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EXAMPLES

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2. Books: Mann JI, Pyorala K, and Teuscher A. Diabetes in epidemiological perspective. London: Churchill Livingstone. 1983.
3. Chapter in book: Phillips SJ, and Whisnant JP. Hypertension and strock. In: Laragh JH, and Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

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

CONTENTS

Case Report

Editorial:

Toward more Objective Teaching
Small Gruop Teaching

Hikmat Abdul Rasuol *FRCS.*

Small Group Teaching:

Small group teaching is one of variety of education method for promoting student learning and can be more wording experience.

This learning modality is indicative of the movement from a teacher-centred approach to a more student-centred approach.

Its needs to be planed carefully and to develop skills in group management.

The organizer of a course or program has to be clear about the rationales for using small group work and the outcome expected of this method.

The use of 50min lectures and small group may be complementally to the learning process.

Small group teaching is characterized by student participation and interaction.

Ideally effective small group work occurs when there are a small number of students.

It is usually difficult to ensure the participation of large number of students, the number of student in each group depend on experience of tutor.

Numbers in small groups are, however frequently fixed by curriculum demands.

Advantages:

There are advantages of small group teaching over large class:-

- It familiarizes the students with an adult approach to learning.
- It encourages students to take responsibility for their own learning.
- It promotes deeper understanding of material.
- It encourage problem – solving skills.
- Encourage participation.
- It develops:-

1. Interpersonal skills.
2. Communication skill.
3. Social team working skills.
4. Presentation skills.

- It encourages an awareness of different views on issues and has the potential to encourage an attitude of tolerance.

Notwithstanding these advantages, small group work should only be adopted when it is the most efficient approach to achieve these benefits /objective.

There are disadvantages of small group including

The tutor expertise.

The role and the tutor can be crucial of to the success of any small group work.

Staff may be more familiar with tradition mode of teaching and may need training with specific role of small group tutor, preparation need more time and expertise.

Types

- Student – centered discussion /dialog group.
- Structured teacher – centered tutorial group usually focusing on an identified task.
- Between these tow tasks there are a lot of creativity courses.
- Seminars.
- Workshops.
- Clinical skills sessions.
- Communication skills sessions.

Head of National Accreditation Committee of Medical Colleges in Iraq

- Problem- based learning tutorials.
- Clinical teaching sessions

- Ward - based.
- Ambulatory care, outpatient- based.
- Community- based.

These sessions should be integral components of the course content and relate appropriately to the tutor learning offer

For example the week's work may be framed around a patient problem; the lectures and small group work, both theoretical and practical contribute to an understanding of patient problem.

These small group activities must complement the institutions overall curricular strategy, address specific course objectives and enhance the educational program.

The sessions should be seen to be an integral component of the course content.

Preparation of tutor and requirement:

Tutors will wish to confirm the details of their role are perceived by the course organizers and ensure that they are properly briefed on the specific objective of the small group session.

The tutor should be the first to appear.

The success of small group learning may be judged by the extent to which trust is created.

The session:

- The tutor will set the scene, state the objective and suggest some basic ground rules and the session at this and subsequent stages of the session.
- The tutor should visualize the student learning needs specifically from their point of view.
- The tutor may merge with the group.
- During discussion of session these issues should be discussed :-
- Participation of all students.
- Encourage critical thinking.
- Articulation of thoughts.
- Encourage team work.
- Review objectives.
- Summary of achievement.
- Feedback to learners is important and is widely regarded as one of strong things of small group teaching.
- In interconnected sessions (e.g. problem-based model) there will be a need to agree on the topics for discussion at the next session.

Student Role:-

- They are the focus and key figure in any learning events.
- To achieve the benefit , there should be :
- Prior reading.

a. Contributing actively to the conduct of the session and Contributing effectively on the issued raised.

b. Have some rules in assessment and evolution.

Evaluation and Assessment:

This needs careful consideration.

The student should be informed on nature of evaluation whether formative, summative or both.

Performance:

To achieve it, it needs:-

1. Students self reporting.
2. Tutor observation and individual development of each student and contribution to discussion and problem solving.
3. External observation assesses the group process which should be in depth analysis of interaction and frequency and contribution by both students and staff.

The assessment should include:

1. Attendance.
2. Contribution and ideas.
3. Research, analysis and preparation of materials.
4. Support and encouragement of team members and cooperation.
5. Practical contribution and end product.

Course Follow-up:

These courses need to be evaluated as any modulation of teaching and this evaluation will be achieved by:-

a. Evaluation of outcome as far as objective is concern by (OSCE-communication skill).

b. Did the entire group share in conducting the task?

c. Did anyone of group dominate?

Conclusion:

This type of teaching is a powerful education tool and method and its benefit achieved when it is conducted carefully and skillfully and obviously the advantages includes:-

I. Encouragement of independent self learning and critical thinking.

II. To make this modality of teaching successful, it needs a trained staff.

III. Development is an important part of the process.

Preferences:

1. Bright, problem-based, small group learning-British Medical Journal, 1995; 311:342-343.

2. Collins, Brown JS, Newman SE, cognitive apprenticeship, teaching the craft of heading, writing and mathematics in (leswick and bled) knowing learning and in structure essay in honor and Robert Glaser. Lawrence Erlbaum, hillsdale, 1989; NJ, pp.453-494.

3. Healths field in 1990 how to assess group work, the time higher education supplement march 26:40-41.

4. Walton HJ, ASME medical education booklet no.1 small group methods in medical teaching medical education, 1997; 31:437-464.

Possible Role of Th-2 Cell-Related Cytokines (IL-6 and IL-10) in Breast Cancer.

Ahmed A .Al-Hassan[1] *PhD*, **Nidhal Abdul Muhymen[1]** *PhD*, **Ala'a Ghany Hussien[2]** *FICMS*, **Nahla G Al-Khayli[3]** *FICMS*.

Abstract

Background: Breast cancer is a complex disease, many etiological agents are proposed to play a role in its pathogenecity, one of these factors is cytokines.

Objectives: In the present study we measured the concentration of IL-6 and IL-10 in serum of breast cancer patients and examined their association with clinicopathological variables including stages of the disease and estrogen/progesterone receptor (ER, PR) expression on tumor cells, to determine whether it associate with the disease progression.

Subjects and Methods: The study included 80 subjects, it comprised of 45 Breast cancer patients, 12 patients with benign breast lesions and 23 apparently healthy controls. ELISA method has been used for estimation the level of IL-6 and IL-10 in serum of three studied groups.

Results: There was an elevation of IL-6 and IL-10 level in the sera of BC patients with significant differences between BC and controls ($p<0.001$), also, this elevation was associated with progression of the tumor. In addition, IL-6 level was found to be inversely related to ER and PR expression ($P< 0.05$) while in regard IL-10 there was no significant differences in the median of IL-10 level between the patients who express positive and negative ER and PR.

Conclusions: These data indicated that elevated IL-6 and IL-10 serum levels are associated with BC and associate with advanced stage of disease. It was feasible that assays for serum levels of IL-6 and IL-10 can be used as predictive tests for tumor progression in BC patients.

Keywords: Breast cancer, IL-6, IL-10.

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Introduction

Both the innate and acquired arms of the immune system are believed to play crucial roles in the anti-tumor response, it is well known that the interactions of tumor cells with their microenvironment may affect tumor growth and metastasis formation, among these, cytokines were suggested to play role in breast carcinoma [1,2].

Interleukin-6 (IL-6) is a multifunctional protein with multiple biologic activities on a variety of cells.

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It is produced by macrophages, T cells (Th2), B cells, endothelial cells and tumor cells, IL-6 levels have been found to be elevated in several cancers including renal carcinoma [3], ovarian and other gynecological tumors [4], lung cancer [5], and breast cancer [6], it is able to promote tumor growth by upregulating anti apoptotic and angiogenic proteins in tumor cells. IL-6 plays a key role in regulating estrogen synthesis in normal and malignant breast tissues. The activities of estradiol 17 Beta-hydroxysteroid dehydrogenase and estrone sulfatase are all increased by IL-6.

Interleukin-10 is the most potent anti-inflammatory cytokine yet identified. It can also be produced by many types of tumor cells such as colon carcinoma, melanoma cells [7]

and breast cancer [8]. Thus, because of its potential 'protective' effects on tumor cells, particularly via inhibition of specific tumor-reactive cytotoxic T lymphocyte, IL-10 production and secretion may be reasonably supposed to be up-regulated in cancer patients. The current study is a trial to estimate IL-6 and IL-10 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:

IL-6 and IL-10 has been estimated by using a solid phase sandwich enzyme linked immuno sorbent assay (ELISA) (BIOSOURCE, Europe S.A., Belgium, Lot No. 053804; 053303/A).

Statistical analysis

All the data have been analyzed statistically using Kruskall-Wallis test and MannWhitney analysis for measuring the differences between the studying groups [9].

Results:

Estimation of serum level of IL-6

The level of IL-6 in sera of BC patients was significantly higher than the healthy control and patients control group (median = 19 pg /ml; 5.1 pg / ml; 6.6 pg / ml), respectively, ($p<0.001$) as shown in table 1.

The highest concentration of IL-6 was recorded in breast cancer patients with advanced stage -stage III- (median=39 pg/ml) in comparison to other stages (stage 0; stage I and stage II) (median=9.2 pg/ml), ($P < 0.001$), Table 2.

Estimation of serum level of IL-10

The level of IL-10 in sera of BC patients was significantly higher than the healthy control and patients control group (median = 41 pg /ml; 11.1 pg / ml; 6.7 pg / ml)), respectively, ($p<0.001$), as shown in Table 3.

The highest concentration of IL-10 were recorded in breast cancer patients with advanced stage -stage III- (median=59 pg/ml) in comparison to other stages (stage 0; stage I and stage II) (median=15.5 pg/ml), ($P < 0.001$), Table 4.

The association of IL-6 with estrogen and progesterone receptors

The results of association between serum IL-6 level and ER and PR expression in breast cancer samples were shown in tables- 5&6. IL-6 level was indeed found to be inversely correlated to ER and PR expression ($p= <0.05$).

The association of IL-10 with estrogen and progesterone receptors

In regard the correlation between serum IL-10 level and the expression of ER and PR, table- 5&6 revealed no significant differences in the median of IL-10 level between the patients who express positive and negative ER and PR.

Table 1: The difference in median levels of serum IL-6 (pg/ml) concentration among the three studied groups.

Serum IL-6	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	2.4	2	1.5	
Maximum	196.3	75.2	65	
Median	19	6.6	5.1	<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-6 (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	2.4	3.8	
Maximum	120	196.3	
Median	9.2	39	<0.001
NO.	28	17	

Table 3: The difference in median levels of serum IL-10 (pg/ml) concentration among the three studied groups.

Serum IL-10	BC cases	BBL control	Healthy control	P (Kruskall-Wallis)
Minimum	2.6	2.4	0	
Maximum	113.4	54.6	44.1	
Median	41	11.1	6.7	<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 IL-10 (pg/ml) according to the stage of disease.

Values	Stage 0, I& II	Stage III	Mann-Whitney
Minimum	2.6	3.2	
Maximum	69	113.4	
Median	15.5	59	<0.001
NO.	28	17	

Table 5: The difference in median levels of serum IL-6 and 10 (pg/ml) according to the estrogen receptors.

	Estrogen receptor		
	Positive (n=21)	**Negative (n=24)**	**P**
Interleukin-6 conc.			
Range	(2.4 – 61.8)	(13 – 196.3)	
Median	7.9	25.2	<0.05
Interleukin-10 conc.			
Range	(2.6 – 99.6)	(1.6 – 113.4)	
Median	25.8	36.5	>0.05

Table 6: The difference in median levels of serum IL-6 and10 (pg/ml) according to the progesterone receptors.

	Progesterone receptor		
	Positive (n=26)	**Negative (n=19)**	**P**
Interleukin-6 conc.			
Range	(2.4 – 74.8)	(16 – 196.3)	
Median	8.4	26.2	<0.05
Interleukin-10 conc.			
Range	(2.6 – 88.5)	(4.4 – 113.4)	
Median	29.8	33.5	>0.05

Discussion

Regarding Th2- cells- related cytokines (IL-6 and IL-10), current results were in agreement with those of other authors who have demonstrated significantly higher levels of those cytokines in sera of patients with BC than those of control groups [10,11].

Moreover, in the present study there was a positive correlation between clinical stage and the serum levels of both cytokines (IL-6 and IL-I0), this result was inagreement with findings of Ordemann and associates, in (2002), and Kozlowski *et al.,in* 2003 who found that a high levels of IL-6 and IL-10 were frequently observed in stage III than in the other two tumor stages (I and II) [12,13].

Regarding IL-10, it is produced at high concentrations by a wide number of tumor cells, including breast carcinoma, it is a dominant cytokine found in the BC cells environment [14]. Kucharzik *et al., in* (1997) have demonstrated that tumor cell derived TGF-β1 and PGE2 are major factors for IL-10 stimulation [15]. IL-10 may play an important role in tumorigenesis since it can suppress Th1 cells ability to secrete IL-2 and IFN-γ, both essential for an optimal cell-mediated anti-tumor activity [14, 16]. IL-10 does not only affect effectors cells but can lead to diminished expression of MHC molecules by the tumor cells via down regulation of Transporter Associated with Antigen Presentation (TAP1) and (TAP2) proteins of the antigen-processing machinery [17].

Both PGE-2 and IL10 have been shown to suppress antigen presentation, to suppress cytotoxic T cell (CTL) responses, and to inhibit cytokine production by T cells and APC, perhaps most importantly IL12 that plays a central role in initiation and potentiation of cellular immune responses [18, 19].

Cytokines produced by Th2 lymphocytes have been proposed to

promote cell survival by influencing the expression of proteins involved in the regulation of apoptosis. Tumor cells have been previously demonstrated to evade death signals generated by immune effectors or by therapeutic drugs through the development of effective antiapoptotic mechanism such as increased levels of caspase inhibitors or Bcl-2-family members [20].

Among the various prognostic factors, lack of estrogen and progesterone receptors has consistently been associated with poorer prognosis [21]. Of particular note, in present study we found an inverse correlation between expression of ER&PR and IL-6 serum levels, which is in agreement with the findings of other studies [22, 23]. On the other hand, we observed that IL-10 was not correlated with ER and PR status, which is in disagreed with findings of some other studies [24, 25].

The inverse correlation between IL- 6 and ER&PR indicates that the high serum levels of this cytokine 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-6 serum levels would predict poor outcome in BC.

This inverse correlation between IL-6 and ER status not only may reflect the greater aggressiveness of this subtype of breast tumors but it could also be the result of a direct regulation of cytokine expression by ER. Several reports have demonstrated a direct down regulation of cytokines by ER in different organs. This is not only the case for IL-6 [26] but also for IL-1 and TNF-α [26]. Progesterone receptors are also known to down regulate the expression of a number of cytokines, including IL-1 [27] IL-6 [28] IL-8 [29] and TNF-α [27].

Purohit *et al., in* (2002) confirmed these studies and claimed that IL-6 secretion is inhibited by estrogen synthesis in peripheral tissues, including normal and malignant breast tissues. Interestingly, they found that macrophages and lymphocytes which invade many breast tumors are important source of factors that can stimulate estrogen synthesis in malignant breast tissues which explains the high concentrations of estrogen present in breast tumors [30].

On the other hand, Chiu *et al., in* (2000), on their study on normal and transformed mammary epithelial cells reported that IL-6 secretion inhibited the growth of ER positive breast cancer cell lines. In contrast, ER negative breast cancer cell lines were resistant to IL-6 mediated growth of normal and transformed human mammary epithelial cells [31].

References

1. Ben-Baruch A. Host microenvironment in breast cancer development: Inflammatory cells, cytokines and chemokines in breast cancer progression reciprocal tumor-microenvironment interactions. Breast Cancer Res 2003; 5:31-36.

2. Chavey C, Bibeau F, Gourgou-Bourgade S, Burlinchon S, Biorence B, Laune D, et al. Oestrogen receptor negative breast cancers exhibit high cytokine content. Breast Cancer Res 2007; 9(1):R15.

3. Blay JY, Negrier S, Combaret V. Serum levels of interleukin-6 as a prognosis factor in metastatic renal cell carcinoma. Cancer Res, 1992; 52: 3317-22.

4. Scambia G, Testa U, Benedetti P. Prognostic significance of IL-6 serum levels in patients with ovarian cancer. Br J Cancer, 1995; 71:354-6.

5. Yamaguchi T, Yamamoto Y, Yokota S. Involvement of interleukin-6 in the elevation of plasma fibrinogen levels in lung cancer patients Japanese. Journal of Clinical Oncology, 1998; pp: 740-744.

6. Kuang, Y, Zhang Z, Zhang X. Interleukin-6 and its soluble receptors in human breast cancer. Chung Hua Chung Liu Tsa Chill, 1998; 20(4): 305-7.

7. Gastl GA, Abrams JS, Nanus DM. Interleukin-l0 production by human carcinoma cell lines and its relationship to interleukin expression. Int J Cancer ,1993; 55: 96-101.

8. Venetsanakos E. High incidence of interleukin 10 mRNA but not interleukin 2

mRNA detected in human breast tumours British. Journal of Cancer, 1997; 75:1826.
9. Sorlie DE. Medical biostatistics and epidemiology: Examination and Board review First ed, Norwalk, Connecticut, Appleton and Lange, 1995; 47-88.
10. Elsasser-Beile U, Kolble N, Grussenmeyer T, Schultze-Seemann W, Wetterauer U, Gallati H, Schulte Monting J, von Kleist S. Th1 and Th2 cytokine response patterns in leukocyte cultures of patients with urinary bladder, renal cell and prostate carcinomas. Tumour Biol (1998); 19:470.
11. Wise GJ, Marella VK, Talluri G, Shirazian D. Cytokine variations in patients with hormone treated prostate. Cancer J Urol, 2000; 164:722.
12. Ordemann J, Jacobic A, Braumann C, Schwenk W, Volk HD, Muller JM. "Immunomodulatory changes in patients with CRC. Int J Colorectal Dis, 2002; 17: 37-41.
13. Kozlowski L, Zakrzewska I, Tokajuk P, Wojtukiewicz M Z. Concentration of interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) in blood serum of breast cancer patients. Rocz Akad Med Bialymst, 2003; 48:82-84.
14. Wong PY, Staren, ED, Tereshkova N, Braun DP. Functional analysis of tumor infiltrating leukocytes in breast cancer patients. J Surg Res, 1998; 76 1, pp 95–103.
15. Kucharzik T, Lugering N, Wide G, Domschke W, Stoll R. Colon carcinoma cell lines stimulate monocytes and lamina propria mononuclear cells to produce IL-10. Clin Exp Immunol, 1997; 110 (2) pp 296–302.
16. Moore KW, O'Garra A, de-Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol, 1993; 11:165–90.
17. Zeidler R, Eissner G, Meissner P, Uebel, S, Tampe, R, Lazis S. Downregulation of TAPI in B lymphocytes by cellular and Epstein- Barr virus encoded interleukin- 10. Blood, 1997; 90, pp 2390-2397.
18. Van-der P, Kraan TC, Boeije LC, Smeenk RJ, Wijdenes J, Aarden LA. Prostaglandin-E2 is a potent inhibitor of human interleukin 12 production J Exp Med, 1995; 181: 775–9.
19. Beissert S, Hosoi J, Grabbe S, Asahina A, Granstein RD. IL-10 inhibits tumor antigen presentation by epidermal antigen-presenting cells. J Immunol, 1995; 154:1280.
20. Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol, 1999; 17:2941.
21. 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.
22. Singer CF, Kronsteiner N, Hudelist G, Marton E, Walter I, Kubista M, et al. Interleukin 1 system and sex steroid receptor expression in human breast cancer: interleukin 1 alpha protein secretion is correlated with malignant phenotype. Clin Cancer Res; 2003; 9:4877-4883.
23. Chiu JJ, Sgagias MK, Cowan KH. Interleukin 6 acts as a paracrine growth factor in human mammary carcinoma cell lines. Clin Cancer Res, 1996; 2:215-221.
24. Carruba G, D'Agostino P, Miele M, Calabro M, Barbera C, Bella GD,et al. Estrogen regulates cytokine production and apoptosis in PMA-differentiated, macrophage-like U937 cells. J Cell Biochem, 2003; 90:187-196.
25. Curran EM, Judy BM, Newton LG, Lubahn DB, Rottinghaus GE, Macdonald R S, et al. Dietary soy phytoestrogens and ERalpha signalling modulate interferon gamma production in response to bacterial infection. Clin Exp Immunol, 2004; 135:219-225.
26. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocr Rev, 2002; 23:90-119.
27. Davies S, Dai D, Wolf DM, Leslie KK. Immunomodulatory and transcriptional effects of progesterone through progesterone A and B receptors in Hec50co poorly differentiated endometrial cancer cells. J Soc Gynecol Investig ,2004; 11:494-499.
28. Kanda N, Watanabe S. 17Beta-estradiol inhibits MCP-1 production in human keratinocytes. J Invest Dermatol ,2003; 120:1058-1066.
29. Loudon JA, Elliott CL, Hills F, Bennett PR. Progesterone represses interleukin-8 and cyclo-oxygenase-2 in human lower segment fibroblast cells and amnion epithelial cells. Biol Reprod, 2003; 69:331-337.
30. Purohit A, Newman SP, Reed MJ. The role of Cytokines in regulating estrogen syntheses: implications for the etiology of breast cancer. Breast Cancer Res, 2002; 4(2): 65-69.
31. Chiu JJ, Sgagias MK, Cowan KH. Interleukin – 6 acts as a paracrine growth factor in human mammary carcinoma cell lines. Clin Cancer Res, 2000; 2 (1): 215-221.

Relationship of Peripheral Blood Lymphocytes Immune Alteration Phenotype to Disease Activity in Rheumatoid Arthritis Patients.

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Abstract

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disorder causing synovitis of diarthroidal joints in which activated T-lymphocytes has a prominent position in RA pathology and may be important in prediction of disease outcome.

Objective: To evaluate cellular expression of certain activation marker on PBLs and their relevance to disease activity pattern of RA patients.

Patients and methods: this study included forty six RA patients, seven patients with Osteo-arthritis (OA) and 10 apparently healthy individuals. The collection of our baseline data based on routine laboratory and clinical assessment of disease activity Score (DAS). Blood sample was taken from each subject in all groups, at the time of attendance. Lymphocytes were separated; slides were prepared fixed on charged slides, foiled, and kept at -20ºC until assayed. CD3 and CD54 expression was detected using Immunocytochemistry staining, while CD71was detected using direct immunoflouresence staining.

Results: The results of CD3 and CD54 revealed a statistically higher percentage of expression in rheumatoid arthritis patients when compared with that of the apparently healthy and OA control groups. The CD71 showed statistically significant higher expression in minimum disease activity group without any correlation with clinical and laboratory disease activity indices.

Conclusions: we provide further evidence of a T-cell differentiation defect in RA, which could explain some of the well-characterized immunologic features of the disease but it is not related to the disease activity state.

Keywords: Rheumatoid arthritis, disease activity, lymphocytes activation, immunocytochemistry, immunofluorescence and CD marker.

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Introduction

Rheumatoid arthritis (RA) is a quintessential autoimmune disease with a growing number of cells, mediators, and pathways implicated in this tissue-injurious inflammation [(1)]. It's strongly suggested that it was driven by specific T-cell-mediated cellular immunity against self-antigens and the T-cell-mediated cellular immunity was proposed to be involved in RA pathology [(2, 3)].

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface molecule that have been expressed upon lymphocyte activation, ICAM-1 gene expression on T cells regulated by Phosphotyrosyl Phosphatase Activity [(4)], and only mitogens or specific cytokines increase its expression on T lymphocytes [(5-7)].

The membrane glycoprotein transferrin receptor (TfR, CD71), In addition to being an iron transporter, it has been shown to play a role in T cell activation. Stimulation of the TfR with specific Abs results in T cell proliferation, IL-2 secretion, and protein kinase C activation. It appears to play as a costimulatory role in T cell activation [(8,9)].

Those markers were reported to play an important role not only in T cell recruitment, but also in T cell activation, proliferation, and in the development of specific immune responses. Furthermore, circulating

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leukocytes provide an important source for biomarker discovery for RA. In contrast to target tissue biopsy based approaches, which are often limited by restricted access to target tissues, profiling peripheral blood cells has emerged as an attractive biomarker discovery strategy [10, 11].

This study aimed to investigate the expression of immune alteration phenotype on PBL, like the expression of CD54 and CD71 and to correlate the results of PBL-alteration phenotype expression with different disease activity patterns.

Materials and methods

Subjects:

The present study groups consisted of 46 Iraqi patients with RA fulfilled the ACR classification criteria [12]. They were recruited from the out-patient clinic at the Department of Rheumatology and Rehabilitation, Al-Kadhumyia Teaching Hospital in Baghdad. Also 7 age-and sex-matched osteo-arthritis patients and 10 apparently healthy controls were enrolled in the study. These controls were healthy blood donors.

The scoring system of the present disease activity was done according to modified DAS28-3. It combines of both clinical and laboratory parameters. The clinical examination of joint swelling and tenderness was performed for 28 joints (include the same joints: shoulders, elbows, wrists, metacarpophalangeal joints, proximal interphalangeal joints and the knees [13].

General laboratory and immunolaboratory assessments included erythrocyte sedimentation rate, C-reactive protein, and RF. Clinical and laboratory characteristics of the patient included in the study are summarized in Table 1.

Blood samples:

A Blood sample (5 ml venous blood) was aspirated from a suitable vein from all patients and unaffected controls. Blood was collected in pyrogen-free silicone-coated tubes with heparin. The blood samples were used for lymphocyte separation according to Isopaque-ficol technique (originally described by Boyum in 1968) [14].

Heparinised peripheral blood was diluted 1/1 with phosphate buffered saline (PBS), and mononuclear cells were isolated by ficoll density gradient centrifugation at 2000 rpm for 20 minutes. Mononuclear cells were washed three times with PBS for 5 minutes, resuspended at $2x10^6$cells/ml, and fixed on poly-L-lysine-coated glass slides, wrapped, and kept at -20°C until assayed.

Immunocytochemistry staining method:

Briefly, the precoated charged slides were removed from freezer, allowed to reach room temperature, unwrapped and then dipping the slides into PBS-filled jar for about 5 minutes and slides were placed on a flat level surface, then endogenous peroxidase was quenched by initial incubation of the smears by enough drops of Peroxidase block for 5 minutes at room temperature then rinse with PBS from a washing bottle. The slides then placed in PBS wash bath for 2 minutes and excess buffer were taped and wiped around smears. Then, enough power block reagent (1/10 diluted in PBS) were applied for 5 minutes and excess blocking reagent was taped but not washed to ovoid non-specific binding of antibodies. Then, the coated lymphocytes were covered by 20 µl of 1/30 diluted mouse monoclonal Ab (primary Ab) specific human CD-marker (CD3 and CD54). Slides then incubated at 37°C for 1hr, and then unreacted monoclonal Ab was removed by three cycle for 2 minutes of washing with PBS. Then they were washed and wiped around the smear.

