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**RESEARCH ARTICLE**

# Estimation of *Toxoplasma gondii* infection by Serological and Immunohistochemical methods in Baghdad City-Iraq.

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## *Abstract*

*Toxoplasma gondii(T. gondii)* is one of the most prevalent infectious agents in human andhas a worldwide distribution.

The current study was conducted to estimate theseroprevalence of anti-Toxoplasma Abs in800 apparently healthy persons of both sexesbyLatex agglutination test (LAT), and evaluate the serological methods (Enzyme-linked Immunosorbent Assay ELISA and Enzyme-linked Fluorescent Assay ELFA) and Immunohistochemical IHC technique in detection of *T. gondii* in135 spontaneously aborted and 13 induced aborted women.

The LAT test indicated that 27.13% (217/800) were seropositive for anti-*Toxoplasma* Abs. No difference in the rate of infection between male (28.33%, 68/240) and females (26.61%, 149/560) was found. Toxoplasmosis was detected in 21.5% (29/135) and in 22.96% (31/135) spontaneously aborted women as investigated by ELISA and ELFA tests respectively. *T. gondii*Ags were confirmed in the placentae of 25.2% (34/135) spontaneously aborted women when IHC technique was used. These results indicatethatthere is no relationship between *Toxoplasma*seropositivity and the gender, but it increase with the age.The three methods ELISA, ELFA and IHC were all specific, but IHC was the more sensitive technique in detection of toxoplasmosis.



## Introduction:-

The protozoan *T. gondii* is an obligate intracellular parasite that infects human and a broad spectrum of vertebrate hosts (Skariah, 2010). The transmission of *T. gondii* occurs by; ingestion of oocysts shed in feline feces, ingestion of *T. gondii* cysts from chronically infected tissues, or by vertical transmission. About 20% to 90% of the world's adult population in different regions are reported to have had contact with the parasite(Firouz*et. al.*, 2014& Abdel-Raouff and Elbasheir, 2014). Between 30 and 50% of the world adult human population is may be chronically infected with *T. gondii* depending on geographic location (Pappas, 2009).*T. gondii* infection in humans is generally asymptomatic and induces a self-limiting disease. The most common clinical manifestation of acute infection is cervical lymphadenitis. Chronic infection before pregnancy does not cause transmission to the fetus, but acute infections in untreated pregnant women may cause severe disease, such as premature birth, permanent neurological damage, and visual impairment, as well as fatal necrotizing encephalitis(Louis, 2013 &YadYad*et al.*, 2014).

Epidemiological studies have identified the following risk factors: owning cats, eating raw or unwashed fruits and vegetables, eating raw or undercooked lamb, beef and minced meat products, animal farming and having contact with soil (Chiang, 2014& Robert, 2012).

Serological studies were showed a considerable variation in the prevalence ofToxoplasma infection from 0-95% in different parts of the world and betweendifferent population groups within the same country (Asthana, 2006 &Yentur, 2015). The serological methods are based on sensitivity and specificity (based on immune complex mechanism) including affinity and avidity(Petersen *et al*., 2005).LAT test was considered as a primary screen test for detection of *Toxoplasamgondii* antibodies globally beside indirect hemagglutination tests immunofluorescence tests (lFAT) and rarely by ELISA (Mahdavi*et al*.,2008 &Firouz*et. al.*, 2014).
Immunohistochemistry can detect the antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues(Ramos-Vara, 2014).

## Materials and Methods:-

A total of 800 apparently individuals referred to Al-Yarmouk Teaching Hospital laboratory unit for marriage testing profile in Baghdad city were the subjects for studying the seroprevalence of anti-Toxoplasma antibody by Latex Agglutination Test (LAT). They were 560 female (age ranged between 15 and 52 years), and 240 males (age ranged between 17 and 66 years). Also135 women attending the obstetrics and Gynecology Department in Al-Yarmouk Teaching Hospital who were admitted for evacuation of spontaneous abortion were the subjects to study the rate of toxoplasmosis infection. In addition, 13 women with induced abortion, due to maternal cardiac disease were considered as a healthy control group.

**Serum samples collection and antibody testing:-**

Five ml of venous blood was collected from all 800 apparently healthy persons and from 135 spontaneously aborted women using a sterile disposable syringe and dispensed into a sterile tube and allowed to clot at room temperature. Then clots were discarded and the sample tubes span at 500 xgfor 10 minutes. Clear sera were carefully collected, aliquot into eppendorf tubes and stored frozen at -20°C until tested. Each sample was tested for the presence of anti-*Toxoplasma* antibodies IgM using commercial ELISA Kit (Bioelisa, Spain) and ELFA (VIDAS® System from BioMerieux Company). All procedures were done according to manufacture instructions.

