Original Article

Toxoplasma Gondii Infection in Females

Prevalence of Toxoplasma Gondii Infection in Females with Bad Obstetric History in Mardan District

Farhat Rehman, Naila Aslam and Fazal Rahim

ABSTRACT

Objective: Toxoplasma gondii infection is a potential threat in pregnant women leading to abortion, intrauterine fetal death and congenital toxoplasmosis in newborns. The study was conducted to determine the prevalence of Toxoplasma gondii infection among pregnant women with history of recurrent pregnancy loss.

Study Design: Cross sectional study.

Place and Duration of Study: This study was conducted at the Gynaecology and Obstetric outdoor department of Mardan Medical Complex and Teaching Hospital, Mardan from October 2016 to April 2017.

Materials and Methods: The study included 360 women of child bearing age from urban and rural areas of Mardan district. Study group comprised of 180 females with recurrent pregnancy loss. The control group included 180 females of approximately the same age and parity. After informed written consent, the obtained sera were examined for Toxoplasma IgM and IgG antibodies by using Enzyme Linked Immunosorbent Assay. Toxo IgM and IgG index of 1 or greater were considered as positive for infection with the protozoan.

Results: The study group showed 6.9% (n=25) seropositivity for Toxo IgM antibodies, IgG antibodies were 15% (n=54), while, 1.9% (n=7) females showed both IgM and IgG antibodies in their serum. Over all seroprevelance was 40.5% among study group as compared to 7.2% among the controls. The incidence of infection was observed as increasing with advancing maternal age. High incidence of both IgM and IgG antibodies was found among women between 31 to 35 years age group.

Conclusion: The seroprevelance is much high as has been reported from other parts of the country. Therefore, it is now the time for further in depth studies regarding this hidden problem which is a potential risk for recurrent fetal loss in pregnant women.

Key Words: Toxoplasma gondii, IgM, IgG, antibodies, ELISA

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INTRODUCTION

Toxoplasma gondii is an obligate intra cellular protozoan belonging to phylum Apicomplexan. It can infect all nucleated cells in various warm blooded animals[1]. Humans are prone to toxoplasmosis by consuming infected meat of the animals or by ingesting its cysts found in the soil, contaminated food and water[2]. Newborns get infected from their mothers by vertical transmission through placenta [3]. This may lead to various complications in fetus including abortion, hydrocephalus, premature births, intrauterine growth retardation, still birth, retinal damage and brain calcification [2,3,4]. The fetal manifestations are related to the time when the infection is contacted. The earlier a mother is infected the worst will be the manifestations in the developing fetus[4,5]. Immuno compromised individuals are more prone to infection specially HIV infected persons.[5,6]

Various studies are conducted worldwide including Pakistan regarding this hidden public health problem especially affecting pregnant women.[7]The infection is usually asymptomatic in healthy individuals. The prevalence rate varies widely. In well developed countries like USA it is 10% among pregnant females.[8] Other countries like Brazil, Thailand, China, Japan, India and Iran shows a much higher rate of 50-80%[8,9,10]. The prevalence rate of Toxoplasmosis among pregnant women in Pakistan also varies. In Punjab it was found to be 63%. In Azad Kashmir 48% and in Khyber Pukhtunkhwa it was found 38% in recent past studies[11]. The diagnosis of the disease requires serological tests to determine anti toxoplasma antibodies including IgM, IgG and IgA. Females with positive IgM antibodies require caution and further serological tests to confirm acute infection. In pregnant females amniocentesis is required to confirm the diagnosis by doing PCR or animal inoculation to detect the infection[12]. Keeping in view the previous studies regarding its prevalence rate

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and its complications in pregnant women and newborns, the study was conducted to determine the seroprevelance among women with previous history of recurrent pregnancy loss in Mardan district.

MATERIALS AND METHODS

Study area and population: The study was conducted at Gynaecology and Obstetric outdoor department of Mardan Medical Complex and Teaching Hospital, from October 2016 to April 2017. It included 360 pregnant women from various rural and urban areas of district Mardan. Study group included 180 females with previous history of recurrent pregnancy loss. Control group included 180 females with no such history of pregnancy loss. An informed written consent was taken and all personal, clinical information and obstetrical data were recorded.

