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IRAQI JOURNAL OF MEDICAL SCIENCES

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ARABIC ABSTRACTS

EDITORIAL

GMC MEANS PEACE

Abdul Hussain M Al-Hadi *PhD.*

The global movement for mothers and children was declared in the late nineties and aims at improving the status of mothers and children all over the world with special emphasis on mothers and children in the developing world.

The WHO report for the year 2005 emphasizes that mothers and children matters so does their health.

In many countries universal access to the care all women and children are entitled still far from realization. This movement aims at meeting the principle requirements for child survival and mother revival.

It is necessary to refocus the technical strategies developed within maternal and child health programs and to put more emphasis on the importance for the often-overlooked health problems of newborn.

Management of child health has become one of the major programs developed to care for children from the under five group. It also aims to make such care more realistic and fruitful through upgrading the performance of health care workers to care for all health problems that are facing children feasibly and appropriately, at the same time the program give special importance to role that can be played by the family in providing care for the child who suffer from these problems.

Environmental sanitation has proven a major role in promotion, protection and maintenance of the health of general population, through ensuring safe water supply and safe environment free from pollution one can ensure good health and high developmental opportunities.

Women and girls need special attention throughout their developmental stages, they both are at high risk of wider range of health problems related to their physiological characteristics. Women need special care during pregnancy, childbirth, postpartum and even more attention to the newborn baby. Through antenatal care, many of the complication that arises during the course of pregnancy and childbirth can be minimized or prevented. At the same time many causes of death of women and newborn babies could be prevented through proper delivery and postpartum care. Nutritional status of mothers and children play a major role in deciding their health status.

Insuring proper *nutrition* of mothers and children is a vital requirement to maintain their good health and to protect them from different health problems, at the same time depriving mothers and children from proper nutritional requirement will expose them to major health problems that can endanger their health and well being.

Short birth interval has proven its adverse effect on both mothers and children, children born after short birth interval are more liable to be born with low birth weight and born prematurely. At the same time children who are followed by pregnancy and childbirth during their first year will be deprived from the care they need because of pregnancy and arrival of the newborn baby, it will be more appropriate to

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have proper appropriate to have proper spacing of births for the sake of both mothers and children.

Iraq has suffered successive wars and civil disturbance away from *peace*, which all together have affected the health of mothers and children.

Sanction imposed on Iraq following the occupation of the state of Kuwait has endangered the health and well being of all Iraqi population, but more have has incurred on mothers and children. This was shown with the progressive deterioration of the health and nutritional status of both mothers and children with steep acceleration of infant, under five maternal morbidity and mortality. Surveys showed that infant mortality risen by 2.8 times during the period Aug 1990 to Aug 1991 as stated by the national survey conducted by a team from Harvard University during Sept 1991.

The *educational status* of mothers showed a positive relation with better health of mothers and children, mothers with higher educational level are more likely to look after their children in a better way, to secure their needs and demands and look after themselves and their families in a more rational manners.

All the health needs of mothers and children could be met with simple and cost effective *appropriate* mechanics and tools.

Through getting *communication* and proper exchange of experience many needs of mothers and children can be met instantly.

Finally the status of women within community is very much related to health and environmental status, through provision for proper opportunity for women to upgrade their status, and to play their role in the developmental process.

Emancipation of women is vital in this respect, through elimination of gender discrimination providing women the share they deserve with community.

Serum sFas in Non-Hodgkin's Lymphoma

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Abstract

Background: Lymphomas are a heterogenous group of malignancies of B or T cells that usually originate in the lymph nodes. They are divided into Hodgkin and non-Hodgkin's lymphomas. Disruption of the physiological balance between cell proliferation and death is a universal feature of all cancers. There has been an increasing interest in the role of apoptosis in tumorogenesis of lymphoma. On the molecular base apoptosis is caused by activation of the caspases through extrinsic and intrinsic pathways. Extrinsic pathway centers on tumor necrosis factor family, where the ligand will bind to the cell surface receptor and this in turn will induce apoptosis. Fas receptor is a member of the TNF/NGF receptor superfamily. Fas family is constituted of the receptor, ligand and soluble form. Soluble Fas will compete with Fas receptor for binding to ligand, thus interfering with Fas-L-mediated apoptosis.

Objective: To measure the level of serum sFas in non-Hodgkin lymphoma (NHL) and to determine the correlations of it with certain clinical and hematological parameters and chemotherapy treatment.

Patients and Methods: This study included 30 patients with NHL (19 males and 11 females), of them 14 patients were newly diagnosed along with 30 apparently healthy controls were involved in this study. The patients were interviewed with history taking, clinical examination and aspirating blood sample for estimation of serum sFas concentration using sandwich ELISA kit from Chemicon. Also serum CRP level, plasma LDH level, hemoglobin concentration, platelets count, WBC count and peripheral blood film were all performed using standard techniques.

Results: This study revealed that the serum sFas concentration was significantly higher in NHL patients than in healthy controls ($p = 0.0001$). This increase was significantly higher in pretreated patients and closely related to the pathological grade of NHL ($p = 0.0002$ and 0.0035 respectively).

Conclusions: Serum sFas is a simple, non-invasive and clinically useful laboratory parameter .It maybe used as an auxiliary marker to assess the prognosis and the therapeutic planning in NHL.

Keywords: Fas, NHL.

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Introduction:

Lymphomas are a heterogenous group of malignancies of B or T cells that usually originate in the lymph nodes, but may originate in any organ of the body, and share the single characteristic of arising as the result of a somatic mutation(s) in a lymphocyte progenitor [1].

Within the broad group of lymphomas, Hodgkin disease (HD) is segregated from all other forms, which constitute the non-Hodgkin lymphomas (NHLs) [2]. Disruption of the physiologic balance between cell proliferation and death is a universal feature of all cancers. The mechanisms and extent of this disruption in lymphoma cells determine the characteristics and evolution of the

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tumor, and this can be achieved through the deregulation of cell proliferation or inhibition of apoptosis [3].

Apoptosis, or programmed cell death, is a genetically regulated process in which the cell is active in producing its own death, a type of cellular suicide for the sake of maintaining homeostasis in the cellular community [4].

Apoptosis is caused by the activation of caspases. Caspases are a family of intracellular cysteine proteases that lie in a latent (zymogen) state in cells but become activated in response to a wide variety of cell death stimuli [5].

Though many pathways for activating caspases may exist, only two have been elucidated in detail; one of these centers on tumor necrosis factor (TNF) family receptors, where the ligand will bind to the cell surface and this will induce apoptosis (Extrinsic). The other involves the participation of mitochondria (Intrinsic), which release caspase-activating proteins into the cytosol, thereby triggering apoptosis [6, 7, 8].

Fas family is constituted of the receptor, ligand and soluble form. Fas (APO-1/CD95) receptor is a member of the TNF/NGF receptor superfamily. It is expressed on a variety of human B and T cell lines, on various normal human tissues and on many different tumor cells [9, 10].

Triggering of Fas receptor by its ligand (expressed in cells that show cytotoxic activity, including activated T-cells and natural killer cells) results in rapid induction of apoptosis by activating the cascade of proteases (caspases) [11, 12].

While the soluble form of Fas (sFas), which is devoid of the transmembrane region, will compete with membrane-associated Fas for binding to ligand, thus interfering with FasL-mediated apoptosis. Serum sFas level is increased in various diseases, including leukemia, lymphoma and SLE [5, 6, 7, 8, 13, 14, 15, 16].

In lymphoma the Fas superfamily can be used as tumor markers in the fields of tumorogenesis, clinical course, treatment and prognosis [7, 9, 10, 11, 12].

Aim of the Study:

To measure the level of serum soluble Fas in non-Hodgkin's lymphoma patients. Furthermore, to determine the relation between serum sFas concentration and certain clinical parameters, hematological markers, stage, classification, prognostic parameters and chemotherapy treatment of non-Hodgkin's lymphoma.

Patients,Control,Methods And aterial:

This study was conducted prospectively on 30 NHL patients, from October 2004 to June 2005, 19 were males and 11 were females, M:F ratio was 1.73:1.

The criteria for inclusion of the patients in this study were the followings:

1. No limitation of age or sex.
2. The patients were randomly selected.
3. The patients were diagnosed, clinically and histopathologically, by a consultant physician and an experienced histopathologist.
4. The patients were either newly diagnosed or already on treatment.

Twenty of these patients were attending the National Center for Hematology; while the rest were attending Al-Khadimiya Teaching Hospital and Baghdad Medical City.

A total of 30 apparently healthy control with an age range of 16-65 year were selectively included in this study. The criteria for inclusion of the control in this study were the followings [9]: They were selected in a way to be age (mean and range) and sex ratio matched for the patient group.

1.They had no fever within 1 week.
2.They are not receiving any medication.
3.They are not known to be pregnant.
4.They did not have a recent history of acute illness or any history of chronic illness.

A sample of venous blood was collected in plain and EDTA tubes.

Serum sFas present in the serum was measured by using sFas ELISA detection kit [from CHEMICON].

Serum CRP was estimated by using semiquantitative technique kit [from BIOKIT]. Plasma LDH was measured by using colorimetric method kit [from RANDOX]. Hemoglobin concentration, platelet count, WBC count, and peripheral blood film were all were performed using standard techniques [17].

The patient is considered anemic if Hb < 13g/dl (PCV < 40%) for males and if Hb < 11.5 g/dl (PCV < 36%) for females [1].

The patient is considered to have thrombocytopenia if Platelet count < $150x10^9$ and to have leucocytosis if WBCs count > $11.0x10^9$ [18].

Results:

Thirty patients with malignant lymphoma, with an age range of 14-67 year and age mean of 46.2 year, were enrolled in this study. 14 of the NHL patients were newly diagnosed. The Descriptive statistics of NHL patients are listed in the following table 1:

Table 1: Descriptive Statistics of NHL patients

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Parameter		NHL patients (n=30)	
		No.	%
B symptoms*	+	12	40
	-	18	60
Number of extranodal sites	0	19	63.33
	1	6	20
	2	5	16.67
Ann Arbor clinical stage	I	0	0
	II	4	16
	III	10	40
	IV	11	44
Anemia	+	24	80
	-	6	20
Platelet count ($x10^9$/l)	< 150	7	23.3
	≥ 150	23	76.7
WBCs count ($x10^9$/l)	> 11	6	20
	≤ 11	24	80
Serum CRP** (mg/l)	0	4	13.33
	0.6	2	6.67
	1.2	5	16.66
	2.4	3	10
	4.8	16	53.34

Plasma LDH* (U/l)**	**Normal**	**5**	**16.66**
	High	**25**	**83.34**
NCIWF** classification for NHL**	**High grade**	**15**	**50**
	Intermediate grade	**8**	**26.7**
	Low grade	**7**	**23.3**
IPI score***	**0**	**0**	**0**
	1	**8**	**26.67**
	2	**9**	**30**
	3	**9**	**30**
	4	**4**	**13.33**
	5	**0**	**0**
Age-adjusted IPI score	**0**	**0**	**0**
	1	**8**	**34.8**
	2	**11**	**47.8**
	3	**4**	**17.4**

* **B** symptoms: fever, night sweat and weight loss
CRP: C-reactive protein
***LDH**: Lactate dehydrogenase
**** **NCIWF**: National Cancer Institute Working Formulation
***** **IPI**: International Prognostic Index

Serum soluble Fas concentration and NHL patients:

The results reported in this study revealed that there was a highly significant difference (p = 0.0001) in serum sFas level between NHL patients mean ± S.E. (4961.9 ± 422pg/ml) with range (1657 to 12430 pg/ml) and normal control mean ± S.E. (1993.8 ± 125.9pg/ml) and range (988–2849 pg/ml) (Table 2).

There was no relation between serum sFas level and sex (p = 0.5338) or age (p = 0.67) of NHL patients.

The level of sFas in pretreated patients was significantly higher than in treated patients (p = 0.0002) (Fig. 1). Also the level of serum soluble Fas concentration was significantly decreased with the number of chemotherapy hours (p<0.0001)(Fig. 2).

There was no relation between serum sFas concentration and the plasma LDH, serum C-reactive protein, the number of extranodal sites, the presence of B symptoms, the Hb concentration, WBC and platelet count, bone marrow involvement, the performance status score, the international prognostic index (IPI) and the age-adjusted IPI score for all NHL patients and for the 14 pretreated patients.

Moreover, there was a highly significant difference between serum sFas level in NHL patients with high and intermediate grade versus those with Low grade lymphoma (p = 0.0035) and although the serum sFas level was higher in the in the high grade compared to the intermediate grade but it did not reach the level of significance (p=0.1786) (Figure 3).

Table2: Concentration of serum sFas in NHL and control groups:

Group	Serum sFas concentration (Range) (pg/ml)	Serum sFas concentration (Mean ± S.E.) (pg/ml)
NHL (n=30)	1657 - 12430	4961.9 ± 422
Control (n=30)	988 - 2849	1993.8 ± 125.9
p-value	0.0001	

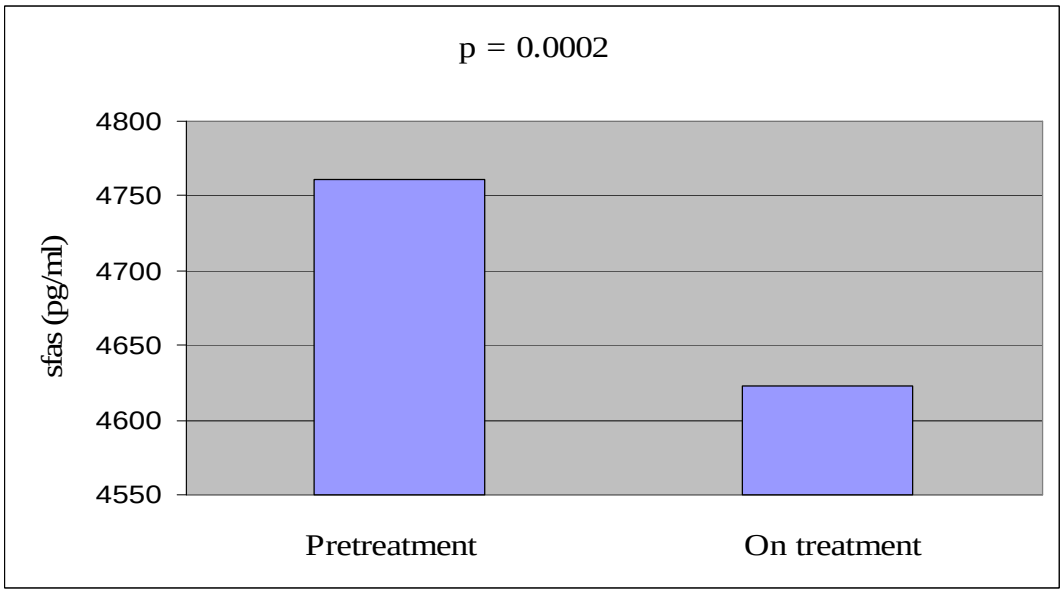

Figure 1: Mean of concentration of serum sFas in pretreatment and on treatment NHL patients.

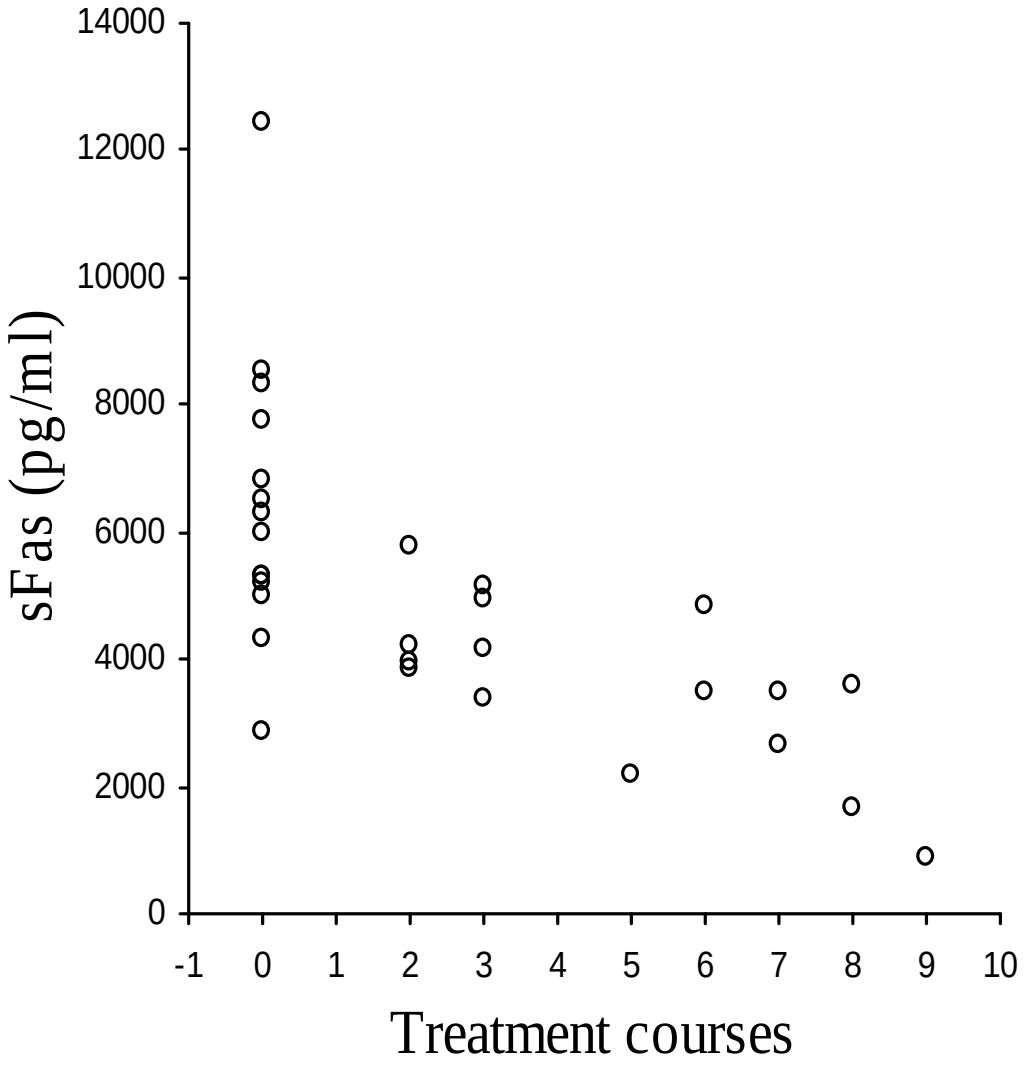

Figure 2: Relation between number of treatment courses and serum sFas level of NHL patients.

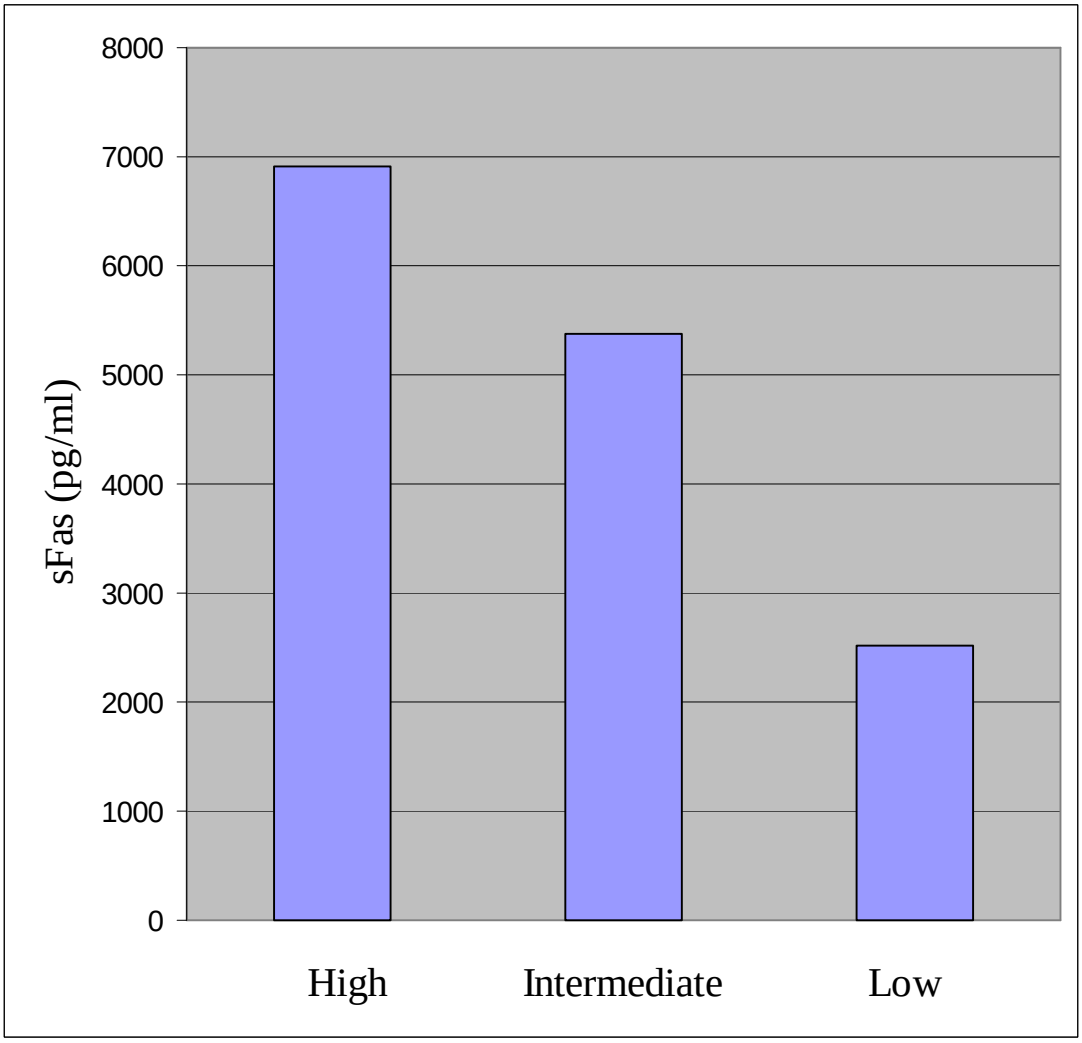

Figure 3: The mean serum concentration of s-Fas in High, Intermediate and low-grade NHL patients.

Discussion:

The present study is a prospective one aiming at evaluating the significance of the apoptotic inhibitor sFas using ELISA technique in patients with non-Hodgkin's lymphoma. The study included the comparison of serum sFas to a number of clinicopathological variables of well-known significance.

The incidence of NHL was higher in males than in females and it was rising with age. These results are similar to those reported by Ministry of health cancer registery [19, 20] and Yasir [21].

However, serum sFas level of NHL patients did not depend on either age or sex, and these findings were in agreement with Niitsu et al [9].

This study demonstrated that the serum sFas level was significantly

higher in NHL patients than in healthy control (Table 2). Similar findings were reported by Sunil et al [7], Niitsu et al [9],Takeshi et al [11], Yufu et al [22], Munker et al [23] and Knipping [24].

The soluble Fas (which is a competitive inhibitor of apoptosis) compete with Fas receptor for binding to Fas ligand, thus interfering with Fas ligand-mediated apoptosis [16,25,26]. Many studies had revealed that the defects in the Fas-mediated apoptotic pathway were associated with lymphoproliferative disorders and particularly lymphoma [27, 28,29], therefore we may proposed that this increase in serum soluble Fas level is part of the disease process and may explain the tumorogenesis of NHL.

The serum sFas level was significantly higher in the more aggressive high and intermediate grade than in the low-grade lymphoma (Figure 3). Similar findings were reported by Sunil et al [7], Niitsu et al [9], Takeshi et al [11], Munker et al [23 and Knipping[29].Morover John CR. and Ian TM. had proposed that the more the aggressive the lymphoma the more the antiapoptotic phenomenon [6, 35], and this may explain the high level of sFas in the more aggressive grade .

This study had revealed that the level of serum soluble Fas concentration was significantly decreased with the number of chemotherapy courses (Figure 3) and it was significantly higher in pretreated patients compared to treated patients (Figure 2) . This was in agreement with Takeshi et al [11] and Niitsu et al(9) who cocluded that serum sFas concentration may be used as a valuable clinical tool and since it is well correlated to the clinical course and to the remission and relapse state therefore it may help in generating a more appropriate treatment plan. Similarly Niitsu et al [9] and Kondo et al [36] had found that NHL patients with a low pretreated serum sFas level had achieved complete remission while those with high serum sFas level were considered resistant to chemotherapy.

In this study there was no relation between B symptoms and serum sFas concentration. This finding was supported by Munker et al [23] and Knipping[24], who concluded that serum sFas level was not related to the clinical symptoms. Also no relation was found between the performance status and serum sFas concentration, which was in agreement with Sunil et al [7].

Moreover, LDH level which is a part of the IPI score that corresponds well with the tumor burden, extent of disease, response to treatment and relapse [32,33], was not related to the serum sFas concentration (p=0.1839). This finding was in agreement with Sunil et al [7], Takeshi et al [11], Munker et al [23] and Knipping et al [24].

Furthermore no relation was found between number of extranodal sites and serum sFas level (p=0.197) which was in agreement with Niitsu et al [9].

Also similar to Takeshi et al[11] study the serum sFas level was not affected by the clinical stage (Ann Arbor staging system).

Since this study had revealed that the serum sFas concentration was not related to the parameters that indicate growth and invasive potential of the tumor, including plasma LDH level, extranodal disease involvement and clinical stage of the tumor, therefore we might propose that sFas was not produced by the tumor cells. This finding is supported by Niitsu et al [9], Takeshi et al [11] and Munker et al [34]. However the cells that produce serum soluble Fas are unclear yet. Takeshi et al [11] had proposed that it may be produced by T lymphocytes, which may reflect a responsive change, or immune activation in lymphoma and

this may protect lymphoma cells from apoptosis [11].

In agreement with Sunil et al [7] and Niitsu et al [9] this study had showed that the IPI score and age-adjusted IPI score were not correlated with the level of serum sFas (p=0.3102 and p=0.5130 respectively).

In this study 32% of NHL patients had bone marrow involvement and this was in agreement with Julie et al [35] and Raad [36], however no relation was found between the bone marrow involvement and serum sFas concentration (p=0.337), which was supported by Sunil et al [7] study.

Also there was no relation between hemoglobin concentration, platelet count or WBCs count and serum sFas concentration (p=0.945 ,p=2456 and p=5457 respectivel) , these findings were in agreement with Takeshi et al [11].

Serum CRP is another simple and valuable prognostic factor for NHL. Its increment is closely related to worsening of clinical course and increase LDH level [37]. In this study there was no relation between CRP and serum sFas concentration (p=0.1456) which was in agreement with Takeshi et al [11], Munker et al [23] and Knipping et al [24].

Conclusions:

1. Since the antiapoptotic serum soluble Fas level was significantly higher in NHL patients, especially those who are pretreated and having the more aggressive disease. Therefore we may concluded that the serum sFas concentration has the prospect to become a clinically useful, simple, noninvasive, time-saving, prognostic and therapeutic planning auxiliary tumor marker test for the more aggressive non-Hodgkin's lymphomas.
2. Serum sFas maybe considered as a complementary independent test to the international prognostic index scores (IPI) for NHL.

References:

1. Ernest B, Barry SC, Marshall AL, Thomas JK, Uri S. Williams's hematology. 6th ed. Vol. 2; McGraw-Hill Scientific publications, New York, 2001.

2. Cotran RS, Kumar V, Collins T. Robbins pathologic basis of diseases. 6th ed.; W.B. Saunders Company, Harcourt publishers, 1999.

3. Margarita SB, Abel SA, Miguel AP. Cell cycle deregulation in B-cell lymphomas; Review article. Blood 2003: 101(4): 1220-1235.

4. Clark WD, George D. Role of calcium in glucocorticosteroid-induced apoptosis of thymocytes and lymphoma cells: Resurrection of old theories by new findings; Review article. Blood 1998; 91(3): 731-734.

5. Aaron DS, David WH, Linda ZP, Mark DM. Receptor- and mitochondrial- mediated apoptosis in acute leukemia: a translational view. Blood 2001; 98(13): 3541-3553.

6. John CR. Mechanism of apoptosis; Review article. American journal of pathology 2000; 157: 1415-1430.

7. Sunil SM, Naresh KN, Partha PM, Srinivas P, Advani SH, Nadkarni JJ. Circulating levels of TNFα and TNF receptor superfamily members in lymphoid neoplasia. American journal of hematology 2000; 65: 105-110.

8. Michael JP, Thomas B, Douglas RG, Tesu L. Fas and Fas Ligand in gut and liver. American journal of physiology-Gastrointestinal and liver physiology 2000; 278: G354-G366.

9. Niitsu N, Sasaki K, Umeda M. A high serum soluble Fas/APO-1 level is associated with a poor outcome of aggressive non-Hodgkin's lymphoma. Leukemia 1999; 13(9): 1434-40.

10. Yusuf AH. Apoptosis and the dilemma of cancer chemotherapy. Blood 1997; 89(6): 1845-1853.

11. Takeshi H, Hisashi T, Nasao T, Hideko G, Toshiki Y, Michio S, et al. Serum-soluble Fas determines clinical symptoms and outcome of patients with aggressive non-Hodgkin's lymphoma. American journal of hematology 2000; 64: 257-261.

12. Roberto C, Elizabeth AR, Philip LC. Fas Ligand interactions are involved in Ultraviolet-B-induced human lymphocyte apoptosis. The journal of immunology 1998; 98: 241-151.

13. Franco S, Marco T, Paola C, Franco D. Fas-L up-regulation by highly malignant myeloma plasma cell: Role in the pathogenesis of anemia and disease progression. Blood 2001; 97(5): 1155-1164.

14. Gitendra RW, Hoffbrand AV. Biochemical and genetic control of apoptosis: Relevance to normal hematopoiesis and hematological malignancies. Blood 1999; 93(11): 2587-3600.
15. Joseph A, Sarkis M, Maria K, Catherine D, Arthur H, Sandra S, et al. Biologically active Fas antigen and its cognate ligand are expressed on plasma membrane-derived extracellular vesicles. Blood 1998; 91(10): 3862-3874.
16. Linda SE, Barry WH. Non-Hodgkin lymphoma, Seminar. Lancet 2003; 362: 139-146.
17. Mitchell SL, Barbara JB, Imelda B. Dacie and Lewis practical hematology. 9th ed.; Churchill Livingstone, Harcourt publishers, China, 2001.
18. Hoffbrand AV, Daniel C, Tuddenham EG. Postgraduate hematology. 5th ed.; Blackwell science publishing, 2005.
19. Ministry of health, Iraqi cancer board, Iraqi cancer registry center. Results of Iraqi cancer registry 1998-2000, 2002.
20. Ministry of health, Iraqi cancer board, Iraqi cancer registry center. Results of Iraqi cancer registry 1995-1997, 1999.
21. Yasir AM. The changing patterns of hematological malignancies (leukemias, lymphoproliferative disorders, myeloproliferative disorders, myelodysplastic syndromes, and bone marrow metastatic diseases) before and after the war (1991). A thesis submitted to the Iraqi council of pathology in partial fulfillment of the requirements for the degree of fellowship of the Iraqi commission for medical specialization in Pathology (Hematology), 1998.
22. Yufu Y, Choi L, Hirase N, Tokoro A, Noguchi Y, Goto T, et al. Soluble Fas in the serum of patients with non-Hodgkin's lymphoma: Higher concentrations in angioimmunoblastic T-cell lymphoma. American journal of hematology 1998; 58: 334-336.
23. Munker R, Younes A, Cabanillas F, Andreef M. Soluble CD95 in the serum of patients with low and intermediate grade malignant lymphomas: absence of prognostic correlations. Leukemia and lymphoma 1997; 27: 517-521.
24. Knipping E. Identification of soluble Apo-1 in supernatants of human B- and T- cell lines and increased serum levels in B- and T- cell leukemia. Blood 1995; 85: 1562-1569.
25. Franco C, Peter GI, Randy DG, Emmanuelle Z. MALT lymphomas. Hematology 2001: 241-258.
26. Papoff G, Cascino I, Eramo A, Starace G, Lynch DH, Ruberti G. An N-terminal domain shared by Fas/APO-1 (CD95) soluble variants prevents cell death in vitro. Journal of immunology 1996; 156: 4622-4630.
27. Frank D, Arnon PK, Tetsuya F, Thomas JK. Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells. Blood 2005; 105(8): 3193-3198.
28. Karl LS, James DF, Marilyn LO, Frank LH, Lou AS. Neutrophils induce apoptosis of lung epithelial cells via release of soluble Fas ligand. American journal of physiology-Lung cell molecular physiology 2001; 280: L298–L305.
29. Kei K, Koichi O, Shigehiko I, Keizo A, Junji S, Masahiro K. Elevated serum soluble Fas ligand in natural killer cell proliferative disorders. British journal of haematology 1998; 103 1164–1166.
30. Ian TM. The non-Hodgkin's lymphoma. 2nd ed.; Arnold scientific publishing, 1997.
31. Wesselborg S, Engels IH, Rossmann E, Los M, Schulze OK. Anticancer drugs induce caspase-8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction. Blood 1999; 93: 3053-3063.
32. Philip R, Sandra M, Raman Q. Clinical oncology. 7th ed.; W.B. Saunders Company, Harcourt publishers, 1993.
33. Michael P, Lorenz T, Marita K, Rudolf S, Alfred CF, Christian R, et al. Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of young patients with good-prognosis (normal LDH) aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. Blood 2004; 104(3): 626-633.
34. Munker R, Midis G, Owen SL, Andreff M. Soluble Fas (CD95) is not elevated in the serum of patients with myeloid leukemias, myeloproliferative and myelodysplastic syndromes. Leukemia 1996; 10: 1531-1533.
35. Julie MV, Brian CH, Bruce DC, Janet D, John W. Update on epidemiology and therapeutics for non-Hodgkin's lymphoma. Hematology 2002: 241-262.
36. Raad JM. The value of bone marrow biopsy in staging of neoplastic disease. A thesis submitted to the college of medicine and the committee of graduate studies at the University of Baghdad in partial fulfillment of the requirements for the degree of Master of Science in Pathology (Haematology), 1991.
37. Legouffe E, Rodriguez C, Picot MC, Richard B, Klein B, Rossi JF, et al. Level of CRP is a valuable and simple prognostic marker in non-Hodgkin's lymphoma. Leukemia and lymphoma 1998; 31: 351-357.

THE VALUE OF NUCLEAR MORPHOMETRY IN BREAST CARCINOMA

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Abstract

Background: Breast carcinoma is the most important malignant tumor in female population. There are many indications for the use of adjuvant chemotherapy in its treatment, which need a special selection of patients especially those with high risk &this is judged by several prognostic parameters such as patient's age, tumor size, histological grade and others.

Nuclear morphometry has shown to be a good objective, quantitative method for the evaluation of prognosis, in which the Mean Nuclear Area &
Standard Deviation of Nuclear Area showed an increase from the baseline value of normal breast epithelium to invasive carcinoma &found to be strongly correlated with the recurrence rate within 2.5 years.

Objective: To evaluate some prognostic parameters of breast carcinoma by the use of computerized nuclear morphometry.

Material &Methods:

Fifty-four cases of a histologically diagnosed invasive breast carcinoma of ductal type with a known tumor grade, size &patient's age were reviewed. In each case an average of 5-10 microscopical fields were screened &30 consecutive nuclei were determined at x400 magnification by the use of an image analysis system. Statistical analysis was performed using ANOVA, Tukey's and t-test.

Results:

The mean values of nuclear area varied between the three histological grades (grade I, II and III) by using analysis of variance ($p>0.01$). These values increased with increasing histological grade, (Tukey's test: $p>0.01$).

Similarly the mean values of nuclear area were significantly higher in tumors measuring more than 5cm in diameter than those less than 5cm in diameter. (t-test :$p>0.01$).

On the other hand, the nuclear area in tumors of patients less than 50 years and more than 50 years of age showed no significant difference. (t-test: $p<0.01$)

Conclusions:

The Mean Nuclear Area was of value in the assessment of the histological grade, in which higher figures seen in higher grades. The same relationship found between tumors more than 5cm and those less than 5cm in diameter, in which higher values seen in tumors more than 5cm in diameter. On the contrary the importance of the Mean Nuclear Area was restricted when comparing tumors in patients less than 50 years and those more than 50 years of age.

In conclusion our data suggest that adapting a nuclear morphometric parameter e.g. nuclear area, which was performed on this study, may be a valuable objective tool in evaluating various prognostic indices.

Keywords: Breast carcinoma, nuclear morphometry , and prognosis.

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Introduction:

Breast carcinoma is one of the most common malignancies in female population [(1)].

There are several indications that early systemic adjuvant chemotherapy improves the treatment of patients with primary breast carcinoma. [(2, 3, 4)] However the side effect of those drugs requires a selection of patient at higher risk for this type of therapy. [(5)]

Several prognostic parameters are included in this selection such as the patient's age, size of the tumor, histological type, stage, histological& nuclear grade, mitotic index & axillary lymph node metastasis.[(6,7,8)]However

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some of these parameters may have a subjective nature ,like the mitotic index , histological and nuclear grade and according to certain studies the interobserver agreement rarely exceed 80-90%. [9, 10]

The advantage of quantitative methods is that they are objective and reproducible. [11, 12]

Nuclear morphometry has shown a prognostic significance in many studies, [13] and that the mean nuclear area (MNA) & standard deviation of the nuclear area (SDNA) is an important prognostic predictor. [5]

Investigators found a gradual increase in MNA from the baseline value of normal breast epithelium through benign diseases to invasive carcinoma [13], and that patients with larger MNA &SDNA have worse prognosis. [5]

Other studies like Sterkvist, et.al [14] work, reported that mitotic frequency &the variance of the nuclear area (NA) were most strongly correlated with recurrence rate within 2.5 years & since the quantitative microscopical studies of breast carcinoma has an objective nature, so NA measurement has a considerable advantage over the mitotic index study which is a value of subjective nature. [5]

The MNA & variation of NA were also of prognostic significance in cytological specimens, [15, 16] and that the study of automated diagnosis & grading of breast carcinoma have been proved valuable. [17]

Further more a significant correlation found between morphometric features and estrogen receptor status. [18, 19]

In the present study we attempted to evaluate additional prognostic value of the nuclear profile to other prognostic variables.

Materials and methods:

Fifty-four cases of breast carcinoma were selected from the files of the histopathological laboratory at Al-Yarmouk Teaching Hospital in Baghdad, and reviewed later by two other pathologists; as invasive breast carcinoma of ductal type. All the examined samples were fixed in 10% formalin, embedded in paraffin; the sections were cut at 5µm & stained with hematoxylin and eosin. Histological grading was performed according to the modified Bloom-Richardson grading system on the basis of tubule formation, nuclear atypia & mitotic activity (grade I tumors have fairly normal appearance, grade III are poorly differentiated &grade II are intermediate between I and III. [20]

Two other prognostic parameters were also investigated; namely the tumor size or diameter (more or less than 5cm) and patient's age whether (more or less than 50 years).

In each case, the most cellular areas were looked for at the periphery of the tumor. Necrotic and inflamed areas were avoided. An average of 5-10 high power microscopical fields was screened & 30 consecutive tumor cells with clear nuclear boarders were outlined. Overlapping nuclei were not measured .In each case these nuclei were examined at x400 (x40 objective magnification, x10 camera ocular), by the use of an image analysis system run by global lab image 2 software GLI2 (data translation Inc., USA). The system composed of personal computer PC with frame grabber (DT3120k-1data translation Inc., USA) attached to the PC and a microscope (Olympus BH, Japan) with a video camera (KGB, cc-8603, Taiwan).

The images from the sections were obtained at 800X600 pixels resolution in BMP format. The digitalized images of the nuclear profile were outlined on the monitor screen using a computer mouse. The morphometric feature assessed was the nuclear area (NA).

The system was calibrated with a micrometer slide before each measurement. The data were transferred to a Microsoft® excel work sheet and were expressed in terms of micrometers

and the differences in terms of morphometric measurement between the three groups studied were statistically tested using analysis of variance, and Tukey's(HSD) and t-test.

Results:

The mean values of nuclear area varied between the three histological grades (grade I, IIand III) by using analysis of variance ($p>0.01$). (Table -1)

These values increased significantly with increasing histological grade, (Tukey's(HSD) test: $p>0.01$) (table -4). Similarly the mean values of nuclear area were significantly higher in tumors measuring more than 5cm in diameter than those less than 5cm in diameter (t-test: $p>0.01$). (Table-3)

On the other hand, the nuclear area in tumors of patients less than 50 years & those more than 50 years of age showed no significant difference. (t-test: $p<0.01$) ,(table-2)

Table 1
ANOVA test for the nuclear area of the three grades

Source of variation	*Sum of squares*	*Degree of freedom*	*Mean squares*	*F calculated*
Between groups	**19288.706**	**2**	**9644.353**	**31.877**
Within groups	**138266.862**	**457**	**302.553**	
total	**157555.569**	**459**		

***F tabulated* =4.652**

Table 2
The mean values of the nuclear area in different age groups (expressed as *mean* ± *one standard deviation)*

Age group	*Mean nuclear area*
Less than 50 years old	**49.1±20**
More than 50 years old	**50.21±18.82**

Table 3
The mean values of the nuclear area in different tumor size (expressed as *mean* ± *one standard deviation)*

Tumor size	*Mean nuclear area*
Less than 5 cm	**46.68±17.29**
More than 5 cm	**54.32±21.89**

Table 4
The mean values of the nuclear area in the three histological grades (expressed as *mean ± one standard deviation)*

Histological grade	*Mean nuclear area*
Grade I	**43.75±14.63**
Grade II	**54.35±18.28**
Grade III	**67.5±15.86**

Discussion:

Breast carcinoma is one of the most important causes of death in female population and in order to predict the tumor's behavior many prognostic parameters have been introduced, some of which are subjectively judged, as the mitotic index, histological and nuclear grade with an interobserver agreement that rarely exceeds 80-90%.

From here the advantage of quantitative analytic methods has been introduced as an objective &reproducible tool for the evaluation of the tumor's behavior.

In this work we attempted to study the value of nuclear morphometry in the evaluation of some of the conventional prognostic parameters, namely; tumor's size, patient's age and histological grade.

There was a significant difference in the value of the MNA between the three histological tumor grades (I, II and III) and between those tumors more than 5cm and those less than 5cm in diameter, while there was no significant difference of the MNA between tumors of (more than 50 years and less than 50 years of age).

This showed that the MNA was of value in the assessment of the histological grade, in those higher figures seen in higher grades. The same relationship found between the tumors more than 5cm and those less than 5cm, in which higher values seen in tumors more than 5cm in diameter. On the contrary the value of the MNA was restricted when comparing tumors in patients less than 50 years and those more than 50 years of age. One explanation for the latter finding might be explained by the improper age recording of the examined patients.

Similar results were obtained by other studies indicating the value of the MNA as an objective morphometric index. [13,21]

In conclusion our data suggest that adapting a nuclear morphometric parameter e.g nuclear area, which was performed on our samples, may be a valuable objective tool in evaluating various prognostic indices.

References:

1- Parkin DM, Bray F, Ferlay J, Psani P. Estimating the world cancer burden. Int J Cancer 2001; 94:153-156.

2- Rossi A, Bonadonna G, Valagussa P, Banfi A, Veronesi U. CMF adjuvant program for breast cancer: Five- years results (Abstr). Proc Am Assoc Cancer Res Am Soc Clin Oncol 1980; 21:404.

3- Fisher B, Redmond C, Fisher ER et al. The contribution of recent NSABP trials of primary breast cancer therapy to an understanding of tumor biology: An overview of findings. Cancer 1980; 46:1009-1025.

4- Bonadonna G, Valagussa P. Dose -response effect of adjuvant chemotherapy in breast cancer .N Engl J Med 1981; 304:10-15.

5- Jan P.A.Baak , Herman Van Dop, Piet H.J.Kurver, and Jo Hermans. The value of morphometry to classic prognosticators in breast cancer.Cancer 1985;56:374-382,1985.

6- Elston CW, Ellis IO. Pathological prognostic factors in breast cancer, the value of histological grades in breast cancer: Histopathology 1991; 19:403-410.

7- Black MM, Barclay THC, Hankey BF. Prognosis in breast cancer utilizing histological characteristics of the primary tumor: Cancer 1975; 36: 2048-2055.

8- fisher ER, Redmond C, and Fisher B. Histologic grading of breast cancer. Pathol Annu 1980; 15/1:239-251.