**Placenta tissue collection and Ag detection:-**

The placentae of aborted women were collected from curettage and placed in 10% formaldehyde under consent of senior and physician gynecologists. Two paraffin embedded blocks were prepared for each aborted woman according toCasciaro*et al* method (Casciaro*et al*, 2009). Haematoxylineand eosin staining was carried out to detect the suitable block that will be introduce in the study (sections containing the trophoblast were chosen). The toxoplasma Ags in infected trophoblast were detected by IHC technique using polyclonal primary Abs (Rabbit anti-human *Toxoplasma Gondi*, US Biological, USA).

**Evaluation of the immunostaining:-**

Evaluation of the immunostaining was carried out with the assistant of two histopathologists, the observer was blind to the clinical diagnosis at the time of assessment. The expression of *Toxoplasma*Ags was measured by scoring system. The extension of the IHC was determined in 10 microscopic fields at 400x magnification. The number of stained cells were counted.

**Statistical Analysis:-**

The statistical package for the social sciences (SPSS, version 14.0) was used for statistical analysis. Independent sample t-test was used for two-group comparison in case of quantitative data, while Chi-square test was performed for the comparison of qualitative data. Pearson correlation was used as a qualitative indicator to express the relative relation among variables. P-value <0.05 was considered statistically significant.

## Results:-

**Epidemiology**

The seroprevalence of anti-*Toxoplasma* Abs, in 800 apparently healthy persons was investigated by LAT. The subjects were arbitrarily divided into four age groups. The overall seroprevalence of *T. gondii* infection was 27.1% (217/800) persons of both sexes (table 1). The highest rate was found in the age group 30-39 (30.3%, 33/109), while a lowest rate was found in the age category<20year (23.4%, 55/235).

**Table 1: The seroprevalence of anti- *T. gondii* antibodies in apparently healthy individuals of different age groups by Latex Agglutination Test (LAT).**

| Age groups | Latex Agglutination (LAT) | | | | | |
|---|---|---|---|---|---|---|
| | Positive | | Negative | | Total | |
| | No. | % | No. | % | No. | % |
| **<20** | 55 | 23.4 | 180 | 76.6 | 235 | 29.4 |
| **20-29** | 125 | 28.5 | 314 | 71.5 | 439 | 54.9 |
| **30-39** | 33 | 30.3 | 76 | 69.7 | 109 | 13.6 |
| **≥40** | 4 | 23.5 | 13 | 76.5 | 17 | 2.1 |
| **Total** | 217 | 27.1 | 583 | 72.9 | 800 | 100 |
| Pearson Chi-Square Test; df; P 4.843; 3; 0.184. | | | | | | |

As regards to the gender, the rate 28.3% (68/240) in males and 26.6% (149/560) in females (table 2) was essentially equally divided seropositivity. A market increase in seroprevalence was noted with an increase in age except in females aged >40 years (table1 and table2). The *P*-value of significance showed no significant difference (*P*>0.1) in seropositive in all age groups.

**Table 2: The seroprevalence of anti-*T gondii* antibodies in healthy persons of different age and sex groups.**

| Age Group | Female | | | | | | Male | | | | | | Pearson Chi-Square Test; df;P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Latex Agglutination Test (LAT) | | | | | | Latex Agglutination Test (LAT) | | | | | | |
| | Positive | | Negative | | Total | | Positive | | Negative | | Total | | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| **<20** | 50 | 23.6 | 162 | 76.4 | 212 | 37.9 | 5 | 21.7 | 18 | 78.2 | 23 | 9.6 | 0.067; 1;P=0.796 |
| **20-29** | 81 | 28.8 | 200 | 71.7 | 281 | 50.2 | 44 | 27.8 | 114 | 72.2 | 158 | 65.8 | 0.018; 1;P=0.894 |
| **30-39** | 17 | 28.3 | 43 | 71.7 | 60 | 10.7 | 16 | 32.7 | 33 | 67.3 | 49 | 20.4 | 0.238;1; P=0.625 |
| **≥40** | 1 | 14.3 | 6 | 85.7 | 7 | 1.3 | 3 | 33.4 | 7 | 66.7 | 10 | 4.2 | 0.0001;1;P=0.999 |
| **Total** | 149 | 26.6 | 411 | 73.4 | 560 | 100 | 68 | 28.3 | 174 | 71.1 | 240 | 100 | 0.001;1; P=0.986 |
| Pearson Chi-Square Test; df; P 2.442; 3; 0.486 | | | | | | | Pearson Chi-Square Test; df; P 2.753; 3; 0.431 | | | | | | |