Inclusion/exclusion criteria

The study group included females of child bearing age having previous history of two or more than two pregnancy loss like abortions, still birth, perinatal/ neonatal death. The control group included females of approximately the same age with no such history of recurrent pregnancy loss. Exclusion criteria included primigravida, pregnancy induced hypertension, gestational diabetes, RH incompatibility and history of recent vaccination.

Sample collection: Observing strict aseptic technique 4 ml of blood from cubital vein was collected and was immediately centrifuged at 4000 revolutions per minute for 7 minutes. The clear transparent serum was transferred to gel tube bearing serial number, date and name of person. It was kept at -20C^{o} in freezer for future serological analysis.

Serological Methods: The collected samples were screened for specific Toxo IgM and Toxo IgG antibodies by using Enzyme Linked Immunosorbent Assay (ELISA) Kit (BioCheck USA).

Interpretation of Test: . Toxo IgM and IgG index less than 0.9 (<321IU/ml) was considered as negative. While Ig index equal or between 0.9-0.99 were considered equivocal. Toxo IgM and Toxo IgG index 1.0 and above (321≥ IU/ml) were considered as positive. Calculations were made by absorbance of each tested sample including negative and positive controls.

Statisticalanalysis: Biostatistical analysis was done by using SPSS version 20(SPSS Inc. Chicago, IL, USA).

RESULTS

Table 1 shows Seroposivity of *Toxoplasma gondii* in study and control groups. Females with bad obstetric history (BOH study group) show overall 40.5 % (n-73) positive cases as compared to 7.2 % (n-13) in control group. In BOH study group IgG antibodies were positive in 43 females (23.9 %), while 11 cases (6.1 %) were found positive in controls. IgM antibodies which depict recent infection were positive in 23 (12.8 %) females of the study group. In the control group only 2(1.11%) cases were found positive with IgM antibodies. Both IgG, IgM positive in study group were 7 (3.88%) females while none was positive in control group. Over all seropositivity in both groups was 47.7 %.Using Pearson chi-square test highly Significant difference was found for the seropositivity between the females of BOH study group and controls with p-value = 0.000.

Table No. 1. Seroprevelance of Toxoplasma gondii in study and control groups

Group	Number of cases	IgG positive	IgM positive	Both IgG IgM positive	Total positive cases	P-value
Study	180	43 (23.9 %)	23 (12.8 %)	7 (3.8%)	73 (40.5 %)	0.000
Control	180	11 (6.1%)	2 (1.1%)	0	13 (7.2%)	
Total	360	54 (15%)	25 (6.9%)	7 (1.9%)	86 (23.89%)	

Table No. 2. Advancing maternal age and seropositivity Toxo IgM antibodies in study group

Age in years	IgM Negative	IgM Positive	Total
≤ 25 yrs %age	5 100%	0 0%	5 100%
26—30 %age	48 90.5%	5 9.4%	53 100%
31—35 %age	57 83.7%	11 16.2%	68 100%
≥ 36 yrs %age	47 87.0%	7 13.0%	54 100%
Total %age	157 87.32	23 12.8%	180 100%

Table 2 shows the relationship between advancing maternal age and its effects on IgM seroprevelance. Females with age ≤ 25 were 5 and all were IgM negative (100%). Females aged 26—30 years included 53, out of them 48 (90.6%) were IgM negative while 05 (9.4%) were IgM positive. Third group included females between age 31—35 years, there were 68 females, among them 57 (83.8%) were IgM negative and 11 (16.2%) were IgM positive. The forth group comprised of females aged 36 years and above. It included 54 females and among them 47(87%) were IgM negative where as 7(13%) females were found IgM positive. The above data showed that the seroprevelance increased with advancing maternal age.

Maximum numbers of IgM seropositive females were 31 to 35 years age.