9- Delides GS, Garas G, Georgouli G et al. Interlaboratory variations in the grading of breast carcinoma. Arch pathol Lab Med 1982; 106: 126-128.

10- Baak JPA, Lindman J, Overdiep SH, Langley FA. Disagreement of histopathological diagnoses of different pathologist in ovarian tumors with some theoretical considerations. Eur J Obstet Gynecol Reprod Biol 1982; 13: 51-55.

11- Weible ER. Stereological methods Vol.I.Practical methods for biological morphometry . London: Academic Press, 1979.

12- Baak JPA, Oort J. A manual of morphometry in diagnostic pathology .Heidelberg , Berlin , New York : Springer ,1983.

13- Offiong Francis Ikpatt, Teijo Kuopio and Yrjo Collan. Nuclear morphometry in african breast cancer. Image Anal Stereol 2002; 21:145-150.

14- Syenkvist B, Bengtsson E, Dahlqvist B et al. Predicting breast cancer recurrence. Cancer 1982; 50:2884-2893.

15- Zajdela A, saravia de la Riva L, Ghossein NA. The relation of prognosis to the nuclear diameter of breast cancer cells obtained by cytological aspiration. Acta Cytol 1979; 23:75-80.

16 Kuenen-Boumeester V, Hop WCJ, Blonk DI, Boon ME. Prognostic scoring using cytomorphometry and lymph node status of patients with breast carcinoma. Clin Oncol 1984; 20:337-345.

17- Pranab Dey, Sushmita Ghoshal,and Sanjib Kumar Pattari, Nuclear image morphometry and cytologic grade of breast carcinoma. Analyt Quant Cytol Histol 2000; 22:483-485.

18- Auer GU, Caspersson TO, Gustafsson SA et al. Relationship between nuclear DNA distribution and estrogen receptors in human mammary carcinomas. Anal Quant Cytol 1980; 2:280-284.

19- Baak JPA, Persijn JP.: In search for the best qualitative microscopical or morphometrical predictor of estrogen receptor in breast cancer. Pathol Res Pract 1984; 178:307-314.

20- Bloom HJG, Richardson WW.: Histological grading and prognosis in breast cancer. Br J Cancer 1957; 2:369-377.

21- Tan PH, Goh BB, Chiang G, Bay BH.: Correlation of nuclear morphometry with pathological parameters in ductal carcinoma in-situ of the breast. Mod Pathol 2001; 14(10): 937-4.

Causes of Partial Epilepsy in a cohort of Iraqi epileptic patients

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Abstract

Background: Partial (focal or localization related) epilepsy is the most common seizure disorder encountered in patients with epilepsy. These seizures are focal at onset that is emanating from localized region of the brain. Certain structural and metabolic abnormalities in the brain will predictably lower the epilepsy threshold. Seizure can result from either primary central nervous system dysfunction or underlying systemic diseases. The incidence of structural abnormalities was higher with increasing age of the onset of seizure and declined with long duration of history of epilepsy

Objectives:

1) Identify the cause of partial seizure.

2) Clarify the association of these causes and the age of the patients.

Study: Prospective cohort study.

Setting: Al-Kademeiyah Teaching Hospital.

Patients: 106 patients presented with partial seizure, the age of them ranged between 6-73 years, 52 males and 54 females

Result: The abnormal neuroimaging occurred in (61%) of patients. Tumors occurred in about (19.7%) of patients most of them below 40 years of age while infarctions in about (25.5%) of patient above this age.

(83.7%) of complex partial seizure patients had temporal lobe foci and (16.2%) in frontal lobe, while (49%) of simple partial seizure patient had frontal lobe foci, (22%) frontoparietal and (13%) had parietal lobe foci.

(75.4%) of patient with simple partial seizure and (35.1%) with complex partial seizure had brain(structural) lesion.

Conclusion:

1. Infarction is a common cause of partial seizure in patients above the age of 40 and below this age was a tumor.
2. Partial seizure is associated mostly with organic brain lesions.
3. The incidence of structural lesion was decrease in patients with long history of partial epilepsy.

Key words: Seizure and Epilepsy

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Introduction:

Epilepsy can be defined as an intermittent derangement of the nervous system due to "an excessive and disorderly discharge of cerebral neurones". This was postulated in 1870 by Hughlings Jackson., and modern electrophysiology offers no evidence to the contrary [1].

Partial (focal or localization related) epilepsy is the most common seizure disorder encountered in patients with epilepsy. These seizures are focal at onset that is emanating from localized region of the brain [2].

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Approximately 5-10 % of population will have at least one seizure during their lifetime, with highest incidence occurring in early childhood and late adulthood. [3]

If consciousness (The awareness of, and ability to respond to the environment) is preserved, the attack is termed, simple. If, however, the activity involves some parts of the brain dealing with awareness (such as the temporal or frontal lobes), then consciousness is affected and a complex partial seizure results. Further spread into the diencephalons and hence throughout the remainder of the cortex leads to a secondarily generalized seizure [4].

In the normally functioning cortex, synchronous discharge amongst neighboring groups of neuron is

limited by recurrent and collateral inhibitory circuit. The inhibitory transmitter is GABA while the excitatory neurotransmitters are acetylecholine, aminoacids glutamate and aspartate [4].

In partial seizure there is paroxysmal depolarization of membrane of a local group of neurons, which corresponds temporally to the finding of a focal spike and wave complex on the EEG [5].

Certain structural and metabolic abnormalities in the brain will predictably lower the epilepsy threshold [6]. Seizure can result from either primary central nervous system dysfunction or underlying systemic diseases. This distinction is critical. Since therapy must be directed at the underlying disorders as well as at seizure control [4].

Seizures before the age of 20 years are rarely associated with tumors. If the patient is between 20-30 y of age the risk of progressive lesions goes up slightly [7]

From 30 to 60 years, the incidence of primary brain tumor peaks, reaching approximately 15 percent in patients with PSs. After the age of 60, the incidence of tumors starts to fall off again as a vascular cause becomes more likely [8]

Approximately 30% of patients with PE have mass lesions like neoplasms or vascular malformations as the cause of their seizure disorders [9].

The incidence of structural abnormalities was higher with increasing age of the onset of seizure and declined with long duration of history of epilepsy [10]

The evaluation of the patients with a probable PS includes a variety of diagnostic and clinical evaluation in addition to the history and physical and neurological examination. These include EEG and neuro-imaging pictures. EEG most commonly used as a diagnostic test in assessment of patients with PE. A yield between 60 and 93 percent has been claimed for this procedure when multiple recordings are used or when recordings are made after 24 hours period of sleep deprivation. Sphenoidal electrodes both the anterior and posterior ones improve the yield in patients with mesial temporal sclerosis whose routine EEG are normal. The CT and MRI are non-invasive studies that can determine the cause of seizure and assist in localizing the epileptogenic area. MRI has been demonstrated to be superior to CT in imaging epileotogenic lesion,like temporal sclerosis and cortical gliosis [12].

The treatment of PE may be either medical or surgical, but medical treatment is the primary approach [13].

Patients and Methods:

We take all patients referred for neurological consultation in Al-Kahdemyia Teaching Hospital, Baghdad Teaching Hospital & Al-Yarmok Teaching Hospital for the period from (November 2001-May 2003).

The patients with partial seizure and partial with secondary generalized epilepsy were enrolled in this study. This was secured through a detailed history, elaborate generalized examination and examination of the nervous system.

All patients had 16 channel EEG recordings (by use of Nihon Kohden Corporation: 4321F), some times more than one recording is needed with emphasis on activating procedure like hyperventilation, Each recording is for 20 minutes.

All patients had neuroimagings like brain spiral CT (Somatotom Plus 4-Siemens ,Version C10B) with and without contrast when needed. MRI (Gyroscan NT 1.5 tesla power. Philips

Medical System) was done in patients with no abnormalities revealed by CT.

Results;

One hundred six patients were collected from those who visited neurological department of Al-Yarmok teaching hospital, Al-Kadhemia teaching hospital /Baghdad Teaching Hospital with symptoms and sign goes with partial epilepsy.

The age of the patients ranged between 6-73y. 52 males & 54 females.

In 65 (61%) patients the neuro-imaging study (MRI & CT) was abnormal, while 41(39%) patients no lesion can be defined.

The association between brain lesion and age of the patient was shown in table (1).

Sixty five patients with partial epilepsy had lesions that were revealed on MRI and CT. 21(32%) patients with tumors: 16 patients with glioma, 3 patient, with maningioma, 2 patients with cystic tumor, both of them had a strocytoma. Twenty-seven (42%) patients complained of infraction, 4 (6%) patients had intra-cerebral hemorrhage. Brain abscess is found in 5 (8%) patients. Encephalities occurred in 2 (3%) patients, while 6 patients (9%) had mesial temporal sclerosis.

In our study we found that there are 19 patients who had a history of febrile convulsions in childhood, 5 (26%) of them had focal area at fronto-parietal area while 14 (74%) patients had focal area of epilepsy at temporal lobe. In those patients focal area of epilepsy with temporal lobe had changes on brain MRI go with mesial temporal sclerosis in 6 (32%) patients.

Neuro-imaging in patients with SPS revealed lesion in 52 (75.3%) patient from 69 patients, while there are lesions in 13 (35.1%) patients complained from complex partial epilepsy.

We found that when there are a long duration of epilepsy the abnormalities on neuro-imaging or structural brain abnormalities decrease

Table 1: Types of lesion in relation to age:

Age	No. of all patient	Brain abscess	Hemorrhage	infarction	Mesial Temporal	Encephalitis	Tumors	No of pt. with Lesion
6-15	23	1		1	1			3
16-25	22			2	3		2	7
26-35	20	3		5	2	1	6	17
36-45	8		1	2			5	8
46-55	10			4		1	2	7
56-65	15	1	1	9			4	15
66-75	8		2	4			2	8
Total	106	5	4	27	6	2	21	65

Table 2: partial seizure and brain structural lesions.

Types of seizure	No. of patient	Associated brain lesion	% Of brain lesion
Simple partial	69	52	75.4
Complex partial	37	13	35.1
Total	106	65	

Table 3: Duration of epilepsy and brain lesions.

Duration of epilepsy	No. of patients	Abnormal MRI and / or CT	%
Less than 1 yr	43	35	81.4
1-2 yr	24	17	70.8
3-5 yr	16	7	43.8
>5 yr.	23	6	26.1
Total	106	65	

Discussion:

In our study there are 65 (61%) patients with partial epilepsy had abnormal neuro-imaging (CT and MRI), while there are no abnormalities in 41 (39%) patients. These results are slightly more than previously (23-55%) reported studies. This could be attributed to the fact that our study was hospital based and more stringent inclusion criteria of the patients in this study [14].

Cerebral vascular accidents or diseases as a cause of partial epilepsy were found in 27 (25.5%) patients and mostly at age above 40 yr., which is more than previous studies [10,15], which revealed that the percentage of vascular disease as a cause of partial seizure is 18%.

While the incidence of tumors a cause of partial epilepsy was found in about 21 (19.7%) patients and most of them are under the age of 40 years in comparison with 20% in previous reported studies and more than percentage reported in other studies (4-12%) [7, 8, 16]. This can be explained by the nature of our study which is prospective with particular attention to find the cause of the partial epilepsy in our patients that were drained from the hospital with their presenting syndrome being partial epilepsy.

We found that the patients with simple partial seizures were 69 (65%) while 37 (35%) patients complained of complex partial seizures these results are comparable previous reported study [17].

There is a high association of structural abnormality in the brain & SPS occurred in about 52 (75.3%), as compared to 13(35.1%) patients with CPS, our figures are comparable (48-71%) to previous reports [14,18]

About febrile convulsion, we found there is increasing in its incidence with temporal epilepsy especially mesial

temporal sclerosis (32%) which was less than that of the previous reports (48%). This finding may be attributed to many patients misdiagnosed by the neuroimiging but there is no relation between a degree of sclerosis and atrophy in MRI and a history of seizure [(19)]. These changes may occur in individual that never had seizure. In other study the identification of mesial sclerosis was incidental by MRI but significantly needs investigation [(20)].

We noticed that when there is a long history of epilepsy the incidence of structural abnormalities of brain lesions decreased while when there is short duration, neuro-imaging abnormalities will increased these result were corresponding to previous reported study [(10,18)].

Conclusion:

1. Infarction is a common cause of partial seizure in patients above the age of 40 and below this age was a tumor.
2. Partial seizure is associated mostly with organic brain lesions.
3. The incidence of structural lesion was decrease in patients with long history of partial epilepsy.

References :

1. Adams DR, Victors M, Ropper HA. Epilepsy and disorder of consciousness. Principal of neurology, 17th ed. New York. McGrew.Hill, 2001, pp 331-363.

2. Cascino GD. Intractable partial epilepsy evaluation and treatment. Myoclin Proc 1990; 65: 15758-1586.

3. Lowen Stein DH . Seizure and epilepsy In : Braunwald E, Hauser SL , Tameson JL et al (eds.) . Harrison's principle of internal medicine, 15th Ed. New York, MC Graw . Hill, 2001, pp 2354-2369.

4. Allen CMC, LueCK CT. Disease of nervous system In: Edwards CRE, Bouchier DA. Davidson's principles and practice of medicine, 18th Ed, UK, Churchill living stone, 1999.

5. Golden Sohn ES , Purpura DP . Intracellular potentials of cortical neurons during focal epileptogenic discharges. Science 1963; 39: 840-842.

6. Latack JT, Abu-Khalil BW, and Siegel GJ, et al. Patients with partial seizure: evaluation by MRI, CT and PET imaging. Radiology 1980; 159: 159.

7. Raynor R, Daine R, Carmicheel E. Epilepsy at late onset. Neurology 1959; 9: 11-7.

8. Shorven SD, Gilliatt RW., Cox TCS; et al . Evidence of vascular disease from CT scanning in late onset epilepsy. Journal of Neurology, Neurosurgery and Psychiatry 1984; 47: 225-230.

9. Babb TL, Brown WJ. Pathological finding in epilepsy. In: J Engel Jr. Surgical treatment of the epilepsies. New York, Raven Press, 1987. Pp 551-540.

10. Yaqub B, Danayiotopoulos CP., Al-Nazha M.; et al . Cause of late onset epilepsy in Saudi Arabian: the rate of cerebral granuloma. J Neural, Neurosurgery, Psychiatry 1987; 50: 90-92.

11. Gibbs EC, Gibb FA. Diagnostic and localizing value of electroencephalographic studies in sleep. Res Nerv ment Dis Proc 1997; 26: 366-376

12. Bergen D, Bleck T, Ramsey R, et al . Magnatic resonance imaging as a sensitive and specific predicter of neoplasm removal for intractable epilepsy. Eplipsia 1989; 30: 318-321.

13. Engel JJr; seizure and epilepsy. Philadelphia, FA Davis Company 1989: 443-474.

14. Young AC, Borg CJ, Moher PD, et al . Is routine computerized axial tomography in epilepsy worthwhile? Lancet 1982; 2: 1446-1447

15. Reisman D, Fitz-Hugh TJr. Epilepsia torda. Anu. Interu Med. 1927; 1:273-282.

16. Woodcock, Cosgrove JBR. Epilepsy after the age of 50: five years follow up-study. Neurology 1964; 14: 34-40.

17. Manford M ., Hart Ym., Sander JE., et al . National general practice study of epilepsy (NGPSE): Partial seizure pattern in general population. Neurology 1992; 42: 1911-1917.

18. MC Gahan JP, Dubin AB, Hill RP. The evaluation of seizure disorders by computerized tomography. J Neurosurg 1979; 50: 328-332.

19. Bower SP, Kilpatrick CJ, Vagrin SJ. Degree of hippocampal atrophy is not related to history of febrile seizure in patients with proved hippocampel sclerosis. J Neurol neurosurg psychiatry 2000; 69: 733-738.

20. Moore KR, Swallow CE, Tsuruder JS. Incidental detection of hippocampel sclerosis on MRI: it is significant. AJNR-Am-J-Neuro-radial 1999; 20 (4): 1609-1612.

A STUDY ON THE BACTERIAL DISSEMINATION AND EXPERIMENTAL PATHOLOGY OF *SALMONELLA PARATYPHI* – AN INFECTION IN WHITE MICE

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Abstract

Background: Paratyphoid infection remains an important public health problem with marked host specificity for humans or higher primates but not naturally virulent for mice except that using high inoculum of the paratyphoid bacilli.

Objective:

1. Study the bacterial dissemination through the organs of white mice.
2. Study the pathological changes associated with this experimental disease process

Methods: One LD50 of the microorganism corresponded to $8x10^7$ bacterial Cell/ml. However 10LD50 doses were intraperitoneally used for mice to produce extensive disease process in mice. Following inoculation of mice the course bacterial dissemination and pathological lesions were studied at specific intervals and for 3 days post inoculation.

Result: The microorganisms were persistent in the spleen and liver for 21 and 17 days postinoculation respectively, where as the microorganisms persisted in the mediastinal lymph nodes and lungs for 9 days and kidney and heart blood for 5 days postinoculation. The main pathological lesions were initiated as a mild infiltration of neutrophils and edema in the spleen liver and lymph node the neutrophils iflteration will be gradully replaced by mononuclear cell infiltration and finally with fibroblasts proliferation.

Conclusion: The Salmonella paratyphi-A is of lower pathogenicity and virulence for white mice, through their dissemination in organs of white mice and associated pathological findings.

Keywords: Salmonella paratyphi-A infection white mice

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Introduction:

Paratyphoid bacilli are enteropathogenic bacteria with marked host specificity, *Salmonella paratyphi* type A, B & C is strictly pathogenic for humans (or higher primates) but not naturally virulent for mice. To kill a mouse, it is necessary to give high inoculum intraperotineally, death occurs within 1-4 days following the development of a toxic syndrome (1). In our country as in other parts of the developing countries, febrile illness due to typhoid and paratyphoid bacilli are common. Although the clinical picture, the epidemiological picture as well as diagnostic procedures of paratyphoid fever have been well studied, some aspects related to the role of bacteria in the disease process and the pathological changes associated with the disease still require some illumination. Thus, the objective of this study was to fulfill a required hypothesis that paratyphoid infection can be simulated in a suitable laboratory animal model that provides a picture for human paratyphoid, the present study aims at the followings:

1- Study the disease process, including the bacterial dissemination through the organs of the white mice

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experimentally infected with *Salmonella paratyphi* - A.

2- Study the pathological changes associated with this experimental disease process in the white mice.

Materials and methods:

White mice, weighing 15-20 Gms, 2 months old, were obtained from Al-Kindi Company for Veterinary drugs and vaccines production. The mice were healthy and reared on concentrated food for two weeks before being used. A local strain of *Salmonella paratyphi* - A, isolated from the febrile patients, in the Ibn-Khatib hospital and their LD50 dose corresponded to $8x10^7$ bacterial cell. A logarithmic phase growth of *Salmonella paratyphi* - A in trypticase soy broth at 37ºc was taken, washed once in phosphate buffer saline, a suspension of a viable count of 10^9 bacterial cell/ml was obtained. Fifty mice were intraperitoneally injected with 0.8 ml of *Salmonella paratyphi* - A suspension containing $8x10^8$ bacterial cells (10 LD50). Two inoculated mice were sacrificed every two days including the dead mice for the period of one month. All the sacrificed and dead mice were studied for the purpose of:

1- Isolation of *Salmonella paratyphi* - A from the different organs of the experimentally infected mice, looking for bacterial dissemination in the organs.

2- For pathological study, small representative pieces from all the organs of infected mice were fixed in 10% neutral buffered formalin, processed routinely, cut at 5-µ thicknesses and stained with hematoxylin and eosin (H&E).

Results:

1- Distribution of *Salmonella paratyphi* - A in the organs of experimentally infected white mice:

During 30 days of experimental infection of mice with *Salmonella paratyphi* - A, an extensive dissemination of this microbe was found in the different organs at different intervals post infection (PI). It is evident (Table – 1) that the spleen and liver found to be the main target organs of invasion, whereas, kidneys and lungs were slightly invaded by this microbe. The spleen had the longest period of infectivity, which lasted for 21 days post infection, whereas, infectivity lasted for 17 days in the liver. Mediastinal lymph nodes and lungs harbored the organism for 9 days. The organisms were isolated from the kidneys and heart blood for 5 days post infection. The brain is free of bacterial isolation.

Table –1: Distribution of *Salmonella paratyphi* - A through the organs of white mice after intraperitoneal inoculation

Intervals (PI) *	Spleen	Liver	Lungs	Media-stinal lymph node	kidneys	Heart blood	Brain
1st day	+	+	+	+	+	+	-
3rd day	+	+	+	+	+	+	-
5th day	+	+	+	+	+	+	-
7th day	+	+	+	+	-	-	-

9th day	+	+	+	+	-	-	-
11th day	+	+	-	-	-	-	-
13th day	+	+	-	-	-	-	-
15th day	+	+	-	-	-	-	-
17th day	+	+	-	-	-	-	-
19th day	+	-	-	-	-	-	-
21st day	+	-	-	-	-	-	-
23rd day	-	-	-	-	-	-	-
25th day	-	-	-	-	-	-	-
27th day	-	-	-	-	-	-	-
29th day	-	-	-	-	-	-	-

*** PI: Post infection.**
**** Two mice were sacrificed for each interval**.

2- The Pathological Findings:

Different pathological findings appeared in this experimental disease processes were as follows:

The spleen:

The earliest lesions were mild infiltration of neutrophils in white and red pulps of the splenic tissue (Fig.-1). These inflammatory cells were gradually replaced by mononuclear cells (lymphocytes and macrophages), and few fibroblasts through the second and third week post infection. Also there is extensive congestion and hyperplasia of white pulp and in the reticuloendothelial cells lining the red pulp. These lesions were completely disappeared through the fourth week postinfection.

The liver:

It showed initially a mild aggregate of neutrophils in the sinusoidal and preisinusoidal areas of the hepatic tissue (Fig.-2), which is extensively congested and these inflammatory cell infiltrations were gradually replaced with few mononuclear cells (Lymphocytes and macrophages) (Fig.-3), through the second week post infection. These lesions were completely disappeared through the third week post infection.

Mediastinal Lymph Node:

There is extensive hyperplasia in the lymphoid follicles of the cortical region and in the reticuloendothelial cells lining the medullary sinuses (Fig.-4). Also there is extensive congestion of the lymphoid tissue.

The lungs:

It shows extensive congestion and mild interstitial pneumonic lesions (Fig.-5).

The Kidneys:

It showed only congestion and mild microthrombi in the renal tissue (Fig.-6).

The brain and other organs showed only congestion.

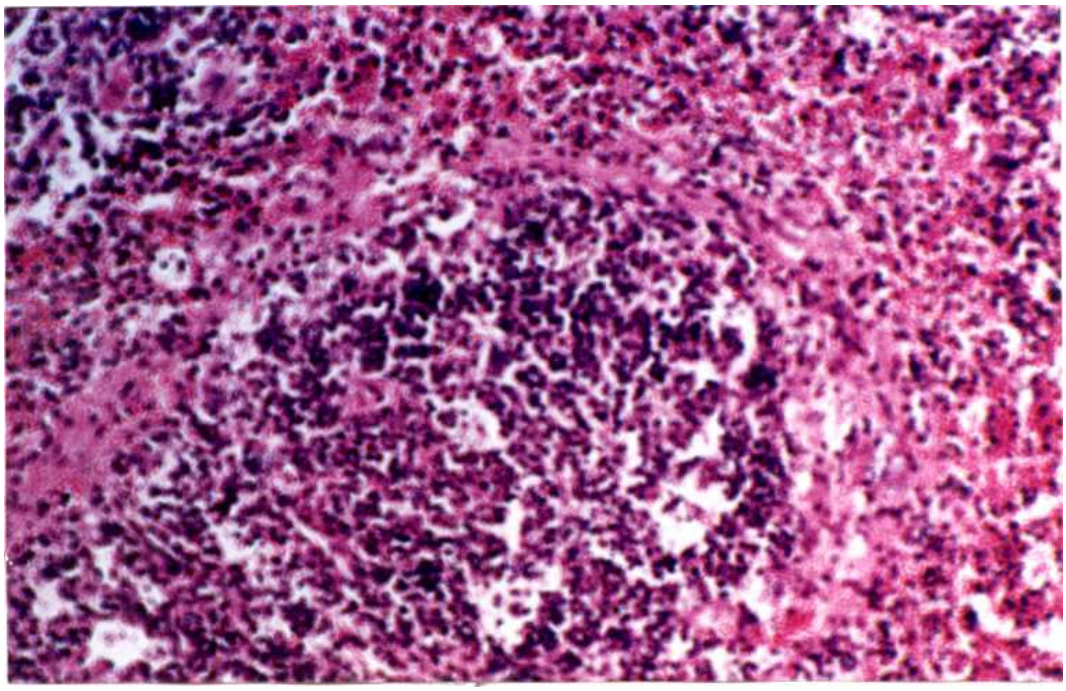

Fig.1: Microscopic section of spleen tissue shows focal aggregate of neutrophils and edema in the white pulp (H&E) X 125.

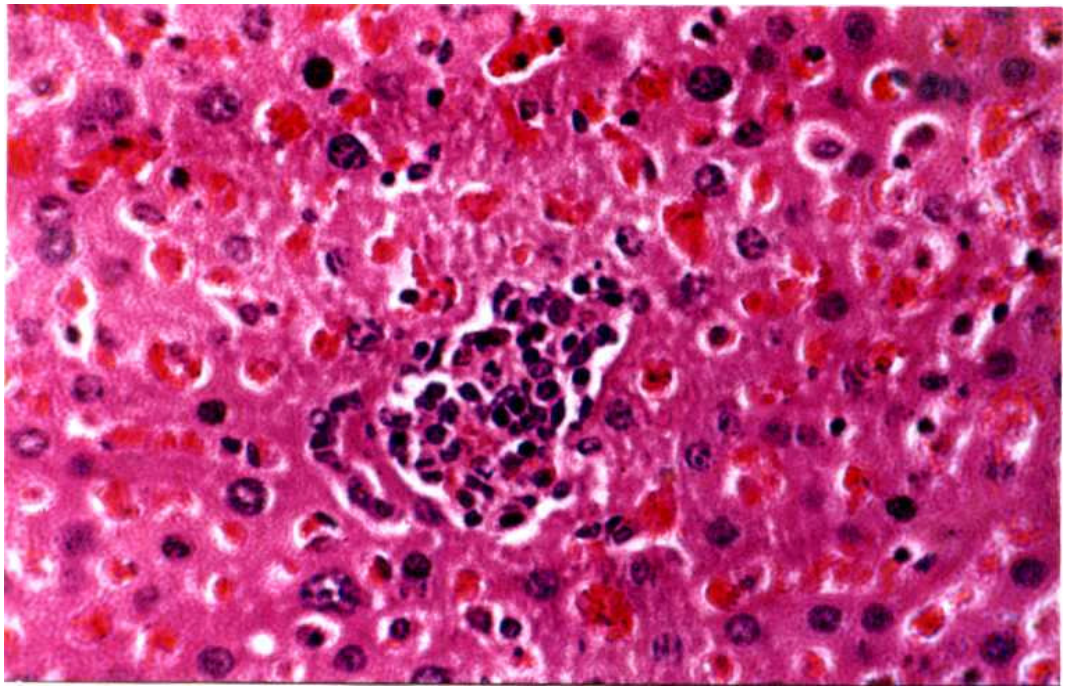

Fig.2: Microscopic section of liver tissue shows the presence of focal aggregate of neutrophils in the area adjacent to the sinusoids (H&E) X 250.

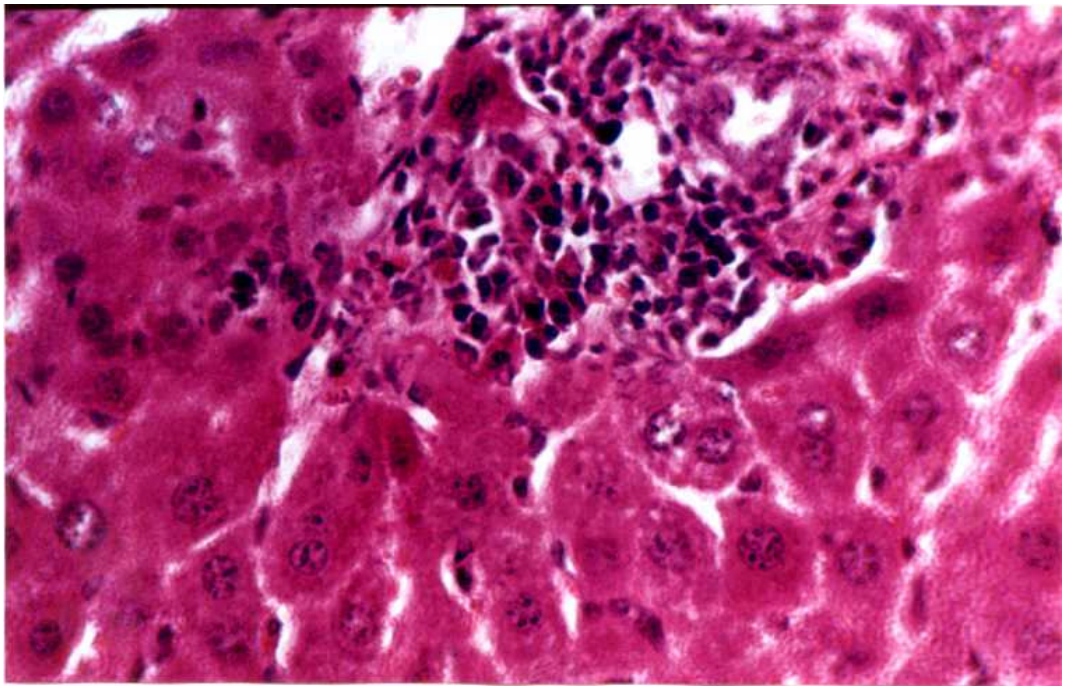

Fig. 3: Microscopic section of liver tissue shows infiltration of lymphocytes and macrophages replacing the neutrophils (H&E) X 500.

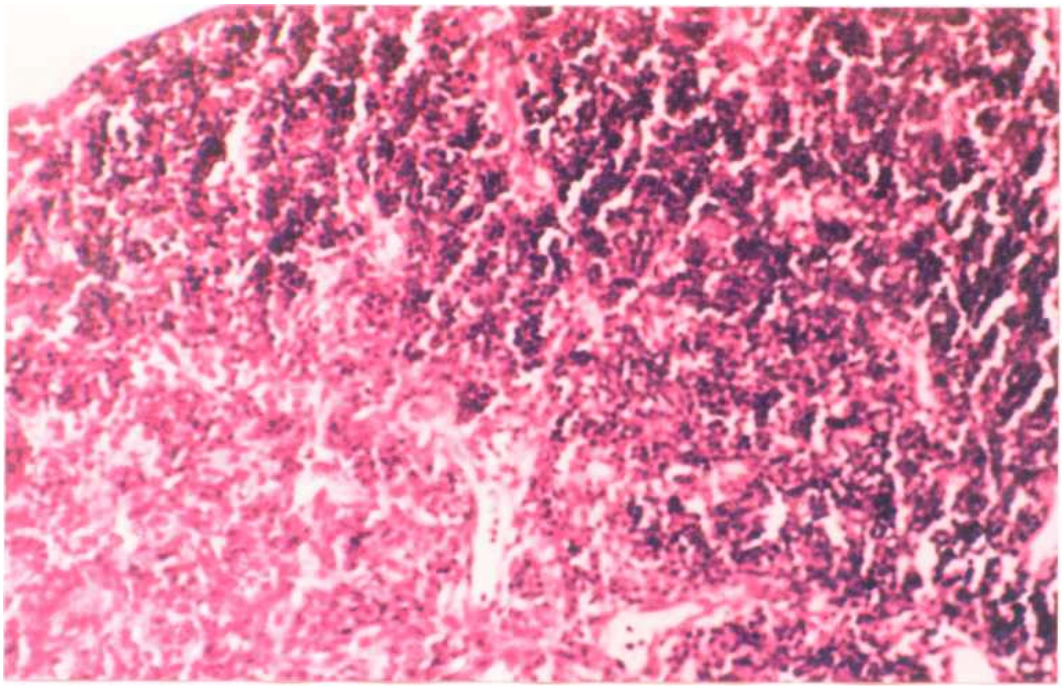

Fig. 4: Microscopic section of mediastinal lymph node shows extensive reactive hyperplasia (H&E). X 125

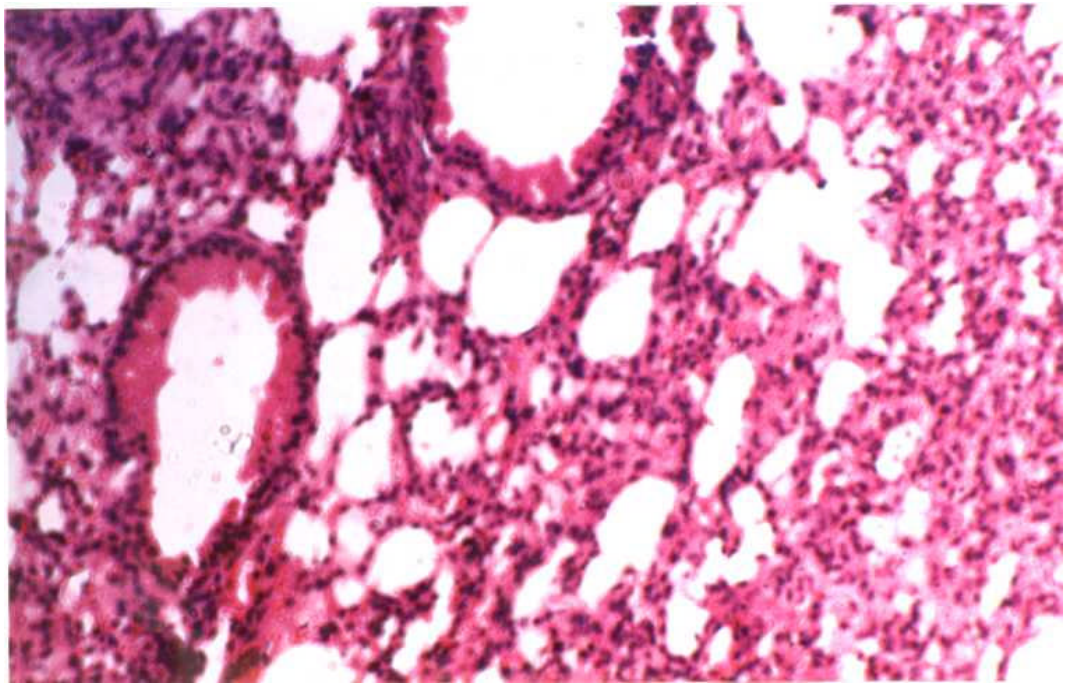

Fig. 5: Microscopic section of pulmonary tissue shows interstitial pneumonic lesions (H&E) X 125.

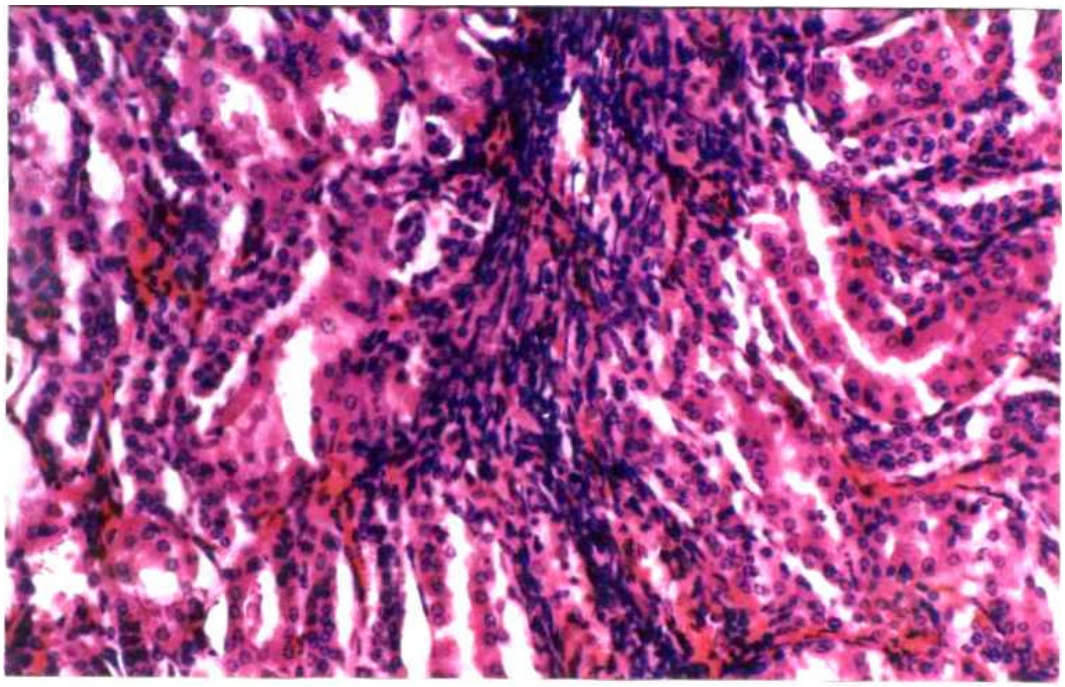

Fig. 6: Microscopic section of renal tissue, shows mild interstitial nephritis (H&E) X125

Discussion:

1- Distribution of *Salmonella paratyphi* - A in the organs of experimentally infected white mice:

Most studies on the typhoid and paratyphoid bacilli in mice have been limited by the fact that these organisms have low pathogencity for this animal species. Thus microorganisms are strictly pathogenic for human being (or higher primates). To kill mice a high

inoculum of these microorganisms should be intraperitoneally given (1). These findings were confirmed by this work, a less extent disease process produced in mice when the inoculum introduced into the peritoneal cavity was $8x10^8$ bacterial cell of *Salmonella paratyphi* - A. Corresponding results were obtained by other workers, who showed that typhoid and paratyphoid like diseases were produced in mice using the paratyphoid bacilli type-B (2) and typhoid bacilli (3, 4 and 5). This study revealed that following the intraperitoneal injection of *Salmonella paratyphi* – A with the massive dose of $8x10^8$ bacterial cell, a less extent growth of this microorganism occurred in the spleen, liver, mediastinal lymph node, lungs and kidneys; the microorganisms reach in to the thoracic duct and to blood through 24 hours post infection. These results go along with those reported by Gerichter, Carter and Collins and AL-Joboury (6, 7, 2), who isolated similar organisms (*Salmonella paratyphi* – A and B) from these organs one day post infection. The difference in the dissemination of this microbe in the different organs of the mice was attributed to the difference in the doses, route of infection and virulence of the microorganism. In this study we used $8x10^8$ bacterial cell injected intraperitoneally, whereas, other studies used 5x109, orally (6), $2x10^6$ intravenously (7) and $5x10^8$ intraperitoneally (2). In the present study following intraperitoneal injection of *Salmonella paratyphi* – A in to the mice, less extent persistence systemic growth occurred in most of the infected mice which harbored the infection in the spleen for 21 days, the liver for 17 days, in the mediastinal lymph node and lungs for 9 days and heart blood and kidneys for 5 days post inoculation, these findings are similar to those reported by Gerichter, Carter and Collins and AL-Joboury (6,7,5 and 2) for typhoid and paratyphoid bacilli.

Pathological Findings:

This study revealed mild lesions, initiated in the liver, spleen and mediastinal lymph nodes and consisted of mild infiltration of neutrophils, edema and congestion of blood vessels. These inflammatory cells infiltrations and edema were demonstrated by other workers (8 & 9) on murine Salmonellosis caused by *Salmonella typhimurium* and in mice infected with typhoid and paratyphoid bacilli (10, 2). Both workers demonstrated extensive neutrophil infiltration forming multifocal microabscesses in different organs, which is not observed in the present study and may be explained on the basis of virulence of the microbe used in the present study. During the second week post infection, the neutrophiles infiltration was gradually replaced by mononuclear cells (Lymphocytes and macrophages and plasma cells) infiltration without granulomatous type lesions which demonstrated by Naconeczna and Hsu (8, 9) on murine Salmonellosis caused by *Salmonella typhimurium* and AL-Joboury (10,2) on mice experimentally infected with typhoid and paratyphoid bacilli. The absence of granulomatous lesions in the spleen and liver in the present study may be explained on the basis of low virulence of the microorganism used in the present study, and therefore, during third and fourth week post infection, most of the infected organs will become free from any lesions. The healing of the lesions through these periods of post infection will indicate that the lesions were originally found at less extent, so easily healed. Other mild lesions such as, hyperplasia of lymphoid tissues, interstitial pneumonic lesions in the lung tissue and micro thrombi in the kidney were demonstrated in the

present study. The occurrence of these lesions in these organs were explained as metastatic type lesions occurred by the microorganisms through, the hematogenous dissemination, such metastatic lesions were also reported in mice experimentally infected by typhoid and paratyphoid bacilli (2, 10) and in typhoid patients (11,12). No brain involvement by *Salmonella paratyphi* – A, is reported in the present study, these findings may be explained on the basis of low virulence of this microbe which did not reach into the brain through the metastasis.

Conclusions:

The results of this study on mice experimentally infected with *Salmonella paratyphi* –A differ from the results of the previous study obtained by AL-Joboury (2) on mice experimentally infected with *Salmonella paratyphi* – B in the followings-:

1- The present study showed focal aggregate of neutrophils without microabscess formation and not accompanied by granulomatous lesion in spleen and liver, comparable to the focal microabscess and granuloma seen in the spleen and liver in the previous study on mice experimentally infected with *Salmonella paratyphi* – B.

2- No involvement of the brain tissue of the mice by the *Salmonella paratyphi* – A in the present study.

3- Mild nonspecific pathological lesions were seen in the lungs and kidneys of the mice in the present study.

4- The above findings indicate that the *Salmonella paratyphi* – A is of lower pathogencity and virulence than of *Salmonella paratyphi* – B and for *Salmonella typhi*.

References:

1- LeMinor L, Richard CL, Molleret,HH, Bercovier,H, Alonso JM. Enterobacteries.In:LeMinor,L.;Veron,M.(eds.) .Bacteriologie Medicale paris: Flammarion, 1982;P.P.: 240-315.

2- Al-Joboury KH. A study on the bacterial dissemination and experimental pathology of *Salmonella paratyphi*-B infection in white mice. The Iraqi J. of Vet. Med., 2000; 24:212-229

3- Parozozovskii,SV, Tsarevskii,CP, Levida,GA, GorelovAL. In vivo experimental modeling of infectious process caused by L-forms of the causative agent of typhoid. Zh. Mikrobiol. Epidemiol. Immunobiol.1985.8: 10-14.

4- Dima,VF, Petrovici,M, Lacxy d. Reaction and response of newborn Guinea pigs to experimental *Salmonella typhi* infection. Arch. Roum-Pathol-Exp Microbiol., 1989,48;(4);299-321 .

5- Al-Joboury KH, Makkawi TA, Khalifa AK, and AL-Falluji M.M. The dissemination of *Salmonella typhi* through the organs of white mice by intrapretoneal infection .The 5th medical AL-Mustansiria, J.Sci.2002; 13:129-134

6- Gerichter CB. the dissemination of *Salmonella typhi* , *Salmonella paratyphi* –A and *Salmonella paratyphi* – B through the organs of white mice by oral infection .J. Hyg. Camb. , 1960; 58:307-319.

7- Carter PB and Collins FM. Growth of typhoid and paratyphoid bacilli in intravenously infected mice.Infect. Immun., 1974; 10;(4): 816-822.

8- Nakoneczna I. And Hsu H S. The comparative histopathology of primary and secondary lesions in murine salmonellosis . Br.J.Exp.Pathol. 1980; 61:76-84.

9- Nakoneczna I And Hsu HS. Histopathological study of protective immunity against murine salmonellosis induced by killed vaccine. Infect. Immun., 1983; 39;(1); 423-430.

10- Al-Joboury KH. , Makkawi TA, Khalifa, K and AL-Fallujii M.M. Typhoid fever: pathological findings associated with *Salmonella typhi* infection in white mice. Proceeding of fifth medical conference, Collage of Medicine and Dentistry and AL-Anbar Health Institute 2000/2001; 17-24

11- Cotran,Kumar and Robbins In: Robbins pathological basis of disease, 14th ed. W.B. Saunders International edition,1989.

