respectively) while female patients seen in B1,A subtypes (11.8% each) similar results were reported [11] Significant correlation was found between gender and Masaoka's staging system and TNM staging system were male patients found in advanced stages while female patients were found in early stages of both staging systems, this again was supported in other studies [11].The only paraneoplastic syndrome in this study was Myasthenia gravis seen in (54.9%) of thymoma patients, this value was within the range of other studies [12].The mean age of Myasthenic thymomatous patients at time of presentation was 36 years (younger than the non- myasthenic thymomatous patients) this was agreed by Kazuo.etal 2003 [13]. Male: female ratio 1.5:1 are in agreement with other results[14].The most frequent histopathological subtypes associated with Myasthenia gravis was the B2, B3 subtypes this was agreed by Pan. et al 2001[15] , Okumora. etal 2001 [6] of the fifty-one patients only [11] patients (21.5%) were asymptomatic this was near the results of Riosa. etal 2001. [16]. the rest of the patients (68.5%) were symptomatic and had different constitutional, locally (tumor related) signs and symptoms or paraneoplastic syndrome this was inconsistent with Moran et al 2001[17] WHO histopathological subtypes correlated with Masaoka's staging system significantly this was agreed by Okumora. etal 2001 [6].

Conclusion

MG was the only paraneoplastic syndrome diagnosed in this study. A significant correlation was found between WHO classification system & Masaoka`s & TNM staging system, and with the sex of the patients.

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Human Cytomegalovirus and Colorectal Adenocarcinoma: Any Association?

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Abstract

Background: Human cytomegalovirus (HCMV) has been implicated to transform many mammalian cells, so we tried to investigate whether HCMV participates in human colorectal carcinogenesis.

Methods: Immunohistochemistry analysis using monoclonal antibody of HCMV early protein was conducted on tissues of 32 colorectal adenocarcinomas and eight colorectal hyperplastic polyps, tissues of normal tumor margin were considered as control.

Results: HCMV early protein was detected in five out of 32 (15.6%) colorectal adenocarcinomas, while none of the eight colorectal hyperplastic polyps and tissues of normal tumor margins was positive for the virus early protein.

Conclusion: the data of this study suggests that HCMV may participate in the process of colorectal carcinogenesis as it is evidenced that HCMV was detected in colorectal cancer tissues only.

Key wards: HCMV, CRC.

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Introduction

Human cytomegalovirus (HCMV) is a member of the herpesvirus family that is a ubiquitous human pathogen worldwide. In the United States, 40% to 90% of adults are seropositive owing to exposure to the virus at some time during life [1, 2]. CMV infection is lifelong, and the latent virus can reactivate to cause serious illnesses when the host is immunocompromised [3, 4]. Various in vitro studies have demonstrated that the gene products of CMV are capable of modulating cell cycle progression and apoptosis by regulating the expression of a number of important host genes [4-7]. For example, CMV infection has been shown to transcriptionally activate the expression of the proto-oncogenes c-*fos*, c-*jun*, and c-*myc* [8, 9].

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The immediate early viral proteins also can block the induction of apoptosis by tumor necrosis factor α or the adenovirus E1A proteins [10]. In addition, CMV has been shown to transform a variety of mammalian cells that are tumorigenic in nude mice [5].

Detection of an infectious agent in human cancers might have important implications in cancer treatment and prevention. To further study whether CMV participates in human colorectal carcinogenesis, we examined colorectal hyperplastic polyps, adenocarcinomas, and normal-appearing colonic mucosa for the presence of one of the HCMV early proteins.

Material and Methods

The specimens included in the study were paraffin embedded blocks of 32 colorectal adenocarcinomas with their normal tumor margin tissues and 8 colorectal hyperplastic polyps. 5µm tissue sections on positive charged slides were subjected for immunohistochemistry analysis to detect HCMV early protein using

monoclonal antibody for HCMV early non-structural protein of 68 KDa (BioGenex, USA) in a dilution of 1:100, refer to the immunohistochemistry procedure in reference [11].

Results

HCMV early protein was detected in colorectal tumor cells in five out of 32 cases (15.6%) (Table 1), with negative normal tumor margin in all of these cases, and none of the 8 colorectal hyperplastic polyps showed positivity for the viral early protein. HCMV positive cells showed dark brown nuclear staining pattern (Figure 1).

Table 1: HCMV early protein positive colorectal cancer cases

Case no.	Tumor Grade	Tumor Stage	Tumor Type	Location	Lymph nodes involvement
1	II	C	Well differentiated	Recto-sigmoid	Positive
2	III	C	Poorly differentiated	Recto-sigmoid	Positive
3	II	B	Moderately differentiated	Colon	Negative
4	II	D	Moderately differentiated	Rectum	Negative
5	II	C	Moderately differentiated	Rectum	positive

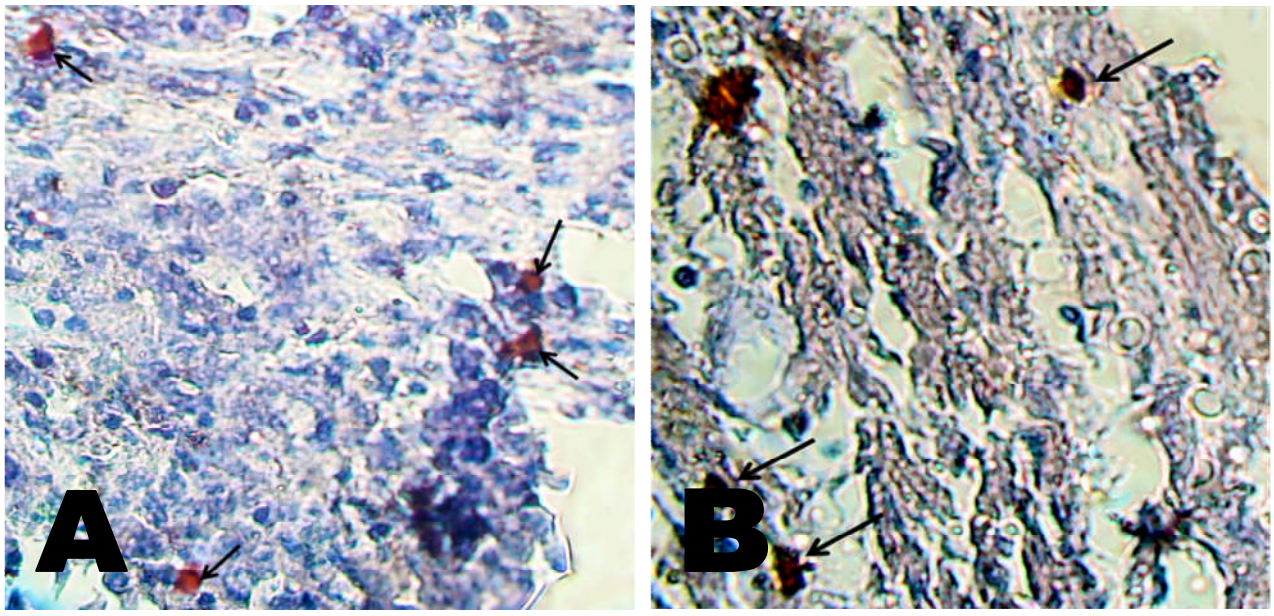

Figure 1: Detection of HCMV early protein by immunohistochemistry in Colorectal Adenocarcinoma. Immunostaining of HCMV by DAB chromogen (dark brown nuclear staining) counterstained with Mayer's heamatoxylin. (**A**) Poorly differentiated, grade III, stage C, colorectal adenocarcinoma show tumor cells positive for the virus early protein. (**B**) Well differentiated, grade II, stage C, colorectal adenocarcinoma show tumor cells positive for the virus early protein. Magnification power of A and B (X400).

Discussion

Despite the rapid advance in the understanding of molecular pathways underlying human colorectal carcinogenesis, the causes that initiate dysregulation of the pathways remain largely unknown. Human cytomegalovirus (HCMV) has been implicated as a potential pathogenic agent [(12)].

In the present study we examined 32 colorectal adenocarcinomas among which five were positive for HCMV early protein which could be supported by many studies and evidences showing that many viral genes and proteins are carcinogenic or participate in the process of carcinogenesis (4-10). One of the viral morphologic transforming regions, mtrII, encodes a 79 amino acid protein that is capable of binding to tumor suppressor p53 to inhibit p53-activated transcription [(13)]. In addition, it was recently reported that the CMV UL82 gene product pp71 stimulates cell cycle progression by inducing protein degradation of another important tumor suppressor Rb and its family members p107 and p130 [(14-16)]. Taken together, these experimental observations strongly suggest CMV to be a potential carcinogenic agent.

Despite the accumulation of in vitro evidence, the role of CMV infection in the development of human cancers has not been established. This is in contrast with other members of the herpes virus family, such as Epstein-Barr virus and human herpes virus 8, that are linked convincingly to several human malignant neoplasms [(17-18)]. The association of CMV with human cancers has been studied in the uterine cervix, prostate, and colorectum, but the data have been conflicting and inconclusive [(5, 12, 19)].

CMV infects a wide range of human cells [(20)], including colonic epithelial cells that give rise to adenomas and adenocarcinomas. The possible association of CMV with human colorectal adenocarcinomas was reported first in 1978 by Huang and Roche [(21)], who detected CMV DNA in 4 of 7 colonic adenocarcinomas by membrane complementary RNA-DNA hybridization. It is interesting that CMV DNA also was detected in 1 of 2 cases of familial adenomatous polyposis but not in normal colonic tissues from the same patients or control cases of Crohn disease [(22, 23)].

In conclusion, HCMV might play a role in the process of colorectal carcinogenesis because it is evidenced now that some of the CMV proteins might have mutagenic potential, which might be expressed only transiently in host cells to induce mutations in cellular genes leading to oncogenic transformation [(24)].

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Detection of IL-10, IFN-γ and IL-8 in sera of patients with recurrent spontaneous abortion

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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

Objective: Estimation of Interleukin-10 (IL-10), IL-8 and **IFN-γ** levels in sera of patients with recurrent spontaneous abortion (RSA) using ELISA method.

Method: 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 blood sample was collected and serum was 58eparated. Estimation of Interleukin-10 (IL-10), IL-8 **IFN-γ** levels in sera of patients was done using ELISA method.

Result: the current study failed to demonstrate a significant difference in circulating levels of IL-8 between RSA and control group (p> 0.05) and no significant different between non-RSA and control (p>0.05) . IFN-γ expression is significantly increased (p<0.001) in women with RSA and non-RSA compared with successful pregnancy. Defective IL-10 expression in women with RSA and non-RSA .The ratio of IFN-γ: IL-10 was found to be highly significant (p<0.001) in aborted women. IL-8 was expressed in high levels in aborted women (RSA and non-RSA) and those with successful pregnancy, but no significant difference (p>0.05) was found when compared between successful pregnancy and RSA or non-RSA, whereas highly significant difference (p<0.001) was found between RSA and non-RSA.

Conclusions: IFN-γ expression is highly significant increased (p<0.001) in women with RSA and non-RSA compared with successful pregnancy, indicating that Thl cytokines might well be implicated in adversely affecting pregnancy. And defective IL-10 expression in women with RSA and non-RSA might be documentary to the previous studies on the possible defect in Th2 cytokines production in these patients.

Key words: Recurrent spontaneous abortion Interleukin-10 (IL-10), IL-, **IFN-γ**, and ELISA.

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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 anatomical 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

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shown to be dependent upon the suppression of T-helper (Th) 1 and T-cytotoxic (Tc)1 cells,which produce interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, and the up- regulation of Th2 and Tc2 cells, 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 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[13].Moreover, during pregnancy, IL-8 is a CXC chemokine that is produced by a variety of cells, mainly monocytes/ macrophages [14].Interleukin (IL-8) has inflammatory and growth-regulating properties [15,16] but is notable for its selective chemotaxis, degranulation , and activation of neutrophils[17]. IL-8 induced activation of neutrophils and elastase activity in the intrauterine environment has been implicated in the mechanisms of rupture of fetal membrane [18], and cervical ripening [19, 20]. **Hence in this study we intend to** determine the concentrations of IFN-γ, IL-10 and IL-8 in circulation of patients using Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

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 subjects 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

Sample collection: Five ml of venous blood was collected from each patient and control group. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at 4 ^{o}C at 450 x g for serum collection. The serum was then aspirated by using a Pasteur pipette and dispensed into sterile glass tubes (1 ml in each) and stored at -20 ^{o}C until used. The repetitive freezing and thawing of serum sample was avoided.

Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of IL-10, IFN-γ and IL-8 in serum:

ELISA was used for the estimation of Interleukin-10 (IL-10), IL-8 and IFNl- γ level in the sera. This ELISA is a two immunological step sandwich type assay. In the first step the cytokine is captured by a monoclonal antibody bound to the wells of amicrotiter plate. In the second step a monoclonal antibody linked to abiotinylated monoclonal antibody is added together with streptavidine.

Monoclonal antibody to IL-10 (mAb9D7, Biotinylated monoclonal antibody 12G8.), **IFN-γ** (monoclonal antibody 1-D1K and Biotinylated monoclonal antibody 7-B6-1) and **IL-8** (monoclonal antibody IM2237) were used in this study; the procedure was according to Cell Com (cellular communication investigations) kit. France**.**

Statistical Analysis

The ANOVA analysis program was used to calculate the values, Mean, Median, Standard deviation and standard error were all used in the analysis.

The chi-square used for the qualitative data.

Results

As shown in table **1** a significant correlation between gestational age and IFN- γ (in circulation detected by ELISA) in group A women. There were no significant correlation among the other combination between gestational age and cytokines.

Table 1: Correlation between gestational age and cytokine tested in this study in sera of group A.

[a]G.A= gestational age

Variables		Correlation Coefficient r =	*P* value
[a]G.A – IL-8		0.027	>0.05
G.A – IL-10		0.297	>0.05
G.A – IFN-γ		**0.228**	**<0.05**

Based on ANOVA test analysis (Table 2) shows, the mean value of serum levels of these cytokines .The results revealed that there was a highly significantly difference (p<0.001) in the mean percentage of IL-8; IFN-γ and IL-10 between group A and group B (865.7± 81.3 versus 190.5±19.5 ; 1850.3±311.4 versus186.3±14.7 and 9.4±1.4 versus 70.1±5.1, respectively) . But the difference was not significant (p>0.05) when we compared the mean value of IL-8 between group A and B with group C; and it was found highly significant difference (p<0.001) in the mean value of IL-10 in sera of women in group C (553±58.9) compared with that of group A (9.4±1.4), and highly significant difference between group C and group B (70.1 ± 5.1). In addition, highly significant difference (p<0.001) was found in the mean value of IFN-γ in sera of women in group A and group C. as shown in (Figure 1).

Table 2: Comparison between the mean values of concentration (pg/ml) of IL-8, IL-10 and IFN-γ (ELISA assay) in sera of studied groups.

Variable	Group		Mean ± SE	F test *p* value	Sig. between groups	
					groups	*P* value
IL-8	A		865.7± 81.3	<0.01	A – B	0.000**
	B		190.5±19.5		A – C	0.996
	C		820±202.8		B – C	0.067[a]
IL-10	A		9.4±1.4	<0.01	A – B	0.000**
	B		70.1±5.1		A – C	0.000**
	C		553±58.9		B– C	0.000**
IFN-γ	A		1850.3±311.4	<0.01	A – B	0.000**
	B		186.3±14.7		A – C	0.000**
	C		7.6 ±0.4		B – C	0.000**

**= highly significant difference (p<0.01); [a]= marginally significant difference

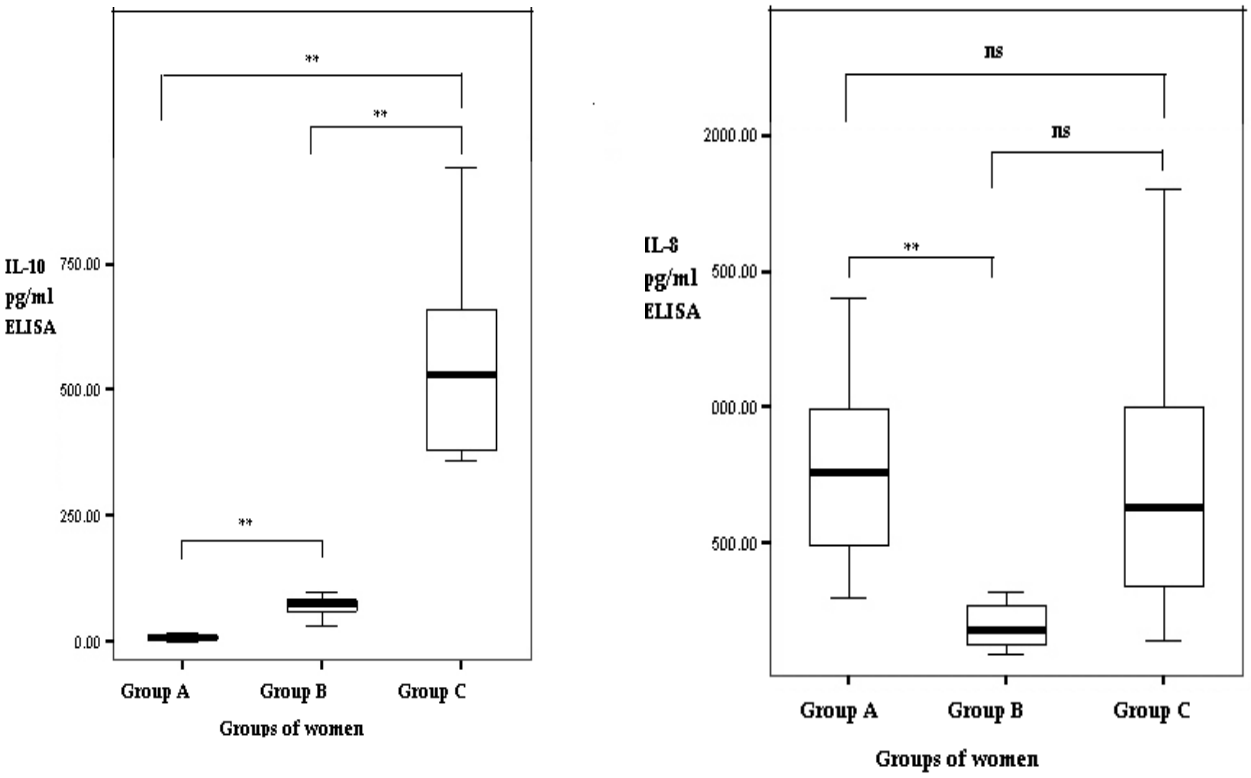

Figure 1: Concentration of IL-8, IL-10 and IFN-γ in sera of investigated women. (ns=not significant *p*>0.05 ;=highly significant *p*<0.001).**

Based on ANOVA test analysis, the results showed a marginally significantly different (0.05<*p*<0.1) when we compared between group A and group C and between group A and B, and a highly significant difference (*p*<0.001) between group Band C, as shown in (Table3).

Table 3: Comparison between IFNγ/IL-10 ratio of the three groups (ANOVA test analysis).

Variable	Group	Mean ± SE	F test *P* value	Sig. between Groups	
				groups	*P* value
IFNγ/IL-10 (ELISA)	A	630.8±263.8	<0.05	A–B	0.068[a]
	B	2.9±0.3		A –C	0.066[a]
	C	0.02±0.002		B –C	0.000**

[a] =marginally significance (0.05<*p*<0.1);** =highly significance difference (*p*<0.001)

IFN-γ and IL-8 expression, showed a significant correlation (*p*<0.05) between them in group A and group B by using ELISA technique, whereas the result revealed that there was no significant correlation (*p*>0.05) between IFN-γ and IL-8 expression in control (group C), (Table 4) .

Table 4: Relation between the mean percent of IFN-γ and IL-8 in serum in group A, B and C.

IFN-γ– IL-8		Correlation Coefficient r =	*P* value
groupA	ELISA–ELISA	**0.279**	**<0.05**
groupB	ELISA–ELISA	**0.501**	**<0.05**
groupC	ELISA–ELISA	-0.629	>0.05

Discussion

The current study, showed a highly significant difference in expression of IL-10 (systemic) (*p*<0.001) between first; second trimester abortion and control groups (successful pregnancy).In addition, no significant difference in expression of IL-10 (systemic) (*p*>0.05) between first trimester and second trimester abortion. These results agreed with other studies that found higher concentrations of IL-10 at delivery than during any other stage of gestation tested, though the reasons for this and its significance were not readily understood [(21)]. Moreover**,** this study, showed that the expression of IL-10 proteins in circulation of women with successful pregnancy (group C) was significantly higher (*p*<0.001) than that of women with RSA (group A) and higher than that in women with non-RSA (group B).this result indicated the systemic immune response might be associated with local cytokine milieu at the feto maternal interface. This significantly higher level of IL-10 with successful pregnancy in this study, could be explained by previous study that showed that IL-10 production was significantly lower in patients with recurrent miscarriage as compared with normal pregnancy or spontaneous

abortion cases [21]. IL-10 plays a positive role in the prevention of spontaneous pregnancy failure in a mouse model; the injection of IL-10 into abortion-prone mice resulted in the prevention of fetal wastage [22]. Results of previous studies [23-25] showed that, IL-10 was produced at higher concentrations by PBMC of women with normal pregnancy than those with a history of unexplained RSA. Thus, IL-10 has emerged as an important Th2-type cytokine in the maintenance of normal pregnancy [26]. 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 [22, 27]. Other study found a lack of cytokine shift in aborted women (RSA or non-RSA) as compared with normal pregnant women at the same time of gestational age [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 expression in women with RSA which was significantly higher (p<0.001) 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 [23, 21, 29, 30].

Hanna and colleagues (2000)[31] examined the expression of IL-10 and its receptor in placental explants or freshly isolated cytotrophoblasts from different gestational ages and compared it with the expression profiles of other cytokines. First and second trimester placental tissues from normal pregnancies predominantly expressed IL-10, whereas the levels of IL-2, IL-4, and IFN-γ were mostly below detection throughout pregnancy.

In the current study, results showed a highly significant difference in expression of IFN-γ (systemic) (p<0.001) between first; second trimester abortion compared with control groups (successful pregnancy). In addition, no significant difference in expression of IFN-γ (p>0.05) between first trimester abortion and second trimester abortion. This result might be explained that IFN- γ associated with pregnancy loss. Other studies showed that the expression of IFN-γ will increase with progress of pregnancy till late first trimester [32]. Furthermore, this study, showed that the expression of IFN-γ proteins in circulation of women with RSA was significantly higher (p<0.001) than that of successful pregnancy (group C) and higher than that in women with non-RSA (group B). This results was in agreement with other study that mentioned the elevated maternal serum levels of interleukin-2 soluble receptor-(IL-2 sR), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) have been associated with pregnancy loss[33].

Results showed no significant difference in expression of IL-8 (systemic) (p>0.05) between first; second trimester abortion and control groups (successful pregnancy).In addition, no significant difference in expression of IL-8 (systemic) (p>0.05) between first trimester abortion and second trimester abortion .This finding supports the proposal that IL-8 may play a maturational role during pregnancy and/or facilitates the process of labor[34,35] or it might be due to the role of Interleukin-8 (IL-8),that has inflammatory and growth-regulating properties during pregnancy [15,16]. 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[13,20,36].

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Bcl_2 overexpression in colorectal carcinoma

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Abstract

Background: Colorectal cancer is a major cause of morbidity and mortality worldwide. Prognostic assessment influences the treatment of patients with colorectal cancer, including decisions about adjuvant therapy. Bcl2 over-expression is a genetic event associated with tumor progression and is a prognostic marker of this disease.

Objective: Colorectal carcinoma is a major cause of morbidity and mortality worldwide. Bcl2 overexpression is a genetic event associated with tumor progression and is a prognostic marker for this disease. The aim of this study is to assess the expression of bcl_2 in colorectal carcinoma and its correlation with other clinicopathological parameters.

Methods: From January 2004- January 2005, thirty –five formalin fixed paraffin embedded tissue samples from patients with colorectal carcinoma were included in this study. Four-micrometer tissue sections were obtained for each case, two of them were stained by H&E and the diagnosis had been revised, and the other two were stained immunohistochemically by using avidin biotin alkaline phosphatase method for evaluating bcl_2 expression. The presence of red cytoplasmic staining in less than 25% of tumor cells was considered a positive expression of bcl_2.

Statistical analysis of all the results were performed using Chi square test at level of significance alpha = 0.05 ($P<0.05$) regarded as statistically significant.

Results: Bcl_2 expression was significantly higher in low grade and early stage colorectal carcinoma .Non mucinous colorectal cancer showed more bcl_2 expression than the mucinous type. An inverse correlation was found between bcl_2 expression with the greatest diameter of the tumor and the lymph node status. Bcl_2 expression was correlated neither with the age nor with the sex of the patient and the tumor location.

Conclusion: Bcl_2 over-expression correlates with many variables as low grade colorectal tumor, early stage, non mucinous type, small tumor size and negative lymph node status.

Key words: bcl2, colorectal carcinoma.

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Introduction

Bcl_2 is a proto-oncogene which codes 26 kd protein that blocks apoptosis and rescues cells from apoptosis [(1)]. Reduction in the capacity of apoptotic cell turnover could be an important step in the development of neoplasia[(2)]. Colorectal carcinoma (CRC) is one of the most common malignancies worlds wide. Several clinical, biological, and genetic parameters have been used to assess the prognosis and to help the clinician in optimizing therapies for CRC patients.

Studies indicate that the most important prognostic variable is the tumor stage [(3)]. however , patients who are apparently at the same pathological stage often have adverse outcome in CRC [(4)], although a lack of correlation have been reported. The role of some cellular oncogenes and tumor suppressor genes in clinical aggressiveness of CRC has been also studied. Point mutations of P53 or K-ras tumor genes occur in about 50% of CRC_s and have been associated with poor prognosis. However, available data are again controversial. Thus recent efforts have focused on prediction of the clinical outcome of CRC patients, with the goal of providing a rational approach for planning specific therapy [(5)].

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Bcl_2 protien expression has been studied in many tumors including CRC. The identification of it as a sensitive prognostic marker may allow the use of adjuvant therapy in a subset of patients with worse prognosis with resultant improvement in their survival [6].

