Sero-Diagnosis of Toxoplasmosis among pregnant women and immunocompromised patients

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دراسة مصلية لداء المقوسات بين النساء الحوامل والمرضى المثبطين مناعيا

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الخلاصة

أجريت الدراسة لاكتشاف انتشار أجسام المضادة لمقوس كوندي بين النِساء الحوامل بالإضافة إلى المرضى المثبطين مناعيا كمرضى السكري ، ومرضى سرطان الدم ومرضى الأورام الخبيثة والفشل الكلوي المُزمن، وذلك باستخدام اختبار ألتراصي باللاتكس (LAT) واختبار تقييم الإنزيم المناعي IgM-EIA, IgG-EIA واختبارالشريط المناعي IgM-DDIA IgG-DDIA. كان العدد الكلي الحالات الايجابية ٢١٠ (٥٢.٢ %) و٧٩ (١٩.٦ %)، ١٩٣ (٤٨ %)،٨٧ (٣١.٩ %) و١٦٣ (٥٩.٩ %) باستعمال اختبار ألتراصي باللاتكسLAT اختبار تقييم الإنزيم المناعيEIA- IgG, IgM واختبارالشريط المناعيDDIA- IgG, IgM على التوالي.

Summary

The study is conducted to detect the prevalence of anti-Toxoplasma gondii antibodies among pregnant women as well as immuncocompromised patients like diabetic patients, leukemic, patients with malignancy and chronic renal failure, with Latex agglutination test (LAT), IgG & IgM Enzyme immunoassay, and IgG & IgM Dipstick dye immunoassay. The total number of positive cases of toxoplasmosis was 210 (52.2%), 79 (19.6%), 193 (48%), 87(31.9%) and 163(59.9%) by LAT, IgM-EIA, IgG-EIA, IgM-DDIA and IgG-DDIA respectively.

Introduction:

Toxoplasma gondii is globally distributed pathogen for human and most of the infected people being clinically asymptomatic, but it causes significant morbidity and mortality in developing fetuses and in immunocompromised patients(1,2) . Toxoplasma gondii is one of the major opportunistic infection agent in immunocompromised cases such as haemodialytic, leukemic, Hodgkin's and non-Hodgkin's disease and diabetic patients, (3). The effect of Toxoplasma infection may differ depending on such factors as individual genetic predisposition, the state of the immune system, the dose, and the virulent of the infecting strain (4).

Clinical signs of toxoplasmosis are non specific and not sufficiently characteristic, so diagnosis of Toxoplasma gondii infection in human is made by biological, serological, histological and molecular methods (5). Latex Agglutination Test (LAT) is a rapid agglutination procedure for the qualitative and semiquantative determination of anti-

Toxoplasma antibodies and it is made by specific agglutination of latex suspension for T. gondii antigens coated polystyrene with Toxoplasma antibodies(6). Enzyme Immune Assay (EIA) could be used in the detection of the isotype IgA, IgM, IgG (7). The fast dipstick dye Immuno assay (DDIA) is simple, cheap, sensitive and specific for the detection of anti- Toxoplasma IgG or IgM antibodies and generally agree closely with the results of EIA(8). The aim of the study is to investigate the prevalence of anti-Toxoplasma gondii antibodies among pregnant women as well as immunocompromised patients by LAT, IgG-EIA, IgM-EIA, IgG-DDIA and IgM-DDIA.

Materials and methods:

The Patients

This study included 61 of healthy individual as control group and 341 patients who were with different diseases that attending AL-Zahraa Maternity and Pediatrics, AL-Hakeem, AL- Furat AL-Awast, and AL-sadder teaching hospitals in Najaf governorate from November 2006 to November 2007.

Group one: Pregnant women who were with or without obstetric problems (habitual abortion, intrauterine death, and congenital anomalies of fetus).

Group two: Patients who were with malignancy and on chemotherapy.

Group three: Patients who were with leukemia and on chemotherapy.

Group four: Insulin nondependent diabetic mellitus patients (type II).

Group five: Patients who had chronic renal failure and on regular haemodialysis.

Blood was drawn from each patient and control groups and collected in sterile serum tube to separate the serum which was stored at -20 till used.

Serological Tests:

A-Latex Agglutination Test (LAT): Complete LAT kits were used, this kit was supplied by Linear Chemicals Barcelona (Spain). and was done according to Johnson (1985) (9).

B- Fast Dipstick Dye Immunoassay (DDIA)

Included: A) IgM-DDIA. B) IgG-DDIA.

These kits were supplied by Quick Check (U.S.A), and was done according to manufacturer's instructions.