12- Rajajee S, AnadiT B, Subha,S, Vatsala ,B R. Pattern of resistant *Salmonella typhi* infection in Infants J Trop.Pediatr. ', 1995; 41;(1): 52-54.

Carrier Detection of Duchenne Muscular Dystrophy by CPK Activity Testing and Conventional Needle EMG.

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) is a dismal disease, which exhibits an X-linked mood of inheritance, characterized by progressive proximal muscular weakness, beginning in early childhood, wheel chair dependency by early teens and death from cardiopulmonary complications by the end of the second or third decade. Although the majority of carriers of DMD and Becker muscular dystrophy (BMD) are asymptomatic but they can be identified through minor clinical changes like limited weakness or occasionally bulky calves, and some may have elevated CPK levels and mild EMG changes, and as DMD is incurable disease, and carrier detection and genetic counseling are an important aspect of the global approach to limiting the number of patients with DMD and BMD and of carriers.

The Objective of the study is to test the value of CPK and conventional needle EMG muscle testing in detecting carriers in a group of Iraqi females with their sons having DMD and BMD.

Patients and Methods: The study was conducted in AL-Kadhimiyah Teaching Hospital Section of Neurology from October 2002 to December 2003, where a group of 20 female carriers of DMD and BMD, from 15 families were studied and compared to other Control group of 20 females randomly picked up. To each female studied full medical history, neurological examination, including manual muscle power testing, and

Pedigree analysis taken, and to each female in the study CPK testing, ECG, with cardiac Echo, and Conventional needle EMG were done.

Results: Only one female carrier (5%) had mild proximal muscle weakness, cardiac involvement only one (5%), had mildly dilated left ventricle but with normal systolic function.

11 (55%) female carriers had mildly elevated CPK above the upper reference range (170 U/L); 10 (58.8%) DMD, and 1 (33.33%) of BMD. And there is significant ($p<0.05$) difference in CPK activity between the two groups.

There is negative correlation between the age of female carriers and the CPK activity.

9 female carriers (45%) total, (52.94%) of DMD had proximal myopathic EMG changes, which were more prominent in the upper limbs. And there is significant ($P< 0.05$) difference in mean amplitudes of motor unit action potentials of Biceps Brachii, and Vastus Medialis muscles.

Conclusion: As the CPK and EMG testings are simple, non costly and readily available tests, and as they can be positive to some extent in a proportions of carriers so they can be performed on all possible carriers in the families of DMD & BMD as a simple screening test, especially the CPK, better to perform at an earlier age, and the EMG at an older age because it requires cooperation.

This has a significant impact on genetic counseling, aiming at preventing the spread of this bleak disease.

Keywords: Duchenne, Becker, Musclar dystrophy, Female carriers, CPK and Conventional EMG.

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Introduction:

Duchene muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive traits. Thus males carrying the gene are affected, whilst heterozygous females are carriers but often unaffected. Carrier females will pass on the condition to 50% of their sons and 50% of their daughters that will be carriers, i.e. a 25% chance that a carrier will have an affected son [(1)].

Gowers was the first to deduce the genetic basis for the disease. In 1986 100 years after Gowers' Kunkel identified the DMD gene and provided molecular

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genetic confirmation of the inheritance pattern [2].

The primary purpose of identifying carriers is to provide accurate information to women and their husbands about the risk that any of their children will inherit the disease [3]. So carrier detection is an important aspect of the care and evaluation of patients with DMD and BMD and their family members [2]. Before the discovery of Dystrophin gene in 1986, carriers can be identified from pedigree information, from physical examination, and from indirect laboratory tests, the simplest and most reliable being the Creatinin Phosphokinase (CPK) test[3].

Mild muscle weakness or enlarged calves are occasionally encountered in heterozygous females [3]. Roses and colleagues have studied the female carriers and report a slight weakness and enlargement of the calves as well as elevated CPK values and abnormalities of the electromyogram (EMG) and muscle biopsy –all-slight in degree- in over 80% of the patients [4]. It has long been known that carriers of DMD may also have symptoms of the disease. Summaries of case reports until 1970 were reported by Penn and colleagues [5]. A separate group known as manifesting carriers is the result of incomplete Lionization of the maternal X-chromosome [1]. The clinical picture of carriers with symptoms can vary from muscle pain and cramp on exertion at one end of the spectrum, to severe muscle weakness leading to wheel chair dependency on the other end [6, 7].

If weakness is present, it is commonly mild, predominantly asymmetric, and proximally distributed [6, 8, 9]. The pelvic girdle is more frequently and earlier affected than the shoulder girdle. Age of onset is also variable, ranging from the first to the fourth decade. Onset before the age of 15 years usually leads to severe involvement [6, 10]. A small minority of female carriers is symptomatic [2]. Clinically apparent muscle weakness occurs in 2.5 to 20 percent of female carriers of a mutated Dystrophin gene [11]. Carriers of BMD rarely have symptoms-since the first report by Moser in 1974 [3], only few instances have been described [8].

A major advance in clinical diagnosis was the discovery by Sibley and Lehninger (1949), amplified by Schapira et al. (1953), that serum activity of the enzyme, aldolase, was greatly increased in boys with progressive muscular dystrophy. After a number of other serum enzymes were found to be elevated, the CPK test introduced by Ebashi et al (1959) proved to be the most sensitive, and it was soon established that young boys with DMD invariably had extremely large elevations of serum CPK that were rarely found in autosomal recessive muscular dystrophy [3]. Serum CK is also increased in approximately 70 and 50 percent of Duchenne and Becker carriers, respectively [12]. The elevations are usually mild, up to three times the upper limit of normal, ranging from 2-10 times the upper limit of normal [11,13].

For many years CPK testing was the best method for carrier detection [2] as about two-third of definite carriers have abnormally increased serum CPK, but a normal CPK test is not conclusive evidence against the carrier state[3]. There is some evidence that the detection rate for carriers is higher in childhood than in adults [3].

EMG In Muscular Dystrophy: Even though not diagnostic, narrows the differential diagnosis by effectively excluding primarily neurogenic processes such as spinal muscular atrophy. In general, the proximal muscles of the lower extremities may exhibit the more prominent EMG findings. The MUAPs in patients with DMD or BMD are typically of short

duration, particularly the simple (i.e, non polyphasic) MUAPs.

MUAPs amplitudes are variable (normal to reduced) and they are typically polyphasic from the variability in muscle fiber diameters, resulting in longer MUAPs durations [4]. Quantitative EMG (using measurement of duration, amplitude, and number of phases of photographed muscle action potentials) has revealed abnormalities in 38 to 42% of definite and probable carriers. Occasionally, the EMG is abnormal in a woman with normal CPK activity [3].

Electrocardiography (ECG) In Muscular Dystrophy: Among patients with DMD extensive fibrosis of the posterobasal left ventricular wall may result in the characteristic ECG changes of tall right precordial R waves with an increased R/S ratio and deep Q waves in leads I , aVL, and V5-6. The disorder is also associated with conduction disturbances, especially intra atrial leading to a variety of atrial arythmias. Intra atrial conduction defects are more common than AV or infra nodal defects in DMD. Since 1967, abnormalities in ECG, like those seen in DMD patients, have been recognized in carriers of DMD [14, 15]. It has become clear that severe cardiac involvement- dilated cardiomyopathy- can also occur in carriers, and may or may not be accompanied by muscle weakness [15, 16, 17].

Patients and methods:

The study was conducted in Al-Kadhimiya teaching hospital section of neurology from October 2002 to December 2003. Where we received families with cases of DMD and BMD, after assessment of their sons and re-evaluation by senior neurologist, CPK testing, EMG and muscle biopsy, we managed to enroll 15 families with clear history and diagnosis of DMD or BMD, 13 families with DMD and 2 families with BMD. From these 15 families 20 mothers of affected sons with DMD or BMD were studied. We called them carrier's group. And for the purpose of comparison we randomly picked other group of 20 females with comparable ages who were mothers of normal sons and had no history of muscle diseases or systemic illness and no family history of muscular dystrophy. And all of them where instructed and informed about the aim of the study we called them control group. A signed consent was taken from all subjects in both groups for inclusion in the study.

Methods:

To each female in the 2 groups' full medical history, neurological examination including muscles strength assessment by manual muscle testing using the medical research council scale, and calf circumference had been measured using special anthropometrics tape adapted from (BSN-JOBST,inc.) also the height and weight had been measured and family pedigree details taken from each mother.

And from each female in the 2 groups, 3ml of venous blood was taken for CPK testing (upper reference limit 170 U/L) and measured according to the RANDOX Enzymatic Colorimetric Method, also to each female ECG taken and in case of any abnormality transthorasic cardiac Echo was done, to each of the female studied a conventional needle electrode EMG, for the Deltoid, Bicepse Brachii, Triceps, and Abducter pollisis brevis (for the upper limbs, and Vastus Medialis, Rectus Femoris, Gastrocnemeus, and Tibialis Anterior muscles, (for the lower limbs) using Medtronic keypoint EMG machine.

Statistical Analysis:

We used the student t test to compare means of calf circumference, weight, height, ages, CPK and mean value of amplitude and duration between carrier and control groups and we used the correlation regression for the relation

between CPK activity and the age of the carriers.

Results:

The Group of Carriers: From the 15 families (13 DMD and 2 BMD), 20 female carriers (17 DMD and 3 of BMD), by family pedigree analysis we found that 12 females (60%) are obligate (definite) carriers and 8 (40%) are of sporadic cases. Their ages ranged (28-60 years), average 36.65, SD 7.52, No one had significant symptoms of muscle weakness, apart from one (5%) who had G 4+ proximal weakness in the pelvic girdle muscle. 4 carriers (20%) had calf hypertrophy (calf circumference >15inches) and only 2 (10%) had rubbery or resilient calf muscle texture. Only one carrier (5%) had history of palpitation on moderate-severe exertion. And 3 carriers (15%) had ECG changes, 2 of them had R-wave in V1 and deep S-waves in V5-6. And only one had LVH and Echocardiographic changes of mildly dilated left ventricle, but with normal ejection fraction (normal systolic function**).** 11 female carrier (55%) had mildly elevated CPK activity above the upper reference limits of 170 U/L, 10 (58.82%) of DMD, and one (33.33%) of BMD.9 carriers (45%), all of them carriers of DMD (52.94%), had proximal myopathic EMG changes of low mean duration and amplitudes of MUAPs and >15% polyphasia as shown in table (1). **The Control Group:** 20 healthy mothers, their age's ranges (20-46 years), average 31.5 SD 8.15 they were age, height and weight matched. No one had calf enlargement (calf circumference. >15 inches). Their mean calf circumference was 14.35 inches range (13.5-15), SD 0.46, compared to 15.05 inches ranges (13.5-17.5), SD 1.05 in the carrier group, but there is no significant difference in calf circumference between the two groups. Only two mothers from the control group (10%) had borderline CPK of 172, 175 U/L. And their mean CPK was 97.9 ranges (36-175 U/L), SD 42.7 Compared to 200.9 U/L as a mean, average (88-303 U/L), SD 74.76 in the carriers' group. And there is significant change in CPK values between the two groups. Also we found a negative correlation between age of carriers and CPK activity. Only 10 females in the control group had done conventional EMG successfully with good cooperation while the others were reluctant to do the test and three of them were uncooperative during the procedure. While all mothers in the carrier group were cooperative during the procedure. The EMG findings were normal and devoid of abnormalities in the control group. While the carrier group 9 females (45%) had low mean duration, and amplitudes of the MUAPs with polyphasic potentials >15%. With no significant difference ($p>0.05$) was found between the carrier group and the control group. But we found a significant difference ($p <0.05$) in the amplitudes of Biceps Brachii, and Vastus Medialis muscles, between the two groups. As shown in table (2) 9 of the carriers (45%) of the total (52.94%) of DMD showed > 15% polyphysia, 5 carriers (25%) only in the proximal upper limb muscles, 3 (15%) had polyphysia in both upper and lower proximal muscles and only 1 (5%) had polyphysia only in the proximal lower limb muscles.

Table (1): the characteristics of carriers

Characteristics of carriers	Carriers of DMD	Carriers of BMD	TOTAL NO.
Sign/symptoms of muscle weakness	1 (5.88%)	0	1 (5%)
ECG changes	3 (17.64%)	0	3 (15%)
Dilated LV	1 (5.88%)	0	1 (5%)
Calf hypertrophy	3 (17.64%)	1 (33.33%)	4 (20%)
Resilient calf	2(11.76%)	0	2 (10%)
CPK> 170 u/l	10 (58.82%)	1 (33.33%)	11 (55%)
Myopathic EMG	9 (52.94%)	0	9 (45%)

Table (2): the difference in the mean duration and amplitude of the MUAPs between carriers and control groups.

Parameter	Muscle	Carriers Mean±SD	Control Mean±SD	P Value
Duration (msec)	Deltoid	8.8±2.3	10.8±0.42	NS
	Biceps	9.12±1.92	10.63±0.38	NS
	Triceps	10.76±1.65	11.94±0.34	NS
	Vastus Med.	9.43±1.8	10.8±0.4	NS
	Gastro.	10.22±0.33	9.98±0.35	NS
Amplitude (mv)	Deltoid	0.529±0.285	0.98±0.113	NS
	Biceps	0.598±0.31	0.975±0.135	S
	Triceps	0.69±0.3	1±0.12	NS
	Vastus Med.	0.68±0.26	1.075±0.17	S
	Gastro.	1.12±0.17	0.945±0.36	NS

NS (not significant) $p>0.05$
S (significant) $p<0.05$

Discussion: In this study we tried to include as many carriers within each family as possible to increase the total number of definite carriers to keep ascertainment bias to a minimum. As four of the carriers came with their sisters who also had affected sons with DMD, and one carrier of BMD came with her daughter who also had an affected son. In our 20 female carriers 12 had definite X-linked pattern by pedigree analysis, and 8 mothers had only one or more affected sons (sporadic cases), in the past they were considered as probable carriers but new techniques to identify carrier status suggest that a

significant proportion of apparently sporadic cases are in fact the offspring of previously unrecognized carriers [1]. So from the 15 pedigrees 8 pedigrees with sporadic cases and 7 pedigrees with familial cases, as it has long been apparent that isolated cases of DMD are very common; Duchenne, himself, failed to recognize the familial nature of the disease in his original ten patients [3]. The question here is whether the female carriers of sporadic case is a true carrier of X-linked recessive or autosomal recessive dystrophy, and in our study all the families taken lacking female affections which excludes this possibility. In this study the cases of DMD were assessed by clinical examination, CPK testing, EMG and Muscle biopsy and we found that all the cases had early involvement around the age of 2-3 years with evident course of progressive muscle weakness and calf hypertrophy, elevated CPK and other query cases were excluded from the study. only one carrier had mild muscle weakness (5%).

the explanation of this low figure, might be due to the relatively small sample , and also we depend on the manual muscle power testing which has the drawback of being highly subjective. While in the Hoogerwaard, et al study they disclose more carriers with muscle weakness by the application of hand held dynamometry [11].

As there is no significant difference in calf circumference between the group of carriers and the control group but still there is relatively high measurements >15 inches in 4 (20%) which still can be a relatively high figure while only two carriers (10%) had rubbery calf muscle texture and this can be explained due to the subcutaneous fat in females which can give a false impression of soft texture. No carrier had cardiac symptoms apart of one who had palpitation on severe exertion, and only three (15%) had ECG changes 2 had R-waves in V1 and relatively deep S in V5-V6 and one had evidence of LVH, but cardiac Echo were completely normal apart from mildly dilated left ventricle but with normal systolic function, this mild cardiac involvement might explained by skewed X-inactivation which is tissue specific.

CPK was in the past the best method for carrier detection, but the results can be difficult to interpret in ethnic and racial groups with normally elevated CPK levels e.g. blacks have a higher reference range than whites; CPK levels of blacks may exceed the laboratory-stated normal limits without the presence of any pathology [3,2], so in our study we take a randomly selected sample of mothers who had normal children and have no history of muscular dystrophy nor of muscle weakness or any systemic illness , for purpose of comparison . As our female sample all are Iraqi, Arabic Muslims, and we only found that the CPK activity in the control group was borderline only in two females (172, 175 U/L) which might be regarded as trivial, but also refer to that it is a non specific test. And many factors affect the results and may give high readings, so we take into consideration these factors like strenuous exercise or muscle trauma, Even I.M injection or EMG needle (so the blood sample should be taken before the EMG examination) also any history of taking drugs that raise the CPK, should be sought in the history (like statins e.g.) Also haemolysed serum will give falsely elevated results.

In our study we found that the CPK, activity was mildly elevated in 11 (55%) of the total carriers, and 10 (58.8%) of carriers of DMD,While it is elevated in only one (33.33%) carrier of BMD. And these findings correlate with the Hoogerwaard et al study [11], also we found that the results of CPK, were statistically significant.

We found that there is a negative correlation between the age and CPK

activity of carriers, which support the opinion that the detection rate of CPK for carriers is higher in childhood than in adults [3] due to the possibility of selective loss of dystrophin-negative fibers with advancing age [13].

The quantitative MUAP analysis was carried out as described by Buchthal [18], in all subjects in the two groups, where we found there is a relatively low mean duration and amplitude, and >15% polyphasia, in the proximal muscles in 9 carriers (45%) in total, while it is (52.94%) in carriers of DMD, while it is normal in the control group and also in the carriers of BMD, which might be explained by the mild involvement in BMD. There is no significant difference in the mean durations and amplitudes between the two groups, but the mean amplitudes of Biceps Brachii, and Vastus Medialis muscles were found to be of significance.

Also we found that polyphasia >15% was found more frequently in the proximal upper limbs muscles (Deltoids, Biceps Brachii) it is found in 8 carriers (40%) , but present in 5 carriers (25%) in the Triceps ,but it was only found in 3 carriers in the Vastus muscle, which is against of what is known of the early and more involvement of the pelvic-girdle muscles in carriers of DMD, but it agrees with the Hoogerwaard et al[11] which showed more involvement of weakness of the shoulder girdle muscles. The EMG had the drawback of being relatively an invasive procedure, and requires patients cooperation, and was difficult in children, so our patients all are adults, and only 10 females from the control group had done the procedure with good cooperation and 7 refused the test and 3 were uncooperative so we consider only 10, while the carrier group all the mothers had good cooperation with the test, which might be that they felt commitment to their affected sons.

Conclusion: As the CPK and EMG testings are simple, non costly and readily available tests, and as they can be positive to some extent in a proportions of carriers so they can be performed on all possible carriers in the families of DMD & BMD as a simple screening test, especially the CPK, better to perform at an earlier age, and the EMG at an older age because it requires cooperation.

This has a significant impact on genetic counseling, aiming at preventing the spread of this bleak disease.

References:

1-David Marsden.,Timothy Fowler J, clinical Neurology, 2nd ed. A rnold, alaondon ; 1998: 110-112.

2-David Altman J, James Gilchrist M. Dystrophinophthies, e-medicine.com Internet: 2002.

3-Lewis Rowland P.,Robert layzer B: X-lnked muscular dystrophies. In: Vinken PJ&Bruyn GW (eds.) Handbook of clinical Neurology, Vol.40 Amsterdam, North Holland Publishing Company, 1979: 349-414.

4-Raymond D Adams, Maurice Victor, and Allan H. Ropper: Principles of Neurology, NY, McGraw-Hill, 2001:1593-1511.

5-Penn AS, Lisak RP, Rowland LP. Muscular dystrophy in Young girls. Neurology 1970; 20: 147-59.

6-Moser H, Emery AEH. The manifesting carrier in Duchenne muscular dystrophy. Clin Genet 1974; 5:271- 84

7-Sewry CA, Sansome A, et al. Manifesting carriers of Xp21 muscular dystrophy ; lack of correlation between Dystrophin expression and clinical weakness. Neuro muscular Disorders 1993; 3: 141-48.

8-Norman A, Haper P. A survey of manifesting carriers of Duchenne and Becker muscular dystrophy in Wales. Clin Genet 1989; 36:31-37.

9-Yoshioka M. clinically manifesting carriers in DMD. Clin Genet 1981; 20: 6-12.

10- Ianasescu VV, Searby CC, Ionasescu R. Manifesting carrier of Becker muscular dystrophy. Clinical and recombinant DNA studies. Acta Neurol Scand 1989; 79 :500- 03

11- Hoogerwaard EM, Bakker E, lppel PE, Oosterwijk JC, Majoor-Krakauer DF, Leschot NJ, et al. Signs and Symptoms of DMD and BMD among carriers in the Netherlands: A cohort study. Lancet 1999; 353:2116

12- Rosalki,SB. Serum enzymes in disease of skeletal muscle. Clin Lab Med 1989; 9: 767

13- Uptodate 9.2 Medical CD, metabolic and inherited disoders, DMD&BMD: 2001.

14- Perloff JK, Roberts WC, Deleon AC, O'Dcherty D. The distinctive electrocardiogram of Duchenne's progressive musclar dystrophy. Am J Med 1967: 179-88.
15- Comili, NigroG, Politano L, Petretta VR. The cardiomyopathy of Duchenne/ Becker consultants. Int J Cardiol 1992; 34: 297-305.
16- Mirabella M, Sevidei S, Manfredi G, et al. Cardiomyopathy may be the only clinical manifestation in female carriers of DMD Neurology 1993; 43: 2342-45.
17- Politano L, Nigro V, Nigro G, et al. Development of cardiomyopathy in female
18- Carriers of Duchenne and Becker muscular dystrophy. JAMA 1996; 275:1335-38
19- Buchthal, F; Electromyography in the evaluation of muscle disease J.Methods in clinical neurophysiology. 1991; 2:25-45.

Association between serum Copper, Oxidized HDL and Glycemic control in patients with type 2 Diabetes Mellitus in relation to Microalbuminuria

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Abstract

Background: diabetes mellitus (DM) is associated with a markedly increased mortality rate from cardiovascular and renal disease, not explainable by traditional risk factors. Although data are not yet conclusive, oxidative stress, dyslipidemia, glycemic control and possibly lipid peroxidation has been increasingly implicated in the pathogenesis of diabetic micro- and macrovascular disease. Little is known, however, about the role of copper in type 2 diabetes.

Aim: The present study includes measurement of free radical activity marker (lipid peroxides expressed as malondialdehyde MDA) along with the serum and urine copper, serum lipid profile, glycated haemoglobin (HbA1c) in addition to urinary protien : creatinine ratio in 55 patients with type 2 DM (T2DM) .

Results: The patients were divided according to the spot urine albumin excretion (urinary albumin ug / mg creatinine ratio) into two groups:- microalbuminurics & normoalbuminurics.

The results were compared with those obtained from 37 age-matched apparently healthy control subjects.

There was a significant elevation in serum malondialdehyde MDA , the percentage of oxidized non high-density lipoprotien (ox. non-HDL%) and serum copper with a significant reduction in the percentage of oxidized high-density lipoprotien (ox. HDL%) in the diabetic patients (particulary in the microalbuminurics) as compared with the control subjects. Serum MDA was significantly and positively correlated with serum copper in microalbuminurics and HbA1c% in both diabetic groups.

LDL size index was significantly increased in microalbuminuric T2DM patients as compared to the controls and normoalbuminurics indicating smaller LDL size in the diabetics in general and in microalbuminuric in particular.

Conclusion: the results of present study suggest an increase in free radical activity , dyslipidaemia and serum copper level favoring atherosclerotic state more in poor glycemic control in type 2 DM particularly in microalbuminurics.

The suggested mechanisms underlying these events are discussed.

Key words: Copper, lipid peroxides, diabetes mellitus., Microalbuminuria.

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Introduction:

Approximately 150 million people worldwide suffer from type 2 diabetes and it has been predicted that this number will double within the next 15 years [(1)], and its incidence is rising in developed and developing countries [(2, 3)].

The prevalence of type 2 DM is growing at an exponential rate [(4)]. T2DM is characterized by insulin resistance coupled with an inability of the pancreas to sufficiently compensate by increasing insulin secretion, with onset generally in middle or old age.

Diabetic nephropathy is the most common cause of renal failure in the Western World [(5)].

Several risk factors have been related to the development of diabetic nephropathy (DN) in type 2 diabetic patients, such as hyperglycemia,

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arterial hypertension, dyslipidemia, and smoking [6,7,8]. Microalbuminuria is a strong predictor of diabetic nephropathy and cardiovascular disease in both type 1 and type 2 diabetes mellitus [9].

The present study will go further to relate oxidative stress events with microaluminuria of type 2 DM.

Material & methods:

A-Subjects:

The study group comprised 55 patients (24 males and 31 females) with type 2 diabetes mellitus (mean age 51.6 +/- 8.1 years) diagnosed according to the WHO definition[10] .The patients were divided into two groups: microalbuminuric (group 1), n = 31 and normoalbuminuric (group 2), n = 24. All patients were recruited from the outpatient Diabetes clinic of the AL-kadhymia Teaching Hospital during the study period from 1 September 2004 to 30 March 2005, .

The main exclusion criteria included any recent illness, impaired thyroid or renal function , diagnosis of renal disease, treatment with estrogen or glucocorticoides, or other drugs except oral hypoglycemic and /or beta blocker antihypertensive drugs & pregnant women, All patients included in the study were nonsmokers; none was taking antioxidant supplements or drugs with known antioxidant activity, The mean duration of diabetes was (7.96 +/- 3.45 years) .

The control group consisted of 37 healthy, sex- and age-matched subjects (48.92 +/- 8.9 years) They were all volunteers recruited from different places and from the staff of the medical college of AL-Nahrain University.

B-Blood samples

About 10 milliliters of venous blood were collected from each subject of the study after a 12- hour fast. Two milliliters of the blood were collected in EDTA containing tubes and sent to the hospital Laboratory for HbA1c assay. The rest was collected in plain plastic tubes; which was centrifuged at 3000 rpm for 7 min within about 30 minutes from the time of collection. The serum was used for subsequent measurement of creatinine, lipid profile (total cholesterol, HDLc, TG), total MDA Level, Oxidized HDLc and copper concentration.

Urine samples:

Random morning urine specimen was obtained from each subject in the study, to quantify albuminuria (albumin-to-creatinine ratio) & creatinine.

Methods:

Serum total MDA was measured by the thiobarbiturate method [11] while serum lipids (Tc, HDL-c and TG) were measured by enzymatic methods using kits from bioMeriux, France.

Serum LDL-c was calculated by Friedewald fomula [12].

Serum oxidized HDL was measured by precipitation of all lipoproteins, except HDL-c, which was measured by phospho-tungstic acid – MgCl2 reagent. The supernatant was used for estimation of oxidized HDL by the same method used for the measurement of total MDA.

Serum copper was measured by flame atomic absorption spectrophotometer after 1: 10 dilution with de-ionized water,

Urine albumin was measured by staining with Ponceau S dye following the method of (Pesce and Strande 1973) [13] and urine creatinine by alkaline picrate kinetic method [14],

Glycated haemoglobin was measured by Variant^{TM} HbA1c ^{program} .[15].

Diabetic patients (n = 5٥) were divided according to urine albumin excreation measuered in ug per mg creatinine (Table 1) into:-

1. patients with albumin-creatinine ratio of 30 - 299 µg/mg were considered microalbuminurics (n = 31)
2. patients with albumin excretion less than 30 µg albumin per mg creatinine were considered normoalbuminurics (n = 24)

Lipid peroxides presented as total MDA and oxidized HDL were measured, then the value of oxidized non-HDL was obtained by subtraction (Total MDA - oxidized HDL = oxidized non-HDL.) as in table (2).

Serum MDA was significantly elevated in the microalbuminuric diabetic patients compared with the normoalbuminuric patients (P = 0.004) and control subjects ($p < 10^{-10}$). MDA was also significantly higher in the normoalbuminuric patients than the control subjects ($P = 6. 10^{-7}$), as shown in table (2).

Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), atherogenic index, AI, (expressed as LDLc / HDLc) and LDL size index (expressed as TG / HDLc) were measured in all groups studied, (table 3).

As expected from the results of serum LDLc and HDLc, both diabetic groups have increased (AI) as compared with the control subjects (table 4). Furthermore, microalbuminurics had significantly higher atherogenic index than the normoalbuminuric diabetics ($P < 0.05$) both diabetic groups showed a significant increase in the LDL size index when compared with controls, Indicating smaller LDL in patient's groups table (4).

Serum copper was higher in the diabetics than the controls being significantly higher in the microalbuminurics than the normoalbuminurics as shown in table (4).

There was a significant positive correlation between serum copper and each of total MDA level and the urine albumin / cratinine in the microalbuminurics only. (Fig 1 & 2)

Discussion:

Most published studies have found increased Lipid Peroxides in T2DM patients [16, 17].

Serum MDA was significantly higher in the microalbuminuric than normoalbuminuric patients. These results confirm earlier reports [18, 19]. A positive correlation between albumin excretion and plasma Thiobarbiturate reactive substance levels was also found [20]

However, not all instances of diabetes result in elevated oxidation. For example, a lower TBA reactivity in tissues of rats with alloxan - induced diabetes [21] and similar levels of MDA in micro-or normoalbuminuric type 1 DM patients were found by others [22, 23]. The suggested biochemical mechanisms for increased lipid peroxide in DM patients are [24] :

- Increased non-esterified fatty acids from increased lipolysis result in an increase in MDA.
- Peroxidative damage of membrane lipids.
- Lipids are more readily oxidized in the presence of increased glucose concentrations.
- Reactive oxygen species (ROS) can also be generated within the kidney by macrophages and polymorphonuclear leucocytes. In inflammatory cells, different sources of ROS have been suggested.
- Transition metals (copper and iron) catalytically activate the oxidation of polyunsaturated fatty acids. An enhancement in plasma transition metal concentration has been noted in diabetic animal models and in

diabetic patients showing complications [25].

The higher atherogenic index (AI) and the presence of smaller LDL particle size in the diabetics were reported to associate the increase in their oxidation susceptibility which is consistent with the present results and agree with previous reports [26-28]. This is further aggravated by the poor glycemic control indicated by the higher HbA1C level.

In another study poor glycemic control correlated significantly with micro- and macroalbuminuria in type 2 DM patients [29]. It may be concluded that poor glycemic control may be considered as a risk factor for the progression from normo- to microprotienuria in type 2 DM [30].

The higher serum copper in diabetics, particularly in the microabuminurics, is thought to be another event of oxidative stress (fig.1).

Serum Cu level was reported to be affected by renal excretion and kidney disease which is one of the major complications of diabetes [31]. In the present study Serum copper correlated positively and significantly with urinary protein excretion in microalbuminurics (fig. 2)

Hypothetically glycated proteins bind transition metals such as copper and iron, and that such `glycochelates' accumulate within the vasculature in diabetes and catalytically inactivate endothelial derived relaxation factor (EDRF) [32].
In the presence of available cellular reductants, copper in low molecular weight forms may play a catalytic role in the initiation of free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates, resulting in wide-ranging impairment in cellular function and integrity [33].

Table (1) displays the clinical characteristics of the study subjects:

	Microalbuminuric	Normoalbuminuric	Controls
Number	31	24	37
Male/female	11 / 20	13 / 11	15 / 22
Age (years) (NS)	49.5 ± 7.6	52.2 ± 8.2	48.9 ± 8.9
Hemoglobin A1C %	8.11 ± 1.16*****	7.68 ± 0.9*****	4.87 ± 1.0
FBG(mmol/L)	8.9 ± 2..4 *	6.3 ± 1.1	5.1 ± 0.3
* P < 0.001, versus the control subjects Mean values are shown, with standard deviations (S.D.)			

Table (2*):* Lipid peroxidation and its fractions percentages in the two diabetic groups (1: microalbuminuric, 2: normoalbuminuric) and control group as Mean ± SD

Groups	S.MDA µmo/L	OX. HDL %	OX. non-HDL %
Group (1) T2DM	0.963 *† ± 0.1	53.9%**‡ ± 15.3	46.1%**‡ ± 15.3
Group (2) T2DM	0.833* ± 0.18	72.0%** ± 11.6	27.97*% ± 11.6
test P-value	0.03	0.0175	0.013
Controls	0.580 ± 0.124	75.5% ± 16.0	24.5% ± 16.0

Student t-test was done between each diabetic group and control (for $p < 0.05$, ** for $p < 0.01$) †Student t-test was done between microalbuminuric and Normoalbuminuric diabetes patients († for $p < 0.05$, ‡ for $p < 0.01$). F test was done between macroalbuminurics and Normoalbuminurics

Table (3): Serum Lipid Profile (mean ± SD) in mmol / L in the diabetic and Control groups.

Groups	*Microalbuminurics*	*normoalbuminurics*	*F test*	*Controls*
T.C	*5.61 ± 1.0 ** *	*5.29 ± 0.78*	*0.04*	*4.40±0.58*
TG .	*1.93 ± 0.2 ** *	*1.63 ± 0.4 ** *	*12 x10^{-5}*	*1.28 ± 0.4*
HDLc	*1.1 ± 0.2 ** *	*1.12 ± 0.18 ** *	*0.81*	*1.48 ± 0.2*
LDLc	*3.6 ± 1.1 * *	*3.46 ± 0.8 ** *	*0.037*	*2.34±0.55*

- **Student t-test was done between each diabetic group and control $p < 0.001$
- F test (one way ANOVA) was done between Microalbuminuric and Normoalbuminuric diabetic patients

Table (4): Serum MDA,copper (Cu), AI(LDL.c/HDL.c), LDL.c size index (TG/HDL.c) and glycated Hb % in the diabetic groups and their controls (mean +/- SD)

Type 2 DM	Hba1c %	MDA µmol/L	S. Cu µmol/L	AI *(LDLc/HDLc)*	LDL.c size index *(TG/HDLc)*
Microalbuminurics (n =31)	8.1* +/- (1.2)	0.96*† +/- (0.1)	27.7**†† +/- (6.2)	3.39** +/- (1.3)	1.79**† +/- (0.42)
Normoalbuminurics (n =24)	7.68* +/- (0.9)	0.833* +/- (0.2)	20.3 +/- (2.9)	3.178** +/- (1.1)	1.49** +/- (0.5)
Controls (n = 37)	4.9 +/- (1.0)	0.580 +/- (0.1)	18.97 +/- (4.4)	1.625 +/- (0.560)	0.9 +/- (0.3)

*p<0.05 versus controls †P<0.05 group (1) versus group (2)
**P<0.001 versus controls ††P<0.001 group (1) versus group (2)

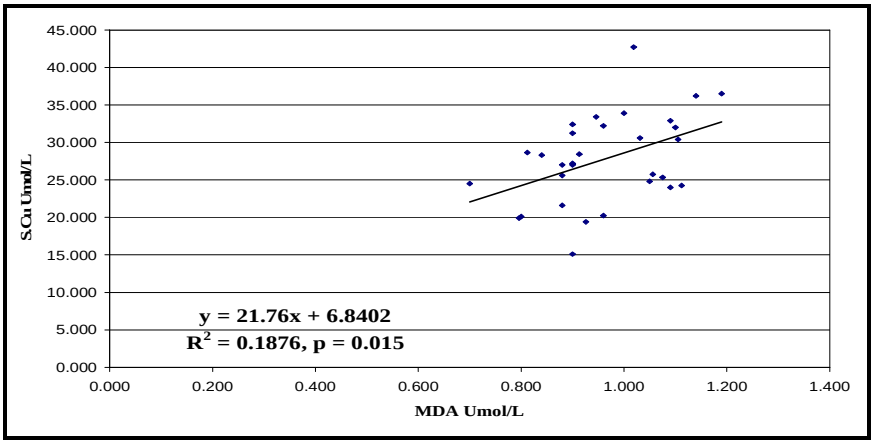

Fig. (1) Correlation between serum copper and total MDA in microalbuminuric type 2 diabetic patients

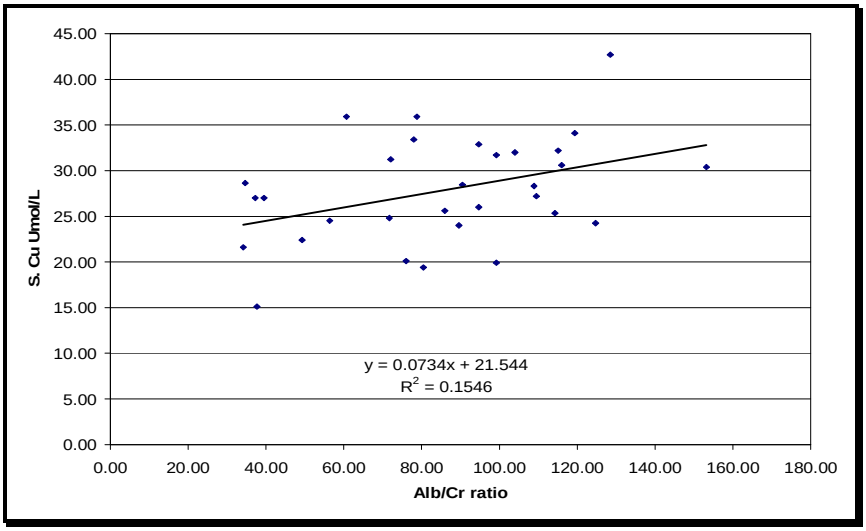

Fig (2) Correlation between serum copper and urine albumin / creatinine ratio in microalbuminuric Type2 diabetic patients.

References:

1- Zimmet P**,** Alberti KGMM, & Shaw J. Global and societal implications of the diabetic epidemic. Nature 2001; 414: 782–7

2- Foster Daniel W. Diabetes mellitus, Harrison's principles of internal medicine, 14th ed. 1998; 2060-2080.

3- Burke JP**,** William K., Gaskill SP et al. Rid rise in the incidence of type 2 diabetes from 1987 to 1996: results from the San Antonio heart study. Arch Intern. Med. 1999; 159 (13): 1450-6.

4- Ludwig, D.S. and Ebbeling, C.B. Type 2 diabetes mellitus in children: primary care and public health considerations. *JAMA*, 2001; 286: 1427-1430.

5-Canadian Organ Replacement Registry (CORR). Annual Report. Ottawa, ON, Canada: Canadian Institute for Health Information; 2001

6-Forsblom CM, Groop P-H, Ekstrand A, et al. Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. Diabetes Care, 1998; 21:1932-1938.

7-Ravid M**,** Brosh D, Ravid-Safran D, Rachmani R et.al.; Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch Intern Med 1998; 158:998-1004.

8-Park JY**,** Kim HK, Chung YE, Kim SW, et al. Incidence and determinants of microalbuminuria in Koreans with type 2 diabetes. Diabetes Care 1998; 21: 530-534.

9-Almdal T**,** Norgaard K, Feldt-Rasmussen B &, Deckert T: The predictive value of microalbuminuria in IDDM. A five-year follow-up study. Diabetes Care.1994; 17: 120-125,

10- Alberti, KG. and Zimmet, PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine*.1998*;* 15, 539-553.

11-Stocks J & Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxides, Brit. J. Haematol 1971; 20: 95 -111

12- Friedwald WT & Levy RI , Estimation of the concentration of LDLc in plasma without the use of preparative ultracentrifuge. Clin.Chem. 1972, 18: 499.

13- Pesce MA & Strande CS. A new micro method for determination of protein in cerebrospinal fluid and urine. Clin. Chem. 1973, 19: 1265-1267.

14- Bartels H, et.al. Clin. Chem. Acta 1972; 37: 193 -197 cited from bioMerieux of France.

15-Rohfing CL. Use of HbA1c in screening for undiagnosed diabetes in the USA population, Diabetes care 2000; 23: 187 -191.

16- Akkus I**,** Kalak S, Vural H, Caglayan O, et.al. Leukocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum and leukocyte vitamin C levels of patients with type II diabetes mellitus. *Clinica Chimica Acta*.1996*; 244*: 221-227.

17-Armstrong AM, Chestnutt JE, Gormley MJ and Young IS. The effect of dietary treatment on lipid peroxidation, and antioxidant status in newly diagnosed noninsulin dependent

diabetes. *Free Radical Biology and Medicine*, 1996; 21, 719-726.

18- Griesmacher A**,** Kindhauser M, Andert, SE, Schreiner W, et al. Enhanced serum levels of thiobarbituric acid-reactive substances in diabetes mellitus. *American Journal of Medicine*, 1995; 98**(5),** 469- 475

19- Collier A**,** Rumley A, Rumley AG, Paterson J.R, et al. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria, Diabetes 1992; 41: 909-913.

20- Knobl P, Schnack G, Pietschmann P , et al .Thermogenic factors are related to urinary albumin excretion rate in type (insulin dependent) and type 2 (non insulin dependent) diabetic patients , *Diabetologia* 1993; 36 : 1045 -1050 .

21- Parinandi NL, Thompson EW & Schmid HHO. Diabetic heart and kidney exhibit increased resistance to lipid peroxidation . *Biochem Biophys Acta* , 1990 ,1047 :63 -69.

22- Leonard, M.B.**,** Lawton, K., Watson, I.D., Patrick, A., Walker, A. and MacFarlane, I. Cigarette smoking and free radical activity in young adults with insulin-dependent diabetes. *Diabetic Medicine* 1995; 12**:** 46-50.

23- Yaqoob M**,** McClelland P, Patrick AW, Stevenson, A, et.al. Evidence of oxidant injury and tubular damage in early diabetic nephropathy. *The Quarterly Journal of Medicine* 1994*;* 87**,** 601-607.

24- Martinez-Cayuela M. Oxygen free radicals and human disease. *Biochimie* 1995; 77: 147–161

25-Trachtman H, Futterweit S, Maesaka J *et al.* Taurine ameliorates chronic streptozocin-induced diabetic nephropathy in rats. *Am J Physiol* 1995; 269: F429–F438

26-Guerci B, Antebi H, Meyer L, Durlach V, et al. Increased ability of LDL from normolipidemic Type 2 diabetic women to generate peroxides. *Clin Chem* 1999; 45:1439-1448.

27- Rabini RA, Fumelli P, Galassi R, Dousset N, et al. Increased susceptibility to lipid oxidation of low-density lipoproteins and erythrocyte membranes from diabetic patients. *Metabolism* 1994; 43**,** 1470-1474

28- Siegel RD, Cupples A, Schaefer EJ, & Wilson PWP. Lipoproteins, apoproteins, and low density lipoprotein size among diabetics in the Framingham Offspring Study Metabolism 1996; 45:1267-1272.

29-Savage S, Nagel NJ, Estacio RO, Lukken N et al. Clinical factors associated with urinary albumin excretion in type II diabetes Am J Kidney Dis. 1995; 25(6), 836-844.

30- Klein R, Klein BEK & Moss SE. Prevalence of microalbuminuria in older-onset diabetes. Diabetes Care, 1993; 16:1325–1330,

31- Al-Shamma GA, Al-Timimi DJ, Al-Ghabban SS & Al-Shamma IA. Changes in serum zinc, copper and magnesium during the nephrotic syndrome. A possible role for copper in hyperlipidemia., Fac Med Baghdad. 1991; 33(3): 345-352

32-Eaton JW, & Qian M**.** Interactions of copper with glycated proteins: possible involvement in the etiology of diabetic neuropathy –Abstracts Alternative Medicine Review, Oct, 2002

33-Britton RS**.** Metal-induced hepatotoxicity. Sem Liv Dis .1996; 16:3-12.

HLA-Class II Risk Alleles Control T-Lymphocyte Proliferation in Response to Enterovirus and Adenovirus Antigens and IgG Antibody Prevalence in Newly Diagnosed T1DM Children

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Abstract

Background: Viral infections are implicated in the pathogenesis of type 1 diabetes mellitus (T1DM) in a number of studies, and playing a role in the initiation of beta-cell damaging process.

Objective: To evaluate the T- cell proliferation in response to enterovirus antigens including coxsackievirus B and poliovirus in addition to adenovirus in an HLA- matched population of children with T1DM and children who were healthy, in addition to screening for specific anti-viral IgG antibodies.

Subjects and methods: A total of 60 Iraqi T1DM children were included in the presents study. They were newly diagnosed diabetics. For the purpose of comparisons, 50 apparently healthy children were selected. HLA typing was measured by microlymphocytotoxicity, while MTT assay was used for lymphocyte proliferation by culturing peripheral blood lymphocytes (PBLs) with Con-A, Coxsackievirus B_5 (CVB5), Adenovirus 3, 4, and 7 serotypes, and Poliovaccine. Serum IgG against these viruses were detected quantitatively with an indirect ELISA.

