Seroprevelance of *Toxoplasma Gondii* Among Aborted Women in Haditha

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Abstract

Toxoplasmosis is a disease caused by the protozoan parasite *Toxoplasma gondii*. Congenital transmission may occur when a pregnant mother acquired *T.gondii* infection for the first time in her life during pregnancy. During a period of six months, 206 blood samples were collected from aborted women who were referred to the Maternity and Child Hospital in Haditha, in addition to fifty (50) samples from apparent healthy women as control. Their age ranged from 15-40 years .All women screened for Toxoplasma IgM antibodies by latex Agglutination Test to study the positive rate of Toxoplasmosis in the community and to investigation the effect of risk factors on the positive rate of the disease. Toxoplasma IgM antibodies were demonstrated by using IFAT and ELFA technique to distinguish between acute and chronic infections. The overall seroprevalence of Toxoplasmosis was 54.2%. The Toxoplasma antibodies increase with age especially in the age group 25-30 years.Disease was higher in area with rural life style 65.4% then that in urban area 39.5%.The frequency rate of abortion was higher in women with one abortion, than those with two or three abortion.The percentage of infected women in the first trimester 67(48.2%) was significantly different from that of other terms of gestation.

Keyword: *Toxoplasma Gondii*, Aborted, Women
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نسبة الإصابة بطفيلي التوكسوبلازما عند النساء المجهضات في مدينة حديثة باستخدام الاختبارات المصلية

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الخلاصة

تهدف الدراسة إلى تقييم نسبة الإصابة بطفيلي التوكسوبلازما بين النساء المجهضات في مدينة حديثة. تم سحب عينات الدم من 206 نساء تعرضن للإجهاض خلال فترة ستة اشهر من اللواتي راجعن مستشفى النسائية والأطفال في مدينة حديثة، إضافة إلى 50 نموذجا جمعت من النساء الأصحاء واعتمدت المجموعة كسيطرة (control) وكانت أعمارهن من 15–40 سنة. فحصت النماذج بطريقة التلازن المباشر Latex Agglutination Test للكشف عن الأجسام المضادة لمقوسات كوندي ودرست نسبة وجود الأجسام المضادة إضافة إلى تقصي بعض العوامل الوبائية المسؤولة على انتشار المرض في المجتمع. استخدمت طريقة الاستشعاع المناعي غير المباشر IFAT وتقنية ELFA لقياس مستوى الكلويولين المناعي للكشف عن الحالات الحادة Acute والمزمنة Chronic. وجدت الأجسام المضادة للطفيلي في 54.2% عينة وتركزت أعلى إصابة في الفئة العمرية 25–30 سنة. يؤثر المستوى الثقافي والصحي والتعرض للحيوانات كثيرا على انتشار المرض حيث ان نسبة وجود الأجسام المضادة في نساء الريف بلغت 65.4% مقارنة مع نساء المدينة 39.5%. كان اعلى تكرار للإجهاض في النساء اللواتي عانين من إجهاض واحد مقارنة مع اللواتي عانين من اثنين وثلاث إجهاضات. لوحظ فرق معنوي بين نسبة وجود الأجسام المضادة وفترة الحمل، فكانت اعلى نسبة إصابة ضمن الأشهر الثلاث الأولى First trimester 67 (48.2%) مقارنة مع بقية الأشهر.

