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## Seroepidemiological aspects for *Toxoplasma gondii* infection in women of Qadisiyah province, Iraq.

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**Abstract :** *Toxoplasma gondii* an intracellular parasite infected large proportion of the world population, but mostly without visible clinical signs in immunocompetent patients. The present study was performed on 125 aborted women as case group and 30 healthy women (15 non pregnant and 15 pregnant women) as control groups in Women's and children Hospital in Qadisiyah province, Iraq from December / 2015 till March / 2016 to investigate *Toxoplasma* infection in women with abortion or not and assess the association between *T. gondii* and some variables such as age, chronic diseases, abortion time, children and frequency of abortions. Five ml of venous blood sample was obtained from each woman under sterile condition. The study were involved the measurement of *Toxoplasma* antibodies (IgG & IgM) in the sera of aborted and normal pregnant women by Elisa test. The outcomes demonstrated the seroprevalence of IgG (latent infection) and IgM (recently acquired infection) anti-*Toxoplasma* antibodies were positive in 55/125 cases (44%) and 5/125 cases (4%) respectively, However the Overall Seroprevalence of anti- *T.gondii* antibodies in aborted women was 48%. Mixed seropositive for IgG and IgM were didn't recorded in this study, whereas the healthy women were 0% for all antibodies, the total seroprevalence of all antibodies in both groups were positive in 60/155 cases (38.7%) and 35.4% for IgG and 3.2% for IgM. Significant relationship ( $P < 0.05$ ) was found between the seroprevalence of *T. gondii* chronic infection and age of the aborted women, several chronic diseases and abortion time, the results was showed no statistically significant association between *Toxoplasma* infection and the ability of women to have children and frequency of abortions.

Keywords: Seroepidemiological, *Toxoplasma gondii*, Qadisiyah province.

### Introduction

*Toxoplasma gondii* is highly prevalent pathogenic protozoa which infect a broad range of warm-blooded animals, including humans<sup>1,2</sup>.

The infection has a worldwide distribution. About one-third of humanity has been exposed to this parasite, but seroprevalence differs greatly between countries (less than 10% to more than 90%) and population group<sup>3</sup>.

*T.gondii* infection in healthy subject is asymptomatic but in immunocompromised patients may have serious disease<sup>4</sup>. All mammals, including humans, and birds are intermediate hosts, whereas Felidae (cats) are intermediate and definitive host, they are the only animals that pass oocyst in their feces . Sheep and goat meats are important infection sources for toxoplasmosis<sup>5</sup>.

The rate of infection in human has been reported differently in various parts of Iraq<sup>6,7,8,9,10</sup> and the world<sup>11</sup>. *Toxoplasma* infection in humans, especially in people with defective immune system, pregnant women, HIV/AIDS patients, children and those with underlying disease could entail serious damage<sup>12</sup>. Almost 70% of the infected population is largely asymptomatic, immunocompromised individuals with this infection show a high rate of morbidity and mortality<sup>13</sup>.

## Materials and methods

### Samples :

Samples were taken from 155 Iraqi women in the current study. The study was carried out during the period from December / 2015 to March / 2016. All patients were obtained from those who had been admitted to Women's Hospital and children in Qadisiyah province from women's advisory department, emergency, blood draw Laboratory and viruses Laboratory .

The samples were divided into three groups :aborted women, healthy pregnant women and healthy non-pregnant women, The first group (aborted women) was divided into two groups based on ELISA IgG test results into (positive IgG and negative IgG).

A questionnaire was designed to obtain the information of women. It contained the Name, Age, Gestational age, Address, Medications taken, history of diseases, number of abortion if occurred previously , have children or not. In addition to date sample collection.

### Sample collection :

Five ml of venous blood sample was obtained from each woman under sterile condition by using disposable syringes, and put into a sterile plain tubes (for serum collection). For serum collection the tubes were let to stand for half to one hour at room temperature, and then centrifugation was done at 3000 rpm for 5 minutes. Then the serum was collected into Eppendorf tubes and was kept at -20° C until use. Freezing and thawing of the sera was avoided and writing the sample sequence on the tube .

### Determination of IgG and IgM *Toxoplasma* antibodies

For the qualitative and quantitative detection of IgM and IgG *Toxoplasma gondii* antibodies in serum of aborted and healthy pregnant and non-pregnant women used two types kits are *Toxoplasma* IgMEIA (enzyme immunoassay) Test Kit and *Toxoplasma* IgG EIA (enzyme immunoassay) Test Kit manufactured by Foresight company, USA.