**Diagnosis:-**

**The infection rate of Toxoplasmosis among spontaneously aborted women detected by ELISA, ELFA and IHC methods.**

*T. gondii* infection among aborted women was investigated by three methods: two serological methods (ELISA and ELFA) and one immunohistochemical method (IHC). IgM antibodies against *T. gondii*were detected in sera of 21.5% (29/135) and 23% (31/135) women suffering from spontaneous abortion using ELISA and ELFA tests respectively. Toxoplasmosis was confirmed in 25.2% (34/135) women as indicated by IHC technique. Those women were found to harbor the *T. gondii*Ags in their placental tissue (table 3). The two cases that equivocal by ELISA test were positive by ELFA and IHC tests. IHC technique was used as a conformation test to discriminate between infected and uninfected women with *T. gondii* in this work. There was no significant difference (*P*>0.05) between ELISA, ELFA and IHC tests in their ability to detect *Toxoplasma* infection in aborted women when these tests were compared with each other.

**Table 3: The incidence of toxoplasmosis among aborted women detected by ELISA, ELFA and IHC techniques.**

| Tests | Positive | | Equivocal | | Negative | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| **ELISA IgM** | 29 | 21.5 | 2 | 1.5 | 104 | 77 |
| **ELFA IgM** | 31 | 23 | 0 | 0.0 | 104 | 77 |
| **IHC Ag** | 34 | 25.2 | 0 | 0.0 | 101 | 74.8 |
| **Pearson Chi-Square** | **ELISA & ELFA P=0.820** | | **ELISA & IHC P=0.514** | | **ELFA & IHC P=0.670** | |

**The sensitivity and specificity of the tests:-**

The sensitivity of IHC was higher (100%) than ELISA and ELFA, while ELISA was the lowest sensitive (85.3%) test for confirming the presence of *Toxoplasma* infection. The three methods (ELISA, ELFA and IHC), were highly specific (100%, 98.1% and 97.1%).

**Scoring system of *Toxoplasma* IHC:-**

Trophoblasts within placental villi infected with *T. gondii*were evaluated by IHC technique, in which, the infected villi were determined in 10 fields. In each field, the total numbers of villi were counted and the extent of cytoplasmic staining of the infected trophoblast cells in a given villus was determined as a percent. Accordingly, it was found that 34/135 (25.2%) spontaneously aborted women had infection with *T. gondii* (group A). From the 101 (remaining of 135) aborted women uninfected*T.gondii*, 36/135 (26.7%) were randomly selected for comparison (group B). In addition, 13 women with induced abortion and without toxoplasmosis (negative for toxoplasmosis by the three tests, ELISA, ELFA and IHC) were considered as control (group C).

The intensity of IHC expression was evaluated for each case and graded into either one of the three grades of expression: grade 1≤ 25%, grade 2 (26-75%) and grade 3 ≥76% (table 4). In group A (aborted women infected with *T. gondii*), trophoblast of seven aborted women (20.6%) expressed grade 1 (weak expression i.e. weak infection), 22 women (64.7%) expressed grade 2 (moderate expression i.e. moderate infection and only 5 women (14.7%) was expressed intense infection (intense expression or grade 3 of expression).

**Table 4: The level of IHC expression for detection of *T. gondii*infected trophoblasts.**

| IHC scoring for *T. gondii* | Group (A) Aborted Women infected with *Toxoplasma* (N=34) | | | Group (B) Aborted Women uninfected with *Toxoplasma* (N=36) | | | Group (C) Induced Abortion (Normal Pregnancy) (N=13) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Grade 1 | Grade 2 | Grade 3 | Grade 1 | Grade 2 | Grade 3 | Grade 1 | Grade 2 | Grade 3 |
| N. | 7 | 22 | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| % | 20.6 | 64.7 | 14.7 | 0 | 0 | 0 | 0 | 0 | 0 |

## Discussion:-

*Toxoplasma gondii* is spread worldwide and infects humans as well as several other mammals(Ghoneim*et al.*, 2009).Serological survey during pregnancy represents a valuable tool for the diagnosis of infection in the neonate and may bring a rapid and effective treatment of an affected child. Thus, all pregnant women should be examined at spot and seronegative women followed at intervals for evidence of seroconversion(Abdel-Raouff and Elbasheir, 2014).

**Epidemiology:-**

The present study is conducted to estimate the seroprevalence of *T. gondii*by LAT in the combined sexes, the seroprevalence was 27.1% (table 1). Differences in the incidence of toxoplasmosis were verified when different diagnostic tests were used.

