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## RESEARCH ARTICLE

### SEROPREVALENCE OF TOXOPLASMA GONDII ANTIBODIES IN ABORTED WOMEN USING ENZYME LINKED IMMUNESORBENT ASSAY (ELISA) IN THI-QAR PROVINCE, IRAQ.

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#### Abstract

Toxoplasmosis is the disease that caused by *Toxoplasma gondii* which can infected all warm blood animals and it is most famous parasite in human.

The study aims is to show the seroprevalence of toxoplasmosis among abortive women in Thi-Qar province. Its include 470 blood samples collected from abortive women in emergency unit of Bent –Al Huda and Al-Hubboby Hospitals and 30 women as a control (women with normal pregnancy and birth without infected with toxoplasmosis). Samples were collected from October 2017 till October 2018 with age (15-40) years.

These samples were first tested with latex agglutination test (LAT) to detect the positive sample, which give agglutinate, then with Enzyme Linked Immunesorbent Assay (ELISA) to detect IgG, IgM antibodies of *Toxoplasma*. It was found from the present study that the (ELISA) test is more specific for *Toxoplasma* diagnosis than (LAT). The seroprevalence for IgG antibody was 36.45%, IgM 4.52% and for both 1.94%. The high seroprevalence of toxoplasmosis was found in urban (26.5%) than rural (16.5%). In age group (20-24) years the seroprevalence was 12.58% found and the high ratio (47.14%) was recorded in the aborted women with first trimester more than second trimester (30.65%).

The purpose of the present study is to evaluate the seroprevalence of *T. gondii* antibodies (IgG, IgM) in aborted women and the association between this parasite and Cytomegalovirus and Rubella. Risk factors (age, sex, time and number of abortion) on infection also investigated.

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#### Introduction:-

*Toxoplasma gondii* is an obligate intracellular parasite that widely prevalent in human and another animals in all countries [1, 2]. It causes toxoplasmosis disease, which infected all warm blood animals [3]. The route of transmission of *Toxoplasma* to human is through ingestion the sporulated oocyst or tissue cyst of infected animal [4]. The congenital toxoplasmosis is most clinically important, its infection to the pregnancy causes death to fetus or severe damage such as hydrocephalus or retinoblastomas [5, 6].

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The seroprevalent of toxoplasmosis in different countries is depend on the various factors such as, Age, nutritional habitation, contact with cats, animals, and geographical condition [7]. The higher rate of seroprevalent was recorder in France 88%, due to eating of raw meat [8]. In the Arabic countries, the seroprevalent show different rate (58.2, 26, 25, 81.4)% in Kuwait,[9], Jordan,[10], Saudi Arabia,[11],and in Egypt [12] respectively.

In Baghdad province the prevalence rate 9.4% of women between (20-61) years old [13]. In Al-Najaf city, the infection rate in women was 40.4% [14]. In Basrah the seroprevalent was (41.1%, 52%) [15].while in Thi-Qar the seroprevalent in the city was 61% by (LAT) test and by ELISA (18% IgM, 32% IgG) [16].

*T. gondii* infection can be diagnosis with different methods such as serological test, Molecular test, skin test, and histological demonstration parasite [17]. The serological test (ELISA) which is a wildy used for detected the antibody of *Toxoplasma*, IgG for a chronic infection and IgM for acute infection [18].

### Material and Methods:-

Blood samples(470) of abortive women were collected from emergency unit of Bent-Al Huda Hospital and Al-Habboby Hospital, in Thi-Qar province during the period from October 2017 till October 2018. Five ml. of blood samples were collected from each abortive woman. Three ml. of blood placed in gel tube, centrifuged and then the collected serum were used in serological test (Latex and ELISA).

Each serum sample collected was tested with latex agglutination (spinarect company from Spain).The result is consider positive if the agglutination appeared after the serum and latex solution added to each other on the slide.

Samples gave agglutination result (310), were tested with ELISA test Kit (forsight company in Germany) to detect the antibodies of *T.gondii* according to the manufacturers 'instructions.

All these samples were tested for CMV and Rubella to differential diagnosis by using (forsightkit, Germany) according to the same manufacture procedure of *Toxoplasma* kit.

### Calculation of results for IgG and IgM antibodies:-

The absorbance value of each sample was divided by the cut-off value, and the results were compared with the following ratios to differentiate between positive and negative sample.

If the result value is more than >1.1 it is positive case and if the result value is less than <0.9 it is negative case.