$Bcl_{2\ protien}$ expression is mainly observed in cell populations with along life and/or proliferating ability such as duct cells in exocrine glands, basal keratinocytes, cells at the bottom of colon crypts, and neurons [7].

The bcl2 produces its effect not by increasing the rate of cell proliferation but by reducing the rate of cell death and thus may contribute to tumorigenesis by keeping the cells alive and lending them vulnerable for further accumulation of gene abnormalities [8].

Although **Bcl_2** expression has been shown in **colorectal** neoplasia, their possible impact on the biologic behavior of the **colorectal** carcinomas is still controversial [9].

In the present study we evaluated the expression of bcl_2 in colorectal carcinoma and its correlation with other clinicopathological parameters.

Materials and Methods

Thirty-five cases with **colorectal** carcinomas that had undergone colectomy were included. The clinicopathologic parameters like age and sex of the patient, tumor grade, tumor stage, tumor greatest diameter, anatomic location, histopathologic type and lymph node status were evaluated as **prognostic** indicators. Histological classification of the tumor was done according to the WHO system. The anatomic localizations were grouped as proximal colon meaning the distance from the cecum up to the splenic flexure and as distal colon beginning from the descending colon to the rectum and rectal tumors.

Four sections (with four micrometer thickness) from formalin fixed, paraffin embedded tissues were obtained, two of them were stained by H&E and revised, and the other two were stained immunohistochemically with anti **Bcl_2** (Chemicon) monoclonal antibody. The IHC select ® immunophosphatase secondary detection system uses biotin avidin alkaline phosohatase complexed antibodies to detect antimouse IgG in the primary antibody. The sample is then incubated with the streptavidin alkaline phosphatase solution, which binds to the biotin labeled secondary antibody present on the tissue. The chromogenic development reagent, the Red violet is then added and reacts with alkaline phosohatase attached to streptavidin biotin antibody complex. The alkaline phosphatase activity on the chromogenic substrate results in the deposit of the red insoluble precipitates at those antigenic sites containing the specific epitopes recognized by the primary antibody. The sections were counter-stained with hematoxylin. The presence of red cytoplasmic reaction at the site of the target antigen is indicative of positive reactivity. Counter stain will be dark blue coloration of the cell nuclei.

The intensity of the immunostaining was evaluated by dividing the staining reaction in four groups [10]:

Weak cytoplasmic staining intensity

Moderate cytoplasmic staining intensity

Strong cytoplasmic staining intensity

Very strong cytoplasmic staining intensity

The quality of the immunostaining was evaluated as follows

0 no positive immunostaining

1 less than 25% of tumor cells showing cytoplasmic positivity

2 25-50% of tumor cells showing cytoplasmic positivity

3 50-75% of tumor cells showing cytoplasmic positivity
4 >75% of tumor cells showing cytoplasmic positivity
A combined score for immunostaining based on both qualitative and quantitative immunostaining was composed by adding both qualitative and quantitative score, which was then divided into 5 main groups:
*No immunostaining score 0
*Weak immunostaining score 1-2
*Moderate immunostaining score 3-4
*Strong immunostaining score 5-6
*Very strong immunostaining score 7-8

Lymphocytes in the stroma and lamina propria were consistently positive and served as internal control and regarded as very strongly positive according to above scoring system [10] (Figure 1). Bcl_2 expression was also positive in the basal cells of the normal colonic crypts (Figure 2).
Statistical analysis was performed using the chi-square test. At level of significance alpha =0.05 and $p< 0.05$ regarded as statistically significant.

Results

Regarding the sex of the patient, bcl_2 expression was more frequently positive in female cases (55.6%) than male cases (25%) but this was not statistically significant (p=0.129) (Table 1). Concerning the age of the patient, bcl_2 expression was more frequently positive in age group more than 40 years (77.8%) than age group ≤40 years (50%) but the results were also statistically not significant (p=0.127) (Table 1). Considering tumor grade, bcl_2 was expressed in all the well-differentiated Adenocarcinoma (100%) and in the moderately differentiated Adenocarcinoma it was (82.6%) and that is more than the poorly differentiated Adenocarcinoma (33.3%). The results were statistically significant (p=0.013), (Figure 3, 4) (Table 2). Bcl_2 expression was found to be positive in early stages of colorectal carcinoma being (100%) in stage A, (86%)positive in stage B while in stage C it was (66%) and (33%) in stage D (Table 3) . although Bcl_2 expression was not significantly correlated to the tumor stage when comparing its expression among all the stages together (p=0.0999), but when taking bcl_2 expression in stage A and B each apart versus stage D , the results were statistically significant (p=0.035 ,p=0.043) respectively (Table 4,5). Regarding the tumor type, non mucinous adenocarcinoma expressed bcl_2 (77.8%) more than the mucinous type (22.2%) and the results were statistically significant (p=0.031) (fig5). There was no correlation between the tumor location and bcl_2 expression (p=0.651), although the distal colon showed more bcl_2 positivity (92.6%) than the rectum (7.4%) (Table 6). The extent of bcl_2 expression by tumor cells decreased significantly with respect to increasing tumor greatest diameter (p=0.036), so (66.7%) of the tumors of ≤5 cm in greatest diameter were positive for bcl_2 compared to (33.3%) of tumors >5cm in greatest diameter (Table 7).
An inverse correlation was found between bcl_2 expression and the lymph node status. (66.7%) of the tumors with negative lymph node metastasis were positive for bcl_2 compared to (33.3%) of the tumors with positive lymph node metastasis and the results were statistically significant (p=0.036). (Figure 6)

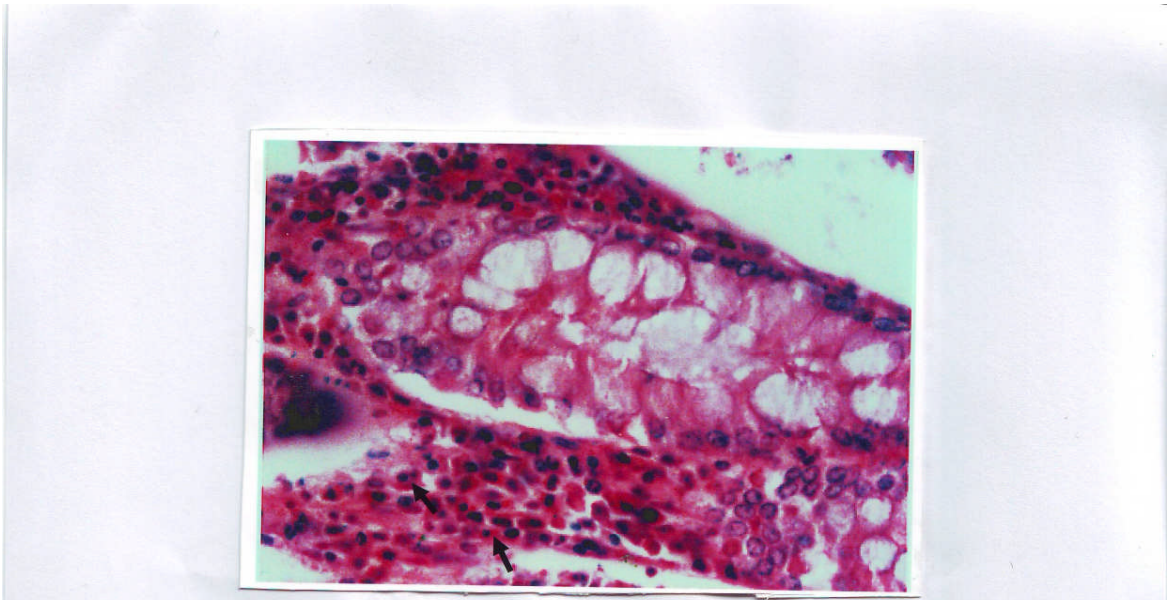

Figure 1: Lymphocytes (arrow) as internal control for bcl$_2$ immunohistochemical staining in a section of colonic mucosal glands (alkaline phosphatase method) (red cytoplasmic staining) 400.

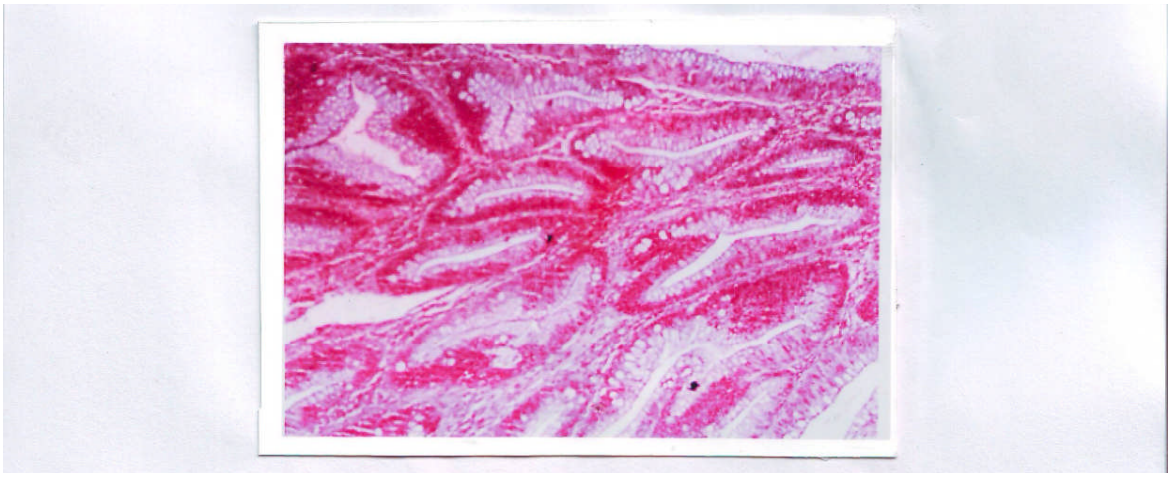

Figure 2: Normal colonic glands showing positive bcl$_2$ expression (alkaline phosphatase method) as red cytoplasmic stain 100.

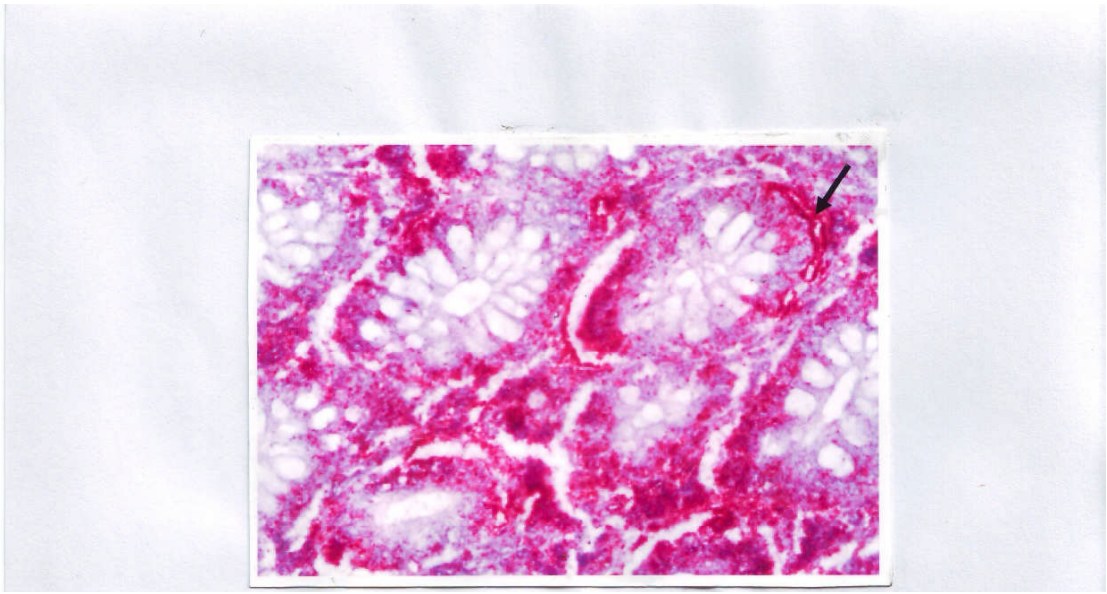

Figure 3: well differentiated colorectal adenocarcinoma showing positive bcl_2 immunostaining (alkaline phosphatase method) (strong reaction) 400.

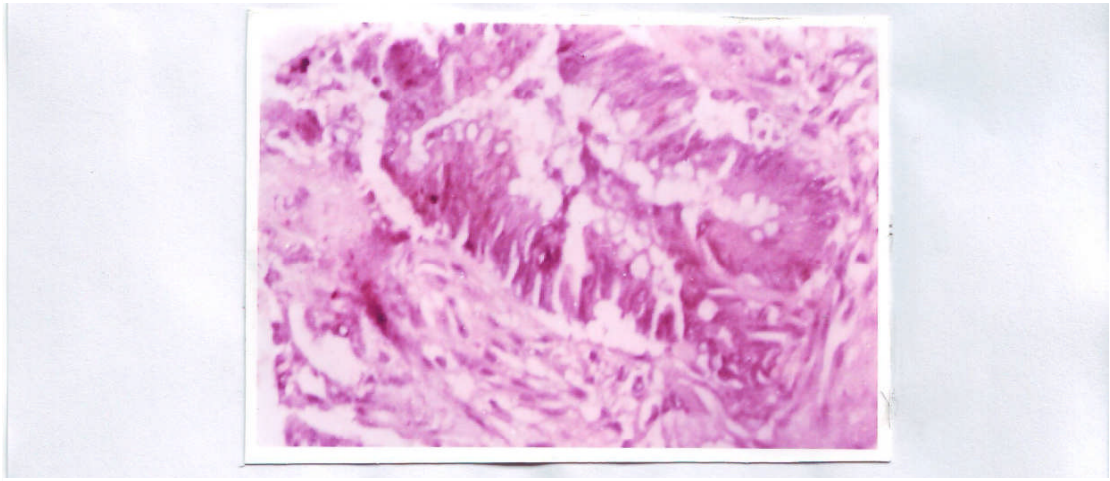

Figure 4: Poorly differentiated colorectal adenocarcinoma showing negative bcl_2 immunostaining (alkaline phosphatase method) 400.

Table 1: Bcl_2 immunostaining in relation to age and sex of the patient

		Bcl_2 immunostaining		P- value
		Negative	Positive	
Sex	Female Male	44.4% 75.0%	55.6% 25.0%	0.125
Age	≤40 y >40 y	50% 22.2%	50% 77.8%	0.127

Table 2: correlation between Bcl_2 positivity and tumor grade in colorectal carcinoma.

		Tumor grade			Total
		well	moderate	poor	
negative	No.	0	4	4	8
	%	0	17.4%	66.7%	22.9%
positive	No.	6	19	2	27
	%	100.0%	82.6%	33.3%	77.1%
Total	No.	6	23	6	35
	%	100.0%	100.0%	100.0%	100.0%

Chi-Square	Value	df	P-value
	8.698	2	.013

P<0.05 significant

Table 3: correlation between Bcl_2 positivity and tumor stage in colorectal carcinoma.

		Tumor stage				Total
		A	B	C	D	
negative	No.		2	4	2	8
	%		25.0%	50.0%	25.0%	100.0%
positive	No.	5	13	8	1	27
	%	18.5%	48.1%	26.9%	3.7%	100.0%
Total	No.	5	15	12	3	35
	%	14.3%	42.9%	34.3%	8.6%	100.0%

Chi-Square	Value	df	P-value
	6.265	3	.099

Table 4: Bcl$_2$ expression in stage A versus stage D

		STAGE		Total
		Stage A	Stage D	
positive	No.	5	1	6
	%	83.3%	16.7%	100.0%
negative	No.		2	2
	%		100.0%	100.0%
Total	No.	5	3	8
	%	62.5%	37.5%	100.0%

Chi-Square	Value	df	P-value
	4.444	1	.035

P<0.05 significant

Table 5: Bcl$_2$ expression in stage B versus stage D

		STAGE		Total
		Stage B	Stage D	
positive	No.	13	1	14
	%	86.7%	13.3	100.0%
negative	No.	2	2	4
	%	66.7%	33.3%	100.0%
Total	No.	15	3	18
	%	83.3%	16.7%	100.0%

Chi-Square	Value	df	P-value
	.720	1	.043

P<0.05 significant

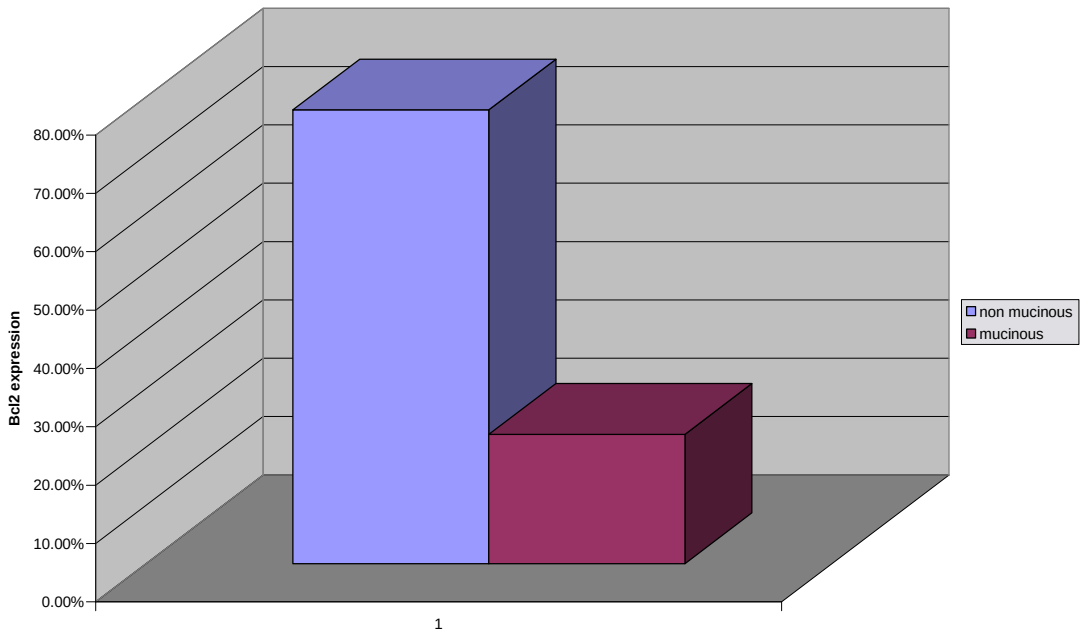

Figure 5: Bar chart of correlation between Bcl$_2$ positivity and tumor type

Table 6: Correlation between Bcl$_2$ positivity and tumor anatomic location in colorectal carcinoma.

		Tumor location		Total
		Distal colon	Rectum	
negative	No.	7	1	8
	%	87.5%	12.5%	100.0%
positive	No.	25	2	27
	%	92.6%	7.4%	100.0%
Total	No.	32	3	35
	%	91.4%	8.6%	100.0%

Chi-Square	Value	df	P-value
	.204	1	.651

Table 7: Correlation between Bcl$_2$ positivity and tumor greatest diameter in **colorectal carcinoma.**

		Tumor size		Total
		≤5 cm	>5cm	
negative	No.	2	6	8
	%	25.0%	75.0%	100.0%
positive	No.	18	9	27
	%	66.7%	33.3%	100.0%
Total	No.	20	15	35
	%	57.1%	42.9%	100.0%

Chi-Square	Value	df	P-value
	4.375	1	.036

P<0.05 significant

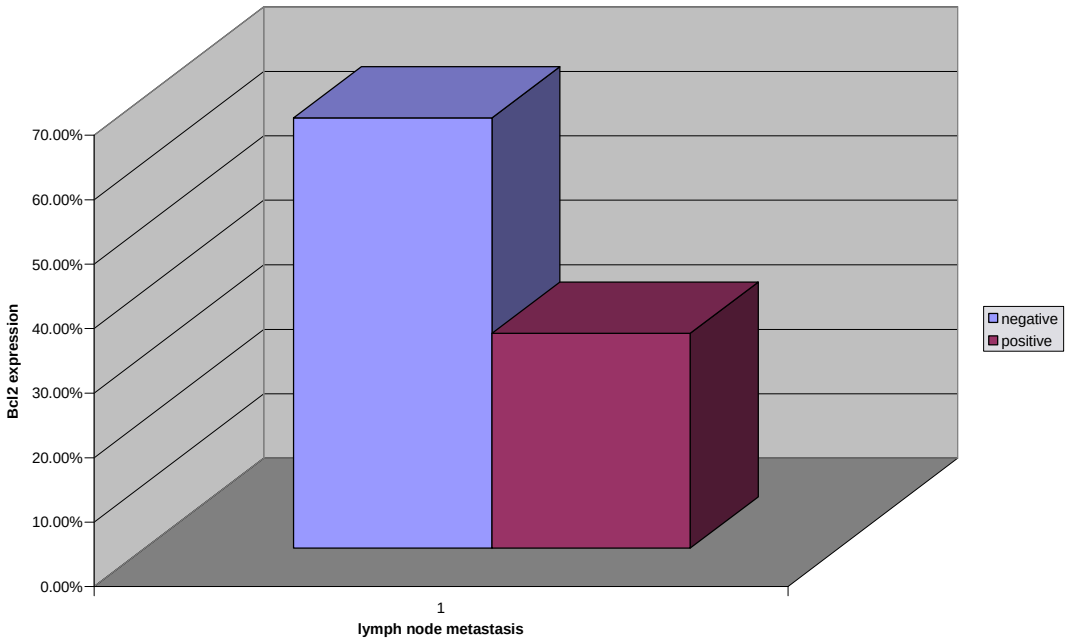

Figure 6: Bar chart of correlation between Bcl$_2$ positivity and lymph node status.

Discussion

In the present study, bcl$_2$ expression was a cytoplasmic staining clearly observed at the base of colonic crypts, as well as in the normal tissue close to the tumor margin.

This matches the observation of Bosari et al., 1995 [11], Barreton et al, 1996 [12], Krajweska et al, 1996[13], Palazzo et al., 1997 [14], and Giatromanolaki et al, 1999 [15].

This may suggest that altered bcl2 expression precedes the development of morphologically recognizable neoplasia.

Regarding bcl$_2$ expression in relation to the age and sex of patients, Bcl$_2$ was found to be more frequently expressed among females and patients of an age group less than 40 years. This agree with the results of Leahy et al., 1999 [16], Tollenaar et al, 1998 [17], Ogura et al, [18], and Ouyang et al, 2005 [19].

The present study revealed that bcl$_2$ expression was significantly correlated with the tumor grade being more frequently expressed in low grade colorectal carcinoma. These results are in agreement with the results of Leahy et al., 1999 [16].

Considering the tumor stage , bcl$_2$ expression was observed in early stages of colorectal adenocarcinoma, although the results were statistically not significant , they agree with the results of Husain et al, 1999 [20] . When we compared bcl$_2$ expression in stage A and stage B each apart versus stage D, a statistically significant correlation was found between high bcl$_2$ expression and early stage tumor. Similarly, Huang et al, 2002 [21], Manne et al, 1997 [22] and Kalklamanis et al, 1998 [23] found a significant correlation of bcl$_2$ expression with the tumor stage. A study made by Meterissian et al, 2001[24] specifically considering bcl$_2$ expression in stage B colorectal adenocarcinoma found that enhanced bcl$_2$ expression in this stage is associated with improved survival. Thus, patients whose tumors do not express bcl$_2$ should be considered for adjuvant therapy.

Taken into consideration the tumor type, bcl$_2$ expression was significantly

higher in non mucinous tumors compared with the mucinous type and this reflects the well known aggressive behavior and bad prognosis associated with the mucinous type. These results are in disagreement with the results of Dursun et al., 2001[25] who found a significant relation of bcl$_2$ expression with the mucinous type tumor and this may be explained by small sample size in the present study. These results are expected since most of the mucinous tumors which were negative for bcl$_2$ expression were associated with high grade and late stage malignancy.

Taking into account tumor greatest diameter , the extent of bcl$_2$ expression by tumor cells decreased significantly with respect to increasing tumor greatest diameter .This result is in agreement with Ofner et al., 1995[26] . This can be explained by the fact that large tumors may be related to other bad prognostic parameters as poor differentiation, advanced stage, high grade, and positive lymph node status.

Regarding the tumor location, none of the cases in the present study were n the proximal colon, this may be due to the fact that tumors in the proximal colon usually present late in the course of the disease and they attain a large size before clinical detection in addition to that they are far from digital and proctosigmoidoscopic examination and they may be beyond surgical treatment on discovery. The relationship between bcl$_2$ expression in the distal colon and the rectum was not statistically significant .These results are in agreement with others as Dursun et al., 2001[25] , Tollenaar et al, 1998 [17] , Husain et al, 1999 [20] and Huang et al, 2002 [21] .

There was a negative correlation between bcl$_2$ expression and lymph node status. These results are in concordance with the results of Dursun et al, 2001[25] and Goussia et al, 2000[27], that bcl$_2$ expression was more in lymph node negative tumors.

Bcl2 expression in colorectal carcinoma was associated with better clinical course especially when p53 expression was absent suggesting that neoplastic transformation related to inhibition of apoptosis results in less aggressive malignancies than those dependent on other oncogenes as p53[26] . An inverse relation between bcl$_2$ and p53 has been observed in other malignancies suggesting that these proteins may interact through opposite mechanisms: inhibition of apoptosis (bcl$_2$) and promotion of apoptosis (p53) [19] .

Conclusion

Bcl2 expression in colorectal carcinoma is correlated with low grade tumor, early tumor stage, non mucinous type, small tumor size and negative lymph node status.

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Immunohistochemical expression of p53 in gastric carcinoma (A Clinicopathological study)

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Abstract

Background: Carcinoma of the stomach is on of the most prevalent cancer types in the world today. P53 is the most notable tumor suppressor gene mutated in human cancers, including gastric cancer. The practical implication of this phenomenon in gastric cancer prognosis or even treatment by restoration of mutated p53 function are yet to be fully exploited.

Objective: To assess the immunohistochemical expression of p53 protein in gastric carcinoma and to study the correlation between p53 protein expression and different clinicopathological variables like: age, gender, site, gross pattern, histological type, grade, and stage of the tumor in gastric carcinoma cases.