In the current study, incidence of authentic toxoplasmosis is 21.5% (29/135), 23.0% (31/135) and 25.2 % (34/135) in spontaneously aborted women when ELISA (for IgM), ELFA (for IgM) and IHC (Ags in trophoblasts) tests respectively were used. Many local studies were conducted to dialing with toxoplasmosis in women who had complicated pregnancy or a history of abortion. In Babylon, by ELISA method, anti -Toxoplasma IgG, IgM, were 18.09% and 9.79%, respectively(Kadhimand Mohammed, 2013).While the rate of Toxoplasma seropositivity in Erbil was higher using LAT 54.46%, followed by ELISA IgG 37.5%, and IgM 9.13%(Hamad and Kadir, 2013).In Zanjan, Northwest of Iran 38.6%, the IgM and IgG were positive in 1.4% and 37.2% respectively(Hajsoleimani*et al*., 2012), while in Ahvaz, Southwest of Iran, 24.6% of the samples with abortion and 21.5% of the samples with normal delivery were positive for IgG antibodies. However, statistical analysis indicated no significant differences at *P*>0.05(Saki, 2015).In Turkey, the prevalence of toxoplasmosis was determined to be 58.3% and 1% for IgG and IgM, respectively(Yentur, 2015).This variation might be attributed to climate, cultural differences regarding hygienic and feeding habits.

Seroprevalence increased with increasing age except in women aged > 40 years. There was no difference in the rate of prevalence in male 28.3% and females of the child bearing age 26.6% (table 2). The same finding was reported by many studies; in Egypt, no significant difference between males (34.7%) and females (35.8%) was found (P>0.05),

and regarding the age, (in Egypt) the highest seropositivity of 45.0% and 41.66% was observed among individuals of 41-50 years and >50 years respectively (Aboelhadid, 2013).
The decline in the rate of infection which is seen in women >40 years may be due to the small sample size. The subjects of this part of the study were healthy individuals referred to Al-YarmoukTeaching Hospital, which is considered as a reference hospital for distant areas of rural and urban quarters. In these Quarters, the relatively high rate of prevalence of toxoplasmosis may be due mainly to distributions of high number of stray cats. Therefore, the close contact with cats, contaminated vegetable and water with cat feces containing oocysts and handling meat containing the bradyzoites may be the major sources of infection in our community. Women of childbearing age have not had previous exposure to *T. gondii* are at risk to acquire acute infection during pregnancy that may result in congenital toxoplasmosis.

**Diagnosis:-**
Current methods for confirmation of toxoplasmosis include the direct detection of the parasites in tissue or body fluids, inoculation and isolation of the protozoan in mice or in tissue culture and of anti-immunoglobulinsIgM, IgG and IgA antibodies in serum or fluids(Flori, 2009).Serological methods may be inconclusive or unreliable in many instances, e.g., patients with underlying disease causing suppressed antibody response, patients taking immunosuppressive therapy and certain cases of congenital toxoplasmosis(Berredjem, 2014 &Fioretti, 2004).IgM antibodies are detectable about 1 week after the infection and remain for several months or years. So the IgM toxoplasma antibody titer does not necessarily mean that the patient has recently been infected. IgA antibodies are considered to be a marker of acute infection, which are produced earlier than IgM, and may persist for several months. Specific IgE antibodies are also produced early and rapidly disappear, may give a greater indication of current infection. The presence of IgG antibodies suggestions the occurrence of infection, but does not provide any information about the timing of infection(Liu,*et al* 2015&Foudrinier, *et al*. 2003).

Correct interpretation of serological test results may be difficult not only because of persistence of IgG, IgM and IgA antibodies but also because commercial kits may be unreliable and may by give false negative or false positive results, especially for IgM antibodies (Montoya and Remington, 2008).

This finding in this study may explain the two equivocal cases by ELISA test, which were found to be positive by ELFA and IHC tests (table 3). It has long been recognized that routine histological examination of the placenta has limitations, especially with regard to the diagnosis of infectious disease and the concomitant cytokine response that may cause severe in utero fetal damage. Immunohistochemical testing of the placenta in such situations can be very useful in terms of identifying the infections agent as well as in demonstrating a market increase in cytokines produced primarily by cells native to the villi and fetal membrane(Nuovo, 2006).

From the results in the present study, that the three methods of diagnosis (ELISA, ELFA and IHC) were specific, but IHC was more sensitive (100%) in documenting of *T. gondii* infection in aborted women. We found that ELFA method was higher sensitive and more specific in detect Toxoplasma antibodies than ELISA and this is agree withGharav*et al*.(Gharav*et al*., 2011).
Regarding to the intensity of IHC expression, high proportion of women from group A (aborted infected women) their placentae produced powerful amount (grade 2) than placentae of other groups (table 4) and this is useful in differentiate infected aborted women from others who are not infected aborted women.

## Conclusions:-
Statistical difference was insignificant between male and females infections by LAT method. Immunohistochemistry technique can be used as the confirmatory test for categorizing the aborted women into aborted women infected with *T. gondii* and aborted women not infected with *T. gondii*.

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