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Serological evidence and associated risk factors of toxoplasmosis among pregnant women in District Mardan, Khyber Pakhtunkhwa, Pakistan

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Abstract

Toxoplasmosis is one of the widely distributed zoonotic disease which is caused by an intracellular protozoan parasite *Toxoplasma gondii*. The objective of this study was to determine the seroprevalence and risk factors associated with infection in pregnant women of District Mardan, Khyber Pakhtunkhwa, Pakistan. Blood sample of 5mL was taken and the serum was tested for *T. gondii* IgG and IgM antibodies using Indirect Enzyme-Linked Immunosorbent Assay (ELISA) technique. Further information was collected by using preplanned questionnaire. A total of 1200 samples were tested for toxoplasmosis, out of which 39.5% were positive. Among seropositive pregnant women the seropositivity for IgG, IgM and both IgG and IgM antibodies was 43.03%, 40.5% and 16.45% respectively. The seropositivity was highest (58.18%) in age group 36-45 whereas lowest (33.92%) in age group 26-35 which was statistically significant. The findings of the present study suggest that *T. gondii* infection is widely spread in the study area which may constitute a significant risk for congenital defects, stillbirths and abortions during pregnancy in women. Screening for the infection among pregnant women can be done twice at least during pregnancy to protect both mother and child from toxoplasmosis. There should be proper awareness in the educational institutes as well as community awareness programs about the risk factors of toxoplasmosis.

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Introduction

Toxoplasmosis is a zoonotic infection which is caused by a protozoan parasite called *Toxoplasma gondii* (Kijlstra and Jongert, 2009). *T. gondii* was first discovered by Manceaux and Nicolle in 1908 in a rodent, *Ctenodactylus gundi* of North Africa. In the same year it was also found by Splendor in Brazil in a rabbit. Its name was based on its morphology (toxo: arc or bow, plasma: life) (Ferguson, 2009). The specie name was patented from a rodent (*Ctenodactylus gundi*) from which parasite was isolated and genus name referring to crescent shape of the parasite (Black and Boothroyd, 2000).

T. gondii is found inside the cell and is worldwide in distribution (Shah *et al.,* 2013). All warm-blooded animals are infected by this parasite (Dubey, 2008). Tachyzoites, bradyzoites, sporozoites are three infectious stages of *T. gondii* (Sibley *et al.,* 2009). It completes its sexual phase of life cycle in wild animals and domestic cats which act as definitive hosts and its asexual phase of life cycle is completed in warm blooded animals and humans which act as definitive hosts. Indirect way of infection is through taking improperly cooked meat or vegetables already contaminated with *T. gondii* (Shah *et al.,* 2014). Man is directly infected by consumption of meat and vegetables, direct contact with contaminated cat litter boxes, blood transfusion (Singh, 2003), transplantation of organs (Dubey and Jones, 2008).

Different studies have shown that warm and humid environment is responsible for the high prevalence of toxoplasmosis (Studeničová *et al.,* 2006). About 33% of the human's population have this parasite (Shah *et al.,* 2013). Infection rate varies geographically in pregnant women. The prevalence rate in Korea is 3.7% and in Saudi Arabia 24.1% (Aqeely*et al.,* 2014), in London 17.3% (Flatt and Shetty, 2012) in Thailand 28.3% (Nissapatorn and Sawangjaroen, 2011), 68.6% in Brazil (Sroka*et al.,* 2010), 92.5% in Ghana (Ayi *et al.,* 2009). The overall prevalence rate of toxoplasmosis in human population in different countries also varies. The prevalence rate in Taiwan is 84%, USA is 10-70%, Iran 55%, Egypt 44%, Peru 38%, Jordan 37%, India 18% (Bari and Khan, 1990).

Infection may lead to pneumonia, pericarditis, and neurologic disorders in immunocompetent hosts (Lee *et al.,* 2012). Infections goes asymptomatic in case of most immune competent individuals and results in chronic persistence of dormant cysts in tissue or the symptoms are mild (Sarkar *et al.,* 2012) but in case of immune-compromised individuals such as in HIV patients, the persons are at risk of producing acute toxoplasmosis. In 10% of HIV patients it results in cervical lymphadenopathy or ocular disease. Mortality rate in USA and Europe due to toxoplasmosis in AIDS patients is about 10% and 30% respectively (Hill and Dubey, 2002).

