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ISSUED QUARTERLY

CONTENTS

Case Report

Editorial:

Safety of health care

Adnan A. Anoze *MRCP*

Patient safety has been high on the national and international agenda in health care. In our country probably more than 50 %of patients experience an adverse event while in hospital a figure which is higher than those around the word.

Considerable efforts have been made to improve safety and it is natural to ask whether these efforts have been well directed.

We believe that the lack of reliable information on safety and quality of care is hindering improvement in safety.

The principle approach to patient's safety has been to establish local and national reporting systems.

These systems invite voluntary reporting of safety incidents with the aim of feeding back the finding in to the system. Reporting systems are valuable component of a safety system

In order to do that we have to choose indicators which are important to patients?

These indicators include hospital mortality by which report the hospital standarised mortality ratios then the mortality after surgery and the surgical subspecialties.

Health care acquired infection is very important the introduction of mandatory reporting and accompanying infection control intiatives are now reducing infection nationally.

The other indicator is drug errors and adverse events which has many causes some of them are undoubtedly preventable and the overall level of adverse drug events would be an important indicator of the safety of any healthcare system.

The lack of reliable data on safety and quality over time hinders improrement efforts at every level of the national health services.

Finally the absence of solid measurement of safety and indeed quality is a worldwide problem.The development of electronic medical records provides considerable potential for obtaining safety data ,but much remains to be done to develop valid approaches for routine monitoring and detection of error and harm.

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

Isolation and identification of Respiratory syncytial virus from Infants with histopathological studies of the isolated virus on experimental animals

Shony M. Odisho[1]*PhD* , Anton S. Al-Bana [1] *PhD* ,Nahi Y. Yaassen[2] *PhD*.

Abstract

Background: Human Respiratory syncytial virus (HRSV) is one of the major causes of severe bronchiolitis and pneumonia in infants.

Objective: Isolation of virus from specimens from infants with severe bronchiolitis and pneumonia, and study the histopathological changes in laboratory animals.

Methods: Specimens collated from infants with lower respiratory tract infection were tested with Respi-Strip kit for the presence RSV antigens; the positive samples were inoculated in HEP-2 for 6-7 passages, and using neutralization test and fluorescent antibody techniques for detection of the isolated virus.

Also 20 mice's 10 weeks old divided into 2 groups one inoculated by dropped in nasal 0.5 ml of 100 TCID50/ml and the other 0.5 ml media as control. The lungs removed for histopathology study and the isolation of the virus in 2-7 days after inoculation.

Results: The Human RSV was successfully isolated in HEP-2 cell line from five specimens collected from infants with respiratory tract infection, previously tested for presence of RSV antigen by using Respi-Strip kits, where viral cytopathic effect (CPE) was first detected on 3rd passage with characteristic giant cell or syncytia type formation after 3 days post inoculation. Viral isolates were identified by using homologous reference antiserum by applying indirect immunofluorescent technique (IFAT) and neutralization test (NT). Experimental infection of mice with the isolated virus revealed histological changes in infected lung mainly characterized with evidence of acute interstitial pneumonia; in addition viruses were re-isolated from infected lung specimens after 2-7 days of experimental infection.

Conclusion: Human RSV replicated well in HEP-2 and cytopathic effect appeared in passage three, also the virus can be isolated and cause pathologic change in lung of infected mice.

Key words: RSV, HEP-2, Neutralization test.

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Introduction

Human Respiratory Syncytial Virus (HRSV) is a member of the Pneumovirus subfamily Paramyxoviridae. It account for approximately 50% of all pneumonia and up to 90% of the reported cases of bronchiolitis in infancy [(1)].

Outbreaks of RSV disease are abrupt in onset and can last up to 5 months; the RSV is mainly associated with bronchiolitis in children suffering from underlying illnesses such as congenital heart disease and broncho-pulmonary dysplasia which are at increased risk for severe infection [(2)]. The HRSV is also an important cause of community acquired pneumonia among hospitalized adults of all age [(3)].The virus was recovered first from children in Baltimore and suggested the name respiratory syncytial virus (RSV) to reflect the giant syncytia which formed in tissue culture[(4)].

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In our country we noticed high percentage of anti RSV and viral antigen in infants under 2 years suffering from lower respiratory infections and bronchiolitis with peak in winter and extends into the spring , and with increasing suspicious of this virus in causing respiratory infections in human; we study the isolation of HRSV from clinical samples of respiratory tract infection from infants in cell culture, study their specific cytopathogenicity, and their histopathological changes in experimentally infected mice.

Materials and methods

A cross-sectional study was conducted by taking nasal and throat swabs from (100) children with respiratory illness during month (December-March) in 2005-2006, from central pediatric Hospital in Baghdad, the swabs were immersed into tube containing 2 ml cooled transport media with fetal calf sera, and examined with Respi-Strip test: this kit used for detection of human RSV in nasopharyngeal specimens. Five strong positive samples for HRSV were clarified by centrifugation at 3000 xg for 30 minutes at 4 ^{o}C, treated for 30 minutes at 37 ^{o}C with 500 I.U. Penicillin, 500ug, Streptomycin/ml and centrifuged again at 4 ^{o}C for 30 minutes at the same speed, the supernatant was used to inoculated into susceptible cells. HEP-2 cell line: were grown in growth medium RPMI with HEPES, supplemented with 10% fetal calf serum, 100 I.U. penicillin and 100 *ug* streptomycine/ml.The cells were grown in 25 cm^2 falcon flasks, after complete monolayer cells then were used for detection virus growth, sample of HEP-2 cells were also grown on cover slips in Leighton tubes for detection of viral antigen by indirect fluorescent antibody technique**.**

The 5 samples were inoculated into HEP-2 cell line as 0.5ml / flask. Control cell cultures were treated with 0.5 ml / flask of maintenance media. Inoculated flasks were incubated at 37 ^{o}C for 1-2 hour for virus adsorption (with continuous rolling every 10 minutes) the cells then washed three times with maintenance media. Inoculated and 2 control cultures were fed with 10ml of maintenance medium and incubated at 34 ^{o}C and were checked daily for virus growth for 5-7 days by detection of cytopathic effect (CPE).

At the end of incubation time, infected culture was frozen and thawed and 0.5ml of medium, cells, and cell debris were used to repassage into new cell culture flask and treated in the same method of first infection at each passage level. This procedure was repeated three times for the secondary HEP-2 until CPE observed. If CPE were detected hemadsorption test was followed.

Leighton tubes were seeded with HEP-2. After complete monolayer cell growth was reached, the medium was discarded and each tube was inoculated with 0.2ml of the isolated virus suspension and after 1 hour incubation at 37 ^{o}C, maintenance medium was added and incubated at 34 ^{o}C for 24-72 hours.

Heamdsorpion test was done. The medium was removed from the monolayer cell culture flasks and the isolated virus suspension was used to infect these cell cultures. After 24 hour of infections, the monolayer cells were washed three times with warm PBS. Guinea pig erythrocytes were washed three times and prepared as 1% suspension; 10ml of these erythrocytes were added to each flask of the monolayer cells and incubated for one hour at room temperature. The supernatant fluid was removed and

flasks were washed twice with PBS then examined by inverted microscope for evidence of adsorption of RBCs to the infected monolayer cells [5].

When the CPE was observed in cell culture after inoculation by isolated viruses, the cells were fixed with buffered 10%formalin and stained with 1% crystal violet for 24 hour. Giemsa stain was also used after fixation of infected monolayer with methanol for 5-10 minutes then added Giemsa 1% was used to stain cells for 24 hours then washed with tap water and examined by inverted microscope.

Florescent test used for identification of isolated viruses. Discarding the medium from Leighton tubes and then washing the cover slips three times with PBS and the cells fixed with cold acetone for 10 minutes at 4 ^{o}C.

Drying of the cover slips in air and treatment with Reference anti-RSV immune serum, incubation for 1 hour at 37 ^{o}C in humid chamber. The cover slips washed three times with PBS for 30 minutes and air dried. Goat Anti- human IgG FITC conjugate was added to the cover slips, incubated for 1 hour at 37 ^{o}C in a humid chamber. Cover slips washed three times with PBS for 30 minutes and air dried. Dried cover slips were mounted on slides by using 50% glycerin in PBS.The slides were examined by fluorescent U.V. light microscope (Olympus).

Micro titration method was used for titration of isolated viruses; flat bottom 96 wells micro-titer plates were used [6]. Cultures of HEP-2 cell line were treated with Trypsin-Versin solution. Sufficient amount of growth medium (about 25 ml for 75 cm^2 flask) was added for each flask. After complete cell dispersion, 0.1ml amount of cell suspension was added for well. When monolayer cell culture complete, growth media was discarded and the virus suspension was serially 10 fold diluted, inoculated into 4 wells with each dilution (50*u*l per well) Control cell wells were also used which inoculated with media instead of virus. The plates were covered with sterile adhesive cover and incubated at 37 ^{o}C for 1 hour for virus adsorption and then 0.1 ml of maintenance medium added and the plate were covered again and incubated at 34 ^{o}C.Virus titer was calculated ([7]), and the titer was expressed as the highest dilution of virus suspension which showed 50% CPE in infected cell cultures.

Reference antiserum for HRSV Imported from DIALAB Company (Germany) was tested against the locally isolated virus. Neutralization test was carried out by using two fold dilution of reference antiserum against 100 TCID50 of the isolated virus. The mixtures (0.5ml of each) were incubated at 37 ^{o}C for 1 hour before inoculation into 4 wells cell cultures per serum dilution. The antibody titer was expressed as the reciprocal of the highest serum dilution which showed neutralization in 50% of infected cell culture wells.

Healthy (20) ten weeks old mice were supplied from the cancer research institute were divided into 2 groups, first group include 15 mice were inoculated intranasal with 10^5TCDI50/ 50*u*l of HRSV, and the second group include 5 mice inoculated normal cell suspension as control. The animals were examined daily and were killed 1-7 days after inoculation. The Lungs were removed and one lobes used for virus isolation which was homogenized with maintenance media and inoculated into susceptible cells .The other lobes will be fixed in buffered 10% formalin for 24 hour for histopathological examination.

The tissue was then embedded in low- melting point paraffin, sectioned at 5 *u*m thickness, and stained with Hematoxylin and Eosin.

Results

HEP-2 cell line was used for isolation of HRSV from the collected five nasal/ throat swabs taken from infants with acute respiratory tract infection which showed previously strong positive results for viral antigen by RSV-Respi test. In first passage all samples showed no cytopathic effect (CPE). But few rounded cell were noticed in second passage, however clear CPE was first noticed on the 3rd passage after three days, which characterized by cell granulation, aggregation, and separation of the infected cells in culture media.

The number of floating and syncytia cells increased in number on 5th and 6 th passage of HRSV isolates in HEP-2, which can be detected in 48h P. I. and syncytia cells increase in size with subsequent passages, accompanied by formation of empty open plaques which fuses to form large empty spaces with excessive floating cells, but such changes were not detected on control cell culture (Figures 1 and 2)

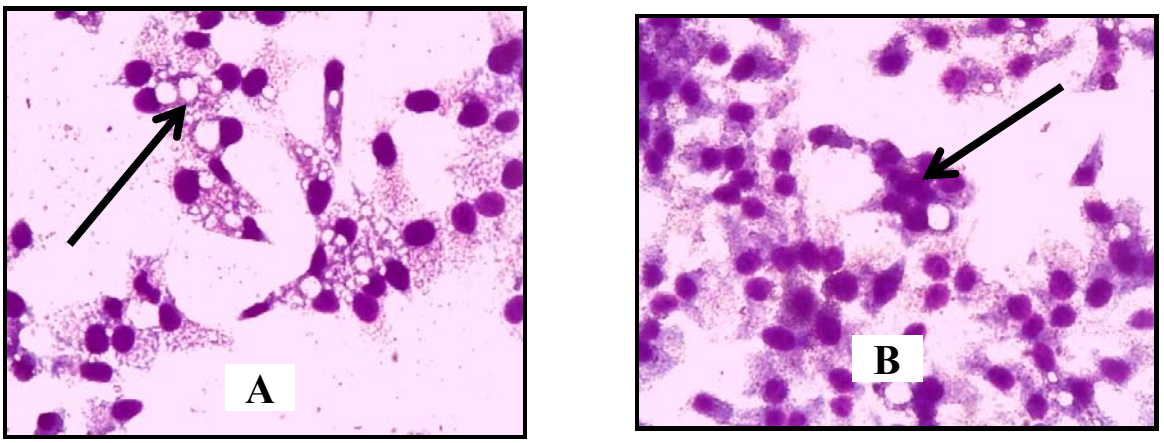

Figure 1: Cytopathic effects in HEp-2 cell line infected with the isolated HRSV, 5 days P.I.(A) Vaculation of cytoplasm (→).(B) Syncytia or giant cells formation (→).(H&E) (x200)

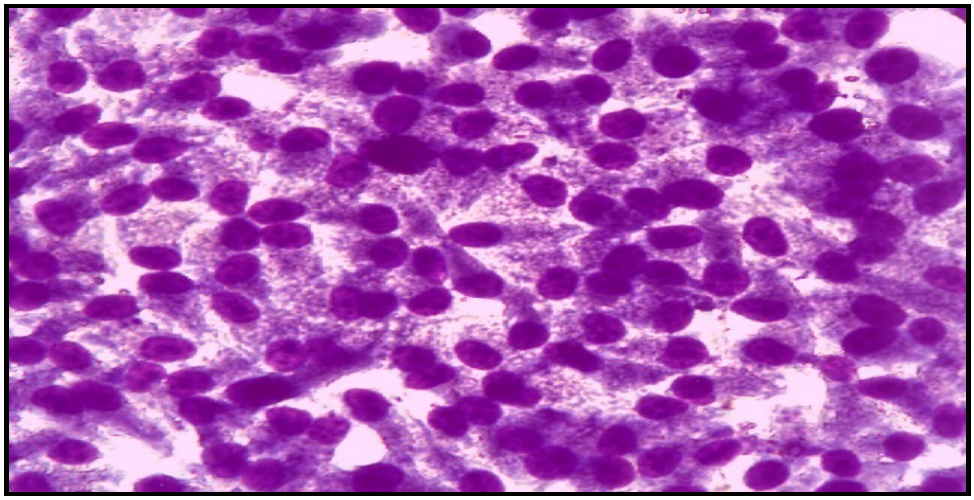

Figure 2: Uninfected HEp-2 cell line (H&E) (x200).

Monolayer cell cultures of HEP-2 infected with the isolated HRSV (passage 5) were used for haemadsorption test. The isolated viruses' infected HEP-2 cells were not able to adsorbed Guinea pig erythrocytes after washing of the infected cells.

The titer of isolated HRSV in infected HEP-2 cell line was $2\times10^{2.4}TCID_{50}$/ml in the 3rd passage; Virus titer increased with further passage and reached the maximum titer $2\times10^{5.4}TCID_{50}$/ml at 6th passage (Table 1).

Table 1: Propagation and titration of HRSV in HEP-2 cell line.

Passage number	Virus titer $TCID_{50}$/ml
2	$2\times10^{1.5}$
3	$2\times10^{2.4}$
4	$2\times10^{3.2}$
5	$2\times10^{5.4}$
6	$2\times10^{5.4}$

The demonstration of specific viral antigen of HRSV in infected cell culture at each passage was accomplished by indirect fluorescent antibody technique (IFAT) of infected HEP-2 cell culture grown on cover slips; few fluorescent cells were detected in second and third passage, but 80% of the monolayer cells. In 5th passage of the infected cells showed bright cytoplasmic fluorescence with predominance around the nucleus and occupied most of the cytoplasm.

Reference HRSV antiserum neutralized the infectivity of the isolated HRSV virus in HEP-2 cell culture by using 100 $TCID_{50}$/0.05 with neutralizing titer of 256.

Human RSV was re isolated in HEP-2 from lung samples collected from experimentally infected mice started from 2nd day post inoculation to 7th day.

Histopathological finding in sections of infected lung collected on 2-7 days P.I. showed evidence of acute interstitial pneumonia which was observed in HRSV- infected lung.

The lesions consisted of an extensive pulmonary edema and congestion accompanied with emphysema. In addition to perivascular leukocytes cuffing, (Figure 4).

In certain section there was acute bronchiolitis characterized by an infiltration of the neutrophils mainly through bronchiolar wall and in the lumen in addition to sloughing of their epithelial lining, (Figure 5). Both of these lesions were developed into acute interstitial pneumonia, which characterized by thickening of alveolar walls due to infiltration of neutrophils and some lymphocytes and extensive congestion of alveolar capillaries leading to narrowing of alveolar lumena,(Figure 6). These pathological finding were not seen in sections of normal mice lung (Figure 3).

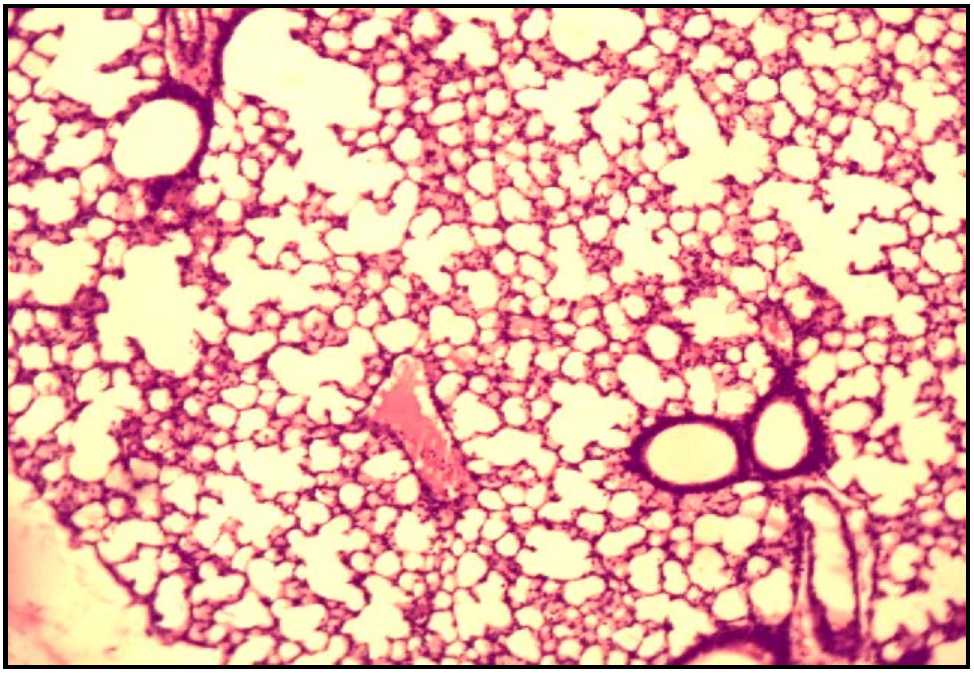

Figure 3: Section of normal mice lung. (H&E) (x100).

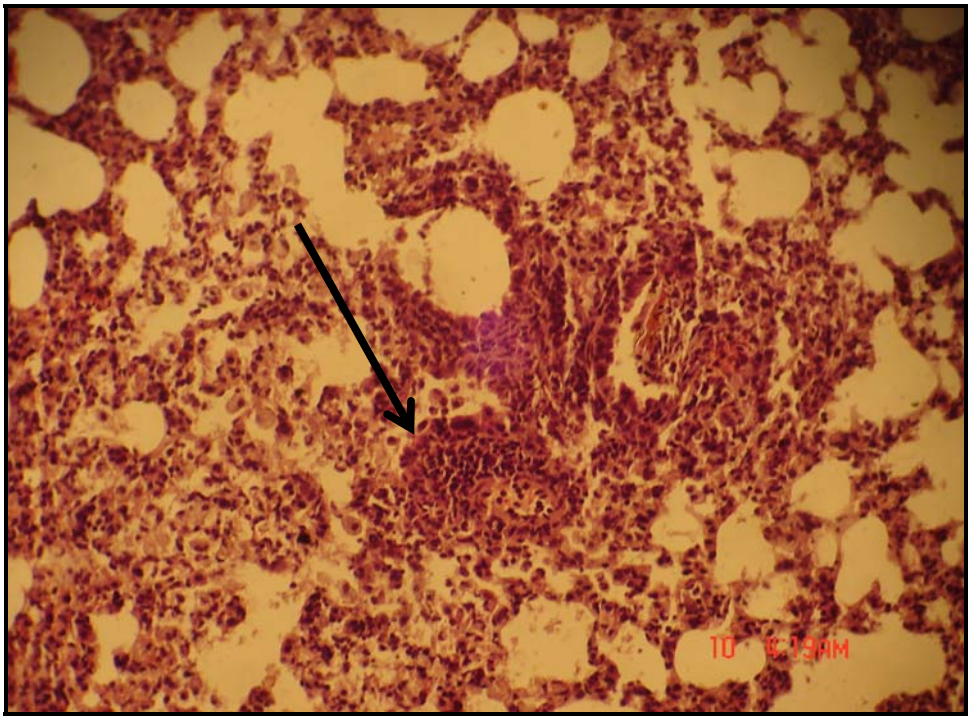

Figure 4: Section of infected lung, pulmonary edema with perivascular leukocytes cuffing (→). (H&E)(x100).

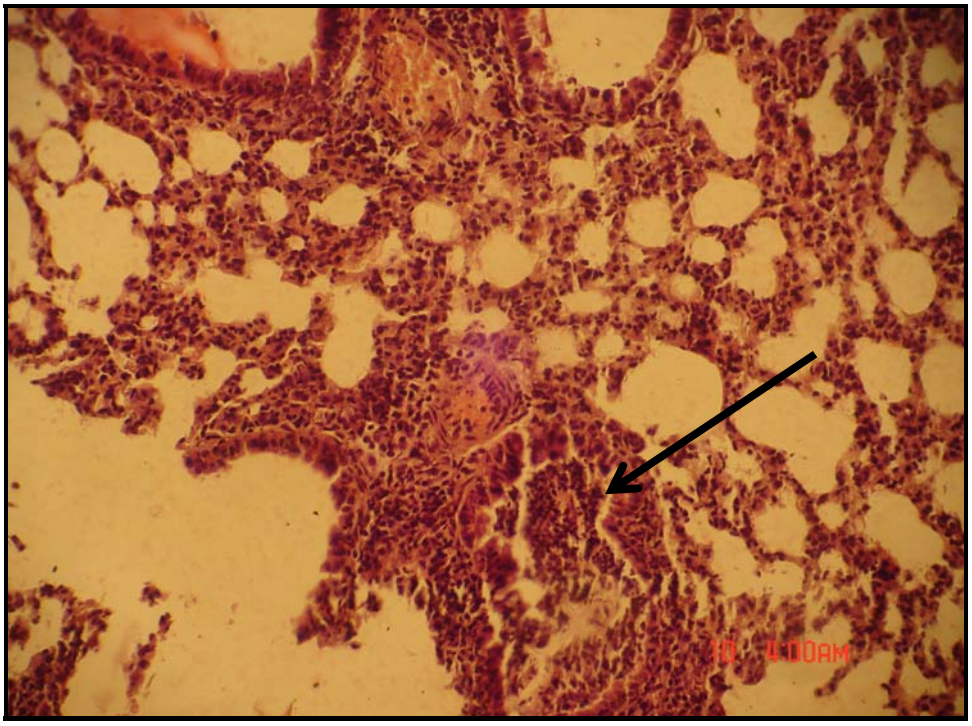

Figure 5: Section of infected lung with acute bronchiolitis (→) characterized by infiltration of neutrophils through bronchiolar wall and lumen. (H&E)(x100).

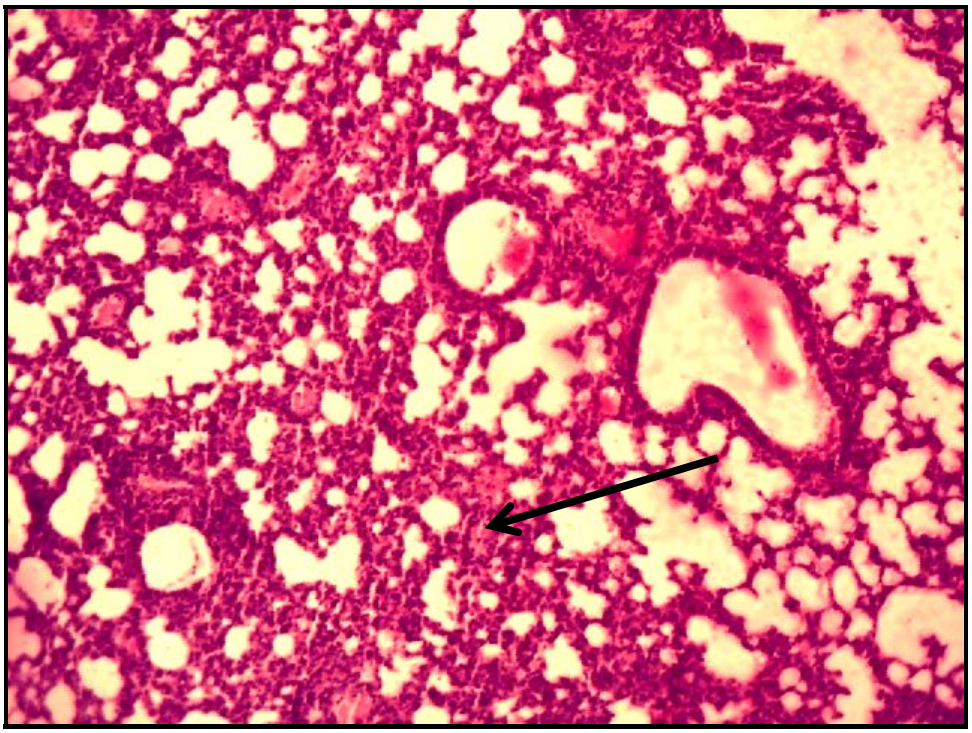

Figure 6: Section of infected lung with acute interstitial pneumonia (→), characterized by thickening of alveolar walls due to infiltration of neutrophils and lymphocytes. (H&E)(x100).

Discussion

The HRSV was isolated from 5 positive specimens' in HEP-2 cell line culture as these cells found to be sensitive for viral isolation,. Our results agreed with other studies which proved that HEP-2 was supporting growth of HRSV and the CPE appeared after sub passages and after 5 days PI [8, 9].

As reported RSV infected HEP-2 cells needs 34°C for incubation to support replication of our viral isolates of HRSV, the virus titer gradually increased with sub passage and reach its maximum titer $10^{(5,4)}$ $TCID_{50}$/ml, our result agree with other studies in increased of titer in subpassge of virus in HEP-2 at 34°c but differ from others who obtained high titer, this difference in virus titer could be due to variation in viral strains or viral subgroup which studies show that group A replicate better than group B in cell culture[8,10]. Also many factors affect rate of virus isolation as timing factor in collecting of the samples, due to loss of viral infectivity because of viral liability which was avoided by immediate inoculation of specimens into susceptible HEP-2 cells, it was found that 90% reduction in the virus titer within 2 hours was observed, but 90% reduction occurred within 24-48 with the virus suspended in medium 199 with 5% rabbit serum [8, 9]. Our result of HRSV isolation is in agreement with others and was successful by using MEM medium supplement with 5% fetal calf serum as transport media [9, 10], also using nasal/ throat swabs for virus isolation was very beneficial in getting positive results in virus isolation which agreed with positive detection of viral antigen by Respi-test. However studies have reported that nasopharyngeal wash was proved to carry 500 fold higher in virus content than nasal swabs specimens [10, 11].

The HRSV, infected cell culture had showed no activity of the viral isolates to haemadsorb guinea pig erythrocytes. This is related to the probable characters of HRSV in absence of heamagglutinin which differ from other paramyxoviruses and also to exclude the presence of such viral infection [12].

Identification of the isolated viruses was performed by indirect immunofluorescent technique which appeared as bright cytoplasmic fluorescence in susceptible infected cells mainly around the nucleus in HRSV , Also virus neutralization test was used for identification the isolated HRSV as indicated in our result that reference antiserum for HRSV neutralized the infectivity of viral isolates[13].

Human RSV was reisolated in HEP-2 from lung samples collected from experimentally infected mice started from 2-7 days PI. These result proved that mice (8-10 weeks) can be experimentally infected with human RSV local isolates as it was indicated by reisolation of virus from target organ with the classical histopathological changes, these agreed with other studies used mice and cotton rats as experimental animals in their investigations [14,15]. These findings agree with several studies which discussed the pathogenicity of HRSV inoculation of various strains of inbred mice, all described the moderate influx of inflammatory cells with reports including lymphocytes and eosinophiles as components of respiratory infiltration[16,17].

References

1. Collins PL, McIntosh K and Chunock RM. Respiratory syncytial virus. Fields virology 3rd Ed. Vol. 1. Lippincott-Raven, Philadelphia, pa. 1996; 1313-1351.

2. Falsey AR. and Walsh EE. Respiratory syncytial virus infection in adults. Clin. Microbiol. Rev. 2000; 13:371-384.

3. Dowell SF, Anderson LJ, Gary HE, Erdman DD, Plouffe JF, File TM, Marston BJ and Breiman RF. Respiratory syncytial virus is an important cause of community acquired lower respiratory infection among hospitalized adults. J. Infect. Dis.1996; 174:456-462.

4. Al-Dahiry T. (2005). Personal communication.

5. Bell DM, Roberts NJ, Madore HP, Hall C and Simons R. Hemadsorption focus assay for growth of influenza and Para influenza viruses in human dermal fibroblasts. Proceeding of the society for experimental Biology and Medicine.1985; 179:377-381.

6. McNulty MS, Allan GM and Mcferran JB. Cell culture studies with cytopathic bovine rota virus. Arch. Virol.1976; 54:201-204.

7. Reed LJ and Muench H. A single method for estimating fifty percent end points.Am.J.Hyg. 1938; 27: 493-497.

8. Falsey AR and Walsh EE. Respiratory syncytial virus infection in adults. Clin. Microbiol. Rev.2000; 13:371-384.

9. Karger A, Schmidt U and Buchholz UJ. Recombinant bovine respiratory syncytial virus with deletions of G or SH genes: G and F proteins bind heparin. J. Gen. Virol.2001; 82:631-640.

10. Hall C B and Douglas RG. Mode of transmission of respiratory syncytial virus. J. Pediatr.1975; 99:100-103.

11. Hambling MH. Survival of the respiratory syncytial virus during storage under various conditions. Br. J. Exp. Pathol.1964; 45:647-655.

12. Smith MH, Frey ML and Dirrks RE. Isolation, characterization, and pathogenicity studies of bovine respiratory syncytial virus. Arch. Virol. 1975; 47:237-247.

13. Wellemans G and Leunen J. The role of V220/69 (bovine respiratory syncytial virus) in respiratory disorder of Belgian cattle. Ann. Med. Vet.1971; 155:35-39.

14. Graham BS, Perkins MD, Wright PF and Karzon DT. Primary respiratory syncytial virus infection in mice. J. Med. Virol. 1988; 26:153-162.

15. Sudo K, Watanabe W, Konno K, Sato R, Kajiyashiki T, Shigeta S and Yokota T. Efficacy of RD3-0028 Aerosol treatment against respiratory syncytial infection in immunosuppressed mice. Anti. Agent. Chemi. 1999; 43:752-757.

16. Graham BS, Bunton LA and Rowland J. Respiratory syncytial virus infection in anti-μ treated mice. J. Virol. 1991; 65:4936-4942.

17. Fischer JE, Johnson JE, Kuli-Zade RK, Johnson TR, Aung S, Parker RA. and Graham SB. Over expression of interleukin-4 delays virus clearance in mice infected with respiratory syncytial virus. J. Virol.1997; 71:8672-8677.

Possible Role of IL-1-α and TNF-α in Breast Cancer

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Abstract

Background: Cytokines have been used as biomarkers in research for prognosis and have been associated with symptoms and adverse outcomes in multiple conditions, including breast cancer.

Objectives: *To estimate the concentration of IL-1-α and TNF-α in serum of breast* cancer (BC) patients compared with control groups and to detect if there is association of serum levels of these interleukins with disease development.

Subjects and Methods: The levels of IL-1-α and TNF-α were measured by ELISA method in sera of 45 BC patients, 12 patients with benign breast lesions and 23 apparently healthy controls.

***Results*:** Present study was demonstrated that IL-1-α and TNF-α levels were significantly elevated in serum of BC patients as compared with controls ($p<0.001$), this elevation were significantly associated with poor prognostic factors including advanced stage and estrogen and progesterone receptors-negative status.

Conclusions: Evaluation the serum level of IL-α-1and TNF-α may be helpful as predictive non-invasive tests for tumor development in breast cancer patients.