Materials and methods: Forty formalin fixed paraffin embedded gastric carcinoma tissue blocks (partial or total gastrectomy specimens) from the archived materials of the Department of Pathology of Baghdad Teaching Hospital and the Center of Gastrointestinal and Hepatic Diseases , and other private laboratories were included in this study. A four micrometer – thick tissue sections were obtained and three slides had been prepared for each case, one was stained with Hematoxylin and eosin (H&E) and then reviewed, while two sections were stained immunohistochemically for p53. Statistical analysis was done using chi-square test for tables with frequencies, percentages, range, mean and standard deviation. Values were considered statistically significant when $P<0.05$.

Results: A clinico-pathological assessment revealed that 28 patients were males and 12 patients were females. Male to female ratio was 2.3 /1. The age of patients ranged between 30-80 years with a mean ± standard error of (55.77±1.88 year). The majority of the gastric carcinoma cases, in this study (70%) were above 50 years of age. Large proportions of gastric carcinoma cases (80%) were located in the antral region while the remaining cases were located in the cardia region. The ulcerative gross pattern was the most predominant gross pattern type(72.5%). Whereas the commonest histological type was the intestinal type (75%). The majority of the gastric carcinoma cases (62%) were moderately differentiated. Most of the gastric carcinoma cases (92.5%) fall in stage lll disease. The overall expression of p53 in gastric carcinoma cases in the present study was (44%). No statistically significant difference was found between p53 overexpression with age and sex of patients ($P>0.05$).Although there was no significant correlation in the relation ship between p53 overexpression with tumor site and gross pattern type,p53 positivity rate was higher in gastric carcinoma cases located in the antrum and in those cases of ulcerative gross pattern type. P53 overexpression was more commonly seen in gastric carcinoma case of intestinal type compared to diffuse type, However, the results were statistically not significant ($P>0.05$). P53 overexpression was more common in gastric carcinoma cases of moderately differentiated type compared to poorly differentiated type, with no statistically significant difference ($P>0.05$) . Although the majority of gastric carcinoma cases which showed positive p53 expression were in stage lll disease, these results were not significant ($P>0.05$).

Conclusion: The overall expression of P53 protein in gastric carcinoma cases in this immunohistochemical study was 44%. There was no significant correlation between p53 overexpression and different clinicopathological variables like: age, gender, gross pattern, histological type, tumor grade and stage.

Keywords: P53, gastric carcinoma, immunohistochemical expression

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Introduction

Understanding the molecular basis of gastric cancer is essential to develop more effective methods of primary prevention and secondary prevention (early diagnosis and treatment). Although the molecular mechanisms in gastric carcinogenesis have not been completely delineated, some important advances in the molecular biology of gastric cancer have been made [(1)].

Several abnormalities in oncogenes , tumor suppressor genes , and growth factor expression have been identified in gastric cancer .P53 is a tumor suppressor gene which is usually mutated up to 67.9% in gastric carcinoma [(1)]; these mutations are usually missense point mutations leading to genetic instability and uncontrolled cell proliferation[(2)].These mutations impair P53 anti-cancer gene inducing effects , so restoring its function would be a major step in curing many cancers , including gastric cancer [(3)],especially the ability of P53 to control apoptosis in response to DNA damage which has important practical therapeutic implications to enhance the effect of radiation and chemotherapy , or even evaluating the effect of adenovirus mediated re-introduction of wild type P53 as a potential clinical utility in gene therapy of gastric cancer [(4)].Thus , it is accepted that P53 plays a fundamental role in tumorgenesis and hence is an obvious choice for therapeutic exploitation .However , conflicting evidence and insufficient knowledge about the P53 pathways in detail and the fact that other mechanisms exist to module P53 activity leave this useful tool a hope for the future as regards use in the clinic [(5)].

So this study aims to assess the immunohistochemical expression of p53 protein in gastric carcinoma and to study the correlation between p53 protein expression and different clinicopathological variables like: age, gender, site, gross pattern, histological type, grade, and stage of the tumor in gastric carcinoma cases.

Patients, materials and methods

From October 2006-May 2007, forty formalin fixed paraffin embedded gastric carcinoma tissue blocks (partial or total gastrectomy specimens) from the archived materials of the Department of Pathology of Baghdad Teaching Hospital and the Center of Gastrointestinal and Hepatic Diseases , and other private laboratories were included in this study.

Clinicopathological parameters as the age and gender, site, gross appearance, histological type, grade and stage of tumor were obtained from histopathological reports.

A four micrometer –thick tissue sections were obtained from representative area, one section was stained with Hematoxylin and eosin (H&E) and then reviewed, while two sections were stained immunohistochemically for p53. The histologic type was classified according to Lauren classification 1965.

The positive control tissue used in the present study was a specimen from poorly differentiated ductal breast carcinoma tissue, which was known to be positive for monoclonal anti p53 protein.

Untreated sections with primary antibody (by omission of the primary antibody)were considered as technical negative control , while normal gastric tissue sections were considered as *tissue negative contro*l and were used for each set of slides. These tissues should show absence of specific staining.

All the slides were examined by light microscope; a random selection of the fields was used for analysis of all cases. Positive p53 results give

intracellular (nuclear) dark brownish color, granular or homogenous precipitate (clear cut) with blue cytoplasm.

The results of p53 positivity in each individual specimen were analyzed according to these independent variables [6, 7]:

-*Intensity of staining*: the intensity of staining of the brownish coloration was considered:

1. Strong (S) if it could be detected very clearly at low magnification (x10).

2. Moderate (M) if it was detected with difficulty at low magnification.

3. Weak (W) if it could only be detected at high magnification (x40).

-*The pattern of staining:* the pattern was considered:

A. Diffuse pattern (D) if the positive cells were distributed through almost all fields.

B. Regional pattern (R) if more than one area of the section showed large number of positive cells.

C. Focal pattern if there were only very few positive cells in the section.

-*Extent of staining*: a minimum of 100 tumor cells were scored (the percentage of positive stained nuclei with p53 protein in malignant cells counted manually at x400 total magnification, in 3-5 neoplastic fields randomly selected, that represent the most positive neoplastic area). P53 immunostaining in at least 10% of the cell nuclei of tumor tissue was regarded as p53 overexpression.

Scoring according to Sophia K.1999 [8] and Roviello F.1999 [9] was done at x40 objective as follows:

*Negative (Score 0) (none of the cells revealed positivity for p53 marker)

*Weak or mild staining (5-<10% positive of tumor cells) (Score +1)

* Moderate staining (<25%) (Score +2)

* Strong staining (>25 %-< 50%) (Score +3)

* Highly strong staining over 50%) (Score +4)

Statistical analysis

Was done using chi-square test for tables with frequencies, percentages, range, mean and standard deviation. Values were considered statistically significant when $P<0.05$.

Results

A total of (40) forty formalin fixed paraffin embedded gastric carcinoma tissue blocks were included in the present study. Clinicopathological assessment revealed that 28 patients were males and 12 patients were females. Male to female ratio was 2.3/1. The age of patients ranged between 30-80 years with a mean ±standard error of (55.77 ±1.88) years. The majority of the gastric carcinoma cases 28(70%) were above 50 years, while 12 (30%) of the cases were below 50 years. Sex distribution of gastric carcinoma cases, showed male predominance 28(70%) compared with female 12(30%). Large proportion of gastric carcinoma cases 32 (80%) were located in the antral region while the remaining cases 8(20%) were located in the cardia region.

Regarding the gross pattern of gastric carcinoma cases, the ulcerative type constituted 29 (72.5%) , while the fungating type constituted 5(12.5%) of the cases, the least gross pattern types were the stenosing 1(2.5%) and polypoidal 1(2.5%) types.

The histological type showed the predominance of intestinal type 30(75%) compared to the diffuse type 10(25%).

Taking into consideration tumor grade, this study revealed that the majority of cases were moderately differentiated type 25(62%) while 15(38%) of the cases were poorly differentiated type.

According to AJCC (TNM) staging system, the majority of gastric

carcinoma cases 37(92.5%) fall in stage III disease.

Positive p53 staining was detected in 18(44%) of gastric carcinoma cases while negative p53 immunostaining was detected in 22(56%) of the cases. (Figure 1)

Twelve cases of gastric carcinoma were below the age of 50 years and 6 cases (50%) of them showed positive P53 expression, while 28 of gastric carcinoma cases were equal or above 50 years of age and 12 cases (43%) of them showed positive p53 expression.

Thirteen cases of gastric carcinoma (46%) out of 28 cases which were of male gender type showed positive p53 expression, while 5 cases(42%) out of 12 cases which were of female gender type showed positive p53 expression. However there was no statistically significant difference in the relationship between p53 overexpression with age and sex, as shown in (Table 1).

Regarding the relation of p53 immunostaining with the tumor site, out of 32 gastric carcinoma cases located in the antrum 15 cases of them (47%) were positive for p53 immunostaining, while out of 8 gastric carcinoma cases located in the cardia , 3 cases of them (38%) were positive for p53 immunostaining. Out of 32 cases located in the antrum , 17 cases of them (53%) were negative for p53 immunostaining , while out of 8 cases located in the cardia , 5 cases of them (63%) were negative for p53 immunostaining, the difference was statistically not significant , as shown in (Table 1).

In regard to the ulcerative gross pattern, 15(52%) gastric carcinoma cases out of 29 cases of ulcerative gross pattern type showed positive p53 expression, followed by fungating type, in which out of 5 cases of fungating type, 2 cases of them (40%) showed positive p53 expression. While out of 29 ulcerative gross pattern gastric carcinoma cases, 14 cases of them (48%) showed negative p53 expression, and out of 5 cases of fungating type, 3 cases of them (40%) showed negative p53 expression (Figure 2).

In consideration to the histological type, out of 10 cases of diffuse type gastric carcinoma, 4 cases of them (40%) showed positive p53 expression (Figure 3), while out of 30 cases of intestinal type gastric carcinoma, 14 cases of them (47%) showed positive p53 expression (Figure 4). Out of 10 cases of diffuse type gastric carcinoma, 6 of them (60%) showed negative p53 expression, while out of 30 cases of intestinal type gastric carcinoma, 16 cases of them (53%) showed negative p53 expression (Figure 5), theses results were also statistically not significant, as shown in (Table 1).

Out of 25 cases of moderately differentiated type gastric carcinoma, 12 cases of them (48%) showed positive p53 expression, while out of 15 cases of poorly differentiated type gastric carcinoma, 6 cases of them (40%) showed positive p53 expression. Out of 25 cases of moderately differentiated type gastric carcinoma, 13 cases of them (52%) showed negative p53 expression, while out of 15 cases of poorly differentiated type gastric carcinoma, 9 cases of them (60%) showed negative p53 expression (Figure 6).

Regarding the relationship between gastric carcinoma cases and stage of disease, out of 37 cases of gastric carcinoma falling in stage III disease, 17 cases of them (46%) showed positive p53 expression, while one case falling in stage IV disease showed positive p53 expression. Out of 37 of gastric carcinoma cases falling in stage III disease, 20 cases of them (54%) showed negative p53 expression; while one case falling in

stage IV disease showed negative p53 expression (Figure 7). There was no statistically significant difference in relationship between p53 protein expression with tumor grade and stage as shown in (Table 1).

P53 immunostaining in at least 10% of the cell nuclei of tumor cells was regarded as p53 overexpression.

The extent of staining of p53 expression in gastric carcinoma in the present study (according to Sophia K. 1999[8]) and Roviello F.1999 [9] was done at x40 objective as follows: (Table 2)

A) 22(55%) of negative cases were within score 0.

B) 7(17.5%) of positive cases were within score 1.

C) 5(12.5%) of positive cases were within score 2.

D) 2(5%) of positive cases were within score 3.

E) 4(10%) of positive cases were within score 4.

The pattern of positive p53 staining was diffuse in 6 (33%), regional in 8(45%), and focal in 4(22%). (Table 2)

The intensity of p53 staining was weak in 6(33%) (Figure8), moderate in 7 (39%), and strong in 5 (28%) (Figure 9) of gastric carcinoma cases (Table 2).

Table 1: Distribution of p53 expression in gastric carcinoma cases in relation to different studied parameters.

		Age		**Gender**		**Site**		**Histological type**		**Histological grade**		**Pathological stage**		
		<50	≥50	M	F	Antrum	Cardia	Diffuse	Intestinal	Moderate	Poor	I	III	IV
P53	positive	6	12	13	5	15	3	4	14	12	6	0	17	1
	negative	6	16	15	7	17	5	6	16	13	9	1	20	1
	total	12	28	28	12	32	8	10	30	25	15	1	37	2
	P-value	**0.701**		**0.812**		**0.709**		**0.471**		**0.870**		**0.653**		

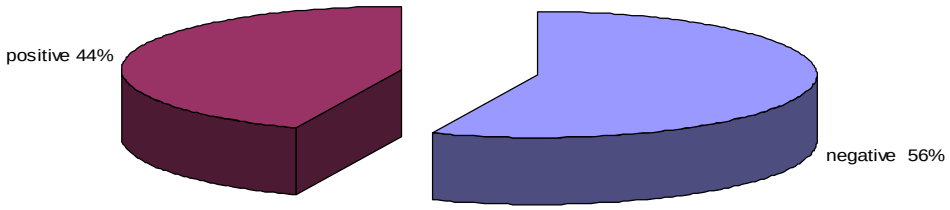

Figure 1: The expression of p53 in gastric carcinoma cases.

Table 2: Distribution of p53 expression in relation to scoring system, staining, and intensity in gastric carcinoma cases.

Scoring of P53 in all cases	0	1	2	3	4	Total
Frequency No. %	22 55%	7 17.5%	5 12.5%	2 5%	4 10%	40 100%
Staining in P53 positive cases	**Diffuse**	**Regional**		**Focal**		**Total**
Frequency No. %	6 33%	8 45%		4 22%		18 100%
Intensity in P53 positive cases	**Weak**	**Moderate**		**Strong**		**Total**
Frequency No. %	6 33%	7 39%		5 28%		18 100%

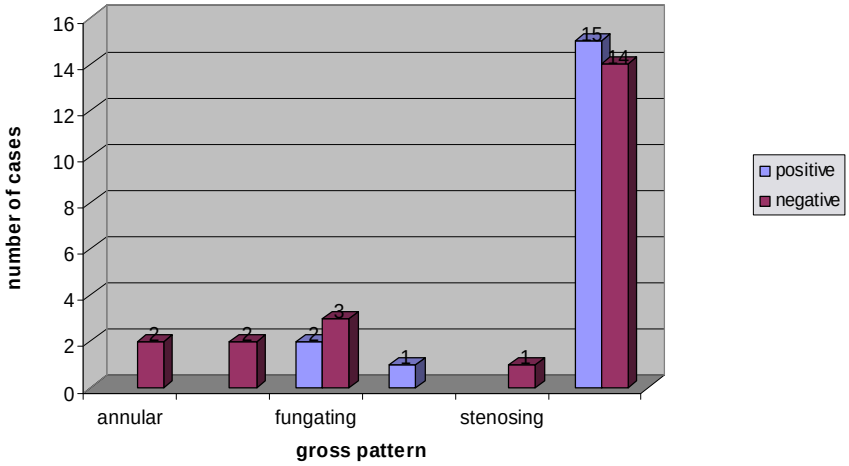

Figure 2: The distribution of p53 expression according to gross pattern.

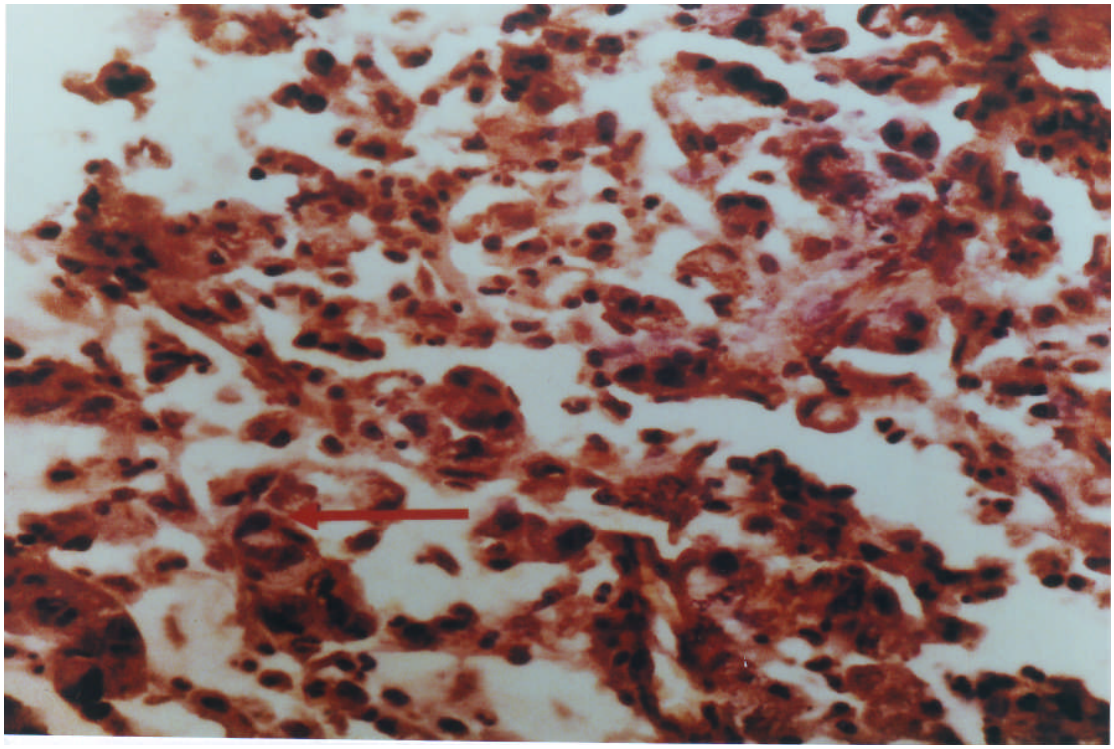

Figure 3: poorly differentiated diffuse type gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method), (Arrow) at (x40).

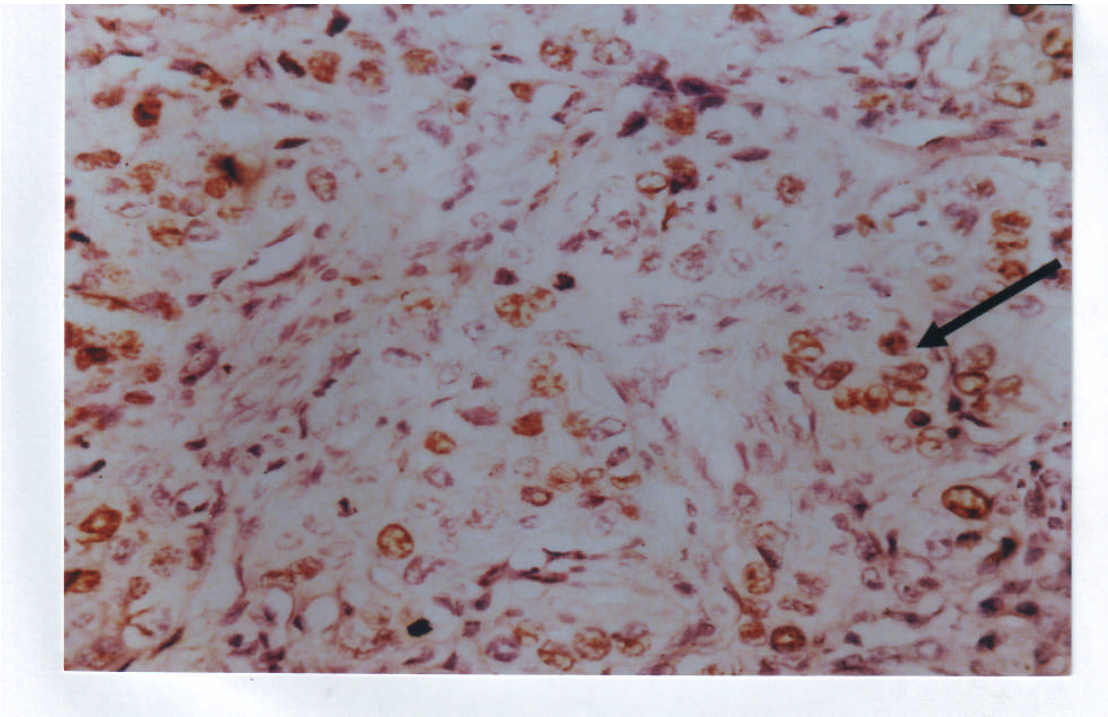

Figure 4: intestinal type gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method), (Arrow) at (x40).

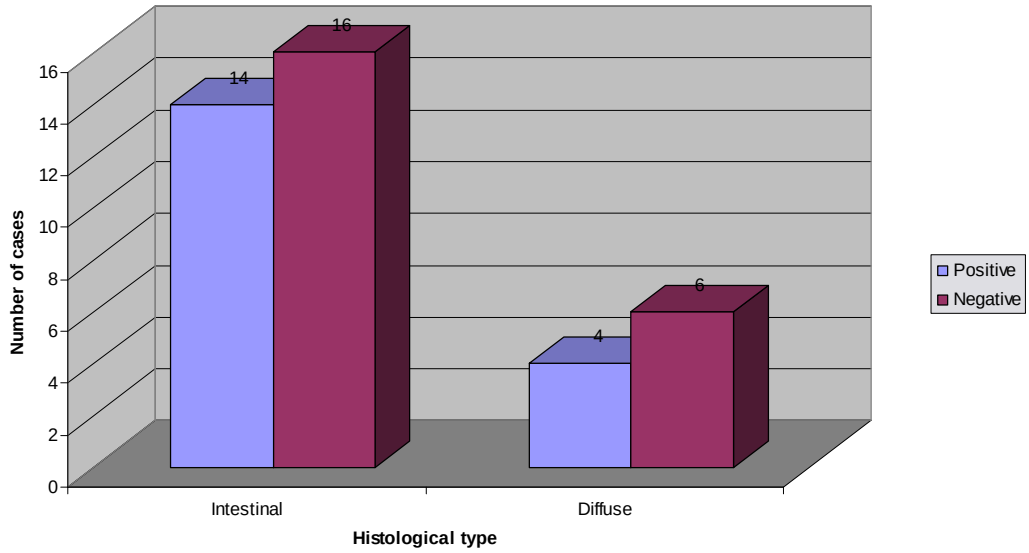

Figure 5: The distribution of p53 expression according to histological type.

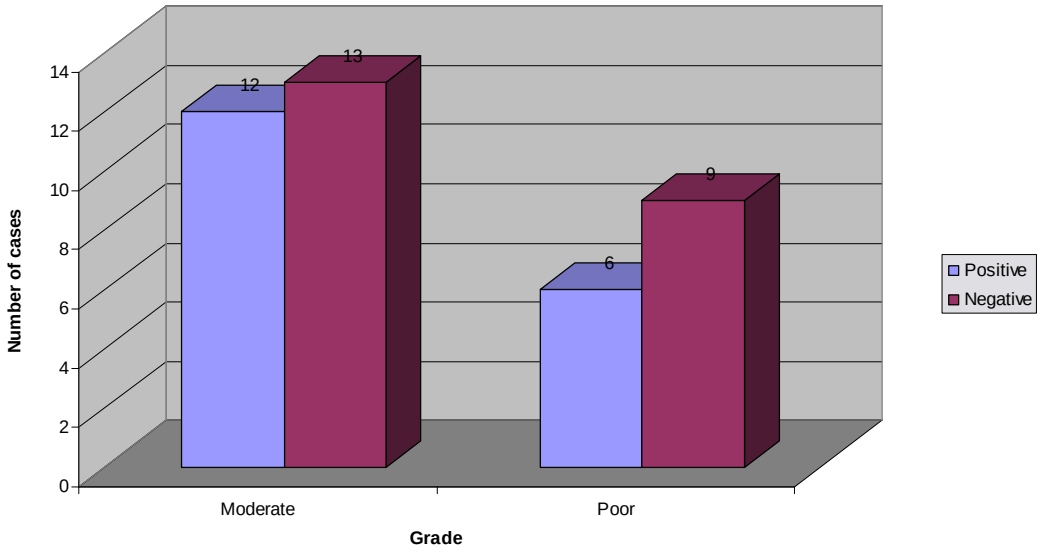

Figure 6: The distribution of p53 expression according to histological grade.

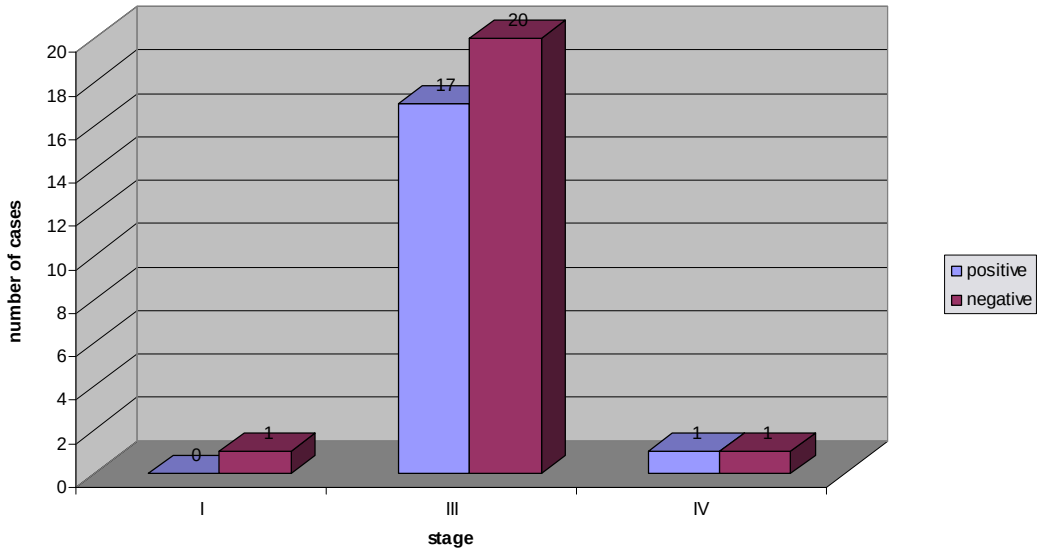

Figure 7: The distribution of p53 expression according to stage.