Results & conclusion: No significant differences were shown in the PBL proliferative percentage in response to Con-A mitogen and tested viruses (CVB_5 and adenovirus) between T1DM and healthy controls, but PBL proliferative percentage of patients showed a significant decline in response to poliovaccine. Strong T-cell proliferation in response to the tested viral antigens were observed and was related to HLA-DR4 and HLA-DQ3 antigens, whereas the HLA-DR3 and HLA-DQ2 alleles were associated with week responsiveness to the same antigens. High significant mean proliferative percentage for all tested viruses were detected in those patients who were sero-positive IgG as compared to the sero-negative IgG diabetic children.

Conclusion: In children with new- onset diabetes, responses were generally decreased, but higher in children who carried risk HLA- class II alleles and who were sero positive to anti- viral IgG antibodies.

Key Words: T1DM, HLA class II alleles, Lymphocyte proliferation, Anti- CVB5 IgG, Anti- polio IgG, Anti-adeno IgG.

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Introduction:

Epidemiological studies indicate that autoimmune diseases such as type 1diabetes mellitus (T1DM) have a strong environmental component to their pathogenesis [1,2]. There is a considerable body of evidence suggesting that involvement of several groups of viruses, but particularly those of the enterovirus genus, in the development and / or acceleration of T1DM [3]. Coxsackie virus B4 (CVB4) - specific IgM responses are more common in newly diagnosed subjects with T1DM than in healthy control subjects [4]. The finding of viral RNA in circulation at the onset of the disease have further support the role of enteroviruses [5].

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Viral infection like other environmental risk factors can probably induce β-cell damaging processes only in individuals with genetic T1DM susceptibility. The most important risk genes are located within the HLA gene complex, where HLA-DQ alleles associated with increased susceptibility to or protection against T1DM can be defined [6]. Enterovirus (EV) infections possibly occur predominantly in individuals with the DQA1*0501. DQB1*02 haplotype, who usually are also positive for HLA-B8 and HLA-DR3 alleles [7, 8]. HLA may also influence immune responses to EV antigens in comparison occurs between patients and control individuals [9].

Few studies have focused on T- cell / virus interaction. In the present study T-cell proliferation in response to enterovirus antigens including coxsackie virus B and poliovirus in addition to adenovirus was analyzed in an HLA-matched population of children with T1DM and children who were healthy, and whether HLA alleles modified the cellular immune responses to the viral antigens.

Subjects, Materials and Methods

Subjects:

Sixty Iraqi T1DM children were subjected to this study. The patients were attending the National Diabetes Center at Al-Mustansiriya University during the period May 2004 - October 2005. Their ages ranged from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). For the diagnosis of Diabetes Mellitus, the criteria as listed in the Expert Committee of Diagnosis and Classification of Diabetes Mellitus, 2003 was used. All the patients were treated with daily replacement doses of insulin at the time of blood sampling. Fifty healthy children were selected, who have no history or clinical evidence of type 1diabetes or any chronic diseases and obvious abnormalities as a control group. These children were compared with T1DM children and matched for sex, age (4-17), and HLA-DR and DQ risk alleles as represented in table -1 and 2. The patient and control subjects were divided into two groups according to their ages, equal or less than 10 years and more than 10 years old.

Collection of Blood Samples:

Ten milliliters of venous blood were collected from each subject. Eight milliliters of blood were put in heparinised test tube (10 U/ml) and used for lymphocyte separation for the detection of HLA polymorphism and lymphocyte proliferation. Heparinised blood was processed as soon as possible. The remaining blood was drawn into plain test tube and the serum was separated by centrifugation at 2500 rpm for 10 min., divided into aliquot and kept at-20°C until used.

HLA Typing: It was carried out by microlymphocytotoxicity assay as described by [11].

Table- 1: distribution of HLA-DR antigens in T1DM children and control groups.

Group	DR3/DR4	DR3	DR4	Others
T1DM (60)	25	7	5	23
Controls (50)	12	6	2	30

Table- 2: distribution of HLA-DQ antigens in T1DM children and control groups.

Groups	DQ2/DQ3	DQ3	DQ2	Others
T1DM (60)	9	15	11	25
Controls (50)	-	12	9	29

Lymphocyte proliferation using MTT assay:

Peripheral blood lymphocytes (PBLs) were isolated using Ficoll-isopaque gradient centrifugation **(**Flow-Laboratories, UK). The washed PBLs were resuspended in complete RPMI-1640 medium (Euroclone, UK) supplemented with 10% heat inactivated AB serum (National blood transfusion center); Hepes; crystalline penicillin (1,000,000 IU) and streptomycin (1gm)(Pharma-intersprl, Belgica), and the final lymphocyte concentration was adjusted to 1-2x10^6 cells / ml.

Triplicate incubations of 100 µl of cell suspension with antigen(s) in 96 flat-bottom microculture plates for 3 days at 37°C in a humidified 5% CO_2 incubator. Then 20 µl of 1-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, Germany) working solution was added to each culture well and the culture were incubated for further 4 hrs. The converted dye was solubilized by adding acidic isopropanol. The absorbancy was read using microculture plate reader using a wave length of 570 nm [12].

The antigens were:

CVB5 antigen solution (1:5 dilution) (KBR-CF antigen Vero, France), poliovirus Trivalent Vaccine (1:5 dilution) (Polioral Trivalent; Chiron), and adenovirus type 3,4,7 solution (1:10 dilution) (KBR-CF antigen type 3, 4, 7, Vero). The final concentration or dilution for the three viral antigens was achieved according to the result of MTT serial dilution run of these antigens.

Con-A mitogen (100 µg/ml) was used as a mitogen positive control.

The percent of proliferative response of lymphocytes was calculated by the following formula:

$$\%\,\text{Proliferation} = \left[\frac{\text{Absorbancy of experimental wells}}{\text{Absorbancy of control wells}} - 1\right] x100$$

Virus antibodies:

IgG class antibody was measured against purified CVB5; adenovirus antigen (serotype 4, 5, 7) and poliovaccin using indirect ELISA method as described by [13, 14]. Sample value lie below the cutoff value (mean negative + 2 SD) were considered negative. Those who were equal or greater than cutoff value were considered positive [15].

Statistical analysis

Regarding of HLA and disease association the frequency distribution for selected variables was done. Student t-test was used to measure the differences between two means; the results were expressed as means ± standard error (SE). The single Factor ANOVA (F-test) was used in this study to find out whether the difference between more than two groups of samples is significant or not. Pearson Correlation (R), which measures to what degree the two variable observations are correlated to each other was employed in addition to Chi Square test.

Results

Lymphocyte Proliferation: This test was performed to study whether the different viral antigens have any association with the proposed cell mediated immune (CMI) activation or not after incubation

with peripheral blood lymphocytes (PBLs) of T1DM patients and healthy controls**.** The results of mean proliferative percentage in response to Con-A were represented in table (3). A similar mean lymphocyte proliferation percentage in response to Con-A mitogen was absorved among patients and control groups, but newly diagnosed T1DM patients tended to have a lower non significant proliferative percentage than control subjects ≤10 years old (83.33 vs. 85.93%, P_1=0.82) and in >10 years old group (86.04 vs. 92.7% , P_1= 0.62).

Role of Viral Antigens in Functional Activation of PBL:

Considering the response to different viral antigen, a lower mean proliferative percentage was seen among patients ≤10 years old in response to CVB_5 compared to controls (36.67 vs 49.16%) and among patients >10 years old than controls (38.87 vs 51.20%). Those differences failed to reach significant levels in both age groups (P_1=0.061, and 0.14 respectively), (Table - 4).

Significant decline of proliferative response against poliovaccine was seen in T1DM patients (34.44%) compared to controls (47.38%) (P_1= 0.045) in ≤10 years old group and >10 years old group (28.30 vs. 40.86%, P_1= 0.004) (Table -4).

A non significant (P_1= 0.82) proliferative percentage decline in response to adenovirus was observed in ≤10 years old patients (19.97%) compared to controls (20.67%) and also in patients >10 years old (23.02%) in comparison with controls (28.61%) (P_1=0.23).

No statistical differences appeared in the mean lymphocyte proliferative percentage between patients in both age groups against CVB_5 (P_2=0.57), poliovaccine (P_2=0.14) and Adenovirus (P_2=0.57).

Relation of HLA Class II Alleles with the PBL Proliferation Percentage in T1DM Patients:

At HLA-class II region, highly significant increased frequencies of DR3 (53.33 vs. 26.25%) and of DR4 (50.0 vs. 12.5%) were observed in the patients compared to controls ($P=9.7x10^{-3}$ and $1x10^{-5}$ respectively) (data was not shown). At HLA-DQ loci, two antigens DQ2 and DQ3 were significantly increased in the patients compared with controls (DQ2: 33.33 vs. 15.0%, P=0.009; DQ3: 40.0 vs. 20.0%, P=0.008).

To find out any relation between the HLA-class II risky alleles (genetic factors) and proliferative percentage of MTT (CMI level), ANOVA test was applied to compare the proliferative percentage in patients with HLA-DR risky alleles (DR3; DR4 and DR3/DR4) with those patients who had other alleles. The results represented in table -5 showed that the mean PBL proliferative percentage in response to different tested viral antigens was significantly higher in the patients with DR4, DR3 and DR3/DR4 serotypes compared with the children carrying other alleles. The significant levels scored P= 0.021 in response to CVB_5, P=0.031 in response to poliovaccine, and P= 0.041 in response to adenovirus. Moreover, the mean proliferative percentage was significantly higher in patients carrying DR4 allele than those patients with DR3 alleles in response to CVB_5 (62.67 vs. 43.32%, P=0.038), to poliovaccine (59.86 vs 38.40%, P=0.031) and to adenovirus (46.02 vs. 22.48%, P= 0.046).

Concerning the HLA-DQ risky alleles (DQ2, DQ3, DQ2/DQ3), our results presented in table -6 showed a significant increase of proliferative percentage in patients carrying different HLA-DQ risky alleles compared with the patients who lack these alleles. The

results scored as significant levels of P= 0.032 in response to CVB_5, P= 0.038 in response to poliovaccine, and (P= 0.042) in response to adenovirus (P= 0.042).

As detected in table -6, the proliferative percentages were significantly higher in patients with DQ3 alleles than in patients with DQ2 alleles in response to all tested viral antigens.

Anti-Viral IgG in T1DM Patients

Seropositivity against the 3 viral antigens was significantly higher in diabetics than controls. Only 12 patients out of 60 were sero-positive (20%) compared to 4 healthy individuals out of 50 (8%) who were sero-positive for anti-CVB_5 IgG. These differences were statistically significant (P=0.048). Nineteen patients (31.67%) were sero-positive for anti-polio-IgG compared to 13 (26%) healthy controls, and no difference appeared between both groups (P = 0.649), whereas only 4 patients were sero positive for anti-adeno IgG (6.67%) compared with the control group who were all sero-negative. This difference was not significant between the two groups.

Relation between Mean Lymphocyte Proliferation Percentage and Anti-Viral IgG in T1DM Patients

To detect any relation that can clarify if the PBLs were primed previously by the same viral antigen. The results represented in table -7 showed a significant increase of mean proliferative percentage in response to CVB_5 in the patients who were sero-positive for anti-CVB_5-IgG compared with the sero-negative patients (50.58 vs 22.99%) (P=0.048).

The mean proliferative percentage for sero-positive and sero negative anti-polio-IgG patients is illustrated in table -8. It was found that the patients who were sero-positive for anti-polio IgG had higher proliferative percentage reading in response to polio-vaccine (31.48%) than those patients who were sero-negative (20.61%) and these differences were significant (P=0.039).

The study also demonstrated increased mean proliferative percentage of PBLs in response to adenovirus in sero-positive anti-adeno IgG patients in comparison to sero-negative anti-adeno IgG patients (30.10 vs. 14.16%) and again these differences reach the significant level (P=0.042) (Table -9).

Moreover, the present findings also revealed a significant positive correlation between the PBL proliferative percentage in response to CVB_5 and anti-CVB_5-IgG (r =0.412). Strong negative correlation was also detected between proliferative percentage in response to adenovirus and anti-adeno-IgG (r =-0.635) while the correlation found with the anti-polio-IgG was weakly positive (r = 0.101).

Table- 3: t-test between controls and T1DM patient groups regarding comparison of MTT proliferation percentage in response to Con-A.

Mitogen	≤10 years					>10 years					P_2
	Groups	No.	Mean	SE	P_1	Groups	No.	Mean	SE	P_1	
Con-A	Controls	21	85.93	10.60	0.82 (NS)	Controls	29	92.70	10.2	0.62 (NS)	0.57 (NS)
	T1DM	36	83.33	5.60		T1DM	24	86.04	8.27		

Table- 4: Comparison of mean proliferation percentage of PBL between controls and T1DM patients in response to CVB_5, poliovaccine and adenovirus.

Viral antigens	≤10 years					>10 years					P_2
	Groups	No.	Mean	SE	P_1	Groups	No.	Mean	SE	P_1	
CVB_5	Controls	21	49.16	5.88	0.061 (NS)	Controls	29	51.20	5.97	0.14 (NS)	0.57 (NS)
	T1DM	36	36.67	3.08		T1DM	24	38.87	5.08		
Polio Vaccine	Controls	21	47.38	5.83	0.045 (S)	Controls	29	40.86	3.28	0.004 (S)	0.14 (NS)
	T1DM	36	34.44	2.79		T1DM	24	28.30	3.28		
Adeno-virus	Controls	21	20.67	2.24	0.82 (NS)	Controls	29	28.61	3.73	0.23 (NS)	0.35 (NS)
	T1DM	36	19.97	1.61		T1DM	24	23.02	3.27		

P_1: T1DM patients vs. control
P_2: T1DM patients ≤10 years vs. patients >10 years old.

Table- 5: Relation of mean lymphocyte proliferation percentage in response to different viral antigens with the HLA-DR risky alleles in T1DM patients.

Viruses	DR3/DR4 (n=25)	DR3 (n=7)	DR4 (n=5)	Others (n=23)	ANOVA F-test	P
CVB_5	40.37	43.32	62.27	29.73	8.585	0.021 (S)
Polio vaccine	34.42	38.4	59.86	25.27	7.689	0.031 (S)
Adenovirus	29.44	22.48	46.02	26.14	5.704	0.041 (S)

Table- 6: Relation of mean lymphocyte proliferation percentage in response to different viral antigens with the HLA-DQ risky alleles in T1DM patients.

Viruses	DQ2/DQ3 (n=9)	DQ3 (n=15)	DQ2 (n=11)	Others (n=25)	ANOVA F-test	P
CVB_5	42.84	60.90	26.41	33.63	7.975	0.032 (S)
Polio vaccine	39.31	48.09	23.21	27.29	6.695	0.038 (S)
Adenovirus	22.26	37.41	31.37	26.74	5.684	0.042 (S)

Table-7: Relation of mean PBL proliferative percentage in response to CVB_5 with the anti- CVB_5 IgG.

CVB_5		No.	Proliferation percentage	SE	P
Anti-CVB_5 IgG	+ve	12	50.58	10.09	0.048 (S)
	–ve	48	22.99	3.27	

t = 2.62

Table-8: Relation of mean PBL proliferative percentage in response to poliovaccine with the anti-polio IgG.

Poliovaccine		No.	Proliferation percentage	SE	P
Anti-Polio IgG	+ve	19	31.48	5.83	0.039 (S)
	–ve	41	20.61	2.92	

t = 3.85

Table-9: Relation of mean PBL proliferative percentage in response to adenovirus with the anti-adeno IgG.

Adenovirus		No.	Proliferation percentage	SE	P
Anti-Adeno IgG	+ve	4	30.10	6.45	0.042 (S)
	–ve	56	14.16	2.22	

t = 2.66

Discussion:

Functional Activity of PBL:

The use of lymphocyte proliferation technique is based on the capability of the lymphocytes for responding to an antigen (specific response), which has induced memory lymphocyte, either by vaccination or by natural infection. These lymphocytes, when they are repeatedly contacted with antigens, have a blastogenic transformation [16].

The proliferative percentage of PBLs has been found lower in T1DM patients than in healthy controls in response to Con-A. Considering the responses to viral antigens, proliferative responses against CVB_5 and adenovirus were tended to have a lower percentage in T1DM patients than controls, but these values were not statistically different, while the proliferative responses against poliovaccine were significantly lower in patients especially in >10 years old group than controls. The low proliferative responses against CVB_5 antigen at disease onset is in agreement with other studies showing reduced T-cell proliferation against CVB_4 [17], while the same investigators found in previous

study, no differences in T-cell proliferation against CVB_4-infected lysate between diabetic patients and healthy-non diabetic individuals [18]. Another report conducted by Juhela *et al.*, 2000 found that PBLs of the children at onset of T1DM had significant weaker responses to purified CVB_4 and non-significant decrease in response to poliovirus type 1 and 3 than healthy children, while the responses to adenoviruse did not differ between patients and controls. Temporary decline in T-cell responsiveness at diabetes onset has also described in GAD peptide that contains the homology region to the CVB_4 2C protein [20].

The results of these studies and the present study are subjected to several interpretations. One explanation is that, decreased responses of PBLs are due to redistribution of virus-specific T-cells, with virus-responder cells presumed to have homed to the pancreas and therefore unavailable for detection in peripheral blood [21], and so T-cell responses to various viral antigens may be suppressed at the onset of the disease. On the other hand, Varela-Calvino *et al.*, 2002 indicated abundance of circulating primed CVB_4 specific responder T-cells that secretes IFN-γ in T1DM patients with relative lack of proliferation. These finding have been related to two broadly defined phenotypes of memory T-cells characterized by [22]. Primed (memory) T-cells with the capacity to proliferate termed as "central memory" TCM cells, and lack immediate effector function and predominantly produce IL-2, the major T-cell growth factor to support proliferation and express CCR_7, a chemokine receptor, that direct homing to lymph nodes. In contrast the primed memory cell subsets that produce the proinflammatory cytokines IFN-γ during an immune response termed "effector memory" subset TEM, those cells do not express CCR_7, present in the circulation at sites of infection or tissue inflammation and release cytokines.

Relation of Lymphocyte Proliferation with HLA:

The present results indicated that stronger T-cell proliferation in response to CVB5, poliovaccine and adenovirus were related to HLA-DR4 allele and HLA-DQ3 allele; whereas the HLA-DR3 and HLA-DQ2 were associated with weak responsiveness to the same antigens. These results are in agreement with a report f Bruserud *et al.*, 1985 who found that DR4, which is in linkage disequilibrium with the HLA-DQB1*0302 allele, is associated with strong T-cell responses; whereas HLA-DR3 associated with HLA-DQB_1*02 allele associates with weak T-cell responses to enterovirus antigens. Another study conducted by Juhela *et al.*, 2000 demonstarted the same observation in T-cell responses to enterovirus antigens in T1DM patients.

Anti-Viral IgG

The present results described finding of IgG antibodies against CVB_5 to be more frequent (20%) in T1DM patients than in controls (8%). A low prevalence of specific CVB-IgG may be due to the use of only one CVB serotype (CVB_5) and there may be another CVB serotype in the sera of T1DM patients, which is not detected. The frequency of IgG antibodies against poliovirus (Oral sabin) was more (31.67%) in diabetic patients than in controls (26%). Also IgG antibodies against adenovirus were detected in only four diabetic children (6.67%).

The presences of CVB5, poliovirus and adenovirus specific IgG antibodies

are evidence of previous infection in T1DM children. This fact was confirmed by measuring the PBLs proliferative percentage in sero-positive IgG diabetic children *in vitro* in response to CVB5, poliovirus and adenovirus, and the results indicated a high significant mean proliferative percentage for all tested viruses in those patients as compared to the sero-negative IgG diabetic children. This means that PBLs of sero-positive IgG patients were boosted earlier either by natural infection or vaccination.

The low prevalence of anti-polio-IgG determined in healthy children may indicate a failure of poliovaccine to enhance the immune system, although these children presumely had taken many boosted doses of oral poliovaccine.

Several studies have found CVB-specific IgM antibodies to be more common in newly diagnosed children compared to healthy individuals (19,23). Others detected an increase of anti-enterovirus antibody levels (both IgM and IgG) preceding the appearance of signs of autoimmunity reflected either by synthesis of several autoantibodies or the development of clinical disease [24]. In contrast Tuvemo *et al.,* 1989 and Emekdas *et al.,* 1992 found no evidence of increased antibody frequencies against CVB1-6 serotypes at the onset of childhood diabetes. A lower antibody titer against CVB3-5 serotypes and adenovirus-7 were also demonstrated in newly diagnosed T1DM children than in healthy controls [27].

Enteroviruses could be involved in the pathogenesis of T1DM. During infection, viruses may reach the pancreatic islet and destroy insulin-producing β-cells by virus-induced cytolysis [28]. Alternatively, β-cell damage might result from virus-induced inflammatory reactions through producing inflammatory cytokines (IL-1β, IFN-α… etc.) [29], in addition β-cell destruction might be based on molecular mimicry, because immunological cross-reactions between enteroviruses and β-cell autoantigens (GAD-65, Tyrosin phosphatease IAR/IA3 can take place at least *in vitro* [14].

In conclusion, the present results show that T- cell proliferation in new onset Type 1 Diabetic children were decreased, but higher in children who carried risk HLA- class II alleles and who were sero positive to anti- viral IgG antibodies.

References:

1. Lönnort M, korpela K, Knip M, *et al.* Enterovirus infection as a risk factor for β-cell autoimmunity in a prospectively observed birth cohort. The Finnish diabetes prediction and prevention study. Diabetes. 2000a;49: 1314-1318.

2. Hyöty H, Hiltunen M, Knip M, *et al.* A prospective study of the role of Coxsackie B and other Enterovirus infections in the pathogenesis of IDDM. Diabetes. 1995; 44: 652-657.

3. Boic B. Diabetes and Autoimmunity.The Journal of International Federation of Clinical Chemistry (JIFCC). 2004; 13(5): 1-9.

4. Helfand RF, Gary HE Jr, Freeman CY, Anderson LJ and Pallansch MA. Serological evidence of an association between enteroviruses and the onset of type 1 diabetes mellitus: Pittsburgh Diabetes Research Group. J Infec Dis. 1995; 172:1206-1211.

5. Lönnort M, Salminen K, Knip M *et al.* Enterovirus RNA in serum is a risk factor for beta cell autoimmunity and clinical type I diabetes: a prospective study. Childhood diabetes in Finland (Di Me) study group. J. Med. Virol. 2000b; 61: 214-220.

6. Thorsby E and Ronningen KS. Particular HLA-DQ molecules play a dominant role in the determining susceptibility or resistance to type 1 (insulin- dependent) diabetes mellitus. Diabetologia. 1993; 36: 371-377.

7. D'Alessio DJ. A case- control study of group B Coxsackievirus immunoglobulin M antibody prevalence and HLA-DR antigens in newly diagnosed case of insulin- dependent diabetes

mellitus. Am. J. Epidemiol. 1992; 135: 1331-1338.
8. Weinberg CR, Dornan TL, Hansen JA, Raghu, PK. and Palmer JP. HLA- related heterogeneity in seasonal patterns of diagnosis in type 1 (insulin-dependent) diabetes. Diabetologia. 1984; 26: 199- 202.
9. Bruserud O, Jervell J and Thorsby E. HLA-DR3 and DR4 control T lymphocyte responses to mumps and coxsackie B4 virus, studies on patients with type 1 (insulin dependent) diabetes and healthy subjects. Diabetologia. 1985; 28: 420- 426.
10. The Expert Committee of Diagnosis and Classification of Diabetes Mellitus: Diabetes Care. 2003; Suppl. 1: S5-S20.
11. Stocker JW and Bernoco D. Technique of HLA typing by complement-dependent lympholysis. In: immunological methods. Academic press incorporation. 1979; PP: 217-1226.
12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. Journal of Immunological Methods 1983; 65: 55-63.
13. Davidkin I, Valle M, Peltola H *et al*. Etiology of measles and Rubella-like illnesses in Measles, Mumps and Rubella-vaccinated children. The Journal of Infectious Disease. 1998; 178: 1567-1570.
14. Härkönen T, Paananen A, Lankinen,H, Hovi T, Vaarala O and Roivainen M. Enterovirus infection may induce humoral immune response reacting with islet cell autoantigens in human. J. Med. Virol. 2003; 69: 426- 440.
15. Voller A, Bidwell, D. and Bartlett, A.: Enzyme-linked immunosorbent assay. In: manual of clinical immunology. 2nd edition. Rose N. R. and Friedman. H. (Editior). Washington DC American Society of Microbiology. 1980; PP: 359-371.
16. Chapel H, Haeney M, Misbah S and Snowden N. Endocrinology and diabetes. In: Essentials of clinical immunology. 4th edition. Blackwell Science. 1999; PP: 269-281.

17. Varela-Calvino R, Ellis R, Sgarbi, G, Dayan, CM and Peakman M. Characterization of the T-cell responses to coxasckie virus B4: evidence that effecter memory cells predominate in patients with type I diabetes. Diabetes. 2002; 51: 1746-1752.

18. Varela-Calvino R, Sgarbi G, Sefina A and Peakman M. T-cell reactivity to the P2C nonstructural protein of a diabetogenic strain of Coxsackie virus B4. Virology. 2000; 274: 56- 64.
19. Juhela S, Hyöty H, Roivainen M *et al*. T-cell responses to enterovirus antigen in children with type I diabetes. Diabetes. 2000; 49: 1308- 1313.
20. Schloot NC, Roep BO, Wegmann DR., Yu L, Wang TB and Elsenbarth GS. T-cell reactivity to GAD_{65} peptide sequences shared with Coxsackie virus protein in recent-onset IDDM, post onset IDDM patients and control subjects. Diabetologia. 1997; 40: 332- 338.

21. Varela-Calvino R and Peakman M. Enteroviruses and type I diabetes. Diabetes Metab. Res. Rev. 2003; 19: 431-441.
22. Sallusto F and Lanzavecchia A. Exploring pathways for memory T-cell generation. J. Clin. Invest. 2001; 188: 805- 806.
23. Yin H, Berg AK, Tuvemo T and Frisk G. Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type I diabetic children at onset. Diabetes. 2002; 51: 1964- 1971.
24. Lönnort M, Knip M, Roivainen, M, Koskela P, Akerblom HK and Hyöty H. Onset of type I diabetes mellitus in infancy after enterovirus infection. Diabet Med. 1998; 15: 431-434.
25. Tuvemo T, Dahliquist G, Frisk G *et al*. The Swedish childhood diabetes study III: IgM against Coxackie B viruses in newly diagnosed type-1 (Insulin-dependent) diabetic children-no evidence of increased antibody frequency. Diabetologia. 1989; 32: 745- 747.
26. Emekdas G, Rota S, Kustimur S and Kocabeyoglu O. Antibody levels against coxsackie B viruses in patients with type I diabetes mellitus. Mikrobiyol. Bul. 1992; 26(2): 116-20.
27. Buschard K and Madsbad S. A longitudinal study of virus antibodies in patients with newly diagnosed type I (insulin-dependent) diabetes mellitus. J. Clin. Lab. Immunol. 1984; 13(2): 65-70.
28. Roivainen M, Rasilainen S, Ylipaasto P *et al*. Mechanisms of Coxsackie virus-induced damaged to human pancreatic β-cells. The Journal of Clinical Endocrinology and Metabolism. 2000; 85(1): 432- 440.
29. Chehadeh W, Kerr-Coute J, Pattou F *et al*. Persistent infection of human pancreatic islet by Coxsackie virus B is associated with alpha interforn synthesis in β-cells. J. Virology. 2000; 74(21):
10153-10164

Oxidized lipoproteins in hypertensive patients on different modalities of treatment.

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Abstract

Background: In addition to hypercholesterolaemia and smoking, hypertension, a chronic disease with many cardiovascular complications, is one of the major risk factors for cardiac ischemia, and the main risk factor for cerebrovascular disease.

Recent studies have demonstrated some unwanted effects on lipid profile and trace element metabolism caused by antihypertensive drugs.

Aim: To elucidate the role of oxidative stress and lipid peroxidation in hypertension and the effect of different medical therapies on these parameters.

Subjects and methods: The present study included measurement of serum lipid profile, total lipid peroxidation, oxidized HDL (Ox-HDL), and urinary protein in 69 patients aged 30-70 years . They were three groups according to the type of therapy: (atenelol, captopril & no pharmacological antihypertensives, NPAHT).

The results were compared with those of 45 apparently healthy controls (age range = 30-66 years).

Results: showed a significant elevation in serum malondialdehyde (MDA) 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 controls. The disturbance in the oxidant / antioxidant balance happened despite the treatment and blood pressure control showing different pictures with different modalities of treatment, being the best with the captopril..

Conclusion: Hypertensives on different modalities of therapy experienced dyslipidemia, (High triglycerides and small dense LDL particles) and oxidative stress presented by changes in the percents of oxidized lipoproteins especially in those on NPAHT.

The suggested mechanisms underlying these events are discussed.

Keywords: hypertension, lipid peroxidation, ox-HDL. captopril , atenelol

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Introduction

Hypertension (HTN) is considered a major health problem that emerges from the wide occurrence of its cardiovascular complications[1] .

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Recent studies indicate that oxidative stress mediated by increased level of reactive oxygen species has a causative role in the pathogenesis of cardiovascular disease (CVDs) such as atherosclerosis and HTN[2], diabetes (particularly in patients with angiopathy), hyperlipidaemia and acutely after myocardial infarction and stroke[3]

The antioxidant and lipid peroxidation products are being extensively studied because of their potential importance

and pathgenetic role in several non-communicable diseases like CVDs and cancer, but the data on HTN is scanty [4]. The present study was undertaken to elucidate the role of oxidative stress, a lipid peroxidation on essential hypertension with the emphasis on the effect of different treatment modalities on these parameters.

Subjects & Methods:

A- Subjects:

The study was conducted on 69 patients aged 30-70 years (mean age = 50.52 ± 8.71 years) with essential hypertension (HTN) attending the medical consultant-clinic at Al-Kadhumia Teaching Hospital, for evaluation of newly diagnosed HTN or re-evaluation of long-standing HTN .

The treated hypertensives were either using pharmacological antihypertensive therapy (PAHT) for a period ranging from two months to nineteen years, or not using PAHT for a period ranging from six months to five years. They were attending the clinic for assessment of control and, if necessary, modulation of therapy. The newly diagnosed patients were first discovered to have HTN during their preliminary attendance to the private clinics few weeks (1-4) before their current visit. During this interval, they had not taken any antihypertensive, with their latest attendance to the consultation clinic was for confirmation of diagnosis and, if HTN persisted, commencement of therapy.

Any patient with other medical illnesses that may have an effect on the measured parameters was excluded from the study, as cardiac, hepatic, endocrine, metabolic diseases, smoking and alcoholism. None of the female patients were pregnant nor on contraceptive pills. Details of clinical state were taken from each subject. Depending on the modality of AHT, the patients were divided into three groups

1-hypertensives on atenelol: They were 27 hypertensive patients on β-blocker (atenolol 50-100 mg/day), age range 34-70 years (mean age = 51.33 ± 8.81 year).

2- hypertensives on captopril: They were twenty-seven hypertensive patients on ACE inhibitor (captopril 25-150 mg/day) , age range 30-65 year (mean age = 50.11 ± 8.53 year)..

3- antihypertensives on no pharmacological antihypertensive therapy (NPHT):

Fifteen hypertensive patients had no pharmacological interference, with age range of 32-64 years (mean age = 49.8 ± 9.33).

Control group:

Forty-five apparently healthy subjects were involved as a control group with matching age and sex to the patient group (mean age was 46.04 + 9.43).

None of them were alcoholic, or on any drug that may interfere with the results of the study. Pregnant females and those on contraceptive pills were excluded.

B- Blood Specimens

Ten milliliters of venous blood were drawn from each patient and control after 12 hours fast. The samples were transferred into clean plane tube, left at room temperature for 15 minutes for clotting, centrifuged, and then serum was separated and divided into 2 parts:

1- for measuring the glucose, urea & creatinine (these were measured at the same day of collection in the laborotaries of Al- Kadhumia Teaching Hospital for TG, TC, HDL-C, and LDL-C. the tubes were stored at 4^{o} C for no longer than

seven days[5]. For measuring the total level of oxidized lipids (measured as total malodialdehyde MDA) and specific levels of oxidized HDL (measured as HDL-MDA). The tubes were stored at –20° C until analysis, which was done within one month after collection[6]

C- Methods :

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

The procedure was also conducted on the fractionated specimens to obtained levels of **oxidized HDL. 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.

Protein in urine was detected by 30% trichloroacetic acid precipitation method [6] to exclude any patient with proteinuria.

Results :

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 (3-1).

Serum MDA was highly significantly increased in HTV patients on atenolol (ATE) and captopril (CAP) *($P=10^{-6}$ & 10^{-5} respectively)* when compared to controls and was also significantly increased in HTV on non-pharmacological antihypertensive therapy (NPAHT) *(P=0.03)* as compared to the controls. There was, also a significant variation between hypertensive groups when compared with each other (Table 1).

Hypertensives on atenelol & NPAHT caused a significant reduction of OX.HDL% fraction *($P=0.05$ & 10^{-4} respectively)*; however, HTV on captopril had insignificant reduction in this fraction *(P=0.4)* when all groups were compared with the controls. Also, there was a significant variation between hypertensive groups when compared with each other (Table 1).

In contrast to OX.HDL%, hypertensives on atenelol and on NPAHT had a significant elevation of OX.non-HDL% *($P=0.05$, 10^{-4} respectively)*; however, hypertensives on captopril had insignificant elevation in this fraction *(P=0.45)* when all groups where compared with controls. There was a significant variation between hypertensive groups when compared to each other *($P=10^{-3}$)* (Table 1).

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) and LDL size index (expressed as TG/HDL-C) are shown in Table 2 .

Table (1): lipid peroxides and oxidized lipoprotein fractions (mean ± SD) in different groups of hypertensive patients and control group.

Groups	n	S.MDA µmol/l	t-test P-value*	OX.HDL %	t-test P-value*	Oxidized nonHDL %	t-test P-value*
HTV on atenalol	27	0.67 ± 0.16	10^{-6}	60.45 ± 11.65	0.05	39.05 ± 11.8	0.05
HTV on captopril	27	0.63 ± 0.16	10^{-5}	70.62 ± 17.1	0.45	29.37 ± 17.1	0.45
HTV on NPAHT	15	0.96 ± 0.36	0.03	55.02 ± 17.09	10^{-4}	44.97 ± 17.09	10^{-4}
ANOVA *P-value*	~	~	10^{-5}	~	10^{-3}	~	10^{-3}
Controls	45	0.46 ± 0.17	~	73.83 ± 17.83	~	26.16 ± 17.83	~

* = Student t-test was done between each HTV group and control group.

Table (2): Lipid profile (mean ± SD) in different hypertensive and control groups.

Group	HTV ON ATE	HTV ON CAP	HTV ON NPAHT	ANOVA *P*-value	CONTROLS
N	27	27	15	~	45
TG(mmol/l)	1.76± 0.67	1.64 ± 0.53	2.21± 0.42	~	1.18 ± 0.54
*t-test P-value**	0.0001	0.0007	0.04	0.009	~
TC(mmol/l)	4.92 ± 1.12	5.1 ± 0.79	5.1 ± 1.2	~	4.34 ± 0.96
t-test P-value*	0.02	0.001	0.01	0.7	~
HDL-C (mmol/L)	1.04 ± 0.23	1.14 ± 0.24	1.09 ± 0.33	~	1.18 ± 0.3
*t-test P-value**	0.04	0.5	0.3	0.3	~
LDL-C(mmol/l)	3.07 ± 1.06	3.03 ± 0.66	2.99 ± 1.31	~	2.66 ± 0.93

*t-test P-value**	0.08	0.15	0.2	0.9	~
Atherogenic index (LDL-C/HDLC)	3.01 ± 0.11	2.87 ± 0.75	2.9 ± 1.33	~	2.45 ± 1.29
*t-test P-value**	0.09	0.19	0.24	0.8	~
LDL size index (TG/HDL-C)	1.79 ± 0.89	1.55 ± 0.78	2.4 ± 0.75	~	1.13 ± 0.79
*t-test P-value**	0.001	0.03	0.000003	0.007	~

*Student t- test was done between each HTV group and control groups.
.ATE : Atenelol , CAP : captopril

Discussion:

The results showed that, oxidative stress increases in hypertensive (HTV) patients regardless the modality of treatment, this is clear from the highly significant elevation of serum MDA level and is in agreement with the results of previous reports [7,8]

This elevation in serum MDA may be due to the association of high blood pressure (BP) with a loss of balance between pro-oxidation and anti-oxidation, energy depletion, and accelerated aging in the target organs, such as heart, kidney and brain [9]

Essential HTV patients are more prone than the normotensive subjects to oxidative stress; because of an increased production of reactive oxygen metabolites and of lipid peroxidation products[10], something that was reported to be frequent among such patients. This may results in a poor control of BP and hence progression of target tissue damage[11]. The latter will be reflected by an elevation in serum MDA as an index of oxidative stress; however, the presence of undesirable side effects of these drugs might contribute to some rise in serum MDA, this contribution varies from one type of therapy to another. Beta-adrenergic blockers were reported to increase cholesterol side effects besides the central nervous system effects. However, ACE inhibitors have fewer side effects in general [12].

It remains to be determined whether these events are simply consequences of tissue damage or strictly involved in primary pathogenic mechanism of HTN. Therefore, a study of oxidant and antioxidant factors seems to be useful in HTV patients in order to evaluate oxidative stress and to correct, if possible, the observed abnormalities with dietetic or pharmacological therapy[13].

The term " ***oxidized non-HDL"*** refers to the oxidized form of VLDL and LDL. The oxidation of LDL appears to be involved in the development of atherosclerosis. The oxidation of LDL leads to alteration of the LDL apolipoprotein B (apoB) recognition site and in the unregulated uptake of LDL by the macrophages via the scavenger receptor. The subsequent accumulation of cholesterol-loaded macrophages (foam cells) in the sub-endothelium

leads to the accumulation of fatty streaks and atherosclerotic plaques [14]*;*

In the in-vitro studies, OX-.LDL was shown to be taken up by the macrophages three to ten times than native LDL[15].

Furthermore, OX.LDL is chemotactic for circulating monocytes, while decreasing the motility of tissue macrophages[15,16], and also stimulates the release of growth factors and cytokines by endothelial cells to further stimulates monocyte migration. The oxidized form of LDL is known to be cytotoxic to endothelial cells and may cause an autoimmune type of response against endothelial cells. Also OX.LDL inhibits endothelial-derived relaxing factor (EDRF)-mediated vasorelaxation. EDRF appears to be crucial in maintaining coronary vasodilation and its activity is impaired in hypercholesterolaemia and atherosclerosis [15,16]

According to the results of the current study, there was a significant elevation of OX.non-HDL in HTV groups on atenolol (ATE) and those on no pharmacological antihypertensive therapy (NPAHT) as compared with the controls, the NPAHT being the highest. This suggests that those patients are more prone to have atherosclerotic changes than those receiving medications with the presence of smaller forms of LDL particles (as evident from higher TG / HDLc molar ratio). On the other hand hypertensives on atenelol have significantly higher level of OX.non-HDL than patients on captopril , who showed insignificant increase in this fraction as compared to the controls; this is due to the fact that ACE inhibitors inhibit LDL oxidation and attenuate atherosclerosis independent of lowering BP and this may due to direct inhibition of angiotensin II-dependent effects[17].

As concerning ***oxidized HDL,*** it has been postulated that HDL has a protective effect against the formation of OX.LDL, and one of the mechanisms is mass transfer of lipid peroxidation products between lipoproteins[18] (i.e. from LDL to HDL) forming OX.HDL which was markedly reduced in the present HTV, mostly those on no medications on the expense of the increase in the atherogenic form of LDL (OX.LDL).

Percentages of OX.HDL were also reduced in HTV people receiving medications but to a lesser extent and with a variable degree depending on the antioxidant properties of the antihypertensive drug being only slightly reduced in those on captopril. This indicates that untreated HTV were more prone to have atherosclerosis than those receiving medications. Also, HTV on atenelol had a higher reduction in percentages of OX.HDL than those on captopril this reveals the antiatherogenic protective effect of captopril against vasculopathy.

The findings of this study, suggest that the studied drugs (atenolol & captopril), in addition to their antihypertensive effect that control Blood pressure, possess antioxidant properties that make them an important means to retard the progression of atherosclerosis in hypertensive peoples.

The antioxidant property of capotril was well evident through the results of MDA, OX.non-HDL and OX.HDL (when results of the above parameters were compared with the healthy controls and other hypertensive groups), and it exceeds that of atenelol and probably overcome its antihypertensive effect[19].

Reported increased nitric oxide (NO)

production, decreased production of thiobarbiturate reactive substances and enhancement of endogenous antioxidant defense during the treatment with capotril. Therefore, the goal of appropriate antihypertensives should not be restricted to achieving normotensive BP values, but should also aim to reduce other risk factors of cardiovascular diseases[20] .

Further improvement in cardiovascular risk can be obtained only with a comprehensive approach to hypertensive patients, including dietary and lifestyle modifications and the use of antihypertensive drugs with beneficial effects on lipid metabolism. It has to be noted that, some of these drugs have divergent effects on lipid metabolism. While β-blockers decrease HDL-C and increase triglyceride (TG), ACE inhibitors do not affect lipid metabolism [20 , 21] .

In the present study, There was also a significant increase in s.TG and s.TC level in the untreated hypertensives. While there was no significant changes in s.HDL-C and s.LDL-C levels, these results are consistent with the fact that untreated HTN has been shown to be associated with a significant elevation in s.TG and s.TC levels[22] . The results of this study and others support the hypothesis that hyperlipoproteinaemia is aften observed in untreated hypertensives patients[23], and when HTN is associated with high plasma lipid levels, it is an atherosclerogenic factor of greator clinical significance causing CVDs [24] .

However, the role of TG in 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 heterogenous entity which is closely related to many factors that may affect coronary risk (hypertension, insulin resistance, sedantarity, and even tobacco consumption). 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 [25].

References:

1- Al-Alwan Aladdin SA, Abou Yousif Z . Iraqi Drug Guide, 1st edition, 1990. NBSD & Central Drug Information Burea, Baghdad. P: 25.

2- Sozmen-EY, Kerry-Z, Uysal-F, Yetik-G. Antioxidant enzyme activities and total nitrite / nitrate levels in the collar model. *Clin- Chem-Lab-Med. 2000; 38(1): 21.*

3- Stringer-MD, Gorog-PG, Freeman-A, Kakkar-VV. Lipid peroxides and atherosclerosis . *Br-Med-J. 1989; 298: 281 -284.*

4-Srinivas-K, Bhaskar-MV, Aruna-Kumari-R, Nagaraj-K, Reddy-kk. Antioxidants, lipid peroxidation and lipoproteins in primary hypertension. *Indian-Heart-J. 2000; 52(3): 285-8.*

5-Buege-JA, Aust-SD. Microsomal lipid peroxidation. *Meth-Enzymol. 1978; 51: 302-310.*

6- Johnson-AM, Rohlfs-EM and Silverman-LM. Protein, In: Burtis-CA, and Ashwood-ER. (Eds.): Tietz Textbook of Clinical Chemistry. 3rd Ed. 1999, Saunders Company, Philadelphia: 477-540.

7- Al-Khazraji-MQ. Oxidative stress in hypertensive patients on different types of treatment. M.Sc. thesis. 2001, College of Medicine, University of Al-Mustanseriah.

8- Al-Khairi-HY. Effects of antioxidants on hypertensives patients with different therapy. M.Sc. thesis. 2000, College of Pharmacy, University of Baghdad.

9-Romero-Alvira-D. Roche-E. High blood pressure, oxygen radicals, and antioxidants. *Med-Hypothesis. 1996; 46(4): 414.*

10-Reddy-KK, Rao-AP, Reddy-TP. Serum vitamins E, A and lipid peroxidation. *Indian-J-Biochem-Biophys. 1999; 36(1): 44.*

11- Oparil-S. Arterial hypertension. In: James-BW and Loyed H. Smith (ed.). Cecil textbook of medicine, 19th edition. USA, W.B. Saunders Company, 1992, pp: 258-266.

12- Boon-NA, Fox-KAA, Bloom field-P. Diseases of Cardiovascular system. In: NA-Boon; JAA-Hunter; C-Haslett; ER-Chilvers (ed.) Davidson's Principle and Practice of Medicine, 18th edition, UK.Churchill Livingstone, 1999: 218-219.

