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MOLECULAR AND SEROLOGICAL DETECTION OF TOXOPLASMA GONDII INFECTION IN KURDISTAN REGION, IRAQ

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Abstract : *Blood samples were collected, from 126 women (aging 18 to 45 years) Clinical examination for women, 78 have given births to full term healthy babies (control group) and 48 women the rest were presented with abortions (study group). during the period between June to December in Zakho hospitals of 2019, used molecular techniques for detection of the organism's DNA using PCR amplification and Serological screening using enzyme linked immunosorbent assay (ELISA), Serological tests resulted in 39.58% (n=19) of the study group members and 26.92% (n=21) of the control group members showed positive ELISA IgG antibodies to T. gondii (Table 2). Of these seropositive samples, 4(21.05%) samples of the study group and 7 (33.33%) samples in the control group showed positive ELISA IgM antibodies.9(39.13%) samples of the study group and 13 (46.42%) samples in the control group showed positive ELISA IgG & IgM antibodies. The overall prevalence rate of PCR amplification resulted in detection of PCR product in (29.16%)% of the study group and 19.13% of the control group. There was no association between positive toxoplasmosis antibodies and abortion, furthermore, the individuals with positive PCR results in the control group did not abort. The aim of the present study was to determine the prevalence of Toxoplasma gondii antibodies in serum samples from two groups of women.*

Keywords*: Toxoplasma gondii, women, PCR, ELISA.*

1. INTRODUCTION:

Toxoplasmosis is an important zoonotic disease that infects all warm-blooded animals, including humans as intermediate hosts. Felids are the key species in the life cycle of this parasite because they are the only hosts that excrete the environmentally resistant stage, the oocyst, in their feces (1). Until relatively recently, latent adult-acquired toxoplasmosis in immunocompetent humans and animals was generally considered to be asymptomatic. In contrast, the causative agent, *Toxoplasma gondii*, represents perhaps one of the most convincing examples of a manipulative parasite of vertebrates. *T. gondii* is a highly successful apicomplexan protozoan capable of infecting all warm-blooded animals worldwide, often at extremely high prevalence levels. Members of the cat family (Felidae) are the only definitive hosts, within which the parasite undergoes full gametogenesis and mating within the intestinal epithelium, culminating in the generation of oocysts containing sporozoites that are shed in the cat's faeces (2) Toxoplasmosis is a neglected tropical disease of poverty caused by the obligated intracellular protozoan parasite, *Toxoplasma gondii* (3, 4). This facultative heterogeneous parasite belongs to the phylum Apicomplexa, class Conoidasida, subclass Coccidiasina, order Eucoccidiorida, family Sarcocystidae, genus *Toxoplasma*, and species *gondii*. Humans can become infected by the ingestion of *T. gondii* cysts present in raw or undercooked meat and by ingestion of oocysts excreted in cat faeces contaminating fruits, vegetables and water, and less frequently by congenital infection, inhalation of oocysts, consumption of raw milk, transfusions or even organ transplantations (5,6,7). The parasite may cross the placenta of an infected woman and may infect the fetus congenitally(8) Congenital infection with *T. gondii* may have severe consequences as miscarriage, fetal death and neurological, ocular and another organ damage in the fetus (9) If the infection occurs in an early phase of pregnancy the rate of transmission is low, but the severity is high if the fetus is

infected; whereas if the infection occurs in a late phase of pregnancy the transmission rate is higher, and the severity would be low (5) On the other hand, infections with *T. gondii* that occur after birth are usually asymptomatic, but the parasite may induce severe disease in immunocompromised patients Toxoplasmosis is a life-threatening disease for transplant recipients under immunosuppression [(10,11)]

Currently, routine diagnosis of toxoplasmosis relies mainly on the use of various serological tests to detect specific antibodies in the serum samples of infected patients. The presence of a recent infection can be determined by detecting seroconversion of immunoglobulin M (IgM) or IgG antibodies, a substantial increase in IgG antibody titer, or a Toxoplasma serologic profile compatible with acute infection (using Toxoplasma serodiagnostic tests, including an IgG avidity test) in sequential serum samples of infected individuals (12, 13). However, this procedure bears limitations in estimating the time of T. gondii infection due to the fact that, in most cases, low IgM titers persist long after the acute phase of disease (14). One approach to improving these tests is to replace the native antigens with recombinant proteins. The major advantages of recombinant antigens for the diagnosis of T. gondii infections are as follows the precise antigen composition of the test is known, more than one defined antigen can be used, and the method can easily be standardized. In addition, selected antigens that are characteristic for the acute or chronic stages of the infection could serve as a tool to discriminate between the two stages.

Hence direct observation of the parasite in biological samples by Polymerase Chain Reaction (PCR) is a major breakthrough for the diagnosis and management of toxoplasmosis (15). Diagnosis of acute toxoplasmosis is achieved primarily by antibody detection and generally only undertaken in pregnant patients with risk factors for trans placental transmission. Positive results in pregnant women must be confirmed at a Toxoplasma reference laboratory. Recent studies have shown that polymerase chain reaction (PCR) testing of amniotic fluid and blood samples is useful for confirmation or exclusion of fetal T. gondii infection (16,17,18).

Aiming the article to study the seroprevalence of T. gondii antibodies and use PCR assay of T. gondii, to confirm recent toxoplasmosis infections in pregnant women's.