After that enough solution of biotinylated secondary antibody (anti-mouse Ab) was applied to cover each smear, distributed evenly over the precoated slides then placed in humid chamber for 1 hour at 37°C and washed in buffer and bathed in PBS for 5 minutes then wiped around smear. Enough solution of streptavidin conjugated peroxidase was applied to cover the smear and slides were placed in humid chamber for 1 hour at 37°C. Then they were washed in buffer, bathed in PBS for 5 minutes and wiped around the wells. Then enough drops of freshly prepared DAB working solution were applied to cover the section at room temperature for 10 minutes or until the color was observed and the reaction was terminated by rinsing gently with distilled water from a washing bottle. Slides then were placed in bath of hematoxyline for 30 seconds at room temperature. Slides were rinsed gently with distilled water from a washing bottle then and under gently running tap water for 5 minutes. A drop of mounting medium (DPX) was placed onto the wet smear and the spot were quickly covered with a cover slip and left to dry.

The slides were examined under 40X-magnification power of light microscope (ZEISS). The dark brown (homogenous or membranous) staining identified positive labeled cells as in figure (3).

Direct immunoflourescence staining method:

The slides were prepared as described previously. Then, the pre-coated slides with lymphocytes were removed from freezer, allowed to reach room temperature, unwrapped and washed with PBS by dipping the slides into PBS- containing jar for about 5 minutes at room temperature. They were laied flat, smear-side up, in humidity chamber, then 20µl of 1/30 diluted fluorochrom (FITC) conjugated monoclonal antibodies were added to each smear, cover chamber and slide were left undisturbed in incubator at 37°C for 50 minutes. Slides then transferred to staining jar filled with PBS at room temperature and PBS replaced twice at 5 minutes intervals, then drained and blotted gently.

Two drops of mounting media [(nine parts glycerol to one part of 0.2M carbonate buffer, pH=9 [15] to enhance fluorescence and retard fading on exposure to UV-light [16] were placed on each smear of slides. Then cover slips were lowered into place slowly to avoid bubbles; cover slips may be sealed around edges with clear nail polish. Slides were examined then with fluorescence microscope at 490 nm; positive cells give green-apple when stained with FITC-labeled antibodies and exposed to UV light.

Statistical analysis

The percentage of each of the tested marker expression on PBLs was calculated by a simple calibration of percentage of reactivity as in following formula:

Percentage of expression= (No. of positive cells/ total No. of cells) ×100%.

Statistical differences in measured values were analysed using independent sample-test. P-values <0.05 were considered statistically significant.

Results

T cell activation phenotype:

Among the studied markers, there is significantly increased percentage of T-cell population in the peripheral circulation of RA patients compared with the percentage found in patients control and healthy individuals. Also, significantly elevated percentage of expression of functional activation antigen (CD54) and early activation antigen (CD71) were found in (table 2) (figure 1, 2).

Patients were divided (based on

modified DAS28-3) in to two groups: high disease activity group (37 patients) and minimum disease activity group (9 patients). There was no statistical significant difference in the mean percentage of cells that express CD3. While, CD54 showed no statistical significant difference with lower percentage in active disease group and CD71 showed statistically significant difference with higher expression in minimum disease activity group (table 3).

Lack of influence of PBLs alteration phenotype with clinical and laboratory indices:

Our results showed no statistical correlation between studied markers and different clinical and laboratory parameters for disease activity (Table 4).

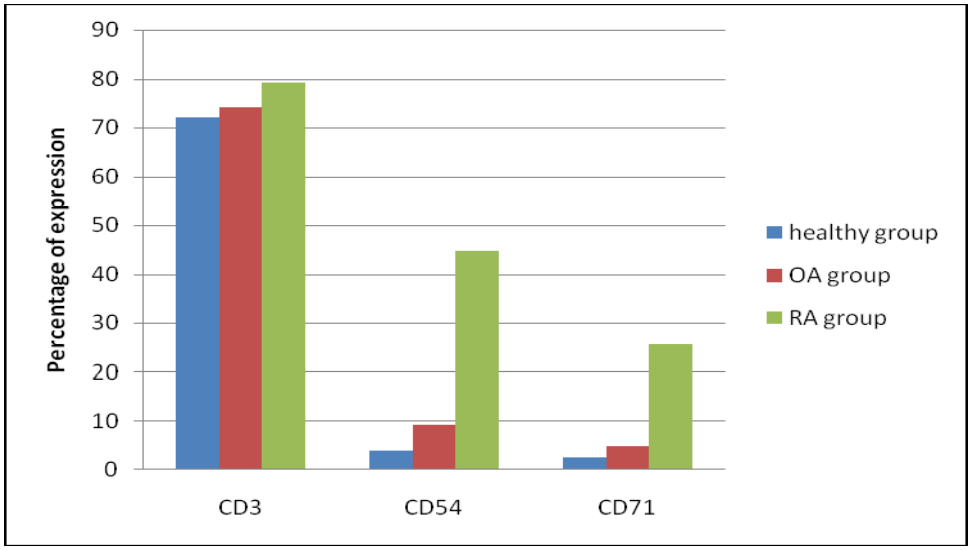

Figure 1: percentage of expression of PBLs markers in different study groups.

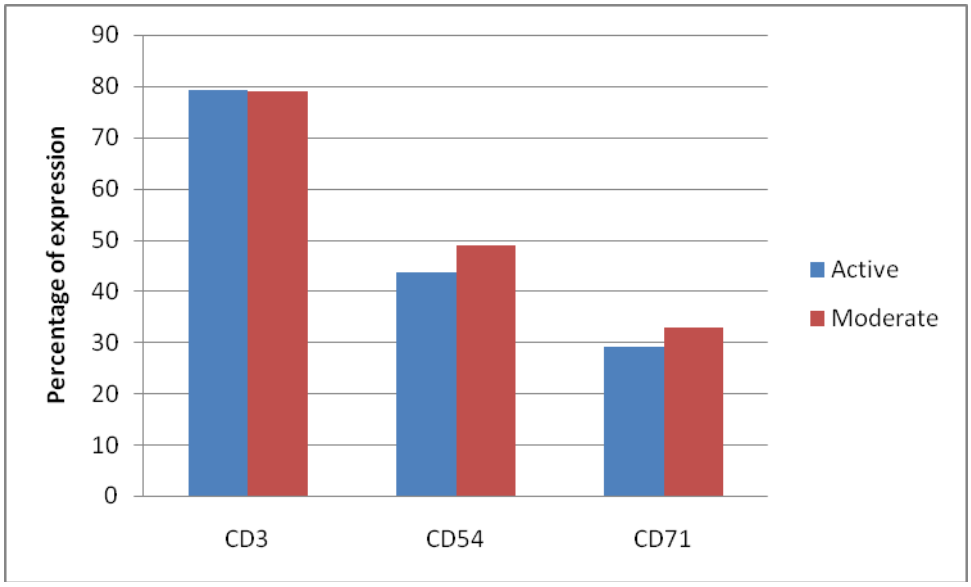

Figure 2: Percentage of expression of PBLs markers in RA patients subgroups.

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

	Controls	Osteo-arthritis group	RA patients	RA patients	
				High disease activity group	Minimum disease activity group
Women/men	9/1	6/1	42/4	34/3	8/1
Age/years	48.6(10)	45.76 (7.6)	47.67(12.09)	48.06(11.96)	46.45(12.97)
Disease duration (months)	----		88.61(72.88)	92.34(68.28)	76.73(92.67)
ESR (mm/1st h	12.50(3.31)	45.6 (12.5)	67.43(20.26)	70.94(19.54)	53(17.33)
CRP (mg/l)	10.20(15.24)	22.4 (16.5)	43.956(55.078)	49.78(59.53)	20(17.75)
Tender joints	-----		10.58(5.42)	12.54(4.62)	4.77(3.19)
Swollen joints	-----		7.35(4.52)	8.63(4.36)	3.66(1.80)
DAS-28(3)	-----		5.77(0.83)	6.11(0.63)	4.844(0.24)
RF sero-positive (No. (%))	2(21.4%)	1 (16.6%)	34 (73.9%)	27(72.9%)	6(63.54%)
Duration of morning stiffness (minutes)	-----		76.41(41.30)	84(41.72)	52.27(30.28)

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

Table 2: descriptive statistics (mean±S.E.) of PBLs markers in different study groups, comparison was done using ANOVA test.

	healthy group	OA group	RA group	ANOVA test
CD3	72.042±1.52	74.3±0.99	79.213±1.4	<0.001 **
CD54	3.7±2	9.142±4.59	44.554±13	<0.001 **
CD71	2.3±1.82	4.571±1.98	25.534±9.59	<0.001 **

**: highly statistical significant difference at the level of p<0.001.

Table 3: Descriptive statistics (mean±S.E.) of PBLs markers in different RA subgroups, comparison was done using independent sample t-test.

	Active	Moderate	Sig. 2 tailed
CD3	79.242±1.39	79.061±1.66	0.761^{NS}
CD54	43.67±12.36	48.85±15.12	0.336 ^{NS}
CD71	29.198±9.08	32.885±9.7	0.039*

^{NS}: no statistical significant difference.

*: statistical significant difference at the level of p<0.05.

Table 4: Correlation matrix between studied PBLs markers and disease activity parameters.

Pearson Correlations	CD 3	CD 54	CD 71
TJC	0.028	-0.079	-0.233
SJC	-0.062	0.116	-0.022
ESR	-0.183	0.008	0.107
RF	-0.022	0.035	-0.023
CRP	0.016	0.092	0.072
DAS-28(3)	-0.065	-0.050	-0.149

Discussion

Our results has made it obvious that the strong up-regulation of both CD54 (functional activation antigen) and CD71 (early activation antigen) give a strong evidence that PBLs were within a state of immune dysregulation. That comes together with a recent study done by Poriadin and his co workers in 2006; who demonstrated that the increased expression of activation induced antigens (including CD54 and CD71) on the PBL from patients with various types of inflammatory disorders. It is abnormal regulation of activation processes of lymphocytes in allergic and autoimmune disease consistence in the absence of lymphocyte activation inhibition [17].

Of note, the persistent PBL expression of activation markers occurs due to impaired activation induced cell death (AICD) with failure in the late cell cycle stages. Tang and coworkers in 2004 tried to demonstrate this abnormality and found that there was a soluble survival signal present in RA patient's serum as well as, synovial fluid produced by $CD14^{+ve}$ cell and its secreted form [18]. In addition, effecter and memory lymphocytes were also reported to accumulate in the peripheral blood and synovium of RA patients [19, 20]. Although, the exact mechanism(s) for accumulation of those atypical T lymphocytes are still incompletely understood; there may be three possibilities for accumulation and expansion of autoreactive lymphocytes in RA patients: first is a continual input of autoreactive lymphocytes into the peripheral lymphocyte pool, certain genetic backgrounds may predispose an individual to accumulate autoreactive T cells in vivo [21,22], the second is a failure to suppress autoreactive lymphocytes via anergy [23,24], the third possibility is a failure to remove autoreactive lymphocytes from the peripheral lymphocyte pool by apoptosis [25]. That may be due to impaired balance between pro-apoptotic (Bax) and anti-apoptotic (Bcl2 and Bclxl) proteins which well known regulator in programmed cell death. Furthermore, the autoreactive lymphocytes were switched from apoptosis-sensitive to apoptosis-resistance state [26, 27].

Our results failed to find a correlation between the expression of CD54 and CD71 with clinical and other laboratory indices. Such a results are in agreement with previous study done by Buckley, 2003 who suggested that the chronic inflammation which occurs may be due to impaired dynamic of inflammatory infiltrate that postulate rheumatoid joint as a "foster home" for PBL in which wrong cells

(PBL) accumulate in the wrong place (joints) at the wrong time (during the resolution of inflammation) leading to improper retention and survival [28].

In summary, we have confirmed and illuminated some of the T-cell differentiation defects in RA. Our data reinforce the importance of early and aggressive therapy for RA. Although T-cell abnormalities may predate clinical signs and symptoms, they appear to be perpetuated by inflammation. Therefore, the control of inflammation, particularly through the use of cytokine blockade, should minimize dysregulation of proliferation.

References

1. Weyand CM and Goronzy J J Pathomechanisms in rheumatoid arthritis time for a string theory? J. Clin. Invest. 2006; 116:869–871.

2. Firestein GS and Zvaifler N J. How important are T cells in chronic rheumatoid synovitis? II. T cell-independent mechanisms from beginning to end. Arthritis Rheum. 2002; 46: 298-308.

3. Skapenko A, Lipesky P and Schulze-Koops H. T cell activation as starter and motor of rheumatic inflammation. Curr Top Microbiol Immunol. 2006; 305:195-211.

4. Roy J, Audette M and Tremblayi M J. Intercellular Adhesion Molecule-1 (ICAM-1) Gene Expression in Human T Cells Is Regulated by Phosphotyrosyl Phosphatase Activity. J of boil chem. 2001; 276: 14553–14561.

5. Dustin M L, Rothlein R, Bhan AK, Dinarello CA and Springer TA. Induction by IL 1 and interferon-gamma, tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol. 1986; 137:245-254.

6. Dustin M L, Singer KH, Tuck DT and Springer TA. Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule-1 (ICAM-1). J. Exp. Med. 1988; 167:1323-1340.

7. Buckle A M, and N Hogg. Human memory T cells express intercellular adhesion molecule-1 which can be increased by interleukin 2 and interferon-gamma. Eur. J. Immunol.1990; 20:337-341.

8. Cano E, Pizarro A, Redondo JM, Sa´nchez-Madrid F, Bernabeu C and Fresno M. Induction of T cell activation by monoclonal antibodies specific for the transferrin receptor. Eur. J. Immunol. 1990; 20:765.

9. Salmeron A, Borroto A, Fresno M, Crumpton MJ, Ley SC and Alarco´n B. Transferrin receptor induces tyrosine phosphorylation in T cells and is physically associated with the TCR ζ-chain. J. Immunol. 1995; 154:1675.

10. Frank R and Hargreaves R. Clinical biomarker in drug discovery and development. Nat Rev Drug Discov. 2003; 2: 566-580.

11. Tsuang MT, Nossova N, Yager T, Tsuang, MM, Guo SC, Shyu KG and Liew CC. Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: A preliminary report. Am J Med Genet B Neuropsychiatr Genet. 2005; 133:1-5.

12. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS,rt al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988; 31: 315-24.

13. Fransen PL and van Riel CM. The Disease Activity Score and the EULAR response criteria Clin Exp Rheumatol. 2005; 23:93-99.

14. Boyum A. lymphocyte seperation, Scan J Clin Lab Invest.1968; 21(97).

15. Batty I. standardization of reagents and methodology in immunology. In Dorothy JF, cathrine Sheehan (eds): principles and laboratory diagnosis, clinical immunology, 1986; P 190-202, Lippincott.

16. Narin RC. Standardization in immunofluorescence. Clin Exp Immunol; 1968; 3:465.

17. Poridian GV, Salmasi ZM and Kasimizkii AN. Activation markers of lymphocytes as indicator of immune system dysregulation in inflammation. Patol Fiziol Eksp Ter. 2006; 1: 2-7.

18. Tang X, Yocum DE, Dejonghe D, Nordensson K, Lake DF and Richard J. Increase activation-induced cell death in peripheral lymphocytes of rheumatoid arthritis patients: the mechanism of action. J Immunol. 2004; 112: 496-505.

19. Thomas ML. The regulation of B- and T-lymphocyte activation by the transmembrane protein tyrosine phosphatase CD45. Curr Opin in Cell Biol. 1994; 6: 247 – 252.

20. Kohem CL, Brezinschek RI, Wisbey H, Tortorella C, Lipsky PE and Oppenheimer-Marks, N. Enrichment of differentiated CD45RBdim, CD27– memory T cells inthe peripheral blood, synovial fluid, and synovial tissue of patients with rheumatoid arthritis. Arthritis Rheum.1996;39:844–54.

21. Kohsaka, H., Nanki, T., Ollier, W.E., Miyasaka, N. and Carson, D.A. (1996) Influence of the rheumatoid arthritis-associated shared epitope on T-cell receptor repertoire formation. Proc Assoc Am Phys. 108:323–8.
22. Griffiths, M.M., Wang, J. and Joe, B. (2000) Identification of four new quantitative trait loci regulating arthritis severity and one new quantitative trait locus regulating autoantibody production in rats with collagen-induced arthritis. Arthritis Rheum. 43:1278–89.
23. Taams, L.S. and Wauben, M.H. (2000) Anergic T cells as active regulators of the immune response. Hum Immunol. 61:633–9.
24. Snijders, A., Elferink, D.G. and Geluk, A. (2001) An HLA-DRB 1- derived peptide associated with protectionagain st rheumatoid arthritis is naturally processed by human APCs. J Immunol 166:4987–93.
25. Schrimer, M., Vallejo, A.N., Weyand, C.M. and Goronzy, J.J. (1998) Persistence apoptosis and elevated expression of Bcl2 in clonally expanded CD4+ CD28- T cells from rheumatoid arthritis patients. J. Immunol. 161: 1018-1025.
26. Wells, A.D., Li, X.C. Li, Y. (1999) Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. Nat Med. 5:1303–7.
27. Ghazi, H.F., Abdulmohymen N., and A. H. Ahmad. (2008): Immunocytochemical detection of some apoptosis regulating proteins (P53 and Bcl-2) in Peripheral Blood Lymphocytes of Rheumatoid arthritis patients. Iraqi J of Med Sciences. 6 (1): 89-98.
28. Buckley, C.D. (2003). Why do leukocytes accumulate within chronically inflamed joints? Rheum. 42: 1433-1444.

Determination some of complement components in infertility women with antisperm antibodies.

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Abstract

Background: Classical activation of complement by antigen and antibody complex leads to formation of membrane attack complex (MAC) that leads to formation holes on the spermatozoa ending in their destruction.

Objective: To determine the complements levels and antisperm antibodies in the sera of infertile women of unknown etiology.

Patients and methods: Study group consisted of 45 infertile women consulting Kammal El-Sammarei Hospital for Infertility and In Vitro Fertilization from Jun -2008 to June-2009. Twenty-four (53.3%) patients had primary infertility and the rest had secondary infertility. Control group: consisted of thirty fertile women. Blood samples were collected from them and anti sperm antibodies in the serum were detected by indirect immunofluorescence test (EURO IMMUNE –GERMENY). In addition to that serum were tested for complement levels (C3 and C4) using single radial immune diffusions test (BINDARID) KIT BIRMINGHAM .UK.

Results: Detections of antisperm antibodies in the serum of infertile women were (64.4%) which is significantly ($p<0.05$) higher from control group using indirect immunofluorescence test. There was a significant ($p=0.000$) difference in the complements levels among infertile women who had ASA positive and ASA negative and control group.

Conclusions: These higher levels of complement components may be due to activation of classical pathway by ASA that directed against sperm antigens ending in defect in function and motility of the sperms.

Key words: Infertility, antisperm antibody, complement.

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Introduction

Complement (C) system is an enzymatic cascade of proteins that forms a vital part of the innate immune system and the end products of complement activation is pores formation by membrane attack complex [(1)]. They present in low concentrations in the serum and once it was activated by any pathways (classical, alternative and Lectin), its levels were increased [(2)]. The presence of antisperm antibodies in the reproductive tracts of some infertile individuals, and presence of complement in cervical and ovarian follicular fluid, suggests that complement-mediated damage of spermatozoa is involved in some cases of infertility. Furthermore, deposition of maternal IgG and complement in the extra fetal tissues indicates that complement activation occurs within the fetoplacental unit [(3)]. Complement and its regulation is important in reproduction, Donev *etal* 2008 reported CD59b was significantly expressed only in testis and played a role in sperm acrosome activation and motility [(4)]. There was no evidence of antibody or complement fixation by viable spermatozoa. It had been found that antibodies present in the serum of women that bind to nonviable spermatozoa(ASA) belong to the IgG and IgM class then Complement fixation occurred via the classical (antibody-mediated) and alternative pathway. This indicated that viable

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spermatozoa may possess antigenic properties different from nonviable spermatozoa. This leads to lack of immunological reaction of women to viable spermatozoa [5]. Anti sperm antibodies could inactivate human sperm motility in the presence of complement, showing that complement-dependent inactivation of sperm motility might be the biological mechanism of female infertility[6], because incubation of motile sperm with complement-fixing immune sera resulted in a significant loss (43-87%) of motility, then activation of (C5b-9) induced alterations in sperm morphology leading to sperm lyses[7].

In this study, we tried to determine the presence of ASA in the sera of infertile women and measure the main complement components (C3 and C4) in the sera of same patients.

Patients and methods

Patients group: consisted of 45 infertile women consulting Kammal El-Sammarei Hospital for Infertility and In Vitro Fertilization from Jun - 2008 to June-2009. The exclusion criteria was women with congenital abnormalities in the uterus, tubes and ovaries , women who ages were more than 45 and less than 20 years , women with defect in ovulation, hormonal disturbances and tube occlusion were excluded. Thus, study group included only women with unknown cause of infertility.

Control group: consisted of thirty healthy fertile women.

Blood was collected from two groups and anti sperm antibodies (ASA) were detected in their serum by indirect immunofluorescence test using kit from EURO IMMUNE – GERMENY. The sites (head, neck and tail) where ASAs directed were also recorded.

Anti-nuclear antibodies were done by indirect immunofluorescence test using kit from EURO IMMUNE – GERMENY for those who had positive antisperm antibodies to sperm head to get rid from cross-reactions.

Serum of both groups were tested for complement levels (C3 and C4) using single radial immune diffusion test (BINDARID) KIT BIRMINGHAM .UK. These tests were done in Immunological department-central public Health.

The study was approved by the Ethical Committee of the Al-Kindi College of Medicine- Baghdad University, Kammal El-Sammarei Hospital for Infertility and Central Public Health. All samples were obtained with informed consent in accordance with Kammal El-Sammarei Hospital for Infertility Declaration. This study was carried out with the approval of the Ministry of Health and District Health Authority Ethical Committee in Baghdad-Al-Resaffa.

Statistical analysis

Student's t-test and ANOVA test used in analysis data statistically by MiniTab statistical software program 13.20. A P- value ≤ 0.05 was considered to be significant.

Results

The patients group consisted from forty-five female patients, their ages ranged from (22-45 years), (median=33). They were complaining from infertility, Twenty-four (53.3%) patients had primary infertility, their ages ranged from (22-40 years) (median =29.9) and the rest (No. =21, 46.7%) had secondary infertility, their ages ranged from (24-45 years) (median =31). The control group their ages were ranged between (17-39 years), median= 30.6.

Detections of antisperm antibodies in the serum of infertile women were (64.4%) which is significantly ($P<0.001$) higher from control group (3.3%) using indirect immunofluorescence test. The highest percentage of antibodies was directed

towards neck (31.3%) as shown in table-1, 2-. Antinuclear antibodies could not be detected in these women.

Complement levels (C3 and C4) in the serum of infertile women as shown in table-3- , figure-1-2-, there were significant (p=0.000) difference between infertile women who had ASA + and ASA- and control group.

Table 1: Percentages of antisperm antibodies in the serum of infertile women and control group using Indirect Immunoflorescence test.

Indirect immunoflorescence Test Control group Number =30		Indirect immunoflorescence Test Infertile women Number =45		Titer		Isotype
No.	%	No.	%	No.	%	
1	3.3	29	64.4	**1:10**		IgG
				15	51.7%	IgM
						IgA
P<0.001						mix
				1:100		
				14	48.2%	

Table 2: Detection sites of antisperm antibodies using Indirect Immunoflorescence test.

Antisperm antibodies directed against											
Head		Neck		Tail		Head+ Neck		Neck+ tail		Head +neck+ tail	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
4	13.7	9	31.03	0	0	7	24.1	7	24.1	2	6.8

Table 3: Serum levels of complement components in infertile women with and without antisperm antibodies compared with control group.

Complement Levels (mg/L)	Infertile women with positive antisperm antibodies Number=29 Means ± SE X ± SEM	Infertile women with negative antisperm antibodies Number=16 X ± SEM	Control fertile women Number =30 X ± SEM
C3	2249.3± 57.5	2059.4±69.7	1216±0.2
C4	526.6±25.2	538.7±34.1	268±0.38

P value = 0.000 using ANOVA test
SE= standard error

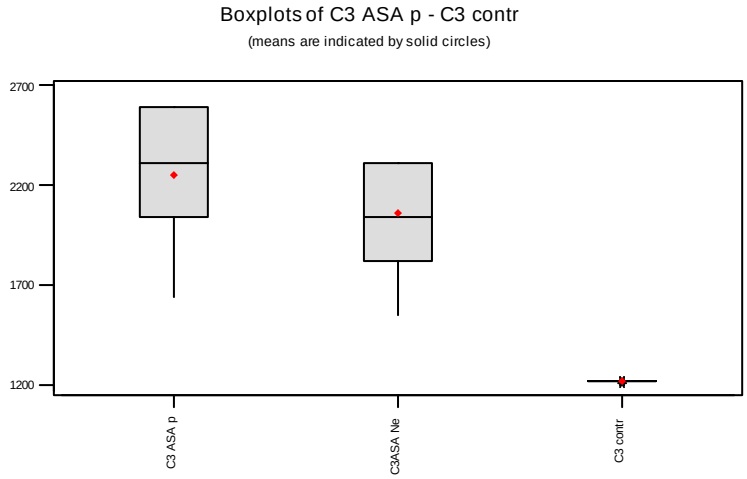

Figure 1: Serum levels of complement components C3 in infertile women with and without antisperm antibodies compared with control group

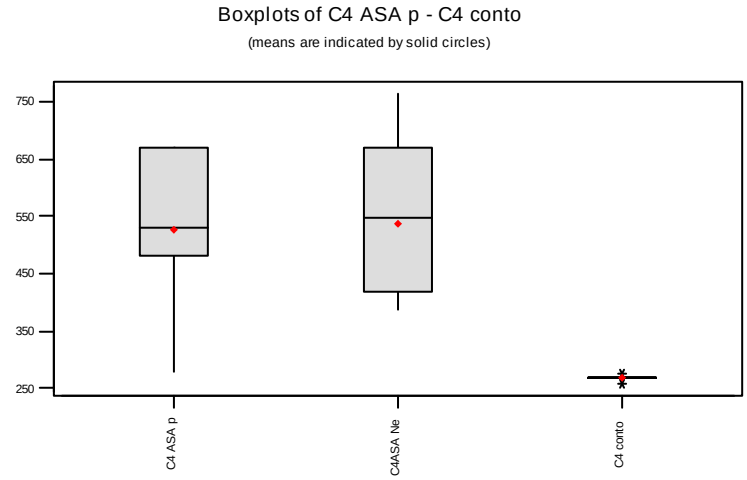

Figure 2: Serum levels of complement components C4 in infertile women with and without antisperm antibodies compared with control group

Discussion

Spermatozoa are cells that must survive transplantation into a foreign host in order to perform their physiological role to reach the oocyte and penetrate it. The biggest hurdle to overcome is innate immune defense that will target the invaders in the female genital tract.

