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Full Length Research Paper

Molecular detection and prevalence of *Toxoplasma gondii* in pregnant women in Sudan

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The aim of the present study was to determine the prevalence of *Toxoplasma gondii* antibodies in serum samples from two groups of women in Sudan and to use molecular techniques for detection of the organism's DNA using B1 gene. The first group (study group) composed of 94 individuals and they aborted during the study, while the second group (control group) also composed of 94 individuals who gave birth to healthy children without complications. Serological screening using enzyme linked immunosorbent assay (ELISA) kits resulted in 39.4% and 35.1% of the women in the control and study group showed positive IgG antibodies to *T. gondii* respectively. PCR amplification of B1 gene resulted in detection of PCR product in 19.1% of the study group and 22.3% 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 usefulness of the B1 gene as a target for toxoplasmosis diagnosis was documented and the possible source of infection in the control and the study groups was discussed.

Key words: *Toxoplasma gondii*, antibodies, women, Sudan, B1 gene, abortion.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite which causes the zoonotic disease toxoplasmosis. It is recognized as a category B priority pathogen by the National Institutes of Health, Bethesda, USA. In several of its hosts, *T. gondii* is associated with congenital infection and abortion (Tenter et al., 2000). In pregnant women, acute infection by *T. gondii* may result in congenital disease that may be prevented or reduced by early treatment (Elnahas et al., 2003; Swai and Schoonman, 2009).

Acute *T. gondii* infection is asymptomatic in most pregnant women, with the most frequent clinical manifestation of infection being lymphadenopathy or congenital disease, causing abortion or severe damages to the foetus at birth or later in life (Luft and Remington, 1984; Alvarado-Esquivel et al., 2002). Infection, however, can result in transmission to the foetus and the risk to the foetus does not correlate with symptoms in the mother. Women who have had *T. gondii* infections, that is, they are seropositive, prior to pregnancy are protected from transmitting the infection to their foetuses. Exceptions to this rule have been reported in women with an immune-compromised state (Minkoff et al., 1997) and acute infection occurring shortly before conception (Gavinet et al., 1997; Hennequin et al., 1997; Vogel et al., 1996). Latent *T. gondii* infection may reactivate in HIV-infected women and result in congenital transmission; these infected children usually have HIV as well (Mitchell et al., 1990).

Seroprevalence rates among pregnant women in Khartoum-Sudan were documented as 27.5 to 34.1% in two independent studies (Elnahas et al., 2003; Satti, 2003). The seroprevalence of toxoplasmosis in an adjacent

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area in Gezira State, however, was found to be as high as 41.7% (Abd Elhameed, 1991). Risk of infection was found to be increased with age, low educational levels and in individuals who have soil-related occupations (Jones et al., 2001).

Diagnosis of acute toxoplasmosis is achieved primarily by antibody detection and generally only undertaken in pregnant patients with risk factors for transplacental 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 foetal *T. gondii* infection (Romand et al., 2001; Guy and Joynson, 1995). The proportion of women at risk of acquiring the infection during pregnancy in Sudan is not well studied and maternal screening is mandatory only in big hospitals and specialised clinics.

In the present study, the seroprevalence of *T. gondii* antibodies in Gezira State was investigated and the utility of a PCR assay targeting B1 gene of *T. gondii* was evaluated, to confirm recent toxoplasmosis infections in pregnant women.

MATERIALS AND METHODS

Samples and serological tests

Blood samples were collected, by venipuncture into plain vacutainer tubes, from 188 women (aging 20 to 40 years) visiting the Maternity Unit of University of Gezira Teaching Hospital in Wad Madani, Sudan. Clinical examination and history of candidates were undertaken by an Obstetrician. Of these women, 94 have given births to full term healthy babies (control group) and the rest were presented with abortions (study group). Serum was separated by centrifugation and stored at –20°C till use. Antibodies against *T. gondii* were determined using ELISA kits (Human Gesellschaft für Biochemica und Diagnostica GmbH- Germany) for both IgG and IgM antibodies.