Keywords: Breast cancer, IL-α-1, TNF-α.

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Introduction

Cytokines are known to have both stimulatory and inhibitory effects on breast cancer growth depending on their relative concentrations and the presence of other modulating factors in the tumor microenvironment. Certain cytokines appear to prevent an effective immune response being mounted, and may contribute to loco-regional and/or metastatic spread, the elevation of the serum concentration of such cytokines, however, might be utilized as a marker of immune status, disease prognosis and monitoring, where as others promote the immune system's anti-tumor capability [(1)].

Interleukin 1 (IL-1) system plays an important role in human pathology and is involved in the local control of malignant disease. Since tumors can be considered 'wounds that never heal', due to their everexpanding tissue invasion and injury. Therefore, it is highly likely that proinflammatory cytokines such as IL-1 are involved in tumor growth and metastasis [(2)]. The IL-1 family of cytokines, and receptors are present within the human breast cancer (HBC) tumor microenvironment and that the IL-1 network of cytokines and receptors within the tumor microenvironment can control tumor cell subpopulation expression of other protumorigenic cytokines such as the angiogenic/growth factor, interleukin-8 and subsequently contribute to angiogenesis, tumor proliferation,and tumor invasion[(2,3)]. As well as the expression of IL-1 correlate with expression of prognostic factors such as estrogen and progesterone receotors (ER/PR) [(2)].

The multifunctional cytokine, tumour necrosis factor (TNF), is involved in the promotion of inflammatory responses and plays a

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critical role in the pathogenesis of inflammatory, autoimmune and malignant diseases [4]. It induces production of chemokines and promotes production of IL-1 and IFN-γ by lymphocytes and macrophages. Initially proposed to have anti-carcinogenic effects [5], TNF was later shown to be tumourigenic in both in vitro [6] and in vivo studies [7]. High plasma TNF levels in cancer patients are associated with a poor disease outcome [8]. TNF is also a key angiogenic molecule that may promote angiogenesis directly by stimulating endothelial cell proliferation and indirectly by modulating expression of other proangiogenic factors [9]. The current study is a trial to estimate IL-1-α and TNF-α level in the patient's sera in comparison with controls. This, however, might open a gate for entrance into the treatment of this disease.

Subjects and Methods

Subjects:

Forty five breast cancer female patients with age range from 28 to 73 years were eligible for this study. They included invasive ductal carcinoma, invasive lobular carcinoma, and in situ ductal carcinoma.The patients were admitted for surgery at Al-Kadhimia Teaching Hospital and nursing home hospital /medical city, for the period between March 2006 till March 2007. Data of estrogen and progesterone receptors status (immunohistochemically) were obtained from medical records of patients and validated by an experienced histopathologist. Controls were consisted of two groups:- A-Patient control group: - Twelve females with benign breast lesions (6 cases with fibrocystic disease and 6 with fibroadenoma) were involved in this study as a patient control group. B-Healthy control group: - A total of 23 healthy females' volunteers who have no history or clinical evidence of any breast lesions and their sex matched with BC patients were selected as a healthy control group. Venous blood samples were collected preoperative.

Methods:

The BioSource Hu IL-1α and TNF-α kits are a solid phase sandwich enzyme linked immuno sorbent assay (ELISA) (BIOSOURCE, Europe S.A., Belgium, Lot No. 053804; 054807). The absorbance of each well was read at 450 nm within 2 hours after adding the stop solution. The absorbance of the standards was plotted on graph paper against the standard concentration to construct the standard curve. The IL-1 α and TNF-α concentration for unknown samples and controls was read from standard curve.

Statistical analysis

The serums cytokines were quantitative variables, but were non-normally distributed as shown by Semirnov-Kolmogorov test, these variables are better to be described by median and the test of significance suitable for them was non-parametric tests. All the data have been analyzed statistically using Kruskall-Wallis test and MannWhitney analysis for measuring the differences between the studying groups [10].

Results

In the current study, the stages of BC for 45 patients (according to TNM system) were 23(51.11%) cases with stages (0, I and II) and 22 (48.88%) cases with stage III.

Estimation of serum level of IL-1α

Table-1 revealed a significant elevation of serum IL-1α level among BC patients (median=19.8 pg /ml) in comparison to that of control groups which include patients with benign breast lesions (BBL) (median=5.8 pg /ml) and healthy control (median=0 pg /ml) ($p<0.001$).

In addition, the median serum level of this cytokine in BC patients increased significantly with advanced stage (P<0.001) (table 2).

Estimation of serum level of TNF-α

Table-3 demonstrated a significant elevation in the level of serum TNF-1α of patients (median=46.4 pg /ml) in comparison to that of patient control (median=11.2 pg /ml) and healthy control (median=8.7 pg /ml) (p<0.001).

Also the present study showed detectable association between TNFα level and the development of disease P<0.001. Table-4 showed that the median serum level of this cytokine in BC patients increased with advanced stage.

The association of IL-1-α and TNF-α with estrogen and progesterone receptors

The results of association between IL-1α and TNFα level with ER and PR expression in breast cancer samples were shown in tables- 5 & 6. IL-1α and TNFα level was found to be inversely associated to ER and PR expression (p= <0.05).

Table 1: The difference in median levels of serum IL-1α (pg/ml) concentration among the three studied groups.

Serum IL-1	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	1.8	1.5	0	
Maximum	53.1	9.6	3.2	
Median	19.8	5.8	0	<0.001
NO.	45	12	23	
P (Mann-Whitney)				
BC X Healthy control <0.001				
BC X BBT <0.001				

Table 2: The difference in median levels of serum IL-1α (pg/ml) according to the stage of disease

Values	Stage 0, I& II	Stage III	P (Mann-Whitney)
Minimum	1.8	4.2	
Maximum	33	53.1	
Median	6.6	27.2	<0.001
N	28	17	

N= number

Table 3: The difference in median levels of serum TNF-α (pg/ml) concentration among the three studied groups.

Serum TNF-α	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	3.6	2.8	1.8	
Maximum	126.5	51.4	42.2	
Median	46.4	11.2	8.7	<0.001
NO.	45	12	23	
P (Mann-Whitney)				
BC X Healthy control <0.001				
BC X BBT <0.001				

Table 4: The difference in median levels of serum TNF-α (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	3.6	3.8	
Maximum	65	126.5	
Median	14.3	62.2	<0.001
N	28	17	

N=number

Table 5: The difference in median levels of serum IL-1α and TNF-α (pg/ml) according to the estrogen receptors.

	Estrogen receptor		
	Positive (n=21)	**Negative (n=24)**	**P**
Interleukin-1 Alfa conc.			
Range	(1.8 – 16.3)	(6 – 53.1)	
Median	5.1	23	<0.05
TNF Alfa conc.			
Range	(3.6 – 35.8)	(13 – 126.5)	
Median	11	63	<0.05

Table 6: The difference in median levels of serum IL-1α and TNF-α (pg/ml) according to the progesterone receptors.

	Progesterone receptor		
	Positive (n=26)	**Negative (n=19)**	**P**
Interleukin-1 Alfa conc.			
Range	(1.8 – 19)	(8– 53.1)	
Median	6	24.2	**<0.05**
TNF Alfa conc.			
Range	(3.6 – 41.2)	(10.4 – 126.5)	
Median	15	66	**<0.05**

Discussion

Evaluation of the role of the immune response in either the development or control of breast cancer is complex. Nevertheless, there is substantial information that in this disease, the immune response is not a host defence reaction and may even serve to facilitate cancer development. Potential mechanisms for these effects include production, by inflammatory cell infiltrates, of direct or indirect modulators of breast cell growth, e.g. cytokines [11].

Our data was in accordance with those of other authors who have demonstrated significantly higher levels of innate cells- related cytokines (IL-1 and TNF-α) in sera of patients with BC than those of control groups, moreover, there was detectable correlation between clinical stage and the serum levels of above mentioned cytokines [12-14]. In contrast to these

results, Green and coworkers in (1997) did not observe any correlation between the cytokines IL-1α, IL-1β, IL-4, IL-6, IL-8, and TNF-α, TNF-β, IL-2, IL-5, IL-7 and tumor histological grade or lymph node metastasis in breast cancer patients [15].

A major tumor-associated macrophages (TAM) derived inflammatory cytokine shown to be highly expressed in breast carcinomas is tumor necrosis factor alpha (TNF-α). It is a multifactorial cytokine, simplied by its name, TNF-α may have cytotoxic and apoptotic activities when administered to breast tumor cell lines [16].

The fact that TNF-α activities vary under different physiological conditions and in a cell-type-dependent manner contributes to a sense of ambiguity regarding its antitumor effects [16]. Indeed, recent investigations strongly suggest that the chronic expression of TNF-α in breast tumors actually supports tumor growth. The number of cells expressing TNF-α in breast carcinoma was found to be correlated with increasing tumor grade and node involvement, and TAM-derived TNF-α expression was suggested to play a role in the metastatic behavior of breast carcinomas [17]. The tumor-promoting functions of TNF-α may be mediated by its ability to induce proangiogenic functions, to promote the expression of matrix metalloproteinases (MMP) and endothelial adhesion molecules, and to cause DNA damage via reactive oxygen, the overall effect of which is promotion of tumor-related processes [11,16].

The role of other inflammatory cytokines (possibly TAM derived), IL-1 was also addressed in breast carcinoma [18]. The role of the IL-1 system in human breast cancer is conflicting. Initial analyses regarding IL-1 indicated that its levels were significantly higher in invasive carcinoma than in ductal carcinoma in situ or in benign lesions, implying that elevated levels of IL-1 are directly correlated with a more advanced disease [19].

In addition, IL-1 has been shown to inhibit growth of breast cancer cells and to promote cellular differentiation in vitro, but it is equally known to stimulate the expression of several proteolytic enzymes in human cancer [20]. The consecutive degradation of extracellular matrix is a key element of local invasion and metastasis [21]. The mitogenic activity by IL-1 can be explained by induction of growth-related oncogene (GRO) gene expression [22] or induction of IL-8 expression via activation of the Nuclear factor κB (NFκB) and activator protein (AP)-1 signal transduction pathways [23]. The robust response of the metastatic- or mesenchymal-appearing breast carcinoma cells to either IL-1 or TNF-α may be because of elevated expression of transcription factors needed for transcription of the IL-8 gene. NF-κB, a transcription factor, which can be activated by either IL-1 or TNF-α, is an example of such a transactivator. Activated NF-κB recognizes and binds to a consensus sequence in the promoter region of the IL-8 gene. This binding is essential, but not sufficient for the induction of IL-8 expression. It is possible that the metastatic breast cell lines have factors working either coordinately or synergistically with activated NF-κB to enhance IL-8 expression [24].

Among the various prognostic factors, lack of estrogen and progesterone receptors (ER&PR) has consistently been associated with poorer prognosis [25]. Of particular note, in present study we found an inverse correlation between expression of ER&PR and cytokines (IL-1-α and

TNF-α) serum levels, which is in agreement with the findings of other studies [2, 13]. The inverse correlation between (IL-1-α and TNF-α) and ER&PR indicates that the high serum levels of these cytokines correlate with low ER&PR expression. Since low ER&PR expression is considered a prognosticator for poor disease outcome in BC, this suggests that the high IL-1 and TNF-α serum levels would predict poor outcome in BC. So, current data suggest that cytokines could be involved in the aggressiveness of ER-negative breast tumors. It was feasible that it can be used to identify patients with a poor prognosis who may benefit from more aggressive management, and this may help us to understanding the pathogenesis of this disease and ultimately may be use in the development of a new therapeutic technique.

References

1. Rao VSR, Dyer CE, Jameel JK. Potential prognostic and therapeutic roles for cytokines in breast cancer (review). Oncology Reports 2006; 15:179-185.

2. Pantschenko AG, Pushkar I, Anderson KH, Wang Y, Miller LJ, Kurtzman SH, Barrows G, Kreutzer DL. The interleukin-1 family of cytokines and receptors in human breast cancer: implications for tumor progression. Int J Oncol 2003; 23:269-284.

3. Kumar S, Kishimoto H, Lin Chua H, Badve S, Miller K, Bigsby RM, Nakshatri H. Interleukin-1α promotes tumor growth and cachexia in MCF-7 xenograft model of breast cancer. American Journal of Pathology 2003; 163(6, 1) pp 2531-2541(11).

4. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. N Engl J Med, 1996; 334:1717-1725.

5. Jaattela M. Biologic activities and mechanisms of action of tumor necrosis factor-alpha/cachectin. Lab Invest 1991; 64:724-742.

6. Komori A, Yatsunami J, Suganuma M, Okabe S, Abe S, Sakai A, Sasaki K, Fujiki H. Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. Cancer Res 1993; 53:1982-1985.

7. Fujiki H, Suganuma M. Tumor necrosis factor-alpha, a new tumor promoter, engendered by biochemical studies of okadaic acid. J Biochem 1994; (Tokyo), 115:1-5.

8. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M. Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. Clin Cancer Res 1998; 4:1743-1748.

9. Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 1998; 77:2246-2251.

10. Sorlie DE. Medical biostatistics and epidemiology: Examination and Board review First ed, Norwalk, Connecticut, Appleton and Lange 1995; 47-88.

11. Stewart TH, Heppner GH. Immunological enhancement of BC. Parasitology, 115 suppl 1997; S 141-S153.

12. Sheen-Chen S-M, Chen W-J, Eng H-L, Chou F-F. Serum concentration of tumor necrosis factor in patients with breast cancer. Breast Cancer ResTreat 1997; 43:211-215.

13. Singer CF, Kronsteiner N, Hudelist G, Marton E, Walter I, Kubista M, Czerwenka K, Schreiber M, Seifert M, Kubista E. Interleukin 1 system and sex steroid receptor expression in human breast cancer: interleukin 1alpha protein secretion is correlated with malignant phenotype. Clin Cancer Res; 2003; 9:4877-4883.

14. Fuksiewicz M, Kaminska J, Kotowicz B, Kowalska M, Rubach M, Pienkowski T. Serum cytokine levels and the expression of estrogen and progesterone receptors in breast cancer patients. Clin Chem Lab Med 2006; 44(9):1092-7.

15. Green AR, Green VL, White MC, Speirs V. Expression of cytokine messenger RNA in normal and neoplastic human breast tissue: identification of interleukin-8 as a potential regulatory factor in breast tumours. Int J Cancer 1997; 72:937-941.

16. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow. Lancet (2001); 357:539-45.

17. Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 1998; 77:2246-2251.

18. Crowther M, Brown NJ, Bishop ET, Lewis CE. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. J Leuk Biol 2001; 70:478-490.

19. Jin L, Yuan RQ, Fuchs A, Yao Y, Joseph A, Schwall R, Schnitt SJ, Guida A, Hastings HM, Andres J, Turkel G, Polverini PJ, Goldberg ID, Rosen EM. Expression of

interleukin-1β in human breast carcinoma. Cancer 1997; 80:421-434.
20. Bourhis XL, Toillon RA, Boilly B, Hondermarck H. Autocrine and paracrine growth inhibitors of breast cancer cells. Breast Cancer Res Treat 2000; 60: 251-258.
21. Woodhouse EC, Chuaqui RF, Liotta LA. (General mechanisms of metastasis. Cancer (Phila), 1997; 80: 1529-1537.
22. Rangnekar VV, Waheed S, Davies TJ, Toback FG, Rangnekar VM. Antimitogenic and mitogenic actions of IL-1 in diverse cell type are associated with induction of gro gene expression. J Biol Chem 1991; 266: 2415-2422
23. Jung YD, Fan F, McConkey DJ, Jean ME, Liu W, Reinmuth N, Stoeltzing O, Ahmed SA, Parikh AA, Mukaida N, Ellis LM. Role of p38 MAPK, AP-1 and NFkB in IL-1 beta induced IL-8 expression in human vascular smooth muscle cells. Cytokin 2002; 18:206-213.
24. Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 1988; 167:1883-1893.
25. Skoog L, Humla S, Axelsson M, Frost M, Norman A, Nordenskjold B, Wallgren A. Estrogen receptor levels and survival of breast cancer patients a study on patients participating in randomized trials of adjuvant therapy. Acta Oncol 1987; 26:95-100.

Efficacy of Different Treatment Modalities Used in Epistaxis

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Abstract

Background: This is a cross-sectional, clinical study, implemented in the Department of Otolaryngology / Sulaimani Teaching Hospital; from July 15th 2004 to April 15th 2005.

Objectives: The aim of the study is to describe the demographic characteristics, aetiological factors and therapeutic measures for epistaxis in Sulaimani region, to improve our experience in the management of this common condition.

Methods: This study included 100 patients of different age and sex who attended ENT department during the period of the study.

Results: At the end of the study we found that most cases of epistaxis can be treated by simple measures like application of lubricants to nose, or cauterization and anterior packing.

Conclusion: Active intervention should be done when indicated.

Keywords: epistaxis, lubricants, cauterization, anterior packing, active intervention**.**

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Introduction

Epistaxis is a common disorder, with most people having experienced one or more episodes in their lifetime. Some estimates suggest that as many as 60% of the population may have suffered from epistaxis at some point in their lifetime, current available data suggests that only approximately 10% of patients seek medical attention for nosebleeds [1, 2]. It occurs in persons of all ages without predilection for sex [3]. Minor recurring nosebleeds are usually caused when the mucosa of the anterior nasal septum becomes compromised as a result of one or more factors. One key factor is a dry external climate or dryness caused by the lack of humidity when heating in the winter. Dryness can also be caused by various medications such as antihistamines and diuretics. These nosebleeds tend to be minor and are relatively easily managed with pressure or stop spontaneously.

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Only a small percentage requires consultation with an otolaryngologist to control the bleeding, suggesting that otolaryngologists see only 0.5% to 1% of the total population who experience nosebleeds [4]. On occasion, however, epistaxis can be quite difficult to manage. This is particularly true in patients who have hypertension, are on anticoagulants or aspirin, or have a familial history of a bleeding disorder such as hemophilia, or have a blood disease like leukemia [4].

The upper parts of the nose are supplied by branches from internal carotid artery (anterior and posterior ethmoidal arteries) and the rest from branches of the external carotid artery (greater palatine, sphenopalatine and superior labial artery which is a branch of facial artery) [5]. In the caudal end of the septum the branches of the two systems anastomose forming Kiesselbach's plexus. The middle turbinate is regarded as the dividing line between the internal and external carotid distributions. Retrocollumelar vein also runs at the caudal end of the septum which sometimes may reveals a tiny area of local ballooning, and this could possibly signify an area of vessel wall weakening which easily bleeds,

perhaps as a result of localized ischaemia and/or trauma [6].

The most common source of bleeding (in about 90% of cases) is Kiesselbach's plexus on the anterior portion of the septum (Little's area), retrocollumelar vein at the caudal edge of the septum is also a frequent site of bleeding in young persons [7], the mucosa in the anterior portion of the septum is very fragile and is tightly adherent to the underlying cartilage, and thus offers little resistance to mechanical or functional stress, so easily irritated via nose picking, cold dry air, cigarette smoking, etc. [8].

The etiological factors for epistaxis include idiopathic, trauma, upper respiratory tract infections, tumors, iatrogenic and disorders of blood vessels and clotting mechanisms [9].

Epistaxis remains a common problem treated by otolaryngologists. Although most cases are managed on an outpatient basis, some require hospitalization for more invasive treatments. Nasal packing is used commonly for epistaxis that requires inpatient management [10]. Sometimes an anteriorly-placed bleeding point is visible with a head mirror and may be easily cauterized; also direct cauterization under endoscopic control appears to be an effective treatment of posterior epistaxis [11]. The elderly population with their associated morbidity often requires more intensive treatment and subsequent admission [12].

Patients and methods

One hundred patients included in this study were consecutively seen in the ENT department at Suleimany Teaching Hospital over a 10 months period from July, 15th 2004 to April, 15th 2005.

A careful history was taken from every patient (or his/her parents or relatives), then a thorough ENT examination was done to locate the bleeding site and to find out any possible anatomical or pathological abnormalities in the nasal cavity.

In active bleeding an attempt was made to arrest the bleeding by local pressure and cautery if necessary, if this failed anterior packing was performed, if failed too then anterior and posterior packing was done. If the bleeding point was inactive then applications of lubricants (in form of gentamicin eye ointment) and/or cautery of the dilated vessels were tried. Admission and blood transfusion was done if clinically indicated.

Any relevant medical illness was treated, like hypertension. The laboratory tests which were done include the hemoglobin level and platelets count, however, when the diagnosis was unclear after history and physical examination, further tests were done like PT, PTT, CT and BT. Sinus X-ray film and X-ray of nasal bones were useful adjuncts if one is considering local trauma, or acute sinusitis. Additional blood investigations, like complete blood picture and blood film, renal and liver function tests were done in indicated cases.

Results

The highest proportion of epistaxis was among individuals less than twenty years of age 50 cases (50%). Sixty three (63%) males were affected as compared to thirty seven (37%) females, giving a male: female ratio of 1.7:1, as shown in figure 1.

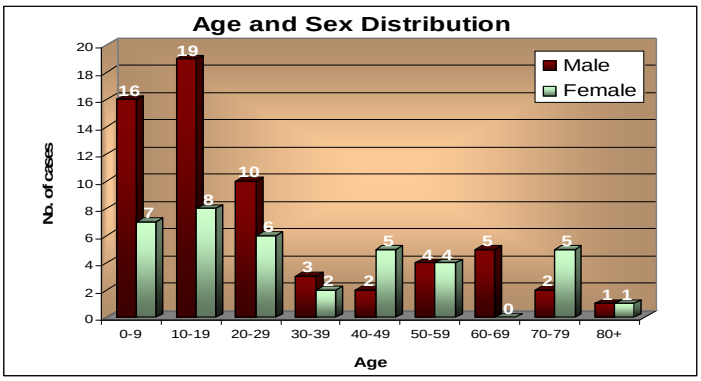

Figure 1: Showing the age and sex distribution.

Eighty patients (80%) were urban inhabitants whereas rural inhabitants were only 20 (20%), as shown in figure 2.

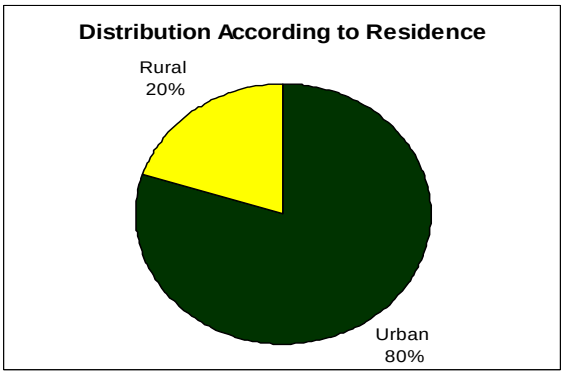

Figure 2: Showing distribution of patients according to residence.

Out of hundred patients who were studied, 59 patients (59%) had active bleeding at the time of examination, as shown in table 1.

Table 1: Showing types of bleeding.

Type of bleeding	No. of cases	%
Active	59	59
Inactive	41	41
Total	100	100

Eleven patients (11%) were smokers, five cases (5%) were alcoholics, and the rest were none (84%), as shown in figure 4.

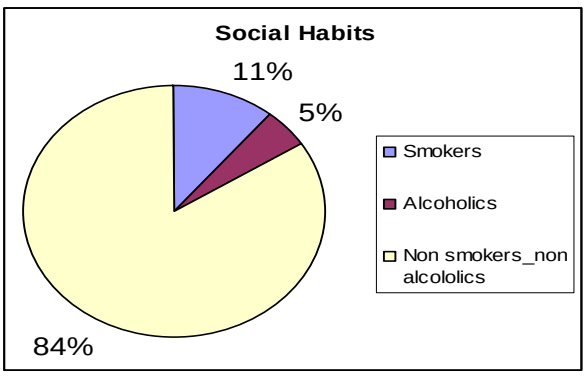

Figure 3: Showing relation of social habits with epistaxis.

Among the local causes, idiopathic group was the commonest (43%), followed by inflammatory causes (30%) and trauma (23%).

Among the general causes; one case had hemophilia and another case was on anticoagulants (Warfarrin).

Only one case of neoplasm (squamous cell carcinoma of the nose and paranasal sinuses) was presented with epistaxis.

One case of chronic renal failure (Uraemia) was presented with epistaxis; the above results are shown in figures (4 and5).

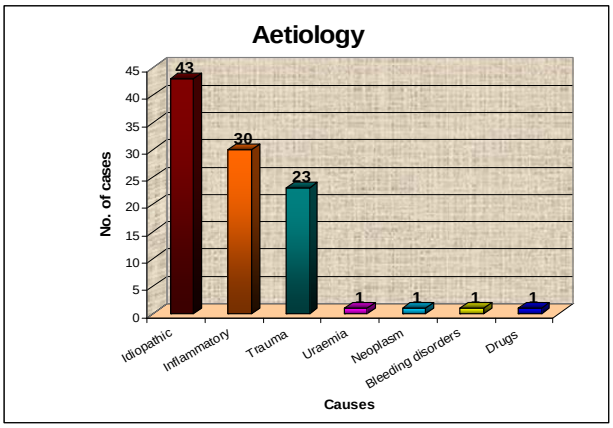

Figure 4: Showing the aetiological factors for epistaxis.

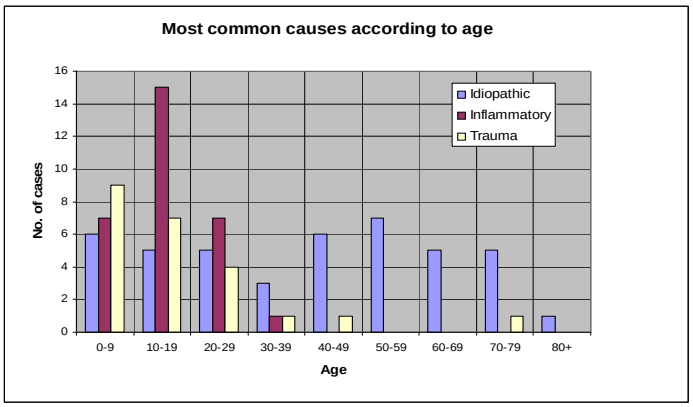

Figure 5: Showing the frequency of most common causes according to age.

The most common traumatic cause was nose picking in 12 cases out 23 cases (52%), followed by accidental trauma in 9 cases (39%), and while surgical trauma was reported in only 2 cases (9%), as shown in figure 6.

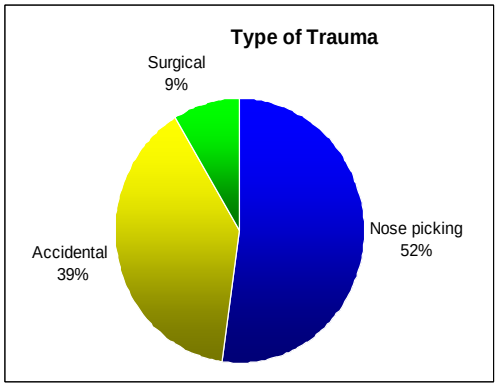

Figure 6: Showing the frequency of traumatic causes of epistaxis.

The bleeding was arising from the right nasal cavity in 48 cases (48%) and from the left in 41 cases (%41), while in 11 cases (11%) was bilateral, as shown in table 2.

Table 2: Showing the distribution of the side of bleeding.

Side of Bleeding	No. of cases	%
Right	48	48
Left	41	41
Bilateral	11	11
Total	100	100

Anterior septum was the commonest site of bleeding (75%). The site of bleeding was not detected in 13 cases (13%), as shown in figure 7.

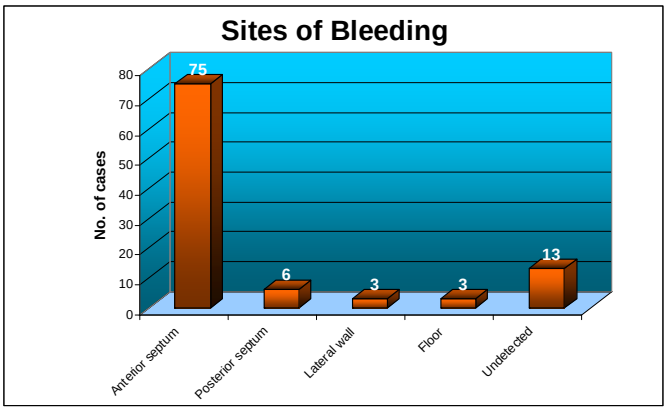

Figure 7: Showing sites of bleeding.

The Little's area was the commonest site of bleeding from anterior septum (67 out of 75 cases , 89.33%), in 4 cases (5.33%) the source of bleeding was from the retrocollumelar vein, and in the rest (4 cases , 5.33%) other sites were detected, as shown in figure 8.

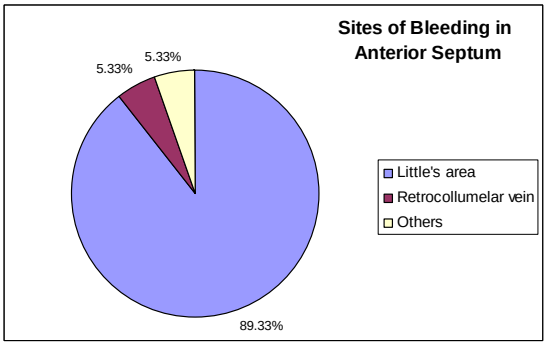

Figure 8: Showing the sites of bleeding in anterior septum.

Fifty five percent of cases had at least one previous attack, as shown in figure 9.

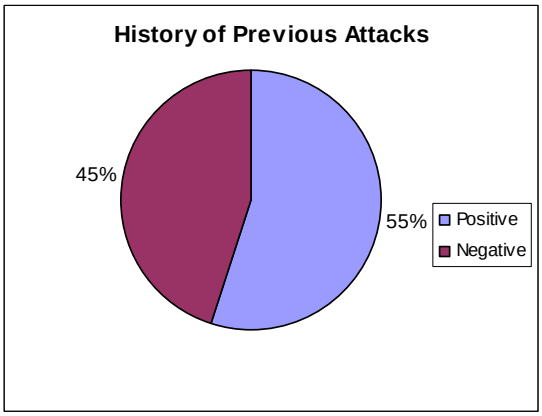

Figure 9: Showing history of previous attacks.

An associated systemic illnesses was present in 20 cases (20%), among them 12 cases had hypertension, as shown in table 3.

Table 3: Showing the frequency of associated systemic illnesses.

Associated systemic illnesses	No.	%
Hypertension	12	12
Diabetes mellitus	2	2
Hemophilia	1	1
Uraemia	1	1
Ischaemic heart disease	1	1
Ventricular septal defect	1	1
Asthma	1	1
Rheumatic fever	1	1
Total	20	20

The haematological investigations and radiological examinations were performed in indicated cases and the results are shown in table 4.

Table 4: showing positive findings in investigations

Investigation	No. of cases	%
Hb<10	5	5
Leukocytosis	7	7
Thrombocytopenia	3	3
X- ray showing fractured nasal bones	6	6
X- ray showing acute sinusitis	1	1
CT scan showing a mass of the nose and paranasal sinuses	1	1

Most of the cases were managed by cauterization of the bleeding point (61 cases, 61%), the second treatment in frequency was anterior packing (19 cases, 19%), while lubricants in form of gentamicin eye ointment were applied to the Little's area in 16 cases (16%).

Only Three cases needed posterior packing, and one case

underwent submucous resection of the septum to remove a spur which was the site recurrent refractory bleeding inaccessible to cautery, as shown in figure 10, and table 5.

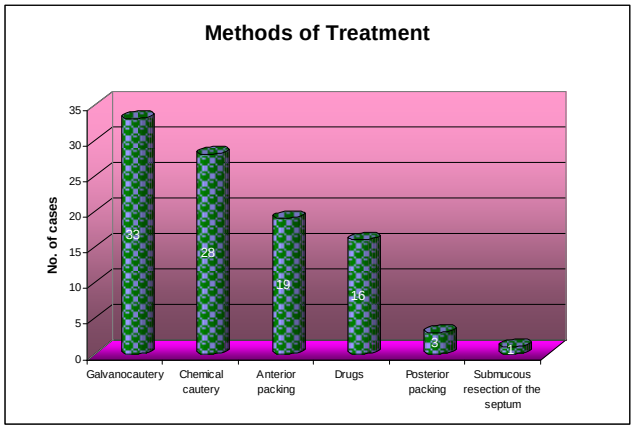

Figure 10: Showing frequency of different methods of treatment.

There is no statically significant difference between the two methods of cauterization regarding efficacy.

