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Estimation of Toxoplasma gondii infection in spontaneous aborted women by Immunohistochemical and Serological method in Holy Karbala /Iraq

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ABSTRACT

Background: Toxoplasma gondii is one of the most important human zoonotic protozoan parasites, infecting one third of the world's population. Objective: to identify the T.gondii by immunohistochemistry (IHC) in 100 spontaneously aborted women and to determine if there is an association exists between results obtained from this method and the results obtained from Rapid Toxo IgG/IgM Chromatographic Immune Assay Test(Cassette). Methods: The patients were divided into three groups, (group 1) 26 positive for Toxoplasma, (group 2) 26 negative for Toxoplasma which consider as control group and (group 3) 17 negative for Toxoplasma (induced abortion group). Result: According to the age the prevalence of toxoplasmosis was significantly higher among the (73.1%) in the age group(15-30)years old ,while the lowest infection rate (26.9%) was recorded in the age group (31-45) years old while the infection rate of Toxoplasma according to residence showed the highest infection(61.5%) in the rural ,while the lowest infection rate (38.5%) in the urban. The occupational status showed a highest infection rate (73.1%) in the house wife, while the lowest infection rate (26.9%) in the employee. The infection rate of Toxoplasma according to history of abortion showed the highest infection rate was (61.5%) in the women had previous abortion, while the lowest infection rate (38.5%) was in the woman had no previous abortion, according to number of gravidities the highest infection rate was (88.5%) in the multigravida, while the lowest infection rate (11.5%) was r in the primigravida. Toxoplasma gondii antigen intensity in placental sections was determined by IHC. The intensity of IHC expression was evaluated for each case and graded into low expression and high expression: low expression (7-30 %) and high expression (30-50%) . In group A (aborted women infected with T. gondii), trophoblast of 13 aborted women expressed low expression and 13 women expressed high expression. The extent of IHC signal positivity was significant in group A with mean percentage (29.10± 2.7). So the comparison in the current study showed that IHC was the more sensitive technique in detection of toxoplasmosis than the traditional technique (cassette).

Keywords: NIL

INTRODUCTION

The protozoan T. gondii is an obligate intracellular parasite that infects human and a wide spectrum of vertebrate hosts. The transmission of T. gondii occurs by; ingestion of oocysts exist with the feline feces, ingestion of T. gondii cysts from chronically infected tissues, or by vertical transmission. About 20% to 90% of the world's adult populations in different regions are recorded to have had contact with the T. gondii [1, 2]. Between 30 and 50% of the world adult human population is may be infected with T. gondii

depending on geographic location [3].The infection with toxoplasmosis is generally asymptomatic in human 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 [4]. Epidemiological studies have performance the following risk factors: pet cats, unwashed fruits and vegetables, eating raw or undercooked beef, lamb and minced meat products, animal farming and having contact with soil [5, 6].

Serological studies were showed a considerable variation in the prevalence of Toxoplasma infection from 0-95% in different parts of the world and between different population groups within the same country [7, 8]. The serological methods are based on sensitivity and specificity (based on immune complex mechanism) including affinity and avidity [9]. Rapid chromatocraphic test was considered as a good test for detection of IgG and IgM anti Toxoplasma gondii antibodies in both a cut and chronic Toxoplasmosis. Immunohistochemistry can detect the antigens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues [10].

Materials and Methods

The type of the study is case control study. Subjects selection one hundred pregnant female patients attending the Obstetrics and Gynecology department of Teaching Hospital in karbala / Iraq, between November - August 2018. They were all admitted to the hospital for spontaneous abortion for evacuation. In addition, 10 women with induced abortion, due to maternal cardiac disease were considered as a healthy control group.

Serum samples collection and antibody testing

3 ml of venous blood were collected from 100 spontaneously aborted women by disinfecting the anticubital fossa with 70% ethanol (Riedel de Haen) and using a disposable syringe and dispensed into a sterile tube. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at 4°C at 450X g for serum collection. The serum was then aspirated by using a Pasteur pipette and aliquot into eppendorf tubes and stored frozen at -20°C until tested

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 to Casciaroet al method [11]. 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 antihuman Toxoplasma Gondi, US Biological, USA).