الكلمات المفتاحية: طفيلي التوكسوبلازما، الإجهاض، النساء

Introduction

Toxoplasmosis is a cosmopolitan zoonotic disease caused by the parasitic protozoan, *Toxoplasma gondii*. This parasite is an obligate intracellular organism and is found in two forms in human(1). The active proliferating tachyzoites are usually seen early as, more acute phases of the infection . The resting forms or tissue cysts are primarily found in the muscle and the brain, probably as a result of the host immune response. *Toxophasma gondii* is a heteroxenous coccidian parasite(2). The definitive hosts are domestic cats and various animal species of wild fields. The intermediate hosts are mammals and brid , A concideration of its transmission route provides an indication of why it may be predicted to alter host behavior, *Toxophasma gondii* has an indirect life cycle, where members of the cat family are the only known definitive hosts able to shed Oocysts with their Faeces(3). These Oocysts contaminate the surrounding environment A 20gr. cat stool can contain between 2-20 million Oocysts and after faecal decomposition, the local soil contamination remains infection for more than one year(4). If Oocysts are ingested by another cat, viable organisms are released which invade the intestinal epithelial cells. Where they undergo both asexual and sexual cycle, if Oocysts are ingested by a non-Feline host(5). Infection is acquired by ingestion of viable tissue cysts in meat and Oocysts are excreted by Cats that contaminated food or water(3). Congenital transmission may occur when an unifected mother acquires primary infection during pregnancy(4). Even though pregnant women that have only mild symptoms of aninfection that may cause spontaneous abortion, or still give birth, with serious foetal damage. The gestational age at which the infection is contracted as a key that variably affecting the clinical foetal outcome(5). The infection has been found in many zoogeographic regions and high infective rates were found among domestic animals-upon ingestion by a non Feline,(6) In secondary host such as a rodent, bird or human, the sporozoite is unable to enter the asexual cycle, which leads to the formation of trophozoite (6). Parasitemia follows, at which point *Toxophasma gondii* can cross the placenta and infects the fetus(7). An immune response is initiated, and the infected organism then acquired long life immunity(5). The acute asexual phase is characterized by the tachyzoite. This form of the organism is very sensitive to extreme temperatures (8). Because of the lack of specific clinical manifestations during the acute infection, *Toxoplasma gondii* can be definitivly diagnosed and is very important in pregnancy because women will benefit mostly from early treatment of prophylaxis against *T.g.* abortion(6), and is based on the detection of specific antibodies in the adult population by Indirect Fluorescent Antibody Test (IFAT) or Latex Agglutination Test (LAT) and Enzyme linked Fluorescent Assay (ELFA) .The purpose of this study was to determine in aborted women by LAT test and ELFA with IFAT test to determine the rate of infection.

Materials and Methods

- **Study group**: A total number of 206 Aborted women attending the Maternity Hospital in Haditha city, participant Consent and hospital approval were obtained. The age of the study groups ranged from 15-40. In addition 50 serum samples were collected from apparent healthy women as a control.
- **Epidemiological assessment**: A questionnaire sheet was designed to assess some of the main risk factors which may influence the prevalence of Toxoplasma infection among the expecting women volunteers. These data were intended to be completed by interviewing each participant during her hospital visit followed by home visits to validate questions related to environmental factors. The influential risk factors

considered in the study include: maternal age, gestational period, and educational level, the level of knowledge was also evaluated.

- **Samples**: A single blood sample was taken from each participant. Samples from all participants were collected over six months. Blood samples were collected in labeled 5ml tubes. Samples were then stored and delivered at 4^{o}c cool containers. The sera were separated by centrifugation and stored in 2ml aliquots at -20^{o}c till testing.
- **Serological testing**:

Kits:

1. Kits used for identification of toxo-antibodies by latex Agglutination. Toxo Test – MT kit (Eiken chemical co, japan).
2. Kits used for IgM IFAT. Bio-Merieux (France).
3. Kits used for IgM-ELFA. Bio-Merieum (France).

- **Serological test:**
- Latex Agglutination (LA) Test: The LA test for toxophasmosis was performed by the procedures Carried out by the manufacture's instruction of Toxo Test-MT kit (Eiken chemical co, Japan). Briefly, serum samples and positive serum were diluted 2- fold serially in a u-shape 96 microtiter plates using dilution buffer (0.2M amino-2-Methyl-1-Propanol) and mixed with Toxophasma antigen-adsorbed polyethylene Latex suspension overnight at room temperature. Antibody titer was determined by the last dilution number of sera which precipitated latex in the middle class dispersion based on the Manufacture's recommendation. Agglutination at dilution of 1:32 or higher was regarded as positive.
- **IFA Method**: This test was performed using Toxophasma whole body antigen (Pasture Institute) for coating the solid phase and revealed by IgM-IFA kits (Bio-Merieuy (France)). Different diluted serum samples (1:20, 1:100, and 1:200) were added to the Solid phase and the bound antibodies were detected with fluorescene-labeled anti-human antibody. Positive samples showed a green fluorescent color.
- **ELFA Method**: This method was performed using IgM ELFA Kits (Biomerieux-France) and VIDAS System. The assay principle combines an enzyme immunoassay method with a final fluorescent detection. In ELFA IgM antibody was detected by membrane and cytophasmic *T. g.* antigens coated on the slide phase receptacle (SPR). The (SPR) was coated by anti-human M chain antibodies. The bound antibody was then incubated with alkaline phosphates-conjugated monoclonal anti-human antibody and the Fluorescent substrate 4-Methy um-belliferyl phosphate. The conjugated enzyme catalyzed this substrate into a fluorescent product (4-Methyl umbelliferone) and the absorbance was measured at 450nm.
- **Statistical analysis**: A descriptive statistical analysis of the serological data in relation to the epidemiological variables were used in the study. Chi-Square test and t test were used and appropriate p cvalue of <0.05 were considered signicant. Data were analyzed using SPSS version 12 soft ware. Data were then presented in table.