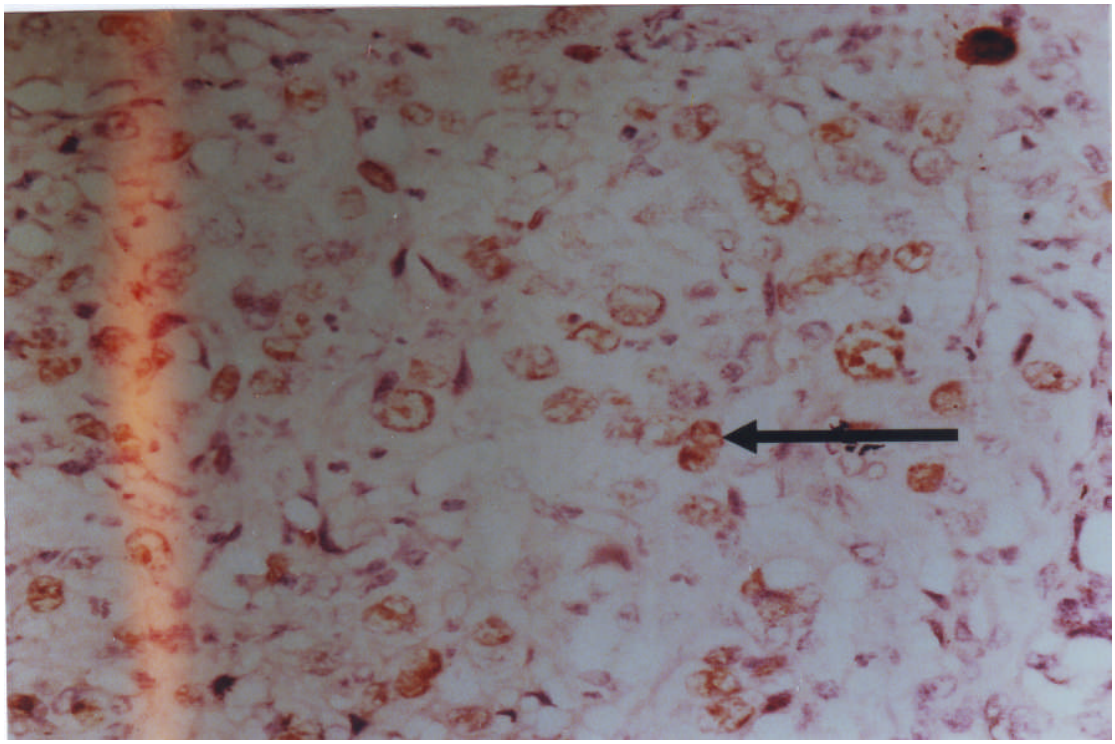

Figure (8) moderately differentiated gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method),(weak expression), (Arrow) at (x40).

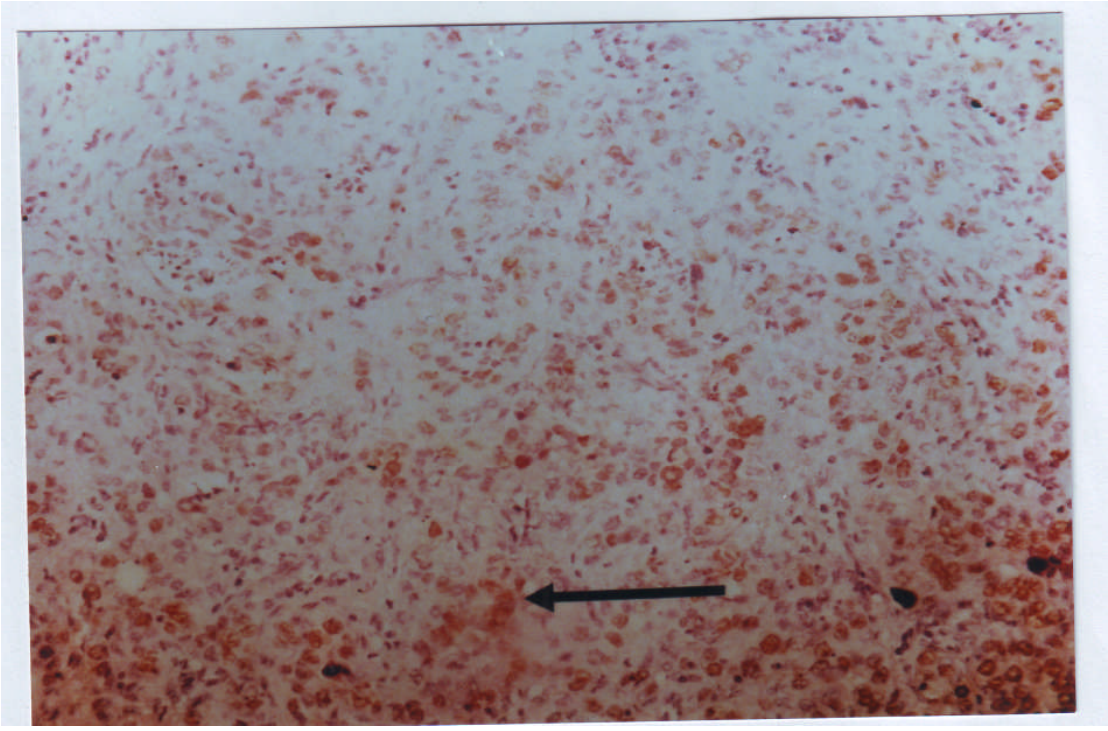

Figure 9: poorly differentiated gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method),(strong expression), (Arrow) at (x10)

Discussion

Worldwide, gastric carcinoma is one of the commonest cancers after lung cancer and a major cause of mortality and morbidity, especially in developing countries [10].

The etiology of gastric carcinoma includes both genetic factors and environmental factors such as H.pylori. Multiple genetic alterations are detected not only in gastric carcinoma, but also in tumors at other sites [11]. In gastric carcinoma, p53 is present solely in tumor cells while it is not so in adjacent normal stomach mucosa. It is also present in dysplastic epithelium surrounding tumor in varying degrees. Joypaul et al reported that there was 20% staining with p53 in severe dyplasia [12].

In this study, p53 overexpression was positive in (44%) of gastric carcinoma cases, which is compatible with other studies in Iraq [13], Iran [14], and Turkey [15].

This study showed that there was no significant correlation between p53 overexpression and patient's age. This result is nearly compatible with the results from Iraq [13], Iran [14], Turkey [15], and also with other studies [11, 16, 17]. Also no significant correlation was found between p53 overexpression and sex of the patient. The slightly higher expression rate in males than females could be attributed to the higher incidence rate of gastric carcinoma in males compared to females, similar results were seen in different studies [11, 13, 14, 15, 16, 17].

Regarding the relation of p53 immunostaining with the tumor site, although the results were statistically not significant , a higher rate of p53 positivity was seen in gastric carcinoma cases located in antrum , this could be explained by the fact that majority of gastric cancers cases(80%) were located in antrum. Fléjou et al found p53 positivity rate was higher in cases located in the cardia and concluded that the tumors located in the cardia exhibited higher rates of aneuploidy than those located in the antrum. They ascribed this difference to different molecular mechanisms leading to malignant transformation in carcinomas located in the cardia and the antrum and proposed that antral tumors developed mostly in response to environmental factors [18] .In other studies, no correlation was found between tumor location and the rate of p53 positivity [16,17, 19].

In the literature, it was reported that there was no significant relationship between p53 positivity rate and growth pattern [11, 16, 17]. This result is similar to a study in Turkey [15], while in this study p53 positivity rate was higher in ulcerative growth pattern type.

In various studies, the rate of p53 positivity was found to be different in varying histological types of gastric carcinoma. The positivity rates were higher in intestinal type gastric carcinoma, varying between (50% and 70%). This rate was lower in diffuse type gastric carcinoma, being (12-27%) [11, 16, 17, 18, 20]. However, some of theses studies had found a significant relationship between p53 overexpression and histological type [11, 18, 20]; others did not [16, 17, 21]. These figures are congruent with those in the literature and suggest that p53 may play a part especially in the formation of intestinal type carcinoma. In a study in Turkey [15], the positivity rates were higher in intestinal type carcinoma, whereas in Iran [14], they found that the positivity rates were higher in diffuse type carcinoma. In the present study, p53 positivity was higher in intestinal type compared to diffuse type.

Regarding the correlation between p53 overexpression and tumor grade,

no significant correlation was found in this study and other studies [14, 17, 18, 20]. In a previous study done in Iraq, p53 protein accumulation was higher in poorly differentiated gastric carcinoma than in moderately and well differentiated ones [13], similar results were also found in other studies [22, 23, 24], this difference in the results obtained from different studies could be due to sample size limitation and different techniques used during work.

In concordance with other studies, there was no statistical significant difference between p53 overexpression and stage of gastric cancer [17, 21, 25].

In conclusion, the overall expression of p53 protein in gastric carcinoma cases in the present study was 44% and there was no significant correlation between p53 overexpression and different clinicopathological variables like: age and sex of patients, site, gross pattern, histological type, tumor grade and stage. However, Intestinal type gastric carcinoma showed a higher p53 expression rate in comparison to diffuse type and also P53 overexpression was more common in moderately differentiated type gastric carcinoma cases than in poorly differentiated type.

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Immunohistochemical Expression of P53 in Invasive Cervical Carcinoma (A Clinicopathological Study)

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Abstract

Background: Cervical cancer is one of the most frequent diseases in women; it comprises approximately 12% of all cancers in women worldwide. P53 is a tumor suppressor gene, functional inactivation of the P53 gene is a key event in tumorigenesis of many human malignancies, in cervical carcinoma this functional inactivation could occur either due to mutations or causes other than mutations like binding and inactivation or degradation by viral proteins.

Objective: To assess the immunohistochemical expression of P53 in invasive cervical carcinoma (squamous cells carcinoma and adenocarcinoma) and to study the correlation between P53 over-expression with clinico-pathological variants (age, grade of tumor and histological type).

Materials and methods: A total of 42 tissue samples of invasive cervical carcinoma (30 cases of squamous cell carcinoma and 12 cases of adenocarcinoma) were included in this retrospective study.

The samples were obtained from archival paraffin embedded blocks covering the years 1998 to 2005 from the histopathology files of al-Kadhimiya Teaching Hospital, Al-Ulwiya Teaching Hospital and from private laboratories. All the clinico-pathological data had been obtained from the files of these patients.

Out of 12 cases of adenocarcinoma, 8 had punch biopsy, and 4 had hysterectomies .For the 30 cases of squamous cell carcinomas, 16 patients had punch biopsy and 14 had hysterectomy.

All cases were analyzed by immunohistochemical staining with P53 tumor marker.

Results: The percentage of P53 over-expression in cervical adenocarcinoma (58.3%) was significantly higher than P53 over-expression in cervical squamous cell carcinoma (16.66%), ($P<0.05$).

P53 nuclear positivity in poorly, moderate and well-differentiated invasive cervical cancers was (50%, 18.18%, and 16.16% respectively), with no significant difference between P53 over-expression in different grades ($P>0.05$).

The percentage of P53 over-expression for the patients below the age of 50 was (32.14%) and for those equal and above 50 was (17.64%), no significant difference was found in P53 over-expression between the two age groups.

From the clinico-pathological assessment, the mean age of cervical adenocarcinoma (38.5 ±1.11 S.D. years) was significantly lower than the mean age of cervical squamous cell carcinoma (47.5±1.94 S.D. years).

No significant difference was found between the grade of the invasive cervical carcinoma and the two histological types.

Conclusion: In this study, a significant correlation has been found between P53 over-expression and the histological type of the invasive cervical carcinoma.

-Although there was no statistical correlation between P53 over-expression and the three grades of the invasive cervical carcinoma, poorly differentiated tumors showed the higher percentage of P53 over-expression.

-No significant difference was found between P53 over-expression and the age of the patient.

Key words: P53, cervical carcinoma, immunohistochemical expression.

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Introduction

Cervical carcinoma is the second leading cause of death in women worldwide.

Cervical cancer affects 13,500 women and accounts for 4,500 death annually in the United States [(1)].In Iraq, the neoplasms of the cervix uteri ranked the 6th among the commonest 10 cancers in female during the period 1976-1985, whereas during the period 1995 – 1997, it ranked the tenth within the leading cancers in females [(2,3)].From the overview of cervical

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cancer situation , we found that cancers of the cervix uteri constitutes 1.4% of the total number of cancers with annual number of 113 new cases of cervical cancer reported in 1995, 1996 and 1997 respectively[3].

P53 is a tumor suppressor gene located on the short arm of chromosome 17[4].Wild "normal" type P53 protein is non- mutant , has tumor suppressor effect because of its ability to inhibit transformation [5].It has a short half-life of about 20 minutes , and can not be detected in the nucleus of most normal tissues [6].The mutant type P53 protein is much more metabolically stable than the wild type and accumulates in the nucleus and has prolonged half-life (up to 9 hours) which renders it more likely to be detected by immunohistochemical assay [7]. P53 gene is capable of modulating the expression of a variety of genes such as genes controlling cell arrest in G1/S phase, genes controlling apoptosis and genes controlling P53 protein [8], so functional inactivation makes the cell turn onto a one way street leading to malignant transformation [9]. This inactivation could occur either due to mutations in the P53 gene (which are the most frequent mutations encountered in human tumors) [10]or causes other than mutations like binding and inactivation or degradation by viral proteins[11] .

Hence this study aims to assess the immunohistochemical expression of P53 in invasive cervical carcinoma (squamous cells carcinoma and adenocarcinoma) and to study the correlation between P53 over-expression with clinico-pathological variants (age, grade and histological type).

Patients and methods

A total of 42 tissue samples of invasive cervical carcinoma (30 cases of squamous cell carcinoma and 12 cases of adenocarcinoma) were included in this retrospective study.

The samples were obtained from paraffin embedded blocks covering the years 1998 to 2005 from the histopathology files of Al-Kadhimiya Teaching Hospital, Al-Ulwiya Teaching Hospital and from private laboratories. All the clinical data had been obtained from the files of these patients. Out of 12 cases of adenocarcinomas, 8 patients had punch biopsies, and 4 had hysterectomies .For the 30 cases of squamous cell carcinomas, 16 patients had punch biopsies and 14 had hysterectomies.

The diagnosis was confirmed by review of two freshly prepared hematoxylin and eosin- stained slides. Two slides had been prepared to be stained immunohistochemically with P53 monoclonal antibody. To determine the signal specificity, negative control slides were included.

In the first run, the negative control slides included sequential omission of reactive component in the test; the primary (monoclonal) antibody, the secondary antibody (the biotinylated link), the conjugate and the substrate. Then, in each immunohistochemistry run, the negative control slides were obtained by omitting the primary antibody and, this was undertaken under identical test conditions [12].

Sections from a breast cancer patient that were known to be immunoreactive for P53 antibodies were used as positive controls for P53 and run with each batch stained [12].

Positive P53 results give nuclear brownish color, granular or homogenous, without any cytoplasmic or artifactual staining as in analytic cells or hemorrhage [13]. The results of P53 positivity in each individual case were analyzed according to the following variables:

1. Semi-quantitative assessment: A minimum of 100 tumor cells were scored. Those cervical tissues with P53 immunostaining in at least 5% of the cell nuclei were considered to have P53 over-expression [14].

Scoring was done at X40 objective as follows [14]:

-Negative (score 0): None of the cells revealed positivity for P53 marker.

-Weak or mild: Staining [(5 %-< 10%) positive of tumor cells] (score +1)

-Moderate: Staining (<25%) (Score +2)

-Strong: Staining (>25 %-< 50%) (Score +3)

-Highly strong: (over 50%) (Score +4)

2. Pattern of staining: The pattern was classified into Diffuse, Regional and Focal. It was considered Diffuse if the P53 positive cells were distributed through almost all the section as homogenous distribution, while considered Regional if more than one area of section showed large number of P53 positive cells, and it was considered Focal if P53 positive cells were localized in only one area of the section [15].

3. Intensity of the staining: The intensity of the brownish coloration was graded as strong, moderate or weak .It is strong if the brownish coloration is detected very clearly even at low power (100) and moderate if it was detected with difficulty at low magnification while if P53 positivity could only be detected at high magnification (1000) it was considered weak [16].

Statistical analysis was done using student t-test were P value of <0.05 was considered significant.

Results

Forty two cases of cervical cancer were included in this study; 30 cases of squamous cell carcinoma their age ranges from (30-65) years with a mean age of (47.50±1.94 S.D.) years. And 12 cases of adenocarcinoma their age ranges from (32-45) years with a mean age of (38.50±1.11 S.D.) years. From the descriptive analysis, there was a significant difference between the mean age of the two histological types; the mean age of adenocarcinoma was significantly lower than that of the squamous cell carcinoma (P<0.0.5). (Figure1)

Histopathologically, there were 30 cases of squamous cell carcinoma: 4(13.33%) cases were well –differentiated, 17(56.66%) cases were moderately –differentiated and 9 (30%) cases were poorly differentiated, and 12 cases of adenocarcinomas: 2(16.66%) cases were well –differentiated, 5(41.66%) cases were moderately –differentiated and 5(41.66%) cases were poorly –differentiated adenocarcinomas, from the descriptive analysis shown in (Table 1) there was no significant difference between the grade of the tumor and the two histological types. (P>0.05)(Figure 2)

Immunohistochemical staining of P53 showed granular brown nuclear staining in positive cases, from the total 42 cases of invasive cervical carcinoma;30 (71.43%) cases were found to be negative immunohistochemical expression of P53 with a (score 0), while 12 (28.57%) cases showed a positive over-expression of P53, 5(11.91%) cases were considered as score 1, 3(7.14%) cases were considered as score 2, 2(4.76%) cases were considered as score 3 and 2(4.76%) cases were considered as score 4 as shown in (Table 2).

The pattern of nuclear immunostaining of the 12 positive cases was in 4 (33.33%) cases having a diffuse pattern, 2 (16.67%) cases having a Regional pattern, and 6(50%) cases having a Focal pattern as shown in (Figures 3 and 4).

The intensity of nuclear immunostaining of the 12 P53 positive cases was Weak in 3(25%) cases, Moderate in 5(41.67%) cases and Strong in 4 (33.33 cases) as shown in (Figures 5 and 6).

There was no significant difference between the age of the patients and P53 over-expression (P>0.05). Also there was no significant difference between the P53 over-expression and the three grades of invasive cervical carcinoma (P>0.05), however poorly differentiated tumors showed high percentage of P53 over – expression as shown in (Table 3).

Immunohistochemical analysis of P53 over-expression in relation to the histological type of invasive cervical carcinoma revealed that P53 over-expression was detected in 5(16.66%) cases out of the 30 cases of squamous cell carcinoma and 7 (58.3%) cases out of 12 cases of adenocarcinoma. A significant correlation was found between the over-expression of P53 and the histological type. The over-expression of P53 in adenocarcinoma was significantly higher than P53 over-expression in squamous cell carcinoma (P<0.05) (Table 4).

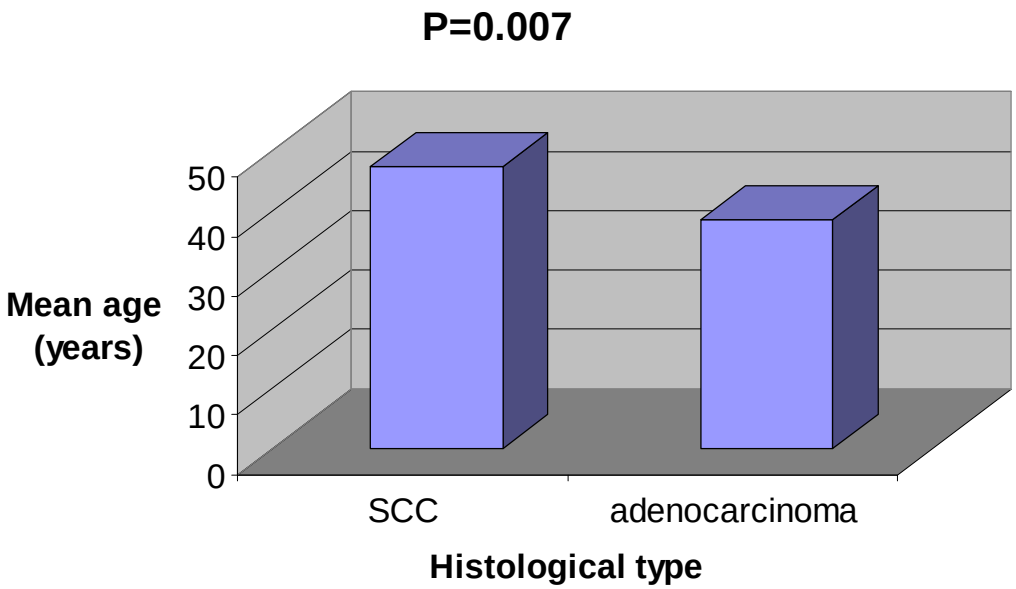
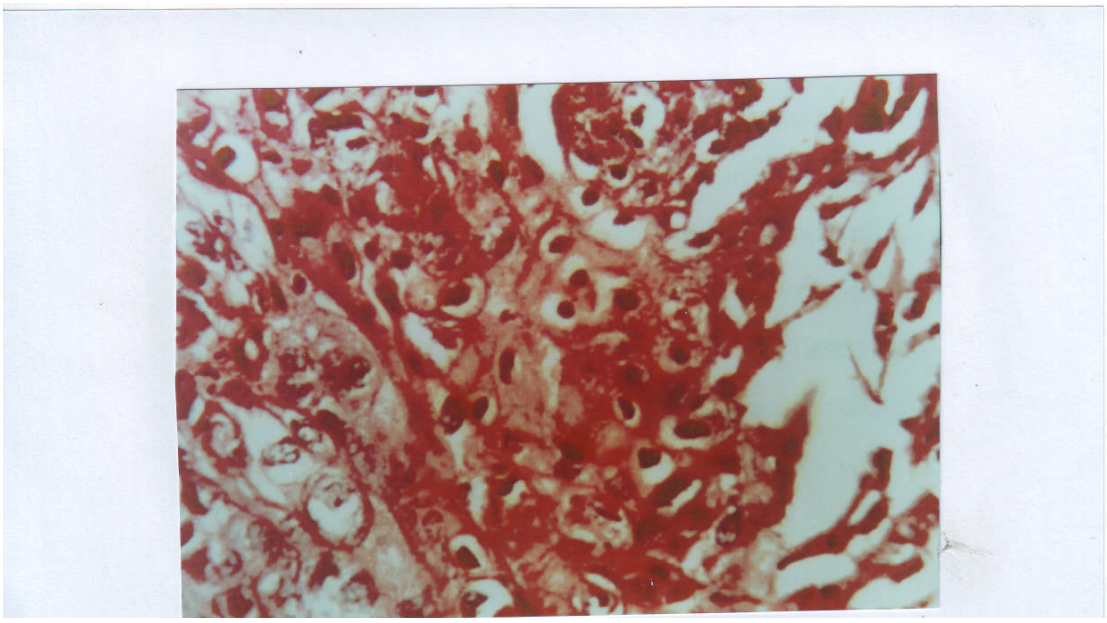


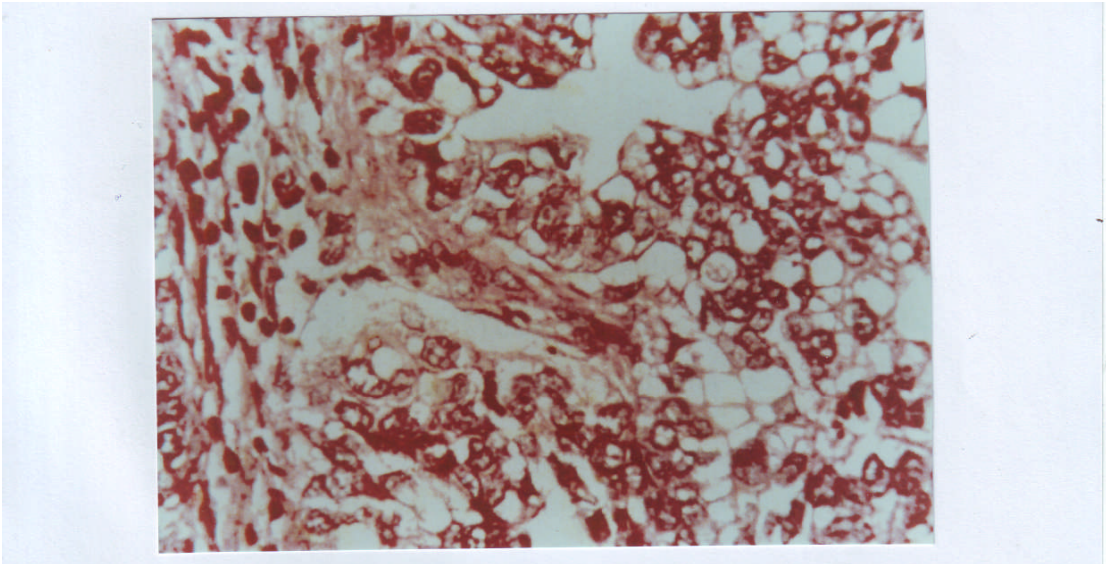
Figure 1: The relation between the age of the patients and the histological type of the invasive cervical carcinoma.

Table 1: Distribution of cases according to the grade of invasive cervical carcinoma.