### Statistical analysis

Statistical analysis was performed with SPSS software package (Version 12 for Windows). For comparison among different groups of study, chi square ( $\chi^2$ ) test was used. A probability value of  $P < 0.05$  indicated a statistically significant difference. One way ANOVA test (LSD value) was also used where appropriate.

### Results

The seroprevalence of IgG and IgM anti-*Toxoplasma* antibodies were positive in 55/125 cases (44%) and 5/125 cases (4%) respectively, However the Overall Seroprevalence of anti- *T. gondii* antibodies in aborted women was 48%. Mixed seropositive for IgG and IgM were didn't recorded in this study, whereas the healthy women were 0% for all antibodies, the total seroprevalence of all antibodies in both groups were positive in 60/155 cases (38.7%) and 35.4% for IgG and 3.2% for IgM. As show in table (1).

**Table (1) Overall Seroprevalence of anti- *T. gondii* antibodies in aborted and healthy women.**

Study groups	N		+ve IgM	%	+ve IgG	%	+ve IgM & IgG	%	total
aborted women	125		5	4	55	44	0	0	48
Healthy women	30	pregnant	15	0	0	0	0	0	0
		Non pregnant	15	0	0	0	0	0	0
<b>Total</b>	<b>155</b>		<b>5</b>	<b>3.2</b>	<b>55</b>	<b>35.4</b>	<b>0</b>	<b>0</b>	<b>38.7</b>

The age range distribution of aborted women infected with anti-Toxoplasma IgG antibody show that significant increase of Toxo- IgG concentration at patients in age range of 15 -24 years followed by 35-44 years and lower level in age range 25 -34 year at LSD value 13.9, although statistical analysis by Chi-square demonstrated significant ( $p < 0.05$ ) association between *Toxoplasma* infection and different age groups. age range 25 -34 year have higher percentage ( 45.5% ) than other age groups , as shown in Table ( 2 ) .

**Table( 2 ) Age range distribution of aborted women in relation to Anti- Toxo. IgG antibody**

Age group and ToxoIgG	N	%	Chi-square and P value	Mean IU/ml	Std. Deviation	LSD Value
15- 24 Years	18	32.7	$X^2=6.9273$ $P=0.031316$ $df=2$ The result is significant at $p < 0.05$	106.36	23.63	13.9
25 - 34 Years	25	45.5		79.78	12.97	
35 - 44 Years	12	21.8		91.62	13.52	
Total	55	100.0		91.06	28.46	

The time of abortion was classified at first, second and third trimester according to time of pregnancy, the result of anti-Toxo. IgG give higher level among the first trimester than other times (second and third trimester) at LSD value 12.24, there are statistical relationship ( $P < 0.05$ ) between abortion time and percentage of infection with chronic toxoplasmosis, the percentage of positive patients at first trimester higher than other ( 52.7 % ), as shown in Table(3).

**Table (3) distribution of infected women in relation to IgG anti-Toxoplasma antibody according to abortion Time.**

Abortion Time and ToxoIgG	N	%	Chi-square and P value	Mean IU/ml	Std. Deviation	LSD value
First Trimester	29	52.7	$X^2=14.1273$ $P=0.000856$ $df=2$ The result is significant at $p < 0.05$	101.10	28.10	12.24
Second Trimester	14	25.5		77.95	15.79	
Third Trimester	12	21.8		82.13	16.86	
Total	55	100.0		91.06	28.46	

A group of the aborted women have other chronic diseases rather than the Toxoplasmosis , such as Hypertension, Diabetic, Hypersensitivity and others, the higher level was shown in aborted women with hypersensitivity followed by hypertension at LSD value 16.78 as well as hypertension with higher significant percentage ( $P < 0.05$ ) were recorded (10.9%) that other chronic diseases, as illustrated in table (4).

**Table (4) Distribution of aborted women with Chronic disease in relation to IgG anti-Toxoplasma antibody.**

Chronic diseases an ToxoIgG	N	%	Chi-square and P value	Mean IU/ml	Std. Deviation	LSD value
No Chronic Disease	42	76.4	$X^2=137.727$ $P=0.00001$ $df=2$ The result is significant at $p<0.05$	96.94	28.26	16.78
Hypertension	6	10.9		62.87	13.08	
Diabetic	2	3.6		5.96	5.81	
Hypersensitivity	3	5.5		133.47	15.35	
Others	2	3.6		73.83	13.90	
Total	55	100.0		91.06	28.46	

The possibility of having children or owning children and their relationship with chronic toxoplasmosis, the our results indicate that Greatest number of aborted women infected with Toxoplasmosis haven't children, but without significant association ( $P>0.05$ ), the level of anti-Toxoplasma IgG antibody was higher in aborted patients with children ( Secondary abortion ) than without children (primary) group although the latter group have higher percentage (54.5%), as shown in table ( 5 ).

