Seroprevalence and risk factors of toxoplasmosis in pregnant women of district Swabi

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Abstract
Toxoplasmosis is parasitic infection that causes severe abnormalities in newborn babies when transmitted from mothers. The present study was conducted to find the seroprevalence and risk factors of toxoplasmosis among the pregnant women of selected samples taken from maternity ward, District Headquarter Hospital Swabi. Furthermore, the selected samples were scrutinized for toxoplasmosis in relation with gestation period, age factor, total number of pregnancies women carried, socioeconomic factors and the source of contamination through which women can be infected. The sera sample was examined for the presence of IgG/IgM anti-Toxo antibodies. For this purpose OnSiteToxo IgG /IgM Rapid test kit was used according to manufacturing protocol CTK biotech (San Diego USA). Overall prevalence of toxoplasmosis was reported to be 12% among which 05 cases were positive for only IgG and remaining 07 were positive both for IgG and IgM. Regarding gestation period, females at the third trimester of pregnancy were at greater risk and about 20% of cases were screened positive. In relation to age factor, women at the age of 31-36 showed high prevalence (25%) for toxoplasmosis. Examining toxoplasmosis in relation to number of pregnancies women carried, high percentage was recorded in women with their first pregnancy (3.96%). The findings also linked the infection with socioeconomic factors i.e. (36.36%) of cases were reported in pregnant women belonging to lower class. Similarly, in reporting results for toxoplasmosis due to contaminated environment, 30% of the cases of toxoplasmosis were mainly caused by the consumption of raw/improperly cooked meat. Other enormous causes included the interaction of women with cats and contaminated soil i.e. 27% each. Proper screening of women at her each trimester of pregnancy and awareness about the infection by health departments may contribute towards the prevention of congenital Toxoplasmosis.

Keywords: Toxoplasma gondii; Toxoplasmosis; Seroprevalence

Introduction
Infection with Toxoplasma gondii, an apicomplexan protozoan, a human pathogenic obligate and intracellular parasite is the causative agent of toxoplasmosis. It is prevalent worldwide in many species of warm-blooded animals and human beings [1-5]. Transmission and spread of infection among humans is largely through inhalation
or ingestion of oocysts discharged in the faeces of infected cats, inoculation of trophozoites through the skin, drinking raw cow’s milk or contaminated water, ingestion of bird’s eggs and by eating raw or undercooked infected meat and vegetables [1, 3, 5].

The parasite persists in three life forms. Tachyzoite is the rapidly dividing and invasive stage. It causes an acute infection by replicating within the cells. This life form of the parasite is responsible for the congenital Toxoplasmosis. It can inhabit and invade several tissues including muscles of heart, liver, spleen, lymph nodes and central nervous system. Bradyzoite is slowly dividing in the tissue cyst and it is responsible for the latent infection. Both the tachyzoite and bradyzoite if present in the meat of infected animals serve as source of infection. The infection can also be caused by consumption of food and water that meets such infected food items [4]. Oocyst is the most infectious stage of this parasite that cannot be destroyed by human digestive enzymes. It is environmentally resistant and under favourable condition sit can remain viable for up to one year [4].

The parasite has a complex and biphasic life cycle. Asexual reproduction takes place in tissues of human and few birds (intermediate host). Sexual reproduction occurs in the digestive tract of domestic and wild cats (definitive host) belonging to family Felidea [6].

A pregnant woman if infected in her first trimester, the placenta would be infected for the rest of her pregnancy acting as a reservoir for the parasite [7]. A pregnant woman suffering from primary acute Toxoplasmosis will transfer the parasite into the offspring via placenta. The permeability of placenta varies throughout gestational period and the transfer of parasite is less in the start of pregnancy while afterwards the permeability of placenta increases. Thus, the infection is highly prevalent in the late period of gestation. A total of 10% cases are recorded in the first trimester whereas, 60% to 70% cases in third trimester of gestation [8]. However, the infection is most severe in first trimester as compare to the third trimester of gestation. Infants that become infected in the first or early second trimester are more severely affected [9, 10].