		Histological Type	
		SCC	Adenocarcinoma
Grade	Well	4	2
	Moderate	17	5
	Poor	9	5
	Total	30	12
	P-value	0.675 (non-significant)	

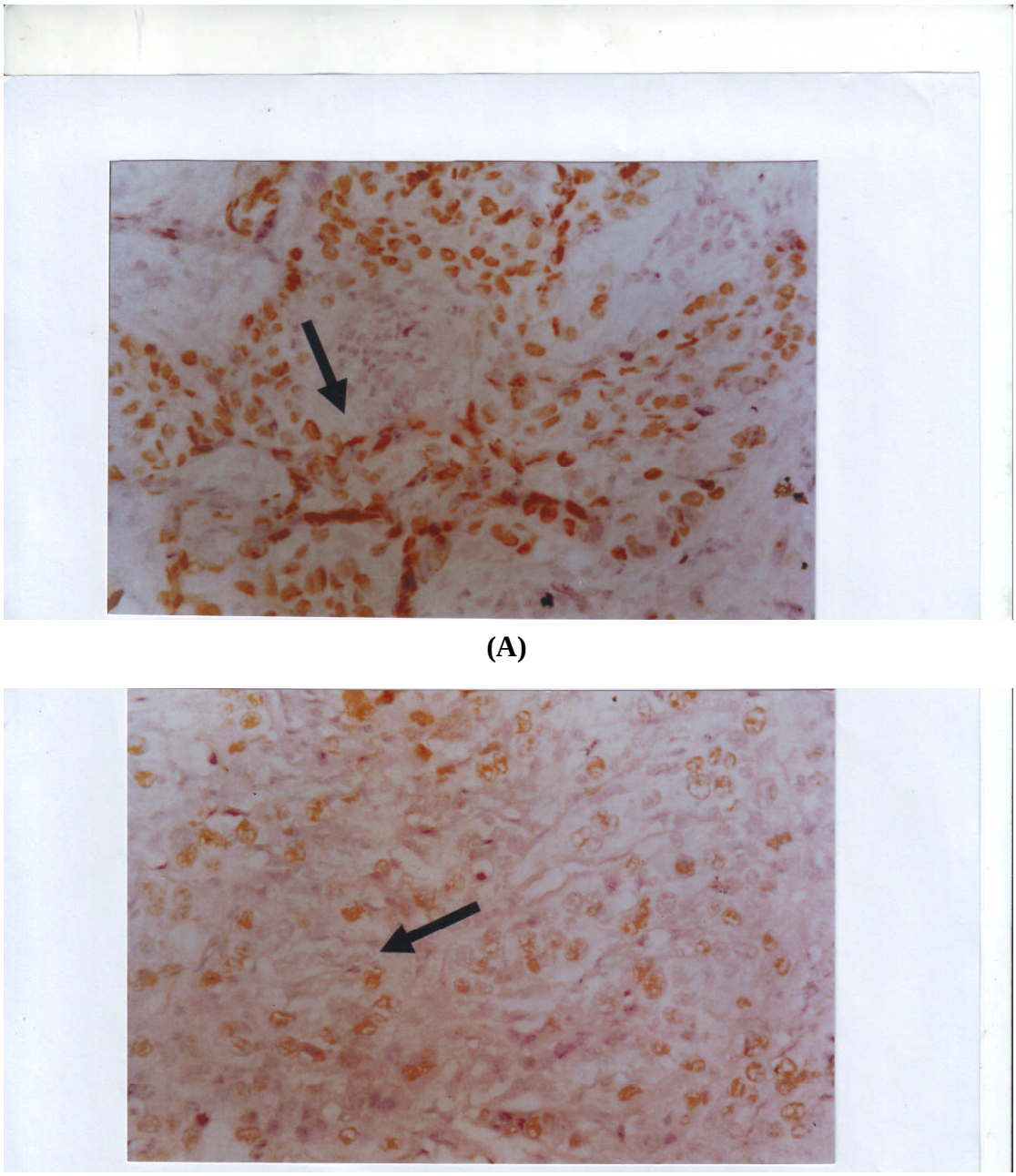


(A)



(B)

Figure 2: Histopathological section of (H&E, 400) [A] Squamous cell carcinoma of the cervix showing nests of squamous tumor cells. [B] Adenocarcinoma of the cervix showing moderately-differentiated glands.



(A)

(B)

Figure 3: Immunohistochemical staining of P53 (200) in cervical squamous cell carcinoma. Staining by DAB chromogen (brownish color) counterstained with Mayer's hematoxylin [A] Squamous cell carcinoma with a regional pattern of P53 nuclear staining (dark arrow) [B] Adenocarcinoma with a diffuse pattern of P53 nuclear staining (dark arrow)

Table 2: Distribution of P53 expression according to Sophia scoring system, pattern and intensity of staining.

Scoring of P53 in all cases	0	1	2	3	4	Total
Frequency No. %	30 71.43%	5 11.91%	3 7.14%	2 4.76%	2 4.76%	42 100%
Staining in P53 positive cases	Diffuse	Regional		Focal		Total
Frequency No. %	4 33.33%	2 16.67%		6 50%		12 100%
Intensity in P53 positive cases	Weak	Moderate		Strong		Total
Frequency No. %	3 25%	5 41.67%		4 33.33%		12 100%

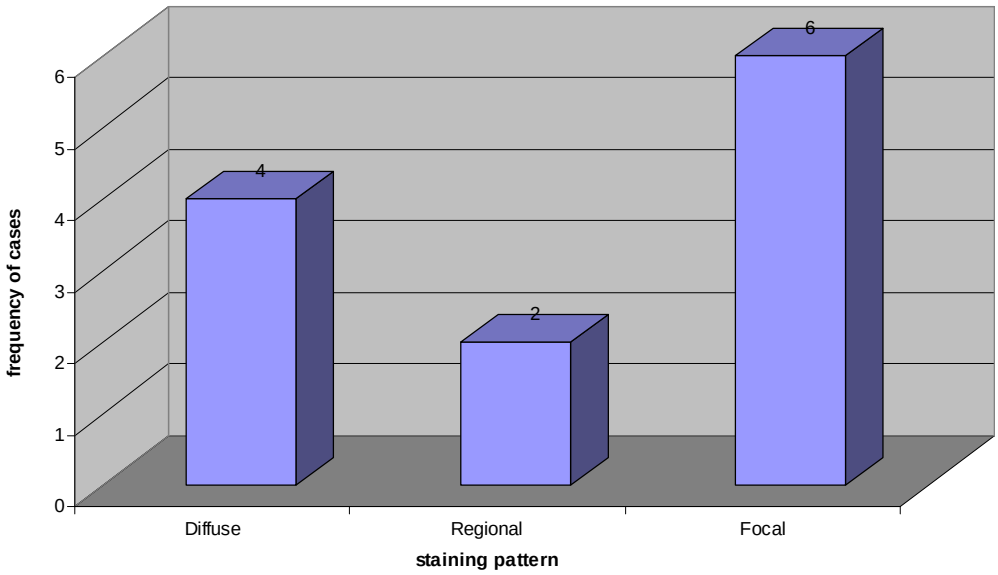
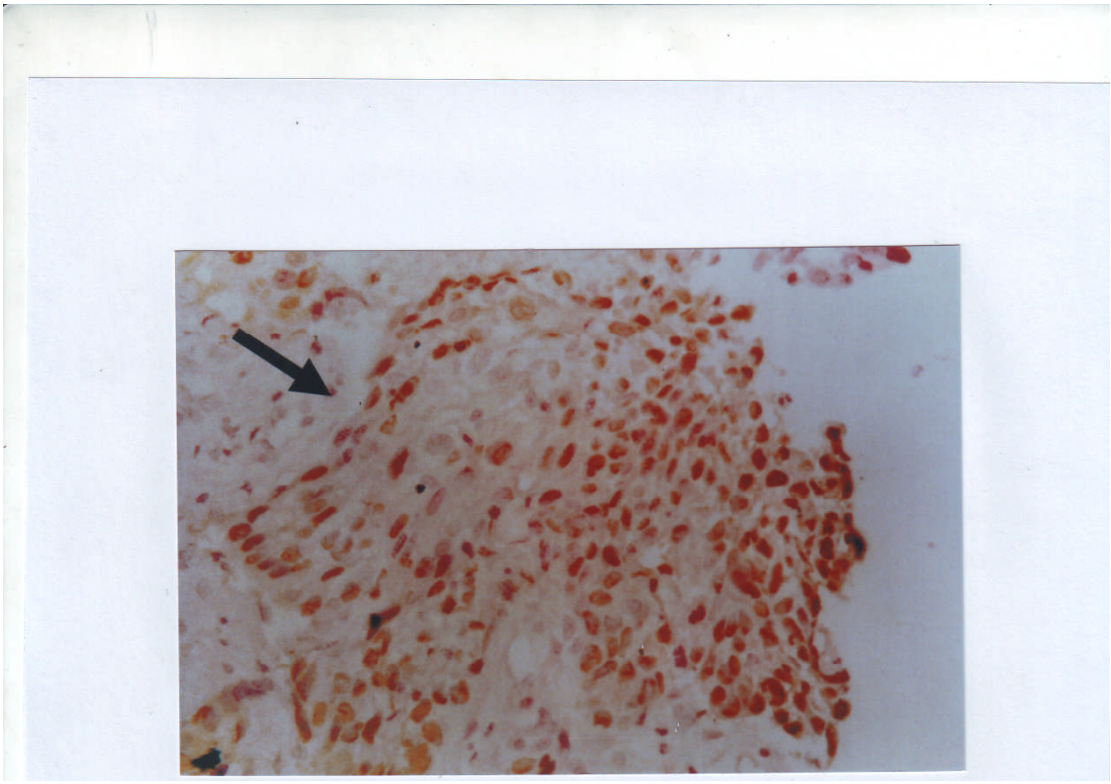
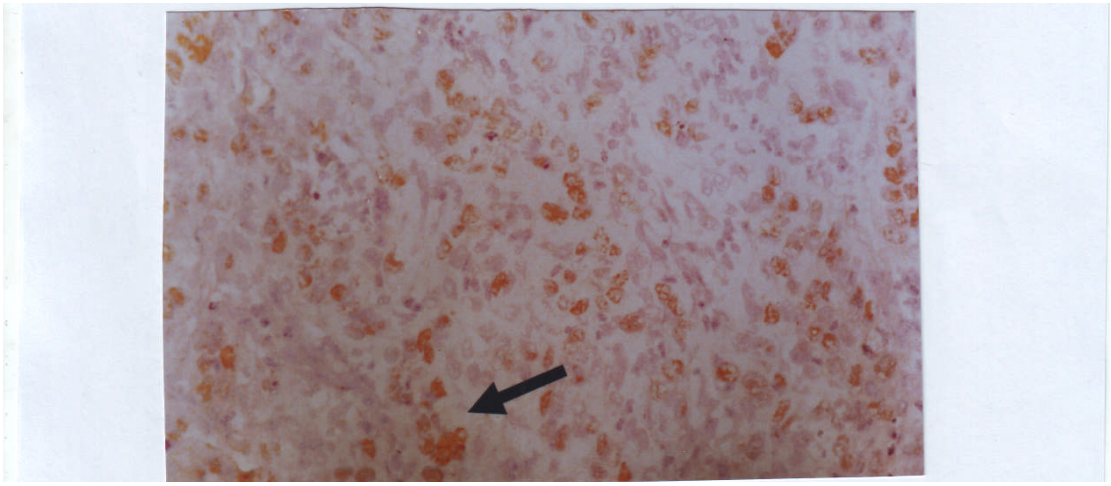


Figure 4: Distribution of P53 expression in invasive cervical carcinoma according to the pattern of staining.



(A)



(B)

Figure 5: Immunohistochemical staining of P53in cervical squamous cell carcinoma. Staining by DAB chromogen (brownish color) counterstained with Mayer's hematoxylin [A] Squamous cell carcinoma with strong P53 staining (>25 %-< 50%) (200) [B] Adenocarcinoma with moderate P53 staining (<25%) of the cells revealed nuclear positivity for P53 marker (dark arrow) (400)

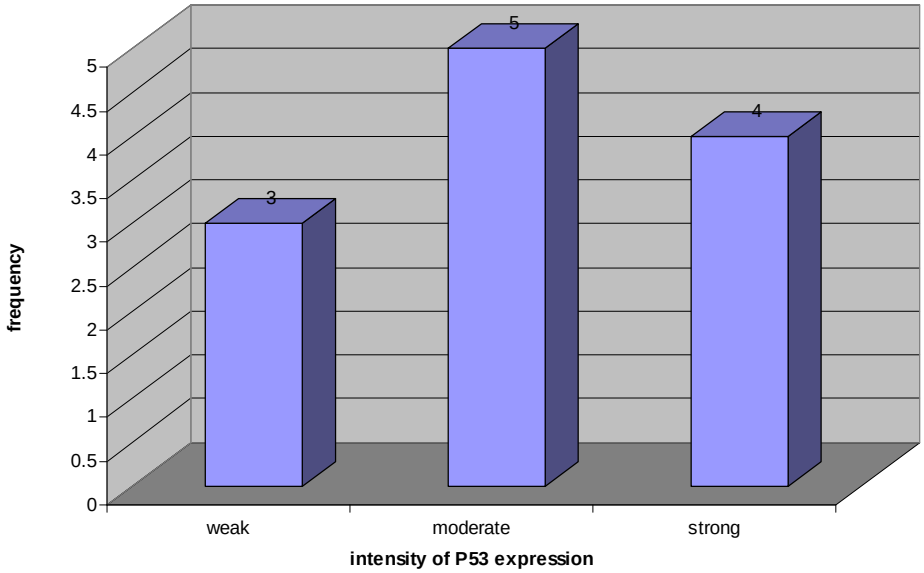


Figure 6: Distribution of P53 expression in invasive cervical carcinoma according to the intensity of staining.

Table 3: Expression of P53 in relation to the grade of invasive cervical carcinoma.

		Grade		
		Well	Moderate	Poor
P53	Positive	1 (16.66%)	4 (18.18%)	7 (50%)
	Negative	5 (83.34%)	18 (81.82%)	7 (50%)
	Total	6	22	14
	P-value	0.094 (non-significant)		

Table 4: Expression of P53 in relation to the histological type of invasive cervical carcinoma.

		Histological type	
		SCC	Adenocarcinoma
P53	Positive	5 (16.66%)	7 (58.3%)
	Negative	25 (83.34%)	5 (41.7%)
	Total	30	12
	P-value	0.02 (significant)	

Discussion

Cervical cancer is one of the most frequent diseases in women, it comprises approximately 12% of all cancers in women worldwide [17, 18].In Iraq, as in developing countries, the lower social conditions, the average age of the first intercourse, the high rate of parity and the lack of the primary care in the health system are important risk factors for the rate of cervical cancer [19].

This study showed that there is a significant difference between the mean age of invasive cervical squamous cell carcinoma and the mean age of invasive cervical adenocarcinoma ($P<0.05$) and this finding is similar to that of Abbas S.M, 2002 [20].

Concerning the relation between P53 over-expression and the age of the patients, the findings of this study were similar to that of Koyamatsu et al., 2002 [21] and Nair et al., 1999[22] which showed that there is no significant difference between P53 over-expression and the age of the patients.

Conflicting data concerning P53 over-expression in relation to the grade of invasive cervical carcinoma have been reported. Cheah and Looi, 2002 [23] showed that there is a significant correlation between P53 over-expression and the grade of the tumor, in such a way that P53 became more frequently expressed with less differentiated tumors. On the other hand, Nair et al., 1999[22] showed that there was no significant correlation between P53 over-expression and the three grades of invasive cervical carcinoma, which is similar to the findings in this study.

Although there was no significant correlation statistically between over-expression and the grade of invasive cervical carcinoma, poorly differentiated tumors showed a high percentage of P54 over-expression (50%).

The evaluation of P53 over-expression in invasive cervical carcinoma was examined in numerous studies, but the obtained results were controversial. In the majority of the studies (Abdulla A.M, 2006[19]; Kersmaekers et al., 1999[24]; Kainz et al., 1995 [25]), the frequency of the P53 over-expression in cervical squamous cell carcinoma was comparable to the results of this study.

P53 over-expression in adenocarcinoma in this study was also comparable to other studies (Abd et al., 1999 [26]; McCluggage et al., 1997 [27]). In concordance with other studies (Cheah and Looi 2002 [23]; Abd et al., 1999 [26]; Quinn MA., 1997[28]; Nagan et al., 1997[29]), this study showed that P53 over-expression in adenocarcinoma was significantly higher than that in squamous cells carcinoma of the cervix.

Some studies (Cheah and Looi 2002 [23]; Tenti P et al., 1998[30]) showed that the higher level of P53 expression in adenocarcinoma compared to squamous cell carcinoma may be due to higher frequency of mutation in adenocarcinoma. Most mutations induce conformational changes causing over-expression of P53 protein, stabilizing it and making it detectable by immunohistochemical analysis (Zheng A et al., 1999 [31]; Berns EM et al., 1998[32];Villuendes R et al ., 1997 [33]).

It has been suggested that P53 over-expression represents a poor prognostic factor [11] .Since P53 expression in adenocarcinoma is significantly higher than that in squamous cell carcinoma of the cervix, this could contribute to the less favorable prognosis of the former than the latter [34].

The current study as other studies (Wang et al., 2004 [35]; Koyamatsu et al., 2002 [21]; Vassallo et al., 2000 [36]) was undertaken with assumption that the immunohistochemical detection of P53 with the monoclonal antibody is almost associated with the presence of the mutated forms of P53 allels, based on wild type P53 protein possessing a short half-life, ranging up to 30 minutes, hence not accumulating to immunohistochemically detectable levels, and mutant forms having longer half-lives providing for immunohistochemical detection in many instances [37] .

Such immunohistochemical detection of P53 has frequently been used as a simpler method than genetic analysis of P53 mutations. However , it is not very specific for evaluation of P53 mutations in human cancers since tissue fixation , the type of antibody and other technical factors may affect the detection [38] .In addition ,not all mutations in the P53 gene result in the increase accumulation of the protein ,frame shift and non-sense mutations can lead to expression of an altered P53 which is undetectable by the available monoclonal antibodies , or some mutations may disrupt the binding sites of the anti-p53 antibody[21].

The absence of antibody reactivity therefore does not rule out genetic alterations of the P53 in human tumors [39].

In conclusion this study showed a significant correlation between P53 over-expression and the histological type of the invasive cervical carcinoma. Although there was no statistical correlation between P53 over-expression and the three grades of the invasive cervical carcinoma, poorly-differentiated tumors showed the higher percentage of P53 over-expression. No significant difference was found between P53 over-expression and the age of the patient.

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Induction of ICAM-1 and ICAM-3 in Women with Recurrent Pregnancy Loss

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Abstract

Background: Recurrent pregnancy loss (RPL) has been found to be associated with increase in the pro-inflammatory cytokines which cause up-regulation of inflammatory mediators including cell adhesion molecules (CAMs) that might act in aggravation of this pathological process.

Objective: To find out whether there is a relation between the pathology of RPL and the expression of intracellular adhesion molecule-1 (ICAM-1) and ICAM-3 at the feto-maternal interface in these patients.

Methods: Immunohistochemistry technique was performed to detect and determine the expression of ICAM-1 and ICAM-3 using paraffin embedded sections of curate samples obtained from 40 women, who where divided into three groups: 24 women with RPL, 10 women with abortion for the first time, and 6 women with induced abortion.

Results: The levels of the expression of both endothelial ICAM-1 and leukocytes ICAM-3 at the feto-maternal interface were found to be significantly up-regulated in the first group as compared with the second and the third groups (p=0.001), with a highly significant positive correlation between these two parameters (r=0.927, p<0.01).

Conclusion: ICAM-1 and ICAM-3 might play an important role in the pathology of RPL by increase adherence and recruitment of inflammatory cells at the feto-maternal interface ending with a pregnancy failure.

Key words: Inter Cellular Adhesion Molecule-1 and Inter Cellular Adhesion Molecule -3, Recurrent pregnancy loss.

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Introduction

Cell adhesion molecules (CAMs) mediate cell–cell interactions and play an important role in cell differentiation [(1)], in the organization of the extracellular matrix and in the recruitment and aggregation of leukocytes from the circulation [(2)]. The immunoglobulin superfamily of which intercellular adhesion molecule (ICAM)-1 and ICAM-2 are members is the most widely distributed family of cell adhesion molecules. ICAM-1 is expressed on leukocytes, epithelial and endothelial cells, ICAM-2 is mainly found on resting endothelial cells and ICAM-3 is constitutively expressed by all resting leukocytes [(3)].

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Cell adhesion molecules are present in human endometrium, where they may play a role in regulating leukocyte trafficking into this tissue [(2, 4, 5)]. It is recognized that the normal endometrium has a population of leukocytes, including macrophages, T-lymphocytes and granulocytes, which are important in the physiology of the endometrium. Furthermore, T cells form 10-15% of lymphocytes in early pregnancy deciduas [(6)], while B cells are professional cells that produce immunoglobulines; their count and population in the endometrium do not change through out menstruation and during pregnancy, and the second major decidual leukocyte population consists of the monocytes/macrophages [(7)].

The expression of ICAM-1 in human endometrium can be stimulated by cytokines including interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-1β [(4, 8, 9)].

However, endothelial, fibroblastic and epithelial cells differ in their response to ICAM-1-inducing cytokines [8, 9]. In contrast to ICAM-1; ICAM-2 expression is not normally augmented by cytokine activation [3].

It was found that these type1 cytokines (like IFN-γ and TNF-α) are up regulated in women with RPL [10, 11]. And as the target of these pro-inflammatory cytokines was found to be mainly vascular associated with inflammatory cells infilterate[7,12,13] . We attempted in this study to explore the expression of endothelial ICAM-1 which is inducible by these cytokines [4], and the expression of leukocytes' ICAM-3, at the feto-maternal interface in these patients to find out whether or not these adhesion molecules play a role in the pathology of pregnancy loss.

Patients and Methods

Patients were divided into three groups; **Group A:** 24 pregnant ladies presented with incomplete first trimester abortion, all of whom gave a history of previous 3-6 consecutive first trimester abortions, with no medical diseases, family history of genetic diseases or uterine anatomical anomaly. Also all of them were negative for acute infection with rubella, HCMV or toxoplasmosis. **Group B:** 10 pregnant ladies presented with incomplete first trimester abortion and had at least three previous normal pregnancies with no previous abortion, and no history of any medical illness, and **Group C:** 6 pregnant ladies with elective termination of pregnancy in the first trimester for a maternal indication under approved consent of two senior gynecologists and a physician. Curate samples of the feto-maternal interface were taken from all these women at the end of evacuation curate operation.

Samples were embedded in paraffin and subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark). Refer to the immunohistochemistry procedure in reference [14], and signal evaluation using CD31 as baseline endothelial marker in blood vessel counting in reference [15], dilution of the monoclonal antibodies was 1:50 for both ICAM-1 and ICAM-3 (DAKO cytomation-Denmark). Negative controls were obtained by omitting the monoclonal antibody and using antibody diluent alone to verify the signal specificity.

Statistical analysis

ANOVA test was used to determine the difference in the expression of ICAM-1 and ICAM-3 among the three groups, and the relationship between these two parameters was measured using the correlation coefficient (*r*). Values of $p<0.05$ were considered as statistically significant.

Results

Figures 1 and 2 shows the percentages of ICAM-1 and ICAM-3 expression respectively in terms of mean ± SE. As shown in figure 1 that the mean percentage of ICAM-1 expression in the first group, which is significantly higher (P=0.001) than that of the second and third groups (using ANOVA analysis), as demonstrated in (Figure 1), and the same was found for ICAM-3 (P=0.001) (Figure2). Additionally, the study showed a highly significant positive correlation between the expression of ICAM-1 and ICAM-3 ($r=0.927$, $p\leq0.01$) in the investigated groups.

Table 1: The expression of ICAM-1 among the studied groups.

ICAM-1	n	Percentage of expression		
		Mean ± S.E.$^{\Psi}$	Min. Value	Max. Value
Group A	24	63.96 ± 3.38	40	90
Group B	10	40.00 ± 3.33	30	55
Group C	6	53.00 ± 1.83	30	40

Different letters: significant difference (P<0.05) between means.

Table 2: The expression of ICAM-3 among the studied groups.

ICAM-3	n	Percentage of expression		
		Mean ± S.E.$^{\Psi}$	Min. Value	Max. Value
Group A	24	55.63 ± 3.41	30	80
Group B	10	31.00 ± 3.06	20	55
Group C	6	25.00 ± 2.58	20	35

Different letters: significant difference (P<0.05) between means.

