

Prevalence of Toxoplasmosis and Relationships With Estradiol Hormone and Vascular Cells Adhesion Molecular-1(VCAM-1)

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Abstract

Sero-epidemiological study were conducted for pregnant and aborted women to investigate the infection person with toxoplasmosis in Babylon province during the period from December 2018 till March 2019 ,ELISA technique for 53 serum samples for pregnant and 67 serum sample for aborted women , according to epidemiological criteria (Residence area , age group , existence of cats in houses , times number of abortion status , number of children , collection samples monthly, trimesters period of pregnancy). Concerning biochemical parameters, the present included determination of estradiol hormone and vascular cell adhesion molecule -1(VCAM-1) in aborted and healthy women that their ages ranged between 20 to 49 years old. it has been found a significant increase ($P<0.05$) in the levels of estradiol hormone in women affected with toxoplasmosis compared to healthy women .VCAM-1 results indicated a significant increase($P<0.05$) in a comparison with those of healthy women it can be concluded that infection with *Toxoplasma gondii* is associated with estradiol hormone and vascular cell adhesion molecules.

Keyword: *Toxoplasmosis, Estradiol, VCAM-1*

Introduction

Toxoplasma gondii is protozoan parasite a compulsory intracellular, which results toxoplasmosis. Its distributed worldwide, and capable infecting virtually all warm-blooded animals ¹ . Its ability of causing acute and life-threatening qualifications in pregnant females (women) and immunocompromised persons ² .In many statuses, the laboratory identification of severe and latent toxoplasmosis depends upon the identification of *T. gondii* especially IgG and IgM antibodies , Most serological tests like ELISA, have been used for the detection of antibodies against the infection that takes place in pregnant women ³ .Ecumenically, the distribution of this disease is extremely variable even inside the countries. In all host species, including humans, Toxoplasmosis is generally acquired either vertically from mother to fetus (congenital infection), or through ingestion of oocysts in contaminated food or water. Rarely, *T. gondii* can transmit through organ

transplantation and the transfusion of infected blood ⁴ . In many cases, laboratory diagnosis of latent and acute *T. gondii* is based on detecting *T. gondii* specific IgM and IgG antibodies, there are several serologic tests for anti-toxoplasma IgM and IgG, among which ELISA has maximum sensitivity and specificity ⁵ . In Arab and regional countries, the prevalence of toxoplasmosis was ranged between 26-81.4%. In Iraq toxoplasmosis studies have reported different prevalence rates, ranged between 19.1-25.2% ⁶ . VCAM-1 is expressed and located on luminal and lateral sides of endothelial cells. During inflammations or infections ,VCAM-1 act to enhance rolling and adhesion of white blood cells before they migrate from blood circulation into affected tissues ¹³ . The present study aims for the following study the prevalence of toxoplasmosis in pregnant and aborted in the Babylon province and Identification of chronic infection and acute infection(IgG, IgM) respectively by using ELISA technique for pregnant and aborted women and study some epidemiological parameter such as residence area and perform correlation between vascular cell adhesion molecule -1 (VCAM-1) with studied parameters (Estradiol and hematological)

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Materials & Method

Blood Samples Collection

1. It had been taking five milliliter of venous blood for both pregnant women and aborted women
2. By using syringe five milliliters as 3 ml. for serological and biochemical tests.
3. Three ml. of the blood samples were put in a gel tube and left standing for 20 minutes at room temperature for a clot, and then the tube was centrifuged at 3000 rpm for five minutes to collect serum.
4. The serum obtained was divided into many portions for different serological tests to avoid the melting of the samples and repeat the freeze because this will affect the quality of the result.
5. All sera were stored in the deep freezing -20C° until being analyzed *Toxoplasma* antibodies.

Enzyme Linked Immunosorbent assay (ELISA)

This assay was performed by using two kits, One for detection of IgG antibodies, and the other for detection of IgM specific antibodies against *Toxoplasma* antigen in the patient 's serum.

Detection of anti - *T . gondii* antibody (IgG) by Enzyme linked Immunosorbent Assay (ELISA) technique .

