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Detection of Phagocytic Activity and T , B and T – Helper lymphocytes in peripheral Blood of Women Infected with Toxoplasmosis in Basrah Province

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Abstract:

A luminol – enhanced chemiluminescence assay was performed to detect the phagocytic activity of polymorph nuclear cells (PMN) in peripheral blood of women infected with *Toxoplasma gondii* . The phagocytic activity of whole blood cells showed high significant differences between the peaks of both patients and control group . In addition immunophentyping analysis was done by using CD3 , CD20 and $CD4^+$ monoclonal antibodies to estimate the mean percentage of T , B and T – helper lymphocytes in peripheral blood of women with toxoplasmosis . Our results indicated that there was no significant differences in mean percentage of T , B lymphocytes between patients and control group . Null cells numbers also did not show any significant differences between the previous studied groups , while the mean percentage of T – helper lymphocytes subset demonstrated a high significant decrease in patients in comparison with control group .

Introduction:

Toxoplasma gondii is a protozoan parasite of the phylum Apicomplexa (1) it is present as an intracellular parasite of different kinds of tissues including muscles and intestinal epithelium, tissues and capable of infecting the nucleated cells of virtually all warm blood animals (2).*T. gondii* infection in human is a widespread through the world . Its seroprevelance is dependent on the local and the age of the population (3).The clinical toxoplasmosis is less common because most of the infection (over 80%) are asymptomatic or mild . Generally the disease has three stages : acute , subacute and chronic (4).

Infection with *T. gondii* causes innate , humoral and cell-mediated adaptive responses which are both needed because the parasite is an intracellular pathogen but it does also move through the extracellular spaces in order to find a new host cell (5).During infection T-cells activated by parasite antigens. Generation of T-lymphocytes possessing parasite-specific activities is a characteristic of both human and murine model . In human both $CD4^+$ and $CD8^+$ T-cells with cytotoxic activity has been isolated from seropositive donors (6).In addition to the resistance conferred by

T-cells , neutrophils also play a role in host resistance to *T. gondii* . Neutrophils can phagocytose and kill the opsonized *Toxoplasma* (7).

Because there is no previous studies evaluating the cellular immune response directed against *T. gondii* infection in Basrah , also the presence of a substantial variation in the degree of pathogenicity in human and animal infections which may have an effect on the immune response, so trials for defining the immunological level were done . According to that the present study aimed to throw the light on :

I. Evaluation of the phagocytic activity in whole blood of the patients and control group using luminol-enhance chemiluminescence .

II. Evaluation of cell mediated immune response (numbers and function) of immunological cells in patients and control group which were included the enumeration of the following cells :

Percentage of T, B,T-lymphocytes by CD3 , CD20, $CD4^+$ monoclonal antibodies respectively , and Null cell by calculation from equation .

Materials and methods :

Eighty venous blood samples from women referred to the dep artment of primary health care in Basrah city and infected with toxoplasmosis confirmed by whole immunoglobulines and IgG indirect fluorescent antibody test a study group (IFAT) . And Twenty five samples from negative cases (control group) were chosen as a control group . The age of women ranged from 16-45 years. Each sample was divided into two parts :

1. The first sample of blood (2 ml) was collected in potassium ethylene diamene tetra acetic acid (EDTA) used for evaluation of phagocytosis using chemiluminescence technique.
2. the second part of blood was incubated in tube containing (10-15) IU/ml lithium heparin and used for the estimation of CD3 , CD20 and $CD4^+$ lymphocytes .

- IFAT was assessed according to (8).
- Chemiluminescence was employed according to (9) . The experiment done by using luminol- enhanced chemiluminescence .
- Evaluation of T , B and T-helper lymphocytes was done by using CD3 CD8 and $CD4^+$ monoclonal antibodies by using mouse anti-human CD3 , CD8 and $CD4^+$ flouresien isothiocynate produced by (Sero Tec.). using fluorescent microscope .
- Null cells percentage was estimated by applying this equation:

$$\text{T-lymphocytes} + \text{B-lymphocytes} + \text{Null cells} = 100$$

$$\text{Null cells} = 100 - (\text{T-lymphocytes} + \text{B-lymphocytes})$$

Preparation of lymphocytes cell suspension

Lymphocytes were separated according to (10)

Statistical analysis

The present results were analyzed by using T-test (11) .