**Table (5) Aborted women with or without children in relation to IgG anti-Toxoplasma antibody.**

With and without Children and ToxoIgG	N	%	Chi-square and P value	Mean IU/ml	Std. Deviation	PValue
No Children	30	54.5	$X^2=0.9091$ $P=0.340356$ $df=1$ The result is not significant at $p<0.05$	90.04	23.07	0.06
With Children	25	45.5		92.29	15.74	
Total	55	100.0		91.06	28.46	

The result of Table (6) show that aborted women with multiple abortion have higher significant level of anti-Toxoplasma IgG antibody in comparison with other group of patients(single and double abortion), most patients have single abortion (43.6 %) followed by double (30.9%) and multiple (25.5%) but without significant value ( $P>0.05$ ).

**Figure (6) Distribution of anti-Toxoplasma IgG in relation to abortion number of aborted women.**

Abortion Number and ToxoIgG	N	%	Chi-square and P value	Mean IU/ml	Std. Deviation	P value of F-Test
Single Abortion	24	43.6	$X^2=4.3091$ $P=0.115956$ $df=2$ The result is not significant at $p<0.05$	80.17	21.75	0.028
Double Abortion	17	30.9		94.71	16.34	
Multiple Abortion	14	25.5		105.31	21.42	
Total	55	100.0		91.06	28.46	

## Discussion

The serological tests are useful for the diagnosis of parasite *Toxoplasma gondii* through the detection of antibodies in the serum samples and determine the stage of infection that were in the acute phase or chronic<sup>14</sup>.

Rates vary prevalence of parasite *Toxoplasma gondii* in women between the different countries of the world depending on the different climatic conditions, food and health habits, economic status, educational level and age<sup>15</sup>.

In current study out of 155 samples, 60(38.7%) were positive for IgG & IgM anti-*Toxoplasma* antibody (4% and 44% for IgG and IgM respectively in aborted women, the Overall was 48%. whereas the healthy women were 0% for all antibodies) by Elisa technique in aborted and healthy women in diwanyia. As to the results are similar with Shallal<sup>16</sup> Results that showed the seroprevalence (IgG) toxoplasmosis was 48% while for (IgM) was 4% in 50 aborted women who had history of spontaneous recurrent abortion in Baghdad city.

Juma and Salman<sup>8</sup> showed that The frequency of toxoplasmosis among women with abortion in Al-Kadhimiya Teaching Hospital in Baghdad, Iraq was found to be 21.67%, using the immunohistochemical analysis for the detection of antigen. However, using the ELISA technique for the detection of specific IgM, the frequency was found to be 19.17% .

Results of Abbas<sup>17</sup> study demonstrated that women with first abortion have high percent for positive cases of IgM antibodies (21.5%) at compare with women without abortion, the positivity rate were 7.5%, by ELISA, which was parallel to the our findings that recorded 4% and 0% in aborted women and healthy women respectively for IgM antibodies , but in this study relatively high percent when compared to the current study may be due to many factors including the region, date and sample size difference these reasons may reflect lower percentage in present study. Previous findings in Babylon province reported prevalence of 41.66% anti-*Toxoplasma* antibody in aborted women, and 0% for control women by Latex agglutination test<sup>18</sup> .

Although frequency of *T.gondii* infection has been reported to increase in older age groups in other studies, this study showed that the highest rate of infection within the age group (25-34 years) and the lowest percentage of infection within the age group (35-44 years), this high rate of seroprevalence in 25-34 age group may belong to higher contact with cats or infected things and vegetables than other age groups.. This finding relatively analogous with Fallahietal.<sup>19</sup> result, they was observed high rate for seropositivity in 25-30 age group in Iran. Previous study in Ghana reported prevalence 29.5% and 27.0% in pregnant women aged 15-25 and 31-40 years, respectively<sup>15</sup>, however in latter study was showed no statistically significant association between *Toxoplasma* infection and age of the pregnant women. While AL-Taei<sup>20</sup> showed in her study that the highest rate of infection within the age group (30-39 years) and the lowest percentage of injury within the age group (10-19 years).