Results

The prevalence of *T.gondii* antibody was measured by latex agglutination test. Among the total 139, (54.2%) were found positive.

Table (1) Number and percentage of patients and healthy control groups against *T.gondii.*

Group	No	No. (+ve)	Negative
Patients	206	139 (54.2)	67 (32.5)
Healthy Control	50	-	50 (19.5)

The correlation between age groups and positive antibody titers is shown in table (2-A).

LAT antibody titer recorded the highest rate of infection 53 (63%) in the age groups 25-30 years. While the results in table(2-B) showed that the most specific assay for IgM antibody detection was IFAT IgM recorded the highest rate of infection 31 (58.4%) in the age groups (25-30) years. In table (2-C) shown the ELFA test recorded the highest rate recorded 42(79.2%) in Same age groups (25-30) years. The difference between antibody titers and age groups were not Significant.

Table (2-A) Results of latex agglutination according to age groups

Age groups	No. Sample	Group Positive	No %	Control Groups No	%
15-20	33	13	39.3	2	4
20-25	53	26	49.0	11	22
25-30	84	53	63.0	19	38
30-35	47	28	59.5	14	28
35-40	39	19	48.7	4	8
Total	206	139	54.2	50	19.5

F = 2.28, DF = 2, P = 0.103

Table (2-B) Results of IFAT test according to age groups

Aga groups	No. Sample	No. Pos. % LAT.	No.Pos. IFAT IgM	%
15-20	13	39.3	6	46
20-25	26	44.0	12	49
25-30	53	63.0	31	58.4
30-35	28	59.5	14	50
35-40	19	48.7	7	36
Total	139	54.2	70	50.3

Table (2-C) Results of ELFA test according to age groups

Age groups	No. Sample	No Pos.% LAT.	No. pos. ELFA IgM	%
15-20	13	39.3	5	38
20-25	26	49.0	17	65
25-30	53	63.0	42	79.2
30-35	28	59.5	14	50
35-40	19	48.7	6	31
Total	139	54.2	84	60.4

In table (3-A) the women from the rural areas registered the highest rate of infection according to LAT test 91(65-4%) as compared with that from urban area 48 (39.5%). The results of chi-square test showed that there is a significant association ($p<0.05$) between infection and residential place. Thus, women who Lived in rural areas are more likely to be in contact with the disease as compared to those in urban area.

Table(3-A)The distribution of *Toxoplasmosis* according to Latex titer and residence

Resident	No. Positive LAT test	%
Rural	91	65.4
Urban	48	39.5
Total	139	54.2

Chi-Square= 7.170, DF = 2, P= 0.028

Table (3-B) The distribution of *Toxoplasmosis* according to IFAT test and residence.

Resident	No. positive IFAT test (IgM)	%
Rural	51	56
Urban	19	39.5
Total	70	50.3

Table (3-C) Thedistribution of *Toxoplasmosis* according to IFAT test and residence

Residence	No. positive IFAT test (IgM)	%
Rural	61	64
Urban	23	47.9
Total	84	60.4

The relationship between the percentage of positive cases with respect to the term of gestation is shown in table 4. The percentages of women in first trimester according to the various toxoplasmosis test in A-for IFAT test recorded 38 (56.7%), second trimester 24 (46.1%), and in the third trimester recorded 8 (40%), While in ELFA test it recorded, in first trimester of gestation 47 (70.1%), which is significant (P<0.05) and different from the percentage of other term of gestation when compared with them by the used of chi-square, for comparing two percentages 31 (59.6%) and 6 (30%) in the second and the third trimesters, respectively.

Table (5) The distribution of total Individual sample according to the term of gestation and various *Toxoplasmosis* tests.