C-Enzyme Immunoassay

Included A) IgM-EIA B) IgG-EIA

These Kits were supplied by Biocheck, (Linear Chemical Barcelona (Spain)), and was done according to manufacturer's instructions.

Determination of the sensitivity and specificity

The sensitivity (i.e. the probability that the assay will be positive when the infection is present) and the specificity (i.e. the probability that the assay will be negative when the infection is absent) were calculated by using the following formulas [10]:

Sensitivity =TP/ (TP + FN) × 100%

Specificity = TN/ (TN + FP) × 100 %

TN =True negative, TP =true positive

FN = False negative, FP =false positive

Statistical Methods:

Chi-square test was applied to find out the significant difference between the data, where the probability (P) was less than 0.05[11].

Results:

Distribution of positive cases according to serological tests:-

The highest percentage of positive cases for toxoplasmosis was (59.9%) by IgG-DDIA and the lowest percentage was (19.6%) by IgM-EIA (Table-1).

Table-1: Distribution positive cases according to serological tests

Tests	NO.	Positive	%
LAT	402	210	52.2
IgM-EIA	402	79	19.6
IgG-EIA	402	193	48
IgM-DDIA	272	87	31.9
IgG-DDIA	272	163	59.9

Distribution of sero-positive cases of toxoplasmosis according to different groups of patients:

This study was shown that the pregnant women and patients with chronic renal failure on regular haemodialysis, were more affected by toxoplasmosis than other groups. ($P < 0.05$), (Table -2).

Table-2: Distribution of sero-positive cases of toxoplasmosis according to different groups of patients:

Tests / Groups	LAT		IgM-EIA		IgG-EIA		IgM-DDIA		IgG-DDIA	
	Positive	%	Positive	%	Positive	%	Positive	%	Positive	%
Pregnant* N=137	89	64.9	60	43.7	83	60.5	45	32.8	81	59.1
Diabetic N=87	48	55.1	13	14.9	39	44.8	23	26.4	26	29.8
Malignant N=16	7	43.7	1	6.2	5	31.2	2	12.5	7	43.7
Leukemia N=7	4	57.1	0	0	1	14.2	0	0	3	42.8
Haemodialysis* N=94	39	41.4	0	0	39	41.4	14	14.8	32	34
Control N=61	23	37.7	5	8.2	26	42.6	3	4.9	14	22.9
Total N=402	210	52.2	79	19.6	193	48	87	31.9	163	59.9

Distribution of Sero-positive Cases According to number of abortions:

The incidence of positive cases in regards to number of abortions was different significantly ($P<0.05$). It was observed that the most positive cases were in pregnant women who had one abortion (table -3).

Table -3: Sero-positive cases of toxoplasmosis in pregnant women according to number of abortion

Tests / No. of abortion	LAT	IgM-EIA	IgG-EIA	IgM-DDIA	IgG-DDIA
1* n=61	42 (68.8%)	23 (37.7%)	34 (55.7%)	9 (14.7%)	35 (57.3%)
2 n=32	22 (68.7%)	16 (50%)	24 (75%)	16 (50%)	22 (68.7%)
3 n=13	11 (84.6%)	9 (69.2%)	11 (84.6%)	13 (100%)	11 (84.6%)
4 n=5	5 (100%)	3 (75%)	5 (100%)	3 (60%)	4 (100%)
No abortion n=26	9 (34.6%)	9 (34.6%)	9 (34.6%)	4 (15.3%)	9 (34.6%)
Total n=137	89 (64.9%)	60 (43.7%)	83 (60.5%)	45 (32.8%)	81 (59.1%)

Distribution of Sero-positive Cases According to Duration of Diseases: A- In diabetic patients

It was found that there was significant differences among sero-positive cases of *Toxoplasma* in diabetic patients in regards to duration of disease, (P<0.05), (Table-4).

Table -4: Seropositive cases of toxoplasmosis according to duration of disease in diabetic patients

Test / years	LAT	IgM-EIA	IgG-EIA	IgM-DDIA	IgG-DDIA
1-5 n=22	17 (77.3%)	4 (18.2%)	17 (77.3%)	7 (31.8%)	13 (59%)
6-10 n=35	18 (51.4%)	4 (11.4%)	18 (51.4%)	7 (20%)	12 (34.3%)
11-15 n=9	4 (44.4%)	0 (0%)	0 (0%)	7 (77.7%)	0 (0%)
16-20* n=17	6 (35.3%)	5 (29.4%)	0 (0%)	2 (11.7%)	0 (0%)
21-25 n=0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
26-30** n=4	3 (75%)	0 (0%)	4 (100%)	0 (0%)	1 (25%)
Total n=87	48 (55.1%)	13 (14.9%)	39 (44.8%)	23 (26.4%)	26 (29.8%)

B-In Chronic Renal Failure:

There was significant differences among sero-positive of *Toxoplasma* in patients with haemodialysis in regards to duration of disease (P < 0.05), (table-5).