This study was approved by the ethical committee of the Gezira University Teaching Hospital in Wad Madani City. The purpose and objectives of the study were explained to all women participated in the study. Furthermore, a written informed consent was obtained from all of them.

DNA extraction and PCR amplification

From both aborted and normally delivered foeti representative samples from the placental tissues attached to the umbilical cord were preserved in Dimethylsulphoxide (DMSO) for molecular studies. DNA was extracted from the placental tissues using Puregene DNA purification kits (Gentra system Minneapolis, Minnesota USA), in accordance to the manufacturer's instructions.

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, as described by Burg et al. (1989) and Buchbinder et al. (2003). The primers used in the first round of the PCR, which produce a fragment of 193 bp, were (5'GGAACTGCATCCGTTCATGAG'3), and (5'TCTTTAAAGCGTTCGTGGTC'3), which correspond to nucleotides 694-714 and 887-868, respectively. The primers used in the second round nested PCR, which produce a fragment of 96 bp, were (5'TGCATAGGTTGCAGTCACTG'3) and (5'GGCGACCAATGTGCGAATAGACC'3), which correspond to nucleotides 757-776 and 853-831, respectively.

Three microliters of template DNA were added to a final volume of 50 µl of PCR mixture consisting of 5 µl of 10×PCR buffer (50 mM Tris-HCl, pH 9.1, 3.5 mM $MgCl_2$), 4 µl of 1.25 mM deoxynucleoside triphosphates, 0.5 µl of *Taq* DNA polymerase (Roche Diagnostics-Germany, 5 U/µl), and 1.5 µl (20 ρmol) of each primer used in the PCR. The amplification was performed in the Gene Amp® PCR system 9700 (Applied Biosystems, California. USA). The cycling conditions for both PCRs were 94°C for 5 min, followed by 40 cycles at 94°C for 45 sec, 59 to 60°C for 45 sec, and 72°C for one min and a final extension at 72°C for 5 min. The annealing temperature was 60°C for the first round PCR while it was 59°C for the nested PCR.

PCR products were subjected to 2% agarose gel electrophoresis in ½TBE buffer, stained with ethidium bromide and visualised in UV transilluminator and photographed using gel documentation system.

Statistical analysis was performed using Chi square test (χ^2) using the computer software (Sigmastat Statistical Software, version 2.03, SPSS Inc.). P values less than 0.05 were considered significant.

RESULTS

Serological investigations resulted in 35.1% (n=33) of the study group members and 39.4% (n=37) of the control group members showed positive ELISA IgG antibodies to *T. gondii* (Table 1). Of these seropositive samples, 5 (15.2%) samples of the study group and 6 (16.2%) samples in the control group showed positive ELISA IgM antibodies. The overall prevalence rate of *T. gondii* antibodies in women in Gezira Province was 37.2%. The difference in the prevalence between the two group was not statistically significant ($\chi^2 = 0.36$, $p > 0.05$).

PCR results from the study group showed that 18 (19.1%) samples gave positive amplification of the partial B1 gene fragment on both the primary PCR and nested PCR (Table 1). The PCR products resulted from the primary PCR and the nested PCR were 193bp and 96bp, respectively. All the PCR positive samples showed positive ELISA IgG antibodies including the 5 samples which also showed positive IgM antibodies. In the control group, however, 21 (22.3%) samples showed positive amplification on both PCRs, giving the same size amplicons reported in the study group (Table 1).

Likewise all the samples which gave positive amplification on PCR were positive to ELISA IgG antibodies including the 6 samples which showed positive ELISA IgM antibodies. There was no significant difference in the PCR results in both groups ($\chi^2 = 0.29$, $p > 0.05$).

In both groups, individuals with positive IgG antibodies were significantly higher than those with positive PCR products ($\chi^2 = 6.05$ (study group), $\chi^2 = 6.38$ (control group), $p < 0.05$). However, individuals with positive PCR products in the two groups together were significantly higher than those with IgM antibodies in both groups ($\chi^2 = 091$, $p < 0.0001$).

Table 1. Serological (IgG and IgM antibodies) and PCR results of screening 188 serum and tissues samples from the control and study groups for the detection of antibodies and B1 gene of *Toxoplasma gondii*.