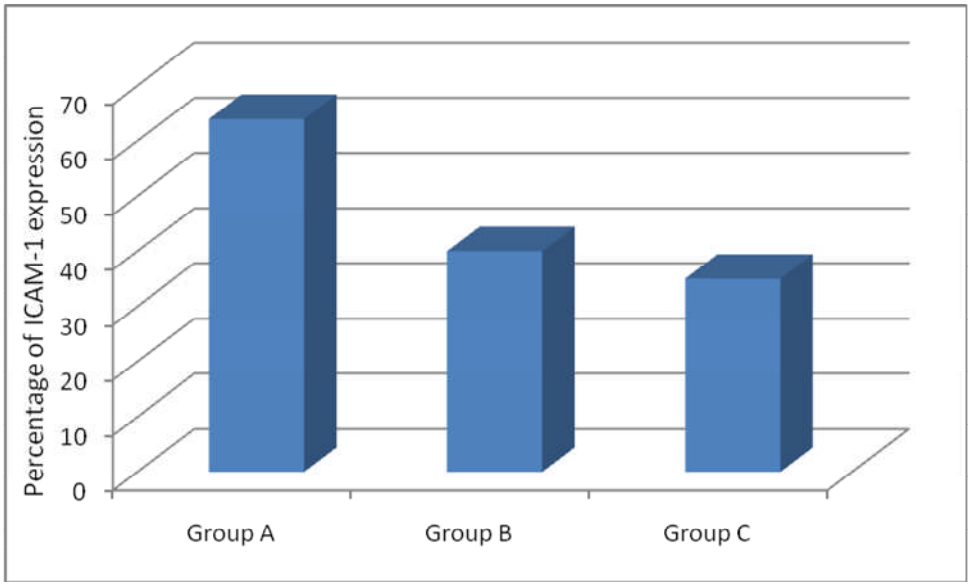

Figure 1: The expression of ICAM-1 among the studied groups

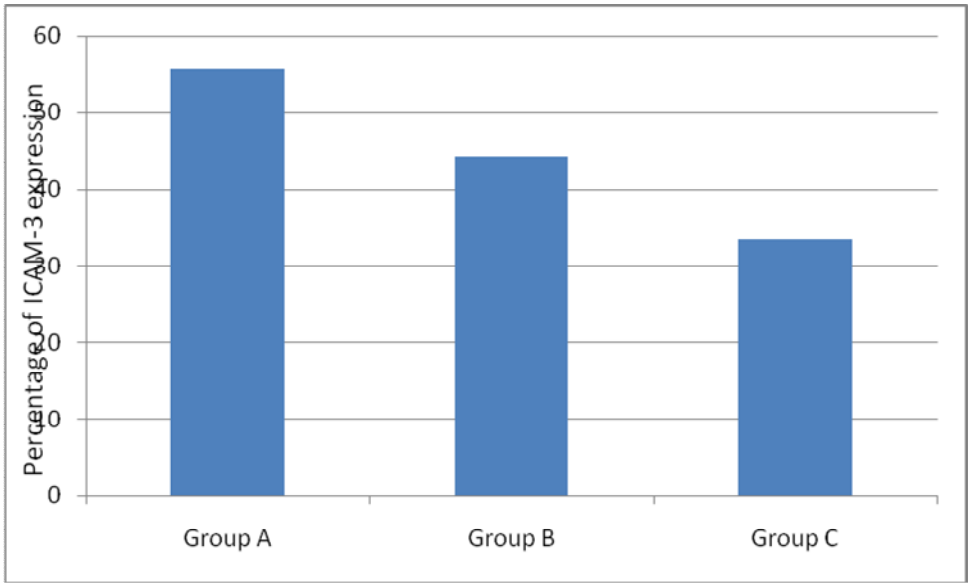

Figure 2: The expression of ICAM-3 among the studied groups

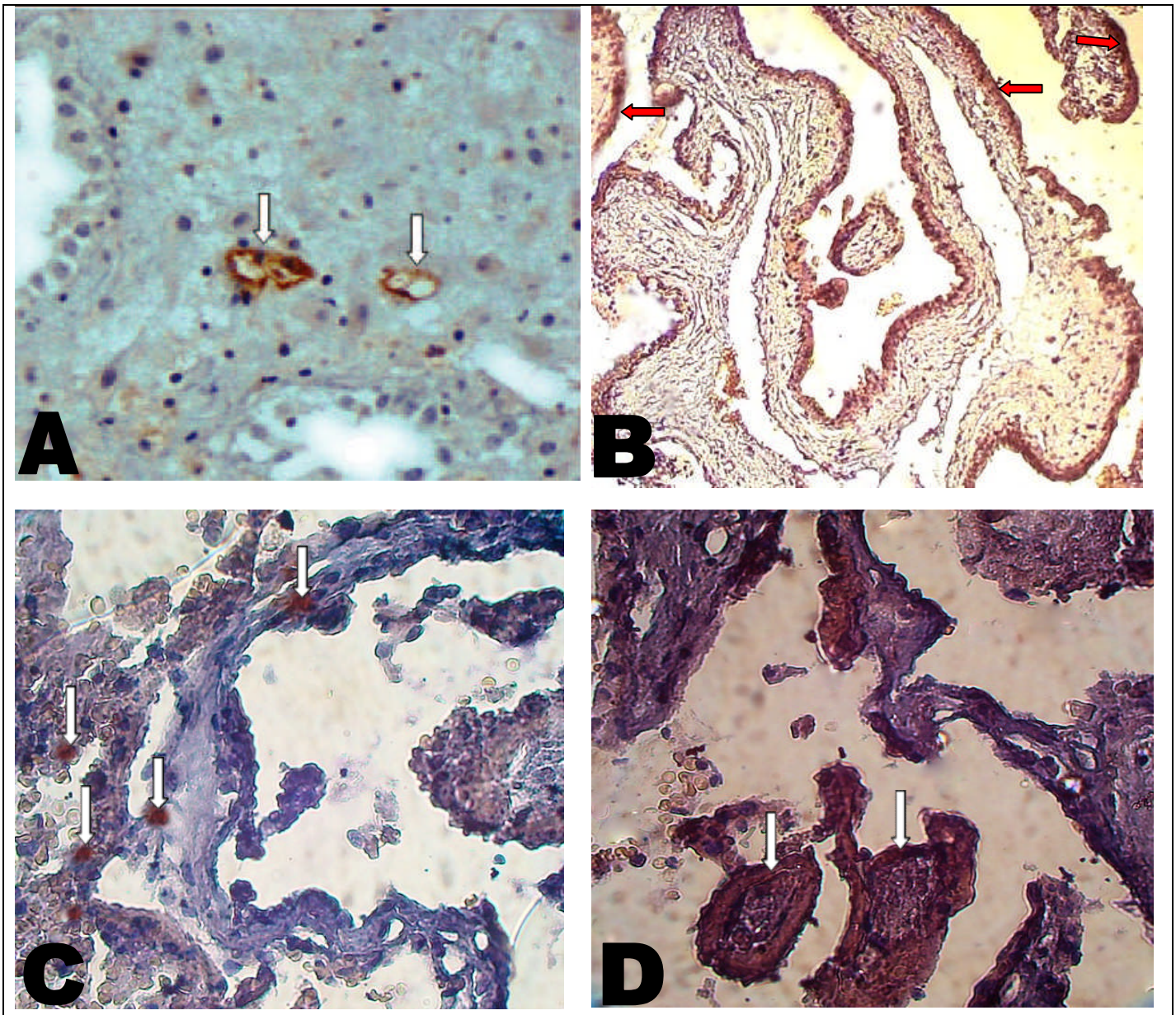

Figure 3:Immunostaining of ICAM-1 and ICAM-3 in women with pregnancy loss.

ICAM-1 was identified on decidual endothelial cells (**A**) (white arrows), and trophoblasts (**B**) (red arrows). While ICAM-3 was identified on tissue infiltrating leukocytes (**C**) (white arrows), and trophoblasts (**D**) (white arrows). Magnification power of A, C and D (X400), B (X100).

Discussion

Spontaneous abortion (resorption) in mice is thought to represent a rejection of the semi-allogeneic feto-placental unit by activated NK cells and activated macrophages [13, 16]. These cells infiltrate maternal mesometrial decidua at the site of implantation and the frequency of implantation sites with such an infiltrate is proportional to the percentage of embryos that resorb.[13] Murine resorptions are characterized by focal necrosis at the junction of the fetal trophoblasts and the decidua, an infiltrate with polymorphonuclear leukocytes at sites of necrosis and along the walls of large vessels in the decidua, and by thrombosis and hemorrhage [17, 18].

There are two main sources of this polymorphonuclear cell (PMN) infiltration; firstly, when thrombin is generated, it will activate IL-8 secretion by endothelial cells, and as a consequences IL-8 recruits PMNs[19,20] .Secondly, pro-inflammatory cytokines like IFN-γ and TNF-α induce endothelial adhesion molecules and increase the transendothelial migration of the recruited leukocytes[2,7,21].

All these studies support the present, which showed increase in the expression of endothelial ICAM-1 and ICAM-3 on tissue infiltrating leukocytes, making these CAMs good indicatos and participating in the pathology of pregnancy loss. Furthermore, recent studies showed that enhanced decidual IL-8 expression interacts with constitutively expressed ICAM-1 in decidual endothelium to modulate neutrophil trafficking into hemorrhagic and inflammatory first trimester deciduas [20]. In addition, other studies showed increase in ICAM-1 surface expression on endothelial cells of preeclamptic women in comparison with pregnant normotensive and non-pregnant women [22] .Therefore, midgestation measurements of circulating ICAM-1 and VCAM-1 (above the cutoff) have a high predictive value and may identify up to 55% of pregnant women who will later develop a severe pregnancy-related complication[23].

Mast cell- and macrophage-derived cytokines engage with their receptors on endothelial cells. This will ultimately leads to activation of nuclear transcription factors that modulate the biosynthesis of endothelial CAMs that mediate leukocyte rolling (E-selectin) and adherence (ICAM-1, VCAM-1)[2]. Which is in line with our previous study on the same groups of women showing significant increase in the transcriptional factor (NF-κB) and the pro-inflammatory cytokine (IFN-γ) in the recurrent loss group as compared with other two groups [24],and significantly higher surface expression of endothelial VCAM-1 in the same group also (unpublished data).

This study also showed that ICAM-1 and ICAM-3 expressed on the trophoblasts in some cases indicating that these CAMs might really have a role in the adherence, implantation and vascular invasion as mentioned in other studies [14, 25].

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Rheumatologic complications of shoulder joint after stroke

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Abstract

Background: Shoulder pain is one of the complications that happened in patient suffered from hemiplegia. There are many pathological processes have been postulated as causes of painful hemiplegics shoulder after stroke. One of the causes is Rotator cuff syndrome. Other causes of shoulder pain are biceptal tendonitis; Subacromial and sub-deltoid Bursitis, Adhesive capsulitis (frozen shoulder) is a common painful condition associated with loss of active movement in the direction of external rotation and abduction.

Objective: The aim of this study is to analyze shoulder pain and its correlation with the different clinical aspects of cerebral dysfunction.

Patients and method: 56 patients affected by different types of stroke were enrolled in this study , each patient was examined by neurologist , CT scan then done and referred to a consultant rheumatologist at alkindi hospital for assessment of his shoulder area , the patient then investigated thoroughly for his or her shoulder pain.

Results and conclusion: The study showed high correlation between shoulder pain and older age patients, aphasia, cortical sensory defects. The shoulder pain development is more common in older age group. Patients with cortical involvement are at high risk to develop shoulder pain. The shoulder pain development is not related to the side of hemiplagia, sex and grading of muscle weakness.

Key ward: shoulder joint, stroke.

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Introduction

Stroke is a sudden neurological dysfunction, resulting from sudden vascular insult involving cerebral vessels [(1)]; it is either ischemic due to cessation of blood supply to the brain [(2)], or hemorrhagic stroke; due to different types of intracranial hemorrhage [(2, 3)]. Shoulder pain is very common problem facing the neurologist when managing patients with stroke [(4)]; 20 – 70% of patient with stroke develop hemiplegics shoulder pain [(5, 6)].

Shoulder pain affects stroke outcome in a negative way; it can cause considerable distress and reduced activity and can markedly hinder rehabilitation resulting in negative interference with recovery after stroke [(7,8)].

The cause of hemiplegics shoulder pain is the subject of considerable controversy [(8)]. There are many pathological processes have been postulated as causes of painful hemiplegics shoulder after stroke. One of the causes is Rotator cuff syndrome, which results from impinging of rotator cuff tendon between acromion and humeral head, resulting in acute pain at the lateral surface of shoulder [(9, 10)].

Other causes of shoulder pain are biceptal tendonitis; which affect large head of biceps resulting in pain at the

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anterior surface of the shoulder joint [10]. Subacromial and sub-deltoid Bursitis, which causes pain at lateral aspect of shoulder. Adhesive capsulitis (frozen shoulder) is a common painful condition associated with loss of active movement in the direction of external rotation and abduction[10]. Other causes include glerohumeral subluxation [11] soft tissue trauma[12] Brachial plexus traction neuropathy [13] and central cortical post stroke mechanism may play a role [7].

Patients and Methods

56 patients; aged from 29 – 73 years; suffered from stroke with hemiplagia during the first [6] months post stroke were studied at Alkindi teaching hospital, between January 2004 – June 2005, all the patients had CT scan of the brain at the onset of the stroke; the patients was classified into ischemic or hemorrhagic stroke according to CT scan results; then neurologist took Full history in details and examined the patient neurologically to diagnose and localize his neurological deficit. Speech was assessed in all patients[14] which includes 1- spontaneous speech assessment 2-comprehension 3-naming objects 4-repetition 5-reading 6- writing the patients with left sided weakness were examined for cortical sensory loss ; astreognosis was examined by asking ability to identify 2 centimeter cube object and 2 centimeter circumference ball we use this a little pit large sizes because of paralysis of the hand and poor hand grip [14].graphesthesia was assessed by drawing the Arabic shape of number 9 and 2 on the patient palm using pencil. Sensory inattention assessed by ability to recognize simultaneous stimuli on both sides of the body the abnormal response is when the patient can recognize only the sound right side of the body at simultaneous stimulation and normal response of both side when examined separately; the patients with aphasia did not involved in cortical sensory examination because of barrier of difficult communication. Muscle power grading was assessed according to medical research counsel scale (MRC) of great British which recorded the power in 6 grades [15] the patients then referred to rheumatologist who examined the patient.

The patient had full blood count, blood sugar, ECG, Chest X-ray, Cervical X-ray Shoulder X-ray and electromyography /nerve conduction study were done by the examining neurologist; the rheumatologist at last diagnosed the cause of the shoulder pain. Patients with Ischemic heart disease, with diabetes mellitus and with any joint problems were excluded from the study. P value < 0.05 was considered significant and was used whenever applied.

Results

56 patients with stroke suffering from shoulder pain were studied; 29 patients had ischemic stroke and 27 had hemorrhagic stroke (intracerebral hemorrhage). 17 of those with ischemic stroke were female (58.6%) and 12 out of 29 were male (41.4%) (Table 1).

10 out of 27 patients with intracerebral hemorrhage were female (37%) and 17 were males (63%). (Table 1)

23 patients aged above 60 years; 18 patients aged between 51-60 years; 10 patients ages were between 41-50 years; 3 patients' ages were between 31-40 years and 1 patient was 29 years old age. (Table 2)

In patients with Ischemic stroke, no patients had shoulder pain in the first month after stroke, 17 out of the 29 developed the shoulder pain in the

second month after ischemic stroke, and 10 patient with ischemic stroke had the shoulder pain in the third month post stroke, one ischemic stroke patient in the 4th and one patient in the fifth month. No ischemic stroke patient in the study developed the shoulder pain in the 6th month post stroke. (Table 3)

In hemorrhagic stroke; one patient had the shoulder pain in the first month, 10 patients in the second month, 10 patients in the third month, 4 patients in the fourth month, one patient in the fifth month and one patient in the sixth month after hemorrhagic stroke (Table 3).

Right-sided weakness was seen in 13 out of 29 patients with ischemic stroke (44.8%) the right-sided weakness was seen in 12 out of 27 (44.4%) patients with intra cerebral hemorrhage. (Table 4)

Left-sided weakness was seen in 16 out of 29 patient with ischemic stroke (45.6%) and seen in 15 out of 27 who had intracerebral hemorrhage (55.6%) (Table 5).

Fifteen of the patients with ischemic stroke have grade 0-1 shoulder muscle power 12 patients have grade 2-3 and 2 patients have grade 4 shoulder muscle weakness. Those with hemorrhagic stroke, 17 patients having grade 0-1, 5 patients having grade 2-3 and 5 patients having grade 4 shoulder muscle power (Table 6) .

Aphasia was found in 21 out of 25 patients those with right sided weakness, Abnormalities of cortical sensory functions (astreognosis, sensory inattention and graphesthesia) was seen in 27 patients out of 31 patients with left sided weakness. (Table 7)

Twenty three patients were diagnosed as frozen shoulder; 20 patients were diagnosed as refereed pain from other sites (neck, elbow); 5 patients had direct trauma to the shoulder by fall on ground; 4 patients had shoulder subluxation and 4 patients had rotator cuff syndrome (Table 8).

Table 1: male/female ratio having shoulder pain in ischemic and hemorrhagic stroke.

	Ischemic stroke.	Hemorrhagic stroke.	Total
male	12	17	29
female	17	10	27
total	29	27	56

P= 0.17 no significant

Table 2: classification of the patients according to age groups

≤ 30 years	31- 40 years	41 – 50 years	51 – 61 years	> 60 years
1	3	10	19	23
1.7%	5.3	17.8%	33.8%	41.1%

P= 0.0001 significant

Table 3: the time of presentation per month

	1st month	2nd month	3rd month	4th month	5th month	6th month
Hemorrhagic stroke	1	10	10	4	1	1
Ischemic stroke	0	17	10	1	1	0
	1	27	20	5	2	1

Table 4: relation of the shoulder pain to the right side of weakness

	Ischemic	hemorrhagic	Total
Right. Side weakness	13	12	25
No	16	15	31
Total	29	27	56

P = 0.81 non significant

Table 5: relation of the shoulder pain to the left sided weakness to shoulder pain

	Ischemic.	Hemorrhagic.	Total
Left. Side weak.	16	15	31
No	13	12	25
total	29	27	56

P= 0.81 non significant

Table 6: relation of shoulder muscle power grade to shoulder pain

	Grad 0 - 1	Grad 2 – 3	Grad – 4	Total
Ischemic.	15	12	2	29
Hemorrhagic.	17	5	5	27
Total	32	17	7	56

P= 0.12 non significant

Table 7: relation of shoulder pain to aphasia and other cortical sensory loss.

	Yes	No	Total	
Aphasia	21	4	25	P <0.005 Sig
Cortical Sensory signs	27	4	31	P <0.005 sig

Table 8: causes of shoulder pain

diagnosis	Frozen shoulder	Referred. pain	Direct trauma	Sub luxation	Rotator cuff
No	23	20	5	4	4
%	41.1	35.7	8.9	7.1	7.1

P=0.0001 significant

Discussion

The shoulder pain is very common problem facing the neurologist in the management of patients with stroke [1 – 6]. This problem occurs in both types of stroke whether hemorrhagic stroke or ischemic stroke.

The present study showed no significant difference between ischemic stroke and intracerebral hemorrhage in the development of shoulder pain, this agreed with. Other studies like Hanukah et al study and Anderson study [5,7].

The present study showed an equal male to female ratio of the shoulder pain in both types of strokes, and this result is in agreement with the results of other studies like Hanukah et al, Anderson study, Jeperson. Jogenson study and Walsh study [5, 7, 8,12].

The nearly equal male/ female ratio of shoulder pain with no significance difference between both stroke type in occurrence of shoulder pain; support the opinion that the shoulder pain is related to the hemiplagia, whatever of its cause and not related to the type of stroke or to the gender difference and this is in agreement with Roy et al study, Hanukah et al study, Anderson study, Jeperson – Jogenson study, Chaca studyand Walsh study [4,5,7,8,11,12].

In the present study the shoulder pain happened more frequently in elderly than in younger aged group; this correlation with older age group is related to already diseased joint, as well as less active life style in elderly patients and this agreed with Walsh study [12].

The present study showed no significant correlation between weakness side (whether right or left sided weakness) with occurrence of shoulder pain and this is in contrast to Roy et al [4]. Who found a significant relationship with non-dominant left sided weakness and in agreement with Walsh who reports no correlation with side of weakness [12].the present study analyze the relation of higher cerebral function, concentrating on aphasia. whatever its type, cortical sensory dysfunction (graphesthesia, astreognosis. and sensory inattention.); We found significant relation between shoulder pain with higher cerebral dysfunction and prove that the patients with cortical involvements are at higher risk for shoulder pain development. This result is agreed with Roy etal study [4].

The study showed that the shoulder pain occurs mostly in the second and third months post stroke in both types of stroke this period may be the time required to clear the effect of muscle stiffness.

The present study showed no significant correlation between muscle power grading and the development of the shoulder pain. And this result is contrasting to Roy study [4], Hanukah etal study [5], Anderson study [7],Jeperson – Jogenson study [8] ,Chaca study [11]and Walsh study [12].

The study showed that frozen shoulder is the most common cause of the hemiplegics shoulder pain; other causes like refereed pain form relatively high percentage (35.7%) from the causes of shoulder pain. Other causes like direct trauma (8.9%) shoulder joint subluxation (7.1%) and rotator cuff syndrome (7.1%). We did not record Brachial plexus traction neuropathy in our patients.

Those finding is in contrast to Walsh [12] and Braus[13] studies which showed high incidence of shoulder subluxation more than other causes, small sized sample in comparison to those studies may explain the last difference in causes of shoulder

pain between the present study and to Walsh[12] and Braus [13] studies.

Conclusion

1) The shoulder pain is very common problem in stroke.
2) The shoulder pain development is more common in older age group.
3) Patients with cortical involvement are at high risk to develop shoulder pain
4) The shoulder pain development is not related to the side of hemiplagia, sex and grading of muscle weakness.
5) Rheumatologist should examine every patient and plan for early exercise should be encouraged.

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HLA- DRB Genotyping of Brain Astrocytomas among Iraqi Patients

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Abstract

Background: The major histocompatiblity complex (MHC) refers to as human leukocyte antigen (HLA). The loss of HLA antigens by neoplastic cells is considerably important for tumor growth and metastasis and expression of certain certain HLA allels may predispose to have certain types of tumors.

Objective: To investigate the genetic susceptibility of HLA-DRB1, DRB3, DRB4 and. DRB5 alleles to brain astrocytomas in Iraqi patients.

Methods: HLA-DRB1, DRB3, DRB4 and, DRB5 allele polymorphisms were typed by polymerase chain- reaction with sequence-specific primers (PCR-SSP) in 30 unrelated patients astrocytomas and 17 unrelated normal control subjects. The association was measured by appropriate statistical tests.

Results: Allele frequency (AF) of HLA-DRB1*10011 and DRB1*10012 was significantly decreased in brain astrocytomas patients than that in normal controls (0.53 vs 0.93) the odds ratio 8.76). There was no association between patients and controls in the rested HLA-DRB1 alleles.

Conclusion: HLA-DRB1*10011 and DRB1*10012 alleles were less common in the patients with brain astrocytomas than in the healthy controls. Individuals carrying HLA-DRB1* 10011 and DRB1*10012 alleles might be considered as protective markers. These protective alleles; might have a role in the degree of malignancy of the tumors and its histological type.

Keywords: PCR-SSP, brain astrocytomas, HLA-DRB

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Introduction

Astrocytic tumors comprise a wide range of neoplasms that differ in their location within the central nervous system (CNS). The majority of tumors had either heterogeneous or positive expression of HLA class I heavy chain (HLA-HC), and β2 microglobulin[1]. The loss of HLA antigens by neoplastic cells is considered important for tumor growth and metastasis [2-4]. Since tumor neoantigens on the surface of aberrant cells are recognized by T-cells only in the context of the HLA "self" antigens, loss of the HLA antigens may allow the tumor to escape immunosurveillance [5]. Defects in the expression and/or function of the human leukocyte antigen (HLA) class I antigen-processing machinery (APM) components are found in many tumor types.

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These abnormalities may have a negative impact on the interactions of tumor cells with host's immune system and on the outcome of T cell–based immunotherapy [6]. The alleles of the HLA system controls a variety of immune functions and influence the susceptibility to more than 40 diseases, many of which have an autoimmune components [7,8] Association of a particular HLA allele with a disease implies that the frequency of the allele is different in the patient population as compared with that of matched control population . A study done by (Angelica etal. 2005) [9] showed that HLA class I antigens were lost in 50% of glioblastoma multiforme (GBM) lesions and in 20% of grade 2 astrocytoma lesions. Selective HLA-A2 antigen loss was observed in 80% of the GBM lesions and in 50% of grade 2 astrocytoma lesions stained. HLA class I antigen loss was correlated with tumor grade. HLA class II antigen expression was detected in 30% of the 44 lesions analyzed. HLA-Dr expressed by brain tumor cells selectively inhibit CD8 subset which participates in immunoreaction against brain tumors in

situ [10].However there has been no report on the association between HLA alleles and brain astrocytomas among Iraqi patents.

In this study, we used polymerase chain reaction with sequence-specific primers (PCR.-SSP) for HLA-DRB alleles typing to investigate the genetic susceptibility of HLA allele polymorphisms in brain astrocytomas of Iraqi patients.

Material and Methods

The brain astrocytoma group (attended Baghdad Neurosurgery Hospital) included thirty unrelated patients (24 men and 6 women), with a mean age of 52.3± 4.55 years, who were evaluated radiologically and surgically. And the diagnosis was confirmed by histopathological examination of the tumor mass at Baghdad Neurosurgery Hospital Laboratories.

The control group consisted of 17 unrelated healthy individuals, matched with patients for sex (14 men and 3 women) and age, with a mean of 50.8 ± 3.44 years.

DNA extraction

Genomic DNA was isolated from leukocytes obtained from anticoagulated peripheral blood of patients and controls, using the salting out method [11].

HLA-DRB1 alleles PCR-SSP typing

For HLA-DRB (HLA-DRB1*01-DRB1*16, DRB3, DRB4, AND DRB5) typing by PCR-SSP, 24 separated PCR reactions were performed for each sample (Biotest-ABDR SSP, Germany). It allowed the detection of 353 HLA-DR alleles. Each PCR reaction mixture contained group-specific- DRB primers and the internal positive control primer pair. HLA-DRB alleles PCR-SSP typed system consisted of 50-100 ng genomic DNA, 5U/ul Taq DNA polymerase (Promega ® USA), and PCR tubes contained dried primer / nucleotide mixtures. PCR amplifications were carried out in thermal cycler (Hybaid LTD® England) according to manufacture instruction. Initial denaturation was made at 94°C for 2 minutes; with 30 cycles each consisting of denaturation at 94° C for 10 seconds, annealing at 65°C for 1 minute. and extension at 72°C for 1 minute. The HLA-DRB alleles typed visualization of amplification was observed using 2% agarose gel electrophoresis. (Promega ® USA), The gels were run for 30 minutes at 8.5 V/ cm in 1X TBE buffer and the bands were visualized using UV illumination and photographed by digital colored camera (Orite, Japan).

Statistical analysis

Odds ratio (OR) was taken to describe the relative risk of a particular HLA-DRB allele. Frequency distribution of the odds ratio for each DRB alleles studied was constructed. Confidence Interval (Cl) was also carried out for the normal distribution of the OR. The chi-square (X^2) test of significant was used to test the departure of the observed frequency from expected which was built on assumption of normal segregation [12].

Results

HLA-DRB1*10011 and DRB1*10012 alleles were significantly present at decreased frequencies in patients with brain astrocytomas, 0.53 vs 0.93, OR = 8.76 (Table 1). Cl: =0.643-0.995. The rested HLA-DRB allele's frequencies showed no significant difference in comparison between patients and the controls. Group DR10 might be associated to disease progression due to its low frequency, when compared with control subjects. The same situation could be seen for HLA-DR15 (frequency in patients 0.77 vs controls 0.97, OR= 5.0), thus, HLA-DRB1 alleles that are associated with increased risk of astrocytomas have decreased frequency in patients compared with controls.

The X2 test of significance was also conducted to examine the association between the HLA-DRB1 risk group alleles. The results indicated that there was a significant association with DRB*10011 and DRB1*10012 alleles ($p<0.05$). The x2 value (5.88) was higher than that of other alleles (2.34) .This was reflected by the significant difference between the observed

and expected values. The reverse was true for the control group. In addition, if we take this comparison into consideration when checking for the observed and expected values for the alleles that showed no significant differences, it was also evident that the observed was higher than the expected for the patients group. In this case, either the non-significant differences might be attributed to the small number of observations or that the contribution of such alleles was too little to be associated with the occurrence of the disease. This would demonstrate a clear picture about the association of these two alleles under the specificity of DR10 with astrocytoma.