Table 5: Showing the outcome of different methods of treatment.

Outcome / Method	Success		Failure		Total		Chi-square	P Value
	No.	%	No.	%	No.	%		
Galvanocautery	27	81.82	6	18.18	33	33	1.5920	0.2070
Chemical Cautery	19	67.86	9	32.14	28	28		
Anterior Packing	16	84.21	3	15.79	19	19		
Drugs	10	62.5	6	37.5	16	16		
Posterior packing	3	100	-	-	3	3		
Submucous resection of the septum	1	100	-	-	1	1		

P value > 0.05,

Only ten cases (10%) were admitted, and four of them had received blood (one Pint for each)in addition to intravenous fluid.

Table 6: Showing number of admitted cases and method of resuscitation.

Admitted cases	Resuscitation			
	Blood Transfusion		Intravenous Fluid Infusion	
	No.	%	No.	%
10	4	4	10	10

The main complications of treatment was seen with the two method of cauterization, specially pain with galvanocautery in 5 cases (15.15% of total cases treated by galvanocautery), and staining of the

area reported in 4 cases out of 28 cases (14.28%) with chemical cautery, only on case of acute sinusitis was complicating anterior packing (5.25%), while one case of acute otitis media was reported with posterior packing (33.33%), treatment by drugs and surgery was not followed by any complication, as shown in table 7.

Table 7: Showing complications of treatment.

complication **Method**	**Pain**	**Temporary staining of vestibule and external nose**	**Acute sinusitis**	**Acute otitis Media**	**Total**	**%**	**chi-square**	**P value**
Galvanocautery	5	-	-	-	33	15.15	0.0580	0.8100
Chemical cautery	1	4	-	-	28	17.85		
Anterior packing	-	-	1	-	19	5.25		
Drugs	-	-	-	-	16	-		
Posterior packing	-	-	-	1	3	33.33		
Submucous resection of the septum	-	-	-	-	1	-		

P value > 0.05 so the difference between the two methods of cauterization is not significant statistically regarding complications.

Discussion

The occurence of epistaxis in our study was most common in the first two decades of life forming 50% of cases (*Figure 1*); this could be explained by more frequent upper respiratory tract infections and a more susceptibility to trauma in this age group. This rate was 43% in the study of Nafi and others in Mousil [13]. The male to female ratio was 1.7:1, which may be attributed to a more liability of trauma in males; another possible explanation is that the female pre-menopausal state may provide a significant protection from epistaxis, the mechanism for this is unknown, but may be secondary to a direct effect of oestrogen on the nasal mucosa or vasculature, this was stated by Tomkinson who showed a significant male predominance (1.6:1) in his study in Wales [14].

Eighty cases (80%) were urban inhabitants while rural's were only 20 cases (20%) (*Figure 2*), this difference may be due to tendency of applying self-treating measures in minor bleeding attacks in areas far from health facilities, or are managed in their local health centers, air pollution in urban areas also contributes to this difference in distribution.

Epistaxis showed higher proportion in winter months (34 cases, 34%), this can be explained by a high incidence of upper respiratory tract infections in winter, also cold dry air in winter months or dryness due to effect of heating all lead to dehydration of the nasal mucosa and subsequent erosion of superficial vessels. Schonweiler conclude from his study on relation of epistaxis to the weather that it is most often occurs between September and March [15]. Other studies in Greece [16] and in Boston [17] showed also an increase in occurrence in winter.

The bleeding was active in 59 cases (59%), and in the rest was inactive (*Table 1*). Those cases who were smokers were only 11 cases (11%), while alcoholics were 5 cases (5%), and the rest were non smokers-non alcoholics (84 cases, 84%) (*Figure 4*), and that is because the majority of our patients were children and adolescents (*Figure 1*).

In the present study the cause of epistaxis was similar to other study reported in Santiago by Vaamonde and others [18]. While in that of Basrah ([19], the traumatic causes was next in frequency to idiopathic group and the inflammatory causes was third. Nose picking was the commonest traumatic causes and was accounted for 52% of all cases with trauma (*Figure* 6) this difference is because of a higher rates of upper respiratory tract infection in our governorate and this inflammation makes nasal mucosa more liable to be injured even by minor trauma, some studies showed even a higher percentage (75% by Razdan) [20].

Localization of the site of bleeding is of utmost importance for subsequent management. In this study we found that in majority of cases the bleeding site was from the anterior part of the septum (75 cases-75%) (*Figure* 7) because this area is more near to the outside and rich in blood supply making it more vulnerable to have epistaxis, of which the Little's area was the commonest site (67 out of 75 cases with anterior epistaxis - 89.33%) (*Figure* 8) because of higher vascularization, the retrocollumelar vein was the source of bleeding in only 4 cases (5.33% of cases with anterior epistaxis) since it causes bleeding when it is dilated. Bleeding from posterior part of the septum was detected in 6 (6%) cases due to its location deep in the nasal cavity making it more protected(4 of them were hypertensive), the lateral wall bleeding occurred in 3 cases (3%) with trauma, and in 3 cases (3%) the bleeding was from the floor. Those cases with undetected site of bleeding were 13 cases (13%) (*Figure* 7). Similar results were reported by Nafi et al in their study in Mosil [13].

Fifty-five cases (55%) had had a similar previous attack indicating persistence of etiological and predisposing factors in these poepole. Most common systemic disease associated with epistaxis was hypertension (12%) (*Table 3*) because of atherosclerosis that makes the blood vessel fragile and looses contractility leading to severe epistaxis sometimes even by simple truama.Similar results was reported by Al-Robaee et.al in Basrah [19].

Regarding investigations, Haemoglobin level was below 10 gm/dl in only 5 cases (5%) and 4 of them had received blood due to recurrent or severe bleeding. Leukocytosis was detected in 7 cases (7%) due to infection and thrombocytopenia in 3 cases (3%) caused by systemic disease. X-ray showed nasal bone fractures in 6 cases (6%) due to severe trauma enough to cause fractured nasal bone, and acute sinusitis in only one case (1%), while CT scan was done in only one case (1%) whom was suspected to have a tumor by his age and clinical presentation and showed a mass in the nose and paranasal sinuses (*Table 4*).

The most used way for treatment was cautery which was applied in 61 cases (61%) with success rate of 75.41% because it was applicable, rapid and effective in controlling the bleeding in most of our patients, chemical cautery was performed for those with inactive bleeding with small vessels in the site of bleeding while galvanocautery for those with active one with larger bleeding vessels in the site of bleeding. The study showed no

statistically significant difference between the two methods of cautery regarding the results (*Table 5)* and complications (*Table 7*) because we have done the procedure according to indication and the need of each patient. The second treatment in frequency was the use of lubricants in form of gentamicin eye ointment by local application for one month which was used in 16 cases (16%) with success rate of 62.5%; it was mainly used in children with inflammatory causes for the bleeding. Anterior packing was used in 19 cases (19%) with success rate of 84.21% due to failure of previous method or being non-appllicable due to severity of bleeding or the bleeding site was not identified. In 3 cases (3%) all the above methods failed to control bleeding and we proceeded to do posterior packing which allowed application of a better anterior pack in addition to packing of more area, of which one of them was done under general anaehtesia . In one case (1%) the bleeding site was a septal spur in which lubricants and cautery failed to resolve the problem, so we were obliged to do submucous resection of the septum under general anaethesia to remove the spur and get axis to bleeding site and the resultant pibrosis also helped to stop bleeding(*Table 5, Figure 10*).

Only 10 cases (10%) were admitted and resuscitated by blood transfusion (4 cases), and intravenous fluid infusion (6 cases). Two cases of all were received general anaethesia (one for posterior packing and the other for submucous resection of the septum), and the rest were done under local anaethesia depending on the condition of each individual patient and his need for specific treatment.

The main complication of treatment was pain in 6 cases with cauterization, especially with galvanocautery (5 cases -15.15% of total cases treated by galvanocautery) due to more depth and area of cauterization by galvanocautery compared to chemical cautery, while in those who underwent chemical cautery, only one case developed pain and in 4 cases the external nose and vestibule were stained by the chemical agent due to its ability to spread which was temporary and disappeared after few days (complications of chemical cautery was 17.85% of total cases treated with that method). Only one case with anterior packing was complicated by acute sinusitis (5.25% of total treated with anterior packing) due to nasal obstruction and impaired ventilation of the sinuses, and an acute otitis media was reported in one case with posterior packing (33.33% of total posterior packing) (*Table 7*) due to of obstruction of a posteriorly located Eustachian tube. No complications were reported in this study with the other two methods of treatment (drugs and surgery) (*Table 7*).

In the study of Razdan and others ([21]) in India on 300 patient's cautery was done in 74% of cases with a success rate of 72.6%, while anterior nasal packing was used in 39% with a success rate of 83.5%, posterior packing was only performed in 7.6% of cases with a success rate of 95.6%. Two (1.1%) patients with anterior packing developed complications in the form of fever and a picture like toxic shock syndrome while 10 (8.5%) patients developed facial oedema. Acute otitis media in patients with anterior packing was noted in 2 (1.1%) patients. One (4.5%) patient with posterior packing showed acute otitis media.

Local non-operative measures control majority of cases with minimum complications, but active intervention should be done when indicated.

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References

1. Dano I, Dangor E, Sichel J, and Eliashar R. Experimental surgical treatment for recurrent epistaxis. *American Journal of Otolaryngology*.1998; l.19: 357–359.

2. Strachan D and England J. First-aid treatment of epistaxis – confirmation of widespread ignorance. *Postgraduate Medicine Journal*. 1998;l.74: 113-114.

3. Buce W. Jafek, and Bruce W. Murrow. ENT Secrets. London:Churchill Livingstone. 2001.

4. Michael S. Benninger , and Bradley F. Marple. Minor recurrent epistaxis; Prevalence and a new method for management. *Journal of Otolaryngology - Head and Neck Surgery*. 2004,131: 317-320.

5. Rolan N J, McRAE RD, and McCOMBE AW. Key Topics in Otolaryngology. London:ELBS. 2001.

6. Alan G. Kerr.Scott-Brown's Otolaryngology. Bath:Butterworth Heinemann.1997.

7. Roger F. Gray, Maurice Hauthorne.Synopsis of Otolaryngology.Cambridge:Butterworth Heinemann.1992.

8. Richard A. Buckingham..Ear, Nose, and Throat Diseases. London:ELBS with Churchill Livingstone.1994.

9. Maran A.G. Logan Turner's Diseases of the Nose, Throat and Ear. Bath:Butterworth Heinmann.1988.

10. John C. Goddard and Evan R. Reiter . Inpatient management of epistaxis; Outcomes and cost. *Journal of Otolaryngology - Head and Neck Surgery*. 2005; 132,:707-712.

11. ALDERSON D. Simple device for chemical cauterization of posterior bleeding points in the nose. *The Journal of Laryngology & Otology*. 2000; 114: 616-617.

12. Pope LE, and Hobbs CG.: Epistaxis; an update on current management. *Postgraduate Medicine Journal*. 2005; l.81: 309-14.

13. Nafi M. Shehab,and Ayad A. Shehab AL-Azawi: Epistaxis, aetiology and management. A study conducted in Mousil, 1995.

14. Tomkinson A, et al. Pattern of hospital attendance with epistaxis. *Rhinology Journal*. .1997**;** 35:129-131.

15. Schonweiler R: Causes of epistaxis in relation to season and weather status. *Journal of Otolaryngology*. 1989; 37**:**198-202.

16. Danielides V, Kontoglannis N, Bartzokas A, Lolis C J, and Skevas A. The influence of meteorological factors on the frequency of epistaxis. *Journal of Clinical Otolaryngology & Allied Sciences*. 2002**;** 27:84-88.

17. Pallen DJ, Chng YM, Mckay MP, Emond JA, Pelletier AJ, and Camargo CA Jr.: Epidemiology of epistaxis in US emergency departments, 1992 to 2001. *Journal of Annual Emergency Medicine*. 2005; 46:77-81.

18. Vaamonde Lago P, Lechuga Garcia MR, Minguez Beltran I, Frade Gonzalez C, Soto Varela A, Bartual Magro J, and Labella Caballero T. Epistaxis: prospective study on emergency care at the hospital level. *Acta Otorrinolaringol Esp*. 2000; 51: 697-702.

19. Hussam Haider S. Al-Robaee, and Ahmed A. Kareem AL-Ansari: Epistaxis, a prospective clinical study. A study conducted in Basrah, 1997.

20. Razdan U, Zada R, and Chaturvedi VN. Epistaxis : study of aetiology, site and side of bleeding. *Indian Journal of Medical Science*. 1999; 53:545-52.

21. Razdan U, Raizada R,and Chaturvedi V.N. Efficacy of conservative treatment modalities used in epistaxis. *Indian Journal of Otolaryngology and Head and Neck Surgery January* .2004**;** 56: 34-39.

Evaluation of the effect of mode of delivery on hematological parameters of healthy full-term newborns

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Abstract

Background: Hematological values at birth encompass broader ranges of normal than at any other time in life, and despite advances in perinatology over the past years, the exact influence of perinatal factors on hematological values in cord blood in normal pregnancy is still unclear. Moreover there was a wide variation and overlap in values between normal and abnormal infants with early symptoms and signs of neonatal sepsis.

Objectives:

1. To obtain the values of hematological parameters including complete blood picture, red cell indices, nucleated red blood cells and reticulocyte count in healthy full-term neonate and compare these values in neonates delivered by normal vaginal delivery and those delivered by cesarean section whether as elective or emergendy cesarean section.

2. To evaluate the effect of the gestational age, duration of rupture of membrane, duration of labor, Apgar score and birth weight on some hematological parameters.

Subjects, Materials & Methods: A total number of 300 healthy full term newborn were included in this prospective study. They were delivered in Al-Khadymia Teaching Hospital / labor room from October 2007 to January 2008. Those newborns were categorized into three groups, including those delivered by normal vaginal delivery (n = 200), by elective cesarean section (n = 80) and by emergency cesarean section (n = 20).

From each newborn 5 ml of venous cord blood was aspirated, and the estimation of hematological parameters was performed by Sysmex (automated hematology analyzer). Calibration of the analyzer was performed manually. Additionally the blood film was stained with Leishman's stain and differential count was done for each slide and reticulocyte count was done by standard method using brilliant cresyl blue stain.

Statistical analysis were done by students ***t*** test and correlation test taking ***P*** value < 0.05 as the lowest limit of significance.

Results: This study revealed that the total white blood cells and absolute neutrophil count were significantly lower in those delivered by elective cesarean section compared to those delivered by normal vaginal delivery (NVD) and emergency cesarean section (CS/L)

Moreover the reticulocyte count and nucleated red blood cells of neonates delivered by ECS and NVD were significantly lower than those delivered by CS/L, while the red distribution width (RDW) was significantly lower in those delivered by NVD than those delivered by ECS and CS/L.

Whereas the duration of rupture of membrane before delivery, duration of labor, gestational age, birth weight and Apgar score had no influence on cord blood hematological parameters and there was no statistical difference between the three groups.

Conclusion:

• This study revealed the total WBC count and absolute neutrophil count in those delivered by ECS were significantly lower than those delivered by NVD and CS/L.

• The mode of delivery had an influence on RDW in that neonates delivered by NVD had significantly lower RDW than those delivered by ECS and CS/L ($p < 0.05$).

• The mode of delivery had an influence on nucleated red blood cell (NRBC) and reticulocyte count in that neonates delivered by ECS and NVD had significantly lower NRBC and reticulocyte count than those delivered by CS/L ($p < 0.05$).

• The mode of delivery had no statistically significant effect on: lymphocyte, eosinophil, monocyte, RBC, Hb, PCV, MCV, MCH, MCHC, platelet, PDW and MPV.

• Duration of labor, duration of rupture of membranes before delivery, gestational age, Apgar scores and birth weight had no influence on cord blood hematological parameters.

Key words: Haematolgical parameters; mode of delivery; newborn.

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Introduction

The hematology of the fetus and newborn is a relatively recent area of study whose development depends upon the evolution of the science of hematology and, especially, upon methods to study the blood and its elements [(1)].

Despite advances in perinatology over the past years, the exact influence of perinatal factors on hematological values in cord blood is still unclear. Many studies describe changes in umbilical hematological parameters in cord blood in complicated pregnancy and in abnormal labor. However, inadequate data are available regarding the influence of perinatal factors on values in cord blood in normal pregnancies [(2)].

Cord blood screening is a useful tool for identification of anemia, sepsis, thrombocytopenia or any hematological diseases that could occur or manifest during the neonatal period. In most cases, the hematological values are frequently determined in the newborn for diagnostic purposes in suspected infection (sepsis), bleeding and hemolytic disorder.

Subjects and methods

Subjects:

This study was done on 300 healthy full-term newborns delivered in Al-Khadymia Teaching Hospital / labor room from October 2007 to January 2008 by draining a blood from umbilical cord.

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Criteria of inclusion of the subjects:-

(1) The newborn should be full-term baby whose gestational age ranges between 38-41 completed weeks, this was dated by last menstrual period of the mother and was confirmed by obstetrical examination and ultrasound done to the mother and the birth weight should be more than 2.5 kg.

(2) The neonates were excluded from study if the mother had any one of the following features: -

Infants born to woman with pre-eclampsia, diabetes mellitus, gestational hypertension, chorionamnionitis (maternal temperature > 38 ^{o}C, uterine tenderness, malodorous vaginal discharge, maternal tachycardia > 100 bpm and fetal tachycardia > 160 bpm), maternal chronic condition (disease of heart, kidney, blood or lung), smoking mothers and twins.

(3) The neonates were excluded if they had perinatal infection, asphyxia at birth (defined as an Apgar score at 1 minutes < 7), with abnormal fetal heart rate monitoring (bradycardia, tachycardia, non reassuring patterns, late or variable decelerations), with a Rhesus or ABO blood group incompatibility, being small for gestational age (below 10th percentile for sex and gestational age), and those delivered from labors complicated by muconium (i.e.) stained amniotic fluid.

The neonates were divided into three groups according to the route of delivery:

(1) First group includes 200 neonates [100 males (50%) and 100 females (50%)] delivered by normal vaginal delivery.

(2) Second group includes 80 neonates [30 males (37%) and 50 females (63%)] delivered by elective cesarean section (breech presentation, cephalo-

pelvic disproportion and repeated cesarean section).

(3) Third group includes 20 neonates [18 males (90%) and 2 femal (10%)] delivered by cesarean section during labour(emergency cesarean section) in which labour was arrested at the first or second stage .

Sample collection

After clamping of the babys' umbilical cord in the labor room, 2 – 3 ml of blood sample was aspirated from venous umbilical cord blood and transferred into an Ethylenediamine-tetra-acetic acid (EDTA) tube. Also a sample of 2 ml of maternal venous blood was collected in EDTA tube.

The following hematological tests were done on the samples from the baby's bilical cord blood:

(1) Complete blood count was done by Sysmex KX-21N (automated hematology analyzer) which was calibrated according to the operator's manual of the analyzer . The parameters obtained from the analyzer were WBC, RBC, Hb, PCV, MCV, MCH, MCHC, PLT, RDW, PDW, MCVand differential WBC.

(2) Blood film was done by using Leishman's stain [3] to assis the RBC, WBC and platelets morphology, to obtain differential WBC count and to calculate the number of NRBC in 200 WBC. [4] Reticulocyte count was done by using brilliant cresyl blue stain and according to standard manual method.

(3) Blood group and Rh was done for the baby and the mother by standard manual method to exclude Rh incompatibility.

Agar score was evaluated immediately after birth in the delivery room and was assist twice, once at 1 minute and again at 5 minutes after birth.

Statistical analysis

The statistical analysis was based on obtaining percentage, ranges, means ± standard deviation (SD) and standard error of mean (SEM), as well as the correlation coefficient and student ***t*** tests. The ***p*** value was determined. ***P*** values of less than 0.05 were considered significant.

Results

This study included 300 healthy full-term newborns with variable mode of delivery.They were collected from October /2007 to January /2008. The newborns were divided into three groups according to the route of delivery, 200 (66.6 %) newborns were delivered by normal vaginal delivery (NVD), 80 (26.6 %) newborns were delivered by elective cesarean section (ECS), and 20 (6.8 %) newborns were delivered by emergency cesarean section (CS/L).

The demographic data and hematological values of the neonates included in this study were summarized in table 1 and 2.

This study revealed that there was no statistically significant difference ($P > 0.05$) between the three group's regardig maternal age, newborn birth weight and Apgar score of the neonates. Only the gestational age of the neonates delivered by ECS was significantly lower compared to the other two groups ($P < 0.05$) as shown in table 3.

Moreover there were no statistically significant differences between the three groups regarding the RBC_S count, Hb, PCV, MCV, MCH and MCHC ($P > 0.05$). While the reticulocyte count and nucleated red blood cells of CS/L neonates were significantly higher than that of NVD and ECS neonates ($P < 0.05$). On the other hand the red cell distribution width of both ECS and CS/L neonates were significantly higher than

that of NVD neonates (P < 0.05). (Tables 4 and 5).

Also there were no statistically significant differences between the three groups regarding the lymphocyte, eosinophil and monocyte (P > 0.05). Whereas the NVD and CS/L neonates showed significantly higher total WBC and absolute neutrophil than their ECS counterpart (P < 0.05) (Table 6).

On the other hand there was no statistically significant difference between the three groups in regard to platelet count, MPV and PDW (P > 0.05) (Table 7).

When using correlation coefficient ,there was no significance influence of duration of labor, duration of rupture of membranes before delivery, gestational age, Apgar scores, and neonate birth weight on cord blood hematological parameters, ***P*** values range from (0.54 – 0.86).

Table 1: Demographic data of 300 neonates included in this study.

Parameter	**Mean ± SD**	**Range**
Maternal age (years)	26.14 ± 6.24	17 – 40
Gestational age (weeks)	39.18 ± 0.77	38 – 41
Birth weight (Kilograms)	3.454 ± 0.29	3.0 – 4.0
Apgar score		
1 minute	8.76 ± 0.45	8 – 9
5 minutes	9.36 ± 0.48	9 – 10
Multiple pregnancies		
Singleton	300 %	
Twin	0	
Gender		
Male	147 (49 %)	
Female	153 (51 %)	

Table 2: Hematologic values of 300 neonates included in this study.

Investigation	**Mean ± SD**	**Range**
Hemoglobin g/L	153.2 ± 8.08	136 – 169
Hematocrit L/L	0.455 ± 0.032	0.40– 0.59
RBC count × 10^{12}/L	4.35 ± 0.47	3.34– 5.24
Reticulocyte count %	3.66 ± 0.63	3 – 6.3
Nucleated RBC × 10^9/L	0.294 ± 0.07	0.1 – 0.48
Mean cell volume fl	104.5 ± 2.91	100 – 109
MCH pg	35.39 ± 1.67	33 – 39.7
MCHC g/L	337.4 ± 9.66	300 – 350
RDW fl	66.86 ± 4.14	60.7– 74.9
Total WBC count × 10^9/L	15.1 ± 3.17	9.2 – 20.7
Absolute neutrophil count × 10^9/L	9.3 ± 2.5	5.1 – 14.5
Absolute lymphocyte count × 10^9/L	4.67 ± 0.9	3.1 – 6.9
Absolute eosinophil count × 10^9/L	0.239 ± 0.185	0 – 0.9
Absolute monocyte count × 10^9/L	0.341± 0.212	0 – 0.9
Platelet count × 10^9 /L	221.8 ± 27.26	150 – 284
PDW fl	12.21 ± 1.34	9.3 – 15.9
MPV fl	10.11 ± 0.62	9 – 11.3

Table 3: Demographic data in relation to mode of delivery

Parameter	NVD(n=200)	ECS(n=80)	CS/L(n=20)	P - value
Maternal age (year)				NVD:ECS> 0.05˚
Mean ± SD	26.8 ± 7.27	25 ± 3.18	23.7 ± 3.03	NVD:CS/L 0.06˚
Range	17 – 40	19 – 30	20 – 32	ECS:CS/L 0.11˚
Gestational age (week)				NVD:ECS 0.00¨
Mean ± SD	39.4 ± 0.76	38.5 ± 0.37	39.2 ± 0.44	NVD:CS/L 0.28˚
Range	39 – 41	38 – 39	38.5 – 40	ECS:CS/L 0.00¨
Birth weight (kg)				NVD:ECS 0.17˚
Mean ± SD	3.43 ± 0.24	3.49 ± 0.34	3.45 ± 0.29	NVD:CS/L 0.76˚
Range	3 – 4	3 – 4	3 – 4	ECS:CS/L 0.68˚
Apgar score (1 minute)				NVD:ECS 0.66˚
Mean ± SD	8.74 ± 0.48	8.8 ± 0.40	8.8 ± 0.41	NVD:CS/L 0.59˚
Range	8 – 10	8 – 9	8 – 9	ECS:CS/L 0.43˚
Apgar score (5 minute)				NVD:ECS 0.61˚
Mean ± SD	9.37 ± 0.48	9.33 ± 0.47	9.35 ± 0.48	NVD:CS/L 0.86˚
Range	9 – 10	9 – 10	9 – 10	ECS:CS/L 0.91˚

¨ Significant ˚ Not Significant

Table4: Red blood cell parameters in relation to mode of delivery in 300 neonates.

Parameter	NVD(n=200)	ECS(n=80)	CS/L(n=20)	P - value
RBC count × 10^{12}/L				NVD:ECS 0.17˚
Mean ± SD	4.37 ± 0.4	4.28 ± 0.5	4.44 ± 0.2	NVD:CS/L 0.44˚
Range	3.36 – 5.1	3.34 – 5.2	4.1 – 4.9	ECS:CS/L 0.23˚
Hemoglobin g/L				NVD:ECS 0.52˚
Mean ± SD	154.2 ± 7.8	152.3 ± 9.3	155.7 ± 5.0	NVD:CS/L 0.15˚
Range	136 – 169	139 – 168	146 – 167	ECS:CS/L 0.15˚
Packed cell volume L/L				NVD:ECS 0.11˚
Mean ± SD	0.45 ± 0.03	0.44 ± 0.03	0.46 ± 0.02	NVD:CS/L 0.17˚
Range	0.40 – 0.59	0.40 – 0.56	0.42 – 0.49	ECS:CS/L 0.05˚
Reticulocyte count %				NVD:ECS 0.59˚
Mean ± SD	3.5 ± 0.59	3.6 ± 0.48	4.8 ± 0.75	NVD:CS/L 0.00¨
Range	3.0 – 4.9	3.0 – 5.0	3.9 – 6.3	ECS:CS/L 0.00¨
Nucleated RBC × 10^{9}/L				NVD:ECS 0.52˚
Mean ± SD	0.28 ± 0.07	0.29 ± 0.07	0.33 ± 0.06	NVD:CS/L 0.01¨
Range	0.10 – 0.4	0.11 –0.39	0.23 –0.48	ECS:CS/L 0.02¨

¨ Significant ˚ Not Significant

Table 5: Red cell indices in relation to mode of delivery in 300 neonates.

Parameter	NVD(n=200)	ECS(n=80)	CS/L(n=20)	P - value
Mean cell volume fl				NVD:ECS 0.63˚
Mean ± SD	104.4 ± 2.9	104.6 ± 2.9	104.4 ± 2.8	NVD:CS/L 0.95˚
Range	100 – 109	101 – 108	100 – 109	ECS:CS/L 0.75˚
Mean cell hemoglobin pg				NVD:ECS 0.09˚
Mean ± SD	35.2 ± 1.67	35.6 ± 1.45	35.6 ± 1.89	NVD:CS/L 0.39˚
Range	33 – 39.4	33 – 39.7	33.9 – 39.5	ECS:CS/L 0.95˚
Mean cell Hb concentration g/l				NVD:ECS 0.91˚
Mean ± SD	337 ± 8.17	337 ± 13.1	337 ± 20.3	NVD:CS/L 0.82˚
Range	300 – 350	300 – 350	320 – 350	ECS:CS/L 0.85 ˚
Red cell distribution width fl				NVD:ECS 0.00¨
Mean ± SD	64.4 ± 2.62	71.8 ± 1.88	71.0 ± 1.0	NVD:CS/L 0.00¨
Range	60.7 – 73.4	68.9 – 74.9	69.7 – 72.6	ECS:CS/L 0.08˚

¨ Significant ˚ Not significant

Table 6: White blood cell and related parameter in relation to mode of delivery in 300 neonates.

Parameter	NVD(n=200)	ECS(n=80)	CS/L(n=20)	P - value
Total WBC × 10^9/L				NVD:ECS 0.00¨
Mean ± SD	15.9 ± 3.4	13.2 ± 1.1	14.3 ± 1.7	NVD:CS/L 0.05˚
Range	9.2 – 20.7	10.6 –16.4	12.4 –17.2	ECS:CS/L 0.00¨
Neutrophil count × 10^9/L				NVD:ECS 0.00¨
Mean ± SD	9.66 ± 2.9	8.37 ± 1.2	9.33 ± 1.9	NVD:CS/L 0.61˚
Range	5.1 – 14.5	5.9 – 10.9	6.3 – 11.8	ECS:CS/L 0.00¨
Lymphocyte count × 10^9/L				NVD:ECS 0.45˚
Mean ± SD	4.63 ± 0.9	4.72 ± 0.8	4.71 ± 0.8	NVD:CS/L 0.17˚
Range	3.1 – 6.9	4 – 6.7	4 – 6.6	ECS:CS/L 0.23˚
Eosinophil count × 10^9/L				NVD:ECS 0.84˚
Mean ± SD	0.24 ± 0.2	0.24 ± 0.1	0.19 ± 0.1	NVD:CS/L 0.17˚
Range	0.0 – 0.9	0.1 – 0.5	0.1 – 0.3	ECS:CS/L 0.08˚
Monocyte count × 10^9/L				NVD:ECS 0.86˚
Mean ± SD	0.33 ± 0.2	0.34 ± 0.1	0.34 ± 0.1	NVD:CS/L 0.84˚
Range	0.0 – 0.9	0.1 – 0.6	0.1 – 0.5	ECS:CS/L 0.84˚

¨ Significant ˚ Not significant

Table 7: Platelet parameters in relation to mode of delivery in 300 neonates.

Parameter	NVD(n=200)	ECS(n=80)	CS/L(n=20)	P - value
Platelet count × 10^9/L				NVD:ECS 0.059˚
Mean ± SD	224.0 ± 29.7	217.3±13.1	218.2± 10.3	NVD:CS/L 0.38˚
Range	284 – 150	161 – 252	201 – 234	ECS:CS/L 0.82˚
Platelet distribution width fl				NVD:ECS 0.07˚
Mean ± SD	12.0 ± 1.34	12.3 ± 1.09	12.7 ± 2.0	NVD:CS/L 0.06˚
Range	9.3 – 14.6	10.7 – 14.4	10.6 – 15.9	ECS:CS/L 0.32˚
Mean platelet volume fl				NVD:ECS 0.38˚
Mean ± SD	10.09 ± 0.59	10.16 ± 0.6	10.11 ± 0.9	NVD:CS/L 0.91˚
Range	9.0 – 11.0	9.0 – 11.3	9.1 – 11.3	ECS:CS/L 0.75 ˚

¨ Significant ˚ Not significant

Discussion

Hematological values of all the neonates included in this study:

Hemoglobin value and Hematocrit value:

This study had revealed that mean Hb level and PCV of all the newborn (n = 300) as shown in table 2, were inagreement with the results of Al-Mossawy study, (n=500) which was done in Baghdad,in 2004 , despite the Hb and PCV estimation were done by cyanmethemoglobin method and microcapillary device respectively and not by automated hematology analyzer used in this study. The mean Hb level and PCV count in this study were similar to an African study done in 1985 by Scott –Emuakpor AB, et al [(6)](n= 402) . However the mean Hb level and PCV in this study were lower than that reported by Walka MM, et al (1998) in Germany, and to African study done by Broadhead R, et al (1995) [(7)] .

This discrepancy in the results may due to the environmental and physiological conditions under which the specimens were obtained, including mode of delivery, the treatment of umbilical vessels (early or late clamping), and the state of physical activity of the baby[(8,9)],also on the ethnic and racial background effect [(8)] .

Reticulocyte count & Nucleated RBC count:

The reticulocyte count and NRBC of all the neonates included in this study (Table 2) were comparable with that of Redźko S, et al (2005) done in Poland [(2)].But they were lower than those reported by Al- Zoubaidi study,(n= 120)which was done in 1998 on neonates' capillary blood, in Baghdad [(10)] and by Walka MM, et al study (n=123) which was done in Germany on umbilical cord blood,in 2004.[(11)] These differences might be attributed to different in mean gestational age since there was a significant inverse relationship between numbers of reticulocytes and NRBC and gestational age,[(12)]or might be due to the number of samples.