If the pregnant women are infected by this parasite it results in toxoplasmic encephalitis, blindness, foetal abnormalities, abortion and even stillbirth (Cook *et al.,* 2000).*T. gondii* infection also results in abortion and infertility in women (Shah *et al.,* 2014). Clinical observations in human include enlargement of the lymph node which results in high fever, headache, muscle ache and sore throat (Hill and Dubey, 2002).
The tachyzoite multiply rapidly. In humans it causes congenital defects and in case of domestic livestock it results in abortion (Chaudhary *et al.,* 2006). There is 40% death chance in fetus if mother is infected during pregnancy (Jones *et al.,* 2001). Congenital toxoplasmosis during the first trimester of pregnancy results in spontaneous abortion and still birth whereas acquired infection in late pregnancy passes asymptomatic in the new-born but may results in retinochoroiditis and even complete blindness (Shah *et al.,* 2013). Symptoms in cats are pancreatitis, pressing of head, teeth grinding, low coordination, voice abnormality, uneasy feelings, circling, weak reflex actions, paralysis (Holzworth,1987)

Transmission of disease depends on environmental conditions, culture, and habits of eating (Kapperud *et al.,* 1996). Toxoplasmosis diagnosis is performed by detection of IgG and IgM antibodies against *T. gondii.* IgG avidity test is carried out for acute infection with low IgG avidity and for chronic infection with high IgG avidity (Liesenfeld *et al.,* 2001). Serologic tests are carried out to find out IgG antibodies for testing infection. Immunoglobulin M (IgM) level is measured

by Avidity test to find out the infection time in pregnant mother. For detection of IgG and IgM antibodies in serum Elisa test is performed (Hasan, 2011)Latex Agglutination Test kit (LAT) is commercially used for IgG antibodies (Tasawar *et al.,* 2012). Sabin-feldmen dye test (SFDT), complement fixation test, intradermal skin test, indirect hemagglutination assay (IHA) and Modified Agglutination Test (MAT) are other serological tests which are carried out to detect the infection (Goz *et al.,* 2007). The parasite can be observed directly in the tissues by staining or the diagnosis can also be made by biopsy. Polymerase Chain Reaction (PCR) is very important, sensitive and efficient method in diagnosis of toxoplasmosis (Aldebert *et al.,* 2011). The objective of the present study is to determine the seroprevalence of anti-*T. gondii* antibodies in pregnant women and to identify the risk factors associated with concern infection in pregnant women of District Mardan, Khyber Pakhtunkhwa, Pakistan.

Materials and methods

Study area

Samples were collected from different tehsiles (Takht Bhai, Katlang and Mardan) of District Mardan.

Sample collection and processing

A total of 5mL venous blood was collected from each individual through sterilized syringes.

Sample processing

The blood was transferred to the blood collecting tubes (EDTA). It was then centrifuged at 3000 rpm for 10 minutes, at 25°C for serum separation from RBCs, in eppendorf tubes.

Serological test for T. gondii antibodies

T. gondii antibodies were detected in the sera of individuals by using an indirect Enzyme-Linked Immunosorbent Assay (ELISA). The kit used was Accu Diag™ Toxo IgM and IgG ELISA Kits (The Diagnostic Automation/Cortez Diagnostics, Inc. *T. gondii* (Toxo) IgM and IgG Enzyme-Linked Immunosorbent Assay (ELISA). The Toxo IgM ELISA Kits had a 100% specificity and a 100% sensitivity, while the Toxo IgG ELISA Kits had a 100% specificity and a 95.3% sensitivity. With each test assay calibrator and controls were run.

Cut-off calibrator value and Immune Status Ratio

For calculation of cut-off calibrator value and Immune Status Ratio (ISR) the optical densities were obtained (OD). The results were interpreted with respect to the ISR values. For IgM, a sample having OD ≤ 0.90 was considered negative, for sample having OD ≥ 1.10 was considered positive, and samples with OD = 0.91–1.09 was considered indeterminate. In case of IgG, a sample with OD < 0.90 was declared as negative, while samples with OD > 1.10 was considered positive, and OD within 0.91–1.09 was considered intermediate (Wam *et al.,* 2016).