DISCUSSION

Toxoplasmosis is a zoonotic disease which is distributed worldwide. Its prevalence in third world countries is high due to their low socioeconomic status and lack of education among females .It is estimated to have affected one third of the world population[2.] . People living in rural areas are at a greater risk. This is due to abundance of the feline (cat) which serve as intermediate host for the parasite. Poor hygienic conditions and consuming contaminated food add to the risk [8,9] . Toxoplasmosis gained attention when it was found in patients with AIDS, and those receiving immuno suppressive therapy causing serious manifestations like meningitis, and focal calcifications in brain [9].Its effects in pregnant females are drastic if the disease is contracted in early trimester[8,9]. It includes abortion in early trimester or may result in congenital toxoplasmosis in newborn. This is due to vertical transmission to fetus from an infected mother.[12,13] The present study shows that females with recurrent pregnancy loss (study group) were having an overall 40.5 % (n-73) seropositive as compared to 7.2 % (n-13) in the control group (Table 1). In the study group IgG antibodies were positive among 43 females (23.9 %), which signify previous infection, while 11 cases (6.1 %) were positive in controls. IgM antibodies which show recent infection were positive in 23 (12.8 %) females of the study group as compared to only 2 (1.11%) in controls. Both IgG, IgM positive in study group were 7 (3.88%) females while none was positive in control group (Table 1). These results are higher as compared to the study conducted by Shahid *et al* in Kohat in 2011[7], which demonstrated an overall 14.4% seroprevelance in pregnant women; this difference could be due to random collection of their samples from pregnant females irrespective of their past history of pregnancy wastage. The prevalence rate in our study is consistent with the studies in neighboring Asian countries. According to a study conducted in India in 2005 found prevalence rate of 45% among pregnant females in New Delhi while in another study conducted at Andra Pradesh in 2012 showed 49.52% prevalence among women with bad obstetric history while the control group had rate of 12.38%.[10,14] Similar study in China conducted by Zhou in 2011 shows 12.3% prevalence rate.[15] Makiko *et al* in2012 in their study showed an overall 10.3% prevalence rate in developed country like Japan.[9,15] The seroprevelance rate was found high among mother of older age group as shown in Table 2. Females between age 31—35 years, were n=68 among them 57 (83.8%) were IgM negative and 11 (16.2%) were IgM positive. Females aged 36 years and above included 54 females, among them 47(87%) were IgM negative where as 7(13%) females were found IgM positive. As compare to young mothers older age group females are more prone to transfer the disease to their newborns. These findings are consistent with other studies conducted in the region. A study by Borkakoty *et al* in Asam India in 2007[16] and by M D Sarkar *et al* 2012[10,16] in Andra Pradesh India, also reported high prevalence rate of 60.7% with advancing maternal age and had found an increase prevalence of toxoplasma infection in mothers of older age group[10,16].These findings can be attributed to the fact that with advancing maternal age the mothers are more exposed to environmental sources of infection. Low immunity state along with anemia due to repeated pregnancies is also contributing factors [17]. These facts may be explained on the basis of low socioeconomic conditions of the general population in the region and poor public health measures taken by the authorities towards provision of clean and safe environment.[18,19] Which increases exposure to the cysts found in raw or under cooked meat and in contaminated water, soil and food.

CONCLUSION

This study shows a high seroprevelance of toxoplasmosis in females of child bearing age. On the basis of the study conducted in this part of Khyber Pukhtonkhwa, further studies are recommended in future in other parts of the country as well. Effective measures are needed to reduce recurrent pregnancy loss and congenital anomalies in newborns. Primary preventive measures are advised to be taken by health surveillance authorities to focus on families specially females from rural areas. It is required to educate them regarding strict personal hygiene, contact and handling of cattles and pets like cats and dogs around them. This will reduce the chances of *Toxoplasma gondii* infection in them.

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Author's Contribution:

Concept & Design of Study:	Farhat Rehman
Drafting:	Naila Aslam and Fazal Rahim
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Final Approval of version:	Farhat Rehman

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