Evaluation of the immune-staining:-

Evaluation of the immune-staining was done by assistant of two histopathologists, the observer was blind to the clinical diagnosis at the time of assessment. The expression of Toxoplasma gondii Ags was measured by scoring system. The extension of the IHC was determined in 10 microscopic fields at 400x magnification. The numbers of stained cells were counted.

Statistical Analysis:-

Statistical analysis was done using Graph Pad prism and SPSS software .The results were expressed as mean ± standard error (mean ± SE) and Chi-square test Categorical data was described as percentage ; comparison done by using Chi-square test. . One way ANOVA- Tukey's Test , One way ANOVA-test and T-test was used to compare parameters in different studied groups. $P \leq 0.05$ was considered statistically significant.

Person correlation coefficient (r) was used to test the correlation among the different parameters in each patients group.

Results:-

1-Demographics study of Toxoplasma gondii

Susceptible infection according to age

The infection rate of Toxoplasmosis according to age showed the highest infection rate was (73.1%) in the

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age group 15-30 years old ,while the lowest infection rate (26.9%) was in the age group (31-45) years old.

Susceptible infection according residence

The infection rate of Toxoplasma according to residence showed the highest infection rate was (61.5%) in the rural, while the lowest infection rate (38.5%) was in the urban.

Susceptible infection according occupational status

The infection rate of Toxoplasma according to occupational status showed the highest infection rate was (73.1%) in the house wife, while the lowest infection rate (26.9%) was recorded in the employee.

Susceptible infection according to history of abortion

The infection rate of Toxoplasma according to history of abortion showed the highest infection rate was (61.5%) in the women had previous abortion, while the lowest infection rate (38.5%) was recorded in the in the woman had no previous abortion.

Susceptible infection according to number of gravidities

The infection rate of Toxoplasma according to number of graviditis showed the highest infection rate was (88.5%) in the multigravida ,while the lowest infection rate (11.5%) was recorded in the primigravida .

2-Scoring system of Toxoplasma IHC:

Trophoblasts within placental villi infected by T. gondii were evaluated expression of immune reaction, thus total numbers of villi were appeared with positive extent staining in cytoplasmic material for infected trophoblastic cells that was shown determined villus as value percent. The extent of IHC signal positivity was significant in group A with mean percentage (29.10± 2.7) (Table 1)

The intensity of IHC expression was evaluated for each case and graded into low expression and high expression: low expression (7-30 %) as showed in (Fig. 1) and high expression (30-50%) as showed in (fig2). . In group A (aborted women infected with T. gondii), trophoblast of 7 aborted women expressed low expression and 19 women expressed high expression of T.gondii (Table 2).