The human immune system is trained during the early postnatal period. In women when become sexually active, their immune system will inevitably contact sperm antigens after coitus. Therefore, once sperm, as an autoantigen, activates the human immune system, an autoimmune response against human sperm will occur and leads to formation of ASA against sperm antigens. This leads to complement activation and complement is a major player in innate immunity. Spermatozoa must therefore evade complement attack if they are wanted to reach their goal.

In order to complement activation needs antibodies and the antibodies in

this study were ASA and were detected in higher percentages in the infertile women 64.4% that is in agreement with other study [8]. The highest percentage was directed against neck of the sperm. Those ASA that directed against head will affect penetration of ova and ASA directed against tail will affect movement of the sperms [9].These directed against neck when there were complement activation will lead to pores formation and damage the sperms especially when the isotype was IgG because IgM produced for only short period about two weeks [10]. We found in this study a higher titer of ASA (1:100) in 48.2% of infertile women. IgG ASAs were capable of activating complement and depositing MC5b-9 on human sperm. Meanwhile, the concomitant detection of sperm-bound IgG and the initial (C3d) and terminal (C5b-9) complement components on the surface of human sperm could be confirmed using a flow cytometric assay [11]. In addition to that, the deposition of activated C3 fragments, the assembly of terminal membrane attack complexes (C5b-9) and oxygen radicals could lead to C3-mediated sperm binding to neutrophils or C5b-9-mediated sperm-motility loss [12]. This show the way to complement activation; we found significant higher levels of C3 and C4 (main components of complements) in the serum of infertile women with ASA. Complement evasion is achieved by the presence of complement regulators both in seminal plasma and on the spermatozoa [13]. Women who have generated an anti-sperm antibody (ASA) response may be particularly at risk because C activation will be enhanced with subsequent spermatozoal damage and destruction and perhaps also inflammatory damage to the female reproductive tract [14,15] and this was in agreement with our results. As a result, impairments of complement components might predispose women to infections and autoimmune diseases that affect fertility [16, 17].

The message of this study is that C and C regulation play important though poorly defined roles in several components. Defects in C regulation may contribute to infertility and manipulation of C at this site may be of benefit either for improving fertility or for contraception.

References

1. Kindt TJ, Goldsby RA and Osborne BA. Kuby Immunology. Sixth edition. WH Freeman and Company. New York .USA. 2007. Pp: 168-185.

2. Delves PJ, Martin SJ, Burton DR and Roitt IM. Essential immunology .11th edition. Blackwell publishing .UK. 2008. Pp: 21-36.

3. Rooney IA, Oglesby TJ and AtkinsonJP. Complement in human reproduction: activation and control.Immun Rese.1993; 12:267-294.

4. Donev RM, Sivasankar B, Mizuno M and Morgan BP.The mouse complement regulator CD59b is significantly expressed only in testis and plays roles in sperm acrosome activation and motility. Mol Immunol.2008; 45:534-542.

5. Vogelpoel FR, teVelde RE, Scheenjes E, Van Kooy R, Kremer J and Verhoef J. Antibody and complement-binding activity of viable and nonviable human spermatozoa. Arch Andro. 1987; 18:189-197.

6. D'Cruz OJ, Toth CA, Haas GG Jr. Recombinant soluble human complement receptor type 1 inhibits antisperm antibody- and neutrophil-mediated injury to human sperm. Biol. Reprod. 1996; 54, 1217-1228

7. D'Cruz OJ, Haas GG Jr, Wang BL, DeBault LE. Activation of human complement by IgG antisperm antibody and the demonstration of C3 and C5b-9-mediated immune injury to human sperm. J. Immunol. 1991; 146, 611-620.

8. Kapoor A, Talib VH and Verma SK.Immunological assessment of infertility by estimation of antisperm antibodies in infertile couples. Ind J Path Microbiol.1999; 42:37-43.

9. Jin-Chun Lu, Feng Huang Yu and Nian-Qing Lu. Antisperm Immunity and Infertility. Expert Rev Clin Immuno. 2008; 4:113-126.

10. Lu J-C, Huang Y_F and Lu N-Q. Antisperm immunity and infertility Exp Rev Clin Immuno.2008; 4:113-126.

11. D'Cruz OJ, Haas GG Jr. Lack of complement activation in the seminal plasma of men with antisperm antibodies associated *in vivo* on their sperm. Am. J. Reprod. Immunol. 1990; 24: 51-57.
12. D'Cruz OJ, Toth CA, Haas GG Jr. Recombinant soluble human complement receptor type 1 inhibits antisperm antibody- and neutrophil-mediated injury to human sperm. Biol. Reprod. .1996; 54: 1217-1228.
13. Harris CL, Mizuno M and Morgan BP. Complement and complement regulators in the male reproductive system. Molecular Immunology. 2006; 43: 57–67.
14. Troedsson M, Liu IK and Crabo B. Sperm transport and survival in the mare: a review. Theriogenology.1998; 50:807–818.
15. Troedsson MH, Loset K, Alghamdi AM, Dahms B and Crabo BG. Interaction between equine semen and the endometrium: the inflammatory response to semen. Anim. Reprod. Sci.2001; 68: 273–278.
16. Wen L, Atkinson JP and Giclas PC.Clinical and Laboratory evaluations of complement difficiency.J Allergy Clin Immunolo.2004; 51:336-340.
17. Boackle SA and Holers VM. Role of complement in the development of autoimmunity.Curr Dir Autoimmune.2003; 6:154-168.

CD14 and Bladder Cancer: is there any correlation.

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Abstract

Background: Epithelial cells have evolved a variety of cell-and tissue-specific mechanisms for bacterial detection to enable cells to modulate the inflammatory response depending on the particular situation in a specific organ. These mechanisms provide a means of maintaining a proper balance between defense, tissue injury and their combined effects on organ function, and the moleculeCD14may have a role to play.

Objective: to find any correlation between CD14 marker expression and bladder cancer.

Material and methods: The immunoexpression of CD14 in paraffin sections from 96- bladder cancer tissues and 36-bladder tissues from patients with other bladder disease rather than cancer was investigated using immunohistochemical assay (IHC). The patients were divided into three groups: Group-1: Newly diagnosed bladder cancer patients, 69(43.9%), Group-2:Post-chemotherapy patients, 27(17.2%), Group-3: Other bladder disorders rather than bladder cancer 36(22.9%). The final diagnosis of patients with bladder cancer was established by clinical examination confirmed by cystoscopy and histopathological examination for bladder tissue specimens.

Urinary tract infections were studied for all groups by culturing urine samples using specific culture media.

Results: The results showed that CD14 protein was over expressed in 68.57% of the patients with approximately equal frequent IHC score among patients(23.8%) for each of weak and intense immunoreactions, and (21.0%) for moderate one , and there was no significant difference in the scores of positive IHC CD14 expression in bladder tissue of the cancer patients when compared with non-cancer patients, but there was significant difference between cancerous patients in correlation to the tumor grades.

Positive urine cultures were detected in 28(40.6%) of group-1, 13(48.1%) of group-2, and 12(33.3%) of group-3, while all healthy subjects were free of infection, and a significant difference between bacterial infected patients with and without bladder cancer, in which there was highly CD14 immunoexpression in bladder tissue in Gram-negative bacterial infected patients .

Conclusion: CD14 expression correlated significantly with Gram-negative bacterial infection, but not with cancer.

Key words: Immunohistochemistry, CD14**,** bladder cancer

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Introduction

CD14, is an LPS receptor [(1)], and plays an important role in the signal transduction causing endotoxic shock and is anchored to the monocyte / macrophage cell membrane via glycosylophatidyl inositol [(2, 3)].

Recent reports have described the presence of mCD14 in epithelial cells, endothelial cells, and fibroblasts [(4-6)]. Bladder epithelial cells express CD14 on their surfaces. This fact was evaluated by several investigators. The assessment of CD14 expression was based on using different bladder carcinoma cell lines that were analyzed by flow cytometry using the well-characterized anti-CD14 monoclonal My4 antibodies [(7)]. They improved that

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the bladder carcinoma epithelial cells express mCD14

A soluble form of CD14 is secreted into the urine of superficial bladder cancer patients who receive intravesical BCG. The measurement of soluble urinary CD14 could be of prognostic significance for the response to immunotherapy [8].

In order to limit and control bacterial infections, the host relies on innate immune defense mechanisms, which initially engage epithelial cells of the mucosal surfaces. These cells are the first to encounter luminally localized bacteria and, they have evolved a variety of cell-and tissue-specific mechanisms for bacterial detection [9]. The early recognition of pathogens by cells of the innate immune system is critical to the survival of the host. Attachment of bacteria to epithelial target cells as well as the action of a secreted toxin triggers mucosal chemokine responses in the urinary tract [10]. However, the predominant mechanism prevents bacteria from gaining access to the sensitive upper urinary tract does not require the presence of bacteria per user; it rather recognizes the presence of bacterial LPS [11]. **For the above mentioned data, we intend to investigate, is there any correlation between bladder cancer and CD14 expression.**

Materials and methods

Subjects: patients groups which enrolled Tissue samples from cases of bladder cancer patients as group 1 (96 newly diagnosed cases and 16 cases who had received chemotherapy), and 10 cases of chronic non-specific cystitis, 10 cases of mild non-specific cystitis, 3 cases of chronic bilharizial cystitis, 7 cases of dysplastic urothelium and 6 normal urothelium as group 2 which represent patients with diseases other than cancer and this group represent group 2.

Normal urothelium samples were taken from patients who did not suffer from bladder carcinoma in the past and had a macroscopically normal bladder mucosa during cystoscopy, and they were taken as control (group3). Tissue were fixed in 10 % buffered formalin and embedded in paraffin wax, were stained with hematoxylin-eosin.

Tumor grade was characterized by 2 independent pathologists

Voided urine samples were collected before cystoscopy or surgery for patients of groups 1-3 and for healthy subjects, as aseptically as possible, in sterile containers. The collected mid-stream specimens were transported to the laboratory within 30 minutes of the collection and cultured on a specific media for bacterial isolation.

Immunohistochemical Detection of CD14 Proteins Expression in Paraffin Embedded Sections: According to procedure mentioned by Celis et *al*., 2005 [12]**.**

The antibodies used were: Biotinylated Link: which is F (ab') rabbit anti-mouse IgG HRP-STAR13B (Serotec, UK), and Sheep anti-rabbit IgG: HRP-STAR54 (Serotec, UK), or secondary Anti-mouse Antibody conjugated with peroxidase enzyme. (Sigma).Mouse anti-human CD14 (Serotec,UK) (MCA596F): Batch No.0798.

Slides were examined and stained cells were counted with the assistance of an experienced histopathologist by light microscope at X400 magnification .Immunostaining was scored according to cut-off value. This cut-off for

positivity was 10% positive cells for CD14 [7].Quantitative IHC scoring was evaluated by counting the number of positive and negative cell nuclei in several randomly selected fields in each section .Tumor reactivity was expressed as the marker percent (i.e., the number of stained tumor cells per 1000 cells in each section).More than 1000 cells evaluated under 40 X high power field and the percentage of positive cells was calculated as follows:

For CD14, the intensity of positivity scored as [7]:

a) 0: No reaction.
b) 5-10 %: Weak reaction.
c) 10-25 %: Moderate reaction.
d) 50-80 %: Intense reaction.

Statistical analysis

Student's *t* test, the chi-square (*x2)* test. Correlation coefficient was used. Probability values of $p<0.05$, and $p<0.01$ were considered statistically significant.

Results

CD14 protein was overexpressed in 72 patients (68.6%) ,with approximately equal frequent IHC score among patients(23.8%) for weak and intense immunoreaction ,and (21.0%) for moderate one . Table 1shows the frequency distribution of CD14 overexpression scores in group subjects.

Positive immunoreaction of CD14 was found in bladder tissue samples of 41 out of 55 newly diagnosed patients with bladder carcinoma, 10 bladder tissue samples of previously diagnosed bladder carcinoma and had received chemotherapy ,and in 21 bladder tissue samples of patients without bladder cancer, including 3 normal urothelium biopsies ,with intense immunostaining reaction being the most frequent score among patients of group-1,and group-2.while weak immunostaining reaction observed in group-3 as the most frequent score.(Table 2) .

Frequency Of CD14 In Patients With & Without Bladder cancer

The CD14 IHC results tissue samples taking together all patients with bladder cancer(Group-1 and Group-2) ,and considering patients without bladder cancer and harboring other urological diseases,were correlated to each other and summarized in table 3, in which 51 out of 71(48.57%) bladder cancer patients showed positive results, while 21 of non-cancerous cases, showed positive results(Figure 1A).Chi-Square test showed that there was no statistical difference between bladder cancer and other urological disease for CD14 IHC Scores in tissue sample taken from each case .

Frequency of CD14 IHC Scores In Bladder Cancer Patients In Correlation To Tumor Grade Of TCC:

CD14 protein immunostaining was evaluated in 105 paraffin-embedded bladder tumor specimens. CD14 identified by positive anti-CD14 reaction which is demonstrated at the lower part of the panel; and the luminal endothelial cells.

Regarding the tumor grade of transitional cell carcinoma, CD14 was detected in 15 out of 25of grade-1, 15 out of 19 of grade-2, and 12 out of 13 of grade-3. There was no CD14 detected in bladder tumor with grade-4.

Chi-square was used to compare the results of frequency distribution of CD14 scores among tumor grades of TCC and it showed a significant correlation between each score and tumor grade ($P<0.05$) , Figure 1 .

Frequency of CD14 Overexpression In Relation To the Bacterial Infection:

Forty out of 105 patients presented with Gram-negative bacterial infection,

in which immunohistochemistry staining of CD14 protein, showed that 33 patients were positive with intense reaction being the most frequent score among them (19.0%) (Table 4).

The results showed highly significant correlation (P<0.01) in CD14 expression with bacterial infection criterion.

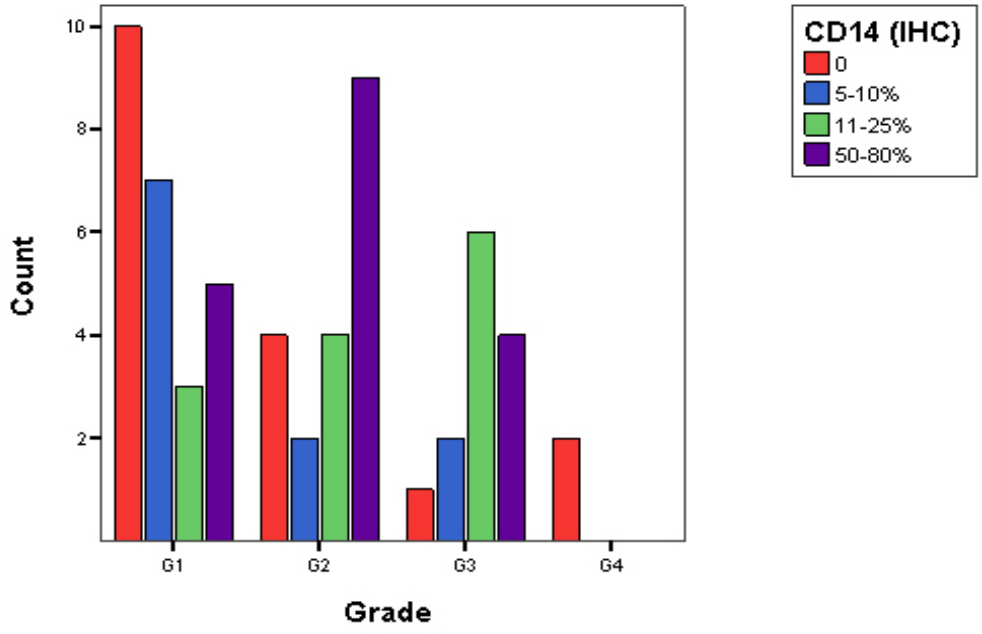

Figure 1: The percentage of CD14 overexpression in bladder carcinoma patients in relation to the tumor grade.

Table 1: Frequency distribution of CD14 Overexpression

CD14 Overexpression	*Patients (%)*
Positive	***72 (68.6)***
_ Weak reaction	25 (23.8)
_ Moderate reaction	22 (21.0)
_ Intense reaction	25 (23.8)
Negative	***33 (31.4)***
Total	**105(100)**

Table 2: Frequency table of CD14 IHC scores in study groups .

CD14 IHC Scores		Groups			
		Group-1	**Group-2**	**Group-3**	**Total**
0	**No.**	14	6	14	34
	%of Total	(25.5)	(37.5)	(40.0)	(31.4)
5-10 %	**No.**	11	3	11	25
	%of Total	(20.0)	(18.8)	(31.4)	(23.8)
10-25%	**No.**	12	3	7	22
	%of Total	(21.8)	(18.8)	(20.0)	(21.0)
50-80 %	**No.**	18	4	3	25
	%of Total	(32.7)	(25.0)	(8.6)	(23.8)
Total	**No.**	55	16	35	106
	%of Total	(52.4)	(15.2)	(33.3)	(100.0)

0: Negative .5-10 % : Weak reaction .10-25 %: Moderate reaction .50-80 %: Intense reaction.

Table 3: Frequency of CD14 IHC scores in patients with & without bladder cancer.

CD14 IHC Scores		Type-CA		Total	Chi-square	Sig.
		Cancer	Non-Cancer			
0	**No.**	20	13	33		
	%of Total	(28.2)	(38.2)	(31.4)		
5-10%	**No.**	14	11	25	7.092	0.071
	%of Total	(19.7)	(32.4)	(23.8)		
10-25%	**No.**	15	7	22		
	%of Total	(21.1)	(20.6)	(21.0)		
50-80%	**No.**	22	3	25		
	%of Total	(31.0)	(8.8)	(23.8)		
Total No.(%ofTotal)		71(67.6)	34(32.4)	**105(100%)**		

Table 4: The percentage of CD14 expression in relation to the bacterial infection

		Infection			Total
		No growth	**Gram-negative**	**Gram-Positive**	
0.0%	**No.**	24	7	2	33
	%of Total	(22.9)	(6.7)	(1.9)	31.4
5-10 %	**No.**	21	4	0	25
	%of Total	(20.0)	(3.8)	(0.0)	23.8
10-25%	**No.**	13	9	0	22
	%of Total	(12.4)	(8.6)	(0.0)	21.0
50-80 %	**No.**	5	20	0	25
	%of Total	(4.8)	(19.0)	(0.0)	23.8
Total	**No.**	63	40	2	105
	%of Total	(60.0)	(38.1)	(1.9)	100.0
Chi-square		31.567			
Sig.		<0.01* *			

** Highly significant correlation.

Discussion

We have used the bladder tissue biopsies to study IHC expression of CD14 in patients with and without bladder cancer.

In the present investigation, we have characterized the immunoexpression of CD14 in bladder tissue of both cancer and non-cancer patients, without significant difference, but its expression in cancer tissue was with significant correlation to the tumor grade. Variation in CD14 expression levels among individuals should correlate with variation in the ability to mount an inflammatory reaction. The factors that contribute to the variable expression level of mCD14 in bladder epithelial cell line, has not been identified [(13)].Variation in mCD14 expression levels among individuals should correlate with variation in the ability to mount an inflammatory reaction.

High mCD14 expression levels were found in grade-1 and grade-2 then in grade-3, while the small size of grade-4 sample showed no CD14 expression .A possible explanation for the down-regulation is that during chronic inflammation, inhibitory feedback mechanisms would decrease the expression of CD14. An alternative explanation is that the down-regulation is a consequence of death and shed of the urothelial cells.

In our study high CD14 IHC expression levels were found in Gram-negative bacterial infected bladder tissue of both cancerous and non-cancerous patients, and it's expression was significantly correlated with this type of bacterial infection (data are not shown) .This is compatible with the fact that CD14, is a cell surface protein involved in LPS binding [(1)], in which LPS is the major constituent of gram-negative bacterial cell wall. It is one member of a group of molecules, called pathogen-associated molecular pattern molecules that are recognized by host

tissue that express pattern recognition receptors. Recognition of microorganisms by this mechanism forms part of the primitive form of defense called innate immunity [(14)]. LPS stimulated an overall increase in mCD14 but specifically induced mCD14 in low mCD14 expression cells [(5)].It has been previously reported that tissue-specific CD14 expression is regulated at the level of transcription, and an 80-kb genomic fragment containing the critical regulatory elements that enhance the tissue-specific expression of CD14 [(15)].LPS specifically up-regulated several genes included CD14 genes [(16)], furthermore, LPS significantly induced CD14 mRNA expression [(17)].

References

1. Wright SD, Ramos RA, Tobias PS, Uleritch RJ,and Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein.Science 1990; 249: 1431-1433.

2. Goyert SM, Ferrero E, Retting WJ, Yenamandra AK, Obata F, and Le Beau MM.The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. Science, 1988; 239:497-500.

3. Hazoit A, Chen S, Ferrero E, Low MG, Silber R, and Goyert SM.The monocyte differentiatio n antigen, CD14 is anchored to the cell membrane by a phosphatidylinositol linkage. J.Immunol.1988; 141:547-552.

4. Jersmann H P A, Hii C S T, Hodge GL, and Ferrante A. Synthesis and surface expression of CD14 by human endothelial cells.Infect.Immunol . 2001; 69:479-485.

5. Putnins E E,Sanaie AR ,Wu Q ,and Firth JD. Induction of keratinocyte growth factor-1 expression by lipopolysaccharide is regulated by CD14 and Toll-Like receptors 2 and 4.Infect.Immunol. 2002; 70:6541-6548.

6. Tamai R ,Sakuta T, Matsushita K, Torii M, Takeuchi O ,Akira S, Akashi S ,and Tanaka H. Human gingival CD14 fibroblast primed with gamma interferon increase production of interleukin-8 in response to lipopolysaccharide through up-regulation of membrane CD14 and MyD88 mRNA expression .Infe.Immunol. 2002;70:1272-1278

7. Schilling J D ,Martin SM ,Hunstad DA ,Patel KP, Mulvey MA, Justice SS,et al.CD14-and Toll-like receptor dependent activation of bladder epithelial cells by lipopolysaccharide and Type 1 piliated Escherichia coli. 2003; 71(3):1470-1480.

8. Jackson A M ,Lien E, Alexandroff AB ,Prescott S, Espevik T, James K, et al. Soluble urinary CD14 after intravesical bacilli Calmette Guerin immunotherapy for carcinoma in situ.Br.J.U, 1997; 80(5):766-71.

9. Bäckhed F and Richter-Dahlfors A.B: Bacteria-induced innate immune responses at epithelial linings. In Intracellular Pathoens: Membrane Interactions and Vacuole Biogenesis.Grovel, J. (ed) .2002; 3(3):153-158.

10. Uhlen P, Lae Stadius A, Jahnukainen T, Soderblom T, Backhed F, Celis G. Alpha-heamolysin of Uropathogenic Escherichia coli induces Ca+oscillations in renal epithelial cells.Nature, 2000; 405:694 -697 .

11. Bäckhed F, Meijer L, Normark S, and Richter-Dahlfors A. A: TLR4-dependent recognition of Lipopolysaccharide by epithelial cells requires sCD14 .Cell. Microbiol. 2004; 4:493-501.

12. Celis JE, Moreira JM A, Gromova T, Cabezon T, Ralfkaier U, Guldberg P, et al. Towards discovery-driven translational research in breast cancer.FEBS Journal, 2005; 272:2-15 .

13. Shimazu R ,Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, and Kimoto M.MD-2,a molecule that confers lipopolysaccharide responsiveness on Toll-Like receptor 4.J.Exp.Med., 1999; 189:1777-1782 .

14. Zhang G and Chosh S. Toll-like receptor-mediated NF-kB activation: a phylogenetically conserved paradigm in innate immunity. J. Clin. Investig. 2001; 107:13-19.

15. Christopher JH, Paul DK, Francesco C, Pu Zhang, Michael SR, James P, and Dong-Er Z.Characterization of human endotoxin Lipopolysaccharide receptor CD14 expression in transgenic mice. The Journal of Immunology .1999; 162:503-509.

16. Marcia RS, Ngoe-Bich N, Timothy GH, and Ricardo S.Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P,and Antigen-stimulation. American Journal of Pathology. 2002; 160:2095-2110.

17. Chomarat P, Banchereau J, Davoust J, and Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat. Immunol., 2000; 1:510-514.

Oxidative and antioxidant status in Smoking men.

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Abstract

Background: Free radicals in cigarette smoke may cause oxidative damage to macromolecules, contributing to cardiovascular diseases and cancer. Decreased plasma antioxidant concentrations in smokers may indicate cigarette smoke–related oxidative stress.

Objective: We compared the effects on serum antioxidant concentrations in confirmed active smokers with those in nonsmokers, independent of differences in dietary intakes and other covariates.

Methods: Serum samples from 60 smokers, and 40 nonsmokers aged 15-60 years were analyzed for ascorbic acid (vitamin C), α-tocopherol (vitamin E), and retinol (vitamin A), by using high performance liquid chromatography (HPLC). The measurement of serum lipid profile, and total lipid peroxidation, oxidized HDL (Ox-HDL) was done as well.

Results: Showed significantly lower serum antioxidant vitamins (A, C & E) concentrations in smokers more than in nonsmokers. Smokers had significant elevation in serum malondialdehyde (MDA) ($p<0.001$) and the percentage of oxidized non high-density lipoprotein (Ox. non HDL %) with a significant reduction in the percentage of oxidized high-density lipoprotein (Ox. HDL %) as compared to the control ($p<0.001$).

Conclusions: These results indicate that cigarette smokers have a significantly lower serum antioxidant status than do unexposed nonsmokers, independent of differences in dietary antioxidant intakes with an increased oxidative stress in smokers' sera.

Key Words: Oxidized HDL, ascorbic acid, α-tocopherol , retinol , cigarette smokers.

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Introduction

Oxidative stress is a condition in which the cellular production of reactive oxygen species (ROS) exceeds the physiological capacity of the antioxidant defense system to render ROS inactive [(1)]. Increased production of ROS involves the oxidation of lipids and lipoproteins, DNA, proteins and other molecules in ways that impair normal cellular function, possibly resulting in impaired health and disease [(2)]. Normal cellular metabolism results in the production of ROS; however, both physical and environmental stressors can further increase ROS production. In this regard, two primary environmental stressors include cigarette smoking and high fat meals [(1)].

Cigarette smoking exacerbates ROS formation and poses a significant oxidant stress in vivo [(3)]. In one puff of a cigarette, a smoker is exposed to more than 1015 free radicals in the gas phase alone [(4)], with additional exposure in the tar phase equal to more than 1017 free radicals per gram. It has been consistently reported that cigarette smokers have elevated biomarkers of oxidative stress compared with non-smokers and this represents a potential mechanistic link between regular cigarette smoking and cardio vascular disease (CVD).