13- Digiesia-V, Oliviero-C, Gianno-V, Fiorillo-C, Rossetti-M, Lenuzza- M, Oradei-A, Nassi-P. Reactive metabolites of oxygen, lipid peroxidation, total antioxidant capacity and vitamin E in essential arterial hypertension. *Clin-Ter. 1997; 148(11): 515-9.*

14-Guerci-B, Antebi-H, Meyer-L, Durlach-V, Ziegler-O, Nicolas-JP, Alicidor-LG, Dovin-P. Increased ability of LDL from normo - Lipidaemic type-2 diabetic women to generate peroxides. *Clin-Chem. 1999; 45(9): 1439-1448.*

15- Harris-LM, Armstrong-D, Broene-R, Aljada-A, Peer-R, Upson-J, Pilla –L, Curl-GR, Ricotta-JJ. Premature peripheral vascular diseases: clinical profile and abnormal lipid peroxidation. *Cardiovascular- Surgery. 1998; 6(2): 188- 93.*

16- Jialal-I, Devaraj-JS. Low-density lipoprotein oxidation, and atherosclerosis. A clinical biochemical perspective. *Clin-Chem. 1996; 42(4): 498-506.*

17- Hayek-T, Attias-J, Coleman-R, Brodsky-S, Smith-J, Breslow-JL Keidar. The angiotensin-converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. *Cardiovasc-Res. 1999; 44(3): 579-87.(PubMid Internet abstract PMID: 8608194).*

18- Parthasarathy-S, Barnett-J, Fong-LC. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *iochim-Biophys-Acta. 1990; 1044 (2): 1275-83. (PubMid Internet abstract PMID: 2344447).*

19-De-Cavanagh-EM, Inserra-F, Ferder-L. Enalipril and captopril enhance glutathion-dependent antioxidant defense. *Am-J-Physicol-Regul-Comp. 2000; 278(3): 572.*

20- Dal-Palue-C, Samplicini-A. The treatment of hypertension in patients with metabolic disorder. *Ann-Ital-Med-Int. 1995; 10 Suppl.: 130S- 132S.*

21-Klein-W. Antihypertensive therapy and modification of metabolic risk factors (glucose and lipid metabolism). *Z-Kardiol. 1992; S1 (6): 294-302. (Midline abstract).*

22-Simons-LA, Simons-J, MacCallum-J, Friedlander. Dubbo Study of the elderly hypertension and lipid levels. *Atherosclerosis. 1992; 92 : 59 (Midline abstract).*

23-Thomas-GW, Maun-JI, Beillin-LJ, Ledingham-JG. Hypertension and raised lipids. *Brit-Med-J.1977; 2: 805.*

24-Weinberger-MH. Antihypertensive therapy and Lipids, Evidence, mechanism, and Implications. *Arch-Intern-Med. 1985; 145: 1102.*

25-Bruckert-E, Emmerich-J, Delahaye-F, Richard-H, Thoma-D. Role of triglycerides in cardiovascular diseases. *Arch-Mal-Coeur- Vaiss. 1992; 85, Spec No.3: 29-35. (Midline abstract).*

PREVALENCE OF PARKINSON'S DISEASE IN AL-KADHIYMIA DISTRICT (BAGHDAD CITY): COMMUNITY-BASED STUDY

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Abstract:

Background: Parkinson's disease is a chronic neurodegerative disorder affects mostly people above 40ys. Studying its prevalence is crucial for health public planning especially as worldwide communities are getting older. There are some worldwide variations in the estimated prevalence rates and the figures are unknown in our country.

Objective: To estimate the prevalence of Parkinson's disease in Al-Kadhiymia district.

Methods: Community-based study was conducted as cross-sectional survey on random sample of the population of the district. Suspected cases of Parkinson's disease identified during home visits were referred to the neurological department at the University Hospital of Iraqi Medical College in order to confirm the diagnosis of the senior neurologist. Diagnosis is made by identifying at least two cardinal features of the disease (resting tremor, bradykinesia, rigidity and postural instability) in the absence of signs of secondary Parkinsonism.

Results: 25 cases of Parkinson's disease collected from a random sample of 22,988 individuals (13 were males, 23 were females. 6 lived in rural areas and 19 in urban). Three cases (12%) were newly diagnosed. Tremor was the predominant symptom of onset (80%). 19 cases had bilateral involvement of the disease, in spite of the unilateral onset of all cases. The crude prevalence rate was 108.75 per 10^5 populations. Age adjusted prevalence rates showed constant increase with age. Gender-adjusted prevalence rates were calculated for male $114/10^5$ populations and for $103/10^5$ populations. Residency-adjusted prevalence rates were 114,3 and 94,3 per 10^5 populations for urban and rural living respectively.

Conclusion: Prevalence rate of Parkinson's disease is just lower than the figures in Europe and North America, but higher than those of Africa and China. It increases constantly with increasing age. There was no significant gender or rural difference in the prevalence rates. The prevalence figure can be applied to the population of Baghdad City because of the similar population structure and characteristics to those of Al-Kadhimiya district.

Keywords: Parkinson disease, prevalence, Baghdad, cross sectional study

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Introduction:

Parkinson's disease (PD) is a progressive neuro-degenerative disorder, which affects the movement or the control of movement including speech and "body language". Four cardinal signs dominate it: bradykinesia, tremor and rest, rigidity and postural instability. It mostly affects elderly people with overall prevalence of about 1.6 percent in persons over 65 years of age[1].

The disease is chronic and progressive. Its life expectancy increased since the introduction of L-dopa treatment. Other medical therapies and some resent surgical techniques providing continuous improvement of the disease disability [2].

In addition, the population of the world, in general, is growing older because of the improving health services. This gives an indication that PD will affect more people i.e. incidence and prevalence of the disease will continue to rise [1]. These facts indicate that the importance of PD as a

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population health issue is expected to increase in the near future.

The prevalence of PD is studied worldwide with some variations in the figures [3-33]. In 1990 worldwide estimated 4 million people were suffering from PD, approximately one million of them in North America [1,2].

In Iraq, there is no local record about the prevalence of PD or the epidemiological factors that affect it. Therefore, we found that it was necessary to study this subject in order to identify the disease extent and to plan useful measures for health care institutions and personnel.

Patients and Methods:

Area of the study

The area chosen for the study was Al-Kadhiymia district, one of the original regions in Baghdad City (Capital of Iraq). It is located north of Baghdad on the western bank of the Tigris River. The total population of Al-Kadhiymia district is 480686 people, according to the Iraqi population survey of 1997 distributed into 38 sectors in urban area and 16 villages in rural one. It was chosen because of:

1. Large number of its population,
2. Its inhabitants are of different social classes,
3. Containing both rural and urban areas,
4. Its proximity to the University Hospital.

All individuals involved in the study were asked to take part in it. Their consent was taken while visiting their families at home.

Sample of the study:

A random sample was determined from the general population of the district as follows:

* For urban area: in each sector, 20% of the families were selected randomly and included in the study.
* For rural area: 20% of the families living in villages were randomly selected to be enrolled in the study.

The sample was about 23 000 individuals described as families in order to involve all age groups. The random sample was determined and designed in cooperation with the Central Statistical Organization authorities that provided us with a detailed account on the names, locations and numbers of the selected streets and sectors. These numbers were listed by local authorities, in marks at beginning and end of streets.

Survey:

This is a community-based study, designed as a cross-sectional survey on the general population. A suitable questionnaire was designed *(see appendix)* in two parts so that the study was run in two phases:

* Phase 1 (screening phase): Families were interviewed at home. One of the researchers did the interviewing to explain the purpose of the study, ask questions about age and gender of each family member and the presence of any of the following:

1- Tremor of the hands "Is there any trembling movement of the hands?"
2- Bradykinesia "Is there any slowness of movement during walking?"
3- Previous diagnoses of PD "Is there any member who has the diagnoses of PD?"
4- Use of antiparkinsonian drugs "Is there any one who is taking L-lopa, Trihexyphenidyl or Bromocriptine tablets?"

Any member who had positive answer would be examined to demonstrate the Parkinsonian signs. Patients excluded from further assessment if they had used anti Parkinsonian drugs for other indications (e.g. Bromocriptine for pituitary adenoma or prophylactic treatment with anticholinergic drugs in

association with dopamine antagonists in patients with psychotic disorder).

* Phase 2 (confirmatory phase): All suspected cases of PD were referred to the neurological department of the Al-Kadhimiya Teaching Hospital where history and neurological examination is performed by senior neurologist.

Patients with confirmed diagnosis of PD would be questioned about: the duration of their symptoms, type of onset, drug ingestion (neuroleptics, antidopaminergic), family history of PD or tremor, presence of medical illness, smoking, number of rooms, family members and total years of education (*see appendix*).

Diagnosis criteria

According to the WHO definition [1] PD was diagnosed when the patient had at least 2 of the cardinal signs (resting tremor, bradykinesia, rigidity and postural instability) with no signs of nervous system involvement, such as corticospinal deficit, cerebral dysfunction, conjugate down or lateral gaze impairment, or prominent early automatic nervous system involvement. Patients with drug induced Parkinsonism were excluded.

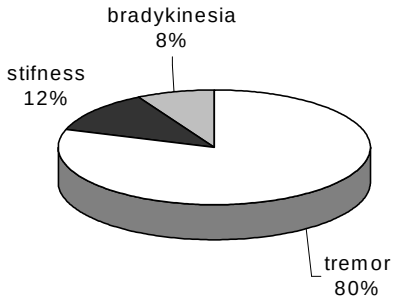

Figure 1: The distribution of onset symptoms in patients with Parkinson's disease

Statistical analysis

Our data was computed in Pentium III system using the statistical analysis system (SPSS version 10.0). We calculated the prevalence rates as crude and category specific (age, gender, and residency). The quantitative data were expressed as mean (X) standard deviation (SD). We determined whether differences in prevalence rates were statistically significant or not by student (t) test and chi-square (X^2) test. A probability limit (p value) of <0.05 was considered statistically significant.

Results:

The cross-sectional survey started from December 1st 2000 till September 15th, 2001. The total population involved in this study was 22988 individuals, 11645 (50.7%) of them were females and 11334 (49.3%) were males. Individuals living in urban areas were 16646 (72.3%) and in rural ones were 6364 (27.7%). 76 individuals with suspected PD were referred to the Al-Kadhimiya Teaching Hospital, 55 (72.3%) of them responded and 21 did not.

Those who did not respond two of them had written certificate of diagnosis of PD by a senior neurologist, two were bedridden with full certificate of PD confirmed by the neurologist, and for the other a second visit was done for reassessment that revealed exclusion of

diagnosis. A total number of 25 cases of PD were collected, 13 males (52%) and 12 females (48%). Three cases (12%) were not diagnosed yet by the time of the survey. Those who were referred but not diagnosed as PD, they had benign essential tremor, dementia or cerebrovascular accident.

The crude prevalence rate of PD was **108.75 per 100 000** population. The results of category specific prevalence rates are as follows:

Age: Table 1 shows the age-specific prevalence rates for the total population of the study. The age is categorized a below 20 years and groups of 10 years age for those 20 years and above. The youngest case was 35 years of age and 94 years was the age of the oldest. The most prominent collection of cases was in the age group of 60-69 years, it contained nine cases representing 36% of the total.

Table-1: Age-specific prevalence rates of Parkinson's disease in Al-Kadhiymia district

Age group	Population	Prevalence per 10^5
<20	12585	0
20-29	3671	0
30-39	2852	35.06
40-49	1693	118.13
50-59	1026	487.33
60-69	699	1287.55
70-79	317	1892.74
>80	113	2068.97
Total	22988	108.75

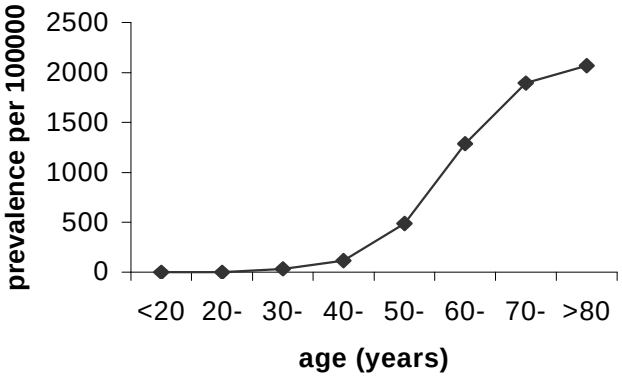

Figure 2: Correlation between prevalence rates of Parkinson's disease with age in Al-Kadhiymia

Gender: The prevalence rate of PD in men ($114.7/10^5$) was slightly higher than in women ($103.05/10^5$), as shown in table-2. This difference was statistically not significant ($X^2 = 0.07$, p=0.789).

Table-2: gender-specific prevalence rates of Parkinson's disease in Al-Kadhiymia district

Age group	Male			Female		
	Total	PD	Prevalence per 10^5	Total	PD	Prevalence per 10^5
<20	6161	0	0	6424	0	0
20-29	1821	0	0	1850	0	0
30-39	1407	1	0	1445	1	69.2
40-49	865	0	115.61	828	1	120.77
50-59	551	4	725.95	475	1	210.53
60-69	315	3	952.38	384	6	1562.5
70-79	150	3	2000	167	3	1769.4
>80	64	2	3125	81	0	0
Total	11334	13	114.7	11645	12	103.05

Residency: Table-3 shows the distribution of cases of PD among rural and urban areas. Nineteen cases were living in urban areas (10 males, 9 females) and six cases in rural one (three males, 3 females). There was slight increase in prevalence rate of PD in urban ($114.29/10^5$) more than in rural ($94.28/10^5$). This difference was of no statistical significance (p=0.68).

Table 3: residency-specific prevalence rates of Parkinson's disease in Al-Kadhiymia district

Area	Cases	Total	Prevalence per 10^5	Significance
Urban	19 (76%)	16624 (72.3%)	114.29	$X^2 = 0.17$ P = 0.68
Rural	6 (24%)	6364 (27.7%)	94.28	
Total	25	22988	108.75	

Onset: The predominant symptom of onset was tremor, which was present in 20 cases (80%), stiffness of the back was the initial symptom in three cases (12%) while bradykinesia was in two cases (8%), as shown in figure-2. All cases had a unilateral onset.

Laterality: The disease was unilateral in six cases (24%), and bilateral in 19 cases (76%). The average duration of the illness in unilateral disease was about half that of bilateral one. This is shown in table-4.

Table 4: The relation between average duration and laterality of illness in patients with Parkinson's disease

Laterality	Cases	Duration (years)	Significance
Unilateral	6 (24%)	2.5±2.41	T = -1.57 P = 0.13
Bilateral	19 (76%)	4.42±2.68	
total	25	3.84±2.68	

Discussion:

Studies on the prevalence of PD had been conducted in many parts of the world, for example: Australia, New Zealand, Japan, United States, United Kingdom and Iceland. These studies generally relied on record of providers of health services (mainly hospitals and medical practitioners) in the identification of cases. Such maneuvers exclude individuals who failed to seek medical attention for their PD symptoms, as well as those who were improperly diagnosed.

This study was a community-based one where the approach of case finding was to go into the community and screen for patients with PD at home. This approach is more accurate especially in our circumstances where medical records are improperly handled and seeking medical advice is incomplete because of ignorance of symptoms, socio-economic difficulties or disease disability.

This is the first community-based study for PD carried out in our country to estimate prevalence of PD.

We found the crude prevalence of PD in the study population was 108 per 100 000 population. This figure is slightly lower than those reported in Europe and North America: London (193)[1], Finland (166)[4], San Morino (152)[5] and Canada (244)[6]. However, it is much higher than African figures: Nigeria (10)[7], Libya (31)[8], as well as China (57)[9].

These differences may be attributed to a different genetic susceptibility in different race [10]. However, other factors should be considered. Different age structures where older age population is more in developed countries may contribute to the higher prevalence rates [11].

Morens et al [12] found that incidence rates were similar in Asian-American men and in white men. This may be explained by more complete ascertainment than the previous studies. Alternatively, it may reflect a real increase in disease frequency among nonwhite U.S. residents because of increased exposure to an environment factor.

Recent prevalence study on PD was conducted in Taiwan on people of similar ethnic group to Chinese. It revealed a prevalence rate similar to European figures, suggesting that environmental factors might be more important than racial factors in the pathogenesis of PD [13].

Age: There was a consistent and rapid increase in prevalence PD with increasing age, even in the highest age categories. It increases about 20 times from age group of forty to that of eighty. This finding was similar to other studies [4,11,13,33]. Three cases (12%) were identified below the age of 50, corresponding to WHO figure [1].

Although PD is intimately related to aging, it has been demonstrated that its underlying process is distinct from natural aging. There is a marked microglial reaction to neuronal damage in PD that is not seen in normal aging [36]. It may be explained by aging-related factors like chronic exposure to neurotoxicants.

Gender: Prevalence of PD regarding gender distribution is controversial. Some studies show higher prevalence rates in men than women [3,4,6,8,19,20,21,22,37]. Other studies [5,10,14,15,24] found equal gender prevalence. In our study, there was no significant gender difference in the prevalence rate.

Residency: Several studies [6,24-26] indicated that rural living is a significant risk factor for PD. As Gorell study [38] we found no association between rural living and PD. It's the individual characteristics of rural living like farming, use of well water and exposure to herbicides and insecticides are the factors associated with PS [24,25]. These factors need to be

evaluated in a specific case-control study with larger sample size.

Characteristics of the disease: The onset symptom was predominately tremor 80%, while stiffness only 12%. This may be explained, as both stiffness and slowness of movement are manifestations of getting older, so the patients were unaware of them.

Three cases were first diagnosed at the time of study representing 12% of total cases. This is agreed with the assumption of Shrag [3] that 10-20% of all community patients remain undiagnosed.

Conclusion:

1- Prevalence of Parkinson's disease in Al-Kadhiymia district is 108.75 per 100 000 population. This figure is slightly lower than European and American figures but more than in Africa and China.

2- There were no significant gender or residency differences in the prevalence of Parkinson's disease.

3- The prevalence figure can be applied to the population of Baghdad City because of similar population structure and characteristics to those of Al-Kadhiymia district.

References:

1. Press Release WHO / 71. Parkinson's disease: A unique survey launched. 14 October 1998. Internet: www. Who. Org

2.Adams RD et al. Principles of neurology. 5th edition, New York, McGraw-Hill, Inc. 1993, chapter 43: p.p. 937-42.

3. Schrag A, Quinn NP, Ben-Shlomo Y. Cross sectional prevalence survey of idiopathic Parkinson's disease and parkinsonism in London. BMJ, 2000; 321: 21-2.

4. Kuopio AM, Moarttila RJ, Helenius H, Rinne UK. Changing epidemiology of Parkinson's disease in southwestern Finland. Neurology 1999; 52: 302-7.

5. Alessandro RD, Gamberini G, Granieri E, Naccarato S, Manzaroli D. Prevalence of Parkinson's disease in the republic of San Marino. Neurology, 1987; 37: 1679-82.

6. Svenson LW, Platt GH, Woodhead SE. Geographic variation in the prevalence rates of Parkinson's disease in Alberta. Can J Neurol Sci, 1993; 20: 307-11.

7. Osuntokun BO, Adeuja AO, Schoenberg BS, Bademosi O, Nottidge VA, Olumide AO. Neurological disorders in Nigerian Africans: a community- based study. Acta Neurol Scand, 1987; 75: 13-21.

8. Ashok PP, Radhakrishnan K, Sridharan R, Moussa ME. Epidemiology of Parkinson's disease in Benghazi, Northeast Libya. Clic Neurol Neurosurg, 1986; 88: 109-13.

9. Li SC, Schoenberg BS, Wang CC, et al. A prevalence survey of Parkinson's disease and other movement disorders in the People's Republic of China. Arch Neurol, 1985; 42: 655-7.

10. Kessler I. Epidemiological studies of Parkinson's disease. A community- based study. AM J Epidemiology, 1972; 96: 242-54.

11. Kusumi M, Nakashima K, Harada H, Nakayama H, Takahashi K. Epidemiology of Parkinson's disease in Yonago City, Japan: comparison with a study carried out 12 years ago. Neuroepidemiol, 1996; 15: 201-7.

12. Morens DM, Davis JW, Gradinetti A, Ross GW, Popper JS, WhiteLR. A prospective study of middle-aged men. Neurology, 1996; 46: 1044-50.

13. Chen RC, Chang SF, Su CL, et al. Prevalence, incidence and mortality of Parkinson's disease: A door to door survey in Ilan Country, Taiwan. Neurology, 2001; 57: 1679-86.

14. DE Rijk MC, Breteler MM, Graveland GA, Grobbee De, Meché FG, Hofman A. Prevalence of Parkinson's disease in elderly: The Rotterdam study. Neurology, 1995; 45: 2143-46.

15. Morgante L, Rocca WA, Rosa AE, etal. Prevalence of Parkinson's disease and other types of Parkinsonism: A door-to-door survey in three Sicilian municipalities. Neurology, 1992; 42: 1901-7.

16. Mutch WJ, Fordyce ID, Downie AW, Paterson JG, Roy SK. Prevalence of Parkinson's disease in a Scottish city. BMJ, 1986; 292: 534-6.

17. Tanner CM, Chen B, Wang W, et al. Environmental factors and Parkinson's disease: A case-control study in China. Neurology, 1989; 39: 660-4.

18. Schoenberg BS, Anderson DW, Haerer AF. Prevalence of Parkinson's disease in the biracial population of Copiah Country, Mississippi. Neurology, 1985; 35: 841-5.

19. Tandberg E, Larsen JP, Nessler EG, Riise T, Aarli JA. The epidemiology of v in the country of Rogland, Norway. Mov Disord, 1995; 10: 541-5.

20. Mayeux R, Marder K, Cote LJ, et al. The frequency of idiopathic Parkinson's disease by age,

ethnic group and sex in northern Manhaten. 1988-1993. Am J Epidemiol, 1995; 142: 820-7

21. Baldereschi M, Carlo A Di, Rocca WA, et al. Parkinson's disease and Parkinsonism in a longitudinal study. Neurology, 2000; 55: 1358-63.

22. Al-Shekhli K, Abdul-Zehra IK. Clinical features and primitive reflexes in Parkinson's disease, thesis 1998.

23. Schoenberg BS, Osuntokun BO, Adeuja AO, et al. Comparison of the prevalence of Parkinson's disease in Black population in the rural United States and in rural Nigeria. Neurology, 1988; 38: 645-6.

24. Ho SC, Woo J, Lee CM. Epidemiological study of Parkinson's disease in Hong Kong. Neurology, 1989; 39: 1314-8.

25. Marder K, Tang MX, Mejia H, et al: Risk of Parkinson's disease among first-degree relatives. Neurology, 1996; 47: 55-60.

26. McCann SJ, Le Couteur DG, Green AC, and et al. The epidemiology of Parkinson's disease in an Australian population. Neuroepidemiol, 1999; 22: 405-8.

27. Wang SJ, Fuh JL, Teng EL, and et al. A door-to-door survey of Parkinson's disease in a Chinese population in Kinmen. Arch Neurol, 1996; 53: 66-71.

28. Wermuth L, Joensen P, Bunger N, Jeune B. High prevalence of Parkinson's disease in the Faroe Island. Neurology, 1997; 49: 426-32.

29. Sveinbjornsdottir S, Hicks A, Jonsson T, et al. Familial aggregation of Parkinson's disease in Iceland. N Engl J Med, 2000; 343: 1765-70.

30. Bennet DA, Beckett LA, Murray AM, et al. Prevalence of Parkinsonian signs and associated mortality in a community population of older people. N Engl J Med, 1996; 334: 71-6.

31. Bharucha NE, Bharucha EP, Schoenberg BS. Pilot survey of the prevalence of neurological disorders in the parsi community of Bombay. Am J Prev Med, 1987; 3: 293-9.

32. Smagiassi A, Mutti A, DE Rosa ADE, palma G, Negrotti A, Calzetti S. A case-control study of occupational and environmental risk factors for Parkinson's disease in the Emilia-Romagna region of Italy. J Neurol Sci, 1997; 143: 157-62.

33. MacDonald BK, Cockerell OC, Shorvon JW. The incidence and lifetime prevalence of neurological disorders in a prospective community-based study in UK. Neurology, 2000; 123: 665-76.

34. Woolson RF. Statistical methods for the analysis for biochemical data. New York, Robert F. Woolson Ed., John Willy & sons, Inc. 1987; p.p: 14-185.

35. Daniel WW: Biostatistics. A foundation for analysis in the health sciences. John Willy & sons, Inc. 1987; Ch. 10: p.p. 343-56.

36. McGeer L, Itagaki S, Akiyama H, McGeer EG. Rate of cell death in Parkinsonism indicate active neuropahtological process. Ann Neurol, 1988; 24: 574-6.

37. Kurtzke JF, Murphy FM: The changing pattern of death rates in Parkinsonism. Neurology, 1990; 40: 42-9.

38. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Richardson RJ: The risk of Parkinson's disease with exposure to pesticide, farming, well water, and rural living. Neurology, 1998; 50: 1346-50.

Clinical plus Color Doppler Assessment of Benign and Malignant Breast Diseases

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Abstract:

Background: Tumor growth and metastases require the development of new vessels (angiogenesis), and the extent of angiogenesis predicts metastases and correlate with early death. Recently developed color Doppler mapping can detect the tumor flow signals in breast cancer and help to distinguish it from benign lesions.

Objective: Evaluation of differences between the blood supply in benign and malignant breast lesions by the use of color Doppler image assessment of the lesions vascularity.

Method: Clinical assessment, ultrasound examination and color Doppler mapping were done for 83 female patients with breast lesions. The following flow data analyses were undertaken; presence or absence of color Doppler signal, number of blood vessels, architectural arrangement and the maximum systolic velocity of blood vessels in the lesions and around it.

Results: Histopathological reveals that 21 cases had carcinoma of the breast and 62 had benign lesions. 27 patients provisionally diagnosed as having malignant lesions; of them 15 cases were truly malignant (sensitivity: 71.5%) and 56 benign one; of them 50 lesions were truly benign (specificity: 80.6%).

An increasing number of blood vessels found in malignant lesions; (85.7%) showed more than 3 vessels in a given lesion, while this figure found only in (8%) of benign lesions. For a cutoff of more than 3 vessels sensitivity and specificity are (85.7%), (91.9%) subsequently.

Architectural differences in the form of penetrating central neoplastic vessels were present in 18 (85.7%) of the malignant lesions with sensitivity (90.4%), while only in 3 (6.1%) of the benign lesions with specificity (93.8%).

The maximum systolic velocity of tumor vessels showed an increasing velocity in malignant lesions, for a cutoff more than 15 cm/s the sensitivity was (90.4%) and specificity was (91.8%).

Combination of the three parameters gave (90.4%) sensitivity and (96.7%) specificity.

Conclusion: Color Doppler imaging considered as an adjuvant primary investigation tool in addition to the ultrasound examination in improving differential diagnosis of breast lesion.

Keywords: Color Doppler, Breast diseases, Benign, Malignant.

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Introduction:

Although an accurate history and clinical examination are important methods of detecting breast diseases, imaging procedure such as mammography and ultrasound which play an established role in the investigation of breast masses [(1)], these methods are limited by their inability to answer questions about the likely growth potential of suspicious breast masses [(2)].

Since advent of color Doppler sonography, however, the visualization of blood flow deep within the breast becomes practicable [(3)].

By demonstrating blood vessel architecture and measuring blood flow parameters, the color Doppler technique has the potential to determine the tumor's tendency towards malignancy [(4)].

Doppler flow in malignant breast lesions is enhanced, so Doppler flow signal can be used to detect increased flow and may further distinguish benign from malignant lesions. Malignant lesions produce signals of high frequency and amplitude with continuous flow through diastole [(5)].

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Vasculature of neoplasm (Tumor angiogenesis) is one of the most important factors other than cell kinetics modifies the rate of tumor growth. Tumor cannot enlarge beyond 1-2 mm in diameter unless they are vascularized, presumably the 1-2 mm zone represent maximal distance across which oxygen and nutrient can diffuse from blood vessel. Neovascularization has dual effect on tumor growth; perfusion supplies nutrients and oxygen, and newly formed endothelial cell stimulate the growth of adjacent cells by secreting polypeptides such as insulin like growth factor, PDGF (Platelet Derived Growth Factor), IL-1 [(6)].

Angiogenesis is a request not only for continued tumor growth; the tumor cells cannot metastasis with out access to vasculature. Several studies have revealed a correlation between the extent of angiogenesis (micro-vessel density) and probability of metastasis in melanomas, lung, colon, prostate, and specifically breast cancers, so vessel density has proven to be significant prognostic indicator [(6)].

In addition the architecture of tumor's vascular supply is a reflection of it's propensity towards malignancy, and is a marker of tumor aggressiveness and correlated with an over all poor prognosis [(7)].

Tumor vessels grow to produce a disorderly tangle and random orientations [(4)]. However, because of focal area of narrowing and dilatation within tumor vessels, focal area of high systolic velocity results [(8)].

Tumor cells in lymph node also stimulate angiogenesis in the surrounded tissue to invade enlarged lymph node resulting in an extrahilar vessel leading to abnormal architectural vasculature that can be assessed by color Doppler for accurate staging of lymph nodes [(9)].

Color flow mapping; two dimensional color flow mapping system which none invasively maps intra organ blood flow in that previously could only be done with angiography

Positive Doppler shift (flow toward the transducer) displays in shades of red through orange to yellow where as negative shifts (flow away from the transducer) are displayed in blue to blue green [(10)].

Materials and Methods:

Eighty-three female patients with breast lesions included in this prospective study were presented to the consultant unit. After a detailed history and thorough physical examination, patients were assessed by imaging and laboratory investigations.

All patients were examined sonographically, then color Doppler image studies were performed using available KRETZ Techink voluson 530 OD machine versa pro utilizing 7.5 MHz linear array prop.

After full examination of breast and axilla, the mass identified and kept with field of color Doppler flow mapping,

The following parameters were assessed

1.Presence or absence of color Doppler signal and counting the total number of blood vessels in and adjacent to the lesion (mass).

2.Vascular architecture in relation to the lesion (mass), vessels were classified as either peripheral as in (Fig. 1) or central penetrating (neoplastic vessel) as in (Fig. 2).

3.Maximal systolic velocity (of that lesion or mass) measured from the time velocity diagram, (Fig.3), (Fig.4).

Color Doppler flow mapping of axillary Lymph Nodes were done for patients with palpable enlarged axillary Lymph Nodes, any abnormal signals were recorded as in (Fig. 5).

Biopsy examinations were done for the lesions after appropriate surgical management depending on clinical, imaging, and cytological examinations.

Statistical Analysis:

Sensitivity measures fraction of patients with malignant breast disease detected by the test under study.

Specificity measures the fraction of patients correctly identified as having

benign breast lesions and proved by histopathology.

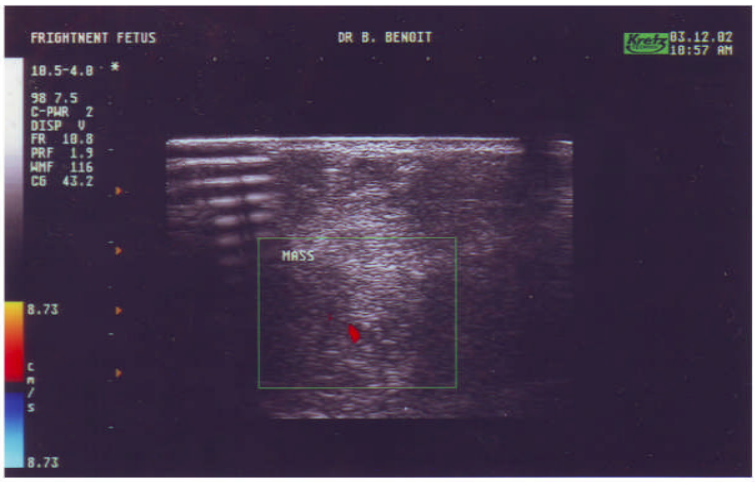

Figure 1: Peripheral feeding vessel (red spot) out side the tumor.

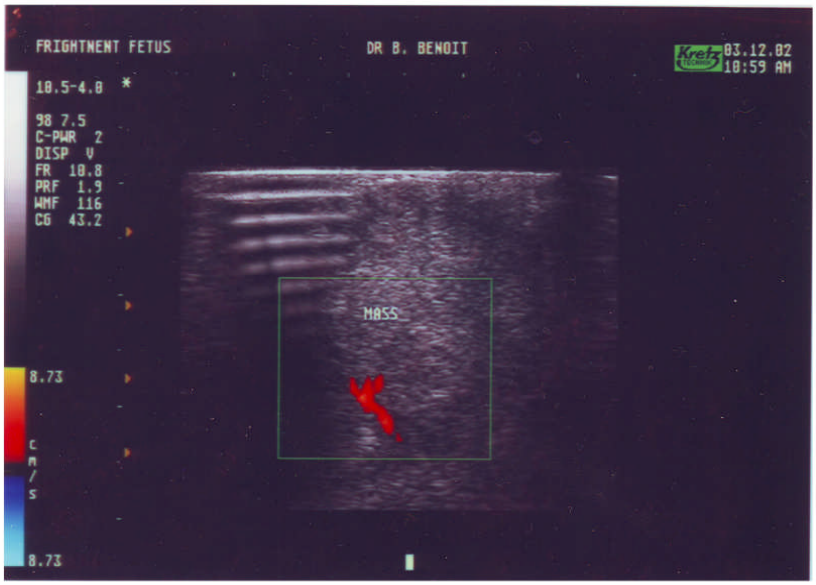

Figure 2: Central penetrating neoplastic vessel (red line) within the nidus of tumor.

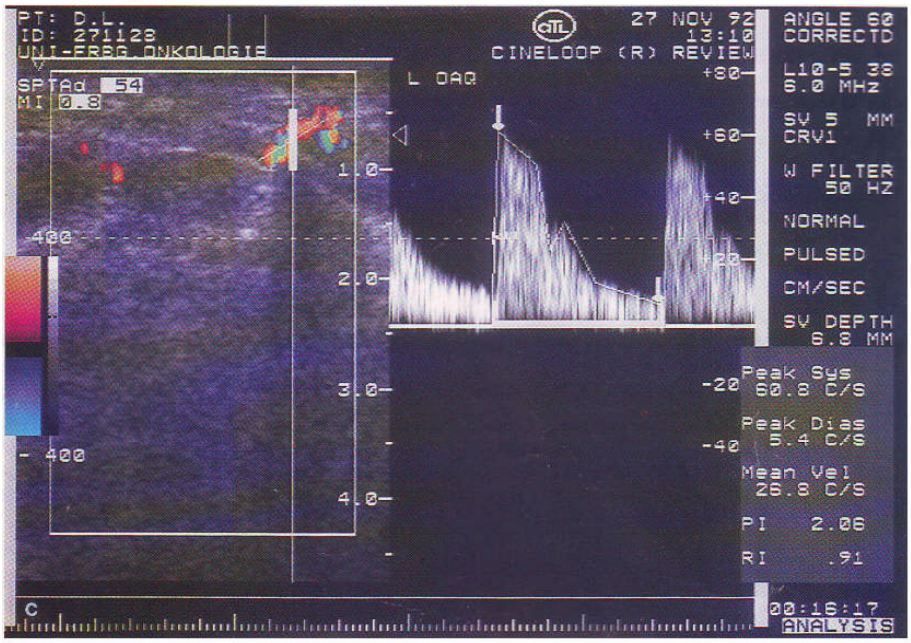

Figure 3: Maximal systolic velocity in malignant breast lesion.

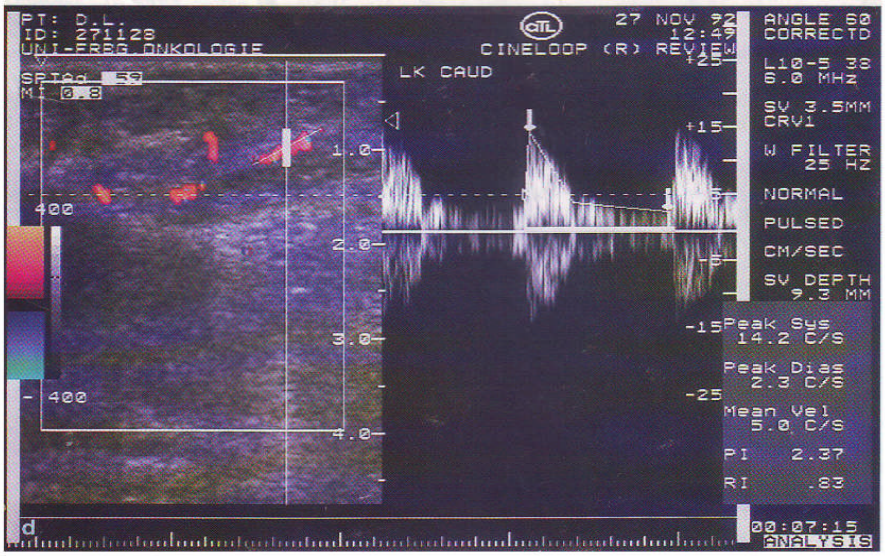

Figure 4: Maximal systolic velocity in benign breast lesion.

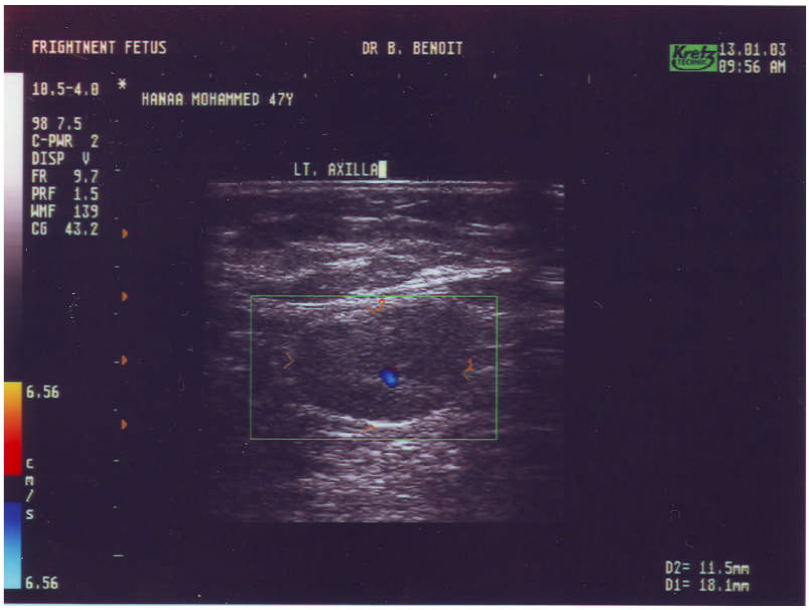

Figure 5: Color Doppler imaging of an enlarged left axillary LN in patient with CA breast showed a central vessel (blue spot).

RESULTS

Three female patients included in this study, histopathological findings showed that 62 samples were benign lesions and 21 were malignant

Based on the clinical findings; 56 patients provisionally diagnosed as having benign lesions, of them 50 patients were truly benign by histopathological examination, whereas 27 patients provisionally diagnosed as having malignant lesions; 15 patients of them proved malignant by histopathology, **Sensitivity** 71.5%, **Specificity** 80.6%

malignant lesions it was central (neoplastic) vessel in 19 lesions & Lesions

; (1 was scrrihous carcinoma & 1well differentiated ductal carcinoma).

The 49 vascular benign lesions detected by color Doppler image showed central neoplastic vessels in only 3 lesions (1 hyperplasia, 1 giant fibroadenoma & 1chronic abscess).

Sensitivity was (**90.4%**), while specificity was (**93.8%**).

3. Maximal systolic velocity; of the lesions blood flow measured by time

Of the eighty

Color Doppler image:

1. Number of blood vessels; in all malignant lesions color Doppler signals were detected, in 18 cases the number of blood vessels were >3, other cases showed <3 vessels, while in benign group; signals were detected in 49 cases, only 5 of them have >3 vessels (Figure. 6).

For a cut off of more than 3 vessels, the sensitivity for detecting cases with carcinoma was (**85.7%**) while specificity was (**91.9%**).

2. Vascular architecture; in peripheral (curvilinear) in the remaining 2

velocity scale in centimeters per second (Figure. 7).

For a cutoff more than 15 cm/s; sensitivity was (**90.4 %**), while specificity was (**91.8 %**).

Color Doppler imaging of axillary lymph nodes; in 16 patients had palpable axillary lymph nodes, signals were positive in 9 cases, all of them were malignant on histological

examination, in the other 7 cases; signals were negative, of them 4 benign and 3 malignant cases, so; Sensitivity **75%,** Specificity **100%.**

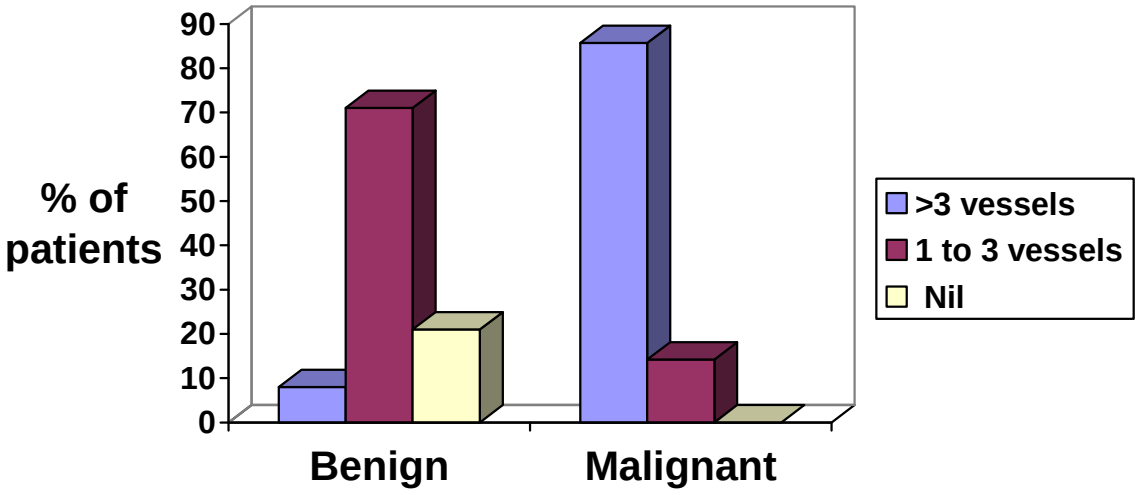

Figure 6: Distribution of the lesions according to the number of blood vessels.

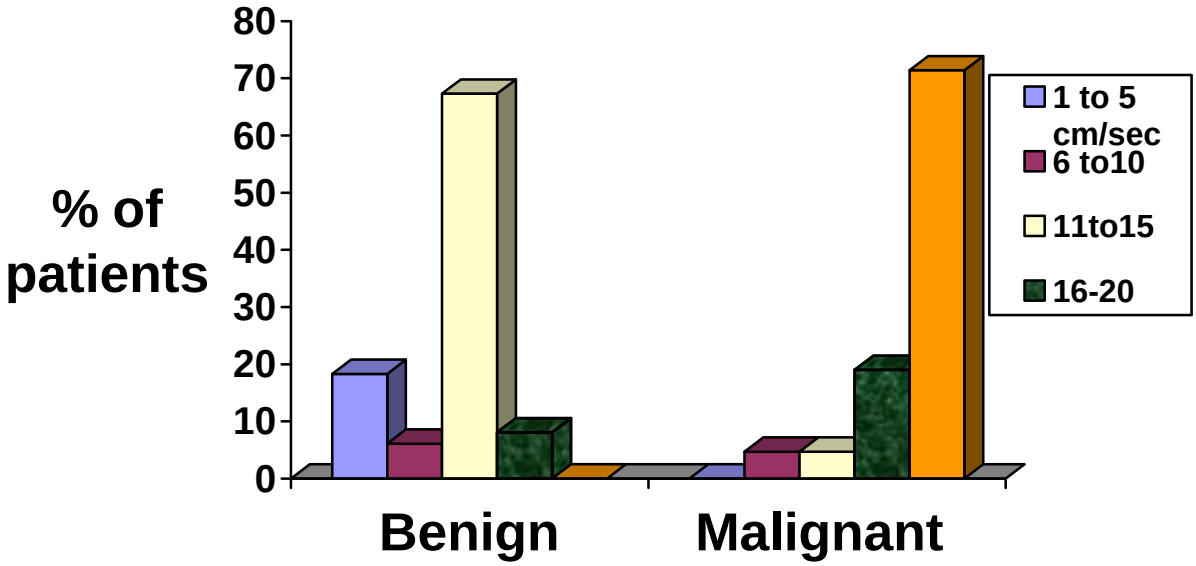

Figure 7:Distribution of lesions according to the maximum systolic velocity cm/s.