Results and discussion:

The results of IFAT showed that eighty sera recorded a positive titer of whole Ig and IgG only not IgM which mean that all infected women during a chronic phase of disease not acute phase.

The results of mean arbitrary unit of chemiluminescence picture confirmed a high significant differences in phagocytosis activity between patients and control group in ($p<0.01$)(table 1) .

Table (1) mean arbitrary unit of chemiluminescence picture of patients and control groups

groups	No.	mean arbitrary unit (cm)	S.D	S.E	C.S. T-test
Patients	80	11.30	4.56	0.15	H.S. 7.18
control	25	6.23	2.14	0.48	

The present results of chemiluminescence postulated that the phagocytosis in patients was higher than that of control group . this means that the polymorphonulclears and macrophages after using luminol (which acting as amplifier and stimulator) were stimulated .

The estimation of chemiluminescence as an indicator of phagocytic efficiency in patients usually carried out using purified granulocytes. It is well known that the course of preparation of purified granulocytes has many influences on granulocytes function . Furthermore , this method requires a large volume of blood , and this sometimes cannot be easily taken from patients with toxoplasmosis . So in current study phagocytic efficiency of whole blood cells was performed using luminol-enhanced chemiluminescence. Moreover , because *T. gondii* was an intracellular parasite that infect phagocytic cells and other cells , a trying was assessed for studying the phagocytosis in whole blood samples of patients infected with parasite by luminol- dependent chemiluminescence .

The correlations between the release of H_2O_2 and the killing of different intracellular protozoan In addition to *T. gondii* like *Trypanosoma cruzi , Leishmania donovani* and tumor cells are evidence of the oxidative metabolism which mentioned previously by (12) ; (13) and (14) . The same findings was also suggested by (15) whoshowed that the $OH^{\cdot}$ and $O_2^{\cdot}$ were toxoplasmacidal.

Another study confirmed that *T. gondii* was susceptible to products of interaction of $O_2^{\cdot}$ and H_2O_2 ($OH^{\cdot}$ and $O_2^{\cdot}$) (16).Reactive intermediate appear to be the mechanism of inhibition of *T. gondii* replication by IFN-γ activated dendritic cells (17) .

the Phenotyping study of lymphocytes showed that the mean percentage of T-lymphocytes number in peripheral blood (estimated by CD3 monoclonal antibodies) of patients and control group were (69.7 and 69.4 %) respectively with no significant differences ($p<0.05$) as in (table 2).

Table (2) : Mean percentage of T-lymphocytes in peripheral blood of patients and control group , estimated by CD3 monoclonal antibody

groups	No.	Range	Mean percentage%	S.D	S.E	C.S. T-test
Patients	80	62-72	69.7	1.75	0.20	N.S. 0.76
control	25	66-72	69.4	1.82	0.36	

N.V.
Range = 65-75
Mean = 70

The absence of significant differences in number of CD3 T-cells between patients and control group was also noted by (18) who emphasized that all infected individuals may have a similar T-cell pattern This pattern of numeration of T-cells is unlike that of these cells when stimulated in vitro (19) In addition (18) showed that *T. gondii* may cause abnormalities in T-cells .The mean percentage of B-lymphocytes number in peripheral blood (estimated by CD20 monoclonal antibodies) of patients was (19.6 %) and showed a quite close value to those of control group (19.2 %) with no significant differences ($p>0.05$) as in (table 3) . This finding indicate that antibodies having no central role in defense against *Toxoplasma* infection , although , it acting through antibody dependent cell mediated cytotoxicity (ADCC) and assist in T-cells activation through secretion of suitable mediators or cytokine (5) . The same results were also documented by (20) who indicated the limiting numbers of B-cells in peripheral blood of seropositive donor .