Red blood cell count & RBC indices:

As shown in table 2 the mean RBC count and RBC indices (MCH, MCV & MCHC) in all the newborns (n=300) were in agreement with the results reported by an African study done by Scott –Emuakpor AB, et al (1985), which was done on 402 neonates [(6)] and a study done by Walka MM, et al in 2004, which was done in Germany on umbilical cord blood of 123 neonates [(11)].

Total WBC & differential count:

This study showed that the mean total and WBC count and the absolute neutrophil count of all the neonates included in this study were in agreement with Al- Mossawy study (n=500), which was done in Baghdad,in 2004 [(62)]and similar to Walka MM, et al study[(11)] which was done in Germany , in 2004.On the other hand it was lower than Al- Zoubaidi study (n = 120), which was done in Baghdad[(10)],in 1998

These differences might be attributed to the difference in the time of collecting blood sample, since there is a marked and rapid increase in the neutrophil count during the first 24 hours after birth [(13)]. Also might be affected by the site of blood sampling, since the samples obtained in Al- Zoubaidi study were capillary blood samples while the samples in this study were cord blood samples and it had been postulated that the total WBC count, absolute neutrophil and lymphocyte count were higher in capillary blood than those obtained from cord blood [(10)].

Platelet count, Mean platelet volume & Platelet distribution width:

As shown in table 2 the mean platelet count, MPV and PDW in all the

neonates (n=300) were comparable with that obtained by Al-Mossawy(2004) (n=500) [14] ,but it was higher than that obtained by Al-Zoubaidi (1998)(n = 120), both studies were done in Baghdad .This may due to the technique used ,since in Al-Zoubaidi study the samples were collected from the heel in a capillary tube and this might cause adhesion of platelet to the site of skin puncture also the platelets were counted manually[10],unlike this study where the samples were collected from cord blood and the platelets were counted by automated haematological analyzer. On other hand the results in this study were lower than that obtained by an African study done in 1995 by Broadhead R, et al (n = 366) [7] and a German study done in 2004 by Walka MM, et al (n = 123), [11] and since all the neonates were healthy and full-term, so low platelet counts in those neonates could be due to racial difference.

***Hemtological values in relation to mode of delivery*:**

Hemoglobin & hematocrit values:

This study revealed that there were no statistically significant difference in the hemoglobin and PCV values between normal vaginal delivery (NVD), elective cesarean section (ECS) and cesarean section after labor (CS/L) neonates (Table 2). These results were similar to that observed by Al-Zoubaidi (1998) in Baghdad [10], Lubetzky R, et al (2000) in Israel [15], Nikischin W, et al (1997) in Germany [16] and Redźko S, et al (2005) in Poland[2] .

Reticulocyte count & Nucleated RBC count (NRBC):

The reticulocyte count and NRBC in ECS and NVD were significantely lower than those in CS/l, however there was no significance difference betweenNVD and ECS. This is because featus born by CS/L are more vulnerable to hypoxia than those delivered by NVD or by ECS and hypoxia is a known causes for increase in NRBC count both in fetal and infant blood through increasing the concentration of erythropoietin, which induces erythropoiesis[18].

Red blood cell count & RBC indices:

In this study there were no statistically significant difference in RBC count, MCV, MCH and MCHC between NVD, ECS and CS/L neonates (Tables 2 and 3), these result were similar to that of Redźko S, et al (2005) in Poland [2]and Nikischin W et al (1997) in Germany[16].

On the other hand RDW in both ECS and CS/L neonates was higher than that found in NVD neonates. This was similar to Redźko S, et al (2005)[2]and it may be explain by increased in the total body fluid in fetuses delivered by CS which may indirectly affect the RDW in cord blood[2].

Total WBC & differential count:

In the current study the total WBC and absolute neutrophil count of neonates who delivered by NVD and CS/L were significantl higher than those delivered by ECS (Table 4). These results were in agreement with that of Al- Zoubaidi (1998) in Baghdad [10], Nikischin W, et al (1997) in Germany [16], Redźko S, et al (2005) in Poland [2] and Chirico G, et al (1999) in Italy [19].

This is because during labor there is a combination of severe stress and physical stimulus, to the mother and periodic physical stress resulting from intermittent episodes of hypoxia during labor, to the featus. This stress causes an increase in circulating catecholamine and cortisol both in mother and infant and since there is a significant correlation between cortisol and leukocytes which is responsible for the

increased WBC and absolute neutrophil count [19, 20]. On the other hand there was no statistically significant difference in absolute lymphocyte, monocyte and eosinophil counts in NVD, ECS and CS/L neonates; similar observation was found by Redźko S, et al (2005) in Poland[2] and Al-Zoubaidi (1998) in Baghdad[10].

Platelet count, Mean platelet volume & Platelet distribution width:

In this study there was no statistically significant difference in platelet count, MPV and PDW between NVD, ECS and CS/L neonates (Table 5), these result were similar to observation of Al-Zoubaidi(1998).[10]in Baghdad, Redźko S, et al(2005) in Poland[2]and Nikischin W, et al (1997) in Germany.[18]

However platelet count in the NVD group was higher than that found in ECS and CS/L but this increase was not statistically significant. This high platelet in NVD may be explained by higher thrombopoietin and cortisol levels observed in vaginally delivered neonates [2].

Hemtological values in relation to demographic data:

In this study there was no influence of duration of labor, duration of rupture of membranes before delivery, gestational age, Apgar score, or neonate birth weight on cord blood hematological parameters, these results were similar to the results of Redźko S, et al (2005) in Poland [2] .

From this study we may conclude that the total WBC count and absolute neutrophil count in those delivered by ECS were significantly lower than those delivered by NVD and CS/L, and the RDW in neonates delivered by NVD was significantly lower than those delivered by ECS and CS/L. Also the NRBC and reticulocytes count in neonates delivered by ECS and NVD had significantly lower NRBC and reticulocytes than those delivered by CS/L. Furthermore the mode of delivery had no statistically significant effect on lymphocyte,eosinophil, monocyte, RBC, Hb, PCV, MCV, MCH, MCHC, platelet, PDW and MPV. Also the Duration of labor, duration of rupture of membranes before delivery, gestational age, Apgar score and birth weight had no influence on cord blood hematological parameters.

Recommendations

1. The hematological reference values for Iraqi newborns need to be confirmed by larger number of blood sampling, collecting the samples in different areas of Iraq and at different ages of neonatal life.

2. Future study to determine the hematological parameters values from the umbilical cord blood in many inherited hematological diseases which presented during the neonatal period.

References

1. Howard A. Pearson, MD. Neonatal hematology: a historical overview, 2005. J:\ Neonatal Hematology - Cambridge University Press. htm.

2. Rodźko S, Przepieść J, Urban J, et al: Influence of perinatal factors on hematological variables in umbilical cord blood. J Perinat Med 2005; 33: 42 – 45.

3. Bain JB, Lewis SM. Preparation and staining methods for blood and bone marrow films. In: Lewis SM, Bain BJ, Bates I (eds). Dacie and Lewis Practical Hematology, 10th edit. Philadelphia, Churchill Livingstone, 2006: pp 61 – 63.

4. Lewis SM, Bain BJ: Basic hematological techniques. In: Lewis SM, Bain BJ, Bates I (eds). Dacie and Lewis Practical Hematology, 10th edit. Philadelphia, Churchill Livingstone, 2006: p 26 – 52.

5. Sue Knowles, Fiona Regan. Blood cell antigens and antibodies. Erythrocytes, platelets and granulocytes. In: Lewis SM, Bain BJ, Bates I (eds). Dacie and Lewis Practical Hematology, 10th edit. Philadelphia, Churchill Livingstone, 2006: p 499.

6. Scott-Emuakpor AB, Okolo AA, Omene AJ, et al: Normal hematological values of the African neonate. Annals of hematology 1985; 51: 11 – 18.
7. Broadhead R, Chingani GW, Dzinyemba WE, et al: Some hematological parameter in Malawian neonates. East Afr Med J 1995; 72(1): 10 – 14.
8. Brugnara C, Platt OS: The erythrocyte and its disorder. In: Nathan DG and Orkin SH (eds). Hematology of infancy and childhood, 5th edit. Philadelphia, W.B. Saunders Company, 1998: pp 21 – 27.
9. Lewis SM. Reference ranges and normal values. In: Lewis SM, Bain BJ, Bates I (eds). Dacie and Lewis Practical Hematology, 10th edit. Philadelphia, Churchill Livingstone, 2006: p 14.
10. Al-Zoubaidi WM: Hematological parameters in healthy neonates delivered by vaginal route and cesarean section. Athesis submitted to the Scintific Council of Pathology, 1998.
11. Walka MM, Sonntag J, Kage A, et al: Complete blood counts from umbilical cords of healthy term newborns by two automated cytometers. Acta Hematolo 1998; 100: 167 – 173.
12. Hord JD, Lukens JN: Anemias unique to infants and young children. In: Lee GR, Forester J, Lulens J, et al (eds). Wintrobe's Clinical Hematology, 10th edit. Baltimore, Williams and Willkins, 1998: pp 1518 – 1537.
13. Xanthou M. Leukocyte blood picture in healthy full – term and premature babies during neonatal period. Arch Dis Child 1970; 45: 242 – 249.
14. Al-Mossawy LS: Complete blood counts and hemoglobin patterns from umbilical cord of healthy full-term newborns. A thesis submitted to Scintific Council of Pathology, 2004.
15. Lubetzky R, Ben-Shachar S, Francis B, et al: Mode of delivery and neonatal hematocrit. Amer J Perinatol 2000; 17: 163 – 166.
16. ematological values in the umbilical vein. Gynecol Obstet Invest 1997; 43(2): 104 – 7.
17. Thomson MA, Edwards AD: Neonatal care for obstetricians. In: D. Keith Edmonds (eds). Dewhurt's Textbook of Obstetrics and Gynaecology, 7th edit. Blackwell publishing, 2007: p83.
18. Szwajcowsky M, Kalinka J, Krajewski P. Nucleated red blood cells as an auxiliary marker of intrauterine infection. J Ped Neonatology 2005; 2(1): NT 15 – 18.
19. Chirico G, Gasparoni A, Ciadelli, et al: Leukocyte counts in relation to the method of delivery during the first five days of life. Fetal and neonatal research 1999; 75: 298 – 299.
20. Mears K, McAuliffe F, Grimes H, et al: Fetal cortisol in relation to labour, intrapartum events and mode of delivery. J Obstet G ynecol 2004; 24(2): 129 – 32

Detection of BCR-ABL protein in chronic myeloid leukemia patients using Immunocytochemistry

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Abstract

Background: Chronic Myeloid leukemia (CML) is a myeloproliferetive disorder associated with chromosomal abnormality, Philadelphia chromosome (Ph), in more than 95% of CML patients. The resulting BCR-ABL fused gene is markers for this type of leukemia. In CML, the product of the fused BCR-ABL gene is typically an oncoprotein termed $P210^{BCR-ABL,}$ a constitutively active tyrosine kinase, activates numerous signal transduction pathways, leading to uncontrolled cell proliferation and reduces apoptosis.

Objective: Primary diagnosis of CML patients by screening the presence of BCR-ABL protein in patients' venous blood lymphocytes using immunocytochemistry technique (ICC).

Method: A total of 42 CML patients, 10 Acute Lymphoid Leukemia(ALL) patients, 2 Acute Myeloid Leukemia (AML) patients, 1 Chronic MyeloMonocytic Leukemia (CMML) patient and 8 healthy individuals were screened. Lymphocyte was separated from heparinized venous blood sample from each subject, smeared and fixed on positive charged slides. Monoclonal antibody specific for BCR-ABL protein was used as primary antibody.

Results: The results showed that all the 42 cases of CML were positive for BCR-ABL protein and all the other cases were negative.

Conclusion: The results indicate that the Immunocytochemistry assay has clinical application as a primary qualitative diagnosis tool of *BCR-ABL* protein.

Key words: BCR/ABL protein - CML-Immunocytochemistry.

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Introduction

Chronic Myeloid leukemia (CML) is characterized most frequently by its association with an abnormal chromosome 22, known as the Philadelphia chromosome (Ph) [1, 2]. It is estimated that at least 95% of CML cases possess the Ph [3]. This abnormal chromosome fuses a central portion of the BCR gene to the second exon of the ABL gene (*BCR-ABL*) [4, 5]. The fusion of BCR and ABL on the Ph chromosome occurs in a head-to-tail manner, with the 3` end of ABL joined to the 5` end of BCR [6].

Regardless, the fusion transcript almost always includes exon 2 of ABL (a2). In contrast, in CML, the break on chromosome 22 is restricted in most patients to an area of 5.8-kb termed the major-bcr (M-bcr). Most breaks occur immediately downstream of exon 2 or 3 of the M-bcr region [6] and result in b2a2 or b3a2 fusion transcripts encoded for protein of 210KiloDalton (KD) ($p210^{BCR-ABL}$) [7]. In acute leukemia, however, the breakage can also occur outside M-bcr in about two third of the cases and usually within the 3' end of intron 1 of the BCR gene termed the minor bcr (m-bcr) , resulting in an e1a2 fusion transcript encode for a protein of 190KD ($p190^{BCR-ABL}$).Also, one third of acute leukemia cases show p210 positive[8]. Other unique breakpoint sites have been found. These include a micro

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3' site termed µ-bcr (BCR exon 19 to BCR exon 20), which encode for a protein of 230KD (p230 $^{BCR-ABL}$). Patients with the e19a2 fusion between BCR exon 19(e19) and ABL exon 2(a2) were classified as having neutrophilic CML [9].

Tyrosine kinase activity is essential to cellular signaling and growth. Constitutively elevated kinase activity has been associated with oncogenic changes in several systems. Both the $p210^{BCR-ABL}$ and the $p190^{BCR-ABL}$ proteins have constitutively activated tyrosine kinase enzymatic activity, with higher levels of activity in the p190 [10].

Interestingly, most of the autophosphorylated tyrosines occur within the BCR segment of BCR-ABL [11]. The tyrosine kinase activity is attributable to the kinase domain found within the ABL segment of the fusion proteins [12]. Indeed, it appears that the degree of transforming activity of BCR-ABL correlates with the degree of tyrosine kinase activity and this activity has been implicated in the growth factor independence that BCR-ABL confers on cells [13].The initial effect of this kinase in primitive hematopoietic stem cells was investigated. It was improve that BCR-ABL protein can regulate protein levels (such as tumor suppressor proteins) by governing secretion through down-regulation of specific tumor suppressor genes [14].

Patients, Materials and methods

Patients:

A total of Sixty three venous blood (VB) samples were included in the study, they were 42 CML, 10 ALL, 2 AML and 1 CMML in addition to 8 samples from healthy individuals were screened as negative control .These samples were screened for the expression of BCR-ABL protein using ICC technique.

Materials and method Methods:

Lymphocytes isolation

Lymphocytes were isolated according to Boyum [15].

Immunocytochemistry procedure

Immunoperoxidase Secondary Detection system (Dako Cytomation, USA, K0673) were used for staining according to the manufacturer instruction.

ICC procedure was done according to Huang *et al* [16].Mouse monoclonal antibody (Mouse anti-Human c-Ab1, BCR-ABL, USBiological, USA) was used as a primary antibody according to the manufacturer instruction. A total of 100cells were counted to determine the percentage of reactivity of BCR-ABL monoclonal antibody. In this study, cells considered as positive when the nucleus was being stained with dark brown color. The percentage of positive cell calculated as following:

$$Percentage\ of\ positive\ cells = \frac{No.\ of\ positive\ cells}{total\ no.\ of\ counted\ cells} \times 100\%$$

The results were scored for the percentage of positive nuclei according to Alessandra *et al.* [17] and as following:

1-Negative: less than 5% positive nuclei.
2-Weak positive: 5%-25% positive nuclei.
3-Moderate positive: 26%-50% positive nuclei.
4-High positive: >50% positive nuclei

Results

The results of immunostained of smears were evaluated on the bases of positive nuclear staining. All CML patients included in this assay were BCR-ABL positive and were consistent with the hematological diagnosis.

CML patients were classified for two groups according to scoring of positive BCR-ABL cells, as in table (1).

Staining pattern of BCR-ABL positive cells was studied using ICC revealed a typical nuclear localization of this protein, figure (1).

No one of ALL, AML, CMML or healthy individuals showed positivity for BCR-ABL cells. That result was consistent with their hematological diagnosis. Also, non- specific binding of monoclonal antibody used in this assay to cell components or the presence of background signal due to endogenous cell biotin were proved through the replacing of the addition of primary antibody to one smear in each slid by phosphate buffer slain. The reproducibility of ICC assay was evaluated by repeating this assay for CML samples that collected at different time points from starting imatinib treatment. The same results were seen.

Table 1: Correlation between score of BCR-ABL positive lymphocytes of CML patients and disease phase and hematological response.

Scoring group	Patients No. (%)	CML -phase		HR	
		CP No. (%)	AP No. (%)	CHR No. (%)	PHR No. (%)
Moderate BCR-ABL positive	12(28.57)	12(100)	---------	12(100)	----------
High BCR-ABL positive	30(71.42)	23(76.66)	7(23.33)	10(33.33)	20(66.66)

-CP=chronic phase, AP=accelerated phase, HR=Hematological response, CHR=complete hematological response, PHR=partial hematological response.

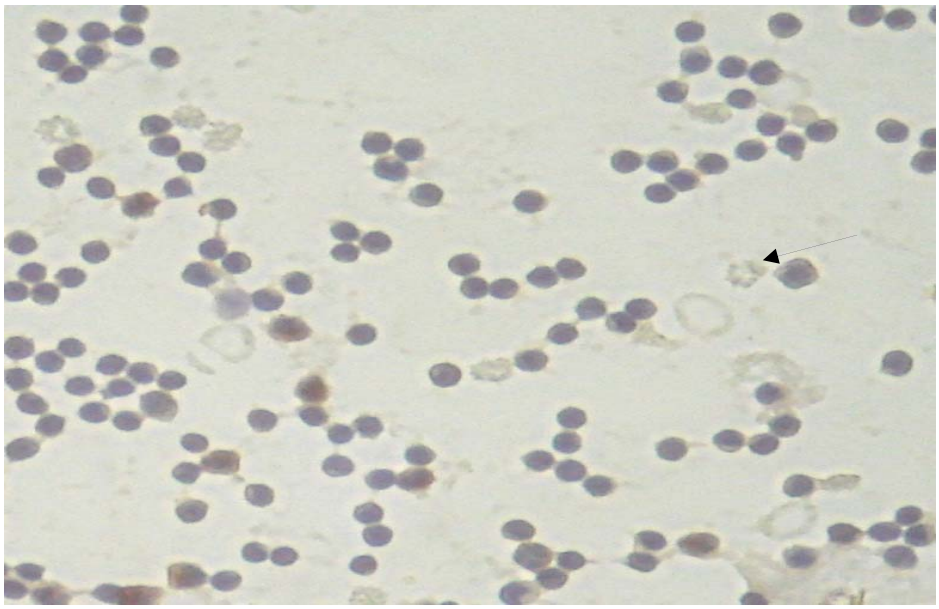

Figure1: Immunocytochemistry staining of BCR-ABL oncoprotein in CML patient's VB lymphocytes using BCR-ABL monoclonal antibody. Antibody binding was visualized by incubating with diaminobenzidine solution (DAB), counterstained with Mayer hematoxylin and evaluated with light microscope. Arrow showed positive BCR-ABL cell. The nuclear stained with dark brown (DAB). High power magnifications of 400X.

Discussion

The genetic alterations in human leukemia and lymphoma results from a creation of hybrid genes which encode chimeric proteins. Those proteins were studied by ICC using antibodies specific for the protein products of gene involved in such alteration [18, 19].

The ICC demonstration of BCR-ABL protein offers several potential advantages over biochemistry techniques such as western blotting or immunoprecipition in which, these techniques are particularly applicable to the study of hybrid genes, since they allow chimeric proteins such as BCR-ABL to be distinguished on the basis of their unique molecular size. However, compared with ICC, biochemical methods are more demanding technically and provide only limited information on subcellular localization of the protein.

Moreover, ICC is rapid and does not require the substantial investment in laboratory equipment [20, 21].

The expression of BCR-ABL was indicated in all CML patients included in this study. That is consistent with what was reported by Volpe *et al.* [22].

Comparing the qualitative results (whether moderate or high) of the immune staining technique showed a full agreement with the results of hematological response. As seen from table (1), all patients (100 %) with moderate BCR-ABL positive cells percentage achieved complete hematological response (CHR), while 33.33% of patients with high BCR-ABL positive cells percentage achieved CHR.

The lack of false positive in patients with other disease or healthy individuals indicate that the BCR-ABL ICC assay has its value as a relative and easy mean for assessing whether or not patients with leucocytosis and /or myeloproliferative syndrome are BCR-ABL positive. The limitations of ICC technique are: (1) the results are semi-uantitative because the amount of visible reaction product is related in a complex manner to the amount of specific antibody binding to the receptor and it is not necessarily a simple linear relationship. (2)Using this assay, the detection of both forms of BCR-ABL protein (p210 or p190) were not possible.

References

1. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science. 1960; 132:1497-501.

2. Rowley J D. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature.1973; 243:290-293.

3. Dreasen O, Rassool F, Sparkes RS. Do oncogenes determine clinical features in chronic myeloid leukemia?. Lancet. 1987; 1:1402-1405.

4. Lifshitz B, Fainstein E, Marcelle C, Shtivelman E, Amson R, Gale RP. *BCR* genes and transcripts.Oncogene.1987; 2:113-117.

5. Shtivelman E, Lifshitz B, Gale R P, Canaani E. Fused transcript of ABL and *BCR* genes in chronic myelogenous leukemia. Nature.1985; 315:550-554.

6. Heisterkamp N, Stam K, Groffen J, de Klein A., Grosveld G. Structural organization of the *BCR* gene and its role in the Ph translocation. Nature. 1985; 315:758-761.

7. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram C R, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, *bcr*, on chromosome 22. Cell. 1984; 36; 93-99.

8. Thomas DA. Philadelphia chromosome positive Acute Lymphoblastic leukemia, anew era of challenges .Hematol.2007; 1:435-443.

9. Pane F, Frigeri F, Sindona M, Luciano L, Ferrara F, Cimino R. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (*BCR-ABL* with c3a2 junction). Blood. 1996; 88:2410-2414.

10. Micho F, Hughes TP, Iwasa Y. Dynamics of chronic myeloid leukaemia. Nature.2005; 435:1267-1270.

11. Liu J, Campbell M, Guo JQ, Lu D, Xian YM, Andersson BS. *BCR-ABL* tyrosine kinase is

autophosphorylated or transphosphorylates p^{160} BCR on tyrosine predominantly within the first BCR exon. Oncogene.1992; 8:101-109.

12. Jing Y, Wang M, Tang W, Qi T, Gu C, Hao S. c-ABL tyrosine kinase activities p21 transcription via interaction withp53. J Biochemis. 2007; 141:621-626.

13. Hughes TP, Deininger M, Hochhaus A, Branford S, Radich J. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors-Review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006; 108:28-37.

14. McCallum L, Price S,Planque N, *et al* .A novel mechanism for BCR-ABL action : stimulated secretion of CCN3 is involved in growth and differentiation regulation . Blood .2006; 108:1716-1723.

15. Boyum A. Lymphocyte separation. Scan J Clin Lab Invest. 1964; 21:97.

16. Huang M.; Dorsey J.F.; Epling-Burnette P.K.; *et al.* Inhibition of BCR-ABL kinase activity by PD180970 blocks constitutive activation of Stat 5 and growth of CML cells.Oncogene,2002;21:8804-8816.

17. Alessandra DB, Karen K, Sharon LM, Thomas GC. Molecular abnormalities in Chronic Myeloid Leukemia: deregulation of cell growth apoptosis. The Oncology.2000;5:405-415

18. Mayerhofer M, Florain S,Krauth M-T, *et al*. Identification of Heme Oxygenase-1 As a Novel BCR/ABL-Dependent Survial Factor in Chronic Myeloid Leukemia . Cancer Research. 2004; 64, 3148-3154.

19. Baccarani M, Saglio G, Goldman JM. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European Leukemia Net. Blood. 2006; 108:1809-1820.

20. Falini B, Mason DY. Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. Blood. 2002; 99:409-426.

21. Orazi A, Ronald C, Dennis M, Magdalena C, Susan LA, Caroline A. Chronic myelomonocytic leukemia; the role of bone marrow biopsy immunohistology. Modern Pathology.2006; 19:1536-1545.

22. Volpe G, Cignetti A, Panuzzo C. Alternative BCR-ABL splice variants in Ph-chromosome positive leukemias result in novel tumor-specific fusion proteins that may represent potential targets for immunotherapy approaches. Cancer Res. 2007; 67:5300-5307.

Calculated Ionized Calcium & Actual Ionized Calcium in Preeclampsia

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Abstract

Background: Preeclampsia is a form of high blood pressure manifested during pregnancy; however, its etiology is unknown. Also, the status of ionized calcium (Cai) during pregnancy and its complication preeclampsia have not been described adequately. In addition to calculation method described for Cai, the calcium-binding dye murexide has become a widely-used tool for measuring changes in the ionized calcium (Cai) concentration in biological systems.

Objective:to demonstrate the level of Cai during preeclampsia with respect to normal pregnancy; and to demonstrate the correlation between calculated Cai and actual Cai.

Subject and methods: the present study is a case-control study conducted during the period from February 2007 until the end of June 2007, which includes measurement of total, corrected and ionized calcium (Cai) in 60 patients with preeclampsia that are classified according to gestational age into preeclamptics in the second trimester G1 (n=30) and preeclamptics in the third trimester G2 (n=30).

The results are compared with 60 apparently healthy pregnants controls that are classified according to gestational age into two groups G3 (n=30) and G4 (n=30).

Results: the serum corrected total calcium; serum calculated ionized Cai and actual Cai are significantly reduced in preeclamptics in the third trimester (G1) compared with normal pregnants (G4) (P<0.001) and even when compared with preeclamptics in the second trimester (G2). The same significant reduction in serum corrected total calcium (P<0.001); but not in serum ionized Ca (calculated and actual Cai) is found in preeclamptics in the second trimester (G2) compared with normal pregnants (G3). Both calculated Cai and actual Cai are significantly correlated (r=0.7, P<0.001 in all study groups apart from preeclamptic in the second trimester where r=0.5, P<0.001).

Conclusion: all preeclamptics have certain factors that reduce vasodilation, enhance vasospasm. This is supported by the finding of low ionized calcium which is essential for the synthesis of endothelial-derived NO. A mathematical equation can be used in clinical practice for expressing ionized calcium.

Key words: preeclampsia, calculated ionized calcium, actual ionized calcium.

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Introduction

Preeclampsia is defined as the onset of hypertension and the presence of proteinuria during pregnancy, usually occurring after the 20th week of gestation in a previously normotensive woman and resolving completely by the sixth week after delivery [1, 2].

The dynamics of calcium homoeostasis are in fact substantially altered in pregnancy [3]. It is generally accepted that the ionized calcium (Cai^{+2}) is the physiologically active form of calcium in the blood [3]. Investigation of Cai^{+2}changes in various disease states have been reported, in which it was measured by ion selective electrod[4], but a few reports of Cai^{+2} measurement in disease are available by EDTA titration method[5]. This paper present data on calcium homeostasis, correlation of ionized calcium measured directly with that calculated from total calcium after correction to total protein and albumin for 60 preeclamptic patients as compared to those of 60 healthy pregnants.

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Subjects & Methods

A case-control study was conducted during the period from February 2007 until the end of June 2007 on sixty patients with preeclampsia (PE) who attending the Obstetric Consultant-Clinic, Antenatal Clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of newly diagnosed PE, or for delivery. Inclusion criteria were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) [(6)] and proteinuria (21.5 mg of urinary protein per mmol creatinine) [(7)].

Depending on the gestational age, the patients were divided into two groups:

1. Preeclamptics in the second trimester (G1):

Includes thirty preeclamptics in their second trimester of pregnancy with an age range was from 18 to 37 years and a gestational age of 20 to 28 weeks as seen in Table 1.

2. Preeclamptics in the third trimester (G2):

Includes thirty preeclamptics with an age range was from 18 to 40 years and a gestational age range from 29 to 40 weeks as seen in Table 1.

Sixty apparently healthy pregnants attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery, matches preeclamptic groups regarding the age, gestational age and depending on the gestational age, the apparently healthy pregnants were subdivided into two groups:

3. Control pregnants in the second trimester (G3):

They were thirty apparently healthy pregnants in the second trimester of pregnancy with an age of 15 to 38 years and a gestational age range of 20 to 28 weeks as seen in Table 1.

4. Control pregnants during the third trimester (G4):

They were thirty pregnants in the third trimester of pregnancy with an age range of 18 to 35 year and a gestational age range of 29 to 40 weeks as seen in Table 1.

Any patients with other medical illnesses that may have an effect on the measured parameters were excluded from the study, such medical illnesses are cardiac, hepatic, endocrine, metabolic diseases, smoking and alcoholism.

Ten milliliters of venous blood were withdrawn into plane test tube from each patient and control, in supine position, without application of tourniquet. The samples were left to clot at room temperature, centrifuged, and the separated sera were transferred into Eppendrof tube and stored at -20^{o} C until analysis of calcium, which was done within one month after collection[(5)].

The total serum calcium levels were measured using atomic *absorption spectrophotometer*[5]. The *Corrected serum calcium* was calculated according to the formula described by Gowenlock[(8)]:

Adjusted calcium (mmol/L) = Measured calcium concentration (mmol/L) + 0.02 [40 – albumin concentration (g/L)].

Instead of obtaining a crude correction for measured calcium, the same data can be used to calculate the *ionized calcium* according to the formula described by Gowenlock [(8)]:

$$\text{Ionized calcium (mmol/L)} = \frac{\text{measured calcium (mmol/L)} - K'/12 \times 60}{K'+60}$$

where

total protein (g/L) + ×K' = 0.19
albumin (g/L).

The *actual ionized calcium* was determined by EDTA complexiметric titration method using murexide as an indicator that was employed by Gowenlock[8].

Results

The serum total corrected and ionized calcium concentrations (actual and calculated) were significantly lowered in the preeclamptic women in third trimester G2 group as compared to healthy controls in the third trimester G4 [P<0.001] and even when compared to the preeclamptic in the second trimester G1 [P<0.001] as seen in Table 2. The same significant reduction in *corrected but not ionized calcium* was noticed in the second trimester group G1 when compared to the healthy pregnants in the second trimester group G3 [P<0.001 for corrected calcium but greater than 0.05 for ionized calcium] as seen in Table 2. There was no significant difference in *corrected and ionized serum calcium* values between healthy pregnants in each group [P > 0.05].

Correlation between calculated Cai and actual Cai:

A significant positive correlation between actual and calculated serum ionized calcium was noticed in different studied groups, in preeclamptics G1 (r=0.7, P < 0.001); and G2 (r=0.5, P < 0.001) also in pregnant control groups G3 and G4 (r=0.7, P < 0.001for both) respectively as in Figures 1, 2, 3, and 4.

Table 1: Demography of different preeclamptics and healthy pregnants groups.

Group	G1	G2	G3	G4
No	30	30	30	30
Age / year (Mean ± SD)	26.1 ± 6.4	25.1 ± 6.9	24.6 ± 4.5	24.8 ± 4.6
Age range (years)	(18-37)	(18-40)	(15-38)	(18-35)
Gestational age / week (Mean ± SD)	26.3 ± 1.5	35.6 ± 1.9	25.5 ± 1.8	34.6 ± 2.1
Gestational age range (years)	(20-28)	(29-40)	(20-28)	(29-40)

Table 2: The mean value & standard deviation of calcium (total, corrected Ca^{+2}, actual ionized Ca^{+2}, calculated ionized Ca^{+2}) in the sera of different preeclamptic and pregnant control groups.

Variable	G1	G2	G3	G4
Total serum calcium (mmol/L)	2.5 ± 0.05	2.4 ± 0.09	2.5 ± 0.1	2.5 ± 0.1
Corrected total serum calcium (mmol/L)	2.3 ± 0.05**	2.2 ± 0.09*§	2.6 ± 0.1	2.6 ± 0.1
Actual serum ionized calcium (mmol/L)	1.9 ± 0.05	1.8 ± 0.12*§	1.9 ± 0.14	2 ± 0.04
Calculated serum ionized calcium (mmol/L)	1.2 ± 0.08	1.1 ± 0.05*§	1.2 ± 0.05	1.2 ± 0.05

G1 & G2: Preeclamptics in the second & third semesters of pregnancy.
G3 & G4: normal pregnants in the second & third semesters of pregnancy.