Congenital Toxoplasmosis has been observed in approximately 3.3–8/10,000 newborns in Brazil [11]. Congenital Toxoplasmosis affects 1 to 10 among 10,000 newborn babies in European countries. Studies have shown that about 4% to 27% develop retinochoroidal lesions leading to 4 permanent unilateral impairment of vision and 1% to 2% of whom developed leaning difficulties or face death [12]. Seroprevalence of Toxoplasmosis was 60% in Saudi Arabia which is the highest ratio recorded [13]. About 15% of women are at risk in USA, at their child bearing age and about 400 to 4000 new cases are reported each year. Half of these cases are mainly caused by eating inadequately cooked meat. In contrast to USA, the Canadian women have very low seropositivity of Toxoplasmosis [14]. In France and some other European countries, the infection is very common but in United Kingdom the prevalence rate is about 9% [15]. Low prevalence ratio of infection is recorded in North America, South East Asia, and Northern Europe whereas high rate of infection has been calculated in Latin America and tropical African countries [16].

Regarding the pathogenic conditions of Toxoplasmosis in Pakistan, several studies have been conducted. One of such survey conducted by [17] showed that T. gondii may cause a wide range of clinical syndrome in congenitally effected children resulting in blindness and mental retardation. Another similar study regarding the ocular Toxoplasmosis conducted by [18] stated that several cases have been recorded in ocular Toxoplasmosis where the parasite reaches the retina, proliferates within host cells, ruptures the host cells and finally invades
into the neighbouring cells to make primary lesions.

Evidences collected from pregnant women by researcher [10] in Mwanza Tanzania, 108 (30.9%) were seropositive out of 350 pregnant women for *T. gondii* specific antibodies. The risk of *T. gondii* infection increases by 7% with a yearly increase in a woman’s age. The seropositivity rate of *T. gondii*-specific antibodies was higher among pregnant women from the urban than those from rural communities. Similarly employed/business women were more likely to get *T. gondii* infection. Likewise per [19] in USA approximately 85% of women of childbearing age were found susceptible to acute infection with *T. gondii*. In pregnant women, acute infection may cause serious health problems to the fetus when it is transmitted from mother to fetus (congenital Toxoplasmosis), including mental retardation, seizures, blindness, and death.

So, in this regard the present study was designed with aims and objectives that to find out the risk factors of toxoplasmosis in pregnant women of District Swabi.

### Materials and methods

#### Study Area

Present study was conducted to find out the seroprevalence and risk factors of congenital Toxoplasmosis in District Swabi, KPK. The data was collected from March to June 2015. Blood samples were collected from pregnant women of maternity ward at District Head Quarter Hospital Swabi. The women visited from the surrounding villages of district Swabi including Anbar, Asota, Baja, Charbagh, Gohati, kernel sherkill,kunda, Maneri, Maine, Marghuz, Manki, Panjpeer, Shewa, Shah Mansoor, Tordher, Zaida etc.

#### Blood Collection

Intra-venous blood was preferred for this purpose. About 2 mL blood was taken by the disposable syringe through vein puncture. Collected blood was centrifuged at 400 rpm for ten minute. After the isolation, the serum was transferred in to sterilized eppendorf tube with the help of sterilized micro pipette. The serum was then stored in freezer at the temperature of -20ºC until the laboratory test and analysis was performed.

#### Serological Tests

All samples were serologically examined for the presence of IgG and IgM. For this purpose, OnSiteToxo IgG/IgM Rapid test kit was used per manufacturing protocol CTK biotech (San Diego USA). All the positive and negative results were recorded and a clinical report was made.

#### Statistics

Statistically the data was analyzed by Ms Excel.

#### Results and discussion

The present study was designed to scrutinize the seroprevalence and risk factors of congenital toxoplasmosis in pregnant women of District Swabi. For this purpose, 100 pregnant women were selected for blood collection which was then analyzed for congenital toxoplasmosis. Congenital toxoplasmosis was examined in the selected samples. Furthermore, it was analyzed in relation with gestation periods, age factor, number of pregnancies, socioeconomic factor and source of contamination from the surroundings.

