

Seroprevalence of *Toxoplasma gondii* among Pregnant Women in Lahore, Pakistan

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ABSTRACT

Toxoplasma gondii a neglected protozoan parasite commonly infects humans worldwide. One third of human population has become victim of *T. gondii* and its infection in pregnant women has serious consequences on women as well as on fetus health. Present study was conducted to assess the seroprevalence of *Toxoplasma gondii* among pregnant women of Lahore, Pakistan.

239 blood samples of pregnant women were collected from Lady Walingdon hospital, Lahore along with other details on associated risk factors of infection. From these samples, 86 were randomly selected to be evaluated for the presence of IgG immunoglobulin by using enzyme-linked immunosorbent assay (ELISA) method. The overall seroprevalence of *T. gondii* in pregnant women was found 22% being IgG positive. The infection rate of *T. gondii* was observed higher (29%) in older age group (29-39 years) as compared to (18%) in younger age group (18-28 years). More seropositive women were found in 3rd trimester (26%) as compared to 1st (22%) and 2nd (15%) trimester of pregnancy. The highest seroprevalence of *T. gondii* was found in women having weight from 40-50kg and lowest rate (9%) was observed in women with 73-83kg weight. Miscarriages were observed in 31% seropositive women. Moreover, the toxoplasmosis rate was higher in socially poor and uneducated women.

The seroprevalence of toxoplasmosis increases as age of mother increases due to accumulated exposure to *T. gondii* during the lifetime. The risk of infection increases in third trimester of pregnancy in women having low body weight.

Key Words: *Toxoplasma gondii*, pregnant women, congenital Toxoplasmosis, ELISA; seropositive.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite commonly infecting humans worldwide (Fox *et al.* 2009). A careful estimate shows that *T. gondii* infects one third of human population globally (Kijlstra & Jongert, 2008). It causes toxoplasmosis that is characterized by clinical syndromes. Its definitive hosts are spread all over the world. The infection rate of *T. gondii* is increasing in human population and it has emerged as health threatening parasite all over the world (Flegr *et al.*, 2014). *T. gondii* was first discovered in 1908 by two working groups in North Africa and Brazil (Black & Boothroyd, 2000) and has been placed in phylum Apicomplexa (Kessler *et al.*, 2008).

There are major three modes of parasite transmission in humans. Firstly, ingestion of infected uncooked or inadequately cooked meat or uncooked foods that have come in contact with

infected meat (Dubey, 1994). Secondly, parasitic transmission can occur from cat excreta containing oocysts to humans either from soil or from litter box. Third, infection can also spread from mother through placenta to its unborn fetus (Jones *et al.*, 2003).

Toxoplasmosis is an international health problem. It is not only restricted to under developed countries but even the developed countries have substantial prevalence of *T. gondii*. In Belgium and France, the infection rate of congenital infection is 2–3 cases per 1000 live births. This infection rate is quite higher than the US reports, where seroprevalence is as low as 1 in 10,000 to 1 in 1000 live births (Dubey & Beattie, 1988; Guerina *et al.*, 1994). Rate of *T. gondii* infection has fallen in many countries like United Kingdom and France over the past few decades (Montoya & Liesenfeld, 2004; Remington, 2006). United States has lowest seroprevalence of *T. gondii* and may show further

decrease of seroprevalence in general population (Smith *et al.*, 1996). But Central America and South America show higher seroprevalence of *T. gondii*, approximately more than 60% (Remington *et al.*, 1970; Remington, 2006). In Colombia, toxoplasmosis is considered a public health problem but still of little concern (Gómez *et al.*, 1997). In Indonesia, it has been worked out that the seroprevalence of *T. gondii* in humans is 58.8% and in pigs is 2.3% only (Tuda *et al.*, 2017).

It is reported that pregnant woman becomes victim of *T. gondii* at large scale due to environmental and demographic conditions. When a woman acquires infection during first or second trimester, then the new born will show severe symptoms of congenital toxoplasmosis (Dunn *et al.*, 1999; Montoya & Remingtons, 2000). The infection of *T. gondii* in unborn babies occurs through placenta. The parasitic infection in fetus leads to serious consequences like jaundice, abortion, brain calcification, microcephaly, hydrocephalus, mental retardation, blindness, and fetal death (Havelaar *et al.*, 2007; Nissapatorn, 2009; Mwambe *et al.*, 2013). There are some effective and efficient methods for diagnosis of *T. gondii*. The common practice of parasite detection is the serological testing of Toxoplasma specific antibodies (IgG and IgM) in serum of patients (Alvarado-Esquivel, 2006; Binnicker, 2010; Mwambe *et al.*, 2013; Cong *et al.*, 2015).