2. MATERIALS AND METHODS :

Blood samples were collected, from 126 women (aging 18 to 45 years) Clinical examination for women, 78 have given births to full term healthy babies (control group) and 48 women the rest were presented with abortions (study group). during the period between June to December in Zakho hospitals of 2019, Serum was separated by centrifugation and stored at –20°C till use. Antibodies against T. gondii were determined using ELISA kits for both IgG and IgM antibodies. The PCR was performed on all DNA samples to amplify a fragment from the B1 gene, which is present in 35 copies and is conserved in the T. gondii genome, PCR. The amplification was performed in the Gene Amp® PCR system 9700, The cycling conditions for both PCRs were 94°C for 5 min, followed by 40 cycles at 94°C for 45 secs, 59 to 60°C for 45 secs, an d 72°C for one min and a final extension at 72°C for 5 min. A nearing temperature was 60°C for the first round PCR while it was 59°C for PCR products were subjected to 2% agarose gel electrophoresis n ½TBE buffer, stained with ethidium bromide and visualized in UV trans illuminator and photographed using gel documentation system.

Statistical analysis was using the computer software and SPSS system, and take Percentage as statistical significance

3. RESULTS:

Samples gave positive amplification of the partial B1 gene fr PCR results from the study group 14 (29.16%) the study group PCR results from group showed that showed that 14 agment on both the primary PCR and nested PCR (Table 1). however, In the control group, 22 (19.13%) samples showed positive amplification on both PCRs, giving of difference size amplicons reported comparison with study group. There was significant difference in the PCR results in both groups.

Table1. PCR test of screening 126 serum from the control and study groups of Toxoplasma gondii.

Group	No.	PCR test	
		+)(	(%)
Study G.	48	14	29.16
Control G.	78	22	19.13
Total	126	36	28.14

In this study Blood samples were collected, from 126 women, Clinical examination for women, 78 have given births to full term healthy babies and 48 women the rest were presented with abortions. during the period between June to December in Zakho hospitals of 2019, Serological tests resulted in 39.58% (n=19) of the study group members and 26.92% (n=21) of the control group members showed positive ELISA IgG antibodies to T. gondii (Table 2). Of these seropositive samples, 4(21.05%) samples of the study group and 7 (33.33%) samples in the control group showed positive ELISA IgM antibodies. The overall prevalence rate of T. gondii antibodies in women's 40 in was 33.25%. The difference in the prevalence between the two group was not statistically significant and seropositive samples, 9(39.13%) samples of the study group and 13 (46.42%) samples in the control group showed positive ELISA IgG&IgM antibodies.

Table 2. Serological tests (IgG, IgM and IgG IgM) of screening 126 serum from the control and study groups for the detection of antibodies of Toxoplasma gondii.

Group	**No.**	**Serology tests**					
		IgG		IgM		IgG&IgM	
		+)(	(%)	+)(	(%)	+)(	(%)
Study G.	48	19	39.58	4	21.05	9	39.13
Control G.	78	21	26.92	7	33.33	13	46.42
Total	126	40	33.25	11	27.19	22	42.77

4. DISCUSSION:

Molecular techniques have shown that it was possible to detect positive toxoplasma infections in some women, which indicated that those women may have acquired the infection during pregnancy. The PCR results, however, detected some individuals who showed positive IgG antibodies as well as those with positive IgM, hence suggesting that some of the women in both groups studied may have acquired infection during pregnancy. The use of the molecular marker B1 or the repeated region of T. gondii presents high sensitivity when applied on clinical samples (19,23,24).

PCR to diagnose active toxoplasmosis is of great importance for immunocompromised patients and congenital toxoplasmosis particularly when serological techniques failed (26). This investigation was based on the combined results of positive serological tests for IgG and/or IgM antibodies, which confirms T. gondii DNA in whole blood and serum samples among different patients (women with history of repeated abortion, positive HIV patients, leukemia and congenital cases). As for all parasitic diseases, the PCR diagnosis of toxoplasmosis is not standardized (27). Therefore, we adjusted PCR conditions to give optimum sensitivity and specificity without appearance of artifacts.

Seroprevalence of T. gondii in women has been determined in two groups of pregnant women until delivery or abortion. The serological diagnosis of toxoplasmosis infections does not represent any interpretative problems for immunocompetent individuals, but it does for pregnant women who acquire the infection during gestation or after conception (25). Antibodies in the con5trol group was higher than the study group, there was significant difference in the seroprevalence in both groups. Most women were exposed to the infection with T. gondii at some stage and they developed immunity, since the majority of the seropositive women in both groups showed IgG antibodies. Only few women in both groups showed IgM antibodies against toxoplasmosis and that was not explained as they are having acute disease or active infection. In the present study, however, all women who showed toxoplasma specific IgM antibodies revealed positive reaction on PCR. This may indicate that these women probably developed recent infection; however, none of the women in the control group did abort whilst those from the study group aborted. 30 to 63% of human infections can be attributed to the consumption of undercooked meat (20). In some areas drinking raw milk particularly camel milk can also constitute an important source of infection (21,22).

5. CONCLUSION :

The use of the molecular techniques in the detection of the T. gondii particles was proven useful in confirming active or recent infection in pregnant women. Present study documented that there was no correlation between abortion and seropositivity to toxoplasmosis.

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