Table 1: Frequency and odds ratios for HLA-DRB1, DRB3, DRB4, DRB5 alleles in patients with astrocytoma in comparison with normal control subjects

HLA-DRB1, DRB3. DRB4, DRB5		FREQUENCY (%)		ODDS VALUE		ODDS RATIO
Group	*Allele*	*Patient*	*Control*	*Patient*	*Control*	*Patient*
DR10(DRB1	10011	53	93	0.87	0.13	8.76
DR10(DRB1	10012	53	93	0.87	0.13	8.76
DR15(DRB1	15011	77	97	0.30	0.06	5.00
DR15(DRB1	15012	77	97	0.30	0.06	5.00
DR15(DRB1	15022	77	97	0.30	0.06	5.00
DR15(DRB1	15023	77	97	0.30	0.06	5.00
DR15(DRB	1503	77	97	0.30	0.06	5.00
DR15(DRB1	1504	77	97	0.30	0.06	5.00
DR15(DRB1	1505	77	97	0.30	0.06	5.00
DR15(DRB1	1506	77	97	0.30	0.06	5.00
DR15(DRB1	1507	77	97	0.30	0.06	5.00
DR52(DRB3)	0207	90	97	0.11	0.06	1.83
DR52(DRB3)	0208	90	97	0.11	0.06	1.83
DR53(DRB4)	01011	90	97	0.11	0.06	0.00
DR53(DRB4)	010111	90	100	0.11	0.00	0.11
DR51(DRB5)	01011	90	100	0.11	0.00	0.11
DR51(DRB5)	01012	90	93	0.11	0.13	0.84

Discussion

Lack of human leukocyte antigens and costimulatory molecules have been suggested as mechanisms by which human malignant gliomas avoid immune recognition and elimination [(12)]. The major finding in this study was that the frequency decreased incidence of HL.A-DRB1*10011 and DRB1*10012 in the Iraqi patients with brain astrocytomas compared with that in healthy controls. HL.A SSP DNA typing on 30 patients revealed a significant decreased of DR10 alleles (DRB1*10011 and DRB1*10012) 0.53 vs 0.93, OR = 8.76, Cl= 0.643-0.995 ($p< 0.05$), X2 = 5.88. In addition to the absence of DRB1*15 alleles in patients compared to controls. None of the tested HLA-DRB alleles occurred at markedly altered frequency between the patients and normal individuals. It is may be the alleles that is associated with genetic susceptibility of this tumors but why? It was entirely unclear up to now; the pathogenesis of genetic association may be linkage disequilibrium (nonrandom association) and/ or changing in the recognized procession of the specific antigen. It is controversial whether or not HLA antigens expression in astrocytoma cells correlates with the development of disease and progression [(14-16)]. As reported in some studies, the reduced expression of HLA antigens in malignant tissues has been proposed as a mechanism thereby tumor-associated proteins cannot be

presented in the T cells[13], therefore the tumor cell proliferates are unperturbed by the immune system and tumor cells protect themselves from host' immunosurveillance. There is possibility that HLA allele genetic association and expression on tumor cells may provide a clue to the understanding of the therapeutic mechanisms of biological response modifiers or immunotheraphy which may cut through the induction of HLA antigens on malignant cells [17-22]. The cells of a given individual may express HLA alleles, which altered binding to tumor peptides, thereby leading to a modified immune response to the tumor. Identification of the mechanism associating HLA-DRB1*10011 and DRB1*10012 with brain astrocytoma could ultimately help target individuals most likely to benefit from cancer screening and prevention strategies and could facilitate novel therapeutic programs for cancer immunoprevention. Further studies with large number of patients with use of nucleotide sequence of targeted alleles may show more clear correlation [23]. The presence of HLA antigen defects in malignant brain tumors may provide an explanation for the relatively poor clinical response rates observed in the majority of the T cell–based immunotherapy clinical trials conducted to date in patients with malignant brain tumors [24].

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Ulcerative colitis in young children, A Case report

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Abstract

Ulcerative colitis is one type of the inflammatory bowel diseases that affect the rectum and the colon. The usual age of presentation is at adolescence and adulthood. It is rarely seen during infancy and in young children. Our case, Mohammed, was presented at the age of 18 months with persistent bloody diarrhea that did not respond to the usual antibiotic and antiparasitic drugs. All the investigations that were done during the period of the illness (more than 9 months) were non conclusive. Then colonoscopy were done which revealed the picture of ulcerative colitis. The patient then was put on oral steroid and salazopyrine with dramatic improvement in the general condition and disappearance of bloody diarrhea. Now the patient is on salazopyrine orally with steroid only in exacerbations.

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Introduction

Inflammatory bowel disease (IBD) is a group of idiopathic, chronic disorders that includes Crohn disease and ulcerative colitis. The cause is poorly understood, and the natural course is characterized by unpredictable exacerbations and remissions [(1)]. The most common time of onset of IBD is during adolescence and young adulthood. A bimodal distribution has been shown with an early onset at 15-25 yr of age and a second smaller peak at 50-80 yr of age. IBD is often reported to be more common in urban areas than in rural areas. In developed countries, these disorders are the major causes of chronic intestinal inflammation in children beyond the first few years of life [(2)].

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Ulcerative Colitis

Ulcerative colitis is a disease that causes inflammation and sores, called ulcers, in the lining of the large intestine. The inflammation usually occurs in the rectum and lower part of the colon, but it may affect the entire colon. Ulcerative colitis rarely affects the small intestine except for the end section, called the terminal ileum [(3)]. Ulcerative colitis may also be called colitis or proctitis. Ulcerative colitis has been noted to present in infancy, although this is very unusual. One needs to be cautious when evaluating reports of ulcerative colitis in infancy because dietary protein intolerance may be easily misdiagnosed as ulcerative colitis in this age group [(4)].

Patient and Result

Mohammad Waly Shihab is a 3 years old boy presented at the age of 1.5 year with persistent bloody diarrhea, admitted to the Department of pediatric, Tikrit Teaching Hospital, which did not respond to both metronidazole and diloxanide furate. The diarrhea comes in attacks and sometimes the patient passed fresh blood per rectum.

There were no other clinical symptoms and signs other than low

grade fever and mild degree of failure to thrive. There was no arthritis or skin rash.

General stool examination revealed semi liquid brown stool with 10-12 pus cells and 8-10 RBC with no evidence of cysts or traphozoites. Stool culture reveals only growth of candida albicans.

Hematological investigations revealed mild hypochromic-microcytic anemia with increased in the total WBC count with neutrophil predominance.

After 9 months from the beginning of illness colonoscopy were done with biopsies were taken from the sigmoid and the colon, which revealed non-specific proctitis with multiple crypt abscesses but no granuloma is seen.

Barium enema was not done in our patient because the dye required was not available. The patient was then diagnosed as ulcerative colitis and start treatment with prednisone 2mg/kg for 3 weeks initially and salazopyrine for 2 weeks then another course of both drugs were given for 45 days.

The patient dramatically responded to the treatment with improvement in the general condition and disappearance of bloody diarrhea.

Now the patient is on salazopyrine orally with steroid treatment during the acute exacerbation.

Fortunately our patient is not in need till now for immunotherapy or surgery because of dramatic response to the initial treatment with sallazopyrine and steroid.

Discussion

Although ulcerative colitis is one of the major causes of bloody diarrhea during the adolescent and the adulthood periods, it is less commonly seen in children. It affects mainly children after the first years of life and it is unusual to see ulcerative colitis in infancy [1].

In spite of the repeated presentation of our patient with bloody diarrhea which did not respond to the usual treatment of amoebic and bacillary dysentery and in spite of negative investigations which were done repeatedly during the 9 months of the illness, the suspicion of inflammatory bowel disease was too late because of unusual presentation of the disease at this age [1, 5].

The major disease of differential diagnosis of bloody diarrhea at this age was cow milk protein intolerance and Crohns disease [1, 2]. Our patient was breast-fed and now on the usual family diet with little infrequent intake of prepared milk. Both cow milk protein intolerance and Crohns disease can be excluded by the typical histopathological findings of biopsies that were taken by colonoscopy and segmoidoscopy which revealed proctitis and crypt abscesses which are typical picture of ulcerative colitis [1]. Colonoscopy also is important in defining the extent of the disease [6]. The colonoscopy also excludes Crohons disease by absence of granulomas characteristic of Crohns disease [1].

What is important to be noticed in our patient is that in spite of long period of the illness (around 9 months) the patient has no other signs of ulcerative colitis like skin rash or features of arthritis [1]. This may be due to that these features usually appear around adolescence and are unusual presentation of ulcerative colitis at this very young age of our patient [4].

The hematological investigation of our case was consistant with the diagnosis of ulcerative colitis. The type of anemia was hypocromic-microcytic which may be either due to continued blood loss from the intestine or due to the fact that microcytic anemia is a

feature of chronic inflammatory disorders due to the misuse of iron during the inflammatory process. The increased WBC count is consistant with ulcerative colitis, which indicates the presence of inflammatory process in the body [7].

General stool examination revealed the presence of blood cell and pus and this picture is seen in ulcerative colitis due to the damage to the bowel mucosa or this picture may be seen due to infection of the bowel that may accompany the inflamed bowel [2].

The definitive diagnosis of ulcerative colitis in our patient is done by segmoidoscopy and colonoscopy with biopsies taken from the sigmoid and the colon which revealed picture suggestive of ulcerative colitis with non specific proctitis and multiple crypt abscesses with no granuloma [1]. This picture confirms the diagnosis of the case and excludes the other differential diagnosis like Crohns disease and cow milk protein intolerance [4].

The rapid response of our patient to the treatment with salazopyrin and steroid orally (initially only) and then continuation on the salazopyrin orally only with improvement in the physical growth and improvement in the appetite and general condition of the patient with rapid disappearance of the bloody diarrhea suggest a mild degree of the disease.

Our patient is now followed clinically by assessment of physical growth (weight, height and weight for height) to identify early features of under growth, which may be due to the disease itself [1], or due to the effect of treatment [8].

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المجلة العراقية للعلوم الطبية
قائمة المحتويات

المقالات

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CONTENTS

EDITORIAL

ARTICLES

تأثيرات كبريتات الخارصين على التهاب القولون المحدث في الارانب

أزهر عبد الحافظ الجمعة ، أديب أحمد الزبيدي

الخلاصة:

خلفية الدراسة : ان فشل ستراتيجية العلاج الحالية في السيطرة على الكثير من حالات داء الامعاء الالتهابي كان دافعاً قوياً للبحث عن نماذج علاجية جديدة.

هدف الدراسة : لدراسة تأثيرات كبريتات الخارصين على التهاب القولون المحدث في الارانب.

طريقة العمل : تم احداث التهاب القولون في عدد من الارانب بواسطة اعطاؤها عن طريق المخرج مزيجا من حامض الخليك - كحول اثيلي (نموذج ١)، أو حامض الخليك (نموذج٢).

تم مقارنة تأثيرات كبريتات الخارصين بتأثيرات كل من الماء المقطر (مجموعة التحكم)، والبردنيزولون من خلال التغير في وزن الحيوان ، و وزن قطعة القولون، و درجة الفحص النسيجي العياني و المجهري. كما قيست تراكيز كلٍ من الخارصين و النحاس في بلازما الارانب في مجموعتي السيطرة و كبريتات الخارصين لكلا النموذجين .

النتائـج : في (نموذج ١)،لوحظ ضرراً شديداً في القولون ثبت عيانياً و مجهرياً. لم تكن درجات الفحص النسيجي العياني والمجهري لمجموعة كبريتات الخارصين تختلف اختلافاً معتداً به عن مجموعة التحكم ومجموعة البردنيزولون .

اما في (نموذج ٢) فقد حدث التهاباً في القولون اقل وخامة، ولكن بالرغم من ذلك لوحظت تأثيرات واضحة.

قلل كلاً من البردنيزولون و كبريتات الخارصين من فقدان وزن الحيوان بالمقارنة مع مجموعة التحكم.

ان الأضرار العيانية والمجهرية قلت بشكل معتد به بعد استخدام كل من البردنيزولون و كبريتات الخارصين.

في كلا النموذجين، لوحظ نقصاناً معتداً به في تراكيز الخارصين في البلازما بعد إحداث التهاب القولون، وان استخدام كبريتات الخارصين قلل هذا النقصان بشكل ملحوظ.

الاستنتـاج: إن نموذج التهاب القولون المحدث بواسطة حامض الخليك في الارانب (نموذج ٢) هو المفضل للتحري عن التأثير المضاد للالتهاب لعقاقير جديدة. وكان لكبريتات الخارصين تاثيراً وقائياً في هذا النموذج.

إن النقصان الملحوظ في مستويات الخارصين في البلازما في كلا النموذجين يشير الى اهمية أعطاء الخارصين في حالات التهاب القولون.

مفتاح الكلمات: داء الامعاء الالتهابي، التهاب القولون المحدث، حامض الخليك، كبريتات الخارصين، البردنيزولون.

فرع الفارم كولوجي [كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٤-١٨

تركيز الدهون لدى مرضى التهاب المفاصل الرثوي المبكر

عدنان عنوز [١] ، شكري فائز ناصر [٢]

الخلاصة

خلفية الدراسة: لاحظ الباحثون في الاونة الاخيرة ازديادالحالات المرضية والوفاة بسبب امراض القلب لدى مرضى التهاب المفاصل الرثوي و عزيت تلك الوفيات الى التصلب العصيدي المتسارع و بعد ارتفاع نسبة الكولسترول وكولسترول البروتين الشحمي ذو الكثافة العالية في الدم من عوامل الخطورة لحوادث التصلب العصيدي .

هدف الدراسة : لقد اجريت هذه الدراسة للاهداف التالية :

١- لتبيان التغيرات في تركيز الدهون في الدم لدى مرضى التهاب المفاصل الرثوي المبكر.

٢- لاظهار الدور المرضي لتغير تركيز الدهون وتغير نسبة التعصد لدى مرضى التهاب المفاصل الرثوي المبكر .

٣- لتبيان العلاقة بين تغير نسبة الدهون وقيم مختبرية اخرى ،خاصة نسبة تثقل كريات الدم الحمراء وبروتين سي المفعل .

طريقة العمل: تم اجراء المعاينة الطبية على خمسة وعشرين مريضاً مصابون بالتهاب المفاصل الرثوي المبكر وفقاً للمعايير المعتمدة من قبل الكلية الاميكية لامراض امفاصل وهم الذين تقل مدة مرضهم عن سنة واحدة لم يستخدموا خلالها اي من ادوية الستيرويدات الجهازية او الادوية المعدلة لامراض المفاصل وتم اجراء فحص نسبة الدهون في الدم (الكوليسترول الكلي وكوليسترول البروتين الشحمي منخفض الكثافة ونسبة كولسترول البروتين الشحمي ذوالكثافة العالية ونسبة ثلاثي الغلسريد)وقياس نسبة التعصد ،كما قيست كذلك نسبة تثقل كريات الم الحمر ونسبة بروتين سي المفعل لكل من المرضى ولعدد مماثل من المجموعة الضابطة .

النتـــــائج: بينت الدراسة ان المرضى المصابون بالتهاب المفاصل الرثوي المبكر اظهروا قيماً عالية من الكولسترول الكلي وكولسترول البروتين الشحمي نخفض الكثافة ونسبة ثلاثي الغلسريد كما تبين ان نسبة كولسترول البروتين الشحمي ذو الكثافة العالية لديهم منخفضة بدلالة احصائية مهمة مقارنة بالمجموعة الضابطة وهذا يفسر ايضاً ارتفاع نسبة التعصد ونسبة قسمة كولسترول البروتين الشحمي منخفض الكثافة /نسبة كولسترول البروتين الشحمي ذو الكثافة العالية لديهم مقارنة بالمجموعة الضابطة .

اظهرت الدراسة ايضاً ان هذه التغيرات في نسبة الدهون في الدم متناسبة مع التغيرات في قيم مختنرية اخرى ،وخاصة نسبتي تثقل كريات الدم الحمر وبروتين سي المفعل مع الدم .

الاستنتاجات والتوصيات: اظهر مرضى التهاب المفاصل الرثوي المبكر طبيعة دهون تعصدية مقارنة بمثلائهم من المجموعة الضابطة ان التشخيص والعلاج المبكرين لهذا المرض مع التقليل من عوامل الخطورة القلبية الاخرى سيكون له تاثير فعال على مسيرة المرض .

مفتاح الكلمات : تركيز الدهون – التهاب المفاصل الرثوي

[١] فرع الباطنية [كلية الطب - جامعة النهرين]
[٢] فرع الباطنية [مستشفى الكاظمية التعليمي]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص١٩-٢٨

التأثيرات النسيجية للميلاتونين على الخلايا الملتهمة الحويصلية للجرذان البالغة

سامية عباس عليوي[١] ، علي هاني عبد [٢]، سلمان شفيق سلمان[٣]

الخلاصة:

خلفية الدراسة: أن هرمون الميلاتونين هو الهرمون العصبي للغدة الصنوبرية وهو يزيد ويقوي المناعة في الأنسان والحيوان. أن الجهاز المتكون من الخلايا أحادية الذواة – والملتهمة ، يمثل وحدة وظيفية مناعية واحدة. وأن الخلايا الملتهمة والموجودة في الحويصلات الرئوية ، هي واحدة من أهم الأعضاء في هذه الوحدة المناعية.

هدف الدراسة: هو معرفة تأثيرجرعات مختلفة من الميلاتونين الغذائي على الخلايا الملتهمة الحويصلية في الجرذان البالغة.

طريقة العمل: تم اعطاءالميلاتونين الغذائي للجرذان البالغة لمدة٣٠يوما متتالية.قسمت الجرذان الى٦مجاميع.المجموعة ١كانت للمقارنة.المجموعة ٢ ، ٣، ٤، ٥ و ٦ أعطيت جرعات من الميلاتونين بمقدار ١٢٥,٢٥٠,٥٠٠,٧٥٠,١٠٠٠ميكروغرام/كغم من وزن الجسم،على التوالي .بعد آخر يوم من المعالجة و تحت تأثير المخدروأزيلت الرئة اليسرى للجرذان وتم أجراء الفحص النسيجي عليها.

النتائج: أظهرت وجود تاثير نافع مهم على الخلايا الملتهمة الحويصلية مع الجرعات الأعتيادية.اما في الجرعات العالية فأنه يؤثر تاثيرا ضارا بليغا.

الأستنتاج: ان هرمون الميلاتونين يحدث تأثيرات نافعة في الخلايا الملتهمة الحويصيلية في الجرذان البالغة في الجرعات الاعتيادية لكنه يسبب ضررا كبيرا في الجرعات العالية.

مفتاح الكلمات: الميلاتونين، المناعة، الخلايا الملتهمة الحويصلية

[١]فرع التشريح والأنسجة والأجنة [كلية الطب – الجامعة المستنصرية]
[٢]فرع التشريح والأنسجة والأجنة [كلية الطب – جامعة النهرين]
[٣]فرع التشريح والأنسجة والأجنة [كلية الطب – جامعة دهوك]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٢٩-٣٥

تأثيرهرمون الميلاتونين علىنسيج البربخ للجرذان البالغة

سامية عياس عليوي١،علي عبد الستار٢، حيدر جواد مبارك ٢

الخلاصة:

خلفيـة الدراسة: إن الحيوانات المنوية تزود بالقابلية المطلوبة لحركتها الطبيعية في البربخ ، وعليه فإن البربخ له دور مهم جدا في الخصوبة الطبيعية . ان هرمون الميلاتونين هو الهرمون العصبي الأساسي للغدة الصنوبرية وهو ينضم الفعاليات الجنسية والتكاثرية في الثديات وبضمنها الأنسان.

هدف الدراسة: من التجربةهو معرفة تأثيرجرعات مختلفة من الميلاتونين الغذائي على نسيج البربخ في الجرذان البالغة.

طريقة العمل: تم إعطاءالميلاتونين الغذائي عن طريق الفم للجرذان البيضاء البالغةلمدة٣٠يوما متتالية.قسمت الجرذان الى٦مجاميع.المجموعة ١كانت للمقارنة.المجموعة ٢ ، ٣، ٤، ٥ و ٦ أعطيت ١٢٥,٢٥٠,٥٠٠,٧٥٠,١٠٠٠ميكروغرام/كغم من وزن الجسم،على التوالي .بعد آخر يوم من المعالجة قتلت الحيوانات تحت تأثير المخدروأزيل البربخ وتم حفظ النسيج في محلول باونز ومن ثم أجريت عليه بقية التحضيرات النسيجية الروتينية اللازمة لأجراء الفحص النسيجي عليه.

النتائـــج: أظهرت وجود تاثير نافع مهم حيث أزداد كل من سمك الجدار لنبيب البربخ، التجويف الداخلي لنبيب البربخ وكذلك تجمع الحيامن داخل التجويف مع الجرعات الأعتيادية.اما في الجرعات العالية فأنه يؤثر تاثيرا ضارا بليغا.

الأستنتاج: ان هرمون الميلاتونين يحدث تأثيرات نافعة في بربخ الجرذان البالغة في الجرعات الاعتيادية حيث أزداد كل من سمك الجدار لنبيب البربخ، التجويف الداخلي لنبيب البربخ وكذلك تجمع الحيامن داخل التجويف لكنه يسبب ضررا كبيرا في الجرعات العالية.

مفتاح الكلمات: البربخ، الميلاتونين، العقم.

[١]فرع التشريح والأنسجة والأجنة [كلية الطب – الجامعة المستنصرية]
[٢]فرع التشريح والأنسجة والأجنة [كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٣٦-٤٣

نبضات القلب اللاإنتظامية عند المصابين بالتهاب الانسداد الرئوي المزمن

جواد كاظم مناتي ،حميد الدليمي

الخلاصة:

خلفية الدراسة : نبضات القلب اللاإنتظامية تحدث في القلب لعدة أسباب ومن هذه الأسباب هو قصور الشرايين التاجية واعتلال عضلة القلب وهنالك عوامل أخرى غير مباشرة كالتدخين تأثر على القلب وذلك بسب قلة الأوكسجين

هدف الدراسة: دراسة العلاقة بين مرض الانسداد الرئوي المزمن الناتج عن التهاب القصبات المزمن والنفاخ الرئوي عند المدخنين ونبضات القلب اللاإنتظامية باستخدام جهاز تخطيط كهر بائية القلب ومراقبة نبضات القلب لمدة ٢٤ ساعة

طريقة العمل: أجريت الدراسة في مستشفى الكاظمية التعليمي –بغداد-العراق على ١٠٠ مريض . في عام ٢٠٠٤ خمسون مريض من العدد الكلي هم مدخنون السكائر لفترة طويلة (المجموعة الأولى) والقسم الأخر غير مدخنون ولا يعانون من أمراض القلب كقصور الشرايين التاجية (المجموعة الثانية). واجريت لهم فحوصات كفحص قياس كهربائية القلب وفحص وظائف الرئة ومراقبة نبضات القلب لمدة ٢٤ ساعة

النتائــج:

١-أن الأشخاص المصابين بمرض الانسداد الرئوي المزمن أكثر عرضة من الآخرين بنبضات القلب الانتظامية

٢-تعتمد كثرة نبضات القلب اللاإنتظامية على شدة المرض وعلى حدوث وذمة رئوية

٣-أكثر من ١٠٠ نبضة بالدقيقة عند ا-كثر من ٨٠% من المصابين بالانسداد الرئوي المزمن وان النبضات المهاجرة الناشى من البطينين ٦٤%. والنبضات المهاجرة الناشى من الأذينين ٧٢%. وارتجاف الأذينين ٢٤%. ورفرفة الأذينين ١٢%. وحصر القلب ١٢%. وتسارع البطينين ١٠%.

الاستنتاجات: أظهرت الدراسة أن التدخين يؤثر على كهر بائية القلب بسب قلة الأوكسجين في الدم وبسب الأدوية التي يتناولها المصاب لتوسيع القصبات مما يؤدي إلى تسارع النبضات والأذينين والبطينين

مفتاح الكلمات: نبضات القلب اللاإنتظامية،نبضات مهاجرة،الإنسداد الرئوي ، التدخين.

فرع الباطنية [كلية الطب - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٤٤-٤٨

ورم الغده الصعتريه .... دراسه سريريه مرضيه في العراق

رنا زهير ناجي[١] ،فائزه عفتان زغير الراوي[٢]

الخلاصه

خلفية الدراسة: ورم الغده الصعتريه هو ورم الخلايا الطلائيه للغده الصعتريه بغض النظر عن وجود او عدد الخلايا اللمفاويه في الغده الصعتريه

هدف الدراسة: دراسه سريريه مرضيه لورم الغده الصعتريه.

المواد و طرق العمل: دراسه استرجاعيه لـ ٥١ حاله من حالات ورم الغده الصعتريه و التي جمعت من مختبرات التحليل النسيجي العامه و الخاصه للفتره من كانون الثاني ١٩٩١ – تشرين الاول ٢٠٠٤.

النتائــج: من مجموع ٥١ حاله كانت هناك ٨ حالات (١٥.٦%) ورم الغده الصعتريه الحميد و ٤٣ حاله (٨٤.٤%) ورم خبيث تم تشخيص ٢٨ حاله (٥٤.٩%) مصابه بداء الوهن العضلي الوبيل حيث كانت نسبه الذكور أعلى من الاناث بنسبه ١.٥ : ١ الاشكال النسيجيه لورم الغده الصعتريه الاكثر شيوعا هي: ب٢ (٣٥.٣ %) ، ب٣ (٢١.٦ %) ثم ب١ (١٩.٦ %) و أ (١٥.٧%) و أب (٧.٨%) أكثر الاشكال النسيجيه شيوعا في الذكور هي ب٢ (٢٧.٥ %) و في الاناث الشكل أ و ب١ (١١.٨ %) لكل منهما.

الإستنتاجات: هناك علاقه ذات أهميه بين الاشكال النسيجيه المختلفه لورم الغده الصعتريه و جنس المريض كذلك بين الاشكال النسيجبه و درجه ارتشاح الورم كذلك وجد إن أكثر من نصف المرضى مصابين بداء الوهن العضلي الوبيل في الذكور أعلى بمره و نصف من الاناث .