In rural and urban areas, people keep cats and other pets which cause environmental contamination with oocysts leading to human infection (Shahzad *et al.*, 2006). No substantial data is available regarding seroprevalence of *T. gondii* in pregnant women in Punjab. It is, therefore, valuable to evaluate the blood serum of pregnant women for *T. gondii* specific antibodies. The objective of the present study is to find the prevalence of *Toxoplasma gondii* in pregnant women of Lahore, Pakistan and to collect information on associated risk factors to prevent toxoplasmosis.

MATERIALS AND METHODS

Blood samples of 239 pregnant women were collected from the Lady Willingdon Hospital Lahore, Pakistan. Detailed information regarding risk factors like owning pets, educational level and socioeconomic conditions was gathered from these women through a designed questionnaire. All healthy pregnant women were included whereas the women with any kind of pathological infection such as HIV +ve, HCV +ve, HBC +ve, Anemia +ve and thalassemia +ve etc, were excluded from this study. Serological Assay:

Out of 239 collected blood samples, 86 samples were randomly selected for serological detection of toxoplasma-specific immunoglobulin, IgG.

ELISA immunoassay technique was employed to analyze the *T. gondii* in blood samples. For diagnosis of toxoplasmosis, a commercially available kit "Toxoplasmosis Latex", manufactured by Antec Diagnostic Products was used. The test was performed according to the procedures described by the kit manufacturer. Cut off value of every sample was determined and thus value index of every sample was calculated (Ahmad & Tasawar, 2016).

The ELISA test results and data obtained through questionnaires were analyzed by one way ANOVA using SPSS version 13.

RESULTS

In the present study the overall prevalence *T. gondii* was 22% as 19 women out of 86 were found positive for IgG antibodies. The seroprevalence of toxoplasmosis was also determined in pregnant women with respect to age. The results showed that the older age group had high rate of prevalence (29%) than the younger age group (18%) (Table I). When the pregnancy stage of the pregnant women was considered in relation to the prevalence, it was found that the women with third trimester had 26%, those with 2nd trimester had 15% and the women with 1st trimester had 22% prevalence of *T. gondii* (Table II).

Table I: Overall seroprevalence of *T. gondii* in pregnant women

Parameters	Total women included	IgG positive cases	Seroprevalence (%)
Age (years)	86	19	22
18-28	55	10	18
29-39	31	9	29

Table II: Seroprevalence of *T. gondii* in women with respect to the stage of pregnancy

Parameters	Total women included	IgG positive cases	Seroprevalence (%)
Trimester	86	19	22
1 st	18	4	22
2 nd	26	4	15
3 rd	42	11	26

The blood pressure is considered another determinant of *T. gondii* infection in pregnant women. A substantial no. of pregnant women (24%) had low blood pressure whereas 66% IgG positive women had high blood pressure and only 14% of women had normal blood pressure. Further the selected samples were divided into four groups with respect to weight. The highest seroprevalence of *T. gondii* (50%) was found in women having weight from 40-50kg and lowest rate (9%) was observed in women with 73-83kg of weight (Table IV). Further, out of 19 IgG positive women, 31% pregnant women had premature miscarriages of their last baby and some of them experienced miscarriages more than once.

Table III: Relationship between Seroprevalence of *T. gondii* and Blood pressure

Parameters	Total women included	IgG positive cases	Seroprevalence (%)
Blood pressure (mm/Hg)	86	19	22
Normal	34	5	14
High	3	2	66
Low	49	12	24

Table IV: Relationship between Seroprevalence of *T. gondii* and body weight

Parameters	Total women included	IgG positive cases	Seroprevalence (%)
Weight (kg)	86	19	22
40-50	22	11	50
51-61	22	3	13
62-72	21	3	13
73-83	21	2	9

DISCUSSION

T. gondii is a common parasite is that transferred in humans by pets owning, blood transfusion and unhygienic environmental conditions. In Pakistan many people are unaware of the need of proper vaccination for their pets. This reality is considered the main cause of spreading infection among the owners. On the other hand, owning a pet does not mean the transfer of *T. gondii* but direct contact with cat feces and improper hand wash after contact causes transfer of parasite (Nissapatorn *et al.*, 2011). Our findings could not

relate to significant association of pet keeping with infection as most of the infected women did not keep pets at home.