Statistical analysis

The data was analyzed by using SPSS software V20 (IBM corp) and was shown in tables. *P*-values less than 0.05 were considered statistically significant.

Results

A total of 1200 pregnant women were approached in which 474 (39.5%) women were seropositive in which the seroprevalence of anti-T. gondii antibodies were calculated, among the seropositive women 204 (43.03%) were seropositive for IgG antibodies, 192 (40.5%) were seropositive for IgM antibodies and 78 (16.45%) were positive for both IgG and IgM antibodies. Regarding the participants residential area, 411 of the study lived in rural area in which 165 (40.41%) were infected by toxoplasmosis (Table 1) while studying the anti-T. gondii antibodies for the suspected rural participant,s 81 (49.1%) were seropositive for IgG, 57 (34.5%) were seropositive for IgM and 27 (16.4%) were seropositive for both IgG and IgM antibodies. Similarly, a total of 789 pregnant women were examined in Urban area, in which 309 (39.16%) were suspected positive and were infected with toxoplasmosis. While studying the seropositivity of antibodies in Urban area 123 (39.8%) were seropositive for IgG, 135 (43.7%) were seropositive for IgM and 51 (16.5%) were seropositive for both IgG and IgM antibodies. Although there is no significant difference of the disease between rural and urban areas but there is high significant difference between the types of infection (Table 2).

Table 1. Overall seroprevalence of toxoplasmosis in pregnant women of District Mardan.

Characteristics Total cases (N) No. of -Ve (%) No. of +Ve (%)	P-Value
Age	
16-25 699 435 (62.2) 264 (37.76)	
26-35 336 222 (66.1) 114 (33.92)	0.000
36-45 165 69 (41.8) 96 (58.18)	
Area	
Urban 789 480 (60.8) 309 (39.16)	0.741
Rural 411 246 (59.9) 165 (40.14)	
Education	
Uneducated 882 537 (60.9) 345 (39.1)	0.650
Educated 318 189 (59.4) 129 (40.5)	

Table 2. Age, area and education-wise prevalence of toxoplasmosis in pregnantwomen of District Mardan.

Characteristics Total cases No. of +Ve (%)	Type of infection			P-Value
	IgG (%)	IgM (%)	IgG+IgM (%)	
Age				
16-25 699 264 (37.76)	117(44.3)	90 (34.1)	57 (21.6)	0.001
26-35 336 114 (33.92)	48 (42.1)	51 (44.7)	15 (13.2)	
36-45 165 96 (58.18)	39 (40.6)	51 (53.1)	6 (6.2)	
Area				
Urban 789 309 (39.16)	123(39.8)	135(43.7)	51(16.5)	0.112
Rural 411 165 (40.14)	81(49.1)	57 (34.5)	27 (16.4)	
Education				
Uneducated 882 345 (39.1)	150(43.5)	135(39.1)	60 (17.4)	0.516
Educated 318 129 (40.5)	54(41.9)	57 (44.2)	18 (14.0)	

The age of the pregnant women which were examined for the toxoplasmosis were between 16 to 45 years. Among pregnant women, 699 examined were having age group 16-25, out of which 264 (37.76%) were infected with *T. gondii* infection in which the seropositivity of IgG anti-*T. gondii* antibodies was 117 (44.3%) and that of IgM antibodies prevalence was 90 (34.1%) and both IgG and IgM antibodies seroprevalence was 57 (21.6%). It shows that the seropositivity of IgG antibodies is more in pregnant women of age group 16-25. Among 1200 pregnant women, 336 were of age group 26-35. Out of which 114 (33.92%) were positive for toxoplasmosis, in which the seropositivity of IgG antibodies is 48 (42.1%) and 51 (44.7%) were seropositive for IgM antibodies and 15 (13.2%) were seropositive for both IgG and IgM antibodies. A total of 165 pregnant women of age group 36-45 were examined, in which 96 (58.18%) were infected with *T. gondii*. The analysis of the infected women of age group 36-45 shows that 39 (40.6%) were seropositive for IgG antibodies, while 51 (53.90%) were seropositive for IgM antibodies and 6 (6.2%) were detected seropositive for both IgG and IgM antibodies. This illustrates that seropositivity of toxoplasmosis is higher in age group 36-45. The disease is highly significant in age groups (Table 2).