The increased oxidative stress observed in smokers may be partly due to the lower blood antioxidant capacity

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routinely observed in smokers [5]. It is possible that the addition of other ROS generators can further promote oxidative stress in cigarette smokers. To our knowledge, no investigation to date has studied the combined effects of cigarette smoking and oxidative stress biomarkers. Therefore, in the present investigation we compared blood antioxidant status and oxidative stress biomarkers in smoking men with those of age-matched control subjects.

Subjects and Methods

A- Subjects:

This study was conducted on 60 smoking men aged 15-60 years (the mean age 46.52□6.21 years) with at least one year of smoking, they were smoking ≥10 cigarettes per day. They were selected from Al- Kadhumia Teaching Hospital, for evaluation of serum lipid profile and antioxidant vitamins. Any smoker with any medicals illness that may affect the measured parameters such as cardiac, hepatic, endocrine, metabolic diseases, and alcoholism were excluded from the study. Details of clinical state were taken from each subject.

Depending on the years of smoking, the smokers were distributed into three groups:

Group 1 (from 1-10 years of smoking): include 20 smokers, age range of 15-30 years (mean 22.52±7.43 years).

Group 2 (from 11-20 years of smoking): include 20 smokers, with an age range of 30-45 years (mean age 36.34± 5.16 years).

Group3 (from 21-30 years of smoking): were 20 smokers, of an age range of 46-60 years (mean age 56.41± 7.12 years).

Control group: Forty apparently age matched healthy non smoking men were considered as a control group (mean age 48.09±9.31 years). None of them was alcoholic, or on any drug that may interfere with the results of the study.

B-Blood specimens:

Ten milliliters of venous blood sample were taken from each smoker and control using plastic disposable syringes after 12 hours fast. The samples were transferred into clean plain test tube, left at room temperature for 15min for clotting, centrifuged, and then serum was separated into two portions:

1- For measurement of total cholesterol, triglycerides, HDL-C, total level of oxidized lipids (measured as total malondialdehyde, MDA) and specific levels of oxidized HDL (measured as HDL- MDA).

2- For measuring the concentration of antioxidant vitamins :- involve determination of serum levels of ascorbic acid (vitamin C), α-tocopherol (vitamin E),and retinol (vitamin A)

All assays were obtained by running duplicates for the test, control, & the standard. The tubes were stored at -20ºC until analysis, which was done within one month after collection.

C-Methods:

High Performance Liquid Chromatography (HPLC), with Octa Decayl Silain (ODS) C-18 Column (250x4.6mm) packed with 5µm particle size (Fisher Company, USA) was used for measurement of the antioxidant vitamin concentration (A, C, &E). They were detected by SPD-10AVP ultraviolet- visible detector, at λ-max 290nm [6].

The thiobarbituric acid (TBA) method of Buege& Aust(1978) was used to measure serum MDA. It is based on the reaction with TBA to give a pink color that is read at 535 nm. The malondialdehyde concentrations were calculated using the molar extinction coefficient of $1.5*10^5$ [7].

The levels of oxidized HDL were obtained after HDL precipitation with Mg-phosphotungestic acid. Oxidized-non HDL (oxidized LDL-VLDL) was obtained by subtracting the value of oxidized HDL from the total oxidized lipids, i.e., oxidized non-HDL =total MDA- oxidized HDL [7].

Results

- Serum lipid profile: Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), atherogenic index (expressed as LDL-C/ HDL-C) & LDL size index (expressed as TG/ HDL-C) are shown in Table 1.
- Lipid peroxides profile: The results of total lipid peroxides, (expressed as s. MDA) and oxidized lipid fractions, which included Ox. HDL (expressed as HDL-MDA) and Ox.non-HDL are described as absolute values (for s. MDA) and as percentages from the total (for oxidized lipid fractions).

These results are shown in Table 2. Serum MDA was significantly increased in smokers group 1& 2 (i.e.) years of smoking 1-10 and 11-20 when compared to the controls (P=0.03 &0.01 respectively) and it was highly significantly increased in group 3 (i.e.) years of smoking 21-30 when compared to the controls P=10^{-5}). There was, also significant variation among smoker groups when compared with each other (ANOVA-P value was 10^{-3}). There was a significant reduction of OX. HDL% fraction (P= 0.05, 10^{-3} & 10^{-4} respectively) when all groups compared with controls. Also, there was a significant variation between smokers groups when compared with each other. There was a significant elevation of Ox .non-HDL% when compared to the control (P=0.05, 10^{-3} &10^{-4} respectively), also there was a significant variation between smokers groups when compared with each other (ANOVA-P value was 10^{-3}).

-Antioxidant vitamins: The concentration of serum antioxidant vitamins (A, C, &E) are shown in table 1. They were significantly decreased in smokers when compared with controls in all groups.

Table 1: Serum lipid profile (mean ± SD) in different smokers and control groups.

Group	Years of smoking			ANOVA P-value*	Control
	1-10	11-20	21-30		
	N=20	N=20	N=20	~	N=40
Triglyceride (mmol/l) **t- test P-value***	1.75±0.65 0.05	1.82±0.52 10^{-4}	2.12±0.36 $7*10^{-4}$	0.01	1.17±0.53
total cholesterol (mmol/l) **t- test P-value***	4.82±1.11 0.05	5.3±0.69 10^{-3}	5.3±1.1 10^{-3}	0.7	4.32±0.93
HDL-C(mmol/l) **t- test P-value***	1.08±0.24 0.04	1.04±0.23 0.5	1.03±0.32 0.3	0.3	1.16±0.3
LDL-C(mmol/l) **t- test P-value***	3.01±1.05 0.07	3.06±0.55 0.05	3.1±1.23 0.05	0.9	2.65±0.92

Atherogenic index (LDL-C/HDL-C) **t- test P-value***	2.74±1.42 0.05	2.89±0.71 0.05	3.02±0.11 0.01	0.8	2.45±1.3
LDL size index (TG/HDL-C) **t- test P-value***	1.67±0.85 10^{-3}	1.74±0.76 10^{-3}	2.2±0.74 10^{-4}	$7*10^{-3}$	1.12±0.75

*Student t- test was done between each smoker and control groups.
*P-value considered significant at 0.05 or less.

Table 2: lipid peroxidation and it[s] fractions (mean±SD) in different smokers group and control.

Years of Smoking	N	S.MDA µ mol/l	t- test P-value*	OX.HDL %	t- test P-value*	OX. non-HDL %	t- test P-value*
1-10	20	0.65±0.05	0.03	65.37±7.9	0.05	33.85±1.01	0.05
11-20	20	0.76±0.2	0.01	70.7±5.95	10^{-3}	28.31±0.12	10^{-3}
21-30	20	0.97±0.25	10^{-5}	7 2.4±11.05	10^{-4}	27.19±1.05	10^{-4}
ANOVA p-value	~	~	10^{-3}	~	10^{-3}	~	10^{-3}
Control	40	0.52±0.13	~	72±14.02	~	29±14.02	~

*Student t- test was done between each smokers group and control.
*P-value was considered significant at 0.05 or less.

Table3: Antioxidant vitamins (mean ±SD) in different smokers group and control.

Years of Smoking	n	Vit. A µmol/l	t- test P-value*	Vit. C µmol/l	t- test P-value*	Vit. E µmol/l	t- test P-value*
1-10	20	1.13±0.12	0.04	24.84±1.76	0.01	10.85±1.01	0.05
11-20	20	0.81±0.09	0.01	18.31±3.19	10^{-3}	8.31±0.12	10^{-3}
21-30	20	0.63±0.11	10^{-3}	17.14±3.08	10^{-3}	7.19±1.05	10^{-3}
ANOVA p-value	~	~	10^{-3}	~	10^{-3}	~	10^{-3}
Control	40	1.56±0.23	~	39.20±1.45	~	18.39±2.08	~

*Student t- test was done between each smokers group and control.
*P-value consider significant at 0.05 or less.

Discussion

In this study oxidative stress (which is expressed as total lipid peroxide and oxidized lipid subfractions) had been measured to demonstrate the relation between smoking and oxidative stress.

The oxidation of LDL is a very complex process. The smoking state alters LDL size and composition. LDL in postprandial state appears to be more susceptible to oxidation than fasting LDL [(8)]. Oxidation of LDL leads to alteration of the apoplipoprotein B (apo B) recognition site and in the unregulated uptake of the LDL by the macrophages via the scavenger receptor, another important factor in LDL oxidation relates to ambient HDL concentrations. HDL carries important antioxidant enzymes, paroxanase and platelet activating factor acetyhydrolase, and also it serves to protect LDL from oxidation in order ways. HDL also

appears to exchange undamaged phospholipids for oxidized phospholipids in LDL; HDL, from smoker subjects is less protective than the control subjects [9].

According to the present results, there was a significant elevation of the oxidized LDL% and reduction of the oxidized HDL% in all smoker groups with increase in the years of smoking. These results are in accordance with the results obtained from Sarafian, *et al.* [10] and Morrow, *et al.* [11]. Serum lipid profiles were seen to be significantly elevated in all smoker groups and as the years of smoking increase, there are more pronounced lipid disturbances, except for the serum HDL-C which was reduced significantly when compared with control group as shown in table 1.

The changes in the serum lipids which were noticed in the smokers in the present study are in accord with previous report [12] while other report showed normal levels of serum LDL-C but of smaller and denser forms, which are more susceptible to oxidation [13].

However, the role of TG in cardio vascular disease (CVD) is a controversial subject. Many epidemiological trials do not identify hypertriglyceridaemia as an independent risk factor when the cholesterol and, in particular the HDL-C level, are taken into consideration. Nevertheless, these results must be interpreted with caution as hypertriglyceridaemia represent a very heterogeneous entity which is closely related to many factors that may affect coronary risk (tobacco consumption, hypertension, insulin resistance and sedantarity). Therefore, hypertriglyceridaemia and hypo-HDL-aemia may be the results of the same primary abnormality, as the HDL-C level is more stable, it is the parameter, which will be identified as a protective factor in epidemiological trials [14].

- Pro-oxidants and antioxidants

The results showed that, oxidative stress increased in smokers, this is clear from the highly significant elevation of serum MDA level and is agreement with the results of previous reports [15-17]. This elevation in serum MDA may be due to the loss of balance between pro-oxidation and anti-oxidation, energy depletion, and accelerated aging in target organs, such as lungs, heart, kidney and brain. Evaluation of parameters for oxidative stress is a well-accepted technique to express the extent of cell damage [16]. Previous studies have demonstrated that MDA levels increase and antioxidant capacity decreases in smokers[18], and this is in agreement with this study which indicate a highly significant increase in serum MDA levels in smokers compared to normal healthy control ($p<0.001$) as shown in table 2. This study demonstrated that current cigarette smokers have higher measures of lipid peroxidation than nonsmokersas shown in table 2. The finding of increased lipid peroxidation in smokers supports the hypothesis that smoking increases free radical–mediated oxidative damage of lipids, a putative risk factor for athero sclerosis cardio vascular disease.

Previous observational studies that assessed the extent of lipid peroxidation in smokers and nonsmokers have yielded inconsistent results. Also in cross-sectional studies that enrolled healthy volunteers, patients with angina, diabetics, and young survivors of myocardial infarction [19]. There are several studies that showed an association between smoking and oxidative damage, including one cross-sectional study that demonstrated an

association between cigarette smoking and autoantibody titer to oxidized LDL cholesterol [20- 22].

Serum levels of vitamin A, vitamin C, and vitamin E have been reported here to be lower in smokers than in nonsmokers as shown in table 3. In studies in which higher measures of lipid peroxidation were found in smokers than in nonsmokers, smokers also had lower serum vitamin E levels, which could account for the reported difference [16]. In other studies, antioxidant vitamin supplements, including vitamin C, vitamin E, and vitamin A [15,17], decreased the extent of lipid peroxidation in smokers to baseline levels of nonsmokers after only a few weeks of supplementation. In a study exclusively of smokers, a combined antioxidant supplement resulted in increased oxidative resistance to lipid peroxidation [23]. Hence, the intake of antioxidants from diet or supplements may have a major influence on the *in vitro* susceptibility of lipids to peroxidation and may account for the reported differences in lipid peroxidation between smokers and nonsmokers independent of the effects of cigarette smoke [19].

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References

1. Bloomer RJ and Goldfarb AH. Anaerobic exercise and oxidativestress: a review. Can J Appl Physiol, 2004; 29, 245–263.

2. Chakravati B and Chakravati DN. Oxidative modification of proteins: Age-related changes. Gerontology, 2007; 53, 128–139.

3. Halliwell B Oxygen radicals: a commonsense look at their nature and medical importance. Med Biol,1984; 62, 71–77.

4. Asmus K & Bonifacic M Free radical chemistry. In Handbook of Oxidants and Antioxidants in Exercise, pp.3–54 [CK Sen, L Packer and O Hanninen, editors]. Amsterdam,The Netherlands: Elsevier, 2000.

5. Alberg AJ .The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. Toxicology, 2002; 180, 121–137.

6. Bird I. High performance liquid chromatography principles and clinical applications. Bio.Med.J. 299, 23, P 783-787, 1989.

7. Buegge-J and Aust-SD. Microsomal lipid peroxidation. Meth Enzymol. 1978; 51: 302 - 310.

8. Burke A, Fitzgerald GA. Oxidative stress and smoking-induced vascular injury. *Prog. Cardiovasc. Dis.* 2003, *46*, 79-90. *Int. J. Environ. Res. Public Health* 2009, *6*

9. Van Oostrom AJ, Sijmonsma TP, Verseyden C, Jansen EH, de Koning EJ, Rabelink T J, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J. Lipid Res.* 2003, *44*, 576-583.

10. Sarafian TA, Marques Magallanes JA, Hungyi Shau, Donald Tashkin, and Michael D. Roth. Oxidative Stress Produced by Marijuana Smoke. Am. J. Respir. Cell Mol. Biol., Volume 20, Number 6, June, 1999 1286-1293.

11. Morrow J D, Frei B, Longmire A W, Gaziano J M, Lynch S M, Shyr Y, and et al. Increase in Circulating Products of Lipid Peroxidation (F2-Isoprostanes) in Smokers-- Smoking as a Cause of Oxidative Damage. N. Engl. J. Med., May 4, 1995; 332(18): 1198 - 1203.

12. Bloomer RJ, Solis AD, Fisher-Wellman KH, Smith WA. Postprandial oxidative stress is exacerbated in cigarette smokers. *Br. J. Nutr.* 2008, *99*, 1055-1060.

13. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med.* 2000; 28:1815–1826. doi: 10.1016/S08915849(00)00344-0.

14. Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis.* 1998; 141:1–15.

15. Ayaori M, Hisada T, Suzukawa M, Strauss W E, Oates J A, Roberts L J, and et al. Plasma levels and redox status of ascorbic acid and levels of lipid peroxidation products in active and passive smokers. *Environ Health Perspect* 2000; 108:105–8.

16. Schectman G, Byrd JC, Gruchow HW. The

influence of smoking on vitamin C status in adults. *Am J Public Health* 1989; 79:158–62.
17. Romero-Alvira-D. Roche-E. High blood pressure, oxygen radicals, and antioxidants.Med-Hypothesis.1996; 46(4):414.
18. Salonen JT, Yla-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen, R,et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet.* 1992; 339:883–887.
19. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 2004; 84:1381–1478.
20. Jalal I. Evolving lipoprotein risk factor: lipoprotein (a) and oxidized low-density lipoprotein. *Clin Chem.* 1998; 44:1827–1832.
21. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem.* 1997; 272:20963–20966.
22. Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis. 1998; 141:1–15.
23. Van der Vaart H, Postma DS, Timens W, Ten Haccken NHT. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax.* 2004; 59:713–721.

The Role of Electroconvulsive Therapy in General Hospital Psychiatric Inpatients Treatment in Baghdad.

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Abstract

Background: Despite the controversy about the role of electroconvulsive therapy (ECT) it is still widely used in the treatment of some psychiatric disorders.

Objectives: The aim of the study is to identify characteristics of the psychiatric inpatients and the role of ECT in the treatment of their disorders.

Methods: Medical records of all inpatients admitted to Al Kadhymia teaching hospital; psychiatric unit were studied carefully. A special form was designed to collect the data. Diagnosis was made according to the International Classification of Diseases, the Tenth Revision (ICD-10).

All the patients were examined physically.

Results: The 145 inpatients included were 76(52.4%) males and 69(47.8%) females with age distribution of 17-75 years. Males were younger than females and 64.7% of the total sample was under the age of 40 years. Average duration of admission was 4.6 weeks.

Schizophrenia made the most frequent diagnosis (41.4%) followed by depression (25.5%) and Mania (7.6%). All patients received psychosocial and psychotropic treatment. Only 13 classical medicines were used.

ECT was received by 42% of the sample. There was higher numbers of males who received ECT than females and the difference was significant. There was no association between ECT and age. Only 5 patients aged between 60 and 66 years and 4 of them aged 17 years had ECT.

The primary usage was for Schizophrenia which represented 69% of ECT recipients followed by depression (23%). It was significant that males received ECT more than females.

Conclusion: The findings suggest that majority of the inpatients can be treated with medications alone but still there is high rate of using ECT for many different disorders including schizophrenia.

Key words: ECT, Psychiatry, inpatients

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Introduction

ECT is a therapeutic procedure in which a tonic clonic seizer is induced by electrical stimulation of the brain. Electroconvulsive therapy (ECT) has a very important role in the treatment of many psychiatric disorders, especially depression [1]. It has been employed in the treatment of schizophrenia in combination with antipsychotic which is more beneficial than either alone [2]. Since being introduced in Italy in 1938, its mode of action has still not been clarified [1].

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Treatment modalities have changed in many ways. Although modified ECT under general anesthesia is performed in most developed countries, still unmodified ECT is used in some places of the world [1, 3, 4]. The rate of use of ECT among psychiatric inpatient ranged from 0.6%to 25% [5, 6]. Among inpatients ECT was performed more to females than males [7]. Most patients who were treated by ECT were 25-40 years old. Very few were above 60 years old [8]. Diagnostic categories were variable. While affective disorders were the most common diagnostic category among inpateints treated by ECT in most centers, schizophrenia was the most common category in others [9, 10, 3] .ECT was

especially used for treatment of depression disorder that is resistant to conventional treatments such as drugs or psychotherapy [11]. The variability in modality of administration, rate of use and diagnostic categories was related to scope of availability of anesthetic service for psychiatric units, multiple including new drugs availability and level of development of the service i.e. presence of adequately trained staff and quality of units[4,6,8,11]. Although the general trend in developed countries is to less use of ECT, the rate is increasing in some developed centers because of pressure of cost of psychopharmacueticals and other forms of treatment [12]. In one study it was found that the attitudes of treating doctors in a developed center were very positive about using ECT but it was not used because of the negative attitudes of the staff and pressure of social and political stereotypy about use of ECT [11].

The aim of the study is to identify the demographic characteristics of psychiatric inpatients in Al Kadhymia teaching hospital and their treatments and the indications of ECT in that treatment.

Patients and Methods

All the patients admitted consecutively to the psychiatric ward of the Teaching Hospital of Al-Nahrain College of Medicine in Al-Kadhymia, during three months period (from 7 February to 7 April 2002) were evaluated.

The hospital is located in the north west of Baghdad involves a psychiatric ward with 25 beds and outpatient clinic and run by two specialist psychiatrists.

Patients were referred to the psychiatric unit by psychiatrists from outside the hospital or by physicians of other different medical specialties. Some of the inpatients were either self referral or brought up by their relatives. During the time of the study there was no compulsory admission and there was no mental health act. In general most of the admitted patients represented the severe cases which could not be treated as outpatients.

The study was retrospective. The medical records of all the patients admitted during the study period were reviewed carefully. Information related to sex, age, diagnosis and type of treatment were collected in a special form and diagnosis was made according to the International Classification of Diseases, Tenth revision (ICD -10)

Exclusion criteria was that patients who left the hospital early before completing their treatment by premature self discharge and those who had deficient information in the records.

All the patients were exposed to medical examination and investigations; including ECG, blood test and X-Ray, which were done routinely. Some patients who had physical problems were referred to specialist physicians for treatment. Fitness for electroconvulsive therapy (ECT) was approved for some patients. The type of ECT used was bilateral and unmodified. Number of ECT sessions was between 4 and 6 sessions. The policy of the psychiatrists working in the hospital that ECT was only given to inpatients. A written consent was taken from the patient or his close relatives.

Simple statistics was used for data analysis.

Results

The evaluated sample included 145 patients; 76 (52.4 %) males and 69 (47.4 %) females. Age distribution was from 17 to 75 years with an average of 35.4 years (mode=37.5) for the total sample, 33.8 years (mode=28) for males and 37.2 years (mode=47.5) for females.

Table 1, shows the distribution of the age of the sample. Patients under the age of 40 years represented 64.7% of the inpatients. Males were younger than females, as they formed 75% of the inpatients under the age of 40. (Table 1)

The duration of admission to hospital ranged between 3 to 16 weeks with average duration of 4.6 weeks.

The final diagnosis of patients was done according to the International Classification of Diseases 10th.Revision (ICD 10) [13] as illustrated in table 2. The most frequent diagnosis was schizophrenia (41.4%) followed by depression (25.5%) and mania (7.6%). (Table 2)

In relation to gender, there was statistical significant association between males and schizophrenia (X=6.5, d.f1, P< 0.05) while no such relationship was found between sex and depression despite the apparent increase of females (14.5%) over males (11%). (X=1.65, df1, P>0.05).

Received Treatment:

In addition to the psychological, social, and other treatments there were two main physical therapies; psychotropic drugs and electroconvulsive therapy (ECT).

i. Psychotropic Medications:

All the inpatients received psychotropic medications, which involved only 13 types of the classical drugs according to their availability during the time of the study due to the UN Sanction on Iraq. The most common drugs for males were chlorpromazine while diazepam was commonly used for females according to diagnosis. (TABLE 3)(Some patients had more than one diagnosis so the total here is 442)

ii. Electroconvulsive therapy (ECT):

Out of the total sample, 61 patients (42%) received ECT while the rest of them were treated by medications and other therapies only.

There was higher percent of males who received ECT (64%) than females (36%). However halve of the females (56%) were never treated by ECT i.e. only 32% of the total female number and 51% of the total male number received ECT. That was statistically significant (X=5.6, df1, P<0.05). Table 4, shows the distribution of age and sex of patients with and without ECT.

For the sake of statistical comparison between age groups of the patients and its association with sex, the sample was divided into two groups i.e. those below and over 40 year old. Results showed that there was no significant association between age group and ECT use. (X=2.46, df1, P<0.05).

Only 5 inpatients (4 males and one female) aged 60- 66 received ECT. That treatment was given to only one male aged 17 and three females aged 18.

Indications of ECT:

ECT was mainly used for schizophrenia; paranoid, catatonic and disorganized types. Schizophrenia represented 69% of ECT recipients while 23% of them were depressed and only 8% of them had mania and primary sever anorexia nervosa complicated with depressive stupor. Seventy percent of the schizophrenic patients received ECT. (TABLE 5)

Statistical analysis showed that ECT was used for patients with schizophrenia more than those with depressive disorders and the difference was statistically significant. (X=9.7, df1, P<0.05).

The same significant association was found among gender, schizophrenia and ECT; as ECT was given more to males (64.3%) than females with schizophrenia (X=6.6, df1, P<0.05). Such association was not significantly recognized in depression (X=1.8, df1, P>0.05).

Table 1: Age distribution of the Sample

Age Groups(years)	Males		Females		Total	
	N	%	N	%	N	%
<19	7	5	3	2	10	7
20-29	32	2.2	19	13	51	35
30-39	18	12.4	15	10.3	33	22.7
40-49	6	4	17	12	23	16
50-59	8	5.5	12	8.3	20	13.8
60>	5	3.5	3	2	8	5.5

N= 145

Table 2: Psychiatric Disorders (ICD-10) of the Inpatients

Psychiatric Disorders	Males	Females	Total	
	N	N	N	%
Dementia	1	1	2	1.4
Alcoholism	5	-	5	3.4
Drug Abuse	-	1	1	0.4
Schizophrenia	39	21	60	41.4
Delusional	-	3	3	2
Schizoaffective	-	1	1	0.7
Depressive episode	16	21	37	25.5
Mania	9	2	11	3.6
Generalized Anxiety	1	3	4	2.8
Panic Disorder	1	2	3	2
Obsessive Compulsive	-	1	1	0.7
Dissociative- Conversional	1	6	7	4.8
Somatoform	2	2	4	2.8
Anorexia Nervosa	-	2	2	1.4
Personality Disorder	2	1	3	2.1
Temporal Lobe Epilepsy	1	-	1	0.7
Total	76	69	145	100

N= 145

Table 3: Psycho tropics used in the treatment of Inpatients

Drugs	Males	Females	Total	
			N	%
Anxiolytics				
Diazepam	25	45	70	12.8
Chlordiazepoxide	6	8	14	3.2
Lorazepam	5	6	11	2.5
antidepressants				
Imipramine	21	38	59	13.3
Amitrypytiline	3	5	8	1.8
Chlomipramine	3	4	7	1.6
antipsychotics				
Chlorpromazine	57	37	94	21.3
Trifluoperazine	22	10	32	7.2
Thioridazine	10	15	25	5.7
Haloperidol	8	6	14	3.2
Fluphenazine Deca.	25	15	40	9
Antimascarinics	37	20	57	13
Carbamazepine	6	5	11	2.4
Total	228	214	442	100

Table 4: ECT Use association with age and sex of Inpatients

Sex	ECT USE	Age Groups						Total
		>19	20-29	30-39	40-49	50-59	60>	
Males	**ECT**	4	15	12	1	3	4	39
	NO ECT	3	17	6	5	5	1	37
Females	**ECT**	1	3	4	3	5	1	22
	No ECT	2	11	11	14	7	2	47
		10	36	33	23	20	8	145

Table 5: Psychiatric disorders treated with and without ECT

Psychiatric Disorders	With ECT			Without ECT			Total	
	Male	Female	Total	Male	Female	Total	N	%
Schizophrenia	27	15	42	12	6	18	60	41.4
Depression	8	6	14	8	15	23	37	25.5
Mania	4	-	4	5	2	7	11	7.6
Complicated Anorexia Nervosa	-	1	1	-	1	1	2	1.4
Others	-	-	-	12	23	35	35	24.1
Total	39	22	61	37	47	84	145	100

Discussion

The findings of this study have some similarities and differences with other studies. First of all that although most inpatients can be treated by drugs alone, the rate using ECT among inpatients is higher than that which is reported in most literature[5,6]. Explanation is that in the period during which the study was performed there was very gross deficiency of drugs in the country caused by the economic sanctions. Most drugs used are of conventional types .New drugs were hardly available. Even old drugs were sometimes not available. This made the treating psychiatrist in critical situation for treatment of sever cases with disturbed behavior that needed admission so ECT became an easy available mode of therapy. Other explanation is that families of patient who perform most nursing function and accompany patients in the wards do not tolerate long period of stay in hospital. In this case treating doctors will resort to using ECT in treatment to shorten period of recovery. The second point is the diagnostic categories. In this study schizophrenia was the most common category treated by ECT while affective disorders were more commonly treated by ECT according to many most literatures[9,10].The same problems of drugs and inability to keep patients for long periods in wards may be taken as explanation. Because cases of schizophrenia present more with agitation and disturbed behavior than affective disorders then ECT was performed more for schizophrenia to control behavior in short period while there was such deficiency of drugs. Most of cases of affective disorders in this study were those of depression that can be treated with drugs alone because such cases are not usually associated with disturbed behavior and there is no urge for using ECT for them except complicated cases or when there is risk of suicide. In fact the most common rate of admission found in this study is for schizophrenia. While this indicate another explanation for the high rate of use of ECT found in this study ,it gives clue that admission to psychiatric unit is kept more to cases with disturbed behavior while other less sever cases such as those with depression can be treated and tolerated at home . Males were more treated by ECT than females while the reverse was found in most studies [7, 9, 10]. In these studies affective disorders specifically resistant depression was the most common diagnostic category treated by ECT.Depression is more common in females and this explain the difference in gender for use of ECT between this study and other studies[9,10]. Some studies had same results to this study and interestingly these studies are done in developing countries that may suffer of same problems of deficiency of drugs and lack of well trained and enough staff to control sever cases without use of ECT [3, 4, 8].This study need to be replicated again after improvement in drug availability nowadays

There is high rate of using ECT among psychiatric inpatients in general hospital in Baghdad.Schizophrenia was most common indication for ECT and this treatment is uses more for males than females. Deficiency of drugs and lack of trained staff is considered responsible for such high rate of use of ECT.

