Study of Toxoplasma infection in women recurrent abortion in First trimester of pregnancy by Indirect immunoflurescent antibody test (IFAT)

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ABSTRACT

During a period of six months, 50 blood samples were collected from women the occupation were Housewife in First trimester of pregnancy who were referred to the Maternity and Child Hospital in Ramadi , in addition to 50 samples from healthy women as control. All women were screened for *Toxoplasma* antibodies by latex agglutination test to study the positive rate of toxoplasmosis in the community and to investigate the effect of the risk factors on the positive rate of the disease.

Toxoplasma -IgM antibodies were demonstrated by using IFAT to distinguish between acute and chronic infections. The overall seroprevalence of toxoplasmosis was 50%. The *Toxoplasma* antibodies increased with age especially in the age groups (26-30-31-35) years. Most of the positive women showed the highest titers 320 IU/ml especially in age group 26-30, 31-35 years. Were recorded high number for abortion (30) from total number(50) .

IFAT test was evaluated for specific IgM when testing 50 sera, 15 cases were with acute infection and 25with chronic toxoplasmosis using IFAT.

Keywords: *Toxoplasma, First trimester, Indirect immunoflurescent antibody test, abortion*

Introduction

Toxoplasmosis is a parasitic disease caused by the protozoan Toxoplasma gondii.[1] The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. Animals are infected by eating infected meat, by ingestion of feces of a cat that has itself recently been infected, or by transmission from mother to fetus. Although cats are often blamed for spreading toxoplasmosis, contact with raw meat is a more significant source of human infections in many countries, and fecal contamination of hands is a greater risk factor[2,3].

During the first few weeks post-exposure, the infection typically causes a mild flu-like illness or no illness. Thereafter, the parasite rarely causes any symptoms in otherwise healthy adults. However, those with a weakened immune system, such as AIDS patients or pregnant women, may become seriously ill, and it can occasionally be fatal. The parasite can cause encephalitis (inflammation of the brain) and neurologic diseases, and can affect the heart, liver, inner ears, and eyes (chorioretinitis) [4,5].

During acute toxoplasmosis, symptoms are often influenza-like: swollen lymph nodes, or muscle aches and pains that last for a month or more. Rarely, a patient with a fully functioning immune system may develop eye damage or nasal lesions from toxoplasmosis. Young children and immunocompromised patients, such as those with HIV/AIDS, those taking certain types of chemotherapy, or those who have recently received an organ transplant, may develop severe toxoplasmosis. This can cause damage to the brain (encephalitis) or the eyes (necrotizing retinochoroiditis). Infants infected via placental transmission may be born with either of these problems, or with nasal malformations, although these complications are rare in newborns [1].

Most patients who become infected with Toxoplasma gondii and develop toxoplasmosis do not know it.[citation needed] In most immunocompetent patients, the infection enters a latent phase, during which only bradyzoites are present, forming cysts in nervous and muscle tissue. Most infants who are infected while in the womb have no symptoms at birth but may develop symptoms later in life [5,6].

While rare, skin lesions may occur in the acquired form of the disease, including roseola and erythema multiforme-like eruptions, prurigo-like nodules, urticaria, and maculopapular lesions.

Newborns may have punctate macules, ecchymoses, or ‟blueberry muffin‟ lesions. Diagnosis of cutaneous toxoplasmosis is based on the tachyzoite form of T. gondii being found in the epidermis. It is found in all levels of the epidermis, is about 6 µm by 2 µm , bow- shaped, the nucleus being one-third of its size. It can be identified by electron microscopy or by Giemsa staining tissue where the cytoplasm shows blue, the nucleus red [7].

Detection of Toxoplasma gondii in human blood samples may be achieved by using the polymerase chain reaction (PCR) [5].

Toxoplasmosis can't be detected with immunostaining. Lymph nodes affected by toxoplasma have characteristic changes, including poorly demarcated reactive germinal centers, clusters of monocytoid B cells and scattered epithelioid histiocytes [8,9].