### Results:-

#### Latex test:-

The current study showed a prevalence infection rate 66% *T.gondii* among 470 examined women who suffering from abortion using latex agglutination test.

#### Seroprevalence of *T. gondii* in aborted women using ELISA test:-

##### According to the antibodies:-

The total percentage of seropositive of *T. gondii* antibodies of aborted women was (43%), while the total percentage of seronegative was 57 %. The percentage of IgG was 36.45%, and IgM 4.52% (Table.1).

**Table 1:-**Seroprevalence of *T.gondii* in aborted women using ELISA test.

Antibodies		IgG	IgM	IgG&IgM	Total	Statistics
Seropositive	No.	113 <sup>a</sup>	14 <sup>b</sup>	6 <sup>c</sup>	133	X <sup>2</sup> =160 DF=2 P≤0.05
	%	36.45	4.52	1.94	43	

The difference in small letter means significant.

**According to the age groups:-**

The high total percentage of *T. gondii* seropositive antibodies was 12.58 % at age group (20-24) year and the lowest value 2.58% at age group >40 year, while the high total percentage of seronegative (IgG-, IgM-) was 14.19% at age group 20-24 year, and the lowest 4.83% at age group >40 year( Table. 2).

**Table 2:-**Seroprevalence of *T.gondii* antibodies by ELISA test according to the age groups in 310 abortive women.

Age group (years)	Positive ELISA		Negative ELISA		Statistics
	No.	%	No.	%	
15-19	13 <sup>b</sup>	4.19	24 <sup>a</sup>	7.74	X <sup>2</sup> =61 DF= 11 P≤0.05
20-24	39 <sup>b</sup>	12.58	44 <sup>a</sup>	14.19	
25-29	32 <sup>a</sup>	10.32	38 <sup>a</sup>	12.25	
30-34	27 <sup>b</sup>	8.71	36 <sup>a</sup>	11.61	
35-39	14 <sup>a</sup>	4.52	20 <sup>a</sup>	6.5	
>40	8 <sup>b</sup>	2.58	15 <sup>a</sup>	4.83	
Total=310	133	43	177	57	

**According to the trimester:-**

Table 3 show the percentage of positive IgG+, IgM+ and both (IgM+&IgG+) of *Toxoplasma* for 1<sup>st</sup> trimester 85%, 11.32%, and 3.77% respectively.

Table 3.Seroprevalence of *T. gondii* antibodies using ELISA test according to the trimester.

Age group (years)	IgG		IgM		IgG+ &IgM+		Statistics
	No.	%	No.	%	No.	%	
1 <sup>st</sup> n=106	90 <sup>a</sup>	85	12 <sup>b</sup>	11.32	4 <sup>c</sup>	3.77	X <sup>2</sup> = 127 DF=2 P≤ 0.05
2 <sup>nd</sup> n= 19	15 <sup>a</sup>	78.94	2 <sup>b</sup>	10.53	2 <sup>b</sup>	10.53	X <sup>2</sup> =6 DF=2 P≤ 0.05
3 <sup>rd</sup> n=8	8 <sup>a</sup>	100	0 <sup>b</sup>	0	0 <sup>b</sup>	0	X <sup>2</sup> =1.00 DF=1 P≤ 1.00 <sup>NS</sup>
Total 133	113 <sup>a</sup>	85	14 <sup>b</sup>	10.52	6 <sup>c</sup>	4.51	X <sup>2</sup> =160 DF=2 P≤ 0.05
Statistics	X <sup>2</sup> =110 DF=2 P≤ 0.05*		X <sup>2</sup> = 7 DF= 1 P≤0.05*		X <sup>2</sup> =0.66 DF=1 P≤ 0.44 <sup>NS</sup>		

**According to miscarriage:-**

The high percentage of IgG+ antibodies of aborted women was 100 % at three miscarriage or more and the lowest 78.57% at single miscarriage. The ratio of IgM+ was recorded at first, and second (15.71%, 8.11%) respectively, while no IgM was recorded at third miscarriage (Table.4).

**Table 4:-**Seroprevalence of toxoplasmosis antibodies using ELISA test according to the miscarriage in 133 aborted women.