References

1- Gelder M, Harrison P, Cowen Ph. Shorter text book of psychiatry.Oxford University Press.2006:565-572.

2- Abraham, Kulhara P. The efficacy of electroconvulsive therapy in the treatment of schizophrenia. A comparative study. Br J Psychiatry. 1987 August; 151:152-5.

3- Motohashi N, Awata S, Higuchi T.A questionnaire survey of ECT practice in university hospitals and national hospitals in

Japan. Journal of ECT. 2004 March; 20(1):21-3.

4- Chanpattana W, Kramer BA Electroconvulsive therapy practice in Thailand. Journal of ECT. 2004 Junuary;20(2):94-8

5- Gazdag G, Kocsis N, Lipcsey A. Rates of electroconvulsive therapy use in Hungary in 2002. Journal of ECT. 2004 March; 20(1):42-44.

6- Little JD. ECT in the Asia Pacific region: what do we knowJournal of ECT? 2003 Junuary; 19(2):93-7.

7- Wood DA, Brgess PM. Epidemiological analysis of electroconvulsive therapy in Victoria, Austuralia. Aust N Z J Psychiatry. 2003 June;37(3):307-11

8- chung KF. Electroconvulsive therapy in Hong Kong: rates of use, indications, and outcome. J ECT. 2003 June; 19(2):98-102.

9- Stromgren LS. Electroconvulsive therapy in the Nordic countries, 1977-1987 Acta Psychiatr Scand. 1991 November; 84(5):428-34.

10- Thompson JW, Blaine JD. Use of ECT in the United States in 1975 and 1980. Am J Psychiatry. 1987 May;144(5):557-62

11- Muller U, Klimke A, Janner M, Gaebel W. Electroconvulsive therapy in psychiatric clinics in Germany in 1995. Nervenarzt. 1998 January; 69(1):15-26.

12- Doessel DP, Scheurer RW, Chant DC, Whiteford HA. Changes in private sector electroconvulsive treatment in Australia. Aust N Z J Psychiatry. 2006 April;40(4):362-7

13- WHO. International classification of disease.1991

Evaluation of Serum Soluble Interleukin -2 Receptor level in Diagnosis of Rheumatoid Arthritis.

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Abstract

Background: soluble interleukin-2 receptor (sIL-2R) is secreted by lymphocytes upon activation and has been used as a marker of immune activation in several diseases.

Objective: This study aimed to assess the potential clinical utility of serum level of serum soluble interleukin-2 receptor (sIL-2R) as a diagnostic tool in rheumatoid arthritis disease (RA). The study investigated also the association between serum sIL-2R levels with other parameters used for assessment of RA such as rheumatoid factor (RF), erythrocytes sedimentation rate (ESR), C-reactive protein (CRP), and uric acid.

Methods: Serum sIL-2R levels, measured by ELISA, were evaluated in 25 RA patients who have positive rheumatoid factor (RF) and compared with those of 25 normal controls. The correlations with the other parameters were analyzed.

Results: Compared with the healthy control group, RA patients tended to have significantly higher serum sIL-2R and ESR concentrations ($P<0.001$). While no significant difference between both groups in serum uric acid was seen. Positive serum CRP (CRP level>6mg/dl) was found in 56% of patients. The sIL-2R level was positively correlated with RF and ESR, while a slight positive correlation with uric acid was noticed. Serum sIL-2R showed a high sensitivity and specificity for the patients with positive RF.

Conclusions: A sIL-2R level is a sensitive and specific marker and can be useful for diagnosis of RA.

Key words: C-reactive protein, Erythrocyte sedimentation rate, Rheumatoid arthritis, Soluble interleukine-2 receptors.

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Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that primarily attacks the synovial membrane of the minor joints leading to joint stiffening, swelling, and loss of function in the joints. Its aetiology is unknown, and definitive diagnosis depends predominantly on characteristic clinical features, typical radiographic findings, the presence of auto-antibodies called rheumatoid factors (RF), elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [1, 2]. Failure to meet these criteria does not therefore exclude the diagnosis, especially during the early stages of the disease. There is no single test for the disease and only few symptoms may be present in the early stages. The common test is rheumatoid factor (RF) is present in 80% of adults who have RA [3], an antibody that is presented eventually in the blood of most people with RA. Rheumatoid factors can bind to normal circulating IgG, forming complexes that are deposited in the joints. These immune complexes can activate the complement cascade, resulting in a hypersensitive reaction, which leads to chronic inflammation of the joints [4].

Other common laboratory tests include complete blood picture, ESR, which measures inflammation in the body. C-reactive protein is another common test that measures disease activity [5] .Recent research has uncovered an important role of cytokines which promote inflammation, such as tumor necrosis factor-α (TNF-α) and interleukin-1ß (IL-1ß). Also, TNF-α and IL-1 are

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considered to be the key cytokines in the development of RA [6]. Soluble interleukin-2 receptor (sIL-2R; previously known as Tac) is a surrogate marker of T-lymphocyte activation and proliferation [7]. A soluble fraction of the IL-2 receptor is released from the cell membrane. It is the released extracellular domain of the IL-2Rα by activated cells during a variety of autoimmune disorders including rheumatoid arthritis (RA) [8], and systemic lupus erythematosus [9]. SIL-2R and CRP increases in RA [10]. In RA, IL-2 protein and the IL-2 soluble receptor (sIL-2R) are preferentially expressed at disease onset, in comparison with later stages of the disease [11]. Studies that have addressed this theme have shown discordant results since they have reported evidence pro and against an association between the current proposed markers of this disease (CRP and ESR) and sIL-2R [11].

The aim of the present study is (i) to determine whether there is a difference in sIL-2R levels between RA patients (who have positive RF) and healthy controls (ii) to estimate the sensitivity and specificity of sIL-2Rin diagnosis of RA, and (iii) to evaluate whether sIL-2R levels correlate with the other parameters used for assessment of RA.

Materials and Methods

1-Subjects

The patients included in the study appeared free of the conditions that may cause raised serum RF including cancerous diseases, cirrhosis, and inflammatory lung diseases. Other connective tissue diseases that raise RF were excluded by serologic tests such as systemic lupus erythematosus.

The patients with RA were recruited from the private clinics and laboratory of outpatient department. Only patients with positive results for RF who fulfilled the American College of Rheumatology diagnostic criteria for RA [12] were selected for inclusion in the study.

Criteria for exclusion. Pregnant women, patients with cancer, diabetes mellitus, or autoimmune illnesses, patients with hepatitis, or patients under dialysis were excluded from the study.

The study includes 25 women with RA in addition to 25 healthy control women. The patients were assessed by a rheumatologist on presentation. Apparently healthy persons were asked to participate as controls and they had negative RF values and an absence of acute and chronic diseases.

Sample collection: Ten milliliters of peripheral blood was withdrawn from each individual. Two milliliters of fresh blood poured in a tube containing 0.4ml of sodium citrate as anticoagulant in order to estimate ESR. Serum for serological tests was obtained by centrifugation at 3000rpm for 10 minutes and coded serum aliquots were stored at -20°C until it was analyzed.

2-Measurements:

Estimation of RF:

The RF-latex kit supplied by Spinreact ® Company-Spain was used for diagnosis of RF in serum of the individuals The RF-latex is a slide agglutination test for the qualitative and semiquantitative detection of RF in human serum. Latex particles coated with human gammaglobulin are agglutinated when mixed with samples containing RF. RF (cut-off, <8IU/ml)

Estimation of serum sIL-2R:

The concentrations of sIL-2Rs in serum samples were measured by the Invitrogen® Human sIL-2Rkit according to the solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA).

Estimation of ESR:

Westergren's method used for estimation of ESR for patients. The

ESR cutoff values, measured by the Westergreen method, are: female< 20mm/h; male< 15mm/h

Estimation of CRP:

The CRP-latex kit supplied by Spinreact ® Company-Spain was used for diagnosis of CRP in serum of the individuals. This kit is a slide agglutination test for the qualitative and semiquantitative detection of CRP in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP. CRP (cut-off value is 6 mg/l),

Estimation of Uric acid:

Uric acid in serum is oxidized by uricase to allantoine and hydrogen peroxide, which under the influence of peroxidase enzyme, 4–aminophenazone, and 2-4 dichlorophenol sulfonate forms a red quinoneimine compound. The intensity of the red color formed is proportional to the uric acid concentration in the sample.

Statistical Analysis

All statistics were carried out using Excell® program-Microsoft Corporation-USA. The results expressed as mean±standard deviation. Correlation coefficient values were estimated by regression analysis. Predictive value, sensitivity and specificity for measured parameters were estimated by the following formulas:

Predictive value of positive result=TP/ (TP+FP)*100%

Specificity=TN/ (TN+FP)*100%

Sensitivity=TP/ (TP+FN)*100%

Where TP=true positive, TN=true negative, FP=false positive, FN=false negative.

Cutoff value for sIL-2Rwas expressed as (mean +2×standard deviation) that equal 1957pg/ml. The difference between groups was estimated using Pooled Student t-test. The difference is said to be difference if p-value is less than 0.05.

Results

The mean sIL-2R concentrations and ESR level were significantly higher ($p<0.001$) in patients with positive RF patients in comparison with healthy controls as shown in Table (1), while no significant difference between both groups in serum uric acid.

Positive serum CRP (CRP level>6mg/dL) were found in 56% of patients (14 / 25). Correlation coefficients (r) values for the patients group showed a positive correlation between the sIL-2R vs. RF (r=0.64) and sIL-2R vs. ESR (r=0.57). A slight positive correlation between sIL-2R vs. uric acid (r=0.34). There is no correlation between each pair of the compared parameters in the control group.

The sensitivity and specificity of the measured parameters are shown in Table (2). Because all patients were positive RF and all controls were negative RF, the sensitivity and specificity for RF=100%. Serum sIL-2R had 84% sensitivity and 96% specificity for the patients with positive RF which is higher sensitivity than other measured parameters. Every sample with values more than the cutoff value of healthy controls (mean + 2×standard deviation) was defined as positive for increased sIL-2Rconcentration. Predictive values showed that the cut off value for sIL-2R=1957pg/ml is an excellent medical decision limit for the prediction of RA.

Table 1: Serum concentration of sIL-2R, ESR, and uric acid of patients and control groups expressed as mean±standard deviation.

Parameter	Patient	Control	p-value
sIL-2R (pg / ml)	2632±1274	935±511	P<0.001*
ESR (mm / hr)	38.3±18.1	8.7±2.6	P<0.001*
Uric acid (mg / dl)	6.1±1.9	4.6±0.9	p>0.05

(*): Significantly different.

Table 2: Sensitivity, Specificity, and Predictive values of the measured parameters.

Parameter	Sensitivity	Specificity	Predictive value
RF	100%	100%	100%*
sIL-2R	84%	96%	94
ESR	78%	96%	95
CRP	64%	92%	86
Uric acid	42%	73%	78

(*): Because all patients have positive RF value and healthy control have negative RF test intentially.

Discussion

Elevated sIL-2Rconcentration in RA patients in comparison with healthy control group (Table 1) is in accordance with many other researches [10, 13]. Iraqi RA patients showed high mean sIL-2Rlevel (2632 ± 1274 pg/ml), while the increase in sIL-2R concentration in other studies were 1532pg/ml [14] and 1855pg/ml [15]. The reason for these differences may be due to the severity of disease or effects of medication on the sIL-2R level in serum as noted in various studies.

Suenaga et al (1998) [16] suggested that sIL-2R measurements to be helpful for the early diagnosis of RA in patients with joint pain, but without symptoms of bone or joint destruction. A high serum sIL-2R level at baseline is a predictor of remission in patients with acute RA [17]. Suenaga et al (1998) [16] have demonstrated that an increased concentration of sIL-2R in the serum of patients with joint pain is a predictor for the future development of RA. Spadaro et al (1997) [18] observed that treatment of RA patients with methotrexate for 6 months was able to decrease the levels of sIL-2R. However, the results of sIL-2R in the present work disagreed with the results of one research. Fro¨de et al (2002) [19] showed that the median levels of sIL-2R did not significantly differ in comparison with those of controls, whereas ESR levels but not CRP were significantly increased. Altogether, these inflammatory indices seem to independently reflect a final pathway of multifactorial events [19]. The reason of the indifference in that study may be due to their low number of patients (n=21) and controls (n=7 only).

An increase of sIL-2R levels during RA has been noted, both in serum/plasma and in synovial fluid [20, 21]. Detailed clinical trials showed that serum sIL-2R levels are related to disease duration and a decline in sIL-2Rconcentration may result from joint improvement [22].

Findings from clinical trials raise a question on whether sIL-2R concentration in serum provides a reliable immunological marker to assess disease activity in RA. Earlier studies reported the possible advantages of sIL-2R measurements for these purposes [23]. Tebib et al (1995) [21] do, however, question the utility of sIL-2R as such a marker, since it is neither specific nor sensitive

to measure disease activity in an outpatient RA population.

The most commonly measured laboratory markers of disease activity in RA are the ESR and CRP. In recent studies, it was reported that CRP is more sensitive than ESR as a marker of disease activity because ESR is additionally affected by several factors, such as age, sex, anaemia, elevated fibrinogen and immunoglobulin levels, renal failure, pregnancy, and abnormal red blood cell morphology [(1)]. Both CRP and ESR give similar information about non-specific inflammation. A high or increasing amount of serum CRP suggests an acute infection or inflammation. CRP appears and disappears more quickly than changes in ESR. Therefore, CRP level may drop to normal following successful treatment, whereas ESR may remain elevated for a longer period [(24)]. As a blood test, CRP is not specific. A high result serves as a general indication of acute inflammation. In cases of inflammatory rheumatic diseases, such as rheumatoid arthritis and lupus, doctors can utilize the CRP test to assess the effectiveness of a specific arthritis treatment and monitor periods of disease flare-up. Its value is as a general indicator, not specific

Some reports indicate relationships between sIL-2R and laboratory markers of inflammation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [(21, 22, 25, 26, 27)]. In patients with RA, IL-2R correlated weakly with ESR (r = 0.24), and CRP (r = 0.24) [(13)]. The finding of our research showed higher r-value for IL-2R with ESR (r=0.57) indicating the reliability of ESR as a diagnostic tool for RA.

The normal serum uric acid in most RA patients are in accordance with general knowledge about serum uric acid in RA[(28)] and may be useful as first step for differentiation between gout and RA.

References

1. Richardson C, Emery P. Laboratory markers of disease activity. J.Rheumatol 1996; 23: 23–30.

2. Tighe H, Carson DA. Rheumatoid factors. In: Kelley WN, Ruddy S, Harris Jr, Sledge CB, eds. Textbook of Rheumatology. Philadelphia, PA: WB Saunders, 1997: 241–249.

3. The Mayo Clinic.com Rheumatoid Factor: Definition.

4. Kuby, Kuby _ Immunology; Ch.20 Autoimmunity: p.467.

5. National institute of arthritis and musculoskeletal and skin diseases (NIAMS); http://www.niams.nih.gov/Health_Info/Rheumatic_Disease/default.asp.

6. Taylor PC. Anti-cytokines and cytokines in the treatment of rheumatoid arthritis. Curr Pharm Des 2003; 9:1095–106.

7. Castillo FM, Romero TA, Est´evez J, et al. Concentrations of cytokines, soluble interleukin-2 receptor, and soluble CD30 in sera of patients with hepatitis B virus infection during acute and convalescent phases. Clinical and Diagnostic Laboratory Immunology. 2002;9(6):1372–5.

8. Wolf RE, Brelsford WG, Hall VC, Adams SB. Cytokines and soluble interleukin-2 receptors in rheumatoid arthritis. J Rheumatol 1992; 19:524-8.

9. Sawada S, Hashimoto H, Iijma S, et al. Increased soluble IL-2 receptor in serum of patients with systemic lupus erythematosus. Clin Rheumatol 1993;12:204-9

10. Vincenzo Pasceri and Edward T. H. Yeh. A Tale of Two Diseases: Atherosclerosis and Rheumatoid Arthritis. Circulation 1999; 100; 2124-6.

11. Corrigan VM, Arastu M, Khan S, et al. Functional IL-2 receptor beta (CD122) and gamma (CD132) chains are expressed by fibroblast-like synoviocytes: activation by IL-2 stimulates monocyte chemoattractant protein-1 production. J Immunol 2001; 166: 4141–7.

12. American College of Rheumatology Subcommittee. Recommendations for the medical management of osteoarthritis of the hip and knee: 2000 update. American College of Rheumatology Subcommittee on Osteoarthritis Guidelines. Arthritis Rheum 2000; 43: 1905–1915.

13. Steiner G, Studnicka-Benke A, Witzmann G, et al. Soluble receptors for tumor necrosis factor and interleukin-2 in serum and synovial fluid of patients with rheumatoid arthritis, reactive arthritis and osteoarthritis. J Rheumatol. 1995; 22(3):406-12.

14. Lee GL, Chen MY, Chuang CY, Chen CY. Serum interleukin-2 receptor in systemic lupus erythematosus and rheumatoid arthritis. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi. 1988; 21(1):16–22. (English abstract).

15. Pountain G, Hazleman B, Cawston TE. Circulating levels of IL-1 β, IL-6 and soluble IL-2 receptor in polymyalgia rheumatica and giant cell arteritis and rheumatoid arthritis. Br J Rheumatol. 1998; 37(7):797–8.

16. Suenaga Y, Yasuda M, Yamamoto M, et al. Serum interleukin-2 receptor for the early diagnosis of rheumatoid arthritis. Clin Rheumatol. 1998; 17(4):311–7.

17. Kuuliala A, Söderlin M, Kautiainen H, et al. circulating soluble interleukin-2 receptor level predicts remission in very early reactive arthritis. Scand J Rheumatol. 2005 Sep-Oct; 34(5):372-5.

18. Spadaro A, Taccari E, Riccieri V, et al. Relationship of soluble interleukin-2-receptor and interleukin-6 with class-specific rheumatoid factors during low-dose methotrexate treatment in rheumatoid arthritis. Rev Rheum Engl. 1997; 64: 89–94.

19. Fro¨ de TS, Tenconi P., Debiasi MR. et al. TNF-α and sIL-2R levels in rheumatoid arthritis. Mediators of Inflammation. (2002)11; 345–349.

20. Nassonov EL, Samsonov MY, Chichasova NV, et al. Soluble adhesion molecules in rheumatoid arthritis. Rheumatology (Oxford). 2000; 39(7):808–10.

21. Tebib JG, Letroublon MC, Noel E, et al. sIL-2Rlevels in rheumatoid arthritis: poor correlation with clinical activity is due in part to disease duration. Br J Rheumatol. 1995; 34(11):1037–40.

22. Rubin LA, Snow KM, Kurman CC, et al. Serial levels of soluble interleukin 2 receptor in the peripheral blood of patients with rheumatoid arthritis: correlations with disease activity. J Rheumatol. 1990; 17(5):597–602.

23. Wood NC, Symons JA, Duff GW. Serum interleukin-2-receptor in rheumatoid arthritis: a prognostic indicator of disease activity? J Autoimmun.1988; 1(4):353–61.

24. Lab Tests Online; American Association for Clinical Chemistry http://www.labtestsonline.org/understanding/analytes/crp/glance.html.

25. Symons JA, Wood NC, Di Giovine FS, DuffGW. Soluble IL-2 receptor in rheumatoid arthritis. Correlation with disease activity, IL-1 and IL-2 inhibition. JImmunol. 1988; 141(8):2612–8.

26. Itoh M, Goto Y, Ohta Y, et al. Relations between surface expression of the interleukin-2 receptor and release of the soluble form of the receptor in cultured mononuclear cells from patients with rheumatoid arthritis or systemic lupus erythematosus. Clin Rheumatol. 1998; 17(1):26–30.

27. Klimiuk PA, Sierakowski S, Latosiewicz R, et al. Interleukin-6, soluble interleukin-2 receptor and soluble interleukin-6 receptor in the sera of patients with different histological patterns of rheumatoid synovitis. Clin Exp Rheumatol. 2003; 21(1):63–9.

28. Richard Ravel, Clinical Laboratory Medicine: Clinical Applications of Laboratory Data. Ch.23 Bone, Joint, and Collagen-Vascular Disorders 6th edition (1995) by Mosby Co USA.

Hepatitis A virus infection in children.

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Abstract

Background: most hepatitis A viral infections in children are asymptomatic or have mild non specific manifestations but some are complicated.

Objective: to evaluate all cases of hepatitis A viral infection in children who were admitted over one year period and enlighten out the abnormal presentations and predict complications.

Patients and methods: during the period from 1st of June 2005- 1st of June 2006, fifty cases of hepatitis A viral infection (who had hepatitis A virus IgM +ve) were studied and analyzed regarding the clinical presentations, coarse of the illness, complications and outcome.

Results: fifty patients enrolled in the study, with male to female ratio of 1.8:1 and 32 (64%) of them presented in the age of 1-5 years. Fulminant hepatic failure was found in 7 (14%) cases, 5 (10%) had prolonged cholestasis , 5 (10%) had exacerbation of pre- existing chronic liver disease, 2 (4%) had recurrent or relapsing hepatitis A viral infection, and 5 (10%) cases had extra hepatic manifestations , 3 (6%) were G6PD deficient patients who had sever hemolysis.

Conclusions: hepatitis A viral infection can present in different ways.

The level of consciousness, presence of ascites and severely abnormal biochemical and hematological values were of great help in predicting complicated cases of hepatitis A viral infection.

Key words: hepatitis A, abnormal presentations, children.

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Introduction

Hepatitis A is the commonest viral hepatitis in the developing world [(1)]. It commonly spreads from person to person by fecal – oral route [(2)]. It often can pass from caregivers to children or adults in settings where there is close contact [(3)]. Travelers to endemic areas are at high risk and may transmit hepatitis A virus (HAV) after infection [(4)]. Most hepatitis A viral infections in children younger than 5 years of age are asymptomatic or have mild, non specific manifestations but some of HAV infections might have complications such as fulminant hepatic failure (which is a clinical syndrome resulting from massive necrosis of hepatocytes or from severe functional impairment of hepatocytes. The currently accepted definition in children includes: biochemical evidence of acute liver injury (usually <8 wk duration); no evidence of chronic liver disease; and hepatic-based coagulopathy in the presence of clinical hepatic encephalopathy) (2) and it occurs in less than 1 in 10000 case [(5)], prolonged cholestasis, recurrent hepatitis, and extrahepatic manifestations or may present on top of already pre- existing chronic liver disease [(1)].

One patient had multiorgan dysfunction including liver failure, hepatic encephalopathy, renal failure, pleural effusion, pericardial effusion and hematological dysfunction as a sequale of hepatitis A infection in otherwise healthy male [(6)].

The aim of this study was to evaluate all cases admitted with HAV infection over one year period and to enlighten the abnormal presentations

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and predict complicated cases of HAV infection.

Patients and methods

This prospective study was done at Children Welfare Teaching Hospital, Medical City in Baghdad in the period from 1st of June 2005 till 1st of June 2006, including cases admitted with hepatitis. Sixty five patients with hepatitis like illness were admitted during the period of the study, only 50 cases with HAV IgM +ve were included in the study. Each case was studied regarding clinical presentations, course of the illness, complications and the outcome. Thorough physical examination and all the necessary investigations were done to the patients in the study.

Results

Fifty patients were studied that had HAV IgM +ve, 32 (64%) males and 18 (36%) female with male to female ratio of 1.8:1.

Thirty-two (64%) patients presented in the age group between 1-5 years (table 1).

All patients included in the study presented with jaundice (100%), 35 (70%) patients had hepatomegaly, 6 (12%) patients had leg edema and 3 of them had additional ascites. Disturbed consciousness was found in 8 (16%) cases, 4 (8%) had typical encephalopathy, 3 of them developed convulsion (Table 2).

Twenty –four (48%) patients had complications: seven (14%) patients presented with fulminant hepatic failure, two of them were died (4%), one after 24 hours of admission and the other after 3 days. five patients (10%) had prolonged cholestasis with mean days of illness of 45 days, one of them had sever hypoglycemia and developed convulsion during the course of the illness. Another five patients (10%) had pre- existing chronic liver disease (2 had Wilson disease and 3 had autoimmune hepatitis), one of the Wilsons patients was semiconscious and had positive family history of same disease. Two of the patients with autoimmune hepatitis presented with disturbed consciousness and generalized edema.

Relapsing hepatitis occurred in 2 patients (4%), both within 4 weeks of the initial infection. Extra hepatic manifestation was found in 5 cases, sever haemolysis was seen in 4(8%) patients, 3 of them were G6PD deficient patients and presented with very dark colour urine, hepatosplenomegaly and sever pallor. two of them required blood transfusion; one of those had impaired renal function. The fifth patient presented with history of convulsion (Table 3)

The mean biochemical and hematological values are important in predicting the outcome of the cases of HAV infection as the mean TSB, S.ALT, S.AST, S. Alkaline phosphatase, PT, and PTT were all higher in complicated cases (Table 4).

Table 1: distribution of patients according to age and sex

Age (years)	Male		Female		Total	
	N0.	%	No.	%	No.	%
< 1	0	0	0	0	0	0
>1-5	20	62.5	12	37.5	32	64
>5-10	10	66.7	5	33.3	15	30
>10-15	2	66.7	1	33.3	3	6
Total	32	64	18	36	50	100

Table 2: Signs and symptoms of the disease at presentation of the patients in order of frequency.