* T-test; G2 versus G4, P < 0.001
§ T-test; G2 versus G1, P < 0.001
* * t-test; G1 versus G3, P < 0.001

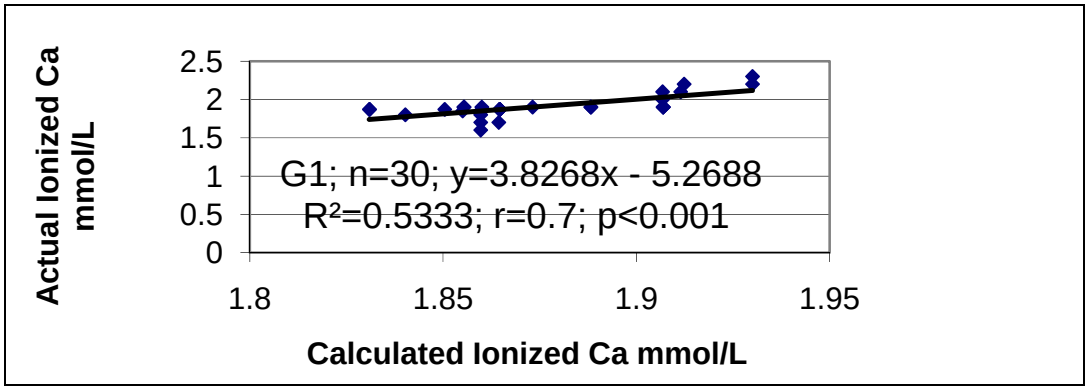

Figure 1: Correlation between actual and calculated serum ionized calcium in second trimester preeclamptics.

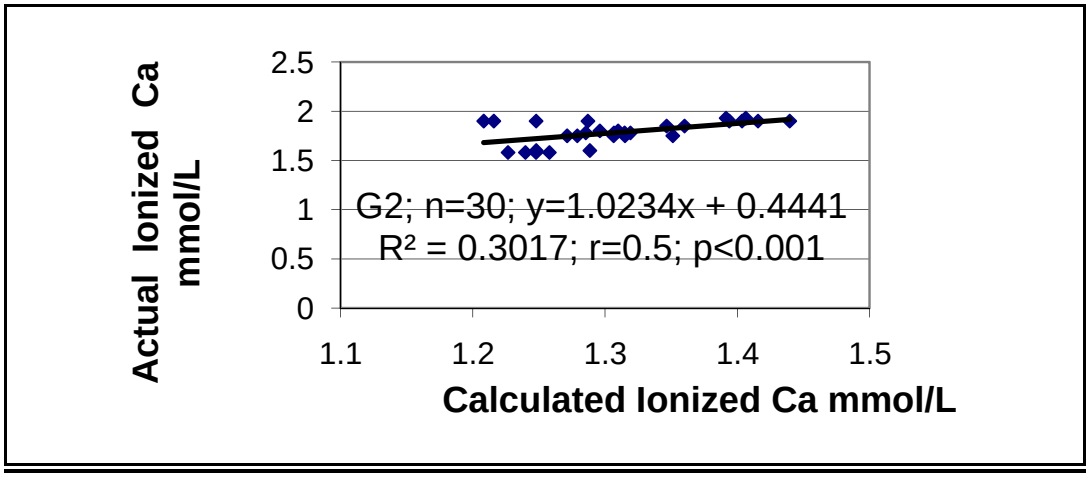

Figure 2: Correlation between actual and calculated serum ionized calcium in third trimester preeclamptics.

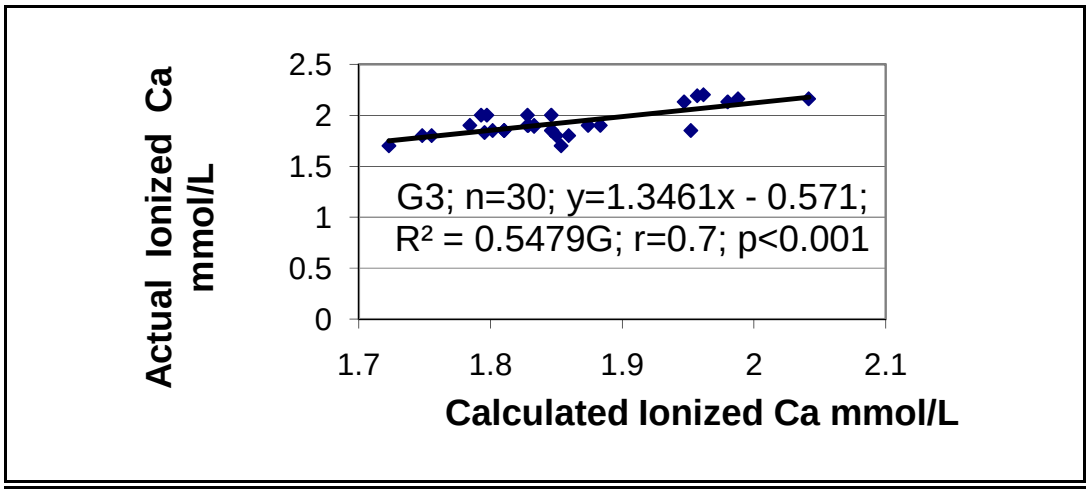

Figure 3: Correlation between actual and calculated serum ionized calcium in second trimester pregnant controls.

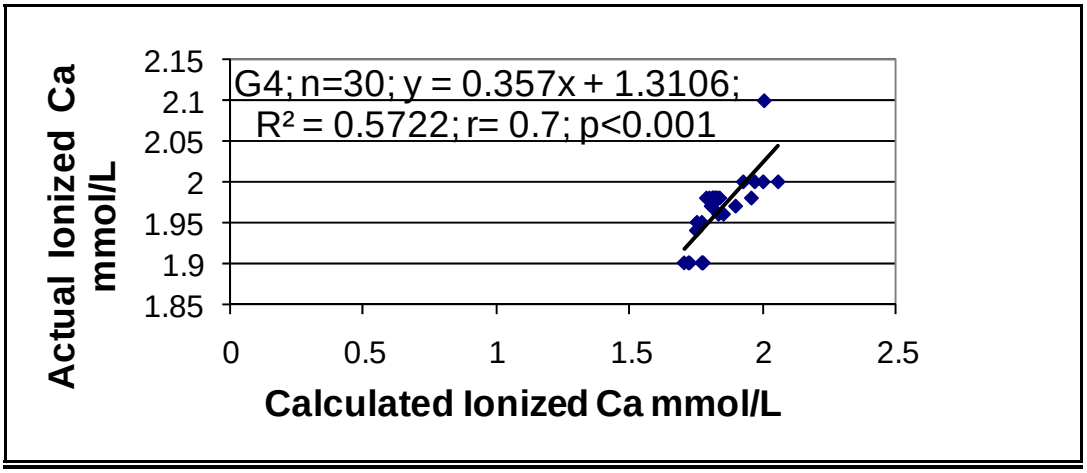

Figure 4: Correlation between actual and calculated serum ionized calcium in third trimester pregnant controls.

Discussion

Regarding the ionized fraction of calcium which is crucial for the synthesis of vasoactive substances in the endothelium as prostacyclin and nitric oxide [9]. The finding of significant reduction in this fraction, as seen in Table 2 is consistent with those reported by Seely et al [10], who revealed that a low level of active vitamin D (1, 25-$(OH)_2$ D) in preeclamptics, may contribute to suboptimal intestinal absorption of calcium during a time of increased calcium demand resulting in lower ionized calcium, increased parathyroid hormone (PTH), and hypocalciuria in preeclampsia[11]. Abnormalities in calcium homeostasis may contribute to the increased vascular sensitivity documented in preeclampsia. In contradiction to the reported difference in ionized calcium between normal and preeclamptic patients, other authors like Sanders et al [12], Siddiqui & Rana[13], Richards et al[14] found no difference in serum ionized calcium.

The question of which-actual ionized calcium or calculated ionized calcium-should be reported to the physician is unresolved, and there is no international recommendation. Bowers et al[15] advocate the use of corrected ionized calcium, because of pH values in venous samples that fell outside the reference limits (loss of CO2 during sample handling lowering the calcium ion concentration in vitro from what it was in vivo), whereas others[16] have question the use of corrected value for ionized calcium, both analytically and clinically.

We have found a close relationship between calculated ionized calcium and the actual values for ionized calcium in patients with preeclampsia. From theoretical and clinical point of view, we expect actual ionized calcium values to be superior to measurement of the calculated ionized calcium because of parathyrin-induced increases in the renal excretion of bicarbonate, thus giving rise to values for pH and ionized calcium different from those at pH 7.4[17]. Our study shows that calculated ionized calcium provides the same clinical information as actual ionized calcium, even in preeclampsia.

In conclusion, biochemical changes in preeclampsia appear to involve

calcium metabolism leading to the appearance of the typical pattern which may cause vasospasm of eclampsia. These changes would include low serum ionized calcium. We also conclude that calculated ionized calcium for a sample is as useful as actual ionized calcium in the evaluation of patients with disorder of calcium metabolism. Clinical superiority of actual ionized calcium is expected only in patients with severe acid-base derrangement. Calculated ionized calcium appears to be a good choice for establishing reference values for healthy adults; however, due to the lack of gold standard method for measuring ionized calcium (ion selective electrode) in Iraqi laboratories; the accuracy (sensitivity and specificity) of complexiметric method for measurement of ionized calcium cannot be determined in this study. Further study of intracellular calcium and calcium pumps to explore their potential role in the pathogenesis of preeclampsia is required for future work.

References

1. Baker PN. Editor. Obstetrics by Ten Teacher. 18th edition. Hodder Arnold**;** 2006, P. 159-161.

2. Parry S, and Marchiano D. Hypertension in pregnancy. In: Mark-M and Sam-S. Editors. NMS (National medical series for independent study) / Obstetrics & gynecology. 5th ed. Lippincott Williams & Wilkins; 2005. P.169.

3. Dunlop W. Normal pregnancy: physiology and endocrinology. In: Edmonds, DK. Editor. Dewhurst Textbook of Obstetrics and Gynecology for postgraduates. 6th ed. Blackwell Science; 1999. P. 81-3.

4. Weeke E and Friis Th. Serum fractions of calcium and phosphorus in uremia. *Acta Med Scand, 1979; 189: 79.*

5. Endres DB and Rude RK. Mineral and Bone Metabolism. In: Carel, AB and Edward, RA. Editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Saunders Company, Philadelphia; 1999. P. 1395-1412.

6. Parry S and Marchiano D. Hypertension in pregnancy. In: Mark-M and Sam-S. (Eds.). NMS (National medical series for independent study) / Obstetrics & gynecology, 5th ed. 2005, P: 169. Lippincott Williams & Wilkins.

7. Yamasmit Water, Chaithongwogwatthana S, Charoenvidhya D, Uerpairojkit B, Tolosa J. Random urinary protein-creatinine ratio for prediction of significant proteinuria in women with preeclampsia. *J-Matern-Fetal-Neonatal-Med. 2004; **16**:257-9.*

8. Gowenlock AH, McMurray JR, McLauchlan D. Editors. Varley's Practical Clinical Biochemistry. 6th ed. Heinemann Medical Books, London; 1984. P. 868-873.

9. Lopez P. Prevention of preeclampsia with calcium supplementation and its relation with the L-arginine: nitric oxide pathway. *Braz J Med Biol Res (BRAZIL). 1996; **29**: 731-41.*

10. Seely EW, Wood RJ, Brown EM, Graves SW. Lower serum ionized calcium and abnormal calciotropic hormone levels in preeclampsia. *J Clin Endocrinol Metab. 1992; **74**:1436-40.*

11. Kisters K, Barenbroka M, Louwenb F, Hausberga M, Rahna KH, Koscha M. Membrane, intracellular, and plasma magnesium, and calcium concentrations in preeclampsia. *AM J Hyperten. 2000; **13**: 765-769.*

12. Sanders R, Koijnenberg A, Huijgen HJ, Wolf H, Boer K, Sanders GT. Intracellular and extracellular ionized and total magnesium in preeclampsia and uncomplicated pregnancy. *Clin-Chem-Lab-Med. 1999; **37**: 55-9.*

13. Siddiqui JA, Rana IA. Mineral and parathyroid hormone inter-relationships in normal pregnancy and pregnancy-induced hypertension. *J Pak Med Assoc. 1993; **43**: 92-5.*

14. Richards SR, Nelson DM, Zuspan FP. Calcium levels in normal and hypertensive pregnant patients. *Am J Obstet Gynecol. 1984; **149**: 168-71.*

15. Bowers JG, Brassard C, Sena SF. Measurement of ion calcium in serum with ion selective electrodes: a mature technology that can meet the daily service need. *Clin Chem. 1986; 32: 1437-47.*

16. Wandrup J, Kancir C, Hyltoft P. Ionized calcium and acid-base status in arterial and venous whole blood during general anesthesia. *Scand J Clin Lab Invest. 1988; 48: 115-22.*

17. Thode J, Holmegaard SN, Transbel I, Fogh Andersen N and Siggaard Andersen O. Adjsted ionized calcium (at pH 7.4) and actual ionized calcium (at actual pH) in capillary blood compared for clinical valuation of patients with disorders of calcium metabolism. *Clin Chem. 1990; 36/3, 41-4.*

Evaluation of markers of oxidative DNA damage in females with breast tumors

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Abstract

Background: DNA damage reflects a balance between oxidative stress and DNA repair ability which associates with breast cancer risk.

Objective: Assessment of the oxidative DNA damage in women with breast tumors using comet tail length (CTL) and comet tail moment (CTM) to measure the extent of single strand DNA breaks in addition to 8-hydroxy deoxy guanosine (8-OHdG) levels and numbers of DNA lesions.

Methods: Blood leukocytes and post operative tumor specimens were taken from 40 females with newly diagnosed breast tumors (age 24-75 years) and leukocytes of 40 healthy controls (age range 24-50 years). The cells were subjected to single cell agarose gel electrophoresis and the severity of DNA damage was quantitated by computer image analysis. The level of the 8-OHdG was measured by ELISA and numbers of DNA lesions was estimated by special formula.

Results: There were highly significant differences($P<0.001$) in the mean levels of leukocyte CTL, CTM, serum 8-OHdG and DNA lesion in benign and malignant breast tumor as compared to the control groups with augmented elevations in these analytes in malignant breast tumor tissues as compared to the benign ones. A Significant increase ($p<0.001$) in the mean tissue 8-OHdG values was reported in the invasive malignant carcinoma as compared with noninvasive subgroup. The leukocyte means of CTL, CTM, 8-OHdG of malignant breast tumor patients with an age $\leq$ 48 years and BMI >24 were significantly higher than their counterparts ($p<0.05$, $P<0.001$, respectively). There were strong positive correlation between both leukocytes ($r=0.71$, $r=0.83$; $P<0.001$) and tissue ($r=0.69$, $r=0.83$; $P<0.001$) CTL, CTM with the concentration of serum 8-OHdG in total breast tumors.

Conclusion: The 8-OHdG and comet assays are useful, sensitive markers for monitoring the severity of DNA modification and damage in breast tumor and could be used to identify persons with increased cancer susceptibility.

Keywords: Comet test, 8-hydroxy deoxy guanosine, oxidative DNA damage, Breast tumors.

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Introduction

Breast cancer is the most common malignant tumor and the leading cause of death in woman world-wide with 1.5 million new cases being estimated in the year 2001 [(1)].

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According to Iraqi Cancer Registry Center, breast cancer is remained the commonest malignant tumor in female population [(2)].

Although tumor initiation and progression are predominantly driven by acquired genetic alterations, yet DNA damage plays a central role [(3)]. These damages are caused by many factors including environmental factors (exposure to chemicals), nutrition, and natural cellular processes [(4)]. Ionizing radiation, an established etiologic agent for breast cancer, and other suspected risk factors, such as chemical

carcinogens, alcohol, estrogen and diet, result in reactive oxygen species (ROS), oxidized bases, bulky DNA adducts and DNA strand breaks. Under these circumstances, women may develop cytogenetic alterations, such as deletions, amplifications and/or mutations in critical oncogenes and tumor suppressor genes, leading to cellular transformation and neoplasm. Higher levels of DNA damage and deficient DNA repair may predispose individuals to breast cancer [5].

Oxidation of DNA in human cells occurs as a consequence of its attack by the free radicals arising endogenously as well as exogenously .Internal sources of the free radicals include ROS released during cellular respiration, or by leukocytes as a part of the defense against foreign organisms .Tobacco smoke and intermediates of xenobiotic metabolism (products of mixed function oxidase reactions) are other sources [6, 7].

Various biomarkers have been used to determine cellular DNA damage. The single cell gel electrophoresis or comet assay is one such state-of- the art technique for quantitative DNA damage and repair *in vivo* and *in vitro* in any eukaryotic cell and some prokaryotic cells. This technique is rapid, non-invasive, sensitive, visual and inexpensive as compared to the conventional techniques and is a powerful tool to study factors modifying mutagenicity and carcinogenicity. It has rapidly gained importance in the fields of genetic toxicology and human bio monitoring [8].

Particular types of ROS are responsible for different kinds of DNA damage. The $OH^{\bullet}$ generated from H2O2 in the immediate vicinity of DNA, is able to damage the deoxyribose backbone of DNA and all of the four DNA bases in various ways, including the generation of 8-hydroxyguanosine), the hydrolytic product of which is the 8-hydroxydeoxyguanosine (8-OHdG) [9]. This 8- OHdG has been used as a "fingerprint" of $OH^{\bullet}$ attack and it is the most commonly measured and the most widely studied DNA oxidation product [10, 11]. The $OH^{\bullet}$ is also responsible for DNA single and double-strand breaks and damage to tumor suppressor genes and other macromolecules [12].

In this study we assayed for the first time the degree of DNA damage and DNA lesions in benign and malignant breast tumors in Iraqi female utilizing the single cell gel electrophoresis (comet assay) and the level of 8-OHdG in the blood and tumor cells. Moreover, we assessed the effect of age, and body mass index on the degree of DNA damage.

Subjects and Methods

The study was part of PhD research that was conducted on 80 females; of these, 40 were newly diagnosed untreated breast tumors with an age range of 24-75years. They were admitted at the Department of Surgery in Al-Khadymia Teaching Hospital during the period of December, 2004 to May, 2006. Moreover, 40 healthy women (age range 24-50 years) with no family history of any type of cancer were involved as controls.

All the subjects were non-smokers non-alcoholic. Personal data on each subject were collected in terms of family history, menstrual history, menopause status, parity, age at first pregnancy, and laterality of the affected breast (Table 1).

Five milliliters (mL) of venous blood were withdrawn pre-operatively from patients and controls into heparinized tube to isolate leukocyte for comet assay. Another three mls of blood

were collected in plain test tube and allowed to clot at room temperature and the serum was separated by centrifugation at 3000rpm (750 Xg) and stored at -20˚C into small aliquots for the measurement of the serum 8-OHdG levels.

From each patient, a piece of tissue was taken from the site of the breast tumor and embedded in formalin, processed in the histopathology laboratory and the stained slides were examined by senior pathologist. According to the histopathological reports, 15 patients were having benign breast tumors and the other 25 were suffering from malignant breast tumors. A second piece of tissue was kept in cold phosphate buffered saline for the comet assay while the third piece was stored at -20 ˚C for the tissue DNA extraction.

Isolation of cells from the blood and breast tissue [13]: Neutrophils were isolated from heparinized blood samples by the method of Nath *et al* [14].

A small piece of the breast tumor tissue was placed in 1-2 mls of ice cold mincing solution containing 20mM EDTA and 10% dimethyl sulphoxide, and then filtered. The filtrate was centrifuged at 4000rpm for 30 minutes at 4ºC to precipitate the remaining intact cells, and the cells viability was determined by 0.1% trypan blue exclusion cell˜s number was adjusted to the concentration of 10^6cells/mL[14].

Extraction of DNA from fresh frozen breast tumor tissue: An organic (phenol) extraction method was used and the optical density of the extracted DNA immediately measured at 260nm using tris EDTA buffer as a blank solution [13]. The concentration of DNA was determined according to the following formula:

[O.D 260nm × Dilution factor × 50µg/ml = µg/mL]

A pure DNA solution gave an A_{260} / A_{280} of more than 1.8 and one absorbance unit indicate a DNA concentration of 50 µg/mL[15].

Agarose Gel electrophoresis of the extracted DNA: The intact DNA was identified using agarose gel electrophoresis in a tri hydroxy methyl amino methane borate buffer (µ= 0.089 M, pH= 8) at 70V (6mA) for 4hr. The gel was stained with an ethidium bromide (10mg/ml) for 20 minutes, visualized under the UV transilluminator and photographed (Figure 1)

Comet assay (single cell gel electrophoresis): The comet assay was performed as described by Singh *et al*., 1988[16] with some modifications. Briefly, regular microscope slides were coated by dipping in solution of 1% high-melting-point agarose. To the coated slides, 75µl of low melting point agarose (37ºC) were mixed with the 5-10µl of cell suspension (~10000cells) and left on ice packs until the agarose layer hardens (3 to 5 minutes). The slide was dipped into cold, freshly made lysing solution (1% Triton-X-100 and 10% dimethyl sulphoxide) for 24 h at 4°C. The slides were placed in a horizontal gel electrophoresis unit containing cold electrophoresis buffer solution (300 mM NaOH, 1 mM Na_2EDTA, pH = 13, 4°C) for 20-60 minutes to allow the DNA to unwind and to resolve alkali-labile sites. The electrophoresis is run at 0.7 V/cm (25 V, 300 mA) for 25 min at 4°C. Following electrophoresis, the slides were neutralized three times (5 min each) with 400 mM Tris–HCl (pH 7.5), stained with 100µl 1X ethidium bromide and scored immediately or dried by placing them in cold absolute alchohol for two minutes then rehydrate in chilled deionized water for 30 minutes, and stain with ethidium

bromide. The slides were analyzed using the LAI Comet Assay Analysis System (Loats Associates, Westminster, MD). The digitized images of a total 50 random nuclei from two duplicate slides were scored. The median comet tail length and moment of 50 cells /slide was determined from duplicate slides [13, 17].

Cells with damaged DNA appear as fluorescent comets with tails of DNA fragments (figure 2a), whereas normal, undamaged DNA does not migrate far from cell origin (figure 2b). Comet tail length represent the distance of DNA migration from the body of the nuclear core and it is used to evaluate the extent of DNA damage. The comet tail moment is defined as the product of the percentage of cellular DNA in the comet tail and the length of DNA tail migration [17, 8].

ELISA of 8- hydroxyl deoxy guanosine (OHdG) concentration: The ELISA trays were coated with a 100µl of 10µg/ml mouse antihuman 8-OHdG monoclonal antibody (*Crescent chemical co., Inc. US*) and kept for an overnight at 4ºC. After thorough washing, 50µl of either filtered serum (0.45µ Millipore filter), or 1µg/ml DNA digested with 0.5ml of 60% formic acid in an evacuated and sealed test tubes (at 140 ºC for 30 min) was added . A standard solution was applied to corresponding wells .To each sample and standard containing wells, add 50µl of reconstituted primary antibody and incubate at 4ºC for overnight. After thorough washing, 100µl of 1/450 of the reconstituted secondary antibody (Horse radish peroxidase-conjugated rabbit anti mouse antibody; *Sigma, USA*) was added to each well. Mix and incubate at room temperature for one hour then add 100µl per well of enzyme substrate solution. Mix uniformly and the trays were incubated at room temperature in dark for 15 minutes. Add 100µl of the reaction terminating solution (1M phosphoric acid)**,** read the absorbance at 450nm. The sample 8-OHdG concentration was calculated from the standard curve by plotting the absorbance versus the logarithm of the standard 8-OHdG concentration [18].

Estimation of DNA lesion: The value of 8-OHdG was converted to the number of lesions/ 10^6DNA bases by assuming that guanine constitutes 21.5% of mammalian DNA using the following formula [18]:-

One lesion/10^5guanines=1/0.465 or 2.15 lesions /10^6DNA bases

Statistical analysis

The data were analyzed using Statistica version 6 and Microsoft Excel. The results were expressed as mean± standard deviation (SD). Student t-test was used to compare the results of patients and control groups. Simple correlation coefficient (r) was performed to test the relation between different markers of DNA damage. Differences were considered statistically significant if the *p* value is lower than 0.05.

Results

Table 2 reveals the mean values of leukocyte comet tail length (CTL), comet tail moment (CTM), number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) of controls, and female patients with benign and malignant breast tumors. The mean ±SD CTL value in leukocytes of the control group was 2.4±0.05µm, while the mean value of CTL in those with benign and malignant tumors was4.7±0.13µm, 15.5±0.27µm, respectively.

Statistical analyses using student t-test showed a highly significant elevation in the mean value of CTL in both malignant and benign groups as

compared to control group(P<0.001). Between group t-test revealed that the mean CTL was higher in the sample of patient with malignant breast cancer as compared to benign breast tumor. The mean ±SD level of CTL for malignant invasive (15.4±0.30µm) is not significantly different from the non invasive tumor values (15.9±0.56µm).

The means ± SD CTM of control leukocyte was 7.8±0.10. In patient with benign and malignant breast tumor the mean ±SD CTM were 12.4±0.14 and 36.1±0.33, respectively. Statistical analyses using student t-test showed significant increase in CTM (P <0.001) in benign and malignant as compared to those of control group. Between groups t-test revealed statistically significant elevation (P <0.0001) of mean CTM in malignant breast tumor as compared to benign breast tumor.

Also in table 2, the mean± SD serum values of 8-OHdG and, number of DNA lesions (expressed as an arbitrary units) of the control group were 16.7±0.54ng/ml, and 0.1±0.004, respectively .While mean values of the benign (27.1±1.91; 0.2±0.01) and malignant breast tumors (152.6±8.06; 1.2±0.06) were significantly elevated above the mean control values (P <0.001). Between breast tumor groups, t-test revealed highly significant elevation (P <0.001) in the mean values of 8-OHdG and, number of DNA lesions in females with malignant tumor as compared to those with benign tumors.

Table 3 demonstrates the mean values of tissue CTL, CTM, DNA lesions, and serum 8-OHdG in female patients with benign and malignant breast tumors .The mean± SD CTL and CTM of malignant breast tissue cells were 4.6 ± 3.66 µm, and 13.9±4.3, respectively .These values were highly significantly increased (P <0.001) above the mean values of benign breast tissue cells values (117.2±6.50; 48.1±14.95, in an order). Between malignant sub groups t-test revealed no significant differences between invasive carcinoma and noninvasive malignant breast carcinoma mean values.

The mean ±SD of tissue 8-OHdG and the numbers of DNA lesions in malignant breast tumor group was 294.8±59.68ng/ml, 2.3±0.42, respectively. Whereas in the benign breast tumor, the mean tissue 8-OHdG level was 29.3ng/ml ±6.95 and the mean of DNA lesions was 0.3±0.06. Student t-test revealed highly significant increase (P <0.001) in the tissue 8-OHdG mean values of the malignant breast tumor as compared to the benign breast tumor subtypes. Yet, there was a significant increase (P <0.001) in the mean tissue 8-OHdG levels in the invasive malignant carcinoma as compared with noninvasive carcinoma subgroups.

Table 4 Clarify the effects of body mass index and age on the mean values of leukocyte CTL, CTM, , number of DNA lesions, and serum 8-OHdG in controls and female patients with benign and malignant breast tumors. Statistical inference revealed no significant differences in the means of the CTL values in control(2.4±0.06 µm) benign (4.6±0.15 µm) and malignant breast tumors(15.0±0.46) patients with an age of ≤48year as compared to their respective mean values of those with a mean age>48(2.5±0.18 µm, 5.1±0.27 µm, 15.8±0.33 µm, respectively).

The mean leukocytes CTL of the controls and females with benign breast tumors with BMI≤24 were 2.4±0.07µm, 4.7±0.14 µm, in an order. These values were comparable and statistically not different from the mean value of those

with BMI>24 (2.4±0.09µm, 5.2±0.39 µm, one by one). In women with malignant breast tumor with BMI≤24, the mean CTL level was 16.4±0.43µm which is significantly higher (P <0.001) than the mean values observed from malignant breast tumor patients with BMI>24 (14.8±0.33µm).

The mean leukocyte CTM of malignant breast tumor patients with an age ≤ 48 years (36.7±0.37) was significantly higher than the mean CTM of the same disease group with an age >48year(33.7 ± 0.68). Furthermore, only females with benign breast tumors with BMI>24 exhibited a significant elevation in mean CTM (13.4±0.4) above the values of those with BMI> 24.

The mean serum 8-OHdG, of controls and those who suffer from malignant breast tumor with an age≤48 year (16.4±0.59ng/ml, and 171.6±12.7 ng/ml, respectively) were significantly increased above the mean 8-OHdG values of those with age > 48 years (P <0.05). Furthermore within the control, benign and malignant breast tumor groups the serum 8- OHdG concentration were significantly elevated in those with BMI> 24 as compared to patients with lower BMI (P <0.01, P <0.05, respectively).

Figure 3 and 4 reveal strong positive correlation between the leukocytes CTL and CTM and concentration of serum 8-OHdG in total breast tumors(r=0.71, r=0.83; P <0.001).Similar significant relationship was also observed between the tissue CTL and CTM and concentration of serum 8-OHdG in total breast tumors(r=0.69, r=0.83; P <0.001).

Table 1: Characteristics of the studied population*

VARIABLE	CATEGORIES	CONTROL N=40	BREAST TUMORS	
			BENIGN N=15	MALIGNANT N=25
Age(years)	Total	36.85±6.76	37.33±16.36	51±5.67
Age at menarche (years)	Total ≤ 12.5 13-14	12.4±0.87 22 (55%) 18 (45%)	11.9±0.89 12 (80%) 3 (20%)	11.92±1.28 17 (68%) 8 (32%)
Parity	≥ 1 Nulliparous Unmarried	23 (57.5%) 3 (7.5%) 14 (35%)	11 (73.3%) 1 (6.7%) 3 (20%)	23 (92%) 1 (4%) 1 (4%)
BMI (Kg/m^2)	Total	25.2± 3.17	22.3± 1.84	25.54± 4.33
Location of tumor	Right breast Left breast Bilateral	- - -	8 (53.4%) 5 (33.3%) 2 (13.3%)	14 (56%) 8 (32%) 3 (12%)

*Results are expressed as mean ±standard deviation.

Table 2: The mean (± standard deviation) values of leukocyte comet tail length, comet tail moment, number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) of female controls, and patients with benign and malignant breast tumors.

		Mean concentration of			
		Comet tail length(µm)	**Comet tail moment**	**8-OHdG (ng/ml)**	**DNA lesions (lesions/10^6bases)**
Control N= 40		2.4±0.05	7.8±0.10	16.7±0.54	0.1±0.004
Benign N= 15		4.7±0.136[a***]	12.4±0.14 [a***]	27.1±1.91 [a***]	0.2±0.01 [a***]
Malignant	**Total N= 25**	15.5±0.27[(a,b)***]	36.1±0.33 [(a,b)**]	152.6±8.06 [(a,b)**]	1.2±0.06[(a,b)***]
	Invasive N= 19	15.4 ±0.3[a(a,b)***]	36.3 ±0.38 [(a,b)***]	145.7±9.16[a***]	1.1 ±0.06[(a,b)***]
	Non Invasive N= 6	15.9±0.56[a***]	35.4±0.65 [(a,b)***]	174.3±14.85[(a,b)***]	1.3±0.01[(a,b)***]

[a]t-test :comparison of the total, benign, malignant breast tumor groups with control: [***]p<0. 001
[b]t-test :comparison of benign group with malignant breast tumor groups: [***]p<0.001
[c]t-test :comparison of invasive malignant breast tumor with non invasive subgroup: Not significant

Table 3: The mean (± standard deviation) values of tissue comet tail length, comet tail moment, number of DNA lesions, and breast tissue 8-hydroxy deoxy guanosine (8-OHdG) in female patients with benign and malignant breast tumors.