Miscarriage	IgM+		IgG+		IgG+ &IgM+	
	No	%	No	%	No	%
Single n=70	11 <sup>b</sup>	15.71	55 <sup>a</sup>	78.57	4 <sup>c</sup>	6.2
Second n=37	3 <sup>b</sup>	8.11	32 <sup>a</sup>	86.48	2 <sup>b</sup>	5.41
More than 3 n= 26	0	0	26 <sup>a</sup>	100	0	0
Total = 133	14 <sup>b</sup>	10.52	116 <sup>a</sup>	90.62	6 <sup>c</sup>	4.7
Statistics	X <sup>2</sup> = 5 DF=1 P≤ 0.05*		X <sup>2</sup> = 12 DF= 2 P≤ 0.05*		X <sup>2</sup> = 0.66 DF=1 P≤ 0.41 <sup>NS</sup>	

**According to residency:-**

**Table 5:-**Show the high percentage seropositive antibodies of *Toxoplasma* 26.5% at urban and the lowest 16.45% at rural area, while the high percentage of seronegative 31.3% at rural and the lowest 25.8% at urban.

Table 5.Distribution of toxoplasmosis antibodies in rural and urban area of 310 aborted women.

Residency	ELISA Positive		ELISA Negative	
	No	%	No	%
Urban n=162	82 <sup>a</sup>	26.5	80 <sup>a</sup>	25.8
Rural n=148	51 <sup>b</sup>	16.45	97 <sup>a</sup>	31.3
Total n=310	133 <sup>b</sup>	43	177 <sup>a</sup>	57
Statistics	$X^2=14$ DF=3 $P \leq 0.05$			

Table 6. Show the high percentage of *T. gondii* antibodies of abortive women (81.25%) at age (25-29) and the lowest 61.5% at age group 15-19 years.No infection with CMV,Rubella alone. In mixed infection (*Toxoplasma* and CMV), the high percentage 38.5% found at age 15 -19 years while the lowest 18.75% at age 25-29 years.

**Table 6:-**The prevalence of *T.gondii*,Cytomegalovirus and Rubella in different age groups of abortive women.

Age (Year)	<i>Toxoplasma</i> only		Mixed <i>Toxoplasma</i> and CMV		Statistics
	No.	%	No.	%	
15-19 n=13	8 <sup>a</sup>	61.54	5 <sup>a</sup>	38.5	$X^2=68$ DF=11 $P \leq 0.05$
20-24 n=39	30 <sup>a</sup>	76.92	9 <sup>b</sup>	23.07	
25-29 n=32	26 <sup>a</sup>	81.25	6 <sup>b</sup>	18.75	
30-34 n= 27	20 <sup>a</sup>	74.07	7 <sup>b</sup>	26	
35-39 n= 14	10 <sup>a</sup>	71.43	4 <sup>b</sup>	28.6	
>40 n= 8	5 <sup>a</sup>	62.5	3 <sup>a</sup>	37.5	
Total n=133	99 <sup>a</sup>	74.44	34 <sup>b</sup>	25.56	
Statistics	$X^2=32$ DF=5 $P \leq 0.05^*$		$X^2=4$ DF=5 $P \leq 0.53^{NS}$		

**Discussion:-**

Blood samples 310 (66%) out of 470 aborted women were examined for Latex agglutination test, and out of 310 positive LAT 133 (43%) samples gave positive with ELISA test. Therefore, the LAT test is less used tests for diagnosis the toxoplasmosis because it gives false positive, in comparison with ELISA test which have high efficiency for detected the acute and chronic infection.This result is consist with Razzak study [19 ] in Dohuk city who used the same test to examined 310 sample, 185 (59%)samples gave positive result, but not agree with Al-Kaysi study [20] in Baghdad who recorded prevalence rates 28% by using the same test.

In 133 samples gave positive result (43%) with ELISA test, the IgG positive was found in 113 case (36. 45%), IgM positive 14 case (4.52%), and (IgM&IgG) in 6 case (1.97% ) from total of 305 examined samples give positive with (LAT). This result agree with Hadeel study in Babylon which showed 48% seroprevalent percentage in pregnant women [17], and Al-Adlaan study in Thi-Qar province who showed the serological test 42.45% [16], but not agree with Kareem in Suliamania city who give seroprevalence ratio 32.6% [18], and Al-Abody study in Thi-Qar who give 23% in pregnant women [21], and Ylmazar study in Turkey who give 30.7% in pregnant women [22].

The rate of infection is raised up in tropical and wet region because it is provide appropriate condition for growth the oocyst and their survival in soil [23,] and also raised up in countries that are consume the under cocked meat [24].