Perhaps this is due to the difference in the type of sample as the current studies included only women while studying AL-Taei included different segments of the sewage workers and farm workers, wholesalers, fruits and vegetables, male and female. The current study did not agree with Al-Obeidi<sup>21</sup> and Al-Ghezy<sup>22</sup> as well as the study of Al-Se'adawy<sup>23</sup> in the Muthanna province, where he scored the highest proportion of injuries in the age group (35-39 years). Also I did not agree with the Al-Abudy<sup>24</sup> as it recorded the highest injury within the age group (36-40 years) and less of the injured in the age group (15-20 years). The current study also differed with the study of both the Al-Ubaydi<sup>25</sup> and Al-Mayahi<sup>26</sup> where he made clear that the highest rate of infection in the age group (11-20 years) and (16-19) respectively.

As for the stages for abortion has current study reported high incidence rates in women suffering from abortion during the first trimester of pregnancy. This results Consistent with the results of Addory<sup>27</sup> study in Salah – Adden province and AL-Mayahi<sup>26</sup> in the city of Kut, Al-Ghurairy<sup>28</sup>, Al-Khashab<sup>29</sup> and Al-Abudy<sup>24</sup> high abortion rates during the first trimester of pregnancy. Also Juma and Salman<sup>8</sup>, found the highest percent of abortion was in the first trimester for those who were positive using monoclonal antibodies against *T. gondii* antigen in tissue. This corresponds with the fact that the injury in pregnancy depends on the degree of the fetus resistance as well as on the immune acquired spontaneously through the placenta, so the fetus more vulnerable to infection during the first trimester of non-immune system is completed<sup>30</sup> and is composed of opposites in the body fetus after the third month of pregnancy<sup>31</sup>. Also other reasons neglect pregnant women along with health

follow-up during the months of pregnancy to check the doctor and make the necessary serological tests for early detection of cases.

While the results of the current study did not agree with the Al-Khanak<sup>32</sup>, with the highest proportion of cases recorded in the second trimester of pregnancy, as well as not consistent with the results obtained Ramsewak *et al.*<sup>33</sup>, as record high proportion of not infected women during the first trimester of pregnancy.

The current study demonstrated a higher frequency of anti-Toxoplasma IgG antibodies in hypertension patients than other groups. many studies have reported an increased prevalence of antibodies against *T. gondii* among several disease, El-Nahas *et al.*<sup>34</sup> suggest that the opportunistic *T. gondii* infection represent a potential significant risk for chronic HCV patients. Previous report about the association of *T. gondii* infection with Chronic heart failure patients showed seropositivity rate for anti-*Toxoplasma* IgG antibodies among patients was significantly higher than in healthy volunteers<sup>35</sup>.

*Toxoplasma gondii* most protozoan causing opportunistic infections in immunocompromised hosts, thus in current study the toxoplasmosis association with other chronic diseases due to the probability of immunodeficiency in this patients.

Our findings about relationship between infection with toxoplasma and the ability to have children elucidated no significant association ( $P > 0.05$ ) in both incidence of infection and concentration of IgG antibody, however this results indicate that women with children have the lowest rate of injury by parasite. There are previous studies indicate that there is a relationship between toxoplasmosis and the occurrence of reproductive problems in the infected hosts, Fux *et al.*<sup>36</sup> observed in histological sections the females of BALBE/C mice during chronic infection with *T. gondii* showed hypertrophy of the endometrium and myometrium of uterus and reduction in folliculogenesis and the formation of corpora lutea in the ovaries when compared to the uninfected mice.

Stahl *et al.*<sup>37</sup> assume that cytokines released in response to the *Toxoplasma* reached the hypothalamus and induced a sequence of events that inhibited the release of gonadotropin-releasing hormone (GnRH), leading to the subsequent weakening of the pituitary-ovarian axis finally develops Ovarian dysfunction, also their observations indicate that absence of the critical preovulatory surge of endogenous luteinizing hormone from the pituitary at infection with toxoplasma parasite leading to impairment of ovulation in mice. Oktenli *et al.*<sup>38</sup> concluded that acute toxoplasma infection in human males may cause temporary hypogonadotrophic gonadal insufficiency regardless of the course of the disease. All of these reasons may interpret the result obtained in the current study.

The current study did not found a significant relationship between the percentage of infection and the number of abortions, although it reported high rates of infection among women who experienced single abortion. The current study agreed with Al-Khashab<sup>29</sup> and Karem<sup>39</sup>, where they found the highest rate of infection among women who have suffered single abortion, while the current study did not agree with Al-Adlan<sup>40</sup> where he did not find a relationship between the injury and the number of abortions. The current study did not agree with Al-Ghurairy<sup>28</sup> it was found that the number of abortions to two times higher than the number of abortions for once.

The reason for the high incidence among aborted women may return to the type of acute injury or reactivate chronic injury due to decrease immunity of pregnant mother's body as the time of the injury during pregnancy has an important role to determine the fate of the fetus<sup>29</sup>.

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