Signs and symptoms	No.	%
Jaundice	50	100
Fever	40	80
Dark colour urine	36	72
Tender hepatomegaly	35	70
Vomiting	30	60
Clay colour stool	20	40
Abdominal distension	12	24
Splenomegaly	9	18
Disturbed consciousness (encephalopathy)	8	16
Leg edema	6	12
Pallor	4	8
Convulsion	4	8
Ascites	3	6

Table 3: Distribution of complicated cases according to clinical presentation.

Types of complication	NO.	%
Fulminant hepatic failure	7	14
Prolonged cholestasis	5	10
HAV on top of pre-existing chronic liver disease	5	10
relapsing hepatitis	2	4
Extra hepatic manifestations: **haemolysis** **convulsion**	 4 1	 8 2
Total	24	48

Table 4: Mean biochemical and hematological values of the patients

Test Presentation	*TSB mean	**S.ALT mean	*** S.AST mean	'ALK.phosph mean	''PT mean	'''PTT mean
Non complicated (NO: 26)	7.8 ± 1	69 ± 4	71 ± 4	65 ± 5	15 ± 2	38 ± 4
Complicated (NO: 24)	16.6 ± 2	89 ± 5	108 ± 8	75 ± 6	42 ± 3	52 ± 5

*total serum bilirubin,
**Serum alanin amino transferase,
*** Serum aspartate amino transferase, 'Alkaline phosphatase, "Prothrombin time, '" Partial thromboplastin time

Discussion

Hepatitis A viral infection accounts for about 50% of the clinically apparent hepatitis, not associated with chronic liver disease, persistent viraemia or intestinal carrier state [2].

Our study included hospitalized patients which were usually ill and jaundiced with hepatitis like illness. We actually isolated every case until proved or disproved to be HAV IgM+ve. There was male predominance which is consistent with Anand AC study [7], while no sex predilection was apparent in Amin J et al study [8].

The most common age group was between 1-5 years (64%) of cases. In the developing nations, the age of acquisitions is usually before 2 years of age while in western societies it is frequent in person's age 5-17 years [9]. No case under one year was found in our series as it is usually uncommon in infants less than one year of age [10]. Splenomegaly plus hepatic enlargement were found in all cases of G6PD deficient patients and all of them had severed anemia and very high total serum bilirubin.

The complicated cases in this study were 48% which is a high percent, it is probably because all cases admitted to the hospital were seriously ill and the mild, ambulant cases usually not admitted. It was reported that fulminant hepatic failure may varies between 0.1% of symptomatic infected children to10 % (1, 9). In our study the most common complication was the fulminant hepatic failure (14%), all those patients were semiconscious, three had ascites and leg edema and two of them died within 3 days of admission. Apart from hepatic insufficiency, the course of HAV infection may be characterized by a relapse following initial improvement (relapsing hepatitis A) and prolonged cholestasis [11]. Although relapse occurs in 3-21% of patients with acute hepatitis A [1, 8, 12], we had 2 relapsing cases (4%) within 4 weeks of the previous infection. Persistent or prolonged cholestasis may follow the acute infection and it may persist for more than 3 months [13]. In this study 5(10%) patients had prolonged course which ranges from 30- 60 days (mean 45 days). One of those patients had protracted hypoglycemia and deep jaundice, he was managed with small doses of steroid and Ursodeoxycholic acid as recommended by one author [14], and he improved and was discharged well.

Although ascites and pleural effusion are possible benign and early complications of acute HAV infection that resolve spontaneously regardless of the illness outcome [2, 3], all 3 cases (6%) seen in this study with ascites proved to have chronic liver disease.

All types of chronic liver disease can present as acute hepatitis [14] but some can be uncovered during the superadded acute HAV infection. Data from literature indicate a high fatality rate during the HAV super infection in patients with chronic hepatitis B and C, particularly those with cirrhosis and in patients with alcoholic cirrhosis [15].

Five (10%) patients had previous chronic liver disease (Wilson's disease was evident in 2 cases (4%) and 3 (6%) had autoimmune hepatitis), the presentation of which was not a straight forward, the children had firm to hard liver texture with features of acute hepatitis like illness, 3 of all had ascites and 2 of them were semiconscious. Family history was the helping clue in the diagnosis of Wilson case.

Immunization with hepatitis A vaccine was recommended in all patients with chronic liver disease by many authors [3, 14,15], but not in India as there was a high prevalence of pre-existing antibodies in these patients [7].

Although extra hepatic manifestations are rare in HAV infection, we had 5 cases (10%), four patients (8%) presented with evidence of hemolytic anemia and three (6%) of those were G6PD deficient, all of them had hepatosplenomegaly, sever anemia and very high serum bilirubin. One of those was a boy 6 years old on the verge of renal failure but fortunately recovered with supportive therapy.

Hemolytic anemia as a complication of acute hepatitis had been reported in up to 23% of patients. However the incidence may rise to 70-87% in patients with G6PD deficiency, massive intravascular haemolysis with renal failure, hepatic encephalopathy and even death have been reported [16, 18].

One patient (2%) had convulsion at the start of the icteric phase but without neck stiffness, and his CSF findings were normal. Looking in the literature, there was only one case report for a five years old child presented with convulsion and neck rigidity, and because HAV RNA was demonstrated in the CSF, it was thought that convulsion might be related to this viral infection [19].

Hepatitis A virus associated mortality world wide is 0.2- 0.4 % (1),but in this study it was 4% which is due to fulminant hepatic failure as our hospital is a tertiary center and we receive terminal cases.

In our study, all the mean hematological and biochemical levels in complicated cases were greater than that in uncomplicated patients (Table 4) which is similar to Sainokami S et al findings [20]. Increased levels of serum transaminases and prolonged PT and PTT gave an idea of the unusual presentation and sometimes bad prognosis.

It was concluded that the Hepatitis A viral infection can present in different ways.

It occurs mostly in the age group (> 1-5 years).

Hepatitis A viral infection on the top of pre-existing chronic liver disease or in G6PD deficient cases could affect the presentation and the course of the disease. The level of consciousness, presence of ascites and severely abnormal biochemical and hematological values were of great help in predicting complicated cases of hepatitis A viral infection.

References

1. Davidson S. Acute Hepatitis in Diseases of Liver and Biliary System in Children by Deirdre A. Kelly; Blackwell publications, second edition. 2004; Ch6:92-104.

2. Yazigi N., Balistreri W., Viral Hepatitis In Kliegman R.M., Behrman R.V., Jenson H.B, Stanton F. Nelson Textbook of Pediatric 18th ed. Philadelphia W.B. Saunders 2007; 355: 1680 - .90.

3. Keefe EB, Iwarsons Mc, Mahon BJ, et al. Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease . Hepatology 1998; 27:881-6.

4. Lemon SM,Thomas DL. Vaccines to prevent viral hepatitis. New Engl.J Med 1997; 336:196-204.

5. Koff RS. Hepatitis A. Lancet 1998; 341:1643-9.

6. Rasheed A, Saeed S. Acute hepatitis A virus infection presenting with multiorgan dysfunction: a case report.Cases J, 2009; July 30(2): 8124.

7. Anand AC, Nagpal AK. Should one vaccinate patients with chronic liver disease for HAV in India? J Assoc Physicians India 2004; 52:785-7.

8. Amin J, Gilbert GL, Escott RG, et al. Hepatitis A epidemiology in Australia. National section 2001; 174(7):338-41.

9. Gilroy R, Mukherjee S. Article on hepatitis A, last updated: August 11, 2004. (E-medicine, internet).

10. Kemmer NM, Miskovisky EP. Hepatitis A.Infect Dis Clin North Am 2000 Sep; 14 (3): 6-9.

11. Durand F, Clinical forms of hepatitis A. Rev Med Intern.2000 Jan; 21(1):50-7.

12. Arslan S, Caksen H, Oner AF,et al. Relapsing Hepatitis A in children. Acta Paediatr Taiwan 2002 Nov-Dec; 43(60):358-60.

13. Ginsber GM, Slater PE, and Shouva LD: cost benefits analysis of a nation wide infant immunity area of intermediate endemicity. J Hepatology 2001 Jan; 34 (1): 92-9.

14. Shepherd R. Complicatios and management of chronic liver disease in Disease of Liver and Biliary system in children by Deirdre A.Kelly. Blackwell Publication, Second Edition 2004;Ch 14:259-79.
15. Lefilliare P, Villeneeuve JP.Fulminant hepatitis in patients with chronic liver diseases. Can. J Pub, Health 2000; 91(3): 168-70.
16. Chau TN, Lai JY. Haemolysis complicating acute viral hepatitis in patients with normal or deficient G6PD activity. Scand. Infec Diseases 1997;29(6):551-3.
17. Sharma D, Sibal A. Making a case for hepatitis A vaccination in G6PD deficient subjects. Indian J of Pediatrics 2005; 72: 640.
18. Gotsman and Muszkat M.G6PD deficiency is associated with increased initial clinical severity of acute viral hepatitis A.J of Gastroenterology and Hepatology 2001; V 16: Issue 11: 1239.
19. Cam S, Ertem D, Koroglu OA et al. Hepatitis A virus infection presenting as seizures. Pediatrics Infect. Dis 2005 July; 24(70): 652-3.
20. Sainokami S, Abe K, Ishikawa K, et al. Influence of load of hepatitis A virus on disease severity and its relationhipwith clinical manifestation in patients with hepatitis A. J Gastroenteral Hepatol.2005 Aug; 20(8): 1165-75.

Morphometric analysis of odontoid process.

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Abstract

Background: The axis vertebra is the strongest cervical vertebrae; the characteristic feature of it is the presence of strong bony element, the dens (odontoid process). Several investigations have shown its relation with certain congenital anomalies.

Objective: The aim of this study was to evaluate the value of some morphometric parameters measured from the odontoid process of the axis vertebra to judge the degree of retroflexion of the odontoid process in relation to the morphological architecture.

Method: 30 randomly selected dry bones of adult second cervical vertebrae were used. These vertebrae were examined grossly and morphometrically, and the data obtained were analyzed by using global lab image/2 computer program.

Results: Morphometric results of this study showed that the retroflexion of the dens should be related to dorsal deviation, if the anteroposterior distance of the dens is fixed. The thicker anteroposterior dens is longer, wider, but less dorsally deviated.

Conclusion: We may be able to consider the dorsally deviated dens and the anteroposterior thickness of the dens as indicator for the degree of retroflexion of the dens.

The study may elaborate the possibility of using the above parameters as markers for the retroflexion of dens in cases of Chiari I malformation. Thus a preliminary diagnosis could be achieved.

Key words: morphometry, dens, congenital anomaly.

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Introduction

Vertebra C2 is the strongest of the cervical vertebrae. C1, carrying the cranium, rotates on C2, as when a person turns the head. The axis has two large, flat bearing surfaces, the superior articular facets, on which the atlas rotates. The distinguishing feature of the axis is the blunt tooth-like dens (odontoid process), which projects superiorly from its body. Both the dens (G. tooth) and the spinal cord inside its coverings are encircled by the atlas. The dens lie anterior to the spinal cord and serves as the pivot about which the rotation occurs. The dens are held in position against the posterior aspect of the anterior arch of the atlas by the transverse ligament of the atlas [(1)].

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The axis, acts as an axle for rotation of the atlas and head around the strong dens (odontoid process), which projects cranially from the superior surface of the body.

The dens are conical in shape. It may be tilted a little, up to 14°, posteriorly, or, less often, anteriorly on the body of the axis: it may also tilt laterally up to 10°. The apex is pointed, and from this point arises the apical ligament. The anterior surface bears an ovoid articular facet for the anterior arch of the atlas [(2)].

Anomalous development of the odontoid is uncommon, and its clinical significance lies in its potential for producing serious neurologic sequelae [(3)]. These anomalous include either odontoid invagination, in which the odontoid process bulges upward into the foramen magnum and compresses the brainstem without deformity of the occipital bone [(4)], or Congenital and developmental osseous abnormalities and anomalies that affect the

craniocervical junction complex which can result in neural compression and vascular compromise and can manifest itself with abnormal cerebrospinal fluid dynamics [5].

Chiari I malformations are often associated with congenital craniocervical anomalies, such as platybasia, basilar invagination, and retroflexion of the odontoid process [6].

The aim of this study was to evaluate the values of some morphometric parameters measured from the odontoid process of the axis vertebra to judge the degree of retroflexion in relation to other morphological architectures.

Material and methods

This study involves anatomical observation of 30 randomly selected dry bones of adult second cervical vertebrae obtain from anatomical museum of the department of human anatomy at Al- Nahrain College of medicine. The odontoid process of these vertebras was examined grossly. Certain morphometric measurements were done on the following parameters:

- The line which connect the upper and lower point of the anterior surface of the body of the axis represented by L1 (figure1).
- The mid-line of the anterior surface of the dens is represented by L2 (figure 2).
- The angle between L1&L2 is called G1. This parameter could indicate the deviation of the anterior surface of dens from that of axis (figure 3).
- The point of deviation of L1 across L2 called P1 (figure 3).
- The most posterior point of the posterior surface of dens is P2(figure 3).
- D1 represents the distance of the horizontal imaginary line between the most posterior point of dens P2 and the L2 (figure3&4).
- D2 represents the distance between P2 and the linear upward extension of L1 (figure3&4).
- G2 represents the deduct value of D2 from D1 (Figure 4).
- The length of dens represents the line between the P1 and its highest apical point (figure 5).
- The width of the dens is widest anterior distance of the dens (figure 5).

Measurements were done on the photographs of the odontoid process of the second cervical vertebra, The photograph of the axis vertebra were done according to the need of experimental analysis of this study regardless of the anatomical position of the second cervical vertebrae. The data obtained from these measurements were analyzed using the global lab-image2 computer program

Global lab image2 software:

Is the keystone in this study by which all the results can be counted and analyzed manually or automatically (with less degree of user interference) [7]. The software is a product from (Data Translation Inc. USA). As any other software, it needs special requirements to be installed on the PC. These requirements are:

- MMX processor (Pentium III or higher).
- Microsoft® Windows 98, ME, or XP.
- At least 128 MB of random access memory (RAM).
- At least 150 MB of available hard disk space.
- CD-ROM drive.
- SVGA monitor.
- Display resolution set to 800x600 pixels or higher.
- Color quality set to highest (32-bit).
- Imaging device or frame grabber board (optional).

Results

The statistical analyses of the dens were as the following:

The first group with significant relation ship these include:

- The correlation between the measurements of the angle (G1) and the (G2) (figure 3, 4), the P value indicates highly significant relationship (table 1).
- The correlation between angle (G1) and the distance between anterior and posterior points of dens (D1) (figure 3, 4), the P value indicates significant relationship (Table 1).
- The correlation between (D1) and length (figure 4, 5), the P value indicates highly significant relationship (table2).
- The correlation between (D2) and the length (figure 4, 5), the P value indicates significant relationship (table2).
- The correlation between (D1) and (D2) (figure 3); the P value indicates significant relationship (table2).
- The correlation between (D2) and (G2) (figure 4); the P value indicates significant relationship (table2).

Table 1: statistical analysis of this study

Type of relation ship	Correlation coefficient (r2)	Significance (P) value	Notes
Angle (G1)with (G2)*	0.872	0.00001	Highly significant relation ship
Angle (G1) with length*	0.001	0.868	Non- significant relation ship
Angle (G1) with side to side *	0.015	0.524	Non- significant relation ship
Angle (G1) with D1#	0.136	0.046	Significant relation ship
Angle (G1) with D2*	0.313	0.058	Non- significant relation ship

The second group with non-significant relation ship these include:

- The correlation between the measurements of the angle (G1) and the length (figure3, 5), the P value indicates non- significant relationship (Table 1).
- The correlation between the measurements of angle (G1) with (D2) (figure 3, 4), the P value indicates non-significant relationship (Table 1).
- The correlation between the measurements of length and G2 (figure5, 4), the P value indicates non- significant relationship (Table 2).
- The correlation between G2 and D1 (figure 4), the P value indicates non-significant relationship (Table 2).
- The correlation between side to side measurements (figure 5) and the angle G1, G2, D1, D2, and the length of dens (figure3, 4), the P value indicates non-significant relationship (Table 2).

Table 2: statistical analysis of this study

Type of relation ship	Correlation coefficient (r2)	Significance (P) value	Notes
D1 and length of dens*	0.419	0.0001	Highly significant relation ship
D2 and length*	0.554	0.006	Significant relation ship
Side to side and length#	0.006	0.697	Non- significant relation ship
G2 and length *	0.192	0.154	Non- significant relation ship
G2 and D1#	0.012	0.73	Non- significant relation ship
Anteroposterior (D1)and D2 *	0.412	0.024	Significant relation ship
Side to side and D2*	0.01	0.757	Non- significant relation ship
G2 and D2 *	0.477	0.013	Significant relation ship
Side to side and (D1) #	0.000	0.961	Non- significant relation ship
G2 and side to side*	0.005	0.832	Non- significant relation ship

Negative correlation (-) *Positive correlation (+)

Discussion

This study concerning with statistical analysis, and morphology with special emphasis on the degree of the inclination of the odontoid process, for functional and clinical manifestations, This inclination is important because The straight odontoid process is only subjected to stress of weight of the skull, but the dorsally inclined dens act to bending stress[8].

The orientations of the parameters and the statistical analysis showed in this study support the view that had been adapted previously in the anomalous dens as a clinical significance in producing serious neurological squeal[3]. Although there are several recognized variations such as (aplasia, hypoplasia, and os odontoideum Congenital absence of the odontoid process, or Duplicated odontoid process),some anomalous may produces a serious clinical manifestations these may be associated with subluxation and neuraxial compression and atlantoaxial instability[9-11],headaches, pseudotumor-like episodes and hydrocephalus)[1], or compression of the upper cervical cord[13].

Chiari I malformations may be associated with changes that may be occurs in the cranio cervical region or herniation of the cerebellar tonsil below the foramen magnum [14]. In addition to that, this malformation is often associated with retroflexion of the odontoid process. These signs confirm prior reports of an increased incidence of Chiari I malformation.

In this study we emphasized on the correlation between the degrees of inclination (retroflexion) in relations with other statistical analysis of the odontoid process.

The line suggested being the anterior vertebral line of the axis vertebra L1 which used for measurements of the degree of angulations (figure 1) is unchanging when the axis vertebra changes from normal anatomical position.

The angle between L1&L2 (G1), this parameter could indicate the deviation of the anterior surface of dens from that of axis (figure 3).G2 represents the deduct value of D2 from D1 this parameter could indicate the dorsal deviation of the anterior surface of dens (L2) excluding its anteroposterior thickness (Figure 4).

There is a significant relation ship with positive correlation between the degree of retroflexion (G1), and the dorsal deviation of the dens (G2), if the antero posterior distance of the dens is fixed so that the degree of retroflexion should be related to the dorsal deviation of the dens.

The significant relation ship with negative correlation between retroflexion (G1), and antero posterior distance of the dens (D1), means that whenever the degree of retroflexion increases, the antero posterior distance (thickness) decreases, however there is a significant relationship with positive correlation between this anteroposterior thickness(D1),thickness with dorsally deviated (D2), and the length of the dens, there is a non-significant relation ship between the length and the degree of retroflexion. This indicates that the longer dens is thicker anteroposterior, with more dorsally deviated. This positive correlation not related to the degree of retroflexion of the dens but it may be related to massive growth of the dens.

Although there is a non-significant relation ship between the retroflexion (G1), with the length, and anteroposterior thickness with dorsally deviated (D2) dens, there is positive correlation between these parameters i.e. the more retroflexed dens, come with increases in the length, thickness with dorsally deviated, and side to side parameters of the odontoid process.

There is a non- significant relation ship with negative correlation between antero posterior thickness of the dens (D1), the dorsally deviated dens (G2), and side to side distance i.e. the thicker and wider dens is less dorsally deviated.

When the length of the dens increases, it may be bulges upward into the foramen magnum and compresses the brainstem and may be of a value in case of basilar invagination and brain stem compression [4].

The study may elaborate the possibility of using the length and width of dens demonstrated in anteroposterior X-ray as a marker for the retroflexion of dens in cases of Chiari I figure 6. Thus, a preliminary diagnosis could be reached, In addition to that evaluation of anterior dens provide essential anatomic data for safer surgical procedures [15].

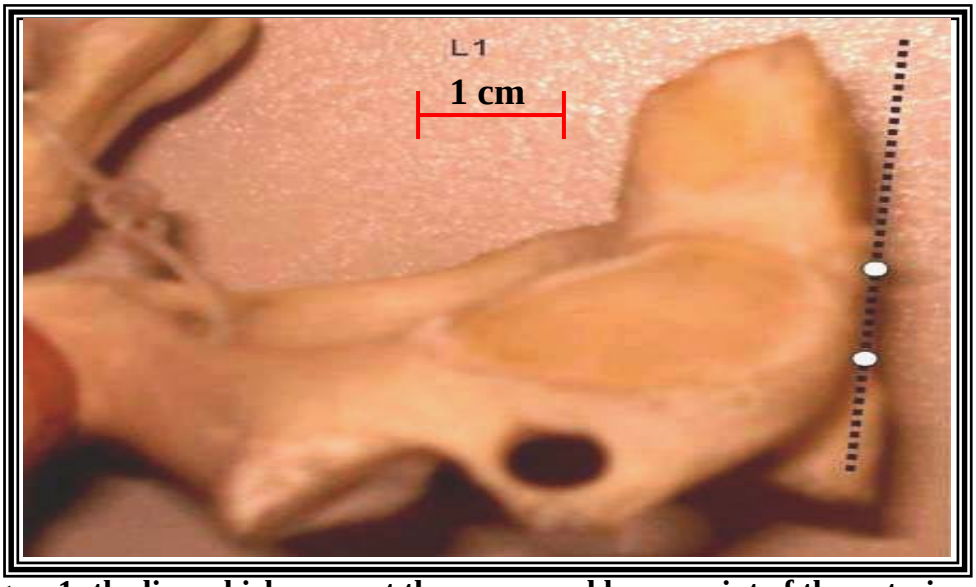

Figure1: the line which connect the upper and lower point of the anterior surface of the body of the axis (L1).

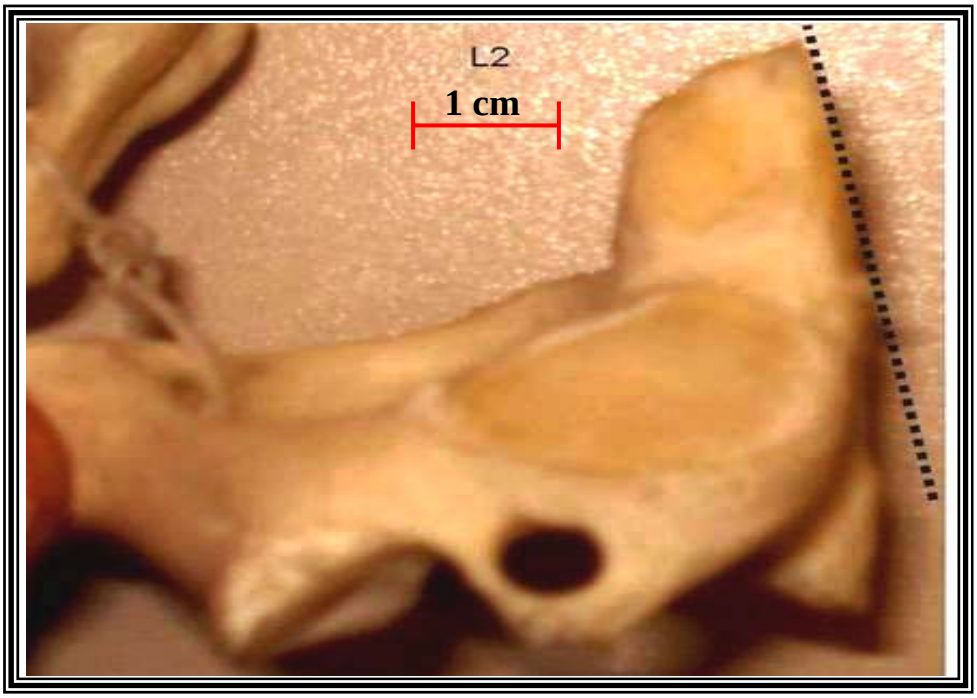

Figure2: the mid-line anterior surface of the dens is L2.

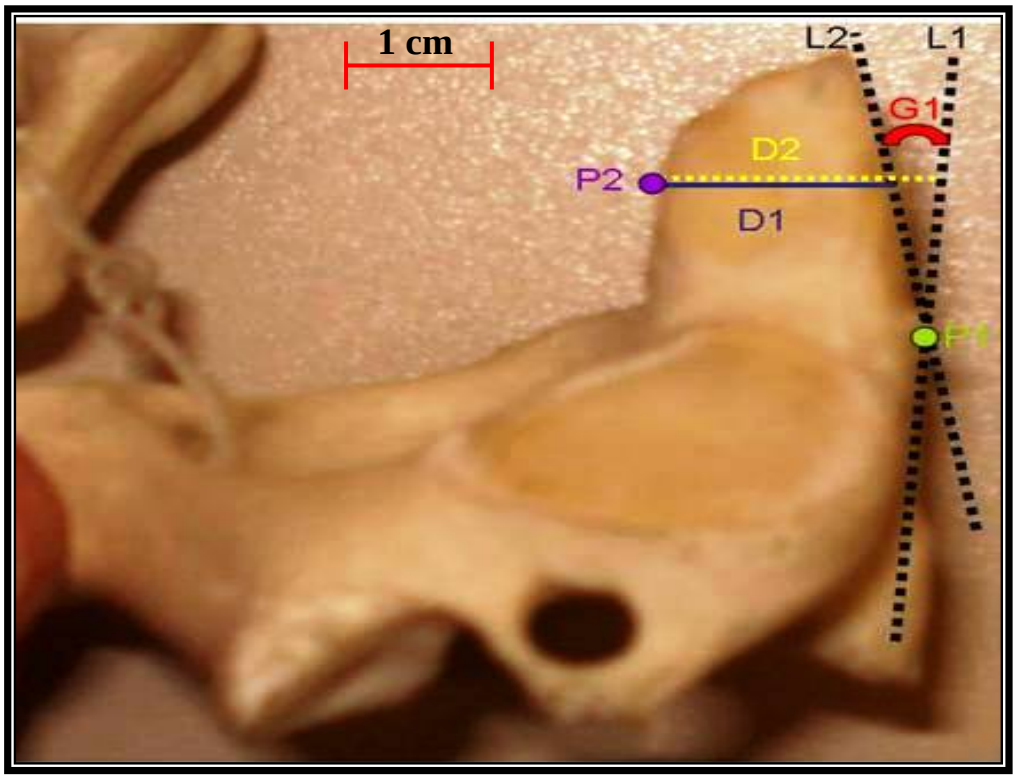

Figure 3: The angle between L1&L2 represented by (G1). P2 represents the posterior point of the posterior surface of dens, D1 represents the distance between the anterior and posterior point of dens P2, and D2 represent the distance between L1&P2.