### Overall prevalence of toxoplasmosis in selected samples of district Swabi

According to the laboratory diagnosis 12% (n=12/100) samples were positive for the toxoplasmosis. Out of the 12 infected pregnant women, 07 were screened positive for recent infection i.e. positive for IgG and IgM and 05 were diagnosed only for IgM i.e. the latent infection, as shown in table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive</th>
<th>Negative</th>
<th>% age</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>12</td>
<td>88</td>
<td>12%</td>
<td>100</td>
</tr>
<tr>
<td>IgM</td>
<td>12</td>
<td>88</td>
<td>12%</td>
<td>100</td>
</tr>
<tr>
<td>IgG</td>
<td>7</td>
<td>93</td>
<td>7%</td>
<td>100</td>
</tr>
<tr>
<td>Both IgM/IgG</td>
<td>12</td>
<td>93</td>
<td>12%</td>
<td>100</td>
</tr>
</tbody>
</table>
Relationship of toxoplasmosis with gestational period

Among the 100 collected samples 35 women were in the last trimester of their pregnancy. Out of which 07 equal to 20% were seropositive for toxoplasmosis and the remaining 28 equal to 80% were normal as they did not show any antibody in the serum. Among 07 seropositive women, 02 women were diagnostic with IgG antibodies while remaining 05 showed both the presence of IgM and IgG antibodies.

Likewise 34 samples were collected from the pregnant women at their second trimester. Among them 03 samples were screened positive with seropositivity equal to 8.8% while remaining 31 sample equal to 91.8% were normal as no antibody was observed in the serum. 02 out these 03 showed recent infection i.e. positive both for IgG/IgM while on 01 sample only IgG antibody was observed. Similarly, 31 samples were collected from women in the first trimester with prevalence ratio 6.45% i.e. two positive cases as shown in table 2.

Table 2. Relationship of Toxoplasmosis with gestational period

<table>
<thead>
<tr>
<th>Gestational period</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>31</td>
<td>2</td>
<td>29</td>
<td>6.45%</td>
</tr>
<tr>
<td>Second trimester</td>
<td>34</td>
<td>3</td>
<td>31</td>
<td>8.8%</td>
</tr>
<tr>
<td>Last trimester</td>
<td>35</td>
<td>7</td>
<td>28</td>
<td>20%</td>
</tr>
</tbody>
</table>

Relationship of toxoplasmosis with Age

To find the age wise distribution, the population was divided into three age groups i.e., from 17-23 years old (n=50), second age group was 24-30 (n=42) and third age group was from 31-36 (n=8) years old. Highest prevalence of infection was observed in the third age group that is 02 out of 08 equal to 25% (n=2/6), both the infected women were positive for IgG anti-Toxo antibodies followed by first age group in which 09 samples equal to 18% were diagnosed positive out of 50 samples (n=9/41). In these 09 positive cases, 04 were positive only for IgG antibodies whereas 05 remaining were seropositive both for IgG and IgM while low prevalence ratio (2%) was documented in the second age group i.e. 01 sample was screened positive for the anti-Toxo antibodies (n=1/41) as shown in table 3.

Table 3. Relationship of toxoplasmosis with age

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total</th>
<th>Infected</th>
<th>Normal</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>First group17-23 years</td>
<td>50</td>
<td>9</td>
<td>41</td>
<td>18%</td>
</tr>
<tr>
<td>Second group24-30 years</td>
<td>42</td>
<td>1</td>
<td>41</td>
<td>2%</td>
</tr>
<tr>
<td>Third group31-36 years</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td>25%</td>
</tr>
</tbody>
</table>

Relationship of toxoplasmosis with number of pregnancies

Hundred samples were divided in five groups based on number of pregnancies a woman carried in her life. Out of hundred samples that were collected, 33 women had their first pregnancy followed by 31, 24, 10 and 02 with their second, third, fourth and fifth pregnancy respectively. Distribution of infection in the first group was 3.96% (n=12/33), 0% in the second group (n=0/31), 0% in the third group (n=0/24), 0% in the fourth and fifth group (n=0/10) and 0% in the fifth group (n=0/2) respectively. Interestingly all the twelve positive cases were reported from the women who had their first pregnancy as shown in table 4.