Term of gestation	LAT No.	(%)	IFAT (IgM) No	%	ELFA (IgM) No	%
First trimester	67	48.2	38	56.7	47	70.1
Second trimester	52	37.4	24	46.1	31	59.6
Third trimester	20	14.3	40	40	6	30
Total	139 (54.2)		70	50.3	84	60.4

chi-Square= 18.9, Df = 2, P = 0.00.

Table (6) The frequency of abortions according to *Toxoplasma* antibody titer in women with one, two, and three recurrent abortions.

Frequency of Abortion	No. of abortion LAT Test No	%	No. of abortion ELFA (IgM) No	%	No. of abortion IFAT (IgM) No	%
One	65	46.7	53	81.5	41	63
Two	38	27.3	24	63.1	17	44.7
Three	29	20.8	6	20.6	9	31
More	7	5.0	1	14.2	3	42
Total	139	54.2	84	60.4	70	50.3

Chi-Square = 25.69, DF = 6, P<0.001

Discussion

The current study is one of the few studies in Haditha city to explore the prevalence of *toxoplasma gondii* infection among one of the most important clinical categories of Toxoplasmosis in immuno complement hosts who are pregnant women. If a women gets an infection with *T. gondii* for the first time in her life during pregnancy, she may pass the infection to her fetus; a situation that ultimately could lead to a very serious fetal damage. Most previous studies have concentrated on the prevalence of *Toxoplasma* infection among general population. The current study is also one of the leading studies that evaluate some environmental and behavioural factors that may influence the infection rate of *Toxoplasma gondii*. The *Toxoplasma* sero prevalence obtained in this study among aborted women in the city was 139 (54.2%). Which is comparable to the results previously reported by(16) who found that the prevalence was 69.7% in Ramadi city and in Baghdad 48.1% (19) and in Abha 31.6% (8). Nearly a similar result was found by (10) who found that 53% percent of *T.gondii* antibody by IFAT, and it was also higher than that found by (15) which was 47.0% among women by using IFAT. Similar studies were conducted in other countries and showed a great variation of Toxoplasmosis, 34% in United Arab Emirates (18), 51% in Saudia Arabia (9) and 81.4% in Egypt (21). Regional variations in the incidence of Toxoplasmosis infection rates from one country to another or even within the same country that has been well documented This variation has been attributed to climate, cultural differences regarding hygienic and feeding habits, Ingestions of cooked meat, food or water contaminated with cat faeces is responsible for the spread of the infections (13, 17). A significant relation is showed in the current study between Toxoplasmosis prevalence rate and the mothers age confirms the fact that seroprevalence of Toxoplasmosis is well known to be

increased with age; the greater the prevalence, when earlier it rises (13). This association does not mean that older age is a risky factor to predisposing the infection but might be explained by the older the person, the longer time being exposed to the causing agent and may retain a steady level of anti-Toxoplasmosis IgM in serum(9). The present data showed that the highest level of sero conversion was among 25-30 age group. The positive titers in women of different age groups indicate a relatively low level of circulative Toxoplasmosis antibody as may be expected in the latent chronic infection groups (14, 19). The difference may be due to the repeated reactivation, the immune response to *T.gondii* will build up and the patient becomes more immune to Toxoplasmosis. So by the increase of age there will be a decrease in the reactivation of the parasite(10). Women with positive cases showed a significant association ($p<0.05$) between residence and Toxoplasmosis antibody positive reaction and was higher in village rural area and low in urban areas. The current results suggested that the educational level of pregnant women may present possible protective measures against toxoplasmosis infection, high educational level may reduce the risk of exposure and increase awareness to adopt appropriate hygenic measures(11). The explanation of these results is related to the fact that farmer women are more exposed to house duties including handling of raw meat and milk products and have low education and less information about the ways of preventing *Toxoplasma.* The percentage of women in the first trimester of the pregnancy was 67 (48.2%) in LAT test, 38 (56.7%) in IFAT test and 47 (70.1%) in ELFA test, while decrease to 52 (37.4%), 24 (46.1%) and 31 (59.6%) in LAT, IFAT and ELFA test, respectively. The percent of age positive case in first trimester was significant ($p<0.05$) and was different from the percentages of other terms of gestation. Nearly, a similar finding was reported by Abbas, (2002) where the highest percentage (81.9%) was at the first trimester,and it was 64% as reported by (20). *T.gondii* crosses the placenta four to eight weeks after the onset of maternal infection, and the positive antibodies at the first trimester are mostly indicating of previous exposure to the parasite(12).

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