Table-5: Sero- positive cases of toxoplasmosis according to duration of disease in patients under haemodialysis.

Tests / Months	LAT	IgM-EIA	IgG-EIA	IgM-DDIA	IgG-DDIA
5-10* n=23	16 (69.5%)	0 (0%)	16 (69.5%)	14 (60.8%)	8 (34.7%)
11-16 n=39	16 (41%)	0 (0%)	16 (41%)	0 (0%)	16 (41%)
17-22 n=16	7 (43.7%)	0 (0%)	7 (43.7%)	0 (0%)	8 (50%)
23-28 n=16	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total n=94	39 (41.4%)	0 (0%)	39 (41.4%)	14 (14.9%)	32 (34%)

Evaluation of Sensitivity and Specificity for Serological Tests:

For 30 positive cases of toxoplasma by IgG-EIA, the sensitivity and specificity of LAT was 73.3%, 60%, respectively (table-6). Whereas for 31 positive cases of toxoplasma by IgM-EIA, the sensitivity and specificity of LAT was 74.2%, 66.7%, respectively (table-7).

Table-6: Sensitivity and specificity of LAT for diagnosis toxoplasmosis versus IgG-EIA results

Test		IgG-EIA		Total
		Positive	Negative	
LAT	Positive	22 True positive	4 False positive	26
	Negative	8 False negative	6 True negative	14
Total		30	10	40

Table-7: Sensitivity and specificity of LAT for the diagnosis of toxoplasmosis versus IgM-EIA results

Test		IgM-EIA		Total
		Positive	Negative	
LAT result	Positive	23 True positive	3 False positive	26
	Negative	8 False negative	6 True negative	14
Total		31	9	40

Discussion

Infection with *T. gondii* which is normally controlled by the host immune system, results in an asymptomatic chronic infection maintained by dormant tissue cysts in immunocompetent individuals[12]. Toxoplasmosis may cause sever disorders in immunocompromised patients and in pregnant women because of the high risk of transplacental transmission and the occurrence of multiple congenital lesions in the fetus[13]. The study applied the following serological tests LAT, IgG-EIA, IgM-EIA, IgM-DDIA and IgG-DDIA for the detection of toxoplasmosis in different groups of patients who were with different types of diseases. The presence of a high *Toxoplasma*-specific IgM antibody titer combined with a high IgG titer probably indicates an cute infection within the previous 3 months. A low to medium IgM titer and a high IgG titer might indicate an acute infection 3-6 months previously, but IgM antibodies have been detected as long as 18 months after initial infection[14]. This study showed that, 210 cases (52.2%) were positive by LAT among suspected serum samples. This ratio is less than the ratio that had been reported by Rai *et al*. (1998) [15]. The ratio of IgG-EIA is nearly in agreement with ratio that had been reported by Jumaian (2005) [16]. The ratio of IgG and IgM-EIA is nearly agreement to the result recorded by Razzak *et al.* (2005) [17]. In this study, the incidence of infection with *Toxoplasma gondii* in haemodialytic patients was investigated by EIA, and the prevalence of *T. gondii* IgG and IgM was 39 (41.4%), 0 (0%) respectively. These findings may be due to that the patients under heamodialysis were immunocompromised and susceptible to infection. The changes in immune response in patients on haemodialysis might be due to impaired monocyte function, uremia and over produce proinflammatory cytokines, such as interleukin-1, tumor necrosis factor-alpha (TNF-∂) and 1L-6 [2]. In the present study the IgG –EIA and IgM-EIA positive cases who were with malignancy were 5 (31.2%), 1 (6.2%) respectively. While Wang *et al.* (2000)[18] recorded that, the rate of IgG in patients with solid malignancies and leukemic patients who were under chemotherapy was 19% and 45.4% respectively. Immunosuppressive patients who with malignant diseases and who had been previously infected with *Toxoplasma gondii* might show reactivation of parasite and increased IgG titer and less frequently increased of IgM titer [19]. The prevalence rate of *Toxoplasma* antibody titer increased with a greater number of previous abortions and only 5.9% had a positive antibody titer of *Toxoplasma*[20], whereas the present study showed that, (34.6%) of normal pregnant women were positive.

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