Discussion

According to the clinical evaluation, our provisional diagnosis revealed a sensitivity (71.6%) and specificity (80.5%), which is comparable to the results of a study done in Baghdad Teaching Hospital in 2001; sensitivity (73.68%) and specificity (89.4%) [(11)].

Color Doppler examination is diagnostically useful in identifying solid nature of markedly hypo-echoic malignant lesion, however the vascularity is highly increased in cases of mastitis where patient management should not be based on Doppler findings, in addition Doppler artifact can cause false positive results and this must be taken into consideration;

movement of the transducer or the patient, breathing, heart motion and fluid movement in a cyst, microcalcification must be differentiated from true signals caused by blood cells movement [12].

In our study the detected signals were demonstrated in all malignant masses (100%), and majority (79%) of benign lesions, in a study done by Wei-Jei Lee et al, in National Taiwan Hospital (department of surgery) showed that signals were detected in (92%) of malignant one and (54%) of benign lesions [13]. Other study showed absence of vascularity only in (4%) of malignant lesions [12].

In this study more than three blood vessels were found in (85.7%) of malignant lesions and only (8%) of benign cases, in comparison with a study [12]; (89%) of malignant tumor showed more than three vessels.

McNicholas et al, evaluate the number of blood vessels; they found that three and more blood vessels present in (83%), (46%) of malignant and benign lesions subsequently [14].

We found that a cut off more than three tumor vessels, the sensitivity, specificity were (85.7%) and (91.9%) subsequently, compared with literature; they were (89%) and (92%) subsequently [12].

Regarding the vascular architecture, a centrally penetrating vessel (neoplastic vessel) is a character of malignancy, it was found in (90.5%), (6.1%) of malignant and benign lesions subsequently. Wei-Jei Lee found that signal was central in 83% of malignant lesions, while all benign lesions show only peripheral signals [13].

The maximal systolic velocity is recommended as the best parameter in the differentiation of benign from malignant tumor by Dock [14], they suggest that systolic peak flow more than 20 cm/s is the best cut off value. In our study; a peak flow of more than 15 cm/s found in 19 of 21 malignant lesions (sensitivity 90.4%, specificity 91.8). Wei-Jei lee found that for a systolic peak of more than 15 cm/s 26 of 28 (93%) patients had malignant lesion [13]. Other study done by Madjar et al, they found that a cut off value more than 15 cm/s has (86%), (91%) sensitivity and specificity respectively [15].

The number of blood vessels detected by color flow mapping has been identified as the easiest parameter for describing tumor vascularity, but diagnostically it is not entirely reliable as the flow velocity [16].

In cases with positive signals axillary lymph nodes; the original primary lesions had a maximal systolic velocity > 20cm/sec; including 3 cases with tumor size about 3 cm. indicating that high velocity is associated with early metastatic potential rather than the size of primary [12].

Conclusion:

Color Doppler examination is a complementary tools to the clinical evaluation and other investigations especially the ultrasound.

Based on vascular architecture, number of blood vessels, systolic velocity within the lesions, a provisional diagnoses whether a lesion is benign or malignant, could be suggested with high accuracy.

The presence of high flow tumor signal in early breast carcinoma is significantly associated with the presence of axillary lymph nodes metastases.

Recommendation :

Standardization of the color Doppler examination technique and equipment parameter is essential for vascularity assessment of tumors.

References:

1. Fornage BD, Sneige N, Faroux MJ, Andry E. Sonographic appearance and ultrasound-guided fine-needle aspiration biopsy of breast carcinomas smaller the 1 cm^3. J ultrasound Med 1990; 9: 559-568.

2. Duda VF, Rode G, Schlief R. Echocontrast agent enhanced color flow imaging of the

breast. Ultrasound Obstet Gynaecol 1993;3: 191- 194.

3. Mattery RF, Steinbach GC. Ultrasound contrast agents: state of the art. Invest Radiol 1991; 26: 5-11

4. Cosgrove DO, Kedar RP, Bamber JC et al. Breast diseases: color Doppler US in differential diagnosis. Radiology 1993; 189: 99-104.

5. Rush BF. Breast, In: Schwarz S.I., Shires GT, Spencer FC, Eds. Principles of Surgery, 7th ed., New York: McGraw Hill Inc., 1999: 546.

6. Ramzis C, Vinay K, Tucker C. Robbins Pathologic basis of diseases, 6th ed, Philadelphia, W. B. Saunders company, 1999: 104-106.

7. Folkman J, Hochberg M. Self-regulation of growth in three dimensions. J Exp Med 1993; 138: 745-753.

8. Arthur C, Fleischer MD, Kevin R et al. Sonographic Depiction of ovarian vascularity and flow, current improvement and future applications of ultrasound in medicine. Journal of ultrasound in medicine March 2001: 20: 241-250.

9. Takako MD, Yukio MD, Yamagishi MD, et al. Color Power Doppler Sonographic Differential diagnosis of superficial lymphadenopathy (Metastases, Malignant lymphoma and benign process). Journal of ultrasound in medicine May 2001:20: 225-231.

10. Edward G, Grant E.Mureen White. Doppler physics. In Duplex Sonography, 1st Ed, New York Inc, Springer-verlay, 1988: 1, 73-74.

11. H Al-Kawaz. The Triple Assessment (Clinical Evaluation, FNAC and Imaging) In Management of Discrete Breast Lesions. A thesis submitted to the Iraqi Commission for Medical Specialization, General surgery, 2002:34-35.

12. Mandjar H, Sauerbrei W, Heinrich J, et al. Color Doppler and Duplex Flow Analysis for classification of Breast Lesions. Gynaecologic Onclogy 1997; 64:392-403.

13. Wei- Jei Lee, Jan-Show Chu, Shyh- Jinn Houng, et al. Breast Cancer Angiogenese: A Quantitative Morphologic and Doppler Imaging Study. Annals of Surgical Oncology, 1995; 2(3):246- 251.

14. McNicholas MMJ, Mercer PM, Miller JC, et al. Color Doppler sonography in the evaluation of palpable breast masses. AJR, 1993; 161: 765-771.

15. Madjar H, Schlief R, Schurmann R, et al. Differentiation of breast lesions by Color Doppler and Duplex measurements. Ultrasound Med Biol 1991; 17: 31-39.

16. Madjar H, Prompeler HJ, Sauerbrei W, et al. Color Doppler flow criteria of breast lesions. Ultrasound Med Biol 1994; 20(9): 849-858.

Ovarian tissue transplantation: A new method and site for induction of folliculogenesis in mice as a model for human female

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Abstract:

Background: Ovarian tissue transplantation is a new method of restoring fertility to women whose ovaries are not functioning normally. Young women who undergo chemotherapy or radiotherapy for cancer face serious consequences to their reproductive health and severely affect the ovarian follicular store, especially.

Objective: the aim of this study was to demonstrate induction of the folliculogenesis from ovarian tissue (OT) transplanted under kidney capsule in the presence or absence of gonadotropins support.

Materials and Methods: Forty-eight healthy female mice were anesthetized and abdominal cavity is open. From one side of the body, small piece (~1 X 1 X 1 mm) of OT was transplanted to the subcapsular membrane of kidney at another side, and surgical operation is closed. Then, female mice were classified into three groups according to the time of gonadotropins injection. Group-1: mice injected with sterile normal saline (control group). Group-2: mice injected with gonadotropins directly for four days. Group-3: mice injected with gonadotropins for four days after eight days of surgical operation. Follicular growth, quality of retrieved ova and histological changes for transplanted OT were assessed.

Results: In general, no deletion for transplanted OT pieces and no side effects post-operation on mice of all groups were recorded. Best follicular growth of transplanted OT was achieved for groups 1 and 2. Graafian follicles were obtained from transplanted OT of group-2, and less degree for group-1. However, least degree for follicular growth of transplanted OT was reported for group-3 as compared to other groups. Immature and mature oocytes with corona and cumulus cells collected by squashing of transplanted OT.

Conclusions: The present data demonstrate that the ovarian tissue transplantation is possible to undergo follicular growth subcapsular of the kidney. Also, physiology of the body supports the ovarian follicular growth in another site other than normal position. Further studies are recommended on *in vitro* maturation and fertilization of retrieved ova and embryo transfer.

Keywords: Mice, Ovary, Transplantation, Folliculogenesis, Gonadotropin.

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Introduction:

Therapeutic advances in the treatment of infertility are leading to improved survival and cure. One problem is that exposure of ovaries and uterus to radiotherapy and chemotherapy in childhood and during the reproductive years predisposes to ovarian failure and permanent uterine damage [1,2]. In addition, a significant number of women experience early menopause due to oophorectomy performed for benign indications [3]. Ovarian tissue transplantation (OTT) has been practiced for over a decade, mainly for experimental purposes. It is now being considered as a potential strategy for preserving fertility in young patients. These strategies have been demonstrated

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in animal models and are now undergoing clinical testing [4,5].

It was suggested that the OTT is a new method of restoring fertility to women whose ovaries are not functioning normally. However, after OTT, follicular development and restoration of hormone secretion have been observed in animal and human studies [6]. It was reported "if ovarian transplantation is proven to be safe and effective in humans, fertility preservation might become readily available for young women who need to delay childbearing for medical or social reasons" [2].

A promising field with respect to preservation of gonadal function and fertility is ovarian cortex transplantation. The ability of ovarian allografts to restore fertility has been demonstrated in several species. Furthermore, OTT was achieved within several sites of the body [7]. In recent years, a resurgence in the field of human ovarian transplantation has occurred, with several teams reporting sporadic cases of both fresh and cryopreserved OTT in different clinical scenarios [8]. Therefore, the aim of this study was to demonstrate induction of the follicular development from ovarian tissue transplanted under capsule of the kidney in the presence or absence of gonadotropins support.

Materials and Methods:

1. Animals

Forty-eight healthy adult female mice of Swiss albino strain (age: 8-10 weeks; weight: 25-28 g) were obtained from the animal house at IVF Institute of Embryo Research and Infertility Treatment / Al-Nahrain University and used in this study. Female mice were kept under suitable environmental conditions such as the room temperature was maintained at $24 \pm 2\ ^{o}C$ and exposed to 14-hour day light program. Tap water and food in the form of pellet were accessible freely, *Ad libitum*, to the animals.

2. Ovarian tissue transplantation (OTT) technique

After induction of general anesthesia with intraperitoneal (IP) injection of 100 µL of sodium pentobarbital (Nembutal, Sanofi, France) diluted 1:4 in normal saline. The procedure of OTT used was modified from the method of Gosden *et al*. [9]. Abdominal cavity of female mouse was opened and small pieces of OT cortex (~1 X 1 X 1 mm) were taken from right ovary. The left kidney was exteriorized and small hole torn in the kidney capsule using fine forceps. Only, one piece of OT was transplanted under capsule of left kidney. Finally, abdominal cavity was closed and Aerospray (Chloranfenical, Calier Laboratories, Spain) and Agrsunt (Sulphanilamide, Agropharm Limited Co., Bucks, UK) were used to cover the wound and prevent microbial infection and inflammation. Then female mouse was placed in clean cage for monitoring and special cure until reversed from anesthesia.

3. Experimental design and ovarian stimulation program

Forty-eight female mice were conducted surgical operation for OTT and randomly divided into three groups according to time of gonadotropins injection. Group-1 (G-1; control group) females injected with 0.1 ml normal saline for four days. Group-2 (G-2) females injected with gonadotropins for four days program post-surgical operation directly. Group-3 (G-3) females injected with gonadotropins for four days program after eight days of OTT and surgical operation. Ovarian stimulation program involves IP injections 5 IU/day FSH (Gonal-F, Serono, Italy) for first three days and 5 IU hCG (Profasi, Serono, Italy) at forth day. Pieces of transplanted OT were recovered after 7-8 hours of last injection for either histological processing and examination or ova retrieval and classification.

4. Histological examination and ovarian follicles assessment

Upon the completion of ovarian stimulation program, six mice were killed by cervical dislocation and the pieces of transplanted OT were recovered and

placed in Bouin's fixative for at least 24 hour. Then, dehydrated by series of ethanol alcohol, cleared by xylene, embedded in paraffin wax (melting point 60 ^{o}C), serially sectioned at 5 μm thick and stained with Erlich haematoxylin and eosin [10].

The sections were examined under light microscope for assessment of different types of ovarian follicles and corpora lutea. Ovarian follicles were classified as follows: primordial, primary, pre-antral and antral follicles according to presence of flattened epithelial cells, granulosa cells and antral cavity [7,11]. Different types of ovarian follicles were counted under high power microscope.

5. Ova retrieval and classification

After 7-8 hours of last injection for 10 females, each piece of transplanted OT was recovered from subcapsular kidney with high care and washed with Minimum Essential Medium (MEM; Sigma, UK) twice. Then, piece of transplanted OT was minced in small Petri dish within MEM enriched with 20% bovine serum albumin (BSA) by using special fine forceps. Ova were collected and washed within MEM enriched with 10% BSA. After microscopically examination, ova were classified into immature, mature and atretic according to morphological features and presence of 1st polar body [12].

6. Statistical analysis

Data presented as mean ± standard error of mean (SEM). The crude data were statistically analyzed using statistical computerized package SPSS (Statistical package of Social Science, version-12) to compare among different means of groups by application of Chi-square analysis and ANOVA test. A *p* value of < 0.05 was considered statistically significant.

Results:

The procedure of OTT subcapsular kidney was easy conducted in mice without real difficulties are faced. However, the results of the present study appeared no lose for pieces of transplanted OT subcapsular kidney (Figure 1 A).

Examination the histological changes in transplanted OT (Figure 1 B and C) appeared best results for follicular development in G-2, and this result in regard to number of primordial, primary and antral follicles non significantly (P>0.05) increased as compared to control group (G-1). Meanwhile, significant (P<0.05) differences in the number of pre-antral follicles were noticed between G-2 and G-1 (Table 1). From the same table, the number of all types of ovarian follicles for G-3 revealed significant (P<0.05) reduction as compared to G-2 and G-1.

The results of ova retrieval and classification were presented in the figure (2). The numbers of immature and mature retrieved ova in the G-2 have the best result in the present study (Figure 1 D). Not significant (P>0.05) differences were noticed in the number of immature ova of G-2 when compared to G-1. In contrast, significant (P<0.05) differences in the number of mature ova were reported between G-2 and G-1. Statistically, significant (P<0.05) reduction in the number of immature and mature ova of G-3 as compared to G-2 and G-1. While, no significant (P>0.05) differences in the number of atretic ova among three groups (Figure 2).

Discussion:

Autotransplantation procedures have historically been limited almost exclusively to non-vascular cortex segments grafted to orthotopic or heterotopic locations [13]. It was reported that the human grafts contained large numbers of germ cells about 11000 primordial follicles, an amount that could provide oocytes for a year [14,15]. Here, we described a successful follicular development after ovarian tissue (OT) transplantation (OTT) subcapsular kidney in mice. Figure (1 A) shows the transplanted OT subcapsular kidney in mice. Performance of OTT was easy and has no problems for general health or no side effects on kidney. The same result was mentioned by Bedaiwy *et al.* [5].

Ovarian transplantation appears to be a relatively simple, novel technique to preserve endocrine function in women undergoing sterilizing cancer therapy or surgery [3,16].

The reproductive outcome of the ovarian transplantation depends on the degree of cell damage inflicted over the transplanted tissues upon harvesting, processing and subsequent transplantation [17]. Furthermore, transplantation of ovarian cortical tissue and hemiovaries gave rise to pregnancies/live birth in sheep [9], mouse intact ovaries [18] and rats [19]. The human trials have shown promising results that have some limitations that require some more research [5,15].

In the present work, female mice were killed after 7-8 hours of last injection for ovarian stimulation program to induce follicular development and maturity of oocytes without spontaneous ovulation (Figure 1 C and D). It was reported low dose of hCG support oocyte maturation, while ovulation needs high dose of hCG and required longer period reach 11-12 hour [20,21]. In our study, best follicular growth at all stages of transplanted OT was noticed in the G-2 whose female mice injected gonadotropins for four days program. However, no significant ($P>0.05$) differences in regard to primordial, primary and antral follicles were reported between G-2 and control group (G-1).

Our results following OTT in mice confirm the results of previous work carried out by Weissman and his colleagues [7], they reported that the follicular development after using combined gonadotropins, FSH and LH. Although it is not known what gonadotropin dose is required for ovarian follicles stimulation [8]. The current data demonstrated that the pre-antral and antral follicles were produced in G-1 from transplanted OT in spite of absence of exogenous gonadotropin support. We assume that this reflects presence of normal endogenous gonadotropin milieu and considered sufficient for follicular development. A similar finding was previously reported about importance of hypothalamic-pituitary-gonadal function on transplanted OT [22,23].

It was certified that the follicles do not progress beyond the stage of two granulosa cell layers without gonadotropin support [24]. However, different follicles of development were observed in the grafted tissue whereas, prior to grafting, only primordial and primary follicles were present [22]. Moreover, limited length of ovarian function in some human ovarian transplant cases using non vascularized grafts may be partially due to ischemic injury until revascularization occurs [5,25]. From the results of the present study, highly reduction in the number of primordial follicles after OTT combined with major loss of oocytes. Same result was noticed on dramatic reduction in the number of ovarian follicles [16]. Approximately 50% of the primordial follicle population survives in isologous grafts in mice [26].

From the results of this study, it is clear that the number of antral follicles is too little, which may be as a result of environmental and site effects on transplanted OT. Weissmen *et al.* [7] believed that the kidney capsules are unlikely to be able to support complete human preovulatory follicular development. Although, they mentioned that the OT under the kidney capsule has been shown to develop apparently normal antral follicles. However, it was reported that the follicular development from transplanted OT less than the normal physiologically status [27].

The results of the present study appeared that the number of pre-antral follicles for G-2 was significantly ($P<0.05$) increased as compared to G-1 and G-3. Also, all types of ovarian follicles and immature and mature ova for G-3 were decreased significantly ($P<0.05$) when compared to G-1 and G-2. The reduction in the number of ovarian follicles may be

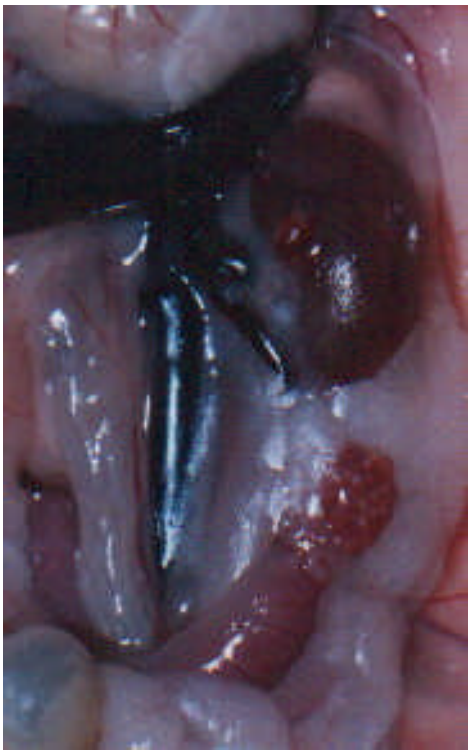
as a result of follicular atresia due to ischemia and apoptosis of primordial and primary follicles, especially [28]. Whether an ischemic insult of the ovarian tissues is associated with comparable alterations in special molecules is still unknown [17,29]. It was known that atretic follicles may reflect the preexisting atretic changes instead of the effect of OTT [5]. Also, it was certified that an important technical limitation of cortical grafting, whether orthotopic or heterotopic, is the potential for follicle atresia during the period of ischemia [2].

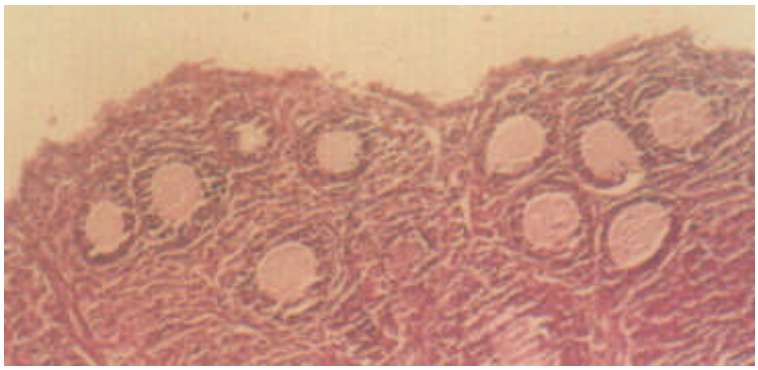
The number of immature ova retrieved from transplanted OT for G-2 was observed non significantly (P>0.05) increased as compared to control group (G-1). Functioning of the graft will be dependent on the initial pool of primordial follicles, the fraction of follicles surviving the grafting procedure, and the local conditions that affect the resting pool of follicles and the fraction of follicles that is recruited from the pool [15]. Knowing that every oocyte reaches full maturity after a fixed timespan and considering the inherent asynchrony in follicle recruitment from the reserve in women, the precise detection of the stage of oocyte maturation becomes an absolute requirement for normal fertilization and embryo development [30].

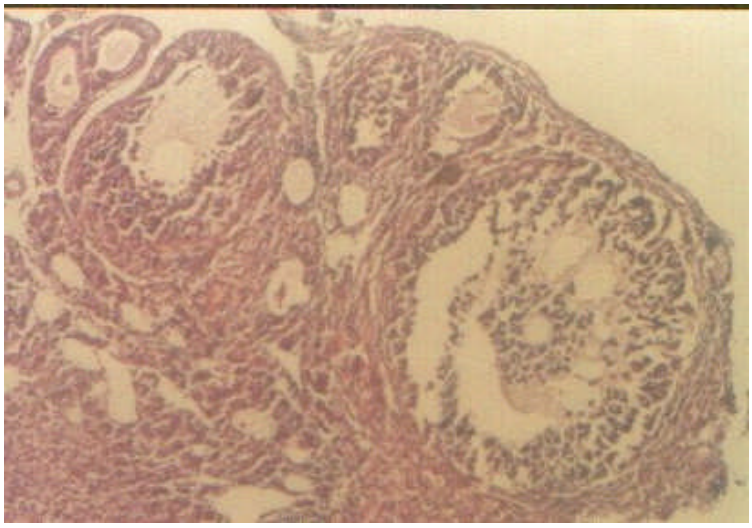
Results of this study appeared that the number of mature ova for G-2 was significantly (P<0.05) increased when compared to G-1 and G-3. Also, significant (P<0.05) reduction was reported in the number of mature ova for G-3 as compared to G-1. The possible explanations for this result are: use of four days ovarian stimulation program causes an increased folliculogenesis and oocytes maturity, but the timing start of this program for G-3 may be reduce outcome and efficiency of transplanted OT as far as from process of OTT. Same result was mentioned by Silber *et al*. [2] who reported that the heterotropic grafts of monkey cortical tissue can generate mature oocytes for IVF. Moreover, several alterations will obviously interfere with the availability of nutrients, hormones and oxygen, which are all essential to obtaining a developmentally competent oocyte [16,27].

Non significant (P>0.05) differences in the number of atretic ova was assessed among three groups of this study. This result may be partially due to technical method for OT recovery and/or retrieval of oocytes. Same result was mentioned by Smitz [15] who reported that the transplant site might it self influence follicle recruitment and atretia. Furthermore, presence of atretic and/or abnormal oocytes, in the present study, may be reflecting wide range of fluctuation of gonadotropins and/or inadequate exposure to gonadotropins which led to abnormal follicular development and subsequently produce abnormal and atretic oocytes. This result is in agreement with results of several investigators [16,18,27]. In contrast, it was mentioned that the elevation level of FSH produces high numbers of abnormal and atretic oocytes [31].

Therefore, the present data demonstrate that the transplantation of ovarian tissue is possible to undergo follicular growth subcapsular of the kidney. Also, physiology of the body supports the follicular development in another site other than normal position. Further studies are recommended on *in vitro* maturation and fertilization of retrieved ova and embryo transfer.







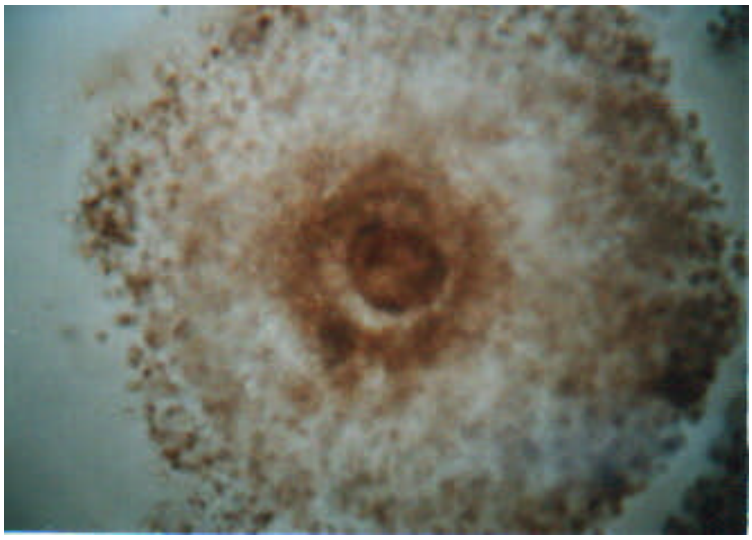


Figure 1: Mice ovarian tissue; (A) transplanted subcapsular kidney, (B+C) processed for histological examination and assessment of follicular development, (D) produced ovum with cumulus cells (Hematoxylin and eosin stain; 100 X).

Table 1: Numbers of different types of ovarian follicles from transplanted ovarian tissues * for three groups of female mice

Types of ovarian follicles	Groups of female mice		
	G – 1; control	G – 2	G – 3
Primordial follicles	7.166 [a] ± 0.771	7.833 [a] ± 0.711	4.50 [b] ± 0.748
Primary follicles	3.33 [a] ± 0.581	4.50 [a] ± 0.339	1.833 [b] ± 0.249
Pre-antral follicles	1.833 [a] ± 0.388	2.666 [b] ± 0.452	0.833 [c] ± 0.249
Antral follicles	1.166 [a] ± 0.249	1.833 [a] ± 0.249	0.50 [b] ± 0.163

*: Number of transplanted OT= 6.
**: Similar letters means non significant (P>0.05) differences.
Different letters means significant (P<0.05) differences.

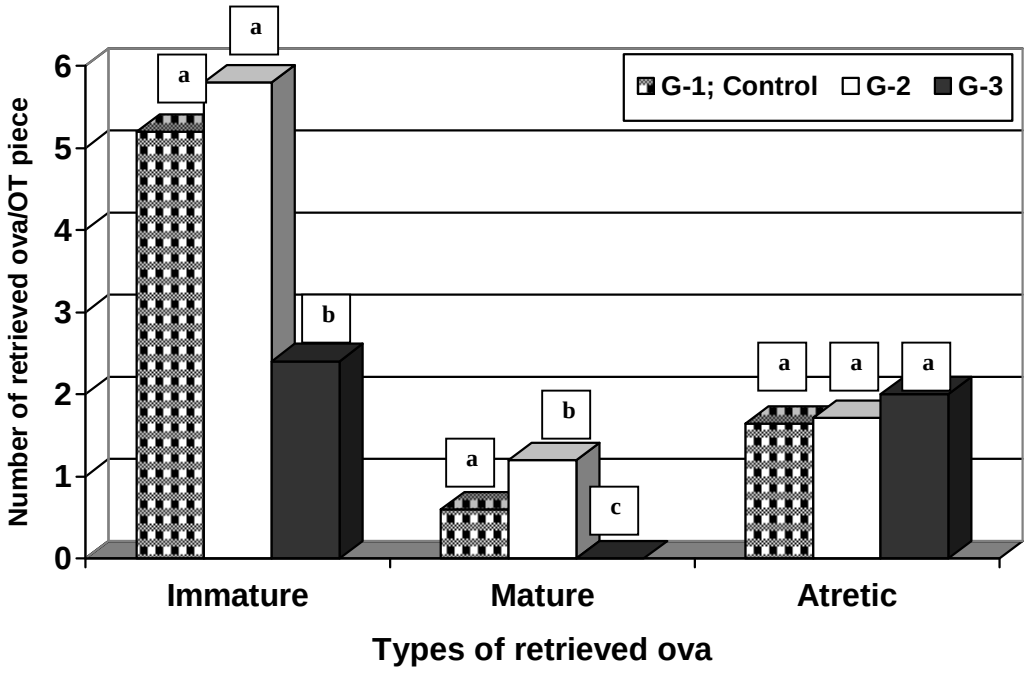


Figure 2: Number of retrieved ova from ovarian follicles of transplanted ovarian tissues * for three groups of female mice

*: Number of transplanted OT= 10.
**: Similar letters means non significant (P>0.05) differences.
Different letters means significant (P<0.05) differences.

References:

1. Critchley HO, bath LE and Wallace WH. (2002). Radiation damage to the uterus-review of the effects of treatment of childhood cancer. Hum. Fertil. 5:61-66.

2. Silber SJ, Lenahan KM, Levine DJ, Pineda JA, Gorman KS, Friez M.J, Crawford EC and Gosden R.G (2005). Ovarian transplantation between monozygotic twins discordant for premature ovarian failure. N. Engl. J. Med. 353: 1-6.

3. Oktay K, Economos K, Kan M, Rucinski J, Veeck L. and Rosenwaks Z. (2001). Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. JAMA. 286:1490-1493.

4. Gosden R. (2001). Gonadal tissue cryopreservation and transplantation. Reprod. BioMed. Online. 4 (suppl.1): 64-67.

5. Bedaiwy MA, Jeremias E, Gurunluoglu R, Hussein MR, Siemianow M, Biscotti C. and Falcone T. (2003). Restoration of ovarian function after autotransplantation of intact frozen-thawed sheep ovaries with microvascular anastomosis. Fert. Steril. 79: 594-602.

6. Torrents E, Boiso I, Barri PN. and Veiga A. (2003). Applications of ovarian tissue transplantation in experimental biology and medicine. Hum. Reprod. Update. 9:471-481.

7. Weissman A, Gotlieb L, Colgan T, Jurisicova A, Greenblatt EM. and Casper R.F. (1999). Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. Biol. Reprod. 60: 1462-1467.

8. Schnorr J, Oehninger S, Toner J, Hsiu J, Lanzendorf S, Williams R. and Hodgen G. (2002). Functional studies of subcutaneous ovarian transplants in non-human primates: steroidogenesis, endometrial development, ovulation, menstrual patterns and gamete morphology. Hum. Reprod. 17:612-619.

9. Gosden RG, Bair DT, Wade JC. and Webb R. (1994). Restoration of fertility to oophorectomized sheep by ovarian autografts stored at – 196 ^{o}C. Hum. Reprod. 9: 597-603.

10. Bancroft J.D. and Stevens A. (1982). Theory and practice of histological techniques. Churchill, Livingston, London, UK. 2nd edition. Pp: 110-111.

11. Jeremias E, Bedaiwy M, Gurunluoglu R, Biscotti CV, Siemionow M. and Falcone T. (2002). Heterotopic autotransplantation of the ovary with microvascular anastomosis: a novel surgical technique. Fertil. Steril. 77: 1278-1282.

12. Veeck L.L. (1986). Morphological estimation of mature oocytes and their preparation for insemination. In: *In vitro* fertilization-Norfolk. Jones H.W.; Jones G.S.; Hodgen G.D. and Rosenwaks Z. (eds.). Williams and Wilkins, Baltimore, MD 21202, USA. Pp: 81-93.

13. Nugent D, Meirow D, Brook PF, Aubard Y. and Gosden R.G. (1997). Transplantation in reproductive medicine: previous experience, present knowledge and future prospects. Hum. Reprod. Update. 3:267-280.

14. Shaw J.M, Bowles J, Koopman P, Wood E.C. and Trounson A.O. (1998). Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum. Reprod. 11: 1668-1673.

15. Smitz J. (2004). Oocyte developmental competence after heterotopic transplantation of cryopreserved ovarian tissue. The Lancet, Comment. 1-2.

16. Baird DT, Webb R, Campbell BK, Harkness LM. and Gosden R.G. (1999). Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at – 196 C. Endocrinol. 140: 462-471.

17. Hussein MR, Bedaiwy MA. and Falcone T. (2006). Analysis of apoptotic cell death, Bcl-2 and p53 protein expression in freshly fixed and cryopreserved ovarian tissue after exposure to warm ischemia. Fertil. Steril. 85:1-12.

18. Candy CJ, Wood MJ. and Whittingham D.G. (2000). Restoration of a normal reproductive lifespan after grafting of cryopreserved mouse ovaries. Hum. Reprod. 15: 1300-1304.

19. Aubard Y, Newton H, Scheffer G. and Gosden R. (1998). Conservation of the follicular population in irradiated rats by the cryopreservation and orthotopic autografting ovarian tissue. Eur. J. Obstet. Gynecol. Reprod. Biol. 79: 83-87.

20. Leonardsson G, Jacobs MA, White R, Jeffery R, Poulsom R, Milligan S. and Parker M. (2002). Embryo transfer experiments and ovarian transplantation identify the ovary as the only site in which nuclear receptor interacting protein 1/RIP140 action is crucial for female fertility. Endocrinology. 143: 700-707.

21. Fakhrildin M-B. M-R, Abdul-Majeed M.R. and Sulaiman B. K. (2006). Effect of different superovulation programs and culture media on quality, *in vitro* maturation and fertilization of mice oocytes. Dirasat, Pure Scienc. 33: 60-69.

22. Gook DA, McCully BA, Edgar DH. and McBain J.C. (2001). Development of antral follicles in human cryopreserved ovarian tissue following xenografting. Hum. Reprod. 16: 417-422.

23. Pakarainen T, Zhang F-P, Poutanen M. and Huhtaniemi I. (2005). Fertility in luteinizing hormone receptor-knockout mice after wild-type ovary transplantation demonstrates redundancy of extragonadal luteinizing hormone action. J. Clin. Invest. 115: 1862-1868.

24. Oktay K, Newton H, Mullan J. and Gosden R.G. (1998). Development of human primordial follicles to antral stages in SCID/hpg mice stimulated with follicle stimulating hormone. Hum. Reprod. 13: 1133-1138.

25. Revel A. and Schenker J. (2004). Ovarian tissue banking for cancer patients: is ovarian cortex cryopreservation presently justified ? Hum. Reprod. 19: 14-19.

26. Liu J, Van der Elst J, Van den Broecke R. and Dhont M. (2001). Live offspring by *in vitro* fertilization of oocytes from cryopreserved primordial mouse follicles after sequential *in vivo* transplantation and *in vitro* maturation. Biol. Reprod. 64: 171-178.

27. Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla J.M. and Balasch J. (2001). Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. J. Clin. Endocrinol. Metab. 86: 4489-4494.
28. Kim S.S. (2003). Ovarian tissue banking for cancer patients: To do or not to do. Hum. Reprod. 18: 1759-1761.
29. Aubard Y, Piver P, Cogni Y, Fermeaux V, Poulin N. and Driancourt M.A. (1999). Orthotopic and heterotopic autografts of frozen-thawed ovarian cortex in sheep. Hum. Reprod. 14: 2149-2154.
30. Smitz J.E. and Cortvrindt R.G. (2002). The earliest stages of folliculogenesis *in vitro*. Reproduction. 123:185-202.
31. Falcone T, Attaran M, Bedaiwy MA. and Goldberg J.M. (2004). Ovarian function preservation in the cancer patient. Fertil. Steril. 81: 243-257.

Study of rubella antibody levels among mothers and their newborn babies following normal delivery versus mothers and their newborn babies following cesarean section

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Abstract:

Background: *Rubella* is generally asymptomatic in healthy adult, but during the first trimester of pregnancy often leads to fetal death or severe congenital defect, so it is considered as an important public health problem.

Objective: This study was conducted to determine *rubella* antibody levels among group of women with normal delivery with their newborn babies and another group of mothers with cesarean section and their newborn babies, and its relation to various epidemiological, medical and obstetric problems

Method: Serum specimens of 166 women with vaginal deliveries and 32 women with cesarean section and their babies were screened for *rubella* antibody levels by Mico ELISA method.

Result: *Rubella* antibody levels were < 1.00 (optical density) in 54.2% of women with vaginal delivery (group one) and 71.9% of women in group two (women with cesarean section), Age (significant for group two), mother education, and crowding index were negatively correlated with antibody levels in both groups. Germen measles vaccination had negative correlation with *rubella* antibody titers in both groups (significant for group one), while weight of newborn babies was significantly correlated with *rubella* antibody titer among babies in-group two.

Conclusion: Weakly positive antibodies were found to be higher among women and their babies following cesarean section than the group of women with normal deliveries and their babies, negative correlation were found between antibody levels and age of mothers, educational level, crowding index.

Keywords: *Rubella* antibody levels and mothers at delivery.

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Introduction:

Rubella infection is generally an asymptomatic childhood disease but during the first trimester of pregnancy often leads to fetal death or severe congenital defect (*congenital rubella syndrome, CRS*) [(1)]. While the inclusion of *rubella* vaccination into routine childhood immunization will decrease *rubella* virus circulating among young children, it will not have immediate impact on the transmission of *rubella* among adults or the occurrence of CRS [(2)].

Worldwide, it is estimated that there are more than 100,000 infants born with *congenital rubella syndrome* (*CRS)* each year. In 1998, standard case definition for surveillance of *CRS* and *rubella* were developed by the World Health Organization (WHO) [(3)]. If applied appropriately, vaccination against *rubella* protects the susceptible person in 95% of the cases [(4)]. Nearly 50% of *CRS* can be prevented via vaccination of the sensitive women, based on the recommendations of Advisory Committee on Immunization Practices (ACIP) [(5)]. Enzyme Linked Immune Sorbent Assay (ELISA) is the most commonly preferred test against *rubella* antibodies in immunized subject [(6)].

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In Iraq *rubella* vaccination had been adapted in the MMR vaccine and also for schoolgirls, but there is no vaccination program for adult women or serological testing for *rubella* antibodies for pregnant women. Since 2003 many programs (vaccination programs is one of them), which were running regularly in our country, were affected by the current situation leading to missing of opportunities for having proper vaccination. Also antibodies following vaginal delivery suppose to be higher than those following cesarean section because of uterine contraction that lead to higher level of antibodies delivered to the baby during vaginal delivery. For these reasons and because of the bad consequence of this disease on the reproductive health of women this study was done to determine *rubella* antibody levels among group of women with normal deliveries and their newborn babies and another group with cesarean section and their newborn babies, and its relation to various epidemiological, medical and obstetric problems

Materials and subject:

The study sample was divided into two groups, group one includes 166 mothers with normal vaginal deliveries and their newborn babies, group two include 32 mothers delivered by cesarean section and their babies. Data on the demographic, socioeconomic characters of the family, medical & obstetric history of the mother during pregnancy was obtained through well-structured questionnaire form. Both groups were taken from the Al-Kadhimiya Teaching hospital during the period from December 2004- July 2005. Blood obtain from both mothers and babies to measure *rubella* specific IgG antibody levels via micro ELISA technique.

Standardization procedures were carried out for the antigen (*Rubella* Ag from Virion), conjugate (Antihuman IgG Fab specific, peroxides conjugate, Sigma), and antisera, the optical dilutions were found to be 1/10, 1/500, 1/2 respectively, ELISA test was used following the WHO standard method [7] using the above antigen in proper concentration for coating micro wells as a solid phase.

The test was carried out on the above samples in the Medical College\ Al-Nahrain University under the supervision of the microbiology department.

Sample values that lie below the cut-off value (mean negative +2SD) were considered negative, and those that were equal to or greater than cut-off value were considered positive. Because we did not have the reference standard to express the results in International units, the antibody levels to *rubella* (absolute optical density values) were divided into the following groups [10].

- Less than 1.00 = weak positive
- 1.00 -1.99 = positive
- Over 2.00 = strong positive

Analysis of data was done using SPSS statistical program version 11.0 to obtain frequencies, percentages, t- test and correlation test were done as test of significant, P value of ≤ 0.05 was considered significant

Result:

Rubella antibody levels were < 1.00 (optical density) in 54.2% of women with vaginal delivery (group one) and 71.9% of women in-group two (women with cesarean section), while it was the reverse for antibody titer of > 1.00 table (1). There were no significant differences between the mean *rubella* antibodies in-group one and two (P > 0.05) and again between babies in the two groups table (2). Majority of mothers in both groups were in the age group 20-29.9 years (58.4%, 50.0% respectively), also higher percentage of mothers in both groups were with education < 6 years (58.4%, 62.5% respectively), women in the second

group had higher crowding index in the 3-5, > 5 than those with normal deliveries (37/5%, 15.6% compare to 24.1% and 8.4%), history of germen measles vaccination, and urinary tract infection during pregnancy were higher in group two than group one (46.9%, 21.9% compare to 30.1, 10.8%), premature deliveries represent 9.0% in the study sample, all of them were in group one, and only one baby had birth weight < 2.5 kg among the second group compare to 10.8% in the first group table (3A&B).

Age (significant for group two), mother education, and crowding index were negatively correlated with antibody levels in both groups, germen measles vaccination had negative correlation with *rubella* antibody titers in both groups (significant for group one), while weight of newborn babies was significantly correlated with *rubella* antibody titer among babies in group two (table 4).

Table (1): Distribution of blood level of *antirubella* antibodies among group of mothers and their newborn babies following normal vaginal delivery and cesarean section

Variables	Normal vaginal deliveries		Cesarean section	
	Freq	Percent	Freq	Percent
Rubella antibody level\ mothers				
< 1.00	90	54.2	23	71.9
1- 1.99	45	27.1	5	15.6
≥ 2.00	31	18.7	4	12.5
Total	166	100.0	32	100.0
Rubella antibody level\ babies				
< 1.00	87	52.4	22	68.8
2- 1.99	47	28.3	5	15.6
≥ 2.00	32	19.3	5	15.6
Total	166	100.0	32	100.0

Table (2): Comparison between blood level of *antirubella* antibodies among group of mothers and their newborn babies with normal delivery and another group with cesarean section

Variables	Normal deliveries	Cesarean section	Significant
Rubella antibody levels\mothers			
Mean	1.23	0.96	T= 1.43
Minimum	0.06	0.05	P > 0.05
Maximum	3.10	2.67	
STD*	0.81	0.72	
ST Error**	0.06	0.13	

Rubella antibody levels\ babies			
Mean	1.27	0.98	T= 1.44
Minimum	0.01	0.20	P > 0.05
Maximum	3.10	3.10	
STD*	0.84	0.80	
ST Error**	0.07	0.14	

*= Standard deviation
**= Standard error

Table (3A): Distribution of mothers following normal delivery and cesarean section according to some demographic and socioeconomic variables

Variables	Normal deliveries		Cesarean section	
	Freq	Percent	Freq	Percent
Age\ years				
< 20	23	13.9	4	12.5
20-29.9	97	58.4	16	50.0
≥ 30	46	27.7	12	21.9
Total	166	100.0	32	100.0
Residency				
Urban	93	56.0	14	43.8
Rural	73	44.0	18	56.3
Total	166	100.0	32	100.0
Mother education				
< 6 years	97	58.4	20	62.5
≥ 6 years	69	41.6	12	37.5
Total	166	100.0	32	100.0
Crowding index				
< 3	112	67.5	15	46.9
3-5	40	24.1	12	37.5
> 5	14	8.4	5	15.6
Total	166	100.0	32	100.0

Table (3B): Distribution of mothers with normal delivery and cesarean section according to some obstetric and medical problems

Variables	Normal deliveries		Cesarean section	
	Freq	Percent	Freq	Percent
History of germen measles vaccination				
Yes	50	30.1	15	46.9
No	116	69.9	17	53.1
Total	166	100.0	32	100.0
URI \ pregnancy				
Yes	18	10.8	7	21.9

No	148	89.2	25	78.1
Total	166	100.0	32	100.0
Weight of newborn /kg				
< 2.5	18	10.8	1	3.1
≥ 2.5	148	89.2	31	96.9
Total	166	100.0	32	100.0
Gestational age / weeks				
≥ 37	151	91.0	32	100.0
< 37	15	9.0	---	---
Total	166	100.0	32	100.0

Table (4): Comparison between *antirubella* antibodies level and different demographic, socioeconomic, medical and obstetric problem among mothers and their newborn babies with normal delivery and cesarean section

Variables	Normal delivery		Cesarean section	
	Rubella antibody levels\ mother	*Rubella* antibody levels\ babies	*Rubella* antibody levels\ mother	*Rubella* antibody levels\ babies
Age\ years				
Person correlation	-.048	-.o74	-.485**	-.470**
Significant	.536	.341	.005	.007
Number	166	166	32	32
Residency				
Person correlation	.011	.020	.021	.066
Significant	.884	.798	.909	.719
Number	166	166	32	32
Mother education				
Person correlation	-.011	.019	-.247	-.157
Significant	.883	.808	.172	.391
Number	166	166	32	32
Crowding index				
Person correlation	-.060	-.034	-.006	-.066
Significant	.442	.666	.974	.719
Number	166	166	32	32
German measles vaccination				
Person correlation	-.175*	-.236**	-.236	-.173
Significant	.024	.002	.194	.343
Number	166	166	32	32
URI \ pregnancy				
Person correlation	-.128	-.084	.141	.107
Significant	.099	.282	.442	.561
Number	166	166	32	32
Weight of newborn				
Person correlation	-.020	.035	.322	.410*
Significant	.795	.657	.073	.020
Number	166	166	32	32

Discussion:

Rubella is generally asymptomatic in healthy adults but leads to *congenital rubella syndrome in* fetus, so it is Considered as an important public health problem [8].