The bio Check *Toxoplasma* IgG ELISA (BC-1087) kit was used The *Toxoplasma* IgG ELISA is intended to evaluate a patient serologic status to *T.gondii* infection.

Principle:

Serum samples were added to patients to dig the plate covered with the pure *Toxoplasma* antigen. In the case of IgG antibodies to Toxoplasmosis in the serum, they will bind to the antigen, All non-washable substances are removed after the addition of the conjugate enzyme is linked to the anti-antigen complex, and remove conjugate enzyme by washing and the base material is added, The plate is incubated to allow hydrolysis of the base material to produce the absorption coefficient of the infected samples.

Detection of anti-*Toxoplasma gondii* antibody

(IgM) by Enzyme linked Immunosorbent Assay (ELISA) technique.

The bioCheck *Toxoplasma* IgM ELISA (BC - 1087) kits were used. The *Toxoplasma* IgM ELISA is intended for using in detection of IgM status to *T.gondii* in human serum and the procedure of IgM antibodies ELISA it's the same methods that used above in IgG procedure.

Biochemical tests :

Blood Samples Collection :

A total number of subjects in this was 65 women of these, 50 women were infected with *Toxoplasma gondii* and suffering from abortion at a least five month ago . The ages of women ranged between 20 to 49 years old . Fifteen healthy fertile women were selected to serve as a control group . The infected women were classified according to their ages into three groups, First group 20-29 years old , Second 30-39 years old , Third group 40-49 years old . All women of this study were free from other chronic diseases and diagnosed by consultant physicians . Five milliliters of blood were withdrawn and then divided into two parts, The first part (2 milliliters) were put in EDTA tubes to complete hematological analyses. The second part of blood sample (3ml) was brought into gel plain tubes and left for /5 minutes to complete blood coagulation . After complete coagulation the samples were transferred into centrifugation at 3000 rpm for 10 minutes and then sera were a spirited by micropipette and transported into Eppendorf tubes for storage at 20 C° for future biochemical analysis.

Estimation of estradiol hormone concentration

Estradiol hormone was estimated by kit supplied from Monobind company (USA) ¹⁴

Estimation of vascular cell adhesion molecular-1(VCAM-1)

The concentration of VCAM-1 factor was estimation according to instructions Monobind company (USA) ¹⁴ .

Results

A total of 120 blood samples (67 pregnant and 53 aborted) women that attending to Maternity and Children's Hospital in the Al-Hilla city, Babylon province, during the period from December 2018 till

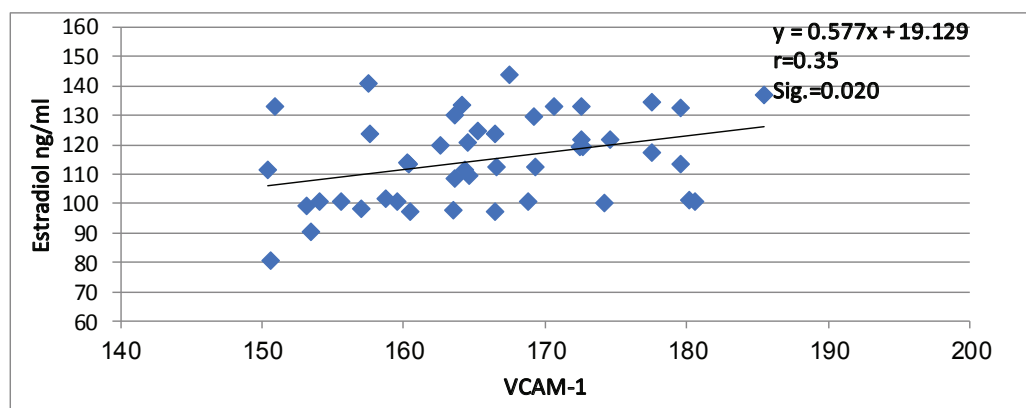
March 2019, their age groups between, (20 years to 49 years) and more than years .