مفتاح الكلمات: أورام الغده الصعتريه داء الوهن العضلي، نظام التصنيف لأورام الغده الصعتريه.و أورام المنصف الصدري.

[١] مختبر الصحه المركزي- بغداد
[٢] فرع علم الأمراض [كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٤٩-٥٣

الفايروس المضخم للخلية و السرطان القولوني-المستقيمي : هل توجد علاقة؟

أسماء باقر العبيدي

الخلاصه

الخلفية وهدف الدراسة: يعتبر الفايروس المضخم للخلية من الفايروسات التي تعمل على تحويل وتغيير خلايا الثديات، لذا فقد حاولنا في هذا البحث دراسة دور هذا الفايروس في عملية التحول السرطاني القولوني - المستقيمي.

المرضى وطريقة الدراسة: اجريت تقنية التصبيغ الكيميائي النسيجي المناعي باستخدام المضادات المناعية الوحيدة النسل للبروتين المبكر للفايروس المضخم للخلية على انسجة من ٣٢ حالة للسرطان القولوني – المستقيمي و ثمانية حالات من الاورام الاصبعية الحميدة في القولون، و قد اعتبرت الانسجة الطبيعية التي على حافة الانسجة السرطانية كسيطرة.

النتائــج: تم الكشف عن البروتين المبكر للفايروس المضخم للخلية في خمسة من ٣٢ حالة للسرطان القولوني – المستقيمي (٦و١٥ %) ، بينما كان الكشف سلبيا في جميع حالات الاورام القولونية الحميدة او في الانسجة الطبيعية التي على حافة الانسجة السرطانية.

الاستنتاج: تقترح نتائج هذا البحث احتمالية اشتراك الفايروس المضخم للخلية في عملية التحول السرطاني القولوني – المستقيمي بسبب الكشف عنه في الخلايا السرطانية فقط.

مفتاح الكلمات:الفايروس المضخم للخلية، سرطان القولون.

فرع الأحياء المجهرية [كلية طب النهرين – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٥٤-٥٧

تحديد المستوى المصلى للانترلوكينات ١٠ ,٨وكاما انترفيرون لمريضات الاجهاض التلقائى المتكرر

نضال عبد المهيمن[١]، امال حسين[٢]

الخلاصة

خلفية الدراسه: ان انتاج المدورات المناعيه مثل انترفيرون كاما من قبل الخلايا التائيه المساعده نوع ١ و الانترلوكين ١٠ من قبل النوع ٢ من تلك الخلايا قد تبين ان له تاثيرات مضره على الحمل و نمو و تطور الجنين.

هدف الدراسه: قياس التركيز الجهازى لبعض من المدورات المناعيه باستخدام تقنية اختبار الاليزا غير المباشرلمريضات الاجهاض التلقائى المتكرر .

المواد و طرائق العمل: تضمنت الدراسه الحاليه مائة وتسع عشْرة إمرأة، تراوحت متوسط اعمارهن بين (٢٣.٩– ٢٨.٥)، تم تقسيمهن الى ثلاثة مجاميع: مجموعة(أ) إجهاض تلقائي متكرّر (RSA) وعددهن ٦٢ إمرأة و متوسط اعمارهن بينَ (٢٨.٥ + ٠.٦٨). مجموعة (ب) - إجهاض تلقائي غير متكرّر (non-RSA) وعددهن ٣٤ إمرأة و متوسط اعمارهن بينَ (٢٦.٤ ± ٠.٨٥). مجموعة(ج) - سيطرة (حمل ناجح): وعددهن ٢٣ إمرأة ومتوسط اعمارهن بينَ (٢٣.٩ ± ٠.٨٨). تم جمع نماذج دم من كل المرضى وكذلك مجموعه السيطره. تم دراسة تاثير IL-10,IFN-γ,IL-8 باستخدام تقنية الاليزا غير المباشر.

النتائج: أظهرت النتائج وجود فرق معنوي عالي ($p<0.01$) بالنسبة لمتوسط النسبة المؤية ل IFN-γ في المجموعة(أ وب) مقارنة بمجموعة السيطرة (ج) .وكذلك وجود فرق معنوي عالي ($p<0.01$) بالنسبة لمتوسط النسبة المؤية ل IL-10 لمجموعة السيطرة (ج) مقارنة بالمجموعة(أ).

كما اكدت نتأئج فحص ELISA أن هناك فرقا معنويا ($0.05<P<0.1$) بين متوسط النسبة المؤيةIFN-γ/IL-10 عند المقارنة بين المجموعة(أ و ب) والمجموعة(ج) .

كما لوحظ ان هنالك زيادة فى نسبةIFN-γ في الإجهاض التلقائي المتكرر وغيرالمتكرر اضافة لذلك، اظهرت نتائج فحص IL-8 عدم وجود فرق معنوي ($p>0.05$) بين متوسط النسبة المؤية ل IL-8 عند مقارنة كل من المجموعة (أ) والمجموعة (ب) مع المجموعة (ج). ولكن اظهرت النتائج ان هناك فرق معنوي ($p>0.05$) بين متوسط النسبة المؤية ل IL-8 عند مقارنة المجموعة (أ) مع المجموعة (ب).

الاستنتاجات: تدعم نتائج هذه الدراسه امكانية وجود الاستجابه المناعيه الالتهابيه والتى تتناغم مع ظاهرة الاجهاض التلقائى المتكررو ذلك بزيادة التعبير عن المدور المناعى كاما اترفيرون مما يعكس دور النوع الاول من الخلايا التائيه وقله فى التعبير عن المدورات المناعيه نوع ١٠لدى مريضات الاجهاض التلقائى المتكرروالذى يبين عدم كافة الخلايا التائيه من النوع الثانى.

مفتاح الكلمات: إجهاض تلقائي متكرّر،**كاما انترفيلاون ، المدورات المناعيه١٠و ٨، اختبار الاليزا.**

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المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٥٨-٦٥

فرط التعبير المناعي للـ Bcl_2 في سرطان القولون والمستقيم

علاء غني حسين

الخلاصة

خلفية الدراسة: يعد سرطان القولون والمستقيم سبب رئيسي للفنائية والمرضية في جميع انحاء العالم . ان التقييم التكهني يؤثر على علاج سرطان القولون والمستقيم بضمنها القرارات حول العلاج المساعد .يعد التعبير المناعي للـ bcl_2 هو حدث جيني مرتبط بتقدم الورم وهو مؤشر تكهني للمرض.

هدف الدراسة: دراسة التعبير المناعي للـ bcl_2 في سرطان القولون والمستقيم وبيان مدى علاقته بالعوامل المرضية والسريرية للورم.

المواد وطرق العمل: للفترة من كانون الثاني ٢٠٠٤ – كانون الثاني ٢٠٠٥ تم اختيار ٣٥ مقطع شمعي لمرضى مصابين بسرطان القولون استعاديا تغطي الفترة من كانون الثاني ٢٠٠٤ الى كانون الثاني ٢٠٠٥ وتم تحضير اربعة شرائح نسيجية بسمك ٤ مايكروميتر، اثنان منها صبغت بصبغة الهيماتوكسيلين والايوسين وتم اعادة فحصها لغرض تشخيصها وتصنيفها نسيجيا والاثنان الاخران صبغت بطريقة التصبيغ الكيميائي النسيجي المناعي للكشف عن الـ bcl_2 . ان وجود التفاعل السايتوبلازمي الاحمر في المقطع اعتبر تفاعلا ايجابيا . بدمج الشدة والكمية للصبغة المناعية فان الصبغة السايتوبلازمية الضعيفة مع كمية من الخلايا الورمية اقل من ٢٥ % اعتبر ايجابيا للتعبير المناعي للـ bcl_2 .

تم التحليل الاحصائي لكل النتائج بأستعمال Chi square واعتبر ت قيمة P اقل من (٠,٠٥) كقيمة ذات مغزى او دلالة احصائيا.

النتائــج: ان التعبير المناعي للـ bcl_2 في الدرجة الدنيا كان ذا مغزى احصائيا . وجد ان تعبير ال bcl_2 كان اكثر في الاورام عالية التمايز النسيجي (او ذات الدرجة الدنيا لتصنيف المرض) وفي المراحل المبكرة للورم . اظهر سرطان القولون والمستقيم غير المخاطي الـ bcl_2 اكثر من النوع المخاطي وكان هذا ذو قيمة او دلالة احصائيا. هناك علاقة معكوسة وجدت بين التعبير المناعي للـ bcl_2 مع اكبر قطر للورم وحالة العقد اللمفاوية . ان التعبير المناعي للـ bcl_2 غيرمرتبط بعمر وجنس المريض وموقع الورم.

الاستنتاج: ان ظهور بروتين bcl_2 في سرطان القولون والمستقيم يترافق مع بعض عوامل المرض كالدرجة الدنيا لتصنيف المرض ، المراحل المبكرة للمرض ، النوع غير المخاطي لسرطان القولون والمستقيم ، صغر حجم الورم والحالة السلبية للعقد اللمفاوية .

مفتاح الكلمات: bcl_2 ،سرطان القولون والمستقيم.

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المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٦٦-٧٦

التعبير المناعي الكيميائي النسيجي ل P53 في سرطان المعدة (دراسة سريرية مرضية)

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الخلاصة

خلفية الدراسة: سرطان المعدة احد اكثر السرطانات انتشارا وفتكا في العالم اليوم. ان جين P53 هو من اكثر الجينات المثبطة للاورام تحولا والملاحظ في السرطانات البشرية بما فيها سرطان المعدة وان التطبيقات العملية لهذه الظاهرة في التكهن بمسار سرطان المعدة او حتى علاجه بواسطة تصحيح وظيفة جين P53 تبقى بحاجة الى استيضاح.

هدف الرداسة : تقييم التعبير المناعي الكيميائي النسيجي لبروتين P53 في سرطان المعدة ودراسة العلاقة بين بروتينP53 مع مختلف المتغيرات السريرية المريضية وتشمل : عمر وجنس المريض ، موقع الورم ، مظهر النمو،النوع النسيجي ، درجة تمايز الورم . ومرحلة الورم في سرطان المعدة.

المرضى ، المواد وطرائق العمل : للفترة من تشرين الاول٢٠٠٦ ولغاية أيار٢٠٠٧ تم اخذ اربعون حالة لسرطان المعدة حيث اخذت الانسجة المطمورة بقوالب شمع البارافين (نماذج من عمليات استئصال المعدة جزئيا او كليا) من أرشيف المواد الخاص بقسم الباثولوجي في مستشفى بغداد التعليمي ومركز الجهاز الهضمي وامراض الكبد وبعض المختبرات الاهلية تم عمل مقاطع نسيجية بسمك اربعة مايكروميتر وبواقع مقطع واحد لصبغة الهيماتوكسيلين والايوسين ، ثم تم اعادة فحصها ، ومقطعين للصبغة المناعية الكيميائية النسيجية لبروتين P53.

ان وجود الصبغة المناعية لـ P53 في ١٠ % على الاقل من نواة النسيج الورمي أعتبر كفرط التعبير لـ P53 ، بينما وجود صبغة قهوائية للنواة في المقطع اعتبر كتفاعل موجب .

تم تحليل المعلومات احصائيا بواسطة اختبار chi-square للجداول ذات التكرارات ، النسب ، المدى والانحراف المعياري . واعتبرت القيمة ذات دلالة احصائيا عندما P<0.05.

النتائــج: إن التقييم السريري المرضي توصل الى ان عدد المرضى الذكور هو ٢٨ وعدد المرضى النساء هو ١٢. ونسبة الذكور الى الاناث كانت ١/٢,٣.

كان مدى العمر للمرضى ٣٠-٨٠ سنة (المعدل ± الخطأ المعياري) يساوي (٥٥,٧٧ ±١,٨٨) سنة . إن غالبية حالات سرطان المعدة عائدة لمرضى الذكور . وكانت نسبة كبيرة من حالات سرطان المعدة ٣٢ (٨٠%) متمركزة في منطقة غار المعدة بينما البقية متمركزة في فؤاد المعدة .

إن سرطان المعدة ذو النوع المتقرح (٧٢,٥%) كان أكثر الانواع شيوعا . بينما كانت غالبية حالات سرطانات المعدة هي من النوع المعتدل التمايز (٦٢%). وكانت اكثر حالات سرطانات المعدة (٩٢,٥%) موجودة في المرحلة الثالثة من المرض.

كان التعبير الاجمالي ل p53 في حالات سرطان المعدة في هذه الدراسة (٤٤%) . لم يكن هناك اختلاف احصائي ذو دلالة في العلاقة بين فرط التعبير ل p53 مع عمر وجنس المريض ، (P>0.05) . على الرغم من انه لم يكن هناك علاقة احصائية ذات دلالة بين فرط التعبير لp53 مع موقع الورم ومظهر النمو للورم ، كان فرط التعبير ل p53 هو اكثر شيوعا في حالات سرطانات المعدة الموجودة في منطقة غار المعدة وفي حالات سرطانات المعدة ذات النوع المتقرح.

كان فرط التعبير لـ p53 هو اكثر شيوعا في حالات سرطانات المعدة ذو النوع المعوي مقارنة بالنوع المنتشر ، ومع هذا لم تكن هناك دلالة احصائية لهذه النتائج ، (P>0.05) .

الاستنتاج: كان التعبير الاجمالي لبروتين p53 في حالات سرطان المعدة في هذه الدراسة المناعية الكيميائية النسيجية هو ٤٤% . لم يكن هناك علاقة ذات دلالة احصائية بين فرط التعبير لـ p53 مع مختلف المتغيرات السريرية المرضية وتشمل : عمر وجنس المريض ، موقع الورم ، مظهر النمو ، النوع النسيجي ، درجة تمايز الورم ، ومرحلة الورم في سرطان المعدة.

مفتاح الكلمات: P53، سرطان المعدة، التعبير المناعي الكيميائي النسيجي

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المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٧٧-٨٩

التعبير المناعي النسيجي الكيميائي لبروتين P53 في سرطان عنق الرحم الاختراقي
(دراسة سريرية مرضية)

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الخلاصة

خلفية الدراسة: سرطان عنق الرحم واحداً من أكثر الأمراض شيوعا في النساء وهو يشكل حوالي ١٢% من السرطانات التي تصيب النساء عالمياً.

يعتبر P53 جين مثبط للأورام وإن فقدانه لهذه الوظيفة يعد مفتاحاً في تولد الكثير من السرطانات البشرية. وسبب الفقدان الوظيفي لجينP53 يعزى أما الى حدوث طفرات في الجين نفسه أو تحلل الأخير بواسطة البروتينات الخاصة ببعض الفيروسات.

هدف الدراسة: تقييم التعبير المناعي النسيجي الكيميائي لبروتينP53 في سرطان عنق الرحم (سرطان الخلايا الحرشفية والسرطان الغدي)ودراسة العلاقة بين فرط التعبير لبروتين P53 مع المتغيرات السريرية المرضية(العمر،درجة التمايز،والنوع النسيجي).

المواد ، وطرق العمل: - أُخِذت ٤٢ عينة نسيجية من سرطانات عنق الرحم شملت (٣٠ حالة من سرطان الخلايا الحرشفية و١٢ حالة من السرطان الغدي)في الرداسة الاسترجاعية .

- أُخِذت الأنسجة الأرشيفية المطمورة في شمع البارافين للمدة بين ١٩٩٨ و ٢٠٠٥ من ملفات مختبرات التحليلات النسيجية من مستشفى الكاظمية التعليمي ومستشفى العلوية وبعض المختبرات الخاصة، وكل المعلومات السريرية المرضية تم إستخلاصها من البيانات الخاصة بالمرضى.

-كل العينات تم التحري عنها بواسطة الصبغة المناعية النسيجية الكيميائية لمعلم P53 للأورام.

النتائــج: - إن نتائج تصبيغ P53 أظهرت وجود علاقة احصائية ذات دلالة بين فرط التعبير المناعي لبروتين P53 في سرطان عنق الرحم الحرشفي (١٦,١٦%) وبين فرط التعبير المناعي في سرطان عنق الرحم الغدي (٥٨,٣%).

- كانت نسبة التعبير المناعي لبروتين P53 في سرطانات عنق الرحم رديئة و متوسطة ، وجيدة التمايز (٥٠% ، ١٨,١٨% ، ١٦,١٦% على التوالي) ولم تكن هناك علاقة ذات دلالة احصائية بين فرط التعبير المناعي ودرجة التمايز.

-ل م تكن هناك علاقة احصائية ذات مغزى بين نسبة فرط التعبير المناعي لبروتينP53 للمرضى تحت سن الخمسين (٣٢,١٤%) وبين نسبة فرط التعبير المناعي لبروتين P53للمرضى في سن الخمسين أوأكثر.

-ان التقييم النسيجي المرضي أظهر وجود علاقة ذات دلالة احصائية بين معدل العمر لسرطان عنق الرحم الحرشفي (٤٧,٥±١,٩٤) وبين معدل العمر لسرطان عنق الرحم الغدي(٣٨,٥± ١,١١).

- لم توجد علاقة احصائية ذات دلالة بين درجة تمايز سرطانات عنق الرحم والنوع النسيجي.

الاستنتاج: - كانت هناك علاقة احصائية ذات دلالة بين فرط التعبير المناعي لبروتين P53 وبين النوع النسيجي.

- على الرغم من عدم وجود علاقة احصائية ذات دلالة بين فرط التعبير المناعي لبروتينP53 وبين تمايز سرطانات عنق الرحم الا ان نسبة التفاعل الموجب لبروتين P53 كانت اعلى في السرطانات رديئة التمايز منها في السرطانات متوسطة وجيدة التمايز.

- لم توجد علاقة احصائية ذات دلالة بين فرط التعبير المناعي لبروتينP53 مع عمر المرضى.

مفتاح الكلمات: P53، سرطان عنق الرحم، التعبير المناعي النسيجي الكيميائي

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المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص٩٠-١٠٢

تحفيز الجزيئه اللاصقه بين الخلايا-١ و الجزيئه اللاصقه بين الخلايا -٣ في النسوه المصابين بالاجهاض المتكرر

نضال عبد المهيمن ، أسماء باقر العبيدي ، حيدر فيصل غازي

الخلاصة

خلفية الدراسة: تتميز حالات فقدان الحمل المتكررة بارتفاع نسبة المدورات المناعية الالتهابية والتي بدورها تعمل على تحفيز العديد من الجزيئات الالتهابية مثل جزيئات التلاصق الخلوية التي قد يكون لها دور مهم في مرضية فقدان الحمل المتكرر.
هدف الدراسة: ايجاد العلاقة بين مرضية فقدان الحمل المتكرر والتعبير الموضعي لجزيئات التلاصق الخلوية ICAM-1 and ICAM-3.
طريقة العمل: استخدمت تقنية التلوين الكميائي النسيجي المناعي لجزيئات التلاصق ICAM-1 و ICAM-3 في عينات الجرف الرحمي والتي تم الحصول عليها من ٤٠ امراة تم تقسيمهن الى ثلاثة مجاميع: ٢٤ امراة حصل لها فقدان حمل متكرر، ١٠ نساء حصل لهن اجهاض تلقائي للمرة الأولى، و ستة نساء أجري لهن عملية انهاء حمل علاجي.
النتائــج: كانت مستويات التعبير الموضعي لجزيئات التلاصق ICAM-1 و ICAM-3 في حالات فقدان الحمل المتكرر ذات زيادة ملحوظة مقارنة مع المجموعتين الثانية والثالثة (p=0.001). فضلاً عن وجود ارتباط معنوي ايجابي كبير بين التعبير الموضعي لهذه الجزيئات (r=0.927, p<0.01).
الاستنتاج: قد تؤدي جزيئات التلاصق ICAM-1 و ICAM-3 دورا مهما في مرضية فقدان الحمل المتكرر من خلال زيادة التصاق وجذب الخلايا الالتهابية مؤدية الى فشل الحمل.
مفتاح الكلمات: الجزيئه اللاصقه بين الخلايا-١ ، الجزيئه اللاصقه بين الخلايا-٣ ، الإجهاض المتكرر

فرع الأحياء المجهرية [كلية طب النهرين – جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٨ م المجلد ٦ العدد ٢ ص١٠٣-١٠٩

ألام ألكتف عقابيل الضربة (الجلطة) الدماغية

زكي نوح حسن[1]،عبد الأمير أبو نايلة[2]،حسن عزيز حسن[3]

الخلاصة

خلفي ة الدراس ة: أل م الكت فِ أح د التعقي داتِ الت ي تحَ دثْ ف ي مرضِ ى الجلط ة الدماغية.

ه دف الدراس ة: إنّ ه دفَ ه ذه الدراس ةِ أنْ تُحلّ لَ أل مَ الكت فِ وإرتباطِه ا بالسماتِ السريريةِ المختلفةِ للجلطة الدماغيةِ.

طريق ة العم ل: اجري ت الدراس ة عل ى ٥٦ م ريض مص ا ب بالجلط ة الدماغي ة بنوعيه ا ا لنزف ي و الفاق ة الدموي ة(١٧).اش تكى ه ولاء المرضـ ى م ن ال م ف ي الكت ف ف ي الجه ة المص ابة بالش لل. ٢٩ م ريض مص اب بالجلط ة الناتج ة ع ن الفاق ة الدموي ة و ٢٧ بالجلط ة النزفي ة، تت راوح اعم ار المرضـ ى ب ين ٢٣-٧٣ سنة .

النت ائج: تب ين م ن خ لال الدراس ة وج ود ارتب اط مه م احص ائيا ب ين الام الكت ف م ع تق دم العم رو م ع اض رار الحس ية المركزي ة(ع دم التميي ز ب اللمس وع دم الاهتم ام الحس ي و ع دم تحس س الرس م ف ى الك ف)(١٧) واص ابات النط ق اللغوي ة(الخ رس)(١٧) .فيم ا ل م تثب ت علاق ة احص ائية م ع ن وع الج نس و جه ة الاص ابة الدماغي ة و ك ذلك م ع ن وع الجلط ة . اثبت ت الدراس ة ان تجم د الكت ف(الالتص اقية المحفظي ة للكت ف)(١٧) ه و الس بب الاكث ر ظه وراً خ لال ه ذه الدراسة .

الإس تنتاج: خرج ت الدراس ة بنتيج ة الاهمي ة الكبي رة للاهتم ام بالاص ابة ب الام الكت ف لم ا لها م ن ت أثير س لبي عل ى اع ادة تأهي ل المـ ريض . و تنصـ ح الدراس ة بمشـ اركة اختصـ اص امـ راض المفاصـ ل و التأهي ل الطب ي منـ ذ الاي ام الاولـ ى للاصابة بالجلطة الدماغية لغرض منع الحالة و تقليل اثارها السلبية

مفتاح الكلمات: الضربه الدماغيه ،مفصل الكتف.

[1]فرع الطب الباطني [كلية طب الكندي جامعة بغداد]
[2]فرع الطب [كلية طب الكندي جامعة بغداد]
[3]فرع الطب الباطني [كلية الطب جامعة النهرين]

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التنميط الجينى لمستضدات خلايا الدم البيضاء الصنف الثانى (HLA-DRB) لسرطان الدماغ النجيمى فى المرضى العراقيين

نضال عبد المهيمن ، عامره هادي

الخلاصة:
خلفية الدراسة: تسمى مستضدات التوافق النسيجي (MHC) والخاصة بالخلايا البشرية بـ(HLA).فقدان الخلايا الورميه لهذه المستضدات ، تعتبر ظاهرة مهمة لنمو و انتشار الورم . كما إن إنخفاض التعبير عن بعض من اليلات هذه المعقدات يمكن ان يهئ للإصابة بانواع معينة من الأورام.
هدف الدراسة: التحري عن امكانية وجود علاقة الإصابة بسرطان الخلايا النجمية في الدماغ وبين وجود اليلات الصنف الثاني من معقدات التوافق النسيجي.
المواد و طرائق العمل: تحديد وجود الليلات HLA-DRB1,DRB3,DRB4,DRB5 باستخدام تقنية تفاعل البلمره المتسلسل نوع (PCR-SSP) **لدى ٣٠ مريض مصاب بسرطان** الخلايا النجمية في الدماغ إضافة إلى ١٧ شخص سليم كسيطرة.
النتائج: وجد ان تكرار اللاليلي لجينات DRB1*10011,DRB1*10012 قد انخفض تواجدهما لدى المرضى مقارنة مع السيطرة (0.93 VS. 0.5). بينما لم نتوصل إلى أي علاقة بين المرض و الاليلات الاخرى.
الإستنتاج: من الممكن اعتبار الاليلات DRB1*10011,DRB1*10012 من العوامل التي توفر حمايه ضد الإصابه بهذا الورم.
مفتاح الكلمات: سرطان الخلايا النجمية في الدماغ ، التنميط الجيني للـ , HLA-DRB تفاعل البلمره المتسلسل .

فرع الأحياء المجهرية[كلية طب _ جامعة النهرين]

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التهاب القولون التقرحي في الاطفال الصغار

(تقرير حالة)

احمد هاشم العاني[١] ، عبد الكريم محمد علي [٢]

الخلاصة

التهاب القولون التقرحي هو واحد من امراض الامعاء الالتهابية التي تؤثر على المستقيم والقولون يظهر المرض غالبا في سن المراهقة والبلوغ ، ومن النادر اني يظهر المرض عند الرضع وصغار الاطفال

حالتنا المرضية (محمد) ظهرت علمات المرض عنده بعمر(١٨ شهر) باسهال دموي مستمر لايستجيب للمضادات الحيوية الاعتيادية والادوية المخصصة ضد الطفيليات . كل التحاليل المختبرية التي اجريت خلال فترة المرض المستمرة لاكثر من تسعة شهورغير محددة اومشخصة لمرض معين . وبعد ذلك نواظير القولون اظهرت صورة التهاب القولون التقرحي . ثم وضع المريض على العلاج المخصص لهذا المرض (Oral steroid and salazopyrine) مع حدوث تحسن ملحوظ وكبير في الحالة العامة للمرض واختفاء الاسهال الدموي .

وعند حدوث نوبات جديدة للمرض يعطى المريض العلاج ذاته المستعمل عند التشخيص الابتدائي .

[١]فرع طب الأطفال [كلية الطب - جامعة تكريت]
[٢]فرع طب الأطفال [كلية الطب - جامعة النهرين]

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