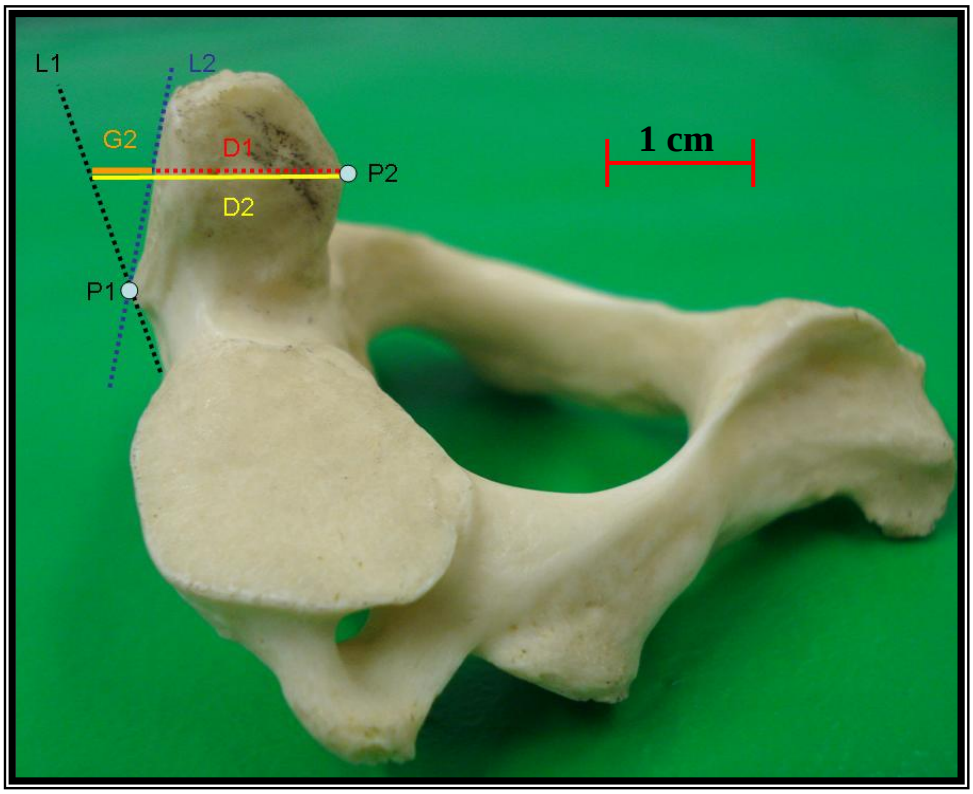

Figure 4: the deduct value between (D2-D1) represented by G2.

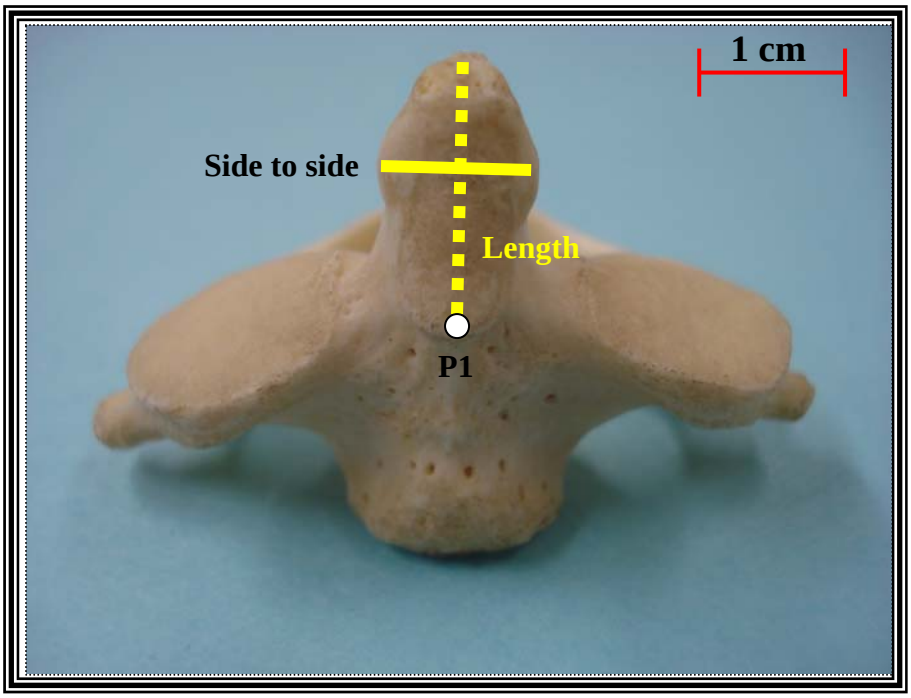

Figure5: the length and the anterior width of the dens (Side to side).

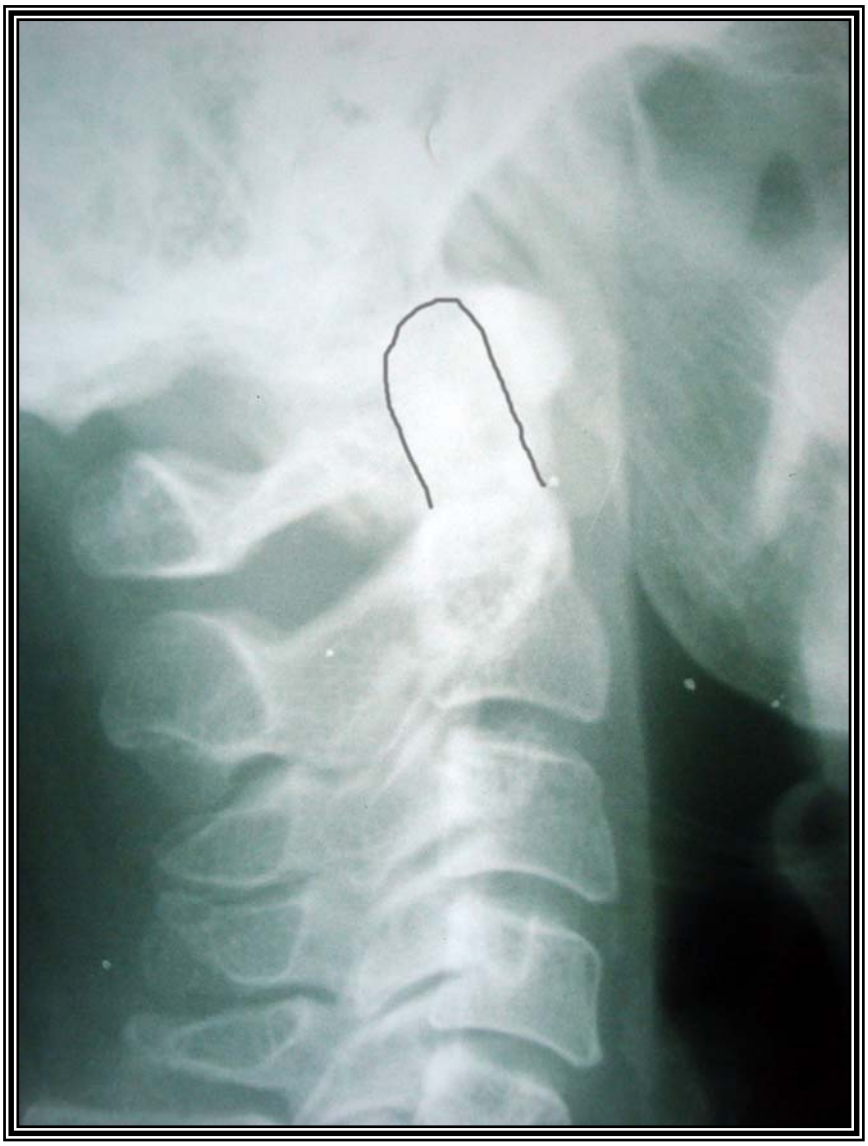

Figure 6: odontoid process in plain X-ray (lateral view).

References

1. Moore Keith L, Dalley Arthur F. Clinically Oriented Anatomy, 5th EditionWilliams & Wilkins, 2006; 486.

2. Susan standring. Grays anatomy the anatomical basis in clinical practice,39th edition Churchill livingstone,2008; 745.

3. Hensinger RN, Fielding JW, Hawkins RJ. Congenital anomalies of the odontoid process Orthop Clin North Am. 1978;Oct;9(4):901-12

4. Kyoshima K, Kakizawa Y, Tokushige K, Akaishi K, Kanaji M, Kuroyanagi T **,** Odontoid compression of the brainstem without basilar impression-- "odontoid invagination". J Clin Neurosci. 2005; Jun;12(5):565-9

5. Menezes AH. Craniocervical developmental anatomy and its implications Childs Nerv Syst. 2008; Apr: 10.

6. Hwang SW, Heilman CB, Riesenburger RI, Kryzanski J. arthrodesis after transoral odontoidectomy and suboccipital craniectomy for ventral brain stem compression in Chiari I patients.Eur Spine,2008; J. Jul 16.

7. Maria SS, Vidal BC and Mello MLS .Image analysis of DNA fragmentation and loss in V79 cells under apoptosis. Genetic and Molecular Biology. 2000; 23:109-112.

8. Koebke J. Morphological and functional studies on the odontoid process of the human axis Anat Embryol (Berl). 1979; Jan 30; 155(2):197-208.

9. Stevens JM, Chong WK, Barber C, Kendall BE, Crockard HA.A new appraisal of abnormalities of the odontoid process associated with atlanto-axial subluxation and neurological disability. Brain Feb; 1994 ;117 (Pt 1):133-48

10. Zadvornov IuN. Variants and developmental anomalies of the odontoid process of the axis Zh Vopr Neirokhir Im N N Burdenko, 1979 ;(1):30-8

11. Garant M, Oudjhane K, Sinsky A, O'Gorman AM. Duplicated odontoid process: plain radiographic and CT appearance of a rare congenital anomaly of the cervical spine. AJNR Am J Neuroradiol. 1997;Oct;18(9):1719-20

12. Milhorat Thomas H, Chou Mike W, Trinidad Elizabeth M M D, Kula Roger W M D, Mandell, Menachem MD, Wolpert, Chantelle MBA,et al. Chiari I Malformation Redefined: Clinical and Radiographic Findings for 364 Symptomatic Patients Neurosurgery. 1999; Volume 44 - Issue 5 - 1005-1017

13. Kremer P, Despaux J, Benmansour A, Wendling D**.** Spontaneous fracture of the odontoid process in a patient with ankylosing spondylitis. Nonunion responsible for compression of the upper cervical cord. Rev Rhum Engl Ed. Jun, 1995; 62(6):455-8.

14. Shane Tubbs' Matthew Bailey, William C. Barrow, Marios Loukas, Mohammadali M. Shoja[4] and W. Jerry Oakes[1], Morphometric analysis of the craniocervical juncture in children with Chiari I malformation and concomitant syringobulbia Neurosurgery vol.25 no.6, 2009; 689-692.

15. Tun K, Kaptanoglu E, Cemil B, Karahan ST, Esmer AF, Elhan A:. A neurosurgical view of anatomical evaluation of anterior C1-C2 for safer transoral odontoidectomy Eur Spine J. 2008 ; Jun;17(6):853-6.

Multiple myeloma with breast masses as extra medullary plasmocytomas,A Case Report.

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Abstract

Background: Breast involvement by immunolymphoproliferative disorders is rare. Primary & secondary malignant lymphomas of the breast are much more common than multiple myeloma, of which only 18 cases were reported in previous literature, till the year 2000.

Case presentation: We report a young female patient presented simultaneously with bilateral multiple breast masses & pathological fracture in right humorous that proved later on via histopathological examination to be a case of multiple myeloma associated with extramedullary plasmocytoma involving breasts.

Discussion and Conclusion: There were diagnostic difficulties caused by the lack of specific radiological & sonographic features that differentiate between primary or secondary breast tumor & breast infiltration by immunolymphoproliferative disorders.

Key words: Plasma cell diseases, plasmocytoma, multiple myeloma, breast mass

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Introduction

Breast involvement in multiple myeloma & solitary extramedullary plasmcytoma is very unusual. In most cases, the manifestation of this disease is usually systemic [(1)].

Multiple myeloma is a neoplastic plasma cell dyscrasia characterized by clonal proliferation of plasma cells & overproduction of paraprotein[(2)].

It is the most common plasma cell disorder representing 1% of all types of cancers [(2)].

Incidence is 4.5/100 000 /year in US. It increases progressively with age.

The mean age at diagnosis of multiple myeloma is 65-70 years, while the onset before 40 years is very rare [(2)].

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Impaired hemopoeisis, renal insufficiency, osteolytic bone lesions, recurrent infections, hypercalcemia are the most common systemic manifestation of the disease.

Extra medullary myeloma (plasmocytoma) is defined as malignant proliferations of plasma cells with or without bone involvement [(2)]. When occur in the soft tissue, it usually involves the upper respiratory tract & oral cavity. Plasmocytoma of breast is very rare [(3)].

This report describes a young female patient with bilateral breast masses with pathological fracture that proved to be part of manifestation of multiple myeloma with extra medullary plasmocytoma involving both breasts.

Case presentation

Thirty four years old, an engineer, female patient presented with thirst lethargy & diffuse bone pain with difficulty in walking of 3 weeks duration. Two months ago she sustained right upper limb pain after a trivial trauma, which proved to be, later on, due to pathological fracture of

the upper end of right humerus by open biopsy (Figure 1), (pieces of bone at that site were sent for histopathological examination & immune histochemical staining) . Blurred vision & headache were reported but no fainting attacks or other system complaint. Menstrual history is normal. She had negative past medical & surgical history.

There is negative family history for breast cancer or other chronic or malignant diseases. She is a mother of one boy, 3 years old, with no use of any contraception or other medications. There is no history of smoking.

On examination, a young female patient ill looking with limited mobility & a POP splint over right upper limb. She is pale dehydrated, afebrile & not jaundiced. Tachycardia 112 beats per minute regular normal volume, blood pressure 100/65 mmHg. There is generalized bone tenderness especially over the limbs. There were no palpable lymph nodes any where. Abdomen, chest & cardiovascular examination were normal.

Breasts examination revealed the presence of 2 masses in the left breast (at upper inner quadrant), largest one around 2.5 x2.5 cm, and another mass in the right breast with no palpable axillary lymph nodes.

After admission a thorough evaluation performed including the basic hematological & biochemical investigations as follows;

PCV 24%, hemoglobin 76 g/l, white blood cell 4.9 X 10^9/l (Neutrophil. 51%, Lymphocyte 44%, Monocyte 3%) ESR 130 mm/hr

Blood film revealed normochromic normocytic with excess roulaux, no abnormal leucocytes and adequate platelet

Blood sugar 110 mg/dl, urea. 36 mg /dl, creatinine 1.2 mg/dl, uric acid 6.5 mg /dl

Total serum protein 11g/dl, S. Ca (serum calcium) 11.5 mg/dl, S. ALP (alkaline phosphatase) 128.8 IU/L & S. LDH (lactate dehydrogenases) 200 IU/ L.

Normal prothrombin time & partial thromboplastin time &liver enzyme tests.

Normal general urine exam with no proteinuria. Urine for bence jones protein was negative

Serum protein electrophoresis: albumin 2.8 g/dl (3.5-5), α-1 0 .23 g/dl (0.1-0.3), α-2 0.72 g/dl(0.6-1.0), β 0.75 g/dl(0.7-1.1) & γ 4.79 g/dl(0.8-1.6) with monoclonal band in γ region &marked reduction at albumin fraction.

Bone marrow aspiration revealed cellular fragment with active normoblastic erythropoiesis, active myelopoiesis sequence maturation no excess in blast. Plasma cells 5%, active megakaryopoeisis.

Bone marrow biopsy showed sections with infiltration of bone marrow by numerous clusters of immature plasma cells with normoblasts in diffuse manner.

Breast ultrasound ; Multiple ill defined complex density non homogenous masses at medial quadrant of left breast largest size 3.5x 3cm , no calcification normal skin, subcutaneous tissue & axillary region, suggestive of malignancy ,another small similar mass in right breast 2.0 x1.5 cm

FNAC (fine needle aspiration cytology) from right breast mass; sheets of lymphocytes &immature plasma cells suggestive of hemopioeitic malignancy infiltrating the breast.

Skull X ray showed 3 ill defined lytic lesions largest is 3x 0.5cm (Figure 2)

Spine X rays & pelvis X rays were normal.

Right shoulder & humerus X ray showed multiple lytic lesion over head, neck &shaft humerus with

subperiosteal reaction & pathological fracture at upper third of humerous.

Bone biopsy from site of fracture showed diffuse infiltrations of bone trabculea by immature plasma cells (plasmablasts) with binucleated forms. Picture consists with multiple myeloma.

Immune histochemical staining of the same specimen revealed sheets of cells showing eccentric nuclei with granular cytoplasm consistent with plasma cell differentiation with frequent mitosis &abnormal nuclear pleomorphism & binucleation. These cells are positive for CD79a, CD 38, and CD 138 with kappa chain restriction. Picture consistent with malignant plasma cell disorder.

The presence of paraproteinemia& monoclonal gammopathy with skeletal lytic changes in addition to plasma cells in bone marrow biopsy are fulfilling major criteria of a diagnosis of multiple myeloma as well as the finding of related organ tissue injury (ROTI) like anemia & hypercalcemia. Breast masses are suggestive of extramedullary plasmocytoma.

After full evaluation, the treatment strategy started in form of zolderonic acid for hypercalcemia, blood transfusion for anemia & then a specific therapy started in form of combination of thalidiomide 100 mg /day orally, continuously, plus VAD protocoal (vincristin 0.5 mg i.v.infusion /day, adriamycin 9 mg/mm^2[16mg] i.v infusion over 24 hours & dexamethason 40 mg /day i.v. for 4 days) every 28 days.

After 4 cycles of VAD protocol with thalidomide over the whole period in addition to zolderonic acid monthly, patient improved concerning her bone pain, general condition, physical capability with healing of her fracture & removal of splint. Bilateral breast masses disappeared on clinical examination ,Her last review of investigations revealed PCV 42%, Hb. 132 g/l, WBC 5.2 x10^9/l with normal differential count & normal platelet &blood film . ESR 23 mm/hr.

Total serum protein is 7.5 g/dl, Albumin 5.0 g/dl with normal serum protein electrophoresis & no M- band. Normal renal function & normal serum calcium level. Breast U/S normal no mass was seen. Skeletal bone survey showed no evidence of any new lesions. Old lesions are smaller in size & less in number.

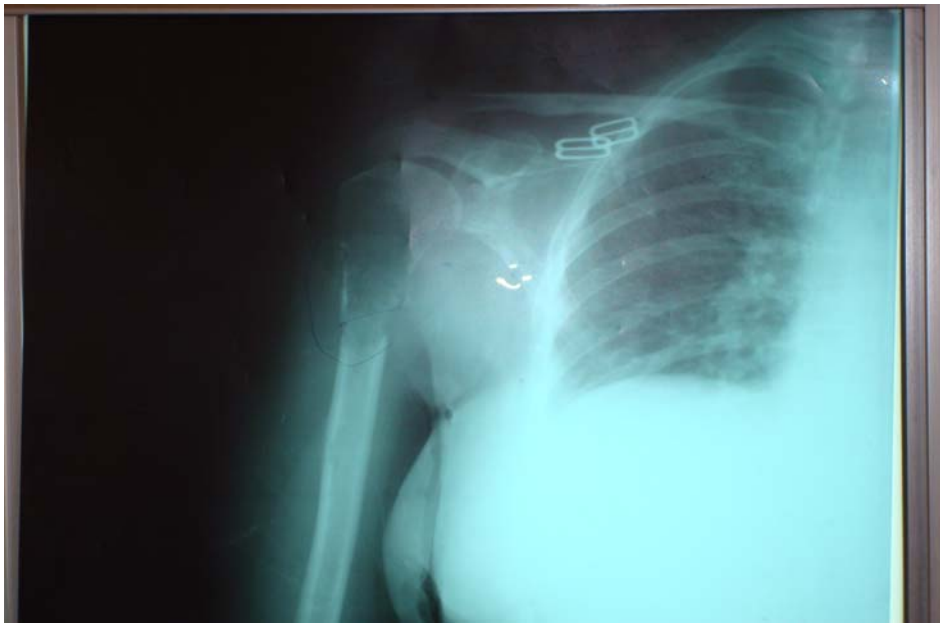

Figure 1: Right humerus X ray

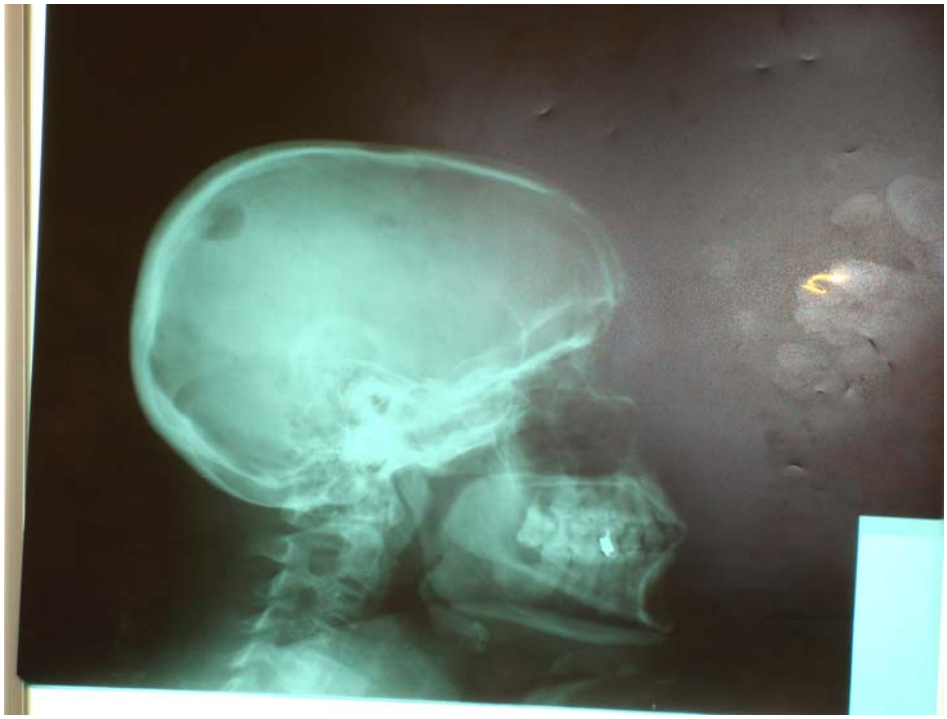

Figure 2:Lateral skull X ray

Discussion

The differential diagnoses for this case is

1. Primary breast carcinoma with bony metastasis (primary synchronous multiple breast cancer).
2. Multiple plasmocytoma with extramedullary plasmocytoma involving breast.
3. Multiple myeloma with extramedullary plasmocytoma
4. Breast non Hodgkin lymphoma with bone involvement.
5. Primary osteosarcoma with breast metastasis.

Final diagnosis settled to be multiple myeloma with extramedullary plasmocytoma

Multiple myeloma is B cell lineage disseminated malignancy, due to clonal proliferation of plasma cells [4].

Clinically apparent extraosseous manifestations are present in <5% of the cases & usually associated with more aggressive behavoiur, resistance to treatment & short survival period [4]. It is a localized growth of plasma cells. It can occur in association with bony structure (medullary) or in other area like in nasopharynx (extra medullary) & it may occur as solitary plasmocytoma without other evidence of multiple myeloma[2].

Clinico-pathological studies shows involvement, in 2/3 of patients, of liver, spleen & lymph nodes while rare cases report breast plasmocytoma in literatures.

Non Hodgkin lymphoma & multiple myeloma are most frequent lymphoproliferative disorders but their localization in breast is quite rare [4].

Few cases of breast multiple myeloma are reported in literatures [4].

Ross et al described one case & review of 10 similar records [5].

Furthermore one case reported by Collins et al [6] & 2 cases by Moulopoulus et al [7]. More recently Kim [8] & Ariad [9] described another 2 cases, in addition to report of 2 cases by Pasquini E. et al [4].

The appearance of breast nodules in patients with immunoproliferative disorders makes it mandatory to differentiate between primary breast cancer & hematological malignancy [10] as well as other benign conditions that may mimic these conditions like pyogenic breast abscess, fat necrosis, lymphocytic mastitis , fibroadenoma or even synchronous breast cancer[3,11].

Rarely multiple myeloma as a systemic disease can involve the breast as extramedullary site (Pasquini E et al reviewed only 16 cases were published in literatures yet)five of them as solitary plasmocytoma, four were synchronous with diagnosis of multiple myeloma , while one case in which breast mass preceedes the diagnosis of systemic diseases. In four patients, the breast involvement followed an established diagnosis of multiple myeloma [4].

Among these cases, distribution of breast nodules was unilateral in 9 patients & bilateral in rest of cases [4].

Review of literatures revealed little help in the use of mammography or ultrasound examination in differentiation between myeloma involving breast & breast cancer, because multiple myeloma may give atypical appearance such as speculated mass that is difficult to be differentiated from primary carcinoma of breast by these investigations unless you have a guided core biopsy [3, 5, 11].

The latest review of diagnostic criteria of multiple myeloma according to the international classification scheme as it is clinico-pathological diagnosis depends on the presence of M protein in serum or urine plus one or more of the following that must be presented which are:

1. Marrow plasmocytoma >5% in absence of underlying reactive process.
2. Tissue biopsy demonstrating replacement & distortion of normal tissue by plasma cells.
3. More than 500 plasma cells /mm^2 in peripheral blood.
4. Osteolytic lesions unexplained by other causes.

In case where the M protein is absent in serum & urine, it must have radiological evidence of osteolytic lesions or palpable tumors & one or more of the following that must be presented:

1. Marrow plasmacytosis >20% from 2 sites in absence of reactive process.
2. Tissue biopsy demonstrates replacement & distrortion of normal tissue by plasma cells [2].

Extramedullary plasmocytoma may represent the initial manifestation of systemic multiple myeloma or otherwise remain solitary for long time [4].

The treatment of solitary plasmocytoma of breast should consisis of local excision followed by brachiotherapy whereas when breast involvement is secondary to disseminated multiple myeloma the treatement should be treatment of the basic disease employing the most widely used schedules like VAD or melphalan based chemotherapy[4] in addition to new immunomodulating agent [2].

References

1. Aysenur M and Esin EU. Plsaocytoma of the breast. Eu. Radiol. 1994;4:500-101

2. Angela D, Martha Q L, and Philip R G. Multiple Myeloma in John P. G. ,John F. , George M. R. , Frixos P. ,Bertil G., Daniel A. A.& Robert T. M. Wintrobe Clinical Hematology, Lippincott Williams & Walkins,12ed.: 2009;99:2372-2375

3. Kaviani A, Djamali- Zavareie M, Neoparast M, Keyhani- Roagha S. Recurrence of primary extramedullary plasmocytoma in brest both simulating breast carcinoma, World Journal of surgical oncology. 2004; 2:29.