Congenital toxoplasmosis is a special form in which an unborn child is infected via the placenta. A positive antibody titer indicates previous exposure and immunity and largely ensures the unborn baby's safety. A simple blood draw at the first pre-natal doctor visit can determine whether or not the woman has had previous exposure and therefore whether or not she is at risk. If a woman receives her first exposure to toxoplasmosis while pregnant, the baby is at particular risk. A woman with no previous exposure should avoid handling raw meat, exposure to cat feces, and gardening (cat feces are common in garden soil). Most cats are not actively shedding oocysts and so are not a danger, but the risk may be reduced further by having the litterbox emptied daily (oocysts require longer than a single day to become infective), and by having someone else empty the litterbox. However, while risks can be minimized, they cannot be eliminated. For pregnant women with negative antibody titer, indicating no previous exposure to T. gondii, as frequent as monthly serology testing is advisable as treatment during pregnancy for those women exposed to T. gondii for the first time decreases dramatically the risk of passing the parasite to the fetus [6,10].

Materials & Methods

Patients:-

A total of 50 patients , the occupation were Housewife in First trimester of pregnancy were investigated in Al-Ramadi Maternity and Child Hospital during the period from 10th to January 10th July 2009. The age ranged between 18-45 years. In addition to 50 serum samples were collected from healthy women as a control. The age of this group is similar to the group of patients studied.

Materials

1-Kits:-

A-Kits used for identification of Toxo-antibodies by latex agglutination. Linear (Spain)

B-Kits used for IgM IFAT Bio-Merieux (France)

Method

1-Latex agglutination test:

Direct agglutination test on the slide for the determination of toxoplasmosis.

Reagent:

1- Toxoplasmosis latex: suspension of latex particles coated with *T.* gondii antigen, sodium azaide 0.1%.
2- Positive control: human serum, sodium azaide 0.1.%
3- Negative control: non-reactive human serum, sodium azaide 0.1.%

Procedure:

1- Bring the reagents and the samples to room temperature. Prepare the sample dilution tubes as in the following table:

Tubes	1	2	3	4	5	6
Saline sol.	-	50 µl	50 µl	50 µl	50 µl	50 µl
Sample	100 µl	Serial dilution of 50 µl				
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
Iu/ml	10	20	40	80	160	320

Placed 50 µl of each sample dilution into the individual circles of the control.Added into each circle one drop of the toxoplasmosis latex reagent, near the sample to be tested. Helped with the little stirrer mix the components covering all the surface of the circle. Rotated the slide slowly either by hand or by means of a mechanical rotator (80 r.p.m) for a period of 5 minutes.

2- Indirect immunoflurescent antibody test (IFAT) for determination of IgM antibodies:

Principle of the method:

The IFAT is based on the use of antiglobulins labeled with fluorescent dyes such as fluorescein isothiocyanate. These fluorochromes emit visible light after excitation by ultraviolet light.

Reagents:

1-Test kit with *toxoplasma* suspension, formalin killed, lyophilized *toxoplasma* suspension medium. Anti-human globulin IgM from rabbits for the *toxoplasma* test, fluorescein-conjugated.

2-Test kit *toxoplasma* controls sera, control serum (human), positive, control serum (human), negative.

3-Phosphate buffer solution concentrate PH=7.2.

4- Packing with 10 glass slides with 12 reaction fields each and 12 cover glasses, ready for use, grease-free.

5- Further reagents:

Glycerol, buffered with phosphate buffer solution (9+1) PH=7.2, distilled water.

Test procedure:

1-Dissolved toxoplasma suspension, lyophilized in 1ml suspension medium, homogenized well a tuberculin or record syringe by drawing and pushing out contents against the flask bottom.

2-Placed approximately 0.01 ml toxoplasma suspension with a syringe or capillary tube on the reaction fields of the grease-free slides which are ready for use. The suspension is not storable.

3-Allowed preparation to dry completely for 60 to 120 minutes in the incubator at 370C. If the prepared slides are kept at about -200C to -300C the toxoplasma preparations are stable for several weeks.

4-Diluted phosphate buffer-solution concentrate, PH 7.2, with distilled water 1+19 (100ml concentrate will give 2000 ml phosphate-buffered saline solution.

5-Prepared a geometric dilution series of the patient's serum with phosphate buffer-solution and load from dilution 1:64 on wards the prepared slide with approximately 0.01-ml of each serum dilution.

6-Placed slide for about 30 minutes in moist chamber.

7-Rinsed slide with phosphate buffer-solution PH=7.2 and adjust for 10 minutes in cuvette with phosphate buffer solution changing the rinsing liquid several times.

8-Placed slide between filter paper and dry up (nowiping) carry on immediately.