		Mean concentration of:			
		Comet tail length(µm)	**Comet tail moment**	**8OHdG (ng/ml)**	**DNA lesion (lesions/10^6bases)**
Benign tumors N= 15		4.6±3.66	13.9±4.31	29.3±6.95	0.3±0.06
Malignant tumors	**Total N= 25**	17.2±6.50 [a***]	48.1±14.95 [a***]	294.8±59.68 [a***]	2.3±0.42 [a***]
	Invasive N= 19	17.3±0.2 [a***]	47.6±0.49 [a***]	290.7±14.91 [a***]	2.3±0.1 [a***]
	Non Invasive N= 6	16.7±0.39 [a***]	49.9±0.83 [a***,b*]	307.6±15.91[a***, b*]	2.4±0.1 [a***]

[a]t-test : comparison of benign group with malignant breast tumor groups: [***]p<0.001.
[b]t-test: Between invasive and noninvasive malignant breast tumor groups t-test: [*]p<0.05.

Table 4: The effect of body mass index and age on the mean (± standard deviation) values of leukocyte comet tail length, comet tail moment, number of DNA lesions, and serum 8-hydroxy deoxy guanosine (8-OHdG) in controls and female patients with benign and malignant breast tumors.

			Mean concentration of:			
			Comet tail length(μm)	Comet tail moment	8OHdG (ng/ml)	DNA lesion (lesions/10^6bases)
Control N= 40	Age (year)	≤48	2.4±0.06	6.9±0.09	16.4±0.59 [a***]	0.1±0.004
		>48	2.5±0.18	7.9±0.23 [a**]	17.5±1.27	0.1±0.01
	BMI (Kg/m^2)	≤24	2.4±0.07	7.5±0.11	17.1±0.68 [b**]	0.1±0.005
		>24	2.4±0.09	7.8±0.14	15.9±0.87	0.1±0.01
Benign tumors N= 15	Age (year)	≤48	4.6±0.15	12.4±0.1	26.9±2.43	0.2±0.01
		>48	5.1±0.27	12.6±0.2	27.5±3.12	0.2±0.02
	BMI (Kg/m^2)	≤24	4.7±0.14	12.3±0.1	27.5±2.11	0.2±0.01
		>24	5.2±0.39	13.4±0.4 [b**]	24.0±5.00 [b**]	0.2±0.03
Malignant tumors N= 25	Age (year)	≤48	15.0±0.46	33.7±0.68	139.9±12.78	1.1±0.09
		>48	15.8±0.33	36.7±0.37 [a***]	171.6±9.34 [a*]	1.3±0.07 [a**]
	BMI (Kg/m^2)	≤24	16.5 ±0.43 [b***]	36.3±0.52	140.1±10.14	1.1±0.07
		>24	14.8 ±0.33	35.9±0.42	162.4±11.64 [b**]	1.2±0.08

[a] Within Age subgroup of benign and malignant breast tumors t-test: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.
[b] Within BMI subgroup of the controls, benign and malignant breast tumors t-test: $^{**}p<0.01$, $^{***}p<0.001$.

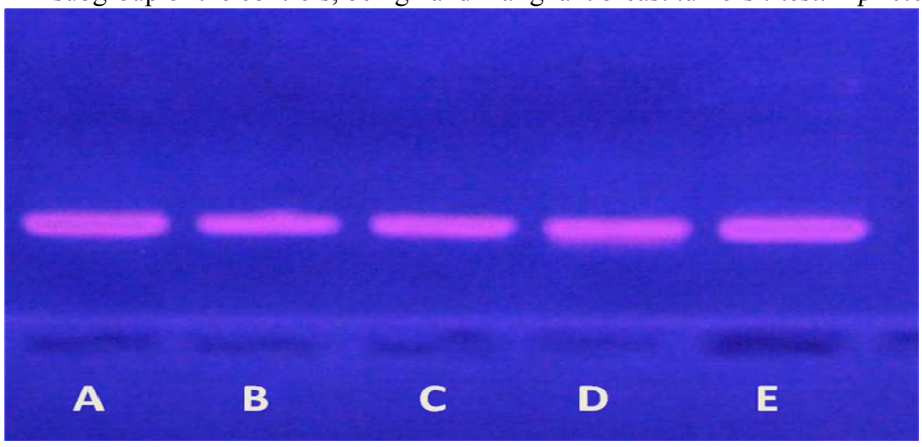

Figure 1: An agarose gel electrophoreticograph of an extracted intact cellular DNA stained with ethidium bromide: *lanes* A and B are for benign breast tumors whereas *lanes* C, D, and E are for malignant breast tumors.

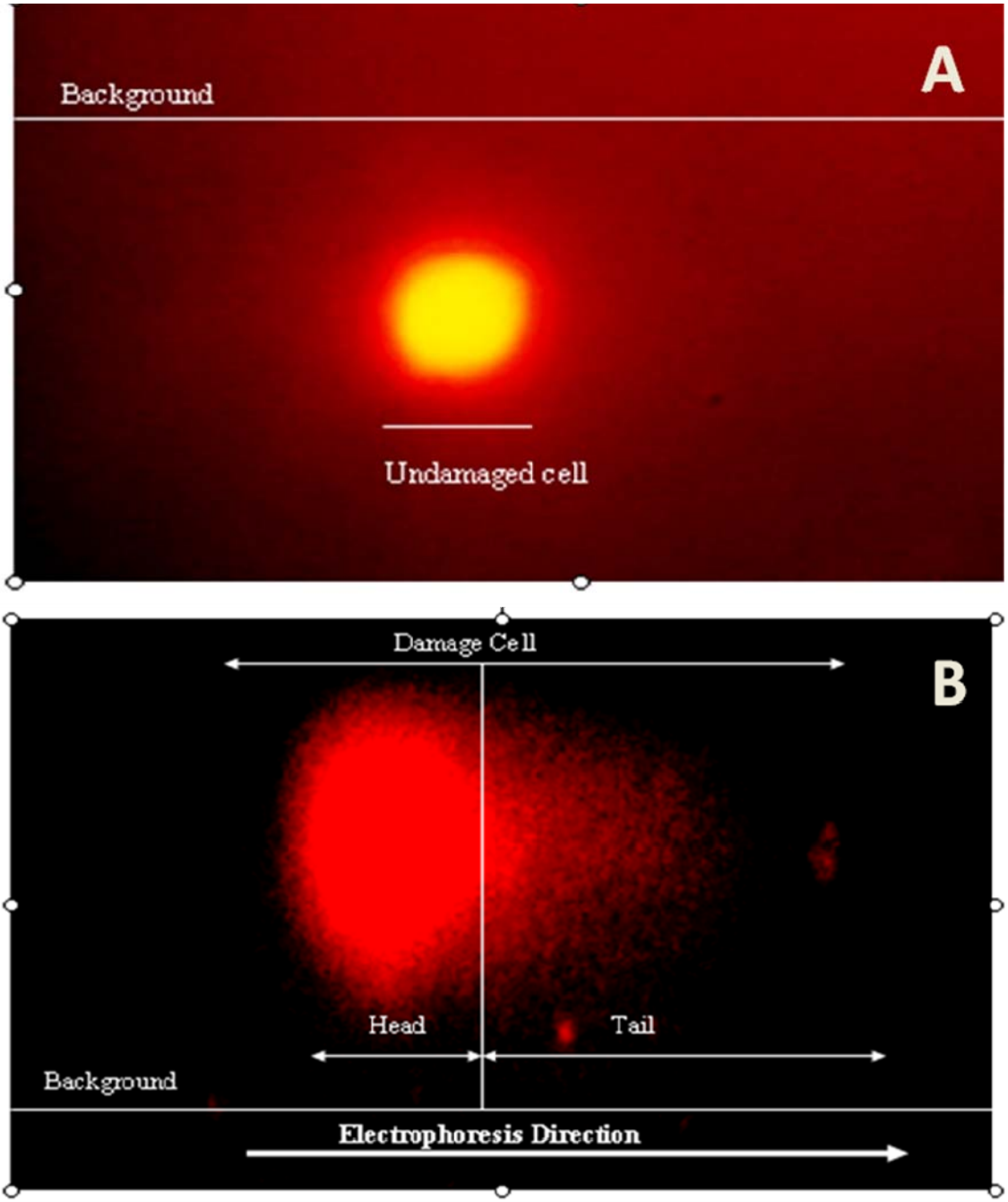

Figure 2: A photograph of a single cell gel electrophoresis. ***A: intact cellular DNA which appears as nucleoids. B: malignant breast tumor cell with extensive DNA damage appear as a typical comet with head and tail.***

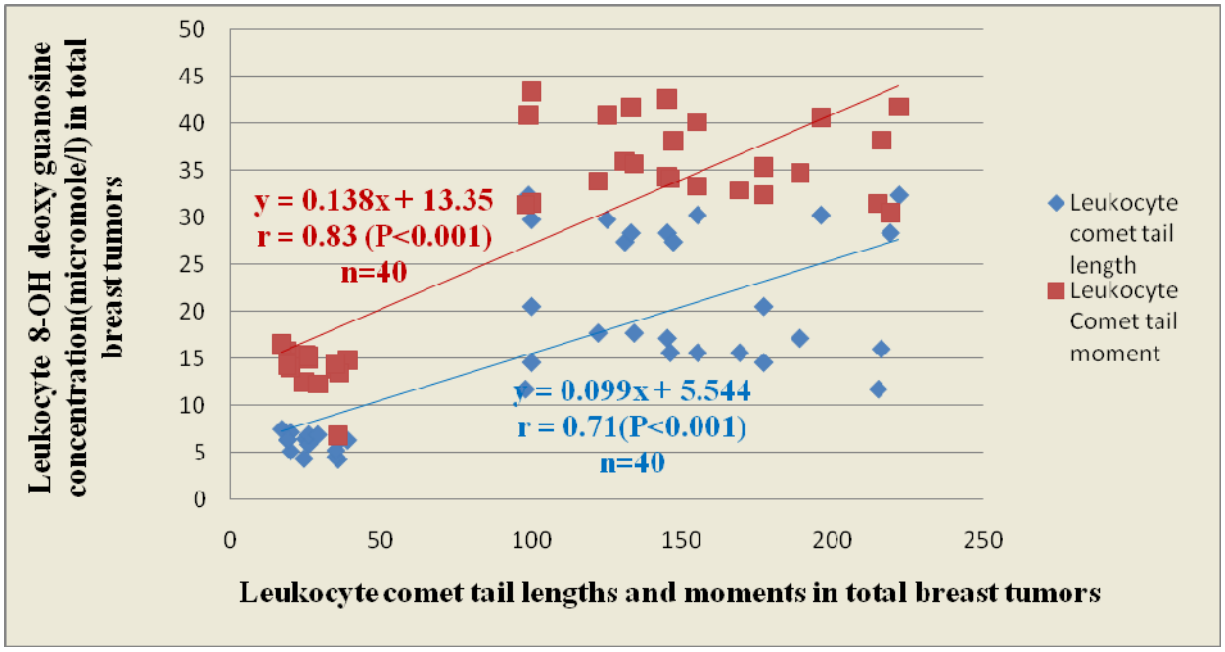

Figure 3: Correlation between Leukocyte comet tail length, comet tail moment, and concentration of serum 8-OHdG in total breast tumors.

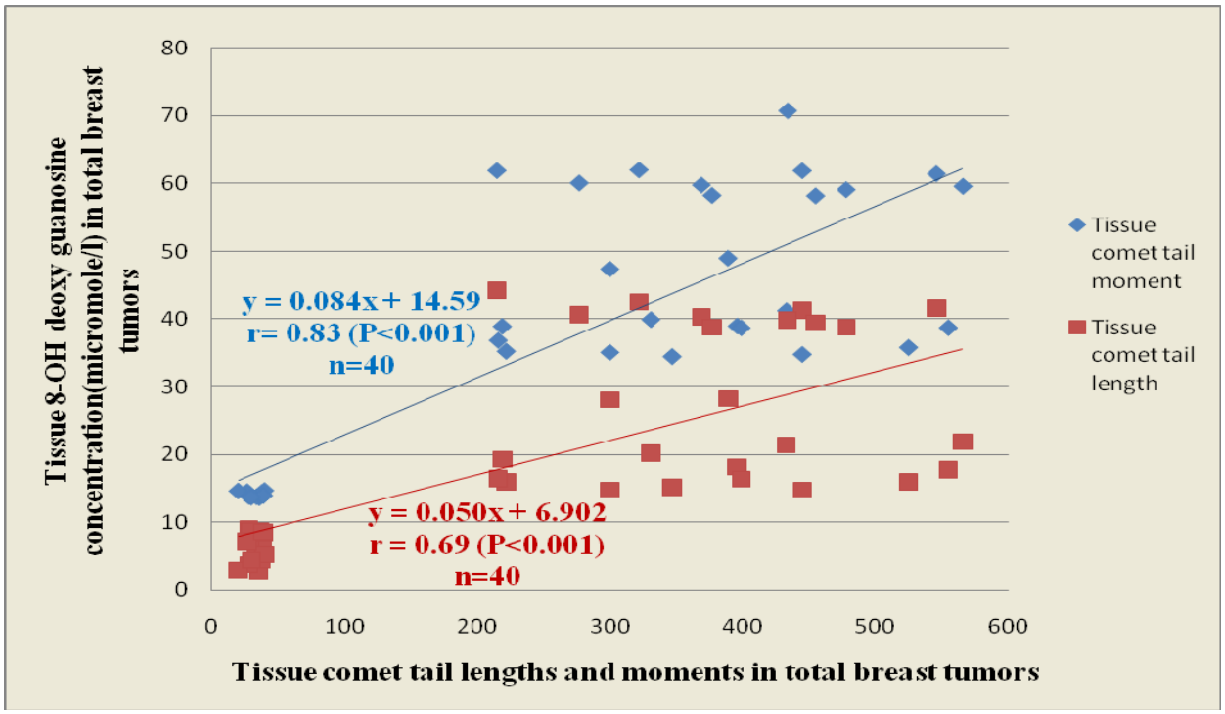

Figure 4: Correlation between Tissue comet tail length, comet tail moment and concentration of serum 8OHdG in total breast tumors.

Discussion

The comet assay protocol used in this study is adequate to detect significant differences in single strand breaks between breast cancer cases and controls. Varieties of tissues have been used in the comet assay. Leukocytes were considered a good marker of actual bodily state as they are more susceptible to the damaging effects of free radicals due to the high percentage of polyunsaturated fatty acids (PUFAs) in their plasma membranes and increased production of free radicals as part of their normal function [7,19] . In this study, DNA damage expressed as comet tail length (CTL) and comet tail moment (CTM) was reported to be higher in leukocytes of benign and malignant breast cases than controls. Similar finding was recorded by Rajeswari *et al.* [20], who observed a 7-fold difference in CTL between breast cancer cases and controls. Furthermore, the means of comet tail length and CTM t were increased in patient with malignant breast cancer as compared to benign breast tumor. Alice *et al.* [21] reported that the lower levels of DNA damage in controls were associated with being long-lived, cancer-free, and without a history of cancer among first degree relatives, supporting the notion of their capacity to control endogenous DNA damage.

DNA damage (expressed as CTM) was increased in malignant breast tumors patients with an age >48 year as compared to those of ≤48years and this may be due to a decrease in DNA repair and an accumulation of DNA damage in malignant breast tumors leading to increased susceptibility to DNA damage .This finding was in line with those of Singh *et al.* [22] who documented that cells from older individuals have less resistance to DNA by *ex vivo* x-ray exposure. Moriwaki *et al.*[23]and Wei *et al.* [24] observed a decrease in DNA repair with increase of age. On the contrary, Ramos *et al.*[25] reported that younger breast cancer patients had a more substantial reduction in capacity to repair UV damage compared with age matched control subjects than did older patients and controls. Moreover, we observed that the age has no effect on the CTL and this finding agrees with cross sectional study on healthy Americans (age range 25- 91 years) that showed no effect of age on the basal level of DNA damage [26] .

In women with malignant breast tumor with BMI≤24 Kg/m^2 the mean CTL level was significantly higher (p<0.001) than those with higher BMI (>24 Kg/m^2) with no effect of BMI on the CTM. This finding disagreed with those of Smith *et al.* [27] who recorded that a high BMI may be associated with increased levels of lipophilic aromatic compounds, such as polychlorinated biphenyls, aromatic and heterocyclic amines, and polycyclic aromatic hydrocarbons, stored in breast adipose tissue, leading to a continuous exposure to DNA-damaging agents. Udumudi *et al*. [28] reported that CTL significantly differed between cancer patients and controls in cervical epithelial cells, as well as in peripheral blood leukocytes. These studies suggested that genetic defects in DNA repair may contribute to higher levels of DNA damage in leukocyte and target tissue in cancer patients.

This study showed that DNA damage (expressed as CTL) was increased in malignant subtypes as compared to controls. This observation agrees with Alice *et al.* [21] finding of a

consistent association of increased endogenous DNA damage (indicated by higher CTL values) with the rise in cancer risk. Statistical analysis revealed no differences in CTL and CTM between the invasive and non invasive malignant breast carcinoma subtypes which mean that women with non invasive carcinoma subtype are under the risk of conversion to an invasive carcinoma type.

In tissue analyses our study showed that the CTL and CTM in malignant breast tissue were increased more than in benign breast tissue. This increase in DNA oxidative damage in malignant breast tissue is probably due to increase proliferation of this type of tissue which leads to increase accumulation of damaged DNA in breast tissue. The malignant noninvasive carcinoma was found to have higher CTM values than invasive carcinoma. A number of epidemiological studies, primarily on lung and skin cancers have suggested that deficiency in DNA repair capacity, accumulation of DNA damage, acceleration of gene rearrangements (deletions, insertions, and amplifications) are involved in human carcinogenesis [29, 30] . Alice *et al.* [21] reported a rise in the risk of breast and thyroid cancers with the increase in the values of CTL and CTM or only CTM, respectively.

The increased serum 8-OHdG level in benign and malignant breast tumors reported herein is consistent with Donghui *et al.*[31],and Zora *et al*. [32] findings who reported high levels of the 8-OHdG in serum and/ or urine of cancer patients. Moreover, elevated levels of 8-OHdG were observed in serum of patients with malignant tumors compared with those with benign tumors. It is possible that an accumulation of damage due to 8-OHdG formation overwhelms the capacity for DNA repair [33].

Our result revealed that the concentration of 8-OHdG was increased in malignant breast tumors patients with an age >48 year above those of lower age. This finding was in line with Kuo *et al* [34] observation who reported that the concentration of 8-OHdG is age dependent. On the contrary, one study found that the accumulation of oxidative DNA damage was unrelated to age or to smoking and drinking habits [35]. Asami *et al.* [36] revealed that the lymphocytic 8-OHdG levels were mainly dependent upon age and smoking status. We observed that the oxidative damage expressed as 8-OHdG was significantly increased in malignant breast tumor tissues of patients with a BMI >24 but were lower in patients with benign breast tumor tissues as compared to those of ≤24 Kg/m^2 BMI. The high BMI associates with increased endogenous estrogen production may explain the association between obesity and breast cancer [37]. Mizoue *et al* ([38] found intensive association between BMI and 8-OHdG levels among smokers with no apparent relations between BMI and 8-OHdG levels among nonsmokers. On the contrary, Trie *et al.* [39] recorded significant negative correlations of the 8-OH-dG level with BMI.

Because 8-OHdG is known to represent one of the major forms of oxidative DNA damage, many researchers have measured 8-OHdG in tissues or urine as a marker of oxidative stress [34] . We observed an increase in the mean concentration of tissue 8-OHdG in malignant breast cancer as compared to the values of the benign breast tumors. This finding agrees with those of Donghui *et al*.[31] who reported

that tissue from breast cancer had significant higher level of 8OHdG than normal control breast tissue. For malignant subtypes there was a significant elevation in the mean concentration of tissue 8-OHdG in the non invasive above the values of invasive malignant breast carcinomas. This result is consistent with those of Parshad *et al.* [40] who revealed a decreased repair of X-ray induced DNA damage in lymphocytes of eight women with pre invasive malignant breast lesions. This observation indicates that oxidative DNA damage may play a role in placing these women at increased risk of conversion to an invasive subtype. Furthermore, the means of numbers of DNA lesion in the sera of patients with benign and malignant breast tumors were significantly higher than the control values. Yet, the increase was much augmented in malignant breast tumors. This result agrees with Donghui *et al.* [32] who found that tissue breast cancer had significant higher level of 8OHdG than control subjects but he found the mean±SD of 8-oxo-dG/10^6 were 3.9±7.2/10^6 and 1.1±1.4/10^6 for cases and controls, respectively. Poirier and Weston [41] found that the concentration of benzpyrene-DNA adduct from malignant tumors taken from smokers to be 0.65-5.33/10^6 DNA bases. Ottender and Lutz [42] work on rat liver, revealed that carcinogen-DNA adduct concentration is associated with a 50% incidence of liver cancer which ranged from 53 to 2083 adducts/10^8 nucleotides for aflatoxin and dimethylnitrosamine carcinogens.

The mean of number of DNA lesions was high in older patients with malignant tumors (> 48 years). Yet, it was not influenced by the increase in patient BMI. In malignant subtypes there were no significant differences in the number of DNA lesions in both sera and tissues of patients with noninvasive as compared to those of invasive malignant breast tumors.

In Conclusion: DNA damage is significantly associated with breast cancer risk. The comet assay could be used for measuring the levels of DNA damage in patients with breast tumors whereas 8-OHdG is a useful marker for DNA modification in the cells. Both tests could contribute to the detection of the degree of DNA damage in persons with breast tumors.

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References

1. Kamangar F, Dores GM, and Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J. Clin. Oncol.* 2006*;* 24:2137–2150.

2. Iraqi Cancer Board. *Results of Iraqi cancer registry* (1990-2000). Baghdad, Ministry of Health, 2000.

3. Polyak K. Breast cancer: Origins and evolution. Sci Med 2007; 117: 3155-3163.

4. McKenna DJ, McKeown SR, McKelvey-Martin VJ. Potential use of the comet assay in the clinical management of cancer. *Mutagenesis* 2008; 23:183-190.

5. Johnson-Thompson MC and Guthrie J. Ongoing research to identify environmental risk factors in breast carcinoma. *Cancer* 2000; 88: 1224–1229.

6. Catherune MG, Susanne PB, Sharon GW, Nicholas JV and Andrew RC. Oxidative stress in humans: Validation of biomarkers of DNA damages .*Carcinogensis* 2002; 23: 1441-1446.

7. Collins A, Cadet J, Epe B and Gedik C. Problems in the measurement of 8-oxoguanine in human DNA .Report of a workshop ,DNA oxidation held in Aberdeen ,UK, 19-21 January .*Carcinogenesis* 1997; 18:1833-1836.

8. WWW.comet assay forum: Indian Industrial toxicology research, India. Last updated on April, 2008.
9. Kasai H. Analysis of a form of oxidative DNA damage (8-hydroxy-2'-deoxyguanosine) as a marker of cellular oxidative stress during carcinogenesis. Mutat Res 1997; 387:147-163.
10. Marnett LJ, Oxyradicals and DNA damage. Carcinogenesis 2000; 21:361-370.
11. Shoji H, Shimizu T, Shinohara K, Oguchi S, Shiga S and Yamashiro Y. Suppressive effects of breast milk on oxidative DNA damage in very low birth weight infants. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2004; 89: F 136-138.
12. Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, *et al*. The role of oxidative stress in chemical carcinogenesis. Environ Health Perspect 1998; 106; Suppl 1:289-295.
13. Bredel M, Bredel C , Juric D , Kim Y , Vogel H , Harsh GR., *et al*. Gene Mapping of Genomic Aberrations from Limited Sources of Fresh-Frozen and Paraffin-Embedded DNA. *J Mol Diagn* 2005; 7: 171-182.
14. Nath N, Chari SN, and Rathi AB. Superoxide dismutase in diabetic polymorphonuclear leukocytes. *Diabetes* 1984; 33:586-589.
15. Sambrook J, MacCallum P, Russel D. Molecular cloning: A Laboratory Manual. Third edition, Chapter 5, Volume one. America. Coldspring Harbor Laboratory Press, 2001.
16. Singh NP, McCoy MT, Tice RR and Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res*. 1988; 175: 184-191.
17. Tice RR, Agurell E, Anderson D, Burline B, Hartmann A, Kobayashi H, *et al*. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol. Mutagen* 2000; 3:206-21.
18. Henry R, Juan J, Jacques L, and Miral D. Comparison of the levels of 8-hydroxyguanine in DNA as measured by gas chromatography mass spectrometry following hydrolysis of DNA by Escherichia coli Fpg protein or formic acid. *Nucleic Acid Res* 2000; 28:e75.
19. Kassie F, Parzefall W and Knasmüller S. Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. *Mutat. Res* 2000; 463: 13–31.
20. Rajeswari N, Ahuja YR, Malini U, Chandrashekar S, Balakrishna N, Rao KV, et al. Risk assessment in first degree female relatives of breast cancer patients using the alkaline Comet assay. *Carcinogenesis* 2000; 21: 557–561.
21. Alice JS, Michael H, Bruce H, Michele M, Cynthia B, Jeffery P, *et al*. DNA damage among thyroid cancer and multiple cancer cases, control, and long-lived individuals. *Mutat Res* 2005; 586:173-188.
22. Singh NP, Danner DB, Tice RR, Pearson JD, Brant LJ, Morrell CH, *et al*. Basal NA damage in individual human lymphocytes with age. *Mutat. Res* 1991; 256*:* 1-6.
23. Moriwaki S, Ray S, Tarone RE, Kraemer KH, and Grossman L. The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased DNA mutability. *Mutat Res* 1996; 364:117–23.
24. Wei Q. Effect of aging on DNA repair and skin carcinogenesis: a mini review of population-based studies. *J Invest Dermatol Symp Proc* 1998; 3:19–22.
25. Ramos JM, Ruiz A, Colen R, Lopez ID, Grossman L, and Matta JL. DNA repair and breast carcinoma susceptibility in women. *Cancer* 2004; 100:1352–7.
26. Singh NP, Danner DB, Tice RR, Brant L, Schneider EL. DNA damage and repair with age in individual human lymphocytes. *Mutat. Res* 1990; 237*:* 123-130.
27. Smith TR, Miller MS, Lohman KK. Case LD and Hu JJ. DNA damage and breast cancer risk. Carcinogenesis 2003; 24: 883-889.
28. Udumudi A, Jaiswal M, Rajeswari N, Desai N, Jain S, Balakrishna N, *et al*. Risk assessment in cervical dysplasia patients by single cell gel electrophoresis: a study of DNA damage and repair. *Mutat. Res* 1998; 412: 195–205.
29. Benhamou S and Sarasin A. Variability in nucleotide excision repair and cancer risk: a review. *Mutat. Res* 2000; 462-58.
30. Berwick M and Vinesis P. Markers of DNA repair and susceptibility to cancer in human: an epidemiologic review. *J.Natl.Cancer Int*. 2000; 92:874-97.
31. Donghui L, Weiqing Z, Jijiang Z, Ping C, Aysegul S, and Eva S. Oxidative DNA damage and 8-hydroxy-2-deoxyguanosine DNA glycosylase/apurinic lyase in human breast cancer. *Molecular carcinogenesis* 2001; 31: 214-223.
32. Zora D, Lance K, Samir L, Egle B, Michael S, and Mary A. Levels of 5-Hydroxymethyl-2'-deoxyuridine in DNA from Blood of Women Scheduled for Breast Biopsy**.** *Cancer Epidemiol Biomarker Prevent* 2001; 10: 147-149**.**
33. Queiroz D, Mendes EN, and Rocha GA. H. pylori strains possessing cagA and vacuolating cytotoxin producers are associated to both types

of gastric carcinoma. *Gastroenterology* 1996; 110: A236 [abstract].

34. Kuo HW, Chang SF, Wu KY, and WU FY. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. *Br Med J* 2003; 60: 590-594.

35. Olmedilla B, Granado F, and Southon S. Serum concentrations of carotenoids and vitamins A, E and C in control subjects from five European countries. *Br. J. Nutr* 2001; 85: 227–238.

36. Asami S, Manabe H, Miyake J, Tsurudome Y, Hirano T, Yamaguchi R, *et al*. Cigarette smoking induces an increase in oxidative DNA damage, 8- hydroxydeoxyguanosine, in a central site of the human lung. *Carcinogenesis* 1997; 18:1763-1766.

37. Berclaz G, Li S, Price KN, Coates AS, Castiglione-Gertsch M, Rudenstam CM, *et al* . Body mass index as a prognostic feature in operable breast cancer: the International Breast Cancer Study Group experience. Ann Oncol 2004; 15:875-884.

38. Mizoue T, Kassi H, Kubo T, and Tokunaga S. Leanness, smoking, and enhanced oxidative DNA damage. *Cancer Epidemiol Biomarkers Prevent* 2006; 15: 582-585.

39. Trie M, Tama K, Iwamoto-tanaka N, and Kassi H. Occupational and lifestyle factors and urinary 8-hydroxydeoxyguanosine. *Cancer Sci*. 2005; 96: 600-606.

40. Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, and Sanford KK. Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br.J. cancer* 1996; 74: 1-5.

41. Poirier MC and Weston A. Human DNA adduct measurements: state of the art. *Environ Health Perspect* 1996; 104: 883-93.

42. Ottender M and Lutz WK. Correlation of DNA adduct levels with tumor incidence: carcinogenic potency of DNA adducts. Mutat. Res 1999; 424: 237-47.

The effectiveness of suburethral sling procedure with autologus rectus fascia in the cure of stress urinary incontinence.

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Abstract

Background: There are many different surgical procedures that can be employed when treating women with stress urinary incontinence. The surgeon is somewhat overwhelmed with choice. Sub-urethral autologous slings have been used since the early 1900s.

Objectives: to evaluate the surgical results after cruciate suburethral sling procedure with autologus rectus fascia in the cure of stress urinary incontinence in females.

Methods: in a prospective study done between June 2005 to January 2007 we enrolled ten women with stress urinary incontinence demonstrated by positive cough test, filling cystometry, and urethral hyper mobility (straining cotton swab $\geq 30^{\circ}$), with different grades of vaginal wall prolapsed underwent cruciate sub urethral sling procedure using rectus fascia flap. Demographic criteria, complications during surgery and post operative period, and subjective cure rate at three months were assessed.

Results: the average age of the patients was 42 years, median parity was 4. The mean operative time was (90±3.8 minute), mean blood loss was 300 ml. there was no bladder or urethral injury. One patient developed acute urinary retention in the fourth post operative day; urinary tract infection occurred in three patients postoperatively. Follow up examinations three months after surgery revealed that 80% of patients report subjective cure and one patient felt that her symptoms improved significantly. Only one patient has remained incontinent.

***Conclusion*:** The cruciate sub urethral sling procedure with autologous rectus fascia is an effective treatment for Stress urinary incontinence in females.

Key words: *suburethral*, sling procedure, autologous rectus fascia, stress incontinence.

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Introduction

A variety of surgical techniques have been described for the treatment of stress incontinence [(1)]. When the problem is primarily due to the loss of support of the bladder neck and urethra, an operation to resuspend these structures and restore normal support is generally successful in eliminating the patient's urine loss. Less commonly, stress incontinence results from failure of the intrinsic sphincteric mechanism itself with or without defective support. McGuire 1981 has labeled this as type III incontinence and notes that it is rarely helped by a repositioning operation alone but usually requires the use of an operation that supports and compresses the deficient sphincteric unit. In most cases this requires the use of the sling beneath the bladder neck to restore normal closure [(2)].

Different panels of experts [(3, 4, 5)] reviewing the long-term data concluded that retro pubic suspension and sling procedures had the best long-term results among all procedures used for stress urinary incontinence. Since the beginning of the 20th century, using a series of materials including muscle, tendon, fascia and synthetic tissues, sling procedure has been used for the treatment of female urinary incontinence [(6)]. The sling operation uses a strip of material to create a support for the bladder neck and proximal urethra. The use of a strip of rectus fascia beneath the bladder neck

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by a vaginal incision and anchored superiorly in the abdominal wall was proposed by Aldridge in 1942 [7].

Sling attachment to the abdominal aponeurosis would provide its movement with the abdominal wall during the increase of the intra-abdominal pressure. During cough or sneeze, the outwards movement of the abdominal wall would draw the sling upwards with consequent increase of the urethral pressure [7]. More recent studies have shown that the endo pelvic fascia has an important function in giving support to the urethra during stress [8, 9]. Thus, the sub urethral sling, instead of raising and actively compressing urethra during the effort, would act in a similar way as the endo pelvic fascia, supporting the urethra and making a passive resistance of urethra possible during the increase of intra-abdominal pressure [6].

Traditionally, slings have been indicated for the treatment of the recurrent stress incontinence, especially in patients who presented a scarred and fixed urethra leading to a defective urethral sphincter function and lower maximum urethral closure pressure. 6 The indication of sling as the first choice for all stress incontinence cases leads to about 90% of cure [10]. The development of less invasive techniques with synthetic material has been responsible for the renovated interest in the use of sling for the treatment of the female urinary incontinence.