Group	Number of samples	PCR results		Serology Results			
				IgG		IgM	
		Positive	Percentage (%)	Positive	Percentage (%)	Positive	Percentage (%)
Study Group	94	18	19.1	33	35.1	5	15.2
Control Group	94	21	22.3	37	39.4	6	16.2
Total	188	39	20.7	70	37.2	11	5.9

DISCUSSION

Seroprevalence of *T. gondii* in women in Gezira State has been determined following two groups of pregnant women until delivery or abortion. 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.

The seroprevalence of toxoplasmosis in Gezira State was within the ranges reported previously by other investigators in Sudan. Previous reports showed antibodies against toxoplasmosis in women ranged between 27 to 34% (Abd Elhameed, 1991; Adnan, 1994; Elnahas et al., 2003). Interestingly, the findings of the present study documented that there was no correlation between abortion and seropositivity to toxoplasmosis. 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 (Remington et al., 2004). In our study, the use of molecular techniques has shown that it was possible to detect positive toxoplsama infections in some women, which indicated that those women may have acquired the infection during pregnancy.

Although the seroprevalence of toxoplasmosis antibodies in the control group was higher than the study group, there was no significant difference in the sero-prevalence in both groups. This can be explained by the fact that 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. It was indicated earlier that one diagnostic problem was associated with toxoplasma specific IgM as these antibodies may occasionally persist for several years (Skinner et al., 1989). Infection in women before conception does not usually put the foetus at risk, but persisting IgM can make it difficult to identify the time of infection in some women presenting at the antenatal clinic. 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.

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 including cotyledons (Owen et al., 1998; Chabbert et al., 2004; Jalal et al., 2004). Placental cotyledons gave a higher sensitivity of detection than brain and mouse inoculation (Owen et al., 1998). During the present study, we succeeded in obtaining positive results from placental tissues (cotyledons) of women, which will undoubtedly make the cotyledons as the tissue of choice for the diagnosis of *T. gondii* infection in pregnant women.

It was unclear how the women who exhibited positive PCR products for the B1 gene of *T. gondii* acquired the infection. Most of them were house-wives and they do not normally keep cats as pets, as this is not a common practice in Sudan, hence excluding the possibility of contact with cats. It is likely that contact with infected meat could be the cause and sometimes consuming un- or under- cooked meat. Risk-factor analysis indicates that 30 to 63% of human infections can be attributed to the consumption of undercooked meat (Cook et al., 2000). However, what kind of meat contributes most to human infections depends on prevalence of *T. gondii* in consumed animals and on eating habits. Eating of some parts of the internal viscera of sheep particularly is very common in Sudan and it is likely this habit may have played a role in the transmission of toxoplasmosis in women. This needs to be confirmed as such parts may not include muscle tissues, which harbour *T. gondii* cysts. In some areas drinking raw milk particularly camel milk can also constitute an important source of infection (Elamin et al., 1992; Hussein et al., 1988). Raw milk from acutely infected animals can contain *T. gondii* parasites. However, it is generally thought that milk will contain mostly tachyzoites, which are less resistant to pepsin digestion (Dubey, 1998) and, therefore, considered less infective. Nonetheless, outbreaks or cases in humans due to consumption of raw milk, specifically goat's milk, have been reported (Riemann et al., 1975; Sacks et al.,

1982; Chiari Cde and Neves, 1984; Skinner et al., 1990), and consumption of raw goat milk was associated with *T. gondii* seropositivity (Jones et al., 2009). Feeding goat whey was also identified as an important risk of infection for pigs (Meerburg et al., 2006).

Population genetic analysis based on restriction fragment length polymorphisms (RFLPs) indicates that *T. gondii* consists of only three clonal lineages, designated types I, II, and III, which occur in both animals and humans with type II associated with more than 70% of human disease cases of toxoplasmosis (Howe and Sibley, 1995). In our study, *T. gondii* type was not determined and it could be one of the other types which are not usually associated with toxoplasmosis in humans. Further work is needed to determine the type of *T. gondii* involved in order to understand the disease and to link it with the lack of abortion in infected women in Sudan.

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