Relationship of toxoplasmosis with socioeconomic factor

03 groups were designed based on financial status. Of these 03 groups the first was upper middle class including 12 females. Second group, was the middle class that have participation of 64 women. Third group included 33 women belonging to lower middle class.
Highest percentage (36.36%) of infection was recorded from lower middle class, i.e., six cases were diagnosed positive (n=6/33). Similarly, six women (18%) were reported positive that belonged to middle class (n=6/64) followed by 0% seroprevalence was recorded in the upper middle class (n=0/3) as shown in table 5.

Table 4. Relationship of toxoplasmosis with number of pregnancies

<table>
<thead>
<tr>
<th>Previous Number of pregnancies</th>
<th>Total sample</th>
<th>+Ve result</th>
<th>-Ve result</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>First pregnancy</td>
<td>33</td>
<td>12</td>
<td>21</td>
<td>3.96%</td>
</tr>
<tr>
<td>Second pregnancy</td>
<td>31</td>
<td>0</td>
<td>31</td>
<td>0%</td>
</tr>
<tr>
<td>Third pregnancy</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>0%</td>
</tr>
<tr>
<td>Fourth pregnancy</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>Fifth pregnancy</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>12</td>
<td>88</td>
<td>12%</td>
</tr>
</tbody>
</table>

Table 5. Relationship of toxoplasmosis with socioeconomic factor

<table>
<thead>
<tr>
<th>Financial class</th>
<th>Total sample</th>
<th>+ve result</th>
<th>-ve result</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper middle</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0%</td>
</tr>
<tr>
<td>Middle</td>
<td>64</td>
<td>6</td>
<td>58</td>
<td>18%</td>
</tr>
<tr>
<td>Lower middle</td>
<td>33</td>
<td>6</td>
<td>27</td>
<td>36.36%</td>
</tr>
</tbody>
</table>

Relationship of toxoplasmosis with source of infection

In the present study, the patients were asked few questions as per the questionnaire to find out the sources of infection. So during interviewed it was concluded that out of twelve positive cases recorded in the present study, nine (27%) women were exposed to cat which is the host of T. Gondii and remaining three (17%) had no such contact. Likewise, out of the total twelve positive cases seven (30%) patients had taken raw/ improper cooked meat whereas remaining five positive cases had the infection from other sources. Similarly, five females (27%), were reported positive for the toxoplasmosis that had a pervious contact with soil, either from farming or gardening as shown in table 6.

Table 6. Relationship of toxoplasmosis with source of infection

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Yes</th>
<th>No</th>
<th>+Ve result</th>
<th>-Ve result</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with cat</td>
<td>43</td>
<td>67</td>
<td>9</td>
<td>3</td>
<td>27%</td>
</tr>
<tr>
<td>Contact with soil</td>
<td>18</td>
<td>82</td>
<td>5</td>
<td>13</td>
<td>27%</td>
</tr>
<tr>
<td>Raw meat</td>
<td>23</td>
<td>77</td>
<td>7</td>
<td>5</td>
<td>30%</td>
</tr>
</tbody>
</table>

Discussion

About 12% seropositive cases were reported in District Swabi. It is in accordance to study conducted by [20] where 19.25% seropositive cases were revealed i.e. slightly higher than the present findings. In this study a total of 805 samples were collected from District Swabi, out of which 155 (19.25%) were positive and 650 (80.75%) were negative for the T. gondii. The age wise distribution shown that the high number of cases i.e. ninety were reported in age group 25-34 years whereas the lowest number of cases, seventeen were reported in age group >34 years. Similarly, the present findings were in more agreement with the findings (14% and 14.4%) of the [21, 22] from district Kohat and Islamabad respectively. About 69% prevalence in Brazil have been observed by [9] while as compared to this finding in USA, low seropositivity was reported by [19]. The seroprevalence was found to vary from 17.5% in the west to
20.5% and 29.2% in the south-Midwest and north-east, respectively. In contrast to USA, a decline was reported in the Toxoplasmosis from UK which was confirmed by the results of [23] that was 9% in 2005.

High percentage of infection was reported in the third trimester, i.e. 20% that was followed by the second and third trimester, i.e. 8.8% and 6.45% respectively. So, the present findings agreed with the conclusions collected by [10] at Helsinki, Finland, showing the increase in infection with increase in gestational period i.e. 20% to 70%. In other studies, conducted by [21] in 2013 at Kohat Pakistan, quite opposite results were recorded; i.e. prevalence was 29% at first trimester, 19% at second trimester and only 2% at last trimester.