4. Pasquini E, Rinaldi P, Nicolini M, Papi M, Fabbri P, Bernardi L, et al. Breast involvement in immunolymphoproliferative disorders : report of 2 cases of multiple myeloma of the breast. Annals of Oncology 2000; 11:1353-1359.

5. Ross J S, King T M, Spector J I, et al. Plasocytoma of the breast. An unusual case of

recurrent myeloma. Arch Intern Med 1987; 147: 1838-1840.

6. Collin C D, Kedar R P, Cosgrove P O, Case Report: myeloma of the breast-appearance on ultrasound & color Doppler Br j Radiol 1994;67: 399-400.

7. Moulopoulus L A, Grandfielf C A, Dimopoulos M A, et al. Extraosseous multiple myeloma. Imaging features. Am J Roentgenol. 1993; 161:1083-1087.

8. Kim E E, Sawwaf Z V, Sneigej N. Multiple myeloma of the breast. Breast Disease 1996; 9:229-233.

9. Ariad S, Lewis D, Cohen R, Bezwoda W R. Breast Lymphoma: A clinical & pathological review & 10 years treatment results. South Africa Med. J. 1995; 85:85-90.

10. Chan N H L, Lam T P W, Yuen J H F, Leong L L Y, Conditions that mimic primary breast carcinoma on mammography & sonography. J H K Col Radiol. 2004; 7:49-55.

11. David S and Garrett T. J. Multiple myeloma masquerading as metastatic breast cancer. Cancer 1986; 57:923-924.

المجلة العراقية للعلوم الطبية
قائمة المحتويات

المقالات

تقرير حالة:

❖ **ورم نقوي متعدد مع كتلة الثدي المكونة من ورم البلازموايات خارج نخاع العظم ، أيلول ٢٠٠٩" تقرير حالة ".**

المجلة العراقية للعلوم الطبية

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الإستشاريين المعتمين للمجلة العراقيـة للعلوم الطبية

الدور الاحتمالي للمدورات الخلوية ذات العلاقة بخلايا تي المساعدة-2 (البين بيضاوي 6 والبين بيضاوي10) في سرطان الثدي

احمد عبد الحسن عباس الحسن[1] , نضال عبد المهيمن[1] , علاء غني حسين[2] , نهلة الخيلي [3]

الخلاصة

خلفية الدراسة: سرطان الثدي هو مرض معقد. الكثير من العوامل افترضت بان لها دور في امراضيته, واحدة منها هي المدورات الخلوية.
هدف الدراسة: في الدراسة الحالية قسنا تركيز البين بيضاوي 6 والبين بيضاوي 10 في مصل مرضى سرطان الثدي لتحديد فيما اذا كان له علاقة بتقدم المرض, وتم فحص مصاحبتها للمتغيرات الامراضية السريرية بضمن ذلك مراحل المرض وسيماء مستقبلات الاستروجين والبروجستيرون على الخلايا الورمية لتحديد اذا كانت مصاحبة لتطور المرض.
الاشخاص وطرائق العمل: تضمنت الدراسة 80 حالة اشتملت على 45 مريضة مصابة بسرطان الثدي, 12 مريضة مصابة بامراض الثدي الحميدة و 23 امراة سليمة ظاهريا كمجموعة ضابطه. استخدم فحص تقنية فحص مترابطة الخميرة بمادة ماصة المناعة ELISA لـ ت ق ي يم مستوى البين بيضاوي 6 والبين بيضاوي 10 في مصل مجاميع الدراسة الثلاثة.
النتائــج: هناك ارتفاع في مستوى البين بيضاوي 6 والبين بيضاوي 10 في مصل مرضى سرطان الثدي مع اختلاف معنوي بين المرضى والسيطرة , كذلك هذا الارتفاع كان مصاحب لتطور المرض. بالاضافة الى ذلك فان مستوى البين بيضاوي 6 كان مرتبط عكسيا مع سيماء مستقبلات الاستروجين والبروجيستيرون بينما بخصوص البين بيضاوي 10 لايوجد فرق معنوي في مستواه بين المرضى الذين يظهرون ايجابا او سلبا للمستقبلات الاستروجينية والبروجيستيرونية.
الاستنتاجات: النتائج اشارت الى ان ارتفاع مستوى البين بيضاوي 6 والبين بيضاوي 10 في المصل تكون مصاحبة لسرطان الثدي ولها علاقة بالمرحلة المتقدمة من المرض. من الملائم القول بان هذه الفحوصات للمستويات المصلية للبين بيضاوي ممكن استخدامها كفحوصات تنبؤية لتطور الورم في مرضى سرطان الثدي.
مفتاح الكلمات:سرطان الثدي ,البين بيضاوي 6 , البين بيضاوي 10 .

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المجلة العراقية للعلوم الطبية 2010 م المجلد 8 العدد 2 ص 4- 9

علاقة التغير المظهري المناعي لخلايا الدم اللمفية المحيطية مع مقياس فعالية المرض في المرضى المصابين بالتهاب المفاصل الرثوي

حيدر فيصل غازي , عبد الرزاق حردان احمد

الخلاصة

خلفية الدراسة: إلتهاب المفاصل الرثوي هو احد امراض ذاتية المناعة يسبب التهاب الغشاء الزلالي للمفاصل ثنائية التركيب, حيث تلعب الخلايا اللمفية المنشطة دوراً مهماً في امراضية التهاب المفاصل الرثوي ولهذا يعتقد بان لها دور مهم في تنبؤ مآل المرض.
هدف الدراسة: تقدير التعبير الخلوي لبعض دوال التنشيط في خلايا الدم اللمفية المحيطية وصلتها بنمط نشاط مرض إلتهاب المفاصل الرثوي.
المواد وطرق العمل: صممت هذه الدراسه للتحري عن نسب اظهار دوال التغير المظهري المناعي في خلايا الدم اللمفية المحيطية في 46 مريض مصاب بالتهاب المفاصل الرثوي, 7 مرضى مصابين بالتهاب المفاصل العظمي و 10 اشخاص اصحاء ظاهرياً. جمعت المعلومات الاساسيه اعتماداً على التقييم المختبري والسريري لفعالية المرض. اخذت عينات الدم من كل الاشخاص ولجميع المجاميع في وقت استشارة الطبيب المعالج, فصلت الخلايا اللمفية وعدت منها مسحات على شرائح زجاجية مشحونه, حفظت بدرجة حراره -20°م لحين وقت التحري. قيست نسبة اظهار المعلمات المناعية 3 و 54 قيست باستخدام طريقة التصبيغ المناعي الخلوي الكيميائي بينما المحدد المناعي 71 فقد قيس نسبة اظهاره باستخدام التصبيغ المناعي الخلوي المتآلق المباشر.
النتائج: ان نتائج المحدد المناعي 3 و 54 قد اظهرت نسبة اظهار عالية في مرضى التهاب المفاصل الرثوي وبمعنوية احصائيه عالية مقارنة بمجموعتي السيطره الصحية والمصابة بالتهاب المفاصل العظمي. اما المحدد المناعي 71 فقد لوحظ فرق احصائي معنوي وبنسبه اظهار اعلى في المجموعة متدنية شدة المرض. عدم وجود اي ارتباط معياري مع دوال المرض المختبرية والسريرية.
الاستنتاج: من نتائج هذه الدراسة, قدمنا ادلة اضافية للخلل التمايزي الحاصل في الخلايا التائيه في مرضى التهاب المفاصل الرثوي والتي من الممكن ان توضح مظاهر مناعية واضحة لهذا المرض والتي ليس لها علاقة بفعالية المرض
مفتاح الكلمات: التهاب المفاصل الرثوي، فعالية المرض، تغير المناعي المظهري، تصبيغ مناعي كيميائي، تصبيغ مناعي متالق و محددات مناعية.

فرع الاحياء المجهرية [كلية الطب – جامعة النهرين]

المجلة العراقية للعلوم الطبية 2010 م المجلد 8 العدد 2 ص 10 - 17

تحديد مستوى بعض مكونات المتمم في النساء اللواتي يعانين من العقم

بتول مطر مهدي[1], وفاء حازم صالح[1], بسمة مكي[1], عاني ادموند كتانو[2], دينا سامي ابراهيم[2]

الخلاصة

خلفية الدراسة: المتمم هو عبارة عن سلسلة من الانزيمات البروتينية, الذى يشكل الجزء الاساسي للجهاز المناعي.تحفيز هذا الجهاز يؤدي في النهاية الى تكوين ثقوب على جدار الخلية.

هدف الدراسة: تحديد مستوى المتمم في مصل النساء اللواتي يعانين من العقم مع تواجد الاجسام المناعية الذاتية للحيامن .

طريقة العمل: تتالف الدراسة من 45 مريضة يعانين من العقم ويراجعن مستشفى كمال السامرائى للعقم للفترة من حزيران-2008 الى حزيران 2009 ومجموعة اخرى تتألف من 30 امرأة من النساء الطبيعيات القادرات على الانجاب بصورة طبيعية. تم سحب الدم من هاتين الفئتين وتم تحديد مستوى المتمم C3,C4 وكذلك تحديد الاجسام المناعية الذاتية الموجهة ضد الحيامن.

النتائج: كان هناك فرق مهم احصائيا بين تلك الفئتين بالنسبة للاجسام المناعية الذاتية للحيامن والمتمم C3,C4.

الاستنتاج: احتمال ارتفاع نسبة المتمم نتيجة لارتفاع نسبة تواجد الاجسام المناعية الذاتية للحيامن ممايودي الى خلل في عمل الحيامن.

مفتاح الكلمات: عقم, الاجسام المناعية الذاتية للحيامن, المتمم

[1]فرع الاحياء المجهرية [كليةطب الكندي– جامعة بغداد]
[2]مختبر الصحة المركزي

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المعلم CD14 و سرطان المثانه:هل هناك اى علاقه؟

نضال عبد المهيمن[1] , زينب عاشور[2] ,عامره خضير[2]

الخلاصة

خلفية الدراسة: ان الخلايا الطلائية قد طورت آليات مختلفة خاصة بالخلايا والانسجة للتعرف على البكتريا وتمكين الخلايا لإحداث فعاليات التهابية تعتمد على الموقع المعين ونوع العضو . هذه الاليات توفر وسيلة للموازنة بين الدفاع واصابة النسيج والتأثير على فعالية العضو ، وان المستضد CD14 له دور في احداث الموازنة.

هدف الدراسة: دراسة التعبير المناعي للمعلم CD14فى نسيج المثانة لمرضى سرطان المثانة في (96) نسيج مثانة لمرضى سرطان المثانة و(36)نسيج مثانة من مرضى يعانون من إضطرابات غير سرطانية في المثانة .

طريقة العمل: قسم المرضى إلى ثلاث مجاميع مرضية شملت:

المجموعة الأولى:مرضى سرطان المثانة المشخصون حديثاً وتضمنت(69حالة مرضية)(43,9%)،

المجموعة الثانية:مرضى سرطان المثانة المشخصون مسبقاًوالمعالجون بالعلاج الكيمياوي وتضمنت (27حالة مرضية)(17,2%)، والمجموعة الثالثة:حالات مرضية أخرى للمثانة عدى السرطان وتضمنت(36حالة مرضية)(22,9%). وقد أختيرت عينات إدرارمن المرضى لتحديد خمج المسالك البولية .

كما تم تقسيم مرضى السرطان(المجموعتين الأولى والثانية) إلى مجاميع نسبة إلى نوع السرطان إلى: سرطان خلاياالمثانة الأنتقالي ،وسرطان خلايا المثانة السطحي واعتمادا على الفحص النسيجي للعينات، استخدمت تقنية التعبير المناعي النسيجي لدراسة التعبير عن CD 14 في نسيج المثانة.

النتائج: أظهرت النتائج إن هذا البروتين موجود في 68،6% من المرضى . لم يكن هناك فرق معنوي في نتائج التعبير المناعي لهذا البروتين بين مرضى سرطان المثانة والمرضى غير المصابين بهذا المرض .

لكن هناك فرق معنوي بين مرضى السرطان مقارنة مع درجة تصنيف الورم بين المصابين بخمج الجراثيم لكل المجاميع المرضية, حيث هناك تعبير عالي للـ CD 14 في نسيج المثانة للمرضى المصابين بالجراثيم السالبة لصبغة غرام .

الاستنتاج: ارتباط التعبير عن المعلم CD14 على الخلايا مع الاصابه بالجراثيم السالبه لصبغة كرام ولكن ليس مع الاصابه بالسرطان.

مفتاح الكلمات: تقنية التعبير المناعي النسيجي,CD14,سرطام المثانه.

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

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حالة المؤكسدات ومضادات الاكسدة عند الرجال المدخنين

شيماء زهراو ندى الساعدي

الخلاصة

خلفية الدراسة: الجذور الحرة في دخان السكائر قد تسبب تدميرا تأكسديا للجزيئات الكبيرة (العملاقة) مما يؤدي الى الاصابة بالامراض القلبية والسرطان. ان النقصان في تركيز مضادات الاكسدة في البلازما قد يشير الى جهد تأكسدي متعلق بدخان السكائر

هدف الدراسة: تمت دراسة تأثير التدخين على تراكيز مضادات الاكسدة في امصال المدخنين ومقارنتها مع مجموعة السيطرة من غير المدخنين من غير الاعتماد على نوع التغذية والتدخلات الاخرى.

الاشخاص وطرق العمل: نماذج من المصل موزعة بين (60) من المدخنين و(40) من غير المدخنين معدل اعمارهم يتراوح من (15-60)سنة استعملت لقياس تركيز كل من فيتامين C (حامض الاسكوربيك),فيتامين E و فيتامين A بأستخدام تقنية كروماتوغرافيا السائل عالي الاداء وقياس مستوى فوق الاكاسيد لشحوم الدم ونمط شحوم الدم في امصال المدخنين ومقارنتها مع مجموعة السيطرة من غير المدخنين.

النتائج: بينت الدراسة انخفاضا معنويا في تراكيز الفيتامينات المضادة للاكسدة (A, C& E) في مصل المدخنين عنه في غير المدخنين, وزيادة معنوية في تركيز المالون ثنائي الالدهايد ونسبة الدهون الغير المؤكسدة عالية الكثافة مع انخفاض معنوي في نسبة الدهون المؤكسدة العالية الكثافة عند مقارنتها مع مجموعة السيطرة.

الاستنتاج: اوضحت النتائج ان لدى المدخنين انخفاضا معنويا في تراكيز مضادات الاكسدة عنهم في غير المدخنين من غير الاعتماد على نوع التغذية مع ملاحظة زيادة في الجهد التأكسدي في مصل المدخنين.

مفتاح الكلمات:البروتين الشحمي عالي الكثافة المؤكسد,حامض الاسكوربيك, فيتامين E, فيتامين A, تدخين السكائر.

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دور العلاج بالرجات الاختلاجية الكهربائية لدى المرضى النفسيين الراقدين في مستشفى عام في بغداد

محمد عبد الحميد السامرائي , عدي خالدعبد الجبار

الخلاصة

خلفية الدراسة: على الرغم من الجدل الدائر حول دور العلاج بالرجات العلاجية الكهربائية في علاج الاضطرابات النفسية فان هذه الطريقة في العلاج لا زالت تستعمل بصورة واسعة في علاج هذه الاضطرابات

هدف الدراسة: تهدف الدراسة الى تحديد دور العلاج بالرجات الاختلاجية الكهربائية في علاج الاضطرابات النفسية لمجموعة من المرضى النفسيين الراقدين مع تحديد مواصفات هؤلاء المرضى

طريقة العمل: تمت المراجعة بعناية للسجلات الطبية لكافة المرضى الراقدين في ردهة الطب النفسي في مستشفى الكاظمية التعليمي في بغداد لمدة ثلاثة اشهر. تم استعمال نموذج خاص لجمع المعلومات وتم التشخيص تبعا للمراجعة العاشرة للتصنيف الدولي للامراض. تمت متابعة الحالة الصحية العامة للمرضى بصورة دقيقة

النتائج:تضمنت الدراسة 145 مريضا راقدا كان منهم76 (62,4%) من الذكور و 69(47,8%) من الاناث و كان توزيع الاعمار بين 17- 75 سنة. الذكور كانوا اقل عمرا من الاناث و74,6% من المرضى كانوا تحت سن 40 سنة. معدل فترة الرقود كان 4.6 اسابيع

كان الفصام الاضطراب الاكثر تكرارا حيث شكل 41,4% من العينة تلاه الاكتئاب(25,5%) ثم الهوس(7,6%).تضمن علاج المرضى استخدام الطرق النفسية الاجتماعية والادوية ذات التاثير النفسي حيث تم استخدام 13 نوعا من الادوية التقليدية

تم استعمال العلاج بالرجات الاختلاجية الكهربائية لـ 42% من العينة وكان الذكور اكثر من الاناث من حيث استعمال هذه الطريقة في علاجهم و بصورة مهمة. لم تكن هناك علاقة ذات اهمية بين العمر واستعمال هذه الطريقة العلاجية. كان هناك 5 مرضى فقط بعمر يتراوح بين 60-66 واربعة بعمر 17 سنة تم استعمال هذه الطريقة في علاجهم.

الاستطباب الرئيسي للعلاج الاختلاجي الكهربائي كان الفصام حيث كان 69% بين المرضى الذين تضمن علاجهم هذه الطريقة يعانون من هذا الاضطراب تلاه الاكتئاب بنسبة(23%).

الاستنتاج: تشير هذه الدراسة الى ان هناك نسبة عالية من المرضى يتضمن علاجهم الرجات الاختلاجية الكهربائية على الرغم من ان معظم المرضى تم علاجهم بالادوية فقط.

مفتاح الكلمات: الرجات الاختلاجية,الكهربائية,المرضى الراقدين .

فرع الباطنية [كلية الطب - جامعة النهرين]

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تقييم مستويات مستقبل الانترلوكين الذائب في المصل في تشخيص التهاب المفاصل الرثوي

إيهام عامر علي

الخلاصة

خلفية الدراسة: يفرز مستقبل الأنترلوكين-2 الذائب من قبل الخلايا اللمفاوية عندما تكون فعالة ويستعمل كمؤشر للاستجابة المناعية لبعض الامراض.

هدف الدراسة: أجريت هذه الدراسة لتقييم الفائدة السريرية المحتملة في مستويات مستقبل الأنترلوكين الذائب في المصل (sIL-2R) كمؤشر ذو حساسية وفعالية لتشخيص مرض التهاب المفاصل الرثوي، كذلك للتقصي عن مدى الترابط ما بين مستويات (sIL-2R) في مصل الدم وبعض المتغيرات المستخدمه في تقدير التهاب المفاصل الرثوي وهي سرعة ترسب كريات الدم الحمر والبروتين الفعال-C (CRP) و العامل الروماتيزمي (RF) و حامض البوليك.

طريقة العمل: قيست مستويات (sIL-2R) بواسطة تقنية Enzyme Linked Immunosorbent Assay (ELISA) في مصل الدم لخمسة وعشرين من مرضى التهاب المفاصل الرثوي وقورنت قيم (sIL-2R) بتلك العائدة إلى (25) شخصا سليما وتم تحليل الارتباط ما بين (SIL-2R) والمتغيرات الاخرى.

النتائج: اظهرت النتائج زيادة معنوية ($p<0.001$) في تراكيز كل من ESR و sIL-2Rالمقاسة في مصل الدم لمرضى التهاب المفاصل الرثوي مقارنة بمجموعة السيطرة، في حين لم تكن هناك اية اختلافات معنوية ما بين كلا المجموعتين لحامض البوليك في مصل الدم.

وجد ان CRP في مصل دم المرضى كان ذا قيمه ايجابيه بمقدار 56% ارتبط مستوى (sIL-2Rبما لا يقبل الجدل مع كل من (RF و ESR) بينما كان الارتباط بقيمه طفيفه مع حامض البوليك. اظهر (sIL-2R) خصوصية وحساسية عاليتين مع المرضى الذين لديهم قيمه أكيدة للعامل الروماتيزمي.

الاستنتاج: يمكن ان يكون تقدير مستويات (sIL-2R) مؤشراً مفيداً ودقيقاً وفعالاً لتشخيص التهاب المفاصل الرثوي.

مفتاح الكلمات:البروتين الفعال-C (CRP) ، سرعة ترسب كريات الدم الحمر، العامل الروماتيزمي (RF)، التهاب المفاصل الرثوي.

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التهاب الكبد الفيروسي نوع(أ) عند الأطفال

سوسن إبراهيم العزاوي[1] , منيب أحمد الزبيدي [1], حيدر أمين عزيز[2]

الخلاصة

خلفية الدراسة: أغلب حالات التهاب الكبد الفيروسي نوع(أ) تكون بدون أعراض أو بأعراض سريريه بسيطة ولكن بعضها يكون معقدا.

هدف الدراسة: لتقييم كل حالات التهاب الكبد الفيروسي عند الأطفال الذين ادخلوا المستشفى خلال مدة سنة واحدة ولإظهار الأعراض السريرية الغريبة والمضاعفات.

الاشخاص وطرق العمل: خلال الفترة من الأول من حزيران 2005 و لغاية الأول من حزيران 2006 تمت دراسة وتحليل خمسون حالة التهاب الكبد الفيروسي نوع(أ) من حيث الأعراض السريرية ومسيرة المرض ومضاعفاته ونتائجه.

النتائج: تم شمول خمسين مريضا بالدراسة وكانت نسبة الذكور للإناث (1,8: 1) واثنان و ثلاثون (64%) مريضا من فئة عمرية بين (1- 5) سنة. وجد عجز الكبد الحاد في سبع حالات (14%) واستمرارية اليرقان عند 5 حالات (10%) و تفاقم حاد لالتهاب الكبد المزمن عند 5 حالات (10%) وكانت هناك حالتين لالتهاب الكبد الفيروسي نوع (أ) المنتكسة (4%) و أعراض سريريه لاتتعلق بالكبد عند 5 حالات (10%) وقد كان ثلاثة منهم (6%) يحملون صفة نقص إنزيم G6PD وعانوا من تحلل الدم الشديد.

الاستنتاج: من الممكن أن تظهر أعراض التهاب الكبد الفيروسي بطرق مختلفة و إن مستوى الوعي ووجود استسقاء البطن وقيم التحليلات الكيميائية و الدموية الغير طبيعية بدرجة كبيرة هي ذات قيمة عظيمة في توقع الحالات المعقدة من التهاب الكبد الفيروسي نوع (أ)

مفتاح الكلمات: التهاب الكبد نوع(أ), أعراض سريريه غريبة , الأطفال

[1]فرع طب الاطفال [كلية الطب ـ جامعة بغداد]
[2]مستشفى حماية الاطفال ـ مدينة الطب

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تحليل القياس الشكلي للناتئ السني

حيدر حمادي عبد الامير

الخلاصة

خلفية الدراسة: الفقره العنقيه الثانيه (المحور) هي من أقوى الفقرات العنقيه ، من الصفات المميزه لهذه الفقره هو وجود بروز عضمي قوي (الناتئ السني) ، الاستقصاءات المتعدده أوضحت عن وجود علاقه بين الناتئ السني والتشوهات الخلقيه.

هدف الدراسة: دراسة وتقييم القيم لبعض القياسات الشكليه من الناتئ السني للفقره العنقيه الثانيه لمعرفة درجة الانثناء الخلفي للناتئ السني بالعلاقه لشكله التكويني.

طريقة العمل: أختير 30 من الفقرات العنقيه الثانيه بشكل عشوائي ، تم أجراء الفحص العياني والقياس الشكلي لها باستخدام برامجية (Global lab image/2) الخاصه بتحليل الصورة بأستجام الحاسوب.

النتائج: نتائج القياس الشكلي اظهرت الانثناء الخلفي يجب ان يكون له علاقة بالأنحرف الظهري للناتئ السني لفقرة المحور اذا كانت المسافة الامامية للناتئ السني ثابته ، زيادة سمك الناتئ السني الامامي الخلفي متوافق مع طول الناتئ السني ، الناتئ السني السميك يكون اطول واعرض لكن قليل الانحراف الظهري

الاستنتاج: يمكن من هذه الدراسة ان نعتبر ان الانحراف الظهري للناتئ السني للمحور وسمك المسافة الامامية الخلفية كدليل لدرجة الانثناء الخلفي ،كذلك يمكن التوصل الى امكانية استخدام القياسات اعلاه كدليل لدرجة الانثناء الخلفي لفقرة المحور في حالة التشوهات الخلقية (Chairi I) لذلك يمكن التشخيص الاولي لهذا المرض

مفتاح الكلمات: القياس الشكلي ، الفقرة العنقية الثانية (المحور) ، التشوهات الخلقية.

فرع التشريح [كلية الطب - جامعة النهرين]

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ورم نقوي متعدد مع كتلة الثدي المكونة من ورم البلازموايات خارج نخاع العظم " تقرير حالة "

أيلول 2009

وسيم فاضل التميمي[1], علاء غني حسين[2],سنان وحيد جاسم[3], أحمد حسين جاسم[3], عبد المهدي عبد الرسول فاتح[3]

الخلاصة

الخلفية : اكتناف او أصابة الثدي بأورام التكاثر اللمفي المناعي نادر الحدوث من خلال الحالات المسجلة علمياً . على العكس فأن حالات سرطان الثدي الاولي أو الثانوي هي الحالات الأكثر تسجيلا" من حالات أصابة الثدي بالورم النقوي المتعدد وبالخصوص بورم البلازموايات خارج نخاع العظم

من خلال مراجعة الحالات المسجلة في المقالات العلمية تبين أن مثل هذه الحالات والتي قد تم تسجيلها ونشرها هي 18 حالة فقط لغاية العام 2000

تقديم الحالة : هنا نسجل حالة شبيهة من حالات الورم النقوي المتعدد مع أصابة الثدي الأيمن وألأيسر بورم البلازموايات خارج نخاع العظم في مريضة عمرها 34 سنة, بعد أصابتها بكسرمرضي في عظم العضد الأيمن مع الام في الجسم و فقر دم متكرر, تم تشخيصها أخيرا بأنها تعود الى مرض ورم نقوي متعدد وقد أستلمت المريضة العلاج المناسب وتحسنت الحالة وأختفت الكتل الثدوية

المناقشة والأستنتاج :أن هناك صعوبة بالغة في تحديد العلامات الشعاعية أو علامات الموجات فوق الصوتية من أجل التفريق بين حالات سرطان الثدي الأولي أو الثانوي و حالات ورم البلازموايات خارج نخاع العظم

مفتاح الكلمات: مرض ورم نقوي متعدد, ورم التكاثر اللمفي المناعي, ورم البلازمويات, ورم الثدي

[1]فرع الطب الباطني [كلية الطب ـ جامعة النهرين]
[2]فرع الباثولوجي والطب العدلي [كلية الطب ـ جامعة النهرين]
[3]مستشفى الكاظمية التعليمي

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