9-Anti-human globulin (Anti-IgM) for the toxoplasma test, fluorescien-conjugated. The globulin lyophilized with an admixture of Evans blue is dissolved with 1 ml phosphate buffer solution.

10-Overlay reaction fields with about 0.01 ml each of anti-human globulin (Anti-IgM) for the toxoplasma test.

11-Kept slide for 30 minutes at room temperature or placed in moist chamber at +370C.

12-Rinsed slide with phosphate buffer solution and adjust in cuvette with phosphate buffer solution.

13-Placed slide between filter paper and dry up (no wiping), carry on immediately.

14-Applied as little buffered glycerol as possible to the dried preparations and close with a cover glass.

15-Left preparation standing for 15 minutes at room temperature.

16-Examined preparations under the microscope.

Result and discussion

The results presented in this study were based on the analysis of a total number of 50 blood samples collected from women suffering from abortion and 10 healthy individuals as controls.

Table 1: Number and percentage of positive, negative, and control groups.

I. Group	N	%
Positive	30	30%
Negative	20	20%
Control	50	50%
Total	100	100%

The prevalence of *T.*gondii antibody was measured by latex agglutination test. Among the total 60, 30 were found positive, while 20 cases were negative (Table 1).

Table 2: Results of Latex agglutination according to age groups.

Age groups	No. Exam	Positive Group(%)	Negative Group(%)	Control Group(%)
15-20	5	2	2	5
21-25	10	5	3	10
26-30	12	8	3	5
31-35	20	11	6	15
36-40	13	4	6	15
Total	60	30	20	100

The correlation between age groups and positive antibody titers is shown in Table 2. The highest rate of infection as recorded in the age groups (31-35) years respectively while the age group (15- 20) showed the lowest rate.

Table 3: Distribution of toxoplasmosis according to Latex titer and age groups with regard to all samples.

Age groups	Total Exam.	Positive		Latex titer IU/ml									
				20		40		80		160		320	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
15-20	5	2	40%	5	5	5	5	5	5	5	5	5	5
21-25	10	5	50%	0	0	0	0	0	0	2	2	2	20
26-30	12	8	66.7%	1	83	2	16.6	3	25	4	33.3	4	33.3
31-35	20	11	55%	0	0	2	10	1	5	0	0	3	15
36-40	13	4	30.8%	1	7.6	0	0	2	15.3	2	15.3	1	7.6
Total	60	30	50%	2	3.3	4	6.6	6	10	8	13.3	10	16.6

Table(3) shows the percentage of infected women corresponding to each titer with respect to the total number of sampled women in the three groups. The antibody titer ranges from 20 IU/ml (1:2) to 320 IU/ml (1:32).The lowest number 2 (3.3 %) was recorded with titer 20 IU/ml and the highest number was 10 (16.6 %) in the titer 320 IU/ml.

Table 4: Distribution of total sample individuals according to the various toxoplasmosis tests.

Sample	Test					
	Latex			IFAT (IgM)		
	Total	+ve	%	Total	+ve	%
Positive	30	30	Loo	30	15	50
Suspected	20	0	0	15	0	0
Control	50	0	0	50	0	0
Total	100	30	30	95	15	15.8

The diagnosis of a recently acquired *T.gondii* infection was based on the detection of specific IgM antibody. Sample groups were screened by the latex agglutination test and the positive cases were confirmed by indirect Immune Fluorescent Antibody Test (IFAT) Regarding positive results 15(50%) of cases were in IFAT, see the table (4).

Table 5: Cross-classification of the categories of the latex test with the categories of the IFAT test

Latex categories	IFAT categories			Total
	0	32	64	
20	2	0	0	2
40	2	0	0	2
80	3	2	1	6
160	3	3	2	8
320	5	5	2	12
Total	15	10	5	30

II. In table 5 , The cross classification of the categories of the latex test with those of the IFAT showed that infected women in the categories (20 and 40) of the latex test are recorded as negative cases in IFAT . In latex agglutination test the positive cases were 30 (50%) cases positive from 60 while the IFAT revealed 15 positive cases out of those latex positive.

In table 6, These results indicated that the total abortions (50) are more likely to occur in age group 31-35 (17) years with high titer 160 and 320 IU/ml 50 while the age group 15-20 years showed the lowest rate(4). In addition to that some women suffered from many abortions during life.

Table 6: Correlation between age, positive antibody titers and number of abortion.