Current findings illustrated high prevalence of infection in the third age group (31-36 years old); two out of eight (25%), followed by first age group (17-23) in which a total of nine (18)% out of fifty females were positive for the anti-Toxo antibodies. Low prevalence ratio had occurred in the second age group (24-30 years) percentage was 2.3%. So, the present results agreed with the findings of [20] which showed that highest prevalence is reported in age group: 25-32 years that was 22.28% followed by another age group 15-24 years, showing 17% seroprevalence. Similarly, same finding was observed in Tanzania by [10] where risk of infection in women was increased with increase in age.

Existing study indicates that infection is more prevalent in women belong to lower and middle class regarding socioeconomic factors, like 36% cases were reported from lower middle class and 18% from middle class whereas no positive case from upper classes have been reported. So, same finding was observed by [10] like high prevalence of Toxoplasmosis was observed in women with low-income 19%. The same [24] showed that 83% of cases were reported from lower class, 62% seropositivity had been reported from middle class and 23% from higher class in Brazil.

Different routes of infection can be possible for Toxoplasmosis. The major one includes contact with domestic and wild cats, contaminated soil and the consumption of raw or improper cooked. In this study, consumption of raw meat was the major source of infection because a large number (30%) of positive cases were reported from pregnant female who had usually taken inadequately/raw cooked meat. Similar result was reported by [25] that 30%-60% cases of toxoplasmosis were recorded because of the consumption of uncooked raw meat in USA and Canada.

In many different countries, females’ interaction with cats is held to be responsible for one of the greater cause of congenital Toxoplasmosis. However, in the present study equal numbers of cases were reported from contact with soil as well i.e. 27% of cases were caused by contact with cat, and the same ratio was reported by contact with soil in their history. Many scientists correlate the contact of soil and cat, because contamination of soil arises mainly by the presence of cat by shedding the parasites oocyst in to the soil through feces. Similarly [26] showed that cats are responsible for infection in Ethiopia. About 27% of positive cases from contact with soil either by living in mud-made houses, gardening or by eating soil in pregnancy due to appetite has been reported in existing results and is also justified by the findings of [10] in Brazil showing high prevalence of Toxoplasmosis caused by soil interaction.

Conclusions and recommendations

Congenital Toxoplasmosis is considered a great reason of abortion and stillbirth in pregnant women of Swabi. In the light of these results and related discussion, it is concluded from the present study that the ratio of prevalence of Toxoplasmosis in this area is threaten towards newborn babies. High percentage has been reported from women carrying their first pregnancy that may leads towards miscarriage. Pregnant
women at last trimester of pregnancy were at higher risk to become infected from *T. gondii*. Regarding age factor, most of cases were reported from women, aged above thirty years. Similarly, the infection was highly prevalent in lower classes as compared to middle and upper middle classes. High ratios of positive cases were reported from the lower middle. The consumption of inadequately cooked meat was a great risk factor of toxoplasmosis in Swabi as compared to other sources because 30% cases of toxoplasmosis were reported due to consumption of raw meat. It is thus advisable that pregnant women must take properly cooked meat to avoid the high-risk factor infection. Contact with cat and other pet animals was secondary risk factor. Similarly contact with contaminated soil through gardening or through other sources also contributed as a risk factor for the Toxoplasma infection in pregnant women of Swabi. Therefore, to prevent the infection in pregnant women proper screening at each trimester is highly recommended. Laboratory facilities thus are required at each maternity home for early diagnosis of parasite. Proper awareness must be given to female at child bearing age, especially about improperly cooked meat and during contact with soil and cats. Hands must be properly washed while eating or handling food. A woman may wear gloves during contact with soil. Pregnant women should be informed about the dangers of eating soil due to appetite in pregnancy.

**Authors’ contributions**

Conceived and designed the experiments: N Shah & Tanzeela, Performed the experiments: N Shah & Tanzeela, Analyzed the data: A Khan & N Shah, Contributed reagents/ materials/ analysis tools: M Adnan, Shahibzada & M Jawad, Wrote the paper: A Khan, N Shah & M Khisroon.

**References**