The data collected was also analyzed on education basis. Out of 1200 pregnant women 318 were educated, in which 129 (40.56%) were infected with *T. gondii* and 882 were uneducated in which 345 (39.11%) were detected seropositive for toxoplasmosis (Table 1).

Discussion

Toxoplasmosis is one of the life-threatening disease (Negash *et al.,* 2008). This study investigated toxoplasmosis by detecting antiToxoplasma antibodies in serum of pregnant women in rural and urban areas in District Mardan. The findings of the present study revealed that the prevalence of anti-*T. gondii* antibodies among pregnant women was 39.5% which was similar to the results of NWFP (38%) but was lower than Punjab (63%), Azad Kashmir (48%) (Bari and Khan, 1990), nijinikon, NW Cameroon 54.5% (Wam *et al.,* 2016).

The present study results were also very lower than the study carried out in Ethopia (85.3%) (Abamecha and Awel, 2016), yaounde, Douela 70% (Njunda *et al.,* 2011). These geographic differences in the seroprevalence may be explained by differences in rural and urban settings. This might explain differences in risk factors of toxoplasma seropositivity as well as climatic changes, geographical location and culture (Shah *et al.,* 2014).

The unexposed people are at risk of getting an acute infection which may be responsible for congenital toxoplasmosis in pregnant women or which may be life threatening in immune-suppressive patients if it reactivated. There are studies that show very low seroprevalence of the infection compared to the present study such as in South Africa 6.4% (Mwambe *et al.,* 2013), Zambia 5.87% (Frimpong *et al.,* 2017). Korea 3.7%, United Kingdom 9% (Aqeely *et al.,* 2014). In Southern Thailand the seroprevalence is 28.3% (Sroka *et al.,* 2010), 24.1% in Saudi Arabia (Aqeely *et al.,* 2014) and 30.9% in Tanzania (Nissapatorn and Sawangjaroen, 2011). Among 474, pregnant women, 43.03% were positive for IgG antibodies having chronic infection and 40.5% were positive for IgM having acute toxoplasmosis, whereas both IgG and IgM seropositivity was 16.45% which was lower than the prevalence in Northern parts of Pakistan where IgG antibodies were 46%, whereas was higher to 27.7% for IgM antibody (Bari and Khan, 1990). It was also lower to latent infection but was higher to acute infection of Cameron whose results for IgG and IgM was 88.7%, 30.9% (Wam *et al.,* 2016). But the present results are also significantly lower to 71.8% and 67.6% respectively obtained from London(Flatt and Shetty, 2012). The latent infection prevalence is also lower to 82.3% in Ethopia whereas the seropositivity for both IgG and IgM in current study is 16.45% which is higher than the work done in Ethopia.Latent infection in the current study is higher than the 3.02% in Ethopia (Abamecha and Awel, 2016). The latent infection prevalence among Pakistani pregnant women studied in Norway which is 17% (Bjerke *et al.,* 2011) is lower to the present study. Moderate prevalence of 30-50% has been found in countries of central and southern Europe (Robert-Gangneux and Dardé, 2012) which shows similar prevalence range in present study. The overall prevalence of toxoplasmosis reported previously in Brazil was 68.37% and the chronic infection prevalence of 63.03% and acute infection of 5.33% (Da Silva *et al.,* 2015) show a higher overall and also chronic infection seroprevalence from present study but show a lower acute infection prevalence than present study. The socio-economic conditions and presence of pets and stray cats, geographic location and age has a great role in high prevalence rate of toxoplasmosis.

Conclusion

The present study findings suggest that *T. gondii* infection is widely spread in the study area which may constitute a significant risk for congenital defects, stillbirths and abortions during pregnancy in women. Screening for the infection among pregnant women can be done twice at least during pregnancy to protect both mother and child from toxoplasmosis. There should be proper awareness in the educational institutes as well as community awareness programs about the risk factors of toxoplasmosis. This is the pioneer study on toxoplasmosis in pregnant women in District Mardan. The present study also open ways for further studies to be carried out in the concerned area. This will further create avenue for management and treatment of this disease in this population.

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Conflict of interests

The authors declare that they have no conflict of interests.

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