Table (1): Percentage of infection of toxoplasmosis among aborted and pregnant women, diagnosed by ELISA technique

Women status	Examined No.	Positive cases for antibody IgG	(%) antibody IgG	positive cases for antibody IgM	(%) antibody IgM	positive cases for antibody (IgG&IgM)	(%) Both antibody (IgG&IgM)
Abortion	67	16	25.8	6	8.9	3	4.4
pregnancy	53	8	15.1	3	5.6	1	1.8
Total	120	24	20	9	7.5	4	3.3
No significance differences at level 0.05		P value= 0.232		P value= 0.496		P value= 0.432	
		X2 calculated= 1.427 X2 tablated= 3.841		X2 calculated= 0.463 X2 tablated= 3.841		X2 calculated= 0.616 X2 tablated= 3.841	

Table (2): Percentage infection of toxoplasmosis among aborted and pregnant women according residence area which diagnosed by ELISA technique.

Residence Area	Examined No.	Positive cases for antibody IgG	(%) antibody IgG	positive cases for antibody IgM	(%) antibody IgM	positive cases for antibody (IgG&IgM)	(%) Both antibody (IgG&IgM)
Rural Area	45	10	22.2	5	11.1	3	4.4
Urban Area	75	14	18.6	4	5.3	1	1.3
Total	120	24	20	9	7.5	4	3.3
No significance differences at level 0.05		P value= 0.637		P value= 0.244		P value= 0.115	
		X2calculated=0.222 X2 tablated= 3.841		X2 calculated= 1.353 X2 tablated= 3.841		X2 calculated= 2.482 X2 tablated= 3.841	



Values with same letters are non-significantly different ($p > 0.05$)

Figure (1): Shows the correlation between estradiol hormone and vascular cell adhesion molecule-1 (VCAM-1) in women affected with *Toxoplasma gondii*

Prevalence of toxoplasmosis among pregnant and aborted women.

The results of a positive sample by using ELISA technique for IgG and IgM and mixed (IgG&IgM) among aborted women 25.8 %, 8.9 %, and 4.4 %, respectively, but the pregnant women that lowest than compared with aborted woman for IgG, IgM and mixed (IgG & IgM) 15.1 %, 5.6 %, and 1.8 % respectively ,these results shows the percentage of infection in the aborted women has high level but do not reveal significant differences ($p>0.05$). The present study agreement with the study of the ¹⁵ in both (IgG&IgM) was 3.7% in aborted women and study of ¹⁶ agree in aborted women for IgG and IgM was 20%, 5% respectively, also the study ¹⁷ for IgM was 6.04%.

While present study disagree with the ¹⁸ for it is antibodies IgG and IgM which were 40%, 29% respectively, in aborted women and also ¹⁷ percentage infection in the aborted women for IgM only it was 6.04%. as well as study ¹⁹ in aborted and pregnant women for IgG were 41.8%, 40.0% respectively and ¹⁶ do not show the percentage IgM in the pregnant women was 0%.

The reason for this fluctuations for the rates of infection may be explain as the antibodies IgM level have been in the first weeks to three weeks from infection are very high and then begin to reduced its concentration and high increased concentration for antibody IgG significantly and that too those seen in aborted women where they have been subjected to infection, , predecessor and thus stimulate the immune system in advance which increases of high concentrations of these antibodies no these fall into chronic infections or may be conformed the most patients in this study have chronic infection (IgG) or sub-acute infection (IgG&IgM) and acute infection (IgM) .

Prevalence of toxoplasmosis among pregnant and aborted Women according to the residence area

By using ELISA test IgG, IgM and mixed (IgG,IgM) in rural area where 22.2% ,11.1% 4.4% respectively , compared to urban area 18.6 %, 5.3%, 1.3% ,respectively any rural more susceptible of urban area to infection , these results shows the rural area have high level but do not reveal significant differences ($P>0.05$).