The high rate seropositive (12.58%), of toxoplasmosis in this study was found in (20-24) age group, this agree with Al-Jubori study in Kirkuk city [25] which showed the high seropositive in the same age group (19-25) , and also agree with study by Mohammed in Waist city [26], which showed that the higher rate of aborted women in age group (20-30) years.The result in current study is not agree with study by Almayahi in Kute city which showed the highest percentage of infection in age group >25 years [27] and Jassem in Baghdad city which showed that high seropositive 60% was found in abortive women at age 35 year and the lowest percentage in age group (19-25) years[28].

The high prevalence of IgM, IgG in age group (20-24) is indicated that the women in this age period are very active in managing household matters and thus are more susceptible to risk factors and pass the infection [29], while the low percentage in age group <40 is due to less contact with causative agent of toxoplasmosis infection, and have low chance to be pregnant in this age.

This study shows the significant differences between antibodies according to the trimester time. The high seroprevalence ratio of IgG 100% found in third trimester higher than second and first trimester (78.94%, 85%), while the IgM ratio was higher in first trimester 11.32% more than second (10.53%). The IgG is the only anti-body that passed from mother to the fetus through the placenta and due to a defect in the process of transport of the anti-body, which leads to accumulation in mother [30]. IgG is remain for long time and it is referred to past infection, its increase may lead to new infection. The IgM antibodies appear during 1-2 week and transform to IgG. The above results is agree with AL-Ubaddy study [31], Al-Harthy study in Sudia Arabia [32], and Akyar in Turkey [33] who recorded infection rate of IgG 24.6% and IgM 1.34%. Fouladvand in Iran showed IgM 3.3 % while IgG 23.4% [34].

The higher percentage of IgG (85%) more than IgM ratio (11.3%) and both (IgG&IgM =10.53%), is not agree with other study in Najaf city [14] which showed the IgG positive (36%) (IgM;IgG) (13.45%) and IgM (5%) respectively. But the present result show the lower ratio than other study by Mohammed in Kuffa city who showed the percentage of IgG and IgM (48%, 19.6%) respectively [35]. It is also not agree with study in Wassit which found the IgG positive (31.6%) [36]. The results of the current study is not agree with AL-Kashaab study in Tikrit city who showed the IgM percentage ratio is more than IgG (58%), (32%) respectively [37]. Al-Garriry study [38] in Diyala show the higher percentage for IgM antibody (23.5%) in compare with IgG (8.6%).

The study show the high rate of seropositive of IgG which is higher in women with recurrent abortive 100%, than single aborted 78.57%. The high ratio of IgM antibodies was found in single aborted women more than (2<sup>nd</sup> & 3<sup>rd</sup>) (15.71%, 8.11%) respectively. This result is agree with Nazakat study in Kirkuk city of pregnant women which showed the same result [39]. The current study is not agreed with Aladlaan study in Thi-Qar who showed there is no significant relationship between the toxoplasmosis and the number of miscarriages [16].

The parasite is an opportunistic organism which remain in the body in a latent form and reactive the dividing in the depression of immunity. During pregnancy, the chronic infection may be transform to acute phase causing miscarriage.

Toxoplasmosis in this study showed significant difference according the residency, the seropositive (ELISA) of abortive women in urban (26.5 %) is higher than rural (16.45%), it might be due to crowding people in the cities center and the environmental pollution compared with rural area which effects the immunity of women, In addition to bad habit for dietary and healthy such as eating fasting food exposes them to infection with toxoplasmosis [40]. The result of the present study is agree with study in AL-Najaf city [37] which showed the higher percent of toxoplasmosis in the urban area (67.6%). and corresponding with study in Baghdad, by Khadime[41]. It is also agree with Al-Wattary study in Mosul city who showed there is significant difference between the infected women in urban more than rural[42].

The current study show the significant differences between the infection of *T. gondii* and mixed with CMV, in aborted women using ELISA test, IgG antibody was recorder with CMV infection but not with IgM antibodies. The prevalent of *Toxoplasma*, CMV, in all countries show (20-70%), (40-100%) respectively[43]. The IgG was recorded in rubella infection in most sample but it is not significant because most the pregnant women take the vaccine against Rubella or the women have past infection [44].

The *Toxoplasma*, CMV, and Rubella infections were among the most common causes for abortion in women but there is no link between them, mixed infection rarely showed together. In immunocompromised, provide opportunistic infection.

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