In this study 54.2% of mothers, 52.4% of babies in the first group and 71.9% of mothers, 68.8% of babies in the

second group had weak *rubella* IgG antibodies, this result is higher than the result found by Turgut et al [2] 2004 in Turkey (17.2% were seronegative for *rubella* antibodies). In 1995-96, WHO conducted a review of *rubella* immunization strategies. Worldwide, 78 countries (more than one-third) reported a national policy of using *rubella* vaccine. This was closely related to each country's economic status. Based on the United Nations country classification, *rubella* vaccine is used in 92% of industrialized countries, 36% of those with economics –in- transition, and 28% of developing countries [9], in 2002 only 124 (58%) of 214 countries or territories around the world had implemented a *rubella* vaccination program [10]. An Australian study found that women born in Asia, sub-Saharan Africa and South America were 5 times as likely as other women to be seronegative for *rubella* virus [11]. Results of the present study are very striking one and might indicate improper vaccination, other point is that *rubella* antibodies decline over time and may increase the risk of reinfection [12]. In a study involving Korean children 18.8% of those who had been vaccinated and 13.8% of those with natural immunity were found to be seronegative for *rubella* virus after 3 years, an Italian study shows that wild-type *rubella* virus reinfected 9.8% of vaccinated girls within 5 years [11]. Obstetricians should always check *rubella* serologies in women of reproductive age if they have been vaccinated, *rubella* serology should also be checked in all pregnancies even if the patients were seropositive during their prior pregnancies [13].

Both mother educational level and crowding index were negatively correlated with antibody levels (except for babies of mothers with normal delivery in association with mother education). This is an expected result since crowded environment and low educational level may be risk factors for diseases such as *rubella* because of easy transmission and inaccurate knowledge about the disease transmission and its impact on health problems. The test used in this study was implemented as a screening test to detect past exposure and no further action was taken for women who had weak or negative *antiarubella* antibodies.

Conclusion and recommendation: Weakly positive antibodies were found to be higher among women and their babies following cesarean section than the group of women with normal delivery and their babies, negative correlation were found between antibody levels and age of mothers, educational level, crowding index.

Because *rubella* antibodies wane over time, screening for *rubella* susceptibility is recommended at the first prenatal visit for all pregnant women, standing orders for *rubella* vaccination after delivery and reinforcement of *rubella* vaccination program may increase immunization status among women in reproductive age

References:

1. Gershon AA. rubella virus (Germen measles). In Mandel GL, Bennet JE, Dolin R. Mandel, Douglas and Bennet's principles and practice of infectious disease. Churchill Livingstone Inc. 5th ed. 2000: 1708-1714.

2. Turgut H, Sacar S, Toprak S, Asan A. Fertile women are still under risk for having *congenital rubella syndrome* infants in Denizli /Turkey. The Internal Journal of Infectious Diseases. 2004; Vol 3, Number 2.

3. Robertson SE, Featherstone DA, Gacic M, Hersh BS. *Rubella* and *congenital rubella syndrome*: global update. Rev Panam Salud Publica. 2003 Nov; 14 (5):306- 15.

4. Centers for Disease Control. Measles, Mumps and *Rubella*- Vaccine Use and Strategies for Elimination of Measles, *Rubella*

and *Congenital Rubella Syndrome* and Control of Mumps: Recommendations of ACIP. MMWR. 1998: 47 (RR-8).

5. Centers for Disease Control. Control and Prevention of *Rubella*: Evaluation and management of suspected outbreaks, *Rubella* in pregnant women, and surveillance for *Congenital Rubella Syndrome*. MMWR. 2001: 50 (RR-12).

6. Balik IR, Topcu AW, Syletir G, Doganay M (ed). Infectious disease and microbiology. Nobel Bookstore, Istanbul, 3rd ed. 2002: 872-875.

7. World Health Organization. Direct ELISA as a secondary test for assaying the potency of vaccines containing tetanus toxoid In: Manual of laboratory Methods for testing of vaccine used in the WHO expanded Programme of immunization. Geneva; WHO, 1997; Publication NO. WHO/ VSQ/ 97.04.

8. Centers for Disease Control. Measles, Mumps, and *Rubella*-Vaccine Use and Strategies for Elimination of Measles, *Rubella*, and *Congenital Rubella Syndrome* and Control of Mumps: Recommendations of ACIP. MMWR. 1998; 47 (RR-8).

9. Robertson SE, Cutts FT, Samuel R, Diaz-Ortega JL. Control of *rubella* and *congenital rubella syndrome (CRS*) in developing countries. Bull World Health Organ. 1997; 75 (1): 69-80.

10. Robertson SE, Featherstone DA, Gacic-Dobo M, Hersh BS. *Rubella* and *congenital rubella syndrome*: global update. Rev Panam Salud Publica 2003; 14 (5): 306-15

11. Banerji A, Jones E, Kelly E, Robinson JL. *Congenital rubella syndrome* despite maternal antibodies. CMAJ. 2005; 172 (13).

12. Bullens D, Smets K, Vanhaesebrouk P. *Congenital rubella syndrome* after maternal reinfection.Clin Pediatr 2002; 39 (2): 113-6.

13.Marret H, Golfier F, Di Maio M, Champion F, Attia-Sobol J, Raudrant D. *Rubella* in pregnancy. Management and prevention. Press Med. 1999 Dec 4; 28 (38): 2117-22.

A RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ARSENIC TRIOXIDE ALONE: CASE REPORT

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Abstract

Acute promyelocytic leukemia is now the most curable subtype of acute myeloid leukemia in adult .It is characterized by 3 main features which are the presence of abnormal promyelocyte in bone marrow, the occurrence of disseminated intravascular coagulopathy and the presence of classical chromosomal translocation {t(15:17)(q22:q21)} or its variant.

The incorporation of ATRA in the induction over the past 25 years represents one of the most important advances in treatment of AML by inducing more differentiation of leukemic cells into mature granulocytes giving higher complete remission rate and resolution of the life threatening coagulopathy as well as decreases the relapse rate in comparison with chemotherapy alone.

In 1990's, investigators from China reported that arsenic trioxide (ATO) induces complete remission (CR) in patients with relapsed or refractory APL in 90% of cases by inducing partial differentiation & apoptosis of leukemic cells.

In Al-Kadhimiya Teaching Hospital, we faced such a case of APL who relapsed for second time within 1 year duration of a previously achieved remission, and fortunately we treat the patient with ATO alone that induced CR within 1.5 month duration of treatment then followed by consolidation courses and maintenance treatment to strengthen this remission state that is still existing.

Keywords: myeloid leukemia, acute promyelocytic leukemia, M3 subtype, relapse, all-transretinoic acid, and arsenic trioxide.

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Introduction

Thirty-one years old female patient who is a married housewife diagnosed as a case of acute promyelocytic leukemia in June 2001 on the base of clinical picture and hematological investigations.

She received classical induction chemotherapy regimen (7 and 3 regimen), which included Ara-c 100 mg/m^2/day (180 mg/day i.v. infusion in 500 ml DW 5% over16-18 hours) and anthracyclin (doxorubicin) in a dose of 45 mg/m^2 (80 mg/day) i.v. infusion in DW 5% in one hour) together with ATRA (all trans retionic acid) capsules in 45 mg/m^2 dose (60 mg/day). The latter was continued for the subsequent 2 m.o. She passed in first remission and received consolidation chemotherapy with ara-c &doxorubicine.

On July 2002, she returned back with early relapse of her disease, a new course of chemotherapy plus ATRA decided to be given similar to first course, but the results was partial remission that necessitated re-induction chemotherapy using 2 cycles of mitoxantran 20 mg/day i.v. infusion in day 1 and Etoposide 100 mg/day i.v. for 3 days.

Remission achieved but on August 2003, the patient re-presented with full blown picture of relapsed disease and considered to be 2nd relapse where decision taken to start supportive treatment as well as using, for the first time in Iraq, a new agent therapy for relapsed cases of APL which is[(Trisenox ®), arsenic trioxide]alone without the use of chemotherapy (according to literatures) in a dose of 0.15 mg/Kg/day (12 mg/day) as i.v. infusion for 3 hours continuing daily until achievement of new remission which occurred at 44th dose of treatment where all hematological parameters were corrected (table 1), hand by hand with careful and close observation for the anticipated adverse effects of this agent

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by repeating electrolytes study, coagulation screen, CBC and ECG monitoring.

Table 1: Follow up of WBC count and differential in this patient

Days of Rx	WBC	Neut.	Lymph	Promyel.	Blast	Notes	B.M.
On Dx	4.5x10^9/L	45%	46%	2%	1%		33% Promyelocytes 5% Blast
1ST	3.6 x10^9/L	41%	35%	16%	4%	Starting Trisenox 0.15 mg/Kg/day	
14th	10.7 x10^9/L	10%	14%	51%	6%	8%Myel. 6%Metamyel.	
18th	10.8 x10^9/L	35%	17%	23%	2%		
27th	4.0 x10^9/L	35%	51%	1%	1%		
32nd	2.6 x10^9/L	60%	31%	1%	zero		
44th						Stop treatment (Complete remission)	5%promyel. 2%blast
Follow up	9.6 x10^9/L	56%	35%	Zero	Zero		
	4.2 x10^9/L	57%	34%	Zero	Zero		
	3.2 x10^9/L	52%	43%	Zero	Zero		
	2.0 x10^9/L	32%	58%	Zero	Zero	Flu like illness	
	7.0 x10^9/L	60%	30%	Zero	Zero	On maintenance treatment	
	7.0 x10^9/L	79%	13%	Zero	Zero		

One month later she received consolidation course of (Trisenox ®) for 3 weeks only in the same dose 12 mg/day by infusion over 3 hours for 5 doses/week, to be followed by intensification chemotherapy and then subsequently by maintenance chemotherapy since that time which includes the following drugs:

1. ATRA every 3 m.o. for 15 days in dose of 45 mg/m^2
2. 6 MP 100 mg/ day
3. MTX 25 mg/ week

She is doing fine till the time of reporting the case on the 5th of October 2005.

Historical Background

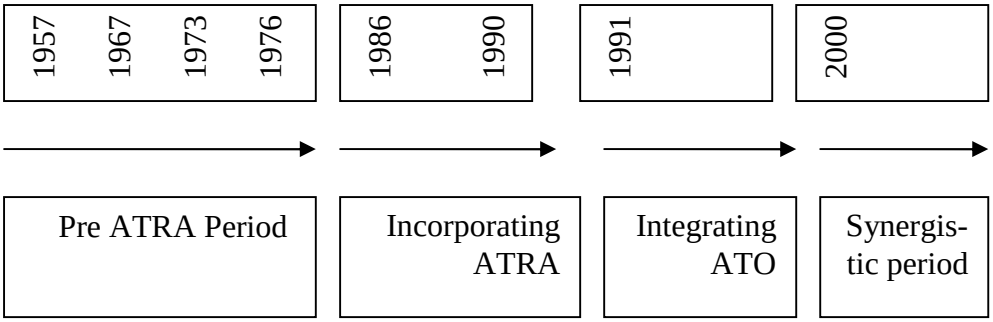
Leif Hillestad[1], the Swedish author, was the first who give the name of acute promyelocytic leukemia (APL), he concluded in 1957 that APL seems to be the most malignant form of acute leukemia. In 1976, the FAB groups assign this disease as M3 subtype of AML.

Also in 1976, a consistent chromosomal change, the balanced reciprocal translocation between the chromosomes 15&17 [t (15; 17)(q22:q21)] was reported by Rowley et al [2]. So far, APL has been shown to be characterized by 3 features: **1.** Presence of abnormal accumulation of promyelocytes in the bone marrow. **2.** The occurrence of afibrinoginemia and DIC that is reversed by chemotherapy, and **3.** Presence of the classical chromosomal translocation or its variant.

The past 3 decades has shown great advances in evolving therapeutic approaches for APL. In the 70s, the APL was treated by chemotherapy &was considered the most devastating subtype of AML. The

introduction of all transretinoic acid (ATRA) and arsenic trioxide (ATO) by Chinese hematologist since mid 1980s opened a new page in the history of leukemia therapy. ATRA is a derivative of vitamin A; which has improved the complete remission (CR) rate and long-term survival of APL patients [3]. On the other hand, the application of ATO further improved the clinical outcome of refractory or relapsed APL [4,5].

Lately, a higher quality remission and survival in newly diagnosed APL were achieved when ATRA was combined with ATO as compared to either monotherapy making APL a curable disease [6]. So the history of APL could be subdivided into 4 periods: pre ATRA period, incorporating ATRA, integrating ATO, and synergisting targeting period.



Since 1996, a large number of reports have shown that ATO induces a CR in 85%to 90% of patients with both newly diagnosed and relapsed APL [7,11,12].

Furthermore, after CR is achieved by ATO, molecular remission (i.e. negative for PML- RARα transcript detected by RT-PCR) is obtainable either with ATO or with ATRA and chemotherapy as a consolidation treatment[9].It seems likely that ATO used in the post remission therapy could prevent recurrence and achieve a longer survival time[8,9].

Mechanism underlying ATO triggered APL cell apoptosis, have been broadly studied. The apoptosis inducing effect is associated with down regulation of Bcl-2[10] which correlates with PML- RAR to block neutrophil differentiation [11].

Role of Arsenic trioxide (As_2O_3):

Investigators from china reported that arsenic trioxide induces CR in patients with relapsed and refractory APL in 90% of cases[4,5,9] (Table 2). Although the mechanisms have not been completely described, preclinical studies suggested that As_2O_3 induces partial differentiation and apoptosis of leukemic cells.

Soignet et al., initiated a pilot study of 12 patients with relapsed APL who were treated with Trisenox® (As_2O_3) at doses ranging from 0.06 to 0.2 mg/Kg/day until leukemic cells were eliminated from the bone marrow as determined by light microscopy (Table 3). 11 patients obtained CR with 8 of the 11 patients who initially tested positive for PML/RAR α fusion transcript later becoming negative (Table 4) [8, 9].

A multicenter trial of 40 patients confirmed the high CR (85%) (9)(Table 3), furthermore, approximately 78% of patients had no evidence of leukemic clone by PCR after 2 courses of Arsenic trioxide (Table 4)[8,9].

The most important toxicities include prolongation of the QTc interval and APL differentiation syndrome. However, two recent reports of sudden cardiac death with (Trisenox ®) indicate that careful monitoring is warranted.

Currently, Arsenic trioxide is considered to treatment of choice for

patients with relapsed disease, particularly in patient exposed to ATRA within the prior 12 months.

Table 2: Patients with relapsed and refractory APL achieving complete response after one course of arsenic trioxide therapy

Study, year	Number	No. CR	%CR
Zhang et al, 1996	42	22	52
Niu et al, 1999	47	40	85
	25	24	96
Soignet et al, 1998	12	11	92
Soignet, 2001	40	35	85

Table 3: Summary of results of 2 trials

Type	Trisenox dose Mg/Kg/day	CR	Time to BM remission (median)	Time to CR (median)	18 m.o. survival
Single center trial (Soignet et al) (N=12)	0.16 (0.06-0.2)	11 (92%)	32 days	54 days	67%
Multicenter trial (N=40)	0.15	34 (85%)	35 days	59 days	66%

Table 4: Cytogentics after As_2O_3 therapy in 2 studies

Type	Conventional cytogentics t (15;17)		RT-PCR for PML/RAR α	
	Absent	Present	Negative	Positive
Single center pilot CR cases=11	8 (73%)	1 (9%)	8 (78%)	3 (27%)
Multicenter trial CR cases=34	31 (91%)	0 %	27 (79%)	4 (12%)

References

1. Hillestod LK. Acute promyelocytic leukemia. Acta med scand 1957; 159: 189- 94.

2. Rowley JD, Golomb HM**,** Vardiman J, et al. Further evidence for a non-random chromosomal abnormality in acute promyelocytic leukemia. Int J Cancer 1977; 20: 869-72.

3. Huang ME, Ye YC, Chen SR, et al. Use of all transretioic acid in the treatment of acute promyelocytic leukemia. Blood 1988; 72: 567-72.

4. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As2O3) in the treatment of acute promyelocytic leukemia APL: II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997; 89: 3354-60.

5. Slack JL, Waxman S, Tricot G, et al. Advances in the management of acute promyelocytic leukemia and other hematologic malignancies with arsenic trioxide. The Oncologist, 2002; 7 (Suppl 1): 1-13.

6. Shen ZX, Shi ZZ, Fang J, et al. All trans retinoic acid /As2 O3 combination yields a high quality remission & survival in newly diagnosed acute promyelocyitc leukemia. Proc Natl Acad Sci USA 2004; 101: 5328-35.

7. Wang ZY. Treatment of acute leukemia by inducing differentiation and apoptosis. Hematology, 2003; 1- 13.

8. Lu DP, Qiu JY, Jiang B, et al. Tetra-arsenic tetra-sulfide for the treatment of acute promyelocytic leukemia :a pilot report. Blood 2002; 99: 3136-43.

9. Soignet SL, Frankel SR, Douer D, et al. United state multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol, 2001; 19: 3852-60.

10. Chen GQ, Zhu J, Shi XG, et al. In vitro studies on cellular & molecular mechanism of arsenic trioxide in the treatment of APL: As2O3 induces NB4cell apoptosis with down regulation of Bcl-2 expression & modulation of PML-RARα/PML proteins. Blood, 1996; 88: 1052-61.

11. Kogan SC, Brown DE, Shultz DB, et al. Bcl-2 cooperates with promyelocytic leukemia retinoic acid receptor and chimeric protein (PML-RARα) to block neutrophil differentiation and initiate acute leukemia. J Exp Med 2001; 193: 531-44.

12. Martin ST, Chadi N, Jane HF et al. Acute promyelocytic leukemia: evolving therapeutic strategies. Blood 2002; 993: 759- 65.

13. Soignet SL, Maslak P, Wang ZG, et al. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med, 1998; 339(19): 1341-8.

Moyamoya Syndrome

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Abstract: Moyamoya syndrome is a chronic progressive occlusive cerebrovascular disorder. We present a 41 years old man who had this syndrome, and presented as intraventricular hemorrhage complicated by communicating hydrocephalus. We describe in this report the clinical features and the diagnostic radiological investigations. The patient was treated by a ventriculoperitonial shunt with full recovery.

Keywords: Moyamoya, Intracranial hemorrhage, Hydrocephalus

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BACKGROUND:

Moyamoya syndrome is a chronic progressive occlusive cerebrovascular disorder that was first described by Suzuki and Takaku. The disease is characterized pathologically by progressive stenosis of the distal internal carotid and basilar arteries and their branches, with concomitant enlargement of perforating collateral arteries at the base of the brain to form the so-called Moyamoya vessels. The disease can be unilateral or bilateral. The name Moyamoya coined by Suzuki and Takaku which describes something hazy, like the puff of cigarette smoke drifting in the air, and refers to the angiographic appearance of the network of collateral vessels at the base of the brain.[1,2] This disease was described initially in Japan but is now recognized worldwide with case reports from Malaysia, Turkey and USA. [3,4] (FIG 1)

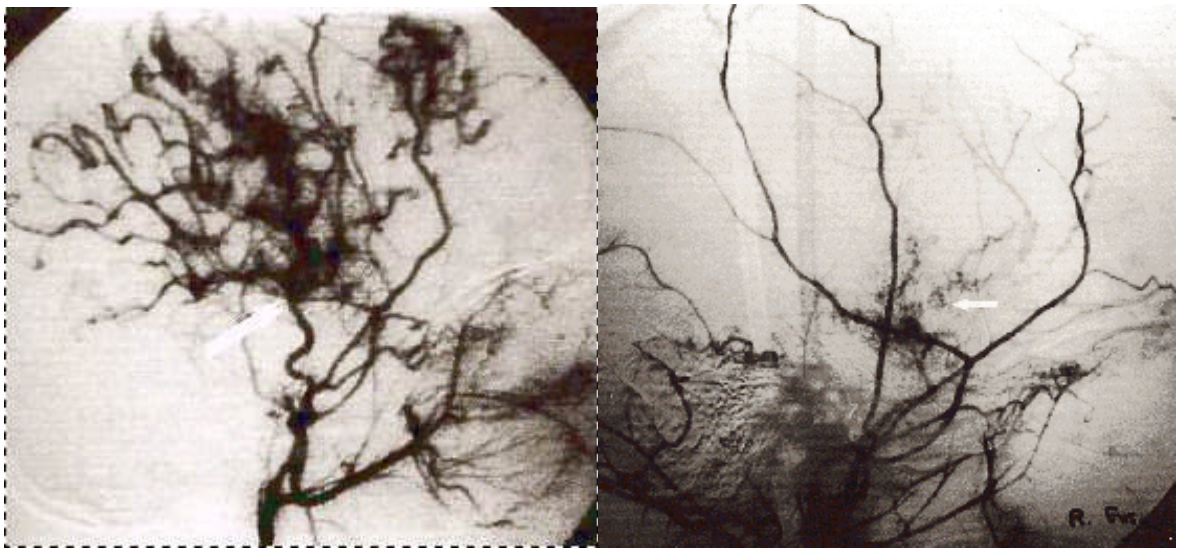

FIG 1,
Shows the characteristic angiographic appearance.

Case reports of occlusion and narrowing of both internal carotids with network of neovasularity at the base of the brain were published since the 1950s. Kudo in 1968 first Introduced the disease in English literature; Nishimoto and takeuchi described an extensive review of 96 cases in the same year. Later in the disease the posterior circulation is involved [5,6,7] with enlargement of extracranial transdural collaterals, including meningeal branches [6]. Eventually there is complete obliteration of the internal carotid artery and the cerebral circulation will depend on the external carotid

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collaterals. The disease might get arrested at any stage or occasionally shows some spontaneous improvement. The exact etiology of the disorder is not known. There is a familial occurrence in Japan of 7%, and the disease has been seen in identical twins. There is also a significant association between moyamoya syndrome and certain human lymphocyte antigen phenotype [8] as well as certain hereditary diseases such as Down syndrome, neurofibromatosis, and sickle cell anemia [9,10]. Moyamoya syndrome is also seen following infectious diseases of the central nervous system, including tuberculosis, meningitis, and leptospirosis. It was also noticed following therapeutic irradiation of the head (especially for sellar and suprasellar lesions), in Fanconi's anemia, connective tissue disorders, renal artery stenosis with hypertension [10], and in patients with type I glycogenesis, craniopharyngiomas, and optic gliomas[10,11] .

Moyamoya disease affects children and adults, with peak incidence in the 1st. and 4th. decades of life. In children the most common symptoms are hemiparesis, speech and sensory disturbances, seizures and transient ischemic attacks (TIA). In adults it usually presents as subarachnoid or intraventricular hemorrhage and acute hydrocephalus. [1,12,13,14] Symptomatology can be intermittent, with light neurological symptoms, or the disease can be progressive with eventual physical and mental deterioration. [9]

The diagnostic evaluation of these patients can be extensive. The initial radiographic study is usually a CT scan, which in some cases may demonstrate the blush of moyamoya vessels after contrast enhancement or, most commonly, small infarcts in various distributions, it also shows any bleeding or hydrocephalus. MRI will confirm these findings, by demonstrating high signal intensity changes in the circle of Willis corresponding to the occlusive vascular changes, and punctuate low signal changes due to the moyamoya vessels themselves throughout the basal ganglia in advanced cases. And high-quality MR angiography is virtually diagnostic [15,16,17,18,19]. However angiography remains the best investigation to demonstrate the site of stenosis and the typical moyamoya vessels.

The electroencephalogram (EEG) shows characteristic changes. However, the EEG results do not aid in the diagnosis or in predicting the natural history of the patient's disease process. Although other tests have been proposed to evaluate these children, such as radioisotope scanning and cerebral blood flow studies, including single photon emission computed tomography (SPECT), positron emission tomography (PET) and MRI perfusion studies, cerebral angiography remains the definitive diagnostic test [20]. It is important that the arteriographer study all circulations, including the external carotid artery system, in order to determine the presence and location of preexisting collateral flow and to determine if surgical therapy feasible.The surgical treatment consists of treatment of hydrocephalus, and revasularization of the brain. The most commonly used procedures are superficial temporal-middle cerebral arterial anastamosis and encephaloduroarteriosynangiosis, which consist of covering the brain with large temporalis muscle flap. However surgical treatment, though improve symptoms due to ischemia, it does not significantly reduce the incidence of stroke.

Key Word; Moyamoya, Intraventricular heamorrhage, Ventriculoperitoneal shunt.

Case report:

We present a 41 years old man who presented with sudden sever frontal headache 40 days before admission, associated with altered level of consciousness, slurred speech and incontinence of urine. By the time he was referred to the Neurosurgical department, the patient had improved, but he still had headache. On examination the patient was conscious, with neck stiffness, and bilateral Papilledema. The rest of his neurological examination was normal, and there was no evidence of neurofibromatosis or any other systemic disease. CT scan, done at the time of the episode shows intraventricular hemorrhage.(FIG 2) A four vessel angiogram was obtained which shows typical findings of terminal Lt. internal carotid artery stenosis, and a tuft of moyamoya vessels in the basal ganglion.(FIG 3)

A new CT scan was done 2 weak later shows resolution of the heamorrhage but there

was marked dilatation of the ventricles of the brain, with periventricular edema, reported as communicating hydrocephalus. (FIG4). A ventriculo-peritonial shunt was inserted, the headache was relieved, the Papilledema resolved, and the patient was discharged with no deficit

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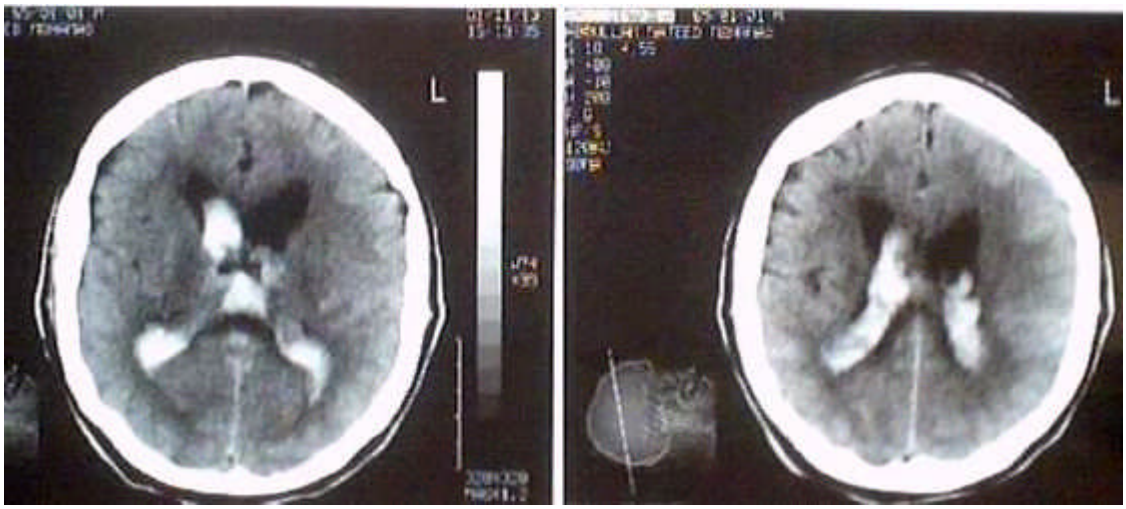

FIG 2.
Axial brain CT showing extensive intraventricular hemorrhage

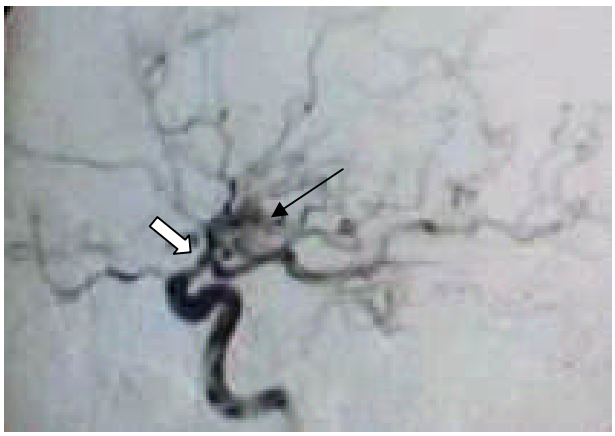

FIG 3.
Digital subtraction angiography showing Stenosis of the terminal Int. carotid And Moyamoya vessels

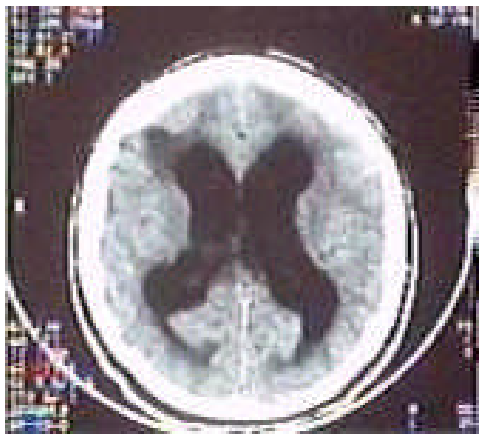

FIG. 4
Axial CT showing advanced hydrocephalus

Discussion

Suzki and Takaku first used this name in 1969 using Japanese name MOYAMOYA to describe the hazy, puff-of-smoke appearance of the neovascularization, which develops, and since then it has been widely known as moyamoya. Histopathologic examination of the diseased vessels reveals intimal hyperplasia and disordered or redundant internal elastic lamina.There is no evidence of inflammatory vasculitis. [(1)] The moyamoya vessels themselves can either be normal or show attenuation of the elastic lamina, fibrin deposition with resultant thrombi, and microaneurysm formation. There is usually a striking absence of inflammatory cells in the walls of vessels. Moyamoya syndrome has demonstrated a probable increase in the amount of basic fibroblast growth factor

in these tissues. This has been suggested to play a role in the development of the arterial wall changes in these patients. Moreover, the response of smooth muscle cells in the vessels of patients with moyamoya syndrome to platelet-derived growth factor, a mitogen of smooth muscle cells, also has been noted to be altered. These changes in the arterial wall lead to intracranial bleeding in adults, while children suffer from recurrent episodes of cerebral ischemia, seizures and stroke. [1,12,13,14]

The angiographic progression of moyamoya syndrome has been well described in the literature. Matsushima and Inaba correlated the angiographic staging proposed by Suzuki with clinical presentations and noted that symptoms occur when there is an imbalance between the spontaneously developing collateral's from the moyamoya vasculature, skull base, dura, and leptomeninges, and the degree of brain ischemia caused by the proximal occlusive disease process. The rate of progression is difficult to predict, but it is generally thought that the syndrome has a progressive phase during which the occlusions occur and the collateral's develop, and a stable phase when all collateral's are fully developed, the occlusive process is completed, and ischemia attacks disappear [11]. Clinical disease progression is unpredictable, with slowly developing neurological deficits.

Medical therapy (e.g., corticosteroids, vasodilators, low molecular weight dextran, antiplatelet agents, and anticoagulants) have been tried, but it efficacy remains questionable.[1] The stenoses continue to progress, usually at an unpredictable rate in a given patient, and medications do not seem to affect this process; however, antiplatelet agents such as aspirin appear to be important in reducing the incidence of symptoms in many of these children, probably because of the many small thromboses and subsequent embolizations that must be occurring distal to the moyamoya stenoses. The authors have noted that in several patients, both pre- and postoperatively, transient ischemic attacks were affected favorably by aspirin intake, and its use is advocated strongly in the treatment of this syndrome. Calcium-channel blockers also have affected the patients' symptoms favorably in several of the authors' cases .

Surgical procedures to treat moyamoya syndrome, with the exception of attempts to denervate the cerebral vasculature proposed by Suzuki and colleagues, have been designed to augment transdural collaterals that are already part of the ongoing moyamoya process. Cervical perivascular ganglionectomy, which involves resection of the stellate ganglion and sympathetic nerves around the carotid arteries, results in only temporary improvement of symptoms by increasing collateral circulation. However, it does not halt the progression of the disease.

Yasargil performed the first superficial temporal artery (STA) to middle cerebral artery (MCA) bypass in a child with moyamoya syndrome. This can very effectively increase hemispheric collateral flow. However, this procedure may be technically difficult in children [21]. Other operative techniques have depended upon the natural tendency of the ischemic brain to form collateral flow from any available source. Encephalomyosynangiosis involves the placement of the inner surface of the temporalis muscle directly on the brain surface following an extensive temporal craniotomy. Recent reports from Japan have documented improved clinical status and dramatic postoperative angiographic results in 70 to 80% of cases [13]. Complications related to the procedure include transient focal seizures probably secondary to the intrinsic muscle activity of the temporalis and the development of chronic subdural hematomas. Alteration in skull configuration following the relocation of the temporalis muscle also may be disfiguring. The large skin incision and dural flap required for the procedure have the potential to interrupt a number of preexisting collaterals to the brain from the dura, and the authors have felt that this procedure has limited application in the treatment of children with moyamoya syndrome .

Other techniques have been used to provide collateral flow in the appropriate patient. Neovascularization was noted initially in a patient in whom the bone flap was not replaced following a STA-MCA bypass. Spetzler and colleagues noted multiple transdural collaterals occurring 6 weeks after an intact STA was sutured to the arachnoid in a patient with no satisfactory recipient vessel at

the time of the intended STA-MCA anastomosis. Matsushima and colleagues transposed the intact STA with a strip of attached galea to a narrow dural opening beneath a linear craniotomy in hope of promoting transdural collateral formation, naming their procedure encephalo-duro-arterio-synangiosis. Subsequent papers by Matsushima and coworkers have demonstrated dramatic enlargement of the donor vessel, increased transdural collateral formation, and impressive changes such as a decrease in moyamoya vessels and increased MCA filling. Clinical and EEG improvement also was noted, although intellectual function appeared unchanged. Matsushima and colleagues recently have suggested that STA-MCA anastomosis combined with Encephalomyosynangiosis may be superior to encephalo-duro-arterio-synangiosis in development of collateral circulation and operative clinical improvement. However, the technical aspects of in anastomoses of small vessels in children remain challenging [2,21,22,23,24,25]

The patient in this study was an adult, and probably passed the active disease stage. The problem in this case was mainly that of hemorrhage and raised intracranial pressure. In such cases ventricular drainage is advised. [1] The treatment was directed at relieving hydrocephalus. We used ventrriculo-peritoneal shunt (VP). The shunt is important in this case because it not only relieves high pressure but it will also improve the cerebral blood flow (CBF) which is an aim in all surgical procedures. Reducing intracranial pressure will improve cerebral perfusion pressure, according to the equation that cerebral perfusion pressure=mean arterial blood pressure minus intracranial pressure (ICP) [26].

References:

1- David G. Piepgras, Keisuke Ueki. Moyamoya Disease. Neurosurgery/ editors, Robert H. Wilkins. Setti S. Rangachary. McGraw- Hill, New York 1996.2nd. Edition. Vol. II. Pp 2125-2129.

2-Spetzler RF, Roski RA, Kopaniki DR. Alternative superficial temporal artery to middle cerebral artery revascularization procedure. Neurosurgery 1980; 7:484.

3-Ng-WK, Tan-CT, George-J, Lee-MK, Loh-TG. A Moyamoya disease in Malaysia: two documented cases, J-Malaysia. 1995 Jun; 50(2): 186-8.

4- Sencer-S, Poyanli-A, Kiris-T, Sencer-A, Minareci-O. Recent experience with Moyamoya diseasein Turkey Eur-Radiol. 2000; 10(4): 569-72

5- Yokoyama-H, Tsutsumi-K, Ichikura-A, Onituka-M, Nakamura-S. Surgical treatment of aneurysms at basilar artery and posterior cerebral artery associated with moyamoya disease: a case report] No-Shinkei-Geka. 1995 Sep; 23(9): 807-11

6- Yamada-I, Himeno-Y, Suzuki-S, Matsushima-Y. Poserior circulation in moyamoya disease: angiographic study. Radiology. 1995 Oct; 197(1): 239-46

7- Moriyasu-H, Yasaka-M, Minematsu-K, Oita-J, Yamaguchi-T. [The pathogenesis of brain infarction in the posterior cerebral artery territory] Rinsho-Shinkeigaku. 1995 Apr; 35(4): 344-51

8- Aoyagi-M, Ogami-K, Matsushima-Y, Shikata-M, Yamamoto-M, Yamamoto-K Human leukocyte antigen in patients with moyamoya disease. Stroke 1995 Mar; 26(3): 415-7

9- Gorrotxategi-P, Reguilon-MJ, Gaztanaga-R, Hernandez-Abenza-J, Albisu-Y. Moya-moya disease in a child with multiple malformations Rev-Neurol. 1995 Mar-Apr; 23(120): 403-5

10-Sameshima-T, Morita-Y, Yanagita-M, Nakano-S, Goya-T. Wakisaka Unilateral middle cerebral artery stenosis in an adult with Down'syndrome -Med-Chir-Tokyo. 2000 Apr; 40(4): 216-9

11- Maruyama-K, Mishima-K, Saito-N, Fujimaki-T, Sasaki-T, Kirino-T. Radiation-induced aneurysm and moyamoya vessels presenting with subarachnoid haemorrhage. Acta-Neurochir-Wien. 2000; 142(2): 139-43

12- Maekawa-M, Nemoto-S, Awaya-S, Teramoto-A. Moyamoya disease with intraventricular hemorrhage due to rupture of lateral posterior choroidal artery aneurysm: case reports No-Shinkei- Geka. 1999 Nov; 27(11): 1047-51

13- Kawasaki-T, Minamida-Y, Fujishige-M, Inaba-K. A case of cerebral aneurysm located at the leptomeningeal artery associated with occlusion of the middle cerebral artery No-Shinkei-Geka 1995 Dec; 23(12): 1141-4

14- Echenne-BP, Leboucq-N, Humbertclaude-V. Ito hypomelanosis and moyamoya disease -Neurol. 1995 Sep; 13(2): 169-71

15- Kumagai-M, Sakai-N, Yamada-H. MR angiography (MRA) No-To-Shinkei. 1995 Aug; 47(8): 723-34

16- Chakraborti-KL, Jena-A, Puri-RK, Goyal-M. Magnetic resonance angiography in Moyamoya disease, Pediatr. 1995 Oct; 32(10): 1099-1100

17- Battistella-PA, Carollo-C, Pellegrino-PA, Soriani-S, Scarpa-P. Angiography in moyamoya disease -Nerv-Syst. 1995 Jun; 11(6): 329-34

18- Fuse-T, Takagi-T. MR angiography with a presaturation method for moyamoya disease] No-To-Hattatsu. 1995 May; 27(3): 251-3

19- Yamada-I, Suzuki-S, Matsushima-Y. Moyamoya disease: comparison of assessment with MR angiography and MR imaging versus conventional Radiology. Radiology 1995 Jul; 196(1): 211-8

20- Kim,-S-K,Wang,-K-C,Oh,-C-W,Kim,-I-O,Lee,-D-S,Somg,-I-C,Cho,-B-K. Evaluation of cerebral hemodynamics with perfusion MRI in childhood moyamoya disease. Pediatr-neurosurg. 2003 Feb; 38(2): 68-75

21- Whitaker,-J. Management of moyamoya syndrome,Arch-Neurol. 2001 Jan; 58(1): 132.
22- Kawamoto,-H, Kiya,-K, Mizoue,-T, Ohbayashi,-N A. Modified burr-hole method 'galeoduroencephalosynangiosis' in a young child with moyamoya disease. A preliminary report and surgical technique Neurosurg. 2000 May; 32(5): 272-5
23- Adelson-PD, Scott-RM. Pial synangiosis for moyamoya syndrome in children -Neurosurg. 1995; 23(1): 26-33
24- Takahashi-A, Kamiyama-H, Houkin-K, Abe-H. Surgical treatment of childhood moyamoya disease--comparison of reconstructive surgery centered on the frontal region and the parietal region Neurol-Med-Chir-Tokyo. 1995 Apr; 35(4): 231-7
25-. Touho-H, Karasawa-J, Ohnishi-H. Cerebral revascularization using gracilis muscle transplantation for childhood moyamoya disease -Neurol. 1995 Feb; 43(2): 191-7; discussion 197-8
26- Abood- S.H, Jasim- A. The relation of intracranial pressure with ventricular size in acquired hydrocephalus,J.fac.Med.Baghdad 2001.Vol.34,No.2

عامل فاس الذائب في مصل دم المرضى المصابين بأورام أللاهودجكن اللمفاوية

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الخلاصة

خلفية الدراسة: الأورام اللمفاوية هي مجموعة غير متجانسة من أورام خلايا باء أو تاء والتي عادة تنشأ في العقد اللمفاوية .وهي تقسم الى أورام هودجكن و اللاهودجكن اللمفاوية . إن تمزيق التوازن الفسيولوجي ما بين تكاثر الخلايا و موتها هو صفة لكل الأورام الخبيثة . لقد كان هنالك اهتمام متزايد بدور موت الخلايا المبرمج في تكوين الورم اللمفاوي. بناء على الأساس الجزيئي إن موت لاخلايا المبرمج هو بسبب تنشيط إنزيم الكاسبيز من خلال الطرق الخارجية و الداخلية .الطريق الخارجي يركز على عائلة عامل تنخر الورم، والتي يرتبط فيها الرابط إلى مستقبل سطح الخلية وهذا بدوره سوف يحدث موت الخلايا المبرمج. إن مستقبل فاس هو أحد أعضاء العائلة العليا لمستقبلات عامل تنخر الورم/ عامل نمو العصب. عائلة فاس تتكون من المستقبل والرابط والشكل الذائب . فاس الذائب سوف يتنافس مع مستقبل فاس للارتباط مع الرابط ، وبذلك سوف يتداخل مع موت الخلايا المبرمج الذي يتوسطه فاس الرابط .

هدف الدراسة : لقياس مستوى فاس الذائب في مصل دم مرضى أورام أللاهودجكن اللمفية . بالإضافة إلى تقرير العلاقة بين تركيز فاس الذائب في مصل الدم ومؤشرات سريريه محددة، ومؤشرات الدم ، والعلاج الكيماوي لورم اللاهودجكن .

المرضى والطرق: شملت الدراسة ٣٠ مريضا" مصابا" بورم اللاهودجكن بضمنهم١٩ ذكرا" و١١ أنثى . من هؤلاء المرضى ١٤ مريضا"شخصوا حديثا" . بالإضافة هناك ٣٠ شخص سليم استخدموا كمجموعة ضبطية . لقد قوبل المرضى وأخذ منهم تاريخهم المرضي وفحصوا سريريا" وسحبت منهم ومن العينة الضبطية عينة دم لتقدير تركيز فاس الذائب في مصل الدم باستعمال عدة شطيرة ألألايزا من شركة كيميكون. وكذلك مستوى بروتين سى التفاعلي في مصل الدم ومستوى الخميرة المخسفجة للاكتات في بلازما الدم وتركيز الهيموغلوبين وعدد الصفيحات الدموية وعدد خلايا الدم البيضاء وفلم الدم المحيطي وقد نفذت جميعها باستخدام التقنيات المعيارية .

النتائج : هذه الدراسة كشفت إن تركيز فاس الذائب كان مرتفعا" بدرجة ذات أهمية إحصائية عند مرضى أورام أللاهودجكن اللمفية مقارنة بالمجموعة الضبطية (٠.٠٠١) وهذه الزيادة كانت أعلى بدرجة ذات أهمية إحصائية عند المرضى قبل أخذ العلاج ومتعلقة بصورة وطيدة بالدرجة الباثولوجية لورم أللاهودجكن أللمفاوي (٠.٠٠٠٢ و٠.٠٠٣٥ على التوالي).