The current study agreement with study ¹⁵ where had percentage infection IgG and mixed (IgG & IgM) 18.2% , 5.5% in rural area while in urban area 13.8%, 4.5% respectively , and the study ²⁰ showed that IgM in rural area was 10% and the urban area for IgG was 28% and agree with ²¹ in rural area for IgM was 9.7%, also, study ²² has percentage IgG and IgM were 23%, 1% in rural area respectively, as well as study ²³ agree with present study where has IgM in rural area and urban area were (4%) and (1.5%) respectively.

The present study disagreement with ²⁴ in Babylon province showed that urban area more than rural area for IgG were 73.1%,26.9% , respectively, but significant differences did not appear in urban areas compared to rural areas. And ¹⁸ who found for (IgG & IgM) 44.8% urban area and 39.6% rural area and as well as study ²⁵ disagree with current where has percentage infection of IgG was (68%) in rural area and (32%) in urban area.

Biochemical Parameters :

Data obtained from the present study indicated significant elevation ($p< 0.05$) of estradiol hormone level in first and second groups of women affected with toxoplasmosis the association between sex-hormone and toxoplasmosis infection is a particular problem in public health ²⁹. The present results were not consistent which indicated that acut infection with Toxoplasmosis is related with significant increase in the levels of estrogen of pregnant and non -pregnant women ³⁰ .

Conclusions

The current study demonstrated increase titer of IgG, IgM in cases of rural area more than of urban area also all the cases fall in the chronic infection because high frequency of IgG and also showed increase levels of cell adhesion molecules VCAM-1 associated with increase estradiol hormone of infected women.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science for Women, University of Babylon, Hilla, Iraq, Iraq and all experiments were carried out in accordance with

approved guidelines.

References

1. Roberts LS , Janovy J. Foundation of parasitology. 7th .Edn., McGraw Hill Higher Education, Boston, New York: 2005;135-137.
2. Cook AJ, Gilbert RE. Sources of toxoplasmosis infection in pregnant women: European multicentre case-control study. *BMJ*. 2000; 321: 142-147
3. Hajsoleimani F , Ataeian A. Seroprevalence of *Toxoplasma gondii* in pregnant women and bioassay of IgM positive cases in Zanjan, Northwest of Iran. *Iran. J., Parasitol.*, 2012;7(2): 82-86.
4. Sadiqui S , Shah SRH , Almugadam BS , Shakeela Q , Ahmad S. Distribution of *Toxoplasma gondii* IgM and IgG antibody seropositivity among age groups and gestational periods in pregnant women. *F1000Research*, 2018; 7.
5. Malary M , Hamzehgardeshi Z , Moosazadeh M , Afshari M , Ahmadi I , Moghaddasifar I. Seroprevalence of *Toxoplasma gondii* infection among Iranian pregnant women : a systematic review and meta-analysis, 2018;24(5)
6. Al-kuraishi AH , Hasan HH , Al-kateeb SMJ. Lipid Profile Changes in Toxoplasmosis Aborted Women Abstract : Introduction : Materials and Methods : *Baghdad Science Journal*, 2013;10(1):168–175
7. Wu BN , O’Sullivan AJ. Sex differences in energy metabolism need to be considered with life style modifications in humans. *J. Nutr. Metab.*, 2011; 391809.
8. Rubstov AV , Rubstova K, Kappler JW, Marrack P. Genetic and hormonal factors in female- biased autoimmunity. *Autoimmun. Rev.* 2010; 9:494-498.
9. Rea IM , Gibson DS , McGilligan V , McNerlan SE , Alexander HD , Ross OA. . Age- and age-related diseases: role of inflammation triggers and cytokines. *Front Immunol.*, 2018;9:586.
10. Riggs BL , Khosla S. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women. *J. Bone Miner. Res.*, 1998;13: 763-773
11. White RE , Gerrity R , Barman SA , Han G. Estrogen and oxidative stress: A novel mechanism that may increase the risk for cardiovascular disease in women. *Steroid*, 2010;75(11): 788-793
12. Hortelano S , Lopez- Fontal R , Traves PG , Villa N , Grashoff C , Bosca L , Lague A. ILK mediates LPS induced vascular adhesion receptor expression and subsequent leucocyte trans-endothelial migration . *Cardiovasc Res.*, 2010;89:283-292.
13. Wittchen ES. Endothelial signaling in paracellular and transcellular leukocyte transmigration. *Front Biosci. Landmark*, 2009;14:2522-2545
14. Abraham GE. The application of natural steroid radioimmunoassay to gynecologic endocrinology . In: Abraham GE, editor . *Radioassay System in Clinical Endocrinology*. Basel : Marcel Dekker , 1981;475-529.
15. Abbas SS , Al-Hamairy AK. Molecular Study of Toxoplasmosis and Its Relationship With Some Parameters (TSP , Globulin and Albumin) Among pregnant and aborted women in the Babylon Province Iraq. *International Journal of PharmTech Research*, 2016;9(9):366–380.
16. Anubhuti¹, Radha Rani Roy², JP Mittra³ SJB. ORIGINAL ARTICLE. *Journal of Evolution of Medical and Dental Sciences*, 2015;4(39): 6763–6768.
17. Al-Hussien EFA. Study of Prevalence and Some Immunological Characteristics of *Toxoplasma gondii*. *Journal of Babylon University*, 2016;24(2): 526–533.
18. Al-Mosawi A. Epidemiological study and immunological infection toxoplasmosis among women in Babylon province ph. D. Thesis. College of Science, University of Babylon : 2012;120.
19. Al-Dulaimy SHF. The effect of toxoplasmosis on serum protein components in pregnant and aborted women, M. Sc. Thesis. College of Science, University of Baghdad, , 2015;115.
20. Al-Rawazq HSAM. Seroprevalence of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) and Risk Factors of Toxoplasmosis for A sample of Pregnant Women in Baghdad. *Al-Kindy College Medical Journal*, 2017;13(2): 43–48.
21. Sharad NA. “Seroepidemiological study for patients with diabetes that infected with *Toxoplasma gondii* in the Babylon province.” *International Journal of Innovation and Applied Studies* 12.1: 2015;183
22. Awad RM. SeroEpidemiological study of Toxoplasmosis in Blood donor in Babylon province. *Iraqi Journal of Biotechnology*, January 2010;9:409–417.