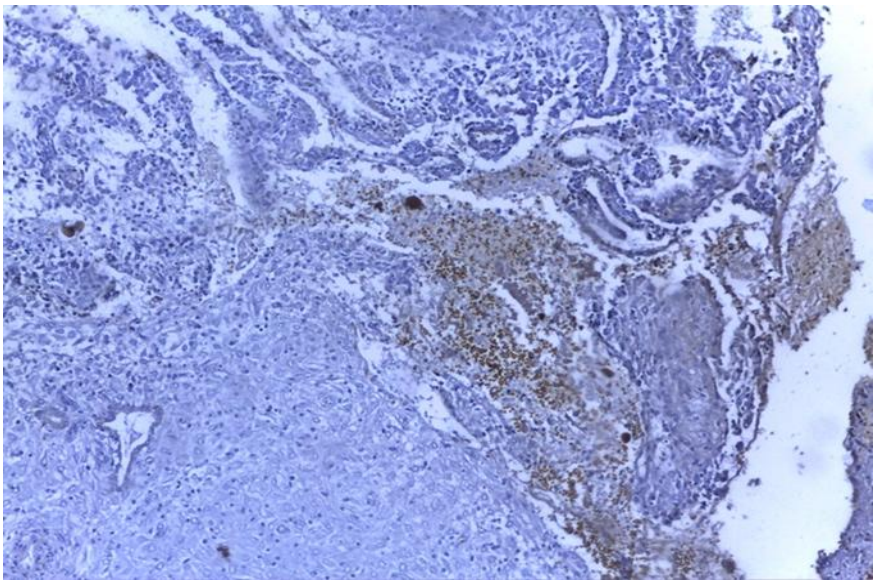

Fig 1: Immunohistochemistry staining of toxoplasma in tropobolastic tissue from women with abortion, explained Low expression of T.gondii (by DAB chromogen. Counter stained with mayer 's hematoxylin). Magnification power (4 X).

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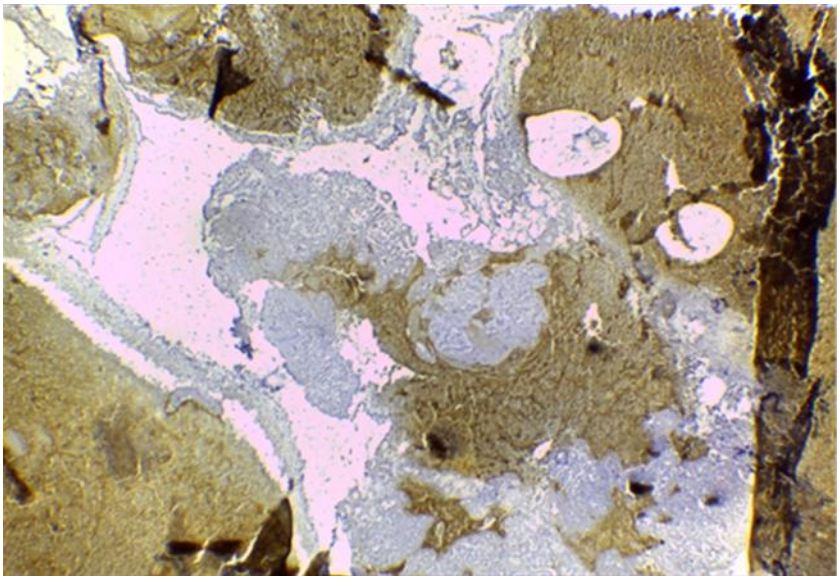

Fig 2: Immunohistochemistry staining of toxoplasma in tropobolastic tissue from women with abortion, explained high expression of T.gondii (by DAB chromogen. Counter stained with mayer 's hematoxylin). Magnification power (4 X).

Table1: Mean percentage ± of infected placental tissue obtained from aborted women by IHC technique:

Group	No.	Mean
A*	26	29.10 ±2.7
B*	26	0.0
C*	17	0.0

*A: Group of aborted women infected with *T. gondii*.

*B: Group of aborted women uninfected with *T. gondii*.

*C: Group of induced abortion (normal pregnancy).

Table 2: The level of IHC expression for detection of T. gondii in infected trophoblasts.

IHC scoring for T. gondii	Group (A) Aborted Women infected with Toxoplasma (N=26)		Group (B) Aborted Women uninfected with Toxoplasma (N=26)		Group (B) Aborted Women uninfected with Toxoplasma (N=17)	
	Low expression	High expression	Low expression	High expression	Low expression	High expression
N.	7	19	0	0	0	0
%	26.93	73.07	0	0	0	0

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Discussion

Toxoplasma gondii is worldwide distributed and infects humans as well as several other mammals [12]. Serological surveys during pregnancy are very important step for early 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 sero-negative women followed at intervals for evidence of sero-conversion (Abdel-Raouff and Elbasheir, 2014).

1-Demographical Distribution of Toxoplasmosis among positive group

Susceptible infection according to age

The results of present study according to age showed the highest infection rate was (73.1%) in young ages, while the lowest infection rate (26.9%) was recorded in older ages .These result agree with [13, 14].

The explanation of these results attributed to the facts that this age group of early marriage among Iraqi women particularly among rural females. Additionally, females may be [according to their work nature] at high contact with contaminated agents which enhance their probability for infection with toxoplasmosis.