Table (3): Mean percentage of B-lymphocytes in peripheral blood of patients and control group , estimated by CD2 monoclonal antibodies

groups	No.	Range	Mean percentage%	S.D	S.E	C.S. T-test
Patients	80	17-23	19.6	1.60	0.18	N.S. 0.91
control	25	18-22	19.2	1.45	0.29	

N.S.
Range = 20-30
Mean = 25

The mean percentage value of null cells on peripheral blood of patients (10.8 %) were of the close value to those of control group (11.4 %) with no significant differences ($p>0.05$) table (4) . Null cells may have been found in the blood of patients with parasitic infection , so the ole is limited in the parasitic infection (21).

Table (4): mean percentage of null cells in peripheral blood of patients and control group

groups	No.	Range	Mean percentage%	S.D	S.E	C.S. T-test
Patients	80	6-20	10.8	2.34	0.26	N.S. 0.27
control	25	8-14	11.4	2.45	0.49	

the present data indicated that the mean percentage of $CD4^+$ (T-helper cells) from patients peripheral blood (29.4 %) was significantly lower than of those of control group (42.2 %) (table 5) This means that there was some immune suppression which also indicated by (22) who revealed that some suppressive factors might be involved in the induction of cytotoxic T-lymphocytes in peripheral blood of patiens with chronic toxoplasmosis the immune suppression induced by *Toxoplasma* infection has been shown to be mediated either by macrophages (17) or suppressor T-cells (23) (24) . Another explanation that $CD4^+$ cells may initially be triggered to release IL-2 , which induced $CD8^+$ T-cells proliferation and ultimately the outgrowth of $CD8^+$ T-cells . Depression of lymphocytes proliferation responsiveness was recorded in some patients with acute and chronic toxoplasmosis (25) .

The lower number of $CD4^+$ cells does not mean that there is no stimulation or no secretion done by these cells because it has been reported that type I cytokine

response are associated with chronic toxoplasmosis in human patients (26) . This data was indicated previously by (27) .

Such type of cellular response proved in this study probably attributed to the role of memory T-cells in recognizing of parasite antigens because all the tested person where in chronic stage of infection characterized by the presence of cyst (28) .

Table (5): Mean percentage of T-helper cells in peripheral blood of patients and control group , estimated by $CD4^+$monoclonal antibody

groups	No.	Range	Mean percentage%	S.D	S.E	C.S. T-test
Patients	80	28-33	29.4	1.13	0.13	H.S. 29.77
control	25	38-44	42.2	42.2	0.41	

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تحديد الفعالية البلعمية و الخلايا اللمفاوية التائية و البائية و الخلايا المساعدة في الدم المحيطي للنساء المصابات بداء المقوسات في محافظة البصرة

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الخلاصة :

لقد تم حساب الفعالية البلعمية باستخدام فحص الاستضاءة الكيميائية المسهل باللومينول للخلايا متعددة الانوية في الدم المحيطي للنساء المصابات بـ *Toxoplasma gondii* و قد اظهرت الفعالية البلعمية لخلايا الدم اختلافات معنوية عالية بين المرضى و مجموعة السيطرة ، فضلاً عن ذلك أجريت الطرز المظهرية المناعية باستخدام الاضداد الاحادية النسيلة CD3 , CD20 , $CD4^+$ لحساب معدل النسبة المئوية للخلايا التائية و البائية و التائية المساعدة في الدم المحيطي للنساء المصابات بداء المقوسات و قد اكدت النتائج الحالية انعدام الفروق المعنوية في معدل النسبة المئوية للخلايا اللمفاوية التائية و البائية بين المرضى و مجموعة السيطرة .و لم تظهر أعداد خلايا Null أي فروقات معنوية بين المجاميع المدروسة السابقة . بينما اوضح معدل النسبة المئوية للخلايا التائية المساعدة وجود نقص معنوي عالي في المرضى بالمقارنة مع مجموعة السيطرة.