ألاستنتاج: إن فاس الذائب هو فحص بسيط ، غير إجتياجي وموفر للوقت . ومن الممكن أن يستعمل كمؤشر مختبري مفيد سريريا" عند مرضى أورام أللاهودجكن أللمفية .

إن فاس الذائب ممكن يستعمل كمؤشر مساعد لتقيم شدّة الورم والتكهن بمسير المرض وكذلك التخطيط لخطة العلاج عند مرضى أورام أللاهودجكن أللمفية .

مفتاح الكلمات : فاس ، اللاهودجكن .

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المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٣-١٢

أهمية الدراسة الشكلية للنواة في سرطان الثدي

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الخلاصة

خلفية الدراسة: يعتبر سرطان الثدي من أهم الأورام الخبيثة لدى الإناث. وهناك دلائل عديدة لاستعمال العلاج الكيميائي المساعد في حالات سرطان الثدي و الذي يحتاج إلى اختيار دقيق للمرضى، خصوصا للذين هم بحاجة شديدة إليه.
ويعتمد اختيار المرضى على عوامل تكهنية عديدة ، مثل عمر المريضة ، حجم الورم ،درجة خبث الورم وعوامل أخرى ، وتعتبر طريقة القياس الشكلي للنواة وسيلة موضوعية وكمية لتقييم العوامل التكهنية للورم ، حيث اظهر فحص مساحة النواة زيادة واضحة عند قياسها في خلايا الثدي الطبيعية ومقارنتها بالخلايا السرطانية.
ولوحظ إن هذه القياسات لها علاقة متبادلة بسرعة معاودة الورم خلال سنتين ونصف من تشخيصه.
هدف الدراسة: تقييم بعض العوامل التكهنية لسرطان الثدي عن طريق القياس الشكلي للنواة بواسطة الحاسوب.
المواد وطرق إجراء الدراسة: تم إعادة فحص أربعة و خمسون حالة لسرطان الثدي القنوي(مشخصة من قبل أخصائي النسيج المرضي) والمعروف حجمه وتصنيفه النسيجي وعمر المريضة أثناء التشخيص. في كل حالة تم فحص ٥-١٠ مقاطع مجهريه وفي كل مقطع تم قياس مساحة ٣٠ نواة باستعمال جهاز التحليل الشكلي تحت تكبير ٤٠٠x.
النتائج: أظهرت التحريات وجود فروق مهمة إحصائيا عند قياس مساحة النواة بين درجات التمايز الثلاثة($p>0.01$). وكذلك بالنسبة للأورام البالغ قطرها 5 سم، حيث ظهرت قيم مساحة النواة أعلى من قيمها في الأورام التي يبلغ قطرها اقل من ٥ سم ($p>0.01$). في حين لم يكن هناك اختلاف إحصائي واضح في مساحة النواة للأورام عند المرضى اكبر من ٥٠ عام أو اقل من ٥٠ عام ($p< 0.01$).
الاستنتاج: إن دراسة التحليل الشكلي للنواة، كاستعمال متغير مساحة النواة قد يكون ذات فائدة موضوعية عند تقييم العديد من العوامل التكهنية لسرطان الثدي.
مفتاح الكلمات: القياس الشكلي للنواة ، العوامل التكهنية ، سرطان الثدي.

[١]فرع علم الأمراض[كلية الطب- الجامعة ألمستنصريه]
[٢]فرع التشريح البشري [وزارة الصحة]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص١٣-١٧

أسباب حدوث الصرع البؤري في مجموعة من المرضى العراقيين

حسن عزيز الحمداني

الخلاصة

خلفية الدراسة : الصرع البؤري هو أكثر الأنواع شيوعا" في المرضى المصابين بمرض الصرع. هذا النوع هو بؤري المنشأ أي انه ينشأ من منطقة محددة من الدماغ. أضرار معينة أيضية أو تركيبية تؤدي إلى زيادة القابلية للإصابة بالمرض. النوبات الصرعية قد تنشأ نتيجة مرض يصيب الجهاز العصبي أساسا" او أجزاء أخرى من الجسم غير الجهاز العصبي. الأضرار التركيبية في الدماغ تزداد احتمالية وجودها مع وجود تاريخ مرضي طويل لمرض الصرع عند المصاب بهذا المرض

هدف الدراسة:

١. اكتشاف أسباب الصرع البؤري
٢. توضيح العلاقة بين المرض و عمر المريض

المرضى وطرق الدراسة : دراسة توقعية ل ١٠٦ مريض في مستشفى الكاظمية التعليمي مصابين بمرض الصرع البؤري تتراوح أعمارهم بين ٦ إلى ٧٣ سنة ، ٥٢ ذكور و ٥٤ نساء

النتائج: لهذه المجموعة من المرضى ٦١% منهم الفحص التصويري كان غير طبيعي. ورم الدماغ وجد عند ١٩.٧ % معظمهم من الأعمار أقل من ٤٠ عاما" بينما وجد احتشاء الدماغ عند ٢٥.٥ % من المرضى و معظمهم من أعمار أكثر من ٤٠ عام ٨٣.٧ % من مرضى الصرع البؤري المعقد وجدت البؤرة المسببة في الفص الصدغي و ١٦.٢ % في الفص الأمامي. بينما وجد إن نسبة وجود البؤرة في الفص الأمامي ٤٩% في مرضى الصرع البؤري البسيط.
٧٥.٤ من مرضى الصرع البؤري البسيط و ٣٥.١ % من مرضى الصرع البؤري المعقد تظهر لهم أعراض تركيبية.

الاستنتاج:

١. احتشاء الدماغ هو أكثر الأسباب شيوعا" لمرضى الصرع البؤري من الأعمار أكثر من ٤٠ سنة بينما ورم الدماغ هي أكثر الأسباب في الأعمار اقل من ٤٠ سنة.
٢. الصرع البؤري مزامل للأضرار التركيبية في الدماغ
٣. التاريخ المرضي الطويل يجعل الارتباط بين المرضى و الأضرار التركيبية أقل وضوحا".

مفتاح الكلمات:الصرع

فرع الطب الباطني [كلية الطب_جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص١٨-٢٢

دراسة انتشار الجراثيم لمرض الباراتايفوئيد نوع -أ- والآفات الناجمة عنها في الخمج التجريبي في الفئران البيضاء

خليل حسن زناد الجبوري

الخلاصة

خلفيةالدراسة : تبقى الإصابة بمرض الباراتايفوئيد ذات أهمية صحية ولكون الإصابة بهذه البكتريا متخصصة في الإنسان و القرود إلا انه في الجرع العالية منها يمكن إصابة الفئران البيضاء وعن طريق الخلب .

أهداف الدراسة:

١. دراسة انتشار عصيات الباراتايفوئيد نوع –أ- في أعضاء الفئران البيضاء
٢. دراسة الآفات الناجمة عنها في مختلف الأعضاء.

طرق العمل: استخدمت ١٠ أضعاف الجرعة النصفية القاتلة في الفئران والتي كانت ٨×١٠ ^{٧} خلية بكتيرية في الملتر الواحد.أعطيت هذه الجرعة عن طريق الخلب للفئران والآفات الناجمة عنها في مختلف الأعضاء ولفترات معينة ولمدة شهر بعد حقن الجراثيم.

النتائج : استمر انتشار الجراثيم لعصيات الباراتايفوئيد نوع –أ- في الطحال والكبد ولمدة ٢١ و ١٧ يوم على التوالي بينما استمر انتشار الجراثيم العقد النصفية اللمفية والرئتين لمدة ٩ يوم وفي الكلى ودم القلب لمدة ٥ يوم .كان أبرز الآفات تجمع العدلات والوذمة في الكبد ، الطحال والعقد اللمفاوية والتي تحل محلها خلايا وحيدة النواة مع تكاثر الأورومات اللمفية في الفترة الأخيرة للمرض .

الاستنتاج: عصيات الباراتايفوئيد نوع –أ- ذات أمراضية واطئة في الفئران البيضاء من خلال توزيعها في أعضاء الفئران البيضاء والآفات الناجمة عنها.

مفتاح الكلمات: الإصابة بعصيات الباراتايفوئيد نوع –أ- في الفئران.

فرع علم الأمراض [كلية الطب البيطري _ جامعة بغداد]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٢٣-٣٠

اكتشاف حاملات مرض دوشن العضلي بفحص فعالية أنزيم CPK مع تخطيط العضلات التقليدي

عبد المطلب عبد الكريم الشيخلي[١] ،سلام فؤاد محمد ربيع[٢] ،حسن عزيز الحمداني[٣]

الخلاصة

خلفية الدراسة: أن مرض دوشن العضلي هو مرض مغم ذو وراثة مرتبطة بالكروموسومx الأنثوي ، ويتسم بضعف عضلي في العضلات الهيكلية القريبة بصورة متفاقمة بدءا من الطفولة المبكرة مرورا في مرحلة المراهقة حيث يكون المريض مقعدا والموت نتيجة العجز التنفسي بنهاية العقد الثاني أو الثالث . بالرغم من إن غالبية حاملات مرض دوشن أو بيكرالعضلي غير ظاهرة عليهم أعراضه لكن بالإمكان معرفتهم من خلال التغيرات السريرية الطفيفة مثل الضعف العضلي المحدود أو بروز عضلات الساق أحياناً بينما نجد لدى بعضهن ارتفاع معدل فعالية الأنزيم العضلي CPK و تغيرات طفيفة في التخطيط العضلي.

ويعتبر مرض دوشن العضلي غير قابل للشفاء في الوقت الحاضر ولحد الآن اكتشاف حاملات المرض والاستشارة الوراثية يعتبران من الجوانب المهمة في الطرق الشاملة لتقليل عدد مرضى دوشن و بيكر العضلي و حملاته.

هدف الدراسة: من هذه الدراسة هو لاختبار قيمة الأنزيم العضلي CPK و تخطيط العضلات التقليدي لأكتشاف حاملات المرض في مجموعة من الأمهات العراقيات لمصابين بمرض دوشن و بيكر العضلي.

المرضى والطريقة: تمت هذه الدراسة في مستشفى الكاظمية التعليمي / قسم طب الجملة العصبية للفترة المحددة بين تشرين الأول ٢٠٠٢ إلى كانون الأول ٢٠٠٣ تمت خلالها دارسه ٢٠ إمرأه حاملة لمرض دوشن و بيكر العضلي ، من خمسة عشر عائلة (ثلاثة عشر عائلة دوشن و عائلتان بيكر) وقد تمت مقارنة النتائج لمجموعة أخرى مكونة من عشرين أمرأه تم جمعها عشوائيا.

لكل إمرأه مشتركة في الدراسة تم أخذ التأريخ المرضي و الفحص السريري الكامل بضمنها فحص قوة العضلات اليدوي وتم دراسة و تحليل شجرة العائلة ، وتم أخذ نماذج من الدم الوريدي لفحص معدل الأنزيم العضلي CPK ، وتخطيط القلب ،فحص القلب بالموجات الصوتية و تخطيط العضلات التقليدي .

النتائج: وجد أن حاملة واحدة فقط (٥%) عندها ضعف بسيط في العضلات الهيكلية القريبة .

وفيما يخص شمول القلب كانت النسبة ضئيلة جدا هو مريضة واحدة فقط (٥%) عندها توسع بسيط في البطين الأيسر للقلب ،.أحدى عشر امرأة (٥٥%) حاملة وجدت لديها ارتفاع معدل الأنزيم العضلي CPK و بأهمية إحصائية.

وقد لوحظ وجود علاقة سلبيه بين عمر المرأة الحاملة للمرض و معدل الأنزيم العضلي CPK .

تسعة حاملات لمرض دوشن العضلي (٥٢.٩٤%) وجدت لديها تغيرات أعتلالية عضلية في العضلات الهيكلية القريبة في فحص تخطيط العضلات وكان هذا جليا في عضلات الأطراف العليا وهو أيضا بأهمية إحصائية .

الإستنتاج: بما أن أنزيم أل CPK وتخطيط العضلات التقليدي هما من الفحوصات البسيطة وقليلة التكاليف ومتوفرة وتكون موجبة الى حد ما في نسبة من الحاملات لذلك يمكن استخدامها لكل الحاملات المحتملين في عوائل لمرض دوشن و بيكر العضلي كفحص استبياني بسيط . وخصوصا فحص أل CPK يفضل أجراءة إلى عمر مبكرة و فحص تخطيط العضلات لعمر أكبر وذلك لتطلبه قدرا من تعاون المريض. وهذا له وقع مهم على الاستشارة الوراثية هادفا لمنع انتشار المرض.

مفتاح الكلمات: دوشن وبيكر العضلي ، الحاملات ، أنزيم cpk ،تخطيط العضلات التقليدي.

[1]فرع الطب الباطني[كلية الطب _ جامعة النهرين]
[2]مستشفى مرجان التعليمي _ دائرة صحة محافظة بابل
[3]مستشفى الكاظميه التعليمي [كلية الطب _ جامعة النهرين]

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الترابط بين النحاس و تأكسد البروتينات الدهنيه عالية الكثافة والسيطرة الأيضيه بالنسبة إلى فرط الزلال الضئيل في الإدرار عند مرضى السكري (نوع ٢).

محمد لطيف ألبياتي[١] ،هاشم مهدي هاشم[٢] ،غسان عبد الأمير الشماع[١]

الخلاصه

خلفية الدراسة: يرتبط داء السكري بنسب عاليه من الوفيات بسبب اعتلالات الأوعية الدموية والكلى التي لا تعود للأسباب الأعتياديه المعروفة ، وبالرغم من عدم كفاية الأدلة فان الجهد ألتأكسدي واختلال الدهون ودرجة السيطرة الأيضيه وكذلك زيادة نسبة فوق الأكاسيد لدهون الدم يجعلها من العوامل ألمسببه لأمراض الأوعية الدموية أما علاقة النحاس بكل ذلك فما هو معروف عنه قليل نسبيا .

هدف الدراسة: هو قياس دلالات زيادة الجذور الحرة واكاسيد الدهون ممثلة بال-MDA واكاسيد البروتينات الدهنيه وتركيز النحاس ونمط الدهون في الدم وتركيز الهيموغلوبين المتسكر (HbA1C)ونسبة الزلال في الإدرار عند ٥٥ مريضا بداء السكري (نوع ٢) ومقارنتها بمثيلاتها في ٣٧ شخصا طبيعيا (مجموعة السيطرة) .

ألنتائج: أدت إلى تقسيم مجموعة المرضى إلى نوعين :

١- المصابين بفرط الزلال في الأدراربدرجة قليله microalbuminuria

٢- المرضى الذين كانت نسب الزلال في الإدرار طبيعيه normoalbuminuria

وبالمقارنة مع نتائج مجموعة السيطرة ظهر وجود زيادة ملحوظة عند مرضى السكري في تراكيز أكاسيد الدهون متمثلة بال-MDA وأكاسيد البروتينات الدهنيه عدا العالية الكثافة حيث أظهرت نقصانا مع وجود علاقة طرديه ملحوظة بين تركيز النحاس وال MDA في المجموعة الأولى من المرضى فقط ومع الهيموغلوبين المتسكر HbA1C في كلي المجموعتين.

وقد تزامنت هذه التغييرات مع صغر حجم البروتينات الدهنيه الخفيفة LDL عند مرضى السكري بصورة عامه والمجموعة الأولى(المصابين بفرط الزلال في الإدرار) بصورة خاصة.

وبينت الدراسة التفسيرات ألمحتمله للظواهر المذكورة.

مفتاح الكلمات: النحاس ، أكاسيد الدهون ، داء السكري ، فرط الزلال الضئيل في الإدرار.

[١]فرع الكيمياء الفسيولوجية[كلية الطب _ جامعة النهرين]

[٢]فرع الطب الباطني[كلية الطب _ جامعة النهرين]

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دور اليلات الخطورة لنظام مستضدات خلايا الدم البيض البشرية- الصنف الثانى فى استجابة الخلايا اللمفية ضد مستضدات الحمات المعوية والغدية وتفشى اضداد IgG هذه المستضدات في الأطفال المشخصين حديثاًبمرض السكر من النوع الأول

إيمان مهدي صالح[١] ، نضال عبد المهيمن[٢]

الخلاصة

خلفية الدراسة: أثبتت العديد من الدراسات أن للفايروسات دورا تؤديه في إحداث أمراضية مرض السكر من النوع الأول وقد يكون لها دوراً في بدء عملية تحطيم خلايا بيتا في البنكرياس.

هـدف الدراسـة: الهدف من الدراسة هو لتقييم الفعالية الوظيفية للخلايا اللمفية بعد تحفيزها بمستضدات الحمات المعوية والتي تشمل فايروس الكوكساكي نوع-B (CVB) وفايروس شلل الأطفال فضلاً عن ألحمة الغدية (Adenovirus) في مجموعة من الأطفال المصابين بمرض السكري من النوع الأول ومجموع من الأطفال الأصحاء المطابقين لمستضدات التطابق النسيجي (HLA) من الصنف الثاني فضلاً عن التقصي عن وجود الكلوبيولينات المناعية نوع IgG ضد هذه الفايروسات.

طريقـة العمـل: شملت الدراسة ستون مريضاً حديثي الإصابة بمرض السكري النوع الأول (مشخصين بالإصابة خلال فترة أقل من خمسة أشهر).والذين تم اختيارهم من المركز الوطني للسكري / الجامعة المستنصرية للفترة من مايس ٢٠٠٤ ولغاية تشرين الأول ٢٠٠٥. ولغرض المقارنة تم اختيار مجموعة من الأطفال (٥٠) طفلاً الاصحاء ظاهريا لغرض التحري عن وجود اليلات الخطورة لمستضدات التطابق النسيجي – الصنف الثاني ثم قياس الفعالية الوظيفية للخلايا اللمفية بعد حضنها مع الفايروسات المذكورة سابقاً باستخدام طريقة MTT فضلاً عن قياس المستويات المصلية للكلوبيولين المناعي IgG ضد الفايروسات الثلاثة باستخدام طريقة ELISA الغير مباشرة .

النتائج: أظهرت الدراسة الحالية انخفاضاً غير معنوياً في الفعالية الوظيفية للخلايا اللمفية كاستجابة للمشطر Con-A وكذلك باستخدام فايروس CVB النوع المصلي (5) وفايروس الـ Adenoللانواع المصلية 3 ، 4 و 7 في الأطفال المرضى مقارنة بالأصحاء. ولكن هذا الانخفاض كان معنوياً فقط عند استخدام فايروس شلل الأطفال ($P < 0.05$). وكانت الزيادة في الفعالية الوظيفية للخلايا اللمفاوية معنوياً ($P < 0.05$) عند المرضى الحاملين للاليلات الخطورة من الصنف الثاني DQ3 - ; DQ2 - ; DR4 - ; HLA-DR3 مقارنة بالمرضى الحاملين للاليلات الأخرى وكذلك عند المرضى الموجبين للكلوبيولينات المناعية نوع IgG مقارنة بالمرض السالبين .

الاستنتاج: انخفضت الاستجابة المناعية عموماً في الأطفال المصابين ولكنها ازدادت في الأطفال الحاملين للاليلات الخطورة HLA من الصنف الثاني و الموجبين للكلوبيولينات المناعية نوع IgG المضادة للفايروسات.

مفاتيح الكلمات: مرض السكر من النوع الأول ، مستضدات التطابق النسيجي - الصنف الثاني ، الفعالية الوظيفية للخلايا اللمفية ، الكلوبيولينات المناعية نوع IgG ضد فايروس الكوكساكي B النوع المصلي 5 وشلل الأطفال والحمى الغدية.

[1]فرع الأحياء المجهرية [كلية طب الكندي - جامعة بغداد]
[2] فرع الأحياء المجهرية [كلية طب النهرين - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٤٧-٥٦

البروتينات الشحمية المؤكسدة لدى المرضى المصابين بارتفاع ضغط الدم الأولي على أنماط علاجية مختلفة

فيصل غازي جاسم ،عبد الرحمن البزاز ،غسان عبد الأمير الشماع.

الخلاصة:

خلفية الدراسة:إضافة إلى فرط كوليستيرول الدم و التدخين، يعتبر ارتفاع ضغط الدم (وهو مرض مزمن مرتبط بالعديد من المضاعفات القلبية الوعائية) واحدا" من عوامل الخطورة الرئيسية لأحتشاء العضلة القلبية و لأمراض الدماغ الوعائية.وأظهرت بعض الدراسات إن بعض الأدوية المضادة أو المهبطة لارتفاع ضغط الدم قد تؤدي إلى حدوث تأثيرات غير مرغوب بها على مستوى الشحوم في الجسم و كذلك أيظ العناصر الضئيلة.

هدف الدراسة: لبيان دور الجهد التأكسدي وفوق اكاسيد الشحوم في ارتفاع ضغط الدم وتأثير مختلف العلاجات الطبية عليها.

ألأشخاص وطرق البحث:الدراسة الحالية تتضمن قياس مستوى فوق الأكاسيد لشحوم الدم ، نمط شحوم الدم ، وفحص الزلال في الإدرار لدى (٦٩) مريضا" مصابا" بارتفاع ضغط الدم تراوحت أعمارهم بين (٣٠-٧٠) سنة ، وتم تصنيفهم الى مجاميع ثلاثة بالاعتماد على نمط العلاج: (الأتينولول و الكابتوبريل و نظام سيطرة بدون عقاقير)
وتم مقارنة النتائج مع نتائج مجموعة من ٤٥ شخصا" سليما" ظاهريا" (مجموعة السيطرة) تراوحت مديات أعمارهم من ٣٠-٦٦ سنة.

النتائج: أوضحت النتائج إن المرضى المصابين بارتفاع ضغط الدم يعانون من ارتفاع معنوي في مستوى المالون ثنائي الألديهايد والنسبة المئوية لأوكسيد البروتين ألشحمي غير عالي الكثافة وانخفاض معنوي في النسبة المئوية لأوكسبد البروتين ألشحمي عالي الكثافة في مصل المرضى مقارنة مع مجموعة السيطرة. ويعزى هذا الاختلال في التوازن ما بين المؤكسدات و مضادات التأكسد إلى ارتفاع ضغط الدم أو إلى الأعراض الجانبية للعقاقير المضادة أو المهبطة لارتفاع ضغط الدم . واختلاف العقاقير المضادة أو المهبطة لارتفاع ضغط الدم اظهر نتائجاً مختلفة لمستوى أكاسيد الشحوم، و النتائج الأفضل كانت للمرضى المعالجين بعقار كابتوبريل ؛ و الاختلاف المعنوي بين مجاميع المرضى يدعم هذه النتيجة.
وأشارت نتائج الدراسة إلى أن فرط شحوم الدم لدى مرضى ارتفاع ضغط الدم يتمثل بزيادة مستوى الكليسيرايد الثلاثي و الكوليستيرول المصحوب بانخفاض البروتين ألشحمي عالي الكثافة في مصل المرضى تحت نظام سيطرة علاجية بعقار اتينولول.

ألاستنتاجات: يعاني جميع مرضى ارتفاع ضغط الدم من خطورة الإصابة بتصلب الشرايين وخصوصا" المرضى بدون نظام سيطرة علاجية بالعقاقير، ووفقا" للنسبة الجزيئية بين الكليسيرايد الثلاثي إلى البروتين ألشحمي عالي الكثافة ظهر وجود دقائق صغيرة كثيفة للبروتين ألشحمي واطئ الكثافة في مصل الدم عند هؤلاء المرضى.

و تمت مناقشة الآليات المقترحة المؤدية إلى تلك النتائج.

الكلمات المفتاحية: ارتفاع ضغط الدم ، الأكسدة الفوقية للشحوم ، البروتين الشحمي عالي الكثافة المؤكسد

فرع الكيمياء الفسيولوجية[كلية الطب _ جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٥٧-٦٤

انتشار مرض باركنسن في منطقةِ الكاظمية (مدينة بغداد): دراسة مجتمعية

عبد المطلب عبد الكريم[١] ،أمجد داود نيازي[٢]،عمار فاضل عبد الله[٣]

الخلاصة
خلفية الدراسة: مرض باركنسن مرض تحطمي مزمن يصيب على الغالب البشر فوق ٤٠ سنة. دِراسَة انتشار المرض حاسمُ لتخطيط الصحةِ العامةِ خصوصاً بعد التقدّمُ في السن ّعالميا. هناك بَعْض الاختلافات العالميةِ في نِسَبِ الانتشار المُخَمَّنةِ والأرقام مجهولة في بلادِنا.

هدف الدراسة: لتَخمين انتشار مرض باركنسن في منطقةِ الكاظمية.
طرق العمل: دراسة مجتمعية أجرتْ كمسح عرضي على العينة العشوائيةِ مِنْ سكان المنطقةِ. الحالات المشكوك فيها لمرض باركنسن ميّزتْ أثناء زيارات بيتيه أحيلتْ إلى قسم العلوم العصبية في مستشفى الكاظمية لكي يُؤكّدَ التشخيصَ. التشخيص يُثبت بتَمييز العلامات الحركية.
النتائج: ٢٥ مِنْ حالاتِ مرض باركنسن جَمعَ مِنْ عينة عشوائية مِنْ ٢٢,٩٨٨ فردِ (١٣ كَانتْ ذكورَ، ٢٣ كَانتْ إناث. ٦ من المناطق الريفية البعيدةِ و١٩ حضريةِ). ثلاث حالاتِ (١٢ %) شُخّصتْ حديثاً. الارتعاش كَانتْ العلامة السائدةَ للبدايةِ (٨٠ %). ١٩ حالة كَانَ عِنْدَها تدخّلُ ثنائيُ مِنْ المرض، بالرغم مِنْ البدايةِ الأحادية الجانبِ لكُلّ الحالات. نسبة الانتشار العامّة كَانتْ ١٠٨.٧٥ لكلّ ١٠٥ سكان.نِسَبَ الانتشار وّضحت زيادةَ ثابتة بالعُمر. نِسَب الانتشار حُسِبَ الجنسَ للذكر ١٠٥/١١٤ للسكان وللاناث١٠٥/١٠٣ سكان. نِسَب الانتشار المعدّلةِ إقامة كَانتْ ١١٤,٣ و ٩٤,٣ لكلّ ١٠٥ للسكان للمعيشة الحضريةِ والريفيةِ على التوالي.
الإستنتاج: نسبة انتشار مرض باركنسن أوطأ مِنْ الأرقام في أوروبا وأمريكا الشمالية، لكن أعلى مِنْ تلك أفريقيا والصين. يَزيدُ بشكل ثابت بزيَادَة العُمر. لم يكن هناك اختلاف هامَّ للجنسَ أو السكن في الريف في نِسَبِ الانتشار. رقم الانتشار يُمْكِنُ أنْ يُقدّمَ إلى سكان مدينةِ بغداد بسبب التركيبِ وخصائص السكان المماثلةِ إلى تلك مِنْ منطقةِ الكاظمية
الكلمات المفتاحية: مرض باركنسن

[١]فرع الباطنية [كلية الطب - جامعة النهرين]
[٢]فرع طب المجتمع [كلية الطب - جامعة النهرين]
[٣]فرع الباطنية [دائرة صحة بابل]

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الفحص ألسريري مع دوبلر الملون لتقييم أمراض الثدي الحميدة والخبيثة

حكمت عبد الرسول حاتم[١] ، بشار عباس[٢]

الخلاصة

خلفية الدراسة: يعتمد نمو وانتقال الأورام الخبيثة على تولد الأوعية الدموية (Angiogenesis)، كما ويعتبر التولد مؤشرا لقابلية انتقال الورم مبكرا.

هدف الدراسة: استخدام إشارات دوبلر الملونة لدراسة وتقييم جريان الدم في أورام الثدي وتمييز الحميدة منها عن الخبيثة.

طريقة العمل: دراسة مستقبلية شملت ٨٣ مريضة، أجري الفحص السريري الدقيق مع فحص دوبلر الملون بواسطة جهاز الأمواج فوق الصوتية للثدي وأخذ بنظر الاعتبار مايلي؛ وجود أو عدم وجود إشارات دوبلر الملونة مع حساب عدد الأوعية الدموية، التوزيع الشكلي للأوعية الدموية و قياس أقصى سرعة لجريان الدم حول وداخل أورام الثدي .

النتائج: بالاعتماد على الفحص ألسريري تم تشخيص ٢٧ حالة مرضية خبيثة و٥٦ حالة مرضية حميدة في الثدي وكانت الحساسية (٧١.٥%) أما النوعية فكانت (٨٠,٦%). وجودت إشارات دوبلر الملونة في جميع الأورام الخبيثة (١٠٠%) و (٧٩%) منها في الأورام الحميدة ، بالإضافة إلى أن عدد الأوعية الدموية في الأورام الخبيثة أكثر مما هي في الأورام الحميدة حيث كانت الحساسية لأكثر من ٣ أوعية دموية (٨٥.٧%) أما النوعية (٩١.٩%). وجد أن شكل الأوعية الدموية في الأورام الخبيثة هي نافذة وسطية بينما هي محيطية في الأورام الحميدة وكانت الحساسية (٩٠.٤%) أما النوعية (٩٣.٨%). وجد أن سرعة جريان الدم في الأورام الخبيثة أعلى مما هي في الأورام الحميدة ،كانت الحساسية (٩٠.٤%) و النوعية (٩١.٨%) لسرعة جريان أكثر من ١٥ سم/ثانية.

وبجمع نتائج فحص دوبلر كانت حساسية التشخيص (٩٠.٤%) أما النوعية فكانت (٩٦.٧%).

الاستنتاج: يعتبر فحص دوبلر الملون مكملا للفحوصات التشخيصية الأخرى لإمراض الثدي .

مفاتيح الكلمات: دوبلر الملون، أمراض الثدي، الحميدة، الخبيثة.

[١]فرع الجراحة [جامعة النهرين_ كلية الطب _ العميد]
[٢]فرع الجراحة [مستشفى الكاظمية التعليمي _ مدرس]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٧٣-٨٠

نقل نُسج مبيضيه: طريقة وموقع جديد لتحفيز نمو الجريبات المبيضية في الفئران كموديل لأنثى الإنسان

محمدباقر محمدرشاد فخرالدين[١] ، فؤاد كاظم الربيعي[٢] ، ابتسام جاسم سوداني[١]

الخلاصة

خلفية الدراسة: تُعد عملية نقل نسيج مبيضي (Ovarian tissue transplantation) طريقة جديدة لاستعادة خصوبة الإناث اللاتي لا تقوم مبايضهن بالوظائف الطبيعية. فالإناث الناضجات اللاتي يخضعن للعلاج من الأورام باستخدام المواد الكيميائية أو الإشعاعية يواجهن مخاطر متتالية تضر بخصوبتهن ومحتوى الجريبات المبيضية (Ovarian follicles) في المبايض بشكل خاص.

هدف الدراسة: هدفت هذه الدراسة إلى إمكانية إدامة تنشيط نمو الجريبات من أنسجة مبيضيه تزرع تحت غشاء الكلية (Subcapsular kidney) سواء بوجود أو غياب إسناد منشطات القند (Gonadotropins).

طرق العمل: تم تخدير ثمان وأربعين أنثى فأر وفُتح تجويفها ألبطني، وتم نقل قطعة صغيرة (1X1X1 ملم) من نسيج قشرة المبيض إلى تحت غشاء الكلية في الجانب المقابل وبعدها يتم إغلاق التجويف ألبطني. تم تقسيم إناث الفئران لاحقاً إلى ثلاثة مجاميع اعتماداً على وقت حقن منشطات القند. مجموعة الإناث الأولى (مجموعة السيطرة G-1) حُقنت بالمحلول الفسيولوجي المعقم، أما المجموعة الثانية (G-2) حُقنت بمنشطات القند لأربعة أيام متتالية بعد العملية الجراحية مباشرةً. في حين حقنت المجموعة الثالثة (G-3) بمنشطات القند لأربعة أيام متتالية بعد العملية الجراحية بثمانية أيام. تم تقييم نمو **الجريبات المبيضية ونوعية البويضات والتغييرات النسيجية للأنسجة المبيضية المنقولة.**

النتائج: أظهرت نتائج مجاميع التجربة الثلاثة عدم فقدان قطع الأنسجة المبيضية المنقولة وعدم ظهور تأثيرات جانبية ضارة على الإناث. تم تحقيق أفضل نمو للجريبات المبيضية لدى إناث المجموعتين الأولى والثانية. تم الحصول على أفضل جريبات مبيضية ناضجة من القطع النسيجية المنقولة لإناث المجموعة الثانية وبدرجة أقل لإناث المجموعة الأولى. ظهرت أقل درجة نمو للجريبات المبيضية من القطع النسيجية للمبايض لإناث المجموعة الثالثة بالمقارنة مع المجموعتين الأولى والثانية. تم الحصول على بويضات غير ناضجة وناضجة محاطة بالركام ألمبيضي من تحطيم الجريبات النامية للأنسجة المبيضية المنقولة.

الاستنتاج: تؤكد نتائج الدراسة الحالية إمكانية نقل أنسجة مبيضيه إلى تحت غشاء الكلية وحصول نمو للجريبات المبيضية. كذلك تُسند فسلجة الجسم نمو وتطور الجريبات المبيضية في موقع آخر غير موقع المبايض الطبيعي. هنالك حاجة لإجراء دراسات أضافية على إنضاج وإخصاب البويضات خارج الجسم وعمليات نقل الأجنة.

مفاتيح الكلمات: الفئران - المبايض - نقل وزراعة الأنسجة - نمو الجريبات المبيضية - منشطات القند.

[١]معهد أبحاث الأجنة وعلاج العقم - جامعة النهرين
[٢]مستشفى الإمام علي (ع) - وزارة الصحة

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دراسة الأجسم المضادة للروبيلا عند الأمهات وأطفالهن بعد الولادة الطبيعية مقارنة مع الأمهات وأطفالهن بعد أجراء العملية القيصرية

إيناس طالب عبد الكريم

الخلاصة

خلفية الدراسة: تعتبر الروبيلا (الحصبة الألمانية) من الأمراض التي لا تسبب أعراض مرضية عند البالغين، ولكن إصابة الحوامل بالمرض في الجزء الأول من الحمل قد يؤدي الى وفاة الجنين أو أحداث تشوهات خلقية عنده، لهذا تعتبر الحصبة الألمانية من الأمراض التي لها أهمية لصحة المجتمع.

هدف الدراسة: أجري هذا البحث لتحديد مستوى الأجسام المضادة للروبيلا عند الأمهات بعد الولادة الطبيعية واطفالهن ومجموعة ثانية من الأمهات بعد أجراء العملية القيصرية لهن مع أطفالهن ومدى علاقتها مع مختلف المشاكل الوبائية والطبية.

طريقة العمل: تم أخذ نموذج دم من ١٦٦ امرأة بعد الولادة الطبيعية وأطفالهن (المجموعة الأولى) وكذالك من ٣٢ امرأة بعد أجراء عملية قيصرية لهن مع أطفالهن (المجموعة الثانية) ثم أجرى فحص الأليزا على نماذج الدم لمعرفة مستوى المضادات للروبيلا عندهم.

النتائج: كان مستوى الأجسام المضادة للروبيلا أقل من واحد عند ٥٢.٢ % من النساء في المجموعة الأولى و ٧١.٩ % من النساء في المجموعة الثانية، كان مستوى الأجسام المضادة للروبيلا ذو علاقة سلبية مع العمر عند الأمهات (ذو مغزى إحصائي عند المجموعة الثانية) وكذلك مستوى التعليم عند الأمهات ومؤشر الكثافة السكانية. وأظهرت النتائج ان أخذ اللقاح ضد الحصبة الألمانية له علاقة سلبية مع مستوى الأجسام المضادة للروبيلا في المجموعتين وذات مغزى أحصائي عند المجموعة الأولى بينما كان وزن الوليد له علاقة ذات مغزى إحصائي عند الأطفال في المجموعة الثانية.

الاستنتاجات: ظهران وجود الأجسام المضادة بشكل موجب وضعيف عند النساء وأطفالهن بعد العملية القيصرية كان أعلى من النساء بعد الولادة الطبيعية وأطفالهن، مع وجود علاقة سلبية بين الأجسم المضادة وعمر الأمهات، التعليم والكثافة السكانية.

كلمات المفتاح: الأجسام المضادة للروبيلا والأمهات بعد الولادة

فرع طب المجتمع [كلية الطب - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٩٠-٩٦

معالجة انتكاس لحالة مصابه بابيضاض الدم النخاعي الحاد(من نوع لوكيميا الخلايا النخاعية الخديج -النوع الثالث)باستخدام مركب ثلاثي اوكسيد الزرنيخ

نبيل سلمان مراد،وسيم فاضل التميمي

الخلاصة

خلفية الدراسة: ابيضاض الدم النخاعي الحاد أو اللوكيميا النخاعية الحادة (AML)مرض ورمي يصيب أنسجة صنع الدم حيث تتسرطن الخلايا الاوليه التي تتطور لتنتج الخلايا غير اللمفاوية بكريات الدم البيضاء وتظهر بأعداد كبيره في النخاع العظمي والدم كخلايا سرطانية قادرة على الانتقال. أن هذا المرض هو من التعقيد بشكل يشمل تفرعات وأنواع متعددة تتطلب معالجات مختلفة تبعا" لنوع المرض واختلافاته الأخرى.

عند اللوكيميا النخاعية ينشأ التسرطن عادة" بخلايا المنشأ (STEM CELL)التي تتطور إلى الخلايا المتعادلة (إحدى أنواع الخلايا الحبيبية(NEUTROPHILS)وقياسا"إلى مدى نضج أغلب خلايا اللوكيميا بالجسم وتماثلها مع الخلايا الطبيعية مع سرعة تكاثرها أو عدوانيتها تصنف بالحادة عندما تكون الخلايا الشاذة قاصرة عن النضج وتبقى غير بالغه وتستمر في التكاثر والاحتشاد بالنخاع بصوره متزايدة دون أن تفنى حسب الاليه الطبيعية لدورة حياة الخلايا الطبيعية.و يعتبر النوع الثالث من اللوكيميا النخاعية الحادة (ACUTE PROMYELOCYTIC LEUKEMIA-M3 SUBTYPE) وهو لوكيميا الخلايا النخاعية الخديج (وهي إحدى المراحل الاوليه المبكرة لتطور ونمو الخلايا المتعادلة)من الأنواع المميزة حيث يتوقف نمو الخلايا الورمية عند نقطه يتشابه فيها مظهرها مع مظهر الخلايا الخديج الطبيعية بالنخاع أي أنها تجمدت عند هذه النقطة وحيث يمكن دفعها إلى النضج وتتم معالجتها بطريقه خاصة مختلفة عن الأنواع الأخرى للحصول على حالة الاستقرار أو الخلو أو الخمود(REMISSION)وهي الحالة بعد العلاج وعودة واستقرار تعدادات كريات الدم البيضاء إلى المستويات الطبيعية وخمود الخلايا الورمية وعدم وجود أية علامات سريريه.وفي حالات معينه تتمكن الخلايا الورمية من العودة إلى فعاليتها عقابيل مرحلة الخمود بعد العلاجات الاوليه وهي مرحلة الرجوع أو التواتر (RECURRENCE OR RELAPSE)

تستهدف المعالجات الوصول إلى مرحلة حصار خلايا اللوكيميا والقضاء عليها (INDUCTION OF REMISSION)وهي مرحلة تحقيق الخلو ومن ثم تأتي مرحلة التقوية وترسيخ الاستقرار (CONSOLIDATION)وتليها مرحلة الوقاية والمحافظة(MIANTENANCE) والتي تتميز فعاليتها في النوع الثالث من اللوكيميا، وتوجد الكثير من التوليفات العلاجية المختلفة المستخدمة في كل من هذه المراحل العلاجية باستخدام مجموعه من العقاقير الكيميائية المتمثلة بالخصوص من مركبات الانثراسايكلين (دوكسوروبسين) ومركب السايتوسين ارابينوسايد (سايتارابين) بالخطة العلاجية الموسومة ببرنامج ٣و٧أيام على التوالي لكل من العقارين.لكن تختلف الخطة العلاجية في لوكيميا الخلايا النخاعيه الخديج (M3)عن باقي المعالجات وهذا الاختلاف يعتمد في تطبيقيه على ما يتميز به النوع الثالث من اللوكيميا النخاعية الحادة من النظرية المؤيدة لنشوء التسرطن في مثل هذه الحالات عند حدوث تبادل بمواقع الصبغيات بين الصبغيين ١٥و ١٧ (CHROMOSOMAL TRANSLOCATION)مسببا" اختلال بعمليات الأيض الخاصة بفيتامين (أ) بأنوية الخلايا و قد أكتشف العلماء أنه باستخدام الحامض الراتنجي المعدل (ALL TRANSRETINIOC ACID_ATRA)وهو أحد مشتقات فيتامين (أ) يمكن إصلاح هذا الاختلال ودفع الخلايا الخديج المتسرطنة الى النضج متحولة إلى خلايا متعادلة، وبطريقة مشابهه لما يؤديه هذا المركب من إصلاح للاختلالات المسببة ،تم اكتشاف

مركب ثلاثي اوكسيد الزرنيخ(ARSENIC TRIOXIDE-ATO) من قبل علماء الصين لأول مره من اجل استخدامه في معالجة النوع الثالث من اللوكيميا النخاعية الحادة وبالخصوص في مثل حالات ألانتكاسه أو الارتداد بالاصابه وقد أثبتت البحوث العلمية المنشورة والحالات المسجلة باستخدام هذا العلاج نجاحا" ملحوظا" في السيطرة على هذه الأنواع من اللوكيميا والتي جعلتها تصنف من الفئات ذات التجاوب الجيد للعلاج من حيث التكهن(PROGNOSIS).

في مستشفى الكاظميه التعليمي تم تسجيل الحالة ألمنشوره في هذا التقرير كونها أول حاله مسجله في العراق تستخدم مركب(ATO) لمعالجة مريضه في الواحد والثلاثين من عمرها أصيبت بحالة الانتكاس في لوكيميا الخلايا النخاعية الخديج للمرة الثانية خلال عامين وكانت قد عولجت سابقا" بالعقاقير الكيميائية مع مركب الأترا(ATRA) وفي هذه المرة تمت معالجتها باستخدام مركب ثلاثي اوكسيد الزرنيخ (ATO) فقط، بجرعة تعادل ٠,١٥مغم /كغم/يوم(١٢مغم/يوم)وقد استمر العلاج حتى اليوم الرابع والأربعون وعنده سجلت حالة الخمود أو الخلو للخلايا الورمية ليتتبعها كورسات التقوية العلاجية وقد تضمنت أضافه الى العقاقير الكيميائية أعادة استخدام نفس المركب(ATO)بنفس الجرعة خمسة أيام في الأسبوع لمدة ٣أسابيع ومن ثم تم الاستمرار بمعالجة المريضة بإعطائها مركب الاترا كل ٣ أشهر لمدة ١٥ يوم وعقار الميثوتركسيت أسبوعيا"و عقار الميركابتوبيورين يوميا" ولا زالت المريضة تحت المتابعة حتى وقت كتابة هذا التقرير.

فرع الطب الباطني و أمراض الدم [كلية الطب - جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٣ ص٩٧-١٠١

متلازمة مويامويا

سمير حسن عبود، عبدالامير جاسم

الخلاصة

متلازمة مويامويا مرض يتميز بانسداد مزمن متطور للأوعية الدموية للدماغ. نقدم في هذا التقرير حالة لرجل في الأربعين من العمر مصاب بهذا المرض، أصيب بنزف في تجاويف الدماغ مسببا استسقاء متصل في تجاويف الدماغ. نصف في التقرير الحالة السريرية ونتائج الفحوص التشخيصية الشعاعية. المريض عولج ببزل تجاويف الدماغ إلى الجوف البريتوني بتحسن تام.

كلمات مفتاحيه: مويامويا، نزف داخل القحف، استسقاء الدماغ.

فرع الجراحة العصبية[كلية الطب- جامعة النهرين]

المجلة العراقية للعلوم الطبية ٢٠٠٧ م المجلد ٥ العدد ٢ ص١٠٢-١٠٧

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المجلة العراقية للعلوم الطبية

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

رقم الإيداع في دار الكتب و الوثائق ببغداد ٧٠٩ لسنة ٢٠٠٠

الهيئة الأستشارية

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