23. Manji ZF, Al-Hamairy AK. Seroepidemiological and Molecular Study of Toxoplasmosis in the Blood Donors and Applicants for Marriage peoples in the Babylon Province, Iraq. *Res. J. Pharm. Bio. Chem. Sci.*, 2015;6(6):1106-1114.
24. Ewadh RM. Seroepidemiological Study Of Toxoplasmosis In Blood Donors In Babylon Province, 2010;409–417.
25. Attieh S. Epidemidogical Study Of Toxoplasmosis Among Pregnant Women In Baghdad City. *Journal of Kerbala University*, 2011;9(2):161–166
26. Roberts LS , Janovy J r. *Foundations of Parasitology*. 6th edn., McGraw-Hill, Dubuque, Iowa: 2000;670.
27. Schwartzman J D. Toxoplasmosis. In Gillespie, S. H and pearson R. D. (Ed.) *Principles and practice of clinical Parasitology*. John Wiley & sons Ltd. Virginia : 2001;113-138.
28. Mortensen PB , Pedersen CB , Westergaard T, Wohlfahrt J , Ewald H. Effects of family history and place and season of birth on the risk of Schizophrenia. *New Eng. J. of Med.*, 1999;340(8): 603-608.
29. Roberts CW , Walker W , Alexander J. Sex-associated hormones and immunity to protozoan parasites. (*lin . Microbiol. Rev.*, 2001;14 : 467-488).
30. Rageb O , Abd El-Maksoud HA, Afaf AD , Fathy KA. Biochemical effect of Toxolpasmosis infestation on hormones and iron in aborted women . *Benha veterinary Medical J.* 2015; 28 (1) :120-124
31. Hussein JH , Al-Aaraji M , Aljorani RH. Study the effect of acut Toxolpasmosis infection one some hormones and the phagocytic activity of neutrophile in pregnant and non - pregnant women befor and after treatment . *Int . J. Eurr . Microbiol . App. Sci*, 2015;4 (10) : 459-465.