Although the success rates for the autologous fascia sling are 65–95%[11,12] the advantages of using synthetic material are there is no need to harvest fascia from the patient, less postoperative pain, cosmetic reasons, and shorter hospital stays. Nevertheless, even though the incidence of complications has decreased significantly in the past decade, complications still cause significant and substantial morbidity, such as mesh erosion and internal organ injuries. Abdel-Fattah and Domingo reported that the erosion rate of synthetic material is 1.72% to 10% in female urinary incontinence surgery [13, 14].

Because of this high incidence of complications associated with synthetic material, in this study, we studied the results of cruciate sub urethral sling with autologous rectus fascia procedures and evaluated the outcomes, intraoperative and postoperative complications; patient's reported subjective outcomes, and their satisfaction.

Material and methods

Between June 2005 and January 2007, a total of 10 consecutive women with stress urinary incontinence (SUI) underwent cruciate suburethral sling procedure with autologous rectus fascia in al kadhymiyah teaching hospital.

Inclusion criteria were primary treatment of stress urinary incontinence (SUI) and showing SUI on filling cystometry without detrussor over activity and a straining Cotton swab $\geq 30^{\circ}$ test indicating urethral hyper mobility.

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

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

All patients received perioperative intravenous antibiotics (e.g. cefoxitin

2 g intravenously every 8 h for 24 h). , Other procedures, e.g. prolapse repair, perineal repair, were permitted and were fully documented.

The following complications were recorded: blood loss of >300 mL, bladder perforation, urethral lesion, and other intraoperative complications. Postoperative complications that were considered were the need for catheterization >24 hours, postoperative bleeding, hematoma, wound infection, urinary tract infection, and temperature rise >38°C.

All patients were asked to restrict any lifting after surgery, including avoiding lifting over 2.5 kg and abstinence from sexual intercourse for 12 weeks.

Follow-up

All patients were asked to come in for a follow-up at the outpatient department 1 week after being discharged.

Postoperative outcome variables were assessed at each office visit included SUI symptoms, de novo or worsening urge incontinence, and urinary retention.

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

Objective assessment of surgical outcome, by filling cystometry was done during the follow up visit. Any leakage from the urethra during an increase in intra-abdominal pressure and in the absence of a detrusor contraction was interpreted as persistent stress incontinence.

Operative technique:

The operation was performed in the following progression in three set stages.

Stage I: preparation of fascial straps.

The patients were placed in the dorsal lithotomy position allowing free access to the perineum and lower abdomen. Emptying the bladder with Foley catheter was done inserted. Lower abdominal transverse incision to the abdominal apponeurosis two fingers above the symphysis pubis was done. The fascial straps were prepared from the rectus fascia and they should be at least 1 cm wide and 10 cm length.

Stage II:attachment of fascial straps to bladder base.

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

A long curved forceps (Robert forceps) was introduced through the sub pubic fossa to bring the fascial strap into vaginal wound, and the straps were drawn down in to the vagina. The two flaps crossed over each other under the urethrovesical junction and thus indicate the cruciate nature of the planned fascial support. Cystoscopy was performed after that to check for any injury in the bladder. Suturing of the fascial straps to the pubo cervical fascia was done.

The excess vaginal skin was removed and the vaginal skin is closed by a series of interrupted no. 1 polyglycolic acid. Vaginal pack was inserted.

Stage III: closure of the abdominal wound.

The triangular shaped areas at the lower ends of the incisions in the

rectus sheath were closed. Abdominal wall was closed.

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

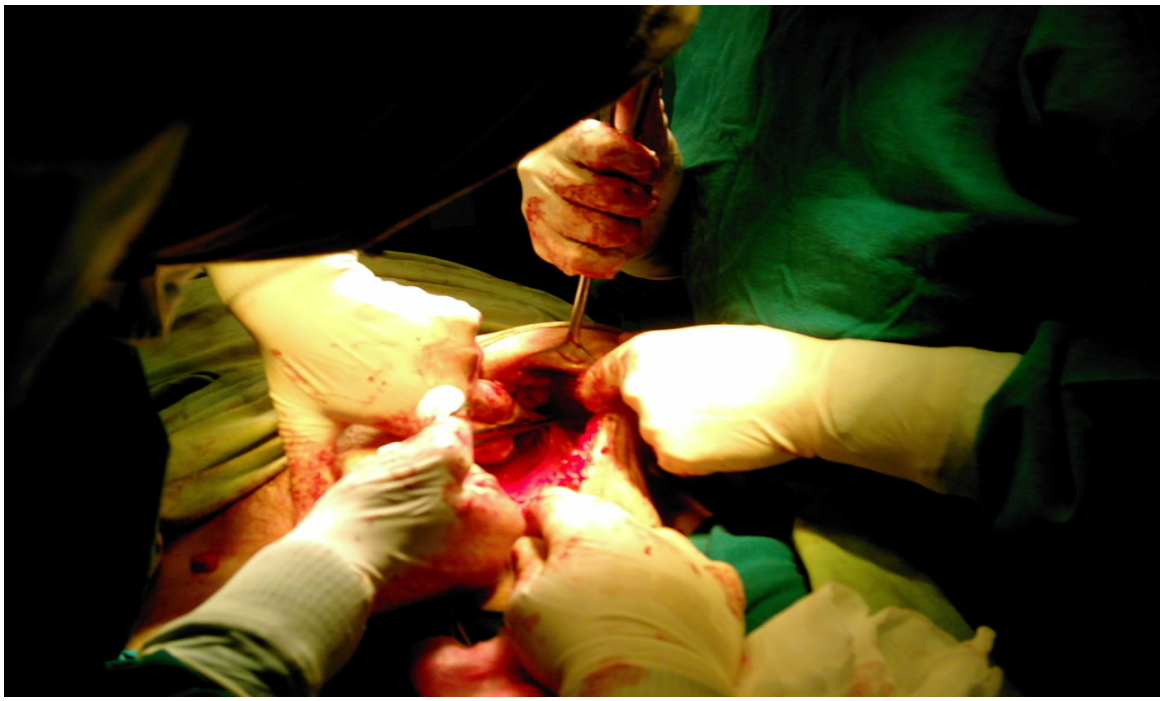

Figure 1: Preparation of the fascial straps

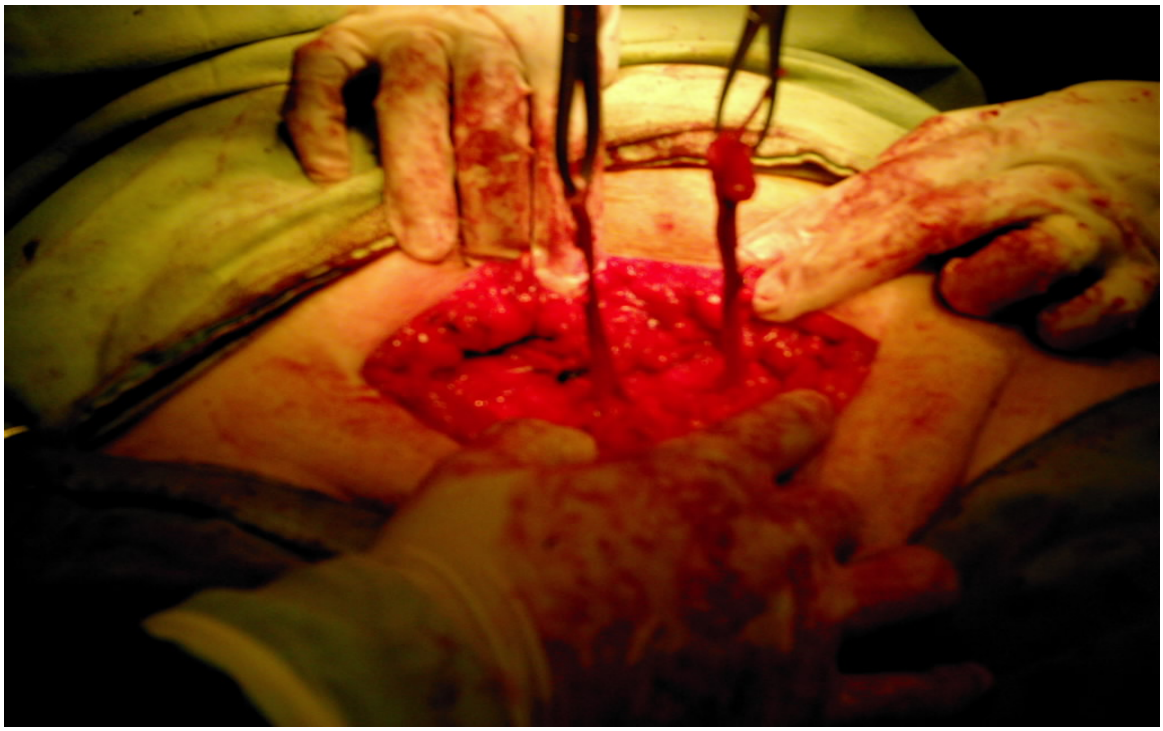

Figure 2: The rectus fascial straps are held with allis forceps

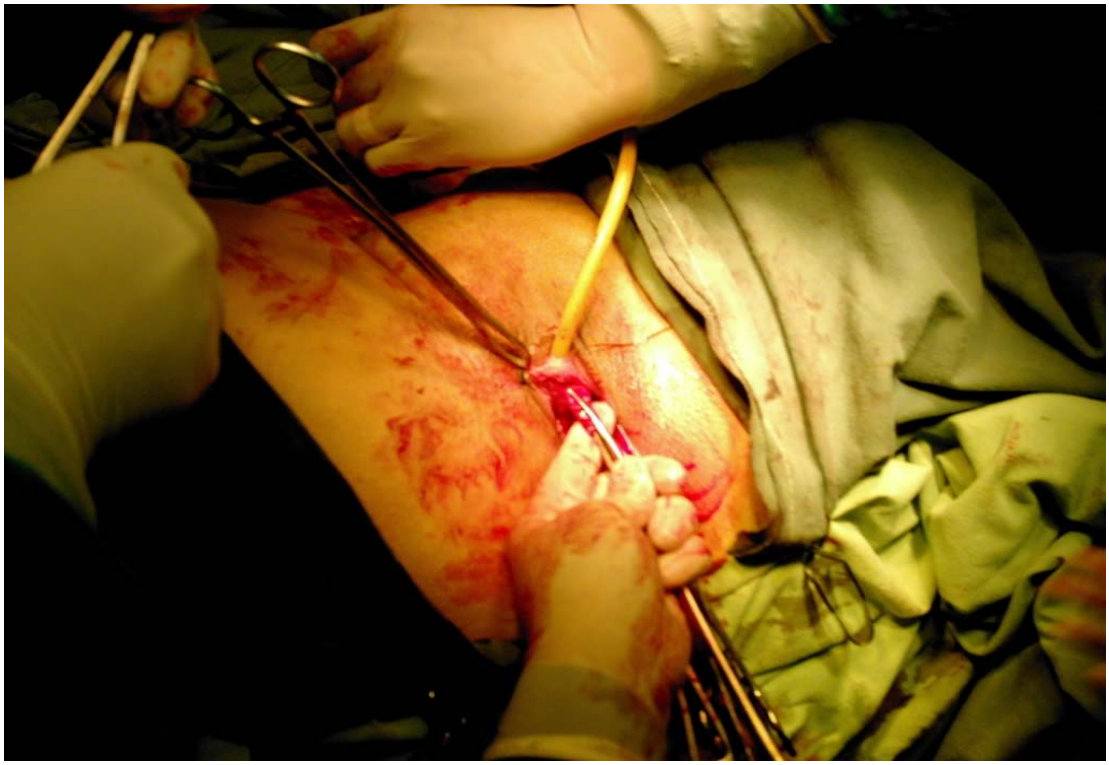

Figure 3: The fascial strap retrieved from the retropubic space held with forceps.

Results

A total of 10 consecutive patients, with a mean age of 42 years (range 36-48) and a median parity of 4(range 2–6) were included in this study. Two patients had undergone prior cesarean section. No patient had undergone prior anti-incontinence surgery. Concomitant surgery was anterior colporrhaphy in 4 patients and colpoperineorrhaphy in seven patients. The mean body mass index at the time of the operation was 25.5 (range 15.4–34.5) kg/m2. 2 (20%) patients were menopausal.

The basic characteristics of the patients are shown in Table 1.

Mean operating time was (90.1 ±3.8 minutes); there was no bladder or urethral injury. Only one patient have blood loss of about 500 cc from dissection of the retropubic space the haemostasis was ensured with suturing of the bleeding areas. One (10%) patient developed urinary retention after being discharged home required catheterization 4 Th postoperative days after taking an allermin tablet. Catheterization was done for her and the catheter removed 24 hours later and voiding trial was successful with posvoid residual volume was 85 cc.

Urinary tract infection occurred in three patients.

Overall SUI was cured in 8 (80%) and improved in 1 (10%) with at least 3 months follow up as shown in table 3.

One patient developed voiding difficulty with a complain of straining to intiate and maintain voiding 7 months following surgery, urine analysis and filling cystometry were done and the post voiding residual urine volume was 50 ml.

Before suburethral sling surgery, 3 patients (30%) had urgency, of which urgency resolved in 2 and persisted in 1 after surgery. De novo urgency appeared in one patient. Urge incontinence appeared in one patient following surgery, as shown in table 4.

Table 5 shows the findings of filling cystometry. It reveals decreased bladder capacity and increased post voiding urine residuals following surgery indicate increased outflow resistance.

None of the patients had functional deficit or an abdominal wall hematoma after the abdominal rectus fascia had been harvested. There was only temporary wound discomfort after the sling procedure.

Table 1: Patient's characteristics (n=10)

Age	42 ±6
Parity	4 ±2.1
BMI	27.1 ±2.5
Postmenopausal state	2 (20%)
Pelvic floor defects	7 (70%)
Prior cesarean section	2(20%)

Table 2: Summary of operative details, perioperative complications, readmissions

Mean operative time (min)	90.1 ±3.8
Mean blood loss (ml)	300
Perioperative complications Bladder injury	0(0%)
Hemorrhage	1(10%)
Urinary tract infection	3(30%)
Urinary retension	1(10%)
Pain at site abdominal wound	1(10%)
Readmissions within 3 months	0 (0%)

Table 3: Procedure outcome (n = 10)

Cure	8 (80%)
Failure	1 (10%)
Improved	1 (10%)

Table 4: Overactive bladder symptoms

Overactive bladder symptoms	**Urgency**	**Urge incontinence**
Before surgery	3	0
After surgery		1
Persistent	1	
Denovo urgency	1	

Table 5: Filling cystometry parameters

Cystometry parameters (n =10)	**before surgery**	**after surgery**
Bladder capacity (mL)	400±25	350±20
Detrusor overactivity	0 (0%)	1(10%)
Postvoiding residual (mL)	40±10	90±20

Discussion

Our results show 80 % succes rate in the cure of SUI with additional 10 % improvements of the pubovaginal sling procedure using autologus rectus fascia. The failure rate was 10 %. Some investigators have reported recurrent urinary incontinence after a pubovaginal slingplasty of over 25% [15, 16]. The risk of failure of sling procedure increases 2.3 times when a cadaveric graft is used, Howden et al. reported that autologous grafts used in pubovaginal slingplasty had superior continence outcomes compared with cadaveric fascia.16 In their study, 153 patients had autologous grafts and 150 had cadaveric grafts. The need for a second operation for recurrent urinary incontinence occurred less in the autologous vs. the cadaveric group (3.3% vs. 12.7%, $P<0.05$) [16].

Muller and his colleagues reported that the success rate of a sling procedure dropped to 37% when patients had proven preoperative urge urinary incontinence [17]. Latini et al. used autologous fascia lata as sling material and had an 85% rate of dry and improved outcomes [18].

Overactive bladder symptoms such as urgency and urgency incontinence can be associated with SUI. In the present study, 3 patients (30%) had urgency as an associated symptom. As possible causes of overactive bladder associated with SUI, several factors are implicated that include weakness of the support provided by the muscle and connective tissue in the bladder neck and urethra, changes of abdominal pressure transmission to the urethra and neuromuscular disorder in the urethra. There has been no convincing theory; however, that explains the difference between SUI with and without an overactive bladder. In the present study, urgency was cured in 2 patients after surgery. This finding indicates that the strengthened support of the bladder neck and urethra by fascial sling may contribute to the resolution of an overactive bladder in some patients. Meanwhile, *de novo* urgency is a perplexing condition that could hamper the therapeutic benefit of anti-incontinence surgery. Bladder outlet obstruction and damage to bladder autonomic innervations by the sling procedure are implicated as possible pathogenesis of *de novo* urgency. However, the precise mechanisms of de novo urgency are poorly understood. In the present study, de novo urgency and de novo detrusor overactivity appeared in only one patient each after surgery.

A comparison of the preoperative and postoperative cystometric parameters indicates an increase in urethral resistance after suburethral sling surgery.

Hilton reported that voiding difficulty increased after a Sling operation compared with a Stamey bladder neck suspension. 12 However, only one patient in our study have been unable to void smoothly after the sling operation. Hilton also found that the sling Operation resulted in a significant reduction in peak urine flow rate and a rise in maximum voiding pressure, suggesting a degree of outflow obstruction [12].

The most important step in the different sling procedures is determining how much tension should be applied to the sling before fixing it or before leaving it free (TVT). Experienced authors [19] report that it is difficult to establish an objective response, because it is subjective and acquired only with experience. McGuire *et al.* [20] noted that tension is not required for supporting the urethra, but on the contrary in the case of sphincter dysfunction more tension

should be applied to increase the coaptation and urethral resistance.

Many methods have been used to evaluate the tension that should be applied to the sling. McGuire and Lytton measured the intraurethral pressure [21]. Yamada *et al.* [22] applied ultrasonography to determine the position of the bladder neck, and Blaivas 19 recommended using a cystoscope to lower the pressure at the moment of suturing the sling. All of these methods are subjective and none of them have confirmed utility with time. In the present study subjective adjustment of tension of the sling by applying it so that it lie comfortably over closed scissor over bladder neck.

As Chapple *et al.* [23] report in their review of surgical treatment for urinary incontinence, the most common complications of sling procedures are voiding difficulty (10.4%), new detrusor instability (7-27%) and lower urinary tract damage (< 3%). Unnecessary tension applied to the sling will clearly affect the first two types of complications mentioned.

Autologous rectus fascial suburethral slings are an effective treatment for the management of stress urinary incontinence in women.

References

1. Wall LL, Norton PA, DeLancey JOL: Surgical management of stress incontinence. In: Practical urogynecology. Baltimore: Williams & Wilkins, 1993; 153-190.

2. McGuire EJ. Urodynamic findings in patients after failure of stress incontinence operations. Prog Clin Biol Res 1981; 78:351-60. MEDLINE)

3. Urinary Incontinence Guidelines Panel: Urinary Incontinence in Adults. Clinical Practice Guideline. *AHCPR*. Publications, no. 92-0038. *Rockville, MO*: Agency for Health Care Policy and Research. Public Health Service. US Department of Health and Human Services, January 2001.

4. Fantt JA, Newman DK, Coiling J et al. Urinary incontinence in adults. Acute and chronic management. Clinical Practice Guideline, no. 2, 1996 Update. AHCPR Publication No 96â€"0682. *Rockville. MO*: US Department of Health and Human Services. Public Health Service. Agency for Health Care Policy and Research, March 1996.

5. Leach GE, Dmochowski RR, Appel RA et al. Female Stress Urinary Incontinence Clinical Guidelines Panel. Summary report on surgical management of female stress urinary incontinence. The American Urological Association. J Urol 1997; 158: 832-839.

6. Bidmead JL. Sling techniques in the treatment of genuine stress incontinence. BJOG. 2000; 107: 147-56.

7. Aldridge AH. Transplantation of fascia for the relief of urinary incontinence. Am J Obstet Gynecol. 1942; 44: 398-411.

8. -DeLancey JOL. Structural support of the urethra as it relates to stress urinary incontinence: the hammock hypothesis. Am J Obstet Gynecol. 1994; 170: 1713-23.

9. Zacharin RF. Abdomino-perineal suspension in the management of recurrent stress incontinence of urine – a 15 years experience. Obstet Gynecol. 1989; 96: 213-20.

10. Chaikin DC, Rosenthal J, Blaivas JG. Pubovaginal fascial sling for all types of stress urinary incontinence: long-term analysis. J Urol. 1998; 160:1312–1316.

11. Albo ME et al. Burch colposuspension versus fascial sling to reduce urinary stress incontinence. N Engl J Med. 2007; 356(21):2143–2155.

12. Hilton P. A clinical and urodynamic study comparing the Stamey bladder neck suspension and suburethral sling procedures in the treatment of genuine stress incontinence. Br J Obstet Gynaecol. 1989; 96:213–220.

13. Abdel-Fattah M, Sivanesan K, Ramsay I, Pringle S, Bjornsson S. How common are tape erosions? A comparison of two versions of the transobturator tension-free vaginal tape procedure.BJU Int. 2006; 98(3):594–598.

14. Domingo S, Alamá P, Ruiz N, Lázaro G, Morell M, Pellicer A Transobturator tape procedure outcome: a clinical and quality of life analysis of a 1-year follow-up. Int Urogynecol J Pelvic Floor Dysfunct,2007; 18(8):895–900.

15. Simsiman AJ, Powell CR, Stratford RR, Menefee SA: Suburethral sling materials: best outcome with autologous tissue. Am J Obstet Gynecol. 2005; 193:2112–2116.

16. Howden NS et al. Comparison of autologous rectus fascia and cadaveric fascia in pubovaginal sling continence outcomes. Am J Obstet Gynecol. 2006; 194:1444–1449.

17. Muller SC, Steinbach F, Maurer FM, Melchior SW. Long term results of fascial sling procedure. Int Urogynecol J Pelvic Floor Dysfunct. 1993; 4:199–203.
18. Latini JM, Lux MM, Kreder KJ. Efficacy and morbidity of autologous fascia lata sling cystourethropexy. J Urol. 2004; 171:1180–1184.
19. Blaivas JG. Pubovaginal sling. In Kursh ED, McGuire EJ eds, Female Urology. *Philadelphia*: JB Lippincott, 1994; 239.
20. McGuire EJ & Wan J. Pubovaginal slings. In Hurt GW ed. Urogynecologic Surgery. : Aspen Publishers Inc, 1992; 97.
21. McGuire EJ, Lytton B. Pubovaginal sling procedures for stress incontinence. J. Urol.1978; 119: 82-4
22. Yamada T, Kura N, Kawakami S et al. Suburethral sling procedure for urinary stress incontinence with special reference to determination of suspension from posturethrovesical angle measured by ultrasonography. Jap J Urol. 1990;81:1351-6.
23. Chapple CR, Ruud Bosch JLH, Hanus T. Female incontinence. Eur Urol 2000; 38: 535-46.

Struma ovarii with literatures review, a Case Report

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Abstract

Struma ovarii is a highly specialized form of ovarian teratoma, characterized by the presence- entirely or predominantly- of mature thyroid tissue. Its most important complications, although rare, are malignant transformation and thyrotoxicosis. In the present 'case report' we describe a case of a 25 year old married woman that seeks medical advice for recurrent lower abdominal discomfort. The diagnosis of complex ovarian cyst confirmed by ultrasound examination of the abdomen, the operative findings were multilocular ovarian cyst treated by oophrectomy. The histological examination demonstrated typical elements of mature thyroid tissue that confirmed the diagnosis of struma ovarii.

Keywords: ovarian teratoma, struma ovarii, thyroid tissue

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Introduction

Teratomas are tumors with more than one cell type which originate from more than one germ layer. These cells may differentiate into any tissue of the body including hair, teeth, fat, skin, muscle and others. These bizarre tumors are usually located at the midline and paraxial regions of the body. One of the most common locations is the ovary [(1)].

Ovarian teratomas make up about one – fifth of all ovarian tumors. Most are cystic and benign; 10 % are bilateral. They occur mostly during the childbearing years, typically in the mid – 30s. About 10% are diagnosed during pregnancy [(2)].

Ovarian teratomas include mature cystic teratomas (dermoid cysts), immature teratomas, and monodermal teratomas eg, struma ovarii, carcinoid tumors, and neural tumors . About 1% to 2% of teratomas may undergo malignant transformation and When malignant transformation occurs, it usually in women older than 40 years [(3)].

Struma ovarii (literally: goiter of the ovary)

Is a rare form of monodermal ovarian teratoma characterized by the presence – entirely or predominantly – of mature thyroid tissue, presenting the same as thyroid gland, with physiological and pathological changes. Simply presence of thyroid tissue with coexistence and predominance of other cell types does not confirm the diagnosis of Struma ovarii [(4)].

Typically, struma ovarii occurs as a part of benign cystic teratomas, but may occasionally be encountered with other ovarian tumors, either germinal as dermoid cysts and carcinoid tumors or nongerminal as serous or mucinous cystoadenomas and Brenner tumors [(5)].

History and presentation of the case

25 years old married woman had 2 children seeks medical advice in May 2009 for lower abdominal pain and discomfort. There was no history of fever, weight loss, jaundice or bleeding per vagina and there were no symptoms of hyperthyroidism.

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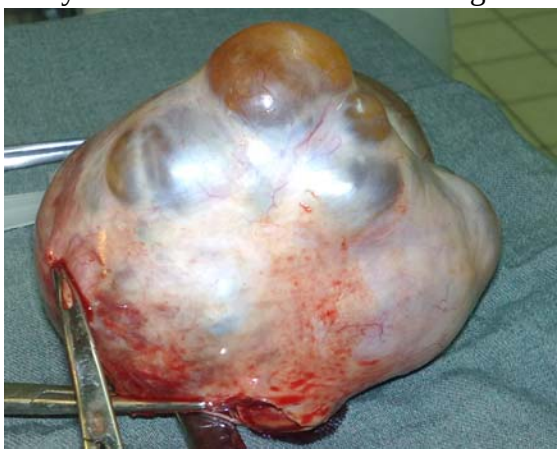
On physical examination there was no pallor, icterus or lymphoadenopathy.

Investigations revealed normal haemogram and serum biochemistry. Chest x ray was normal and diagnosis of multilocular cystic mass confirmed by ultrasonography in AL Karama Teaching Hospital/ Wassit Governorate.

During the intraoperative exploration of the abdominal cavity, a multilocular ovarian cyst of the right ovary was found and a right oophorectomy was performed and biopsy of the left ovary done.

Pathology

Gross examination: right ovary; multilocular cystic mass measured 12* 10 * 9 cm grey smooth slightly thickened outer surface. (Figure 1, Figure 2). Cut surface was multilocular cyst. The cyst filled with grey shiny gelatinous material; the lining of the cyst was smooth. There were no solids areas or intramural nodules within the cyst.



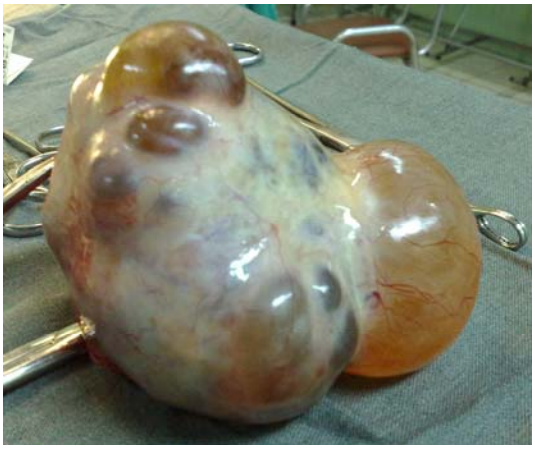
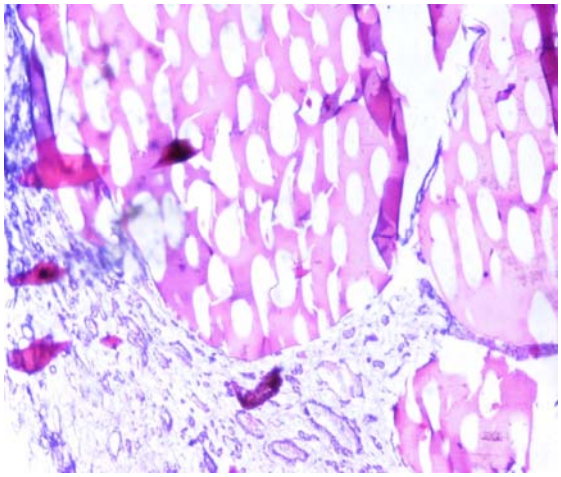


Figure 1&2: multilocular ovarian cystic mass filled with shiny materials.

Microscopical examinations: patient record no. 2622…..May 2009

Compressed ovarian architectures in few sections with replacement of the rest of the ovarian parenchyma by benign colloid filled thyroid follicles (Figure 3&4). No cytological feature of malignancy was seen and no cartilage or skin or neural tissue was seen. Diagnosis in favor of mature cystic teratomas with benign thyroid follicles contain colloid materials (mnodermal teratma; struma ovarii)



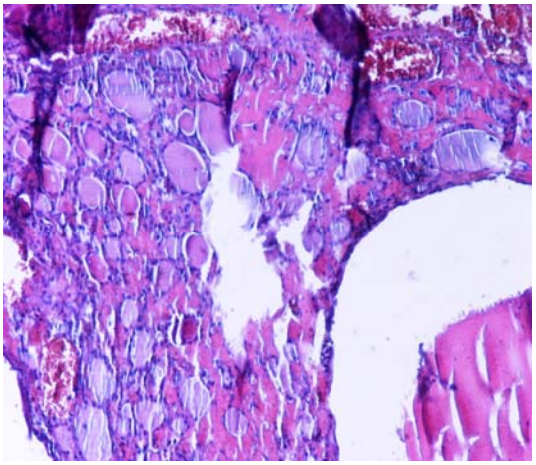


Figure 3&4: Microscopical examinations; thyroid follicles filled with colloid

Discussion

Struma ovarii is a variant of ovarian teratoma in which thyroid tissue components is the major constituent. Thyroid tissue is observed not uncommonly in 5-15% of teratoma, but to qualify as a struma ovarii tumor the thyroid proportion must comprise more than 50% of the overall tissue[6]. Struma ovarii is a relatively rare tumor which comprises less than 1% of all ovarian tumors and 2.7% of all dermoid tumors [7].

This tumor was first described in 1889 by Boettlin, who observed the presence of thyroid follicular tissue in ovaries, and further reports thereafter were published by Gottschalk [8]. However, due to rarity of this type of tumor there has been a paucity of data in the past literature pertaining to diagnosis and treatment of this tumor. Its pathogenesis remains controversial. Today, it is considered that struma ovarii is composed of mature thyroid tissue growing within ovarian teratomas. Although approximately 15% of ovarian teratomas contain a small, non-significant focus of thyroid tissue, only 0.8-3% are characterized by the presence of functional thyroid tissue or thyroid tissue occupying most of the mass, classified as struma ovarii [9].

Its incidence varies in different studies. In study of Higuchi et al, published in 1960, reports 3 cases among 1000 solid ovarian tumors (0.3%). In a recent review of 282 ovarian tumors, 2 cases of struma ovarii have been reported (0.7%) [10]. Struma ovarii is usually presented during reproductive life and rarely before puberty. The tumor always occurs as a pelvic mass, which may be palpable on physical examination, depending on size and location. Most cases are incidentally found during clinical and imaging examination or laparotomy. Preoperative diagnosis of struma ovarii is reported rarely, usually in patients with symptoms of hyperthyroidism. The diagnosis can be made by radiological work-up, including CT scan, MRI and I^{131} sintigraphy. At this point must be underlined that struma ovarii presents some characteristic MRI findings of a multilobulated complex mass with thickened septa, multiple cysts of variable signal intensities and enhancing solid components [11]. Struma ovarii is a rare tumor in its pure form, but its true incidence is hard to estimate because of all the variation in the diagnostic criteria due to the fact that some authors reported it as within a teratoma and others only in its pure form. In general, it is an asymptomatic tumor, "benign-like" in most of the cases, and the diagnostic is based only on the histopatological findings [12]. Struma ovarii in our patient was benign and unilateral involving the right ovary and the thyroid hormonal status was in normal range after operation. The tumor is usually nonfunctional and only 8% of patients' present symptoms and signs of hyperthyroidism, as a result of autonomous activation of its thyroid tissue. The surgical removal of struma ovarii in such cases usually results in disappearance of symptoms, although in rare cases may lead to exacerbation of hyperthyroidism because of the release of TSH receptors stimulating antibodies postoperatively [13]. Malignant transformation of struma ovarii is rare (5-10%). Malignancy is defined by various criteria in different studies, principally differing on classifying struma ovarii as either an ovarian or as a thyroid tumor. Most cases of malignant struma ovarii have been diagnosed on the basis of histological criteria alone, with only about 20 cases presenting clinically appreciable metastatic disease. The

diagnosis of malignancy relies on the basis of cytologic atypia, vascular or capsular invasion, or metastases, like in other ovarian neoplasms, has not been universally accepted, since most authors advocate that malignancy should follow the same guidelines as those for thyroid cancer.