The findings of existing study showed that the overall prevalence of *T. gondii* in pregnant women was 22% being positive for IgG which was even higher (29%) in older age group. The prevalence in our study is quite higher than the seroprevalence found in pregnant women of other countries such as Zambia, (5.87%), (Frimpong *et al.*, 2017), united kingdom, 9% (Aqeely *et al.*, 2014), South Africa 6.4% (Mwambe *et al.*, 2013) and korea 3.7% (Aqeely *et al.*, 2014). There are studies which showed relatively high rate of prevalence of *T. gondii* in other countries, for instance 18.7% in Mozambique (Sitoe *et al.*, 2010), 30.9% in Tanzania (Nissapatorn *et al.*, 2011) and 92.5% in Ghana (Ayi *et al.*, 2009). Globally, the prevalence of toxoplasmosis is 24.1% in Saudi Arabia, (Aqeely *et al.*, 2014), 28.3% in Southern Thailand (Sroka, 2010) and 17.3% in London (Kistiah, 2011). The seroprevalence of *T. gondii* in the present study was quite low as compared to that in Brazil, (68.37%) but comparable to that in Mexico (6.1–27.9%) and USA (17.5–29.2%) (Jones, 2001; Galvan-Ramirez *et al.*, 2012; Silva *et al.*, 2015).

There is a strong association between the risk of infection and the age of pregnant. It has been reported that risk of infection increases significantly with the increase of age (Nissapatorn *et al.*, 2011; Singh *et al.*, 2014). Our findings are in agreement to the study conducted on 955 pregnant women and results revealed that the prevalence of infection increased implicitly with increasing age (Rosso *et al.*, 2008). It appears that as the age increases the risk of infection increases because time of exposure to toxoplasmosis increases as the one gets older (Ertug *et al.*, 2005). There are some studies which revealed that age was not significantly associated with the infection (Ayi *et al.*, 2009).

The transmission of *T. gondii* also occurs by blood transfusion. Different studies were conducted in Iran to find out the possibility of *T. gondii* in individuals who donate their blood to patients. The results showed that 102 out of total 270 samples were positive (37.8%) for IgG and none were IgM-positive (Tappeh *et al.*, 2017). ELIZA method is the most appropriate method for the detection of toxoplasmosis even after two weeks of infection. The presence of IgG and IgM antibodies in the serum indicates the incidence of infection. Some studies have reported the presence of IgM antibodies but in others studies consistent with our results did not find any IgM in serum (Mwambe *et al.*, 2013; Sakae *et al.*, 2013).

Our studies demonstrated that there was no significant association between gestational stage and the incidence of toxoplasmosis. However, the present results showed that the rate of toxoplasmosis was higher in third trimester as compared to first and second trimester of pregnancy. These findings are in agreement with the studies reported elsewhere (Ayi *et al.*, 2009). Premature miscarriages were seen in toxoplasma IgG positive women in our study. Some pregnant women had with their previous baby died after birth while others experienced premature death of the fetus. Our data is in line with the study conducted in Norway, where 35940 women were investigated for toxoplasmosis and the results revealed that *T.gondii* infected women had miscarriage at the end of first trimester (Jenum *et al.*, 1998).

There is valid association between parasite infection and socioeconomic status of the pregnant women. In our study, most of the seropositive pregnant women belonged to backward rural areas of Lahore where educational rate was very low (especially in women) due to poverty and low living standards. The findings of our study are consistent with the results of Frimpong *et al.* (2017), Andiappan *et al.*, (2014) and Jones *et al.* (2009) who revealed that the prevalence of *T.gondii* was high in pregnant women with low socio-economic status as compared to the women with high socio-economic status. Similarly, in Bangladesh, the incidence of *T.gondii* was more common in poor people as compared to people belonging to upper socioeconomic status (Ashrafunnessa *et al.*, 1998). This high prevalence of infection in low socio-economic group is due to unhygienic practices, poor living standards and low education.

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