Susceptible infection according to residence

Geographic location, place of residence in particular has been found to be another form of disparity. The present study indicated that T. gondii infection was more predominant in rural (61.5 %) compared to urban (38.5 %).However, findings in this study is agreement with other study [15, 16].

The aborted women with toxoplasmosis are more common in rural area. This is due to the human in rural area were contact with animals either in house or farms by working and can be contaminated with cat and other animals feces in soil of garden or farms or unwashed fruits and vegetables. Also toxoplasmosis can be transmitted by uncooked or inadequate cooked food or meat in addition to that the cats found in the household or inside the house of farmers than others in urban region. But it is not consistent with a study conducted in thi-qar by [17], who has found a higher prevalence among the city resident (26.5%) than the rural one (16.5%).

Susceptible infection according to occupational status

Occupation plays a key role in the transmission of the disease when occupation is related to direct contact with meat, soil, and animals. The majority of women in this study were housewives (73.1%) and few of them are working in jobs (26.9%).The results of the present study agree with [18-20].

The explanation was that the housewives were more exposed to cat litter and handling raw meat and that housewife was likely to have low education and have less information about the ways of prevention and control of toxoplasmosis.

The results of the present study in contrary with (Kalaby, 2008) who said that, the officials were more affected with toxoplasmosis. Likewise, Mwambe said that, business women and employed pregnant women had higher infection rates with T. gondii than housewives. The occurrence of congenital toxoplasmosis is still a problem in our community and that the best prevention is the prompt and adequate examination of pregnant women for the presence of Toxoplasma gondii infection and required more attention from the health institution..

Abortion proportion in affected cases

It has been proposed that during pregnancy, systemic maternal immune response is biased in favor of a TH2 cytokines [21], and also in women with first abortion [22]. Moreover, TH2 cytokines pattern of pregnancy induces the susceptibility to toxoplasmosis infection, together with the risk of placental infection and congenital transmission [23]. Consequently, the result of the current study confirms previously mentioned data in which the majority (61.5%) of the positive group was found to have no previous abortions, while patients with previous abortions constituted a smaller percent.These results disagree with [24].

Susceptible infection according to gravidity number

The results of present study according to number of graviditis showed the highest infection rate was (88.5%) in the multigravida ,while the lowest infection rate (11.5%) was recorded in the primigravida .These results agree with [25].

2- Toxoplasma Antigen scored by IHC

From the results in the current study, the IHC was more sensitive (100%) in documenting of T. gondii infection in aborted women and this is agree with

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(Khalil et al.,2016) .Regarding to the intensity of IHC expression,the immunopathological effect for both low and high expression of Toxoplasma gondii antigen in the placenta lead to abortion, Human placental studies suggest that the extravillous trophoblasts (EVT) that anchor the placenta to the uterus are much more vulnerable to infection than are the syncytiotrophoblasts that are bathed in maternal blood [26]. Studies in animals have shown that initial infection occurs in the uterus [26]. Thus, it is likely that after primary infection a woman becomes parasitemic, leading to intracellular infection in the uterus, which then gradually leads to EVT infection, as the tachyzoites move from cell to cell, with eventual infection of the fetus. It is also possible that direct movement of tachyzoite infected maternal leukocytes to or across the placenta contributes to fetal infection. It seems likely that multiple mechanisms are involved; which could explain the apparent time delay between primary maternal infection and fetal infection.

Conclusion

According the results of current study the toxoplasmosis distribution according to age groups for study it was found that the age groups of (15-30) years (37.5%) are more likely to be infected than the age group(31-45)years (26.9%) also the majority of aborted women from rural residency in comparison with those of Urban reign .The current study showed that the highest infection rate was (73.1%) among the house wife comparison with those employee .Majority of women had no previous abortion , and highest infection rate in the multigravida ,while the lowest infection rate (11.5%) was recorded in the primigravida. The result appeared that the Immunohistochemistry technique is the best method than others traditional techniques that usually used in diagnosis of T. gondii because this technique depended on using of tissue sample and specific antibodies for this parasite.

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