Metastatic spread, following pattern of ovarian cancer, occurs in about 5% of malignant cases. In these patients, there may be local implantations, lymphatic metastases to the omentum, liver or mesentery, as well as distal blood metastases to bones, brain or lungs [(14)].

Struma ovarii generally appears as a multilocular, encapsulated mass, solid and/or cystic on gross examination. The microscopic examination reveals typical rounded thyroid follicles filled with pink staining, homogenous, gelatinous colloid, lined with monoptychial cuboid or columnar epithelium and separated with internal septations.

In some cases, microfollicles of fetal adenomas type may be found. Malignant transformation of the thyroid tissue may be follicular, papillary, or mixed in pattern, and in rare cases can include elements of cystadenocarcinoma, Brenner tumor, carcinoid or melanoma. The positive immunohistochemical staining for thyroglobulin, T3 and T4 confirms the diagnosis of struma ovarii [(15)].

Because of its rarity, there is no consensus on struma ovarii treatment. Each case must be managed individually. Definitive therapy depends on the extent of the disease and the future childbearing wishes of the patient.

Simple salpingooophororectomy is the therapy of choice for the vast majority of patients, since most cases are unilateral and benign. Total hysterectomy with bilateral salpingooophorectomy is indicated for bilateral tumors or in postmenopausal patients. In cases of malignant transformation, a combination of complete tumor resection, total thyroidectomy and adjuvant I^{131} ablation is usually mandatory; since there is evidence that struma ovarii behaves like its thyroid counterparts [(14)].

If evidence of peritoneal metastases is present, appropriate debulking is indicated.

Fertility-sparing surgery should be considered in patients who desire preservation of fertility, if disease is confined to the ovary. In these cases, the initial approach must be followed, after completion of childbearing, by definitive surgery. [(15)]

It was concluded that Struma ovarii is a rare mature ovarian cystic teratoma and the preoperative diagnosis of which is difficult. To our knowledge it is the first reported case in our territory.

References

1. Crum CP. Female genital tract—ovarian tumors. In Cohan RS, Kumar V, and Collins T (eds) Robbins Pathologic Basis of Disease. Translation of the sixth English language edition. W.B.Saunder Co, Philadelphia, 1999. pp. 963–964.

2. Fox H, and Langley F A. Tumors of the Ovary. London, Heinemann.1976. p 210.

3. Eric K. Outwater, Evan S. Siegelman, and Jennifer L. Hunt. Ovarian Teratomas: Tumor Types and Imaging Characteristics. Radiographics. 2001; 21:475-490.

4. Serov SF, Scully RE, Sobin LH. Histological typing of ovarian tumors. International histological classification of tumors. No. 9. World Health Organization: Geneva 1973.

5. 5.C. Papanikolaou, K. Fortounis, K. Biba, I. Venizelos, a. Permekerlis, A. Papadopoulos et al : Struma Ovarii. The Internet Journal of Surgery. 2007 volume 9 no. 2.

6. Seung-Chul Yoo, Ki-Hong Chang, Mi-Ok Lyu, Suk-Joon Chang, Hee-Sug Ryu, Haeng-Soo Kim. Clinical characteristics of struma ovarii. J Gynecol Oncol**,** June 2008 **.**Vol. 19, No. 2:135-138

7. Kim SJ, Pak K, Lim HJ, Yun KH, Seong SJ, Kim TJ, et al. Clinical diversity of struma

ovarii. Korean J Obstet Gynecol 2002; 45: 748-52.

8. Vandana Rana, V Srinivas, S Bandyopadhyay, SK Ghosh, Yashpal Singh. Bilateral benign non functional struma ovarii with Pseudo-Meigs' syndrome. Indian J of pathology and microbiology 2009; 52(1): 94-96.

9. Zaloudek C. Tumors of ovary. In: Fletcher Christopher DM editor. Diagnostic histopathology of tumors. 2 nd ed. Churchill Livingstone; 2000. p. 614.

10. Gango Pilli S, Suneeta KP, Dhaded AV, Yenni VV. Ovarian tumours: A study of 282 cases. J Indian Med Assoc 2002; 100: 420-424.

11. Kim JC. Kim SS, Park JY. MRI findings of struma ovarii. Clin Imaging 2000; 24: 28- 33.

12. Niculescu S, Crauciuc E, Gargu G, Niculescu D, Filip V, Ene D et al. struma ovarii. Rev Med Chir Soc Med Nat Iasi. 2008 Apr-Jun; 113(2):406-10. [Medline].

13. Mimura Y, Kishida M, Masuyama H, Suwaki N, Kodama J, and Otsuka F et al. Coexistence of Graves's disease and struma ovarii: Case report and literature review. Endocr J 2001; 48: 255- 260.

14. Dardik RB, Dardik M, Westra W, Montz FJ. Malignant struma ovarii: Two case reports and a review of the literature. Gynecol Oncol 1999; 73: 447- 451.

15. Tennvall J, Ljungberg O, Hogberg T. Malignant struma ovarii with peritoneal dissemination. Histopathology 1997; 31: 289-290.

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قائمة المحتويات

المقالات

تقرير حالة:

المجلد الثامن، العدد الأول، ١٤٣١ هـ، ٢٠١٠م

المجلة العراقية للعلوم الطبية

تعنون المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد ١٤٢٢٢ بغداد، العراق. تلفون و فاكس (٥٢٢٤٣٦٨-١-٩٦٤).

رقم الإيداع في دار الكتب و الوثائق ببغداد ٧٠٩ لسنة ٢٠٠٠

الهيئة الأستشارية

عزل وتشخيص فايروس الخلية العملاقة من الاطفال الرضع مع دراسة الامراضية النسيجية للفايروس المعزول

شوني ميخائيل اوديشو [1] , أنطوان صبري البنا [1] , ناهي يوسف ياسين [2]

الخلاصة

خلفية الدراسة: فايروس الخلية العلاقة التنفسي من المسببات الرئيسية للاصابات التنفسية في الاطفال حديثي الولادة ويعتبر من العوامل المؤدية للربو نتيجة الاصابة .
هدف الدراسة: هو عزل الفايروس من حالات التهاب القصبات الحاد وإلتهابات رئوية لاطفال حديثي الولادة ودراسة الامراضية في الحيوانات المختبرية.
طريقة العمل:مررت نماذج مرضية موجبة لوجود المستضد الفايروس باستعمال الفحص السريع في خلاية الزرع النسيجي للتمرير السادس واستعمل التعادل المصلي ولاستشعاع المناعي لتشخيص الفايروس المعزول.
الفئران عمر 10 اسابيع قسمت الى مجموعتين الاولى حقنت 100TCID50 من الفايروس المعزول والثانية ببيئة زرعية كسيطرة حيث تم تقطير 0.5 مل من النماذج في الانف ثم اخذت النماذج من الرئة لدراسة الامراضية وعزل الفايروس من اليوم الثاني الى اليوم السابع بعد الحقن.
النتائــج: تم عزل فايروس الخلية العملاقة التنفسي البشري في خلايا الخط الزرعي من نماذج مرضية جمعت من اطفال يعانون من اصابات تنفسية شديدة، حيث كانت النماذج موجبة لوجود المستضد الفيروسي في فحص Respi-Strip، وتم ملاحظة التاثيرات المرضية لاول مرة في التمرير الثالث التي تميزت بظهور الخلية العملاقة بعد مرور 3 أيام من الحقن والتي تطورت بالتمرير في الخلايةالمصابة في التمرير الخامس والسادس من الحقن. كما تم تشخيص الفيروس وباستعمال امصال ممنعة قياسية وبواسطة فحص الاستشعاع المناعي غير المباشر والتعادل المصلي .
ولقد اظهرت الاصابة التجريبية في الفئران بفيروس الخلية العملاقة التنفسي البشري تغيرات نسجية مرضية في الرئات المحقونة تميزت بظهور علامات الاصابة بالتهاب رئوي حاد ، بالاضافة الى اعادة عزل كلا الفيروس من النماذج الرئوية المصابة في الايام من 2-7 من الاصابة التجريبية.
الاستنتاج:ان فايروس الخلية العملاقة يتكاثر في خلاية HEP-2 حيث تظهر التاثيرات المرضية في التمرير الثالث, كم ان الفايروس يصيب الفئران ويؤدي الى ظهور تاثيرات نسيجيةمع امكانيةعزل الفايروس.
مفتاح الكلمات:فايروس الخلية العملاقة, HEP-2 ,التعادل المصلي.

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الدور الاحتمالي للمدورات الخلوية البين بيضاضي 1 –الفا وعامل تنخر الورم-الفا في سرطان الثدي

احمد عبد الحسن عباس الحسن[1] , نضال عبد المهيمن[1] , علاء غني حسين[2] , لين خلوق مصطفى[3], إيمان شاكر العبيدي[3]

الخلاصة

خلفية الدراسة: تستخدم المدورات الخلوية كعلامات بايولوجية في البحث لبيان مال المرض وارتبطت بالاعراض والنتائج المضادة في حالات عديدة من ضمنها سرطان الثدي.
تستخدم المدورات الخلوية كعلامات بايولوجية في البحوث لبيان مأل المرض حيث وجد بان لها علاقة بالاعراض السلبية في العديد من الحالات المرضية من ضمنها سرطان الثدي.
هدف الدراسة: لتقييم تركيزالبين بيضاضي1- الفا وعامل تنخر الورم-الفا في مصل مرضى سرطان الثدي مقارنة بمجموعتي السيطرة ولتحديد فيما اذا كان هناك مصاحبة بين المستويات المصلية لهذه المدورات الخلوية مع تقدم المرض.
الاشخاص وطرائق العمل: قيست المستويات المصلية للبين بيضاضي1- الفا وعامل تنخر الورم-الفا بتقنية فحص مترابطة الخميرة بمادة ماصة المناعة (ELISA) لـ 45 مريضة مصابة بسرطان الثدي, 12 مريضة مصابة بامراض الثدي الحميدة و 23 امراة سليمة ظاهريا كمجموعة ضابطه.
النتائج:أظهرت الدراسة الحالية ارتفاع احصائي مهم للمستويات المصلية للـ البين بيضاضي1- الفا وعامل تنخر الورم-الفا في مرضى سرطان الثدي مقارنة بمجموعتي السيطرة ($p<0.001$) , هذا الارتفاع كان مصاحبا وبشكل مهم احصائيا للمراحل المتقدمة للمرض والحالة السالبة لمستقبلات الاستروجين والبروجيستيرون.
الاستنتاجات: تقييم المستوى المصلي للبين بيضاضي1- الفا وعامل تنخر الورم-الفا قد يكون مساعد كفحوصات غير متدخلة تنبؤية لتطور الورم في مرضى سرطان الثدي.
مفتاح الكلمات:سرطان الثدي, البين بيضاضي1- الفا, عامل تنخر الورم-الفا .

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فعالية مختلف الطرق المستعملة في علاج الرعاف

هيوا أسعد عبد الكريم

الخلاصة

خلفية الدراسة: هذه دراسة مستقبلية، عشوائية، سريرية أجريت في قسم الأذن والأنف والحنجرة، في مستشفى السليمانية التعليمي، للفترة من 15 تموز 2004م إلى 15 نيسان 2005م.

هدف الدراسة: الهدف من الدراسة هو تحليل التوزيع السكاني، العوامل المسببة و سبل علاج الرعاف في منطقة السليمانية، لتطويرخبراتنا في مجال علاج هذه الحالة الشائعة.

طريقة العمل: شملت هذه الدراسة 100 مريض من مختلف الأعمار ومن الجنسين، الذين راجعوا قسم الأذن والأنف والحنجرة ضمن فترة الدراسة.

النتائج: وقد تبين لنا في نهاية الدراسة أن معظم حالات الرعاف يمكن علاجها بوسائل بسيطة مثل الأستعمال الموضعي للمرطبات، أو كوي و حشو الأنف، بأقل المضاعفات0

الاستنتاج: يجب إجراء تداخلات أخرى اكثر فعالية عند الضرورة.

مفتاح الكلمات: الرعاف, المرطبات , كوي الانف , حشوة الانف الامامية, التداخل الفعال .

فرع الجراحة/ أذن وأنف وحنجرة[كلية الطب ـ جامعة السليمانية]

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تقيم تأثير طريقة ألولادة على ألمؤشرات الدموية عند 300 طفل صحي حديث الولادة فى بغداد

صبح سالم المدلل[1] , منى عبد المعين الحبوبى[2]

الخلاصة

خلفية الدراسة: ان القيم الدموية عند الولادة تشمل مدى واسع و طبيعي اكثر من اي وقت آخر من الحياة. و بالرغم من التطور الحاصل في علم حوالي الولادة في السنوات الأخيرة, إلا ان تأثير عوامل ما حول الولادة على القيم الدموية من الحبل السري في الحمل الطبيعي غير واضحة.

هدف الدراسة:

1- وضع حقائق أوليه للمؤشرات الدموية لحديثي الولاده الأصحاء.

و مقارنة النتائج بين حديثي الولاده المولودين بصورة طبيعية أو عملية قيصرية أو عمليه قيصريه بعد فشل الولاده الطبيعيه

2- تقييم تأثير مدة الحمل , تمزق الأغشية , مدة الولادة و الوزن عند الولادة على بعض المؤشرات الدموية.

المواد وطرق العمل: أجريت هذه الدراسة المنظورة على 300 طفل عراقي معافا حديثي الولادة , ولدوا في مستشفى الكاظمية التعليمي في الفترة ما بين تشرين الأول /2007 و كانون الثاني /2008 , و قد قسموا الى ثلاث مجاميع:-

1- المجموعة الأولى و تضم 200 طفل حديثي الولادة ولدوا بصورة طبيعية [100 ذكور (50%) و 100 إناث (50%)].

2- المجموعة الثانية و تضم 80 طفل حديثي الولادة ولدوا بعملية قيصرية منتخبه [30 ذكور (37%) و 50 إناث (63%)].

3- المجموعةالثالثة و تضم 20 طفل حديثي الولادة ولدوا بعملية قيصرية بعد فشل الولادة [18 ذكور (90%) و 2 إناث (10%)].

من كل وليد تم سحب خمسة ملي لتر من الدم الوريدي للحبل السري و اجريت فحوصات المؤشرات الدموية بواسطة جهاز المحلل الدموي التلقائي, صورة الدم , تعداد الكريات الشبكية و فصيلة الدم للطفل و الأم (يدويا), بالأضافة الى المسح الأحصائي الذي اجري على نتائج هذه الدراسة.

النتائج: لقد بينت هذه الدراسة ان هناك نقصان حقيقي هام في العدد الكلي للكريات البيض و الكريات شريطية النواة العدلة عند حديثي الولادة بعملية قيصرية منتخبه عن عددها عند حديثي الولادة بصورة طبيعية أو بعملية قيصرية بعد فشل الولادة.

علاوة على ذلك ان تعداد الكريات الشبكية و تعداد الكريات الحمراء ذوات النواة عند حديثي الولادة بعملية قيصرية منتخبه أو بصورة طبيعية كان هناك نقصان حقيقي هام عن عددها عند حديثي الولاده بعملية قيصرية بعد فشل الولادة. بينما كان هناك نقصان حقيقي هام في (RDW)

عند حديثي الولادة بصورة طبيعية عن عددها عند حديثي الولادة بعملية قيصرية منتخبه أو بعملية قيصرية بعد فشل الولادة.

لم تكشف هذه الدراسة عن وجود علاقة هامة بين [مدة الحمل , تمزق الأغشية , مدة الولادة, Apgar scores و الوزن عند الولادة] و المؤشرات الدموية للحبل السري.

الاستنتاج:

1- لقد بينت هذه الدراسة ان هناك نقصان حقيقي هام في العدد الكلي للكريات البيض و الكريات شريطية النواة العدلة عند حديثي الولادة بعملية قيصرية منتخبه عن عددها عند حديثي الولادة بصورة طبيعية أو بعملية قيصرية بعد فشل الولادة.
2- ان طريقة الولادة لها تأثيرفي نقصان حقيقي هام في (RDW) عند حديثي الولادة بصورة طبيعية عن عددها عند حديثي الولادة بعملية قيصرية منتخبه أو بعملية قيصرية بعد فشل الولادة.
3- ان طريقة الولادة لها تأثيرفي نقصان حقيقي هام في تعداد الكريات الشبكية و تعداد الكريات الحمراء ذوات النواة عند حديثي الولادة بعملية قيصرية منتخبه أو بصورة طبيعية كان هناك نقصان حقيقي هام عن عددها عند حديثي الولاده بعملية قيصرية بعد فشل الولادة.

مفتاح الكلمات: المؤشرات الدموية , طريقة الولادة , حديثى الولادة

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الكشف عن بروتين BCR-ABL باستخدام تقنية التصبيغ المناعي الخلوي

ميساء عبد الرزاق ضاحي[1] , نضال عبد المهيمن[1] , نبيل سلمان مراد[2]

الخلاصة

خلفيـة الدراسـة: يترافـق ابيضـاض الـدم النخـاعيني المـزمن مـع خلـل كروموسـومي يعـرف بكروموسوم فيلادلفيا (Philadelphia chromosome) الذي يتواجد بنسبة اكثر من 95% في مرضى ابيضاض الدم النخاعيني المزمن . يعد كروموسوم فيلادلفيا و الجين المدمج (BCR-ABL fusion gene)الناتج عن هذا الخلل الكروموسومي كمؤشرمميزلهذا النوع من ابيضاض الدم , حيث يشفر هذا الجين المدمج لبروتين مسرطن يعرف(BCR-ABL oncoprotein) وهو ذو فعالية انزيمية مستمرة النشاط لانزيم تيروسن كاينيزتؤدي الى تنشيط مستمرلعدد من مسارات نقل الاشارة داخل الخلية مما يؤدي الى انقسام خلوي غير مسيطر عليه وتثبيط للموت المبرمج للخلية المنتجة لهذا البروتين.

هدف ألدراسة: التحري عن وجود بروتين (BCR-ABL) في عينات دم من مرضى ابيضاض الدم النخاعيني المزمن و عينات من مرضى انواع اخرى من ابيضاض الدم باستخدام تقنية التصبيغ المناعي الخلوي.

طريقة العمل: جمعت عينات من الدم الوريدي من مرضى ابيضاض الدم النخاعيني المزمن(42 عينة), مرضى مصابين بابيضاض الدم اللمفي الحاد(10عينة) ، مرضى مصابين بابيضاض الدم النخاعيني الحاد (2 عينة)، مريض مصاب بابيضاض الدم النخاعيني لوحيدات الخلايا المزمن(1 عينة) و8 عينات لأشخاص اصحاء. عند البدء، عزلت الخلايا اللمفية من الدم الوريدي المحفوظ بالهيبارين وحضرت مسحات منها وثبتت على الشرائح المشحونة بشحنة موجبة. استخدمت الاجسام المضادة وحيدة النسيلة النوعية لبروتين BCR-ABL كضد اولي للكشف عن وجود هذا البروتين.

النتائج: اظهرت النتائج ان جميع مرضى ابيضاض الدم النخاعيني المزمن موجبين لهذا البروتين بينما المرضى المصابين بأنواع اخرى من ابيضاض الدم والاشخاص الاصحاء سالبين لهذا البروتين.

الإستنتاج : يمكن استخدام تقنية التصبيغ المناعي الخلوي في التشخيص النوعي الاولي لوجود البروتين المسرطن في المرضى الذين يشك باصابتهم بابيضاض الدم النخاعيني المزمن.

مفتاح الكلمات: ابيضاض الدم النخاعيني المزمن , بروتين BCR/ABL , تقنية التصبيغ المناعي الخلوي.

[1]فرع الأحياء المجهرية [كلية الطب - جامعة النهرين]
[2]فرع الطب الباطني [كلية الطب - جامعة النهرين]

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الكالسيوم الآيوني المُقاس و الكالسيوم الآيوني الحقيقي لدى الحوامل المصابات بارتفاع ضغط الدم (قبل الشنج)

فيصل غازي الربيعي

الخلاصة

خلفية الدراسة: ضغط الدم العالي لدى الحوامل (بريِ إكلامبسيا أو قبل الشنج) هو نوع من إرتفاع ضغط الدم يظهر أثناء الحمل؛ ومع ذلك سبب هذا الارتفاع غيرمعلوم. علاوة على ذلك، فأن حالة الكالسيوم الايوني خلال الحمل ومضاعفاته (إرتفاع ضغط الدم المصاحب للحمل " بريِ إكلامبسيا أو قبل الشنج") لم توصف بشكل كامل. اضافة الى الطريقة الحسابية المستعملة لقياس الكالسيوم الايوني، يعتبر ارتباط صبغة الميوركساید بالكالسيوم وسيلة مستعملة بشكل واسع لقياس تغيرات تركيزالكالسيوم الايوني في الأنظمة الأحيائية.

هدف ألدراسة: هو لبيان مستوى الكالسيوم الايوني في حالة ارتفاع ضغط الدم المصاحب للحمل (بريِ إكلامبسيا أو قبل الشنج) وعلاقته مع الحمل الطبيعي؛ ولبيان الأرتباط بين الكالسيوم الايوني المقاس و الكالسيوم الايوني الحقيقي.

طريقة العمل: تتضمن دراسة لعينة ضابطة اجريت خلال الفترة من شباط 2007 لغاية حزيران من نفس السنة وتشمل قياس الكالسيوم الكلي-المصحح, والآيوني لدى 60 حاملا" مصابة بارتفاع ضغط الدم المصاحب للحمل (مجموعة الأختبار) وتم تصنيفهم حسب عمر الحمل الى حوامل مصابات بارتفاع ضغط الدم المصاحب للحمل (قبل الشنج) خلال الفصل الثاني من الحمل (العدد30 مريضة) وحوامل مصابات بارتفاع ضغط الدم المصاحب للحمل (قبل الشنج) خلال الفصل الثالث من الحمل (العدد30 مريضة).

تم مقارنة النتائج مع نتائج 60 حاملة" سليمة" ظاهريا"(مجموعة السيطرة)، وتم تقسيم مجموعة السيطرة اعتمادا" على عمر الحمل الى حوامل اصحاء ظاهريا" خلال الفصل الثاني من الحمل (العدد30 مريضة) وحوامل اصحاء ظاهريا" خلال الفصل الثالث من الحمل (العدد30 مريضة).

النتائج: أظهرت انخفاضا" معنويا" في مستوى الكالسيوم الكلي-المصحح والآيوني في المصل لدى الحوامل ذوات ضغط الدم العالي المصاحب للحمل (قبل الشنج) خلال الفصل الثالث من الحمل لدى مقارنتهم بمجموعة السيطرة المناظرة. نفس الانخفاض المعنوي في مستوى الكالسيوم الكلي- المصحح وليس الآيوني في المصل لوحظ لدى الحوامل ذوات ضغط الدم العالي المصاحب للحمل (قبل الشنج) خلال الفصل الثاني من الحمل لدى مقارنتهم بمجموعة السيطرة المناظرة.كما اظهرت النتائج ارتباطا معنويا" بين الكالسيوم الايوني المقاس و الكالسيوم الايوني الحقيقي.

الإستنتاج: أن الحوامل ذوات ضغط الدم المرتفع المصاحب للحمل (قبل الشنج) توجد لديهم بعض العوامل التي تثبط توسع الاوعية الدموية وتزيد من انقباضها ومما يدل على هذا وجود انخفاض معنوي في الكالسيوم الآيوني (الذي يعتبر ضروريا" لتكوين اوكسيد النتريك المشتق من بطانة الاوعية الدموية). كما يمكن استعمال معادلة رياضية في الممارسة السريرية لحساب الكالسيوم الآيوني.

مفتاح الكلمات: قبل الشنج، الكالسيوم الآيوني المُقاس، الكالسيوم الآيوني الحقيقي.

فرع الكيمياء و الكيمياء الحياتية [كلية الطب - جامعة النهرين]

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تقييم مؤشرات الضررالتأكسدي للحامض النووي د ن أ في اورام الثدي لدى النساء

استبرق عبد الرسول الواسطي [1],نجاة عبد الرزاق حسن[1],أنعم رشيد الصالحي[2]

الخلاصة

خلفية الدراسة: إن مستوى الضررِ في الـ د.ن.أ. يَعْكِسُ ألتوازن بين ألإجهادِ ألتأكسدي والقابلية على ترميمِ الـ د.ن.أ. المصاحب لسرطان الثدي.

هدف ألدراسة: تهدف الدراسةِ إلى تقييمَ ضررِ الـ د.ن.أ. المتمثل بمدى الضرر في شريط الـ د.ن.أ. المفرد من خلال إستخدام إختبار "المذنب" و مستويات 8-هيدروكسي 2-دي أوكسي ألكوانوسين و التي هي ضرب من ضروب ضرر الـ د.ن.أ. التأكسدي ومقياس عددي لضرر الـ د.ن.أ.

طريقة العمل: أجريت الفحوصات على نماذج من كريات الدم البيضاء والورم المستحصل من (40) حالة مشخصة حديثا و غير معالجة سابقا لدى المصابات بأورام الثدي تتراوح أعمارهن بين 24-75 سنة. كما وأجريت فحوصات مماثلة على كريات الدم البيضاء لمجموعة سيطرة مكونة من (40) أمرأة صحيحة .

تضمن إختبار المذنب, الترحيل الكهربائي للخلايا و اعتماد تحليل الصورة بالحاسوب لغرض قياس طول ذيل المذنب و مقدار عزم ذيل المذنب. أما تركيز 8-هيدروكسي 2-دي أوكسي الكوانوسين فقد قيس بتقنية الممتز المناعي المرتبط بألانزيم وتم إحتساب إعداد الضررفي الـ د.ن.أ. مقاسا نسبة الى قواعد الـ د.ن.أ وفق معادلة خاصة.

النتائج:أظهرت النتائج فروقا معنوية بين مجموعة السيطرة و مجموعة الدراسة في ما يتعلق بطول و عزم المذنب ومستويات 8-هيدروكسي2-دي اوكسي الكوانوسين, اضافة الى تضرر الـ د.ن.أ. في كريات الدم البيضاء بالمقارنة بمجموعة السيطرة وعلى وجه ألخصوص فقد كانت الفروق واضحة بين اورام الثدي الحميدة ($P<0.001$) وأورام الثدي الخبيثة ($P<0.001$).

كما سجل ارتفاعا في مستويات 8-هيدروكسي2-دي اوكسي الكوانوسين بشكل معتمد في أورام ألثدي ألخبيثة المغيرة بالمقارنة مع أورام الثدي الخبيثة غير المغيرة ($P<0.001$) وأن تلك المستويات تفوق قيمتها في الاورام الخبيثة مقارنة بألاورام الحميدة.

أن معدلات طول و عزم المذنب, و مستويات 8-هيدروكسي2-دي اوكسي الكوانوسين في كريات الدم البيضاء اظهرت ارتفاعا بيّنًا في الاعمار (48 ≤سنة) و دليل كتلة الجسم (24<) مقارنة مع ألقياسات ألمماثلة بالعمر أو دليل كتلة الجسم ,$p<0.05$ ($P<0.001$ على التوالي).

كما وجد ارتباطا معنويا بيّنا لمستويات ال 8-هيدروكسي2-دي اوكسي الكوانوسين مع مستويات طول و عزم المذنب في كل من كريات الدم البيضاء ($r=0.71, r=0.83; P<0.001$) وخلايا النسيج السرطاني ($r=0.69, r=0.83; P<0.001$) في جميع أورام ألثدي.

الاستنتاجات:إن إختبار المذنب و قياس تضرر الـ د.ن.أ. مقاسا إلى قواعد الـ د.ن.أ. هي مؤشرات مفيدة وحساسة للضرر التاكسدي الحاصل في الـ د.ن.أ. وبالذات تكسرات الشريط المفرد للـ د.ن.أ. في أورام الثدي, وبالامكان تطويرها لتكون أساسا لتحديد نمطية الاستعداد لسرطان الثدي.
مفتاح الكلمات: إختبار المذنب, مستويات 8-هيدروكسي 2-دي أوكسي ألكوانوسين , ضرر الـ د.ن.أ. التأكسدي , أورام الثدي.

[1]فرع الكيمياء و الكيمياء الحياتية [كلية الطب ـ جامعة النهرين]
[2]مركز أبحاث الأجنة وعلاج العقم ـ جامعة النهرين

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فعالية طريقة تعليقة تحت الاحليل باستخدام لفافة العضلة المستقيمة في علاج سلس البول الاجهادي

لقاء رياض الخزاعي

الخلاصة

خلفية الدراسة: هناك طرق جراحية متعددة في علاج سلس البول الاجهادي عند النساء تجعل الجراح محتارا في اختيار العملية المناسبة .

هدف ألدراسة: هو تقييم النتائج الجراحية بعد عملية تعليقة تحت الاحليل باستخدام شريحة من لفافة العضلة المستقيمة في شفاء سلس البول الاجهادي عند النساء.

طريقة العمل : تم اجراء دراسة تطلعية في الفترة ما بين حزيران 2005 و كانون الثاني 2007 باجراء عملية تعليقة تحت الاحليل على عشر نساء يشتكين من سلس البول الاجهادي مبين بفحص السعال الموجب و فحص قياس ضغط المثانة بطريقة الملأ و الاحليل المفرط الحركة باستخدام طريقة الاجهاد مع وضع ماسحة القطن في الزاوية بين الاحليل و المثانة بوجود زاوية مساوية او اكثر من 30 درجة مع الخط الافقي .

اجريت عملية التعليقة المصلبة تحت الاحليل باستخدام شريحة من لفافة العضلة المستقيمة . تم تقييم مواصفات المريضات, المضاعفات خلال و بعد العملية و تقييم درجة الشفاء الشخصي لسلس البول بعد ثلاثة اشهر من العملية .

النتائج : معدل العمر كان 42 سنة و متوسط الانجابية كان اربعة, احتباس البول الحاد حدث في مريضة واحدة فقط.التهاب المجاري البولية الحاد حصل في ثلاث مريضات بعد العملية. الفحص التتبعي بعد ثلاثة اشهر اظهر ان 80 % من النساء اقررن بالشفاء من سلس البول . احدى المريضات لاحظت تحسن في حالتها. مريضة واحدة فقط لم يتم شفاء سلس البول الانضغاطي لديها.

الاستنتاجات: تدل هذه الدراسة على فعالية التعليقة المصلبة تحت الاحليل باستخدام شريحة من العضلة المستقيمة في علاج سلس البول الاجهادي عند النساء.

مفتاح الكلمات : تعليقة تحت الاحليل, لفافة العضلة المستقيمة , سلس البول الاجهادي.

فرع النسائية والتوليد [كلية الطب ـ جامعة النهرين]

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حالة ستروما المبيض مع مراجعه للبحوث

" تقرير حالة "

أمير كاظم ظاهر[1], مهى عاصم كريم[2],رمزي كاظم البياتي[3]

الخلاصة

ستروما المبيض هو نوع خاص جدا من مثاني المبيض(تراتوما) ويتميز بأحتوائه كليا او جزئيا على نسيج الغده الدرقيه. ومن مضاعفاته المهمه ولو كانت نادره هي تحوله الى ورم خبيث او قد يؤدي الى فرط افراز الغده الدرقيه. في هذه الحاله نصف امرأه متزوجه عمرها 25 سنه كانت تعاني من الم متكررفي اسفل البطن وتم تشخيص كيس معقد للمبيض بواسطة فحص البطن بجهاز الامواج الصوتيه (السونار).وكانت نتيجة العمليه كيس معقد للمبيض واجري لها عملية استئصال المبيض ومن خلال الفحص النسيجي تبين وجود نسيج ناضج للغده الدرقيه والذي اثبت تشخيص ستروما المبيض.

مفتاح الكلمات: مثاني المبيض, ستروما المبيض, نسيج الدرقيه

[1]فرع الجراحة [كلية الطب ـ جامعة واسط]
[2]فرع النسائية [كلية الطب ـ جامعة واسط]
[3]دائرة صحة واسط ـ مستشفى الكرامه التعليمي

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