Age groups	Total abortions	Latex titer IU/ml									
		20		40		80		160		320	
		No.	%	No.		No.	%	No.	%	No.	%
15-20	4(8)	0	0	0	0	0	0	1	25	3	75
21-25	7(14)	0	0	1	14.3	2	286	2	286	2	286
26-30	13(26)	1	7.6	2	15.4	2	15.4	4	30.8	4	30.8
31-35	17(34)	3	17.6	2	11.8	3	17.6	4	23.5	5	294
36-40	10(20)	0	0	1	10	2	20	3	30	4	40
Total	50100)	4	8	6	12	9	18	14	28	18	36

III. The result of this study revealed that toxplasmosis is highly endemic in Ramadi city among aborted women since the seropositivity of disease was 30(50%) which is much higher than that recorded in Kirkuk 24.5% [11]. and some similar result was found by Al Dulaimi[12]. who found that prevalence was 69.74% in Al-Anbar.while less than in Egypt(81.4%) [13].

IV. Al-Dulaimi, (2004) has reported similar results, he found that toxoplasma antibodies increased with age especially in the age groups 21-25,26-30 years (85.7%,81.1%) respectively. Similar results were recorded by many authors [14 , 15, 16,17] Other studies recorded contrast results [18,19]. These results were similar to that reported [20]. who used ELISA and IFAT in Northern Tunisia.

Women with positive antibodies showed a significant association between age groups and titers with respect to the number of abortions.

Thus women in high age groups are more likely to get abortion if they were in acute stage of the disease (titers 160 and 320 IU/ml). found that the risk of acute infections and abortions seems to be higher in the youngest ones as showed by the proportion of high antibodies titers observed in(31-35) years age group compared to that observed after 30 years using ELISA and IFAT. [20]. All he patients under test (60) who were diagnosed previously by latex agglutination test (Table 5) in IFAT showed different results depending on the stage of the disease revealing variable picture of sensitivity and specificity of those tests.

This level decreased when the disease was resolved by treatment or limited itself [21]. Acute toxoplasmosis was detected during this study by measurement of specific IgM in 55 of women. IFAT showed (15) positive , while The IgM antibodies appear faster than IgG (as early a five days after infection) and disappear sooner than IgG antibodies. It was found that IgM - IFAT antibody was increased rapidly and fall to low titers [22].

The specific immune response to parasite leads to the production of antibody, infection by *T.gondii* is associated with the production of the immunoglobulins IgG and IgM. Antibodies can bind to the surface of parasites and cause direct damage, or by interacting with complement lead to cell lysis [23].

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Study of Toxoplasma infection in women recurrent abortion in First trimester of pregnancy by Indirect immunoflurescent antibody test (IFAT)

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دراسة داء المقوسات في النساء ذات الإجهاض المتكرر في الثلث الأول من الحمل بطريقة الأستشعاع المناعي عير المباشر

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الخلاصة

جمع 50 نموذجا من دم نساء تعرضن للاجهاض خلال فترة ستة أشهر من اللواتي راجعن مستشفى النسائية والأطفال في مدينة الرمادي، أضافه إلى 50 نموذج جمعت من النساء الأصحاء واعتمدت مجموعة كسيطرة (Control) .

فحصت النماذج بطريقة التلازن المباشر Latex agglutination test للكشف عن الأجسام المضادة لمقوسات كوندي *Toxoplasma gondii* واستخدمت طريقة الأشعاع المناعي غير المباشر IFAT لقياس مستوى الكلوبيولين المناعي IgM للكشف عن الحالات الحادة Acute و المزمنة Chronic .

وجدت الأجسام المضادة للطفيلي في 30 تينة وركزت أعلى إلاصابات عند الفئة العمرية (26-30-31-35) وأظهرت غالبية النساء أعلى معيارية 32:1 (320 وحدة دولية /مل) وخصوصا في الفئات العمرية 26-30,31-35 سنة حيث سجل أعلى عدد للاجهاض من العدد الكلى (50) .

ولأهمية قياس الكلوبيولين المناعي IgM تم استخدام IFAT حيث تم فحص 30 امرأة وأظهرت النتائج فحصا موجبا للعينات باستخدام IFAT لشبة 50% أي إن15 مريضة كانت أصابتهم من النوع الحاد Acute و25 مريضة من النوع المزمن.