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Seroprevalence of *Toxoplasma gondii* in Pregnant and Non-Pregnant Women of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Toxoplasma gondii is a globally found intracellular parasite that infected a large proportion of the world population, it remained asymptomatic in immunocompetent patients but the acquisition of the infection during pregnancy can lead to abortions and other congenital defects. The present study aimed to find the seroprevalence of *T. gondii* among suspected women who visited local hospitals in Khyber Pakhtunkhwa (KP), Pakistan. Sera of 425 suspected women were screened by latex agglutination test (LAT) and enzyme-linked immunosorbent assay (ELISA) was performed to measure the IgG and IgM antibodies level. Univariate analysis and odds ratios were calculated to determine the strength of association. The overall *T. gondii* infection was 60% with LAT, 52% IgG, and 56.26% with IgM ELISA. The seroprevalence was 7.2%-15% among the five study districts of KP province. The toxoplasmosis was found significantly ($p<0.001$) higher in 34.7% to 44.2% of pregnant women as compared to non-pregnant. The high prevalence of infection was recorded among women belonging to the 20-30 years and 31-40 years age group (16.78%-36.45), 1st and 3rd trimesters (33.2%- 40.3%), and housewives (28.8%-37.4%). The mean of IgG and IgM antibodies titer was observed higher than >1.3 in pregnant women and in the 2nd and 3rd trimesters. The present study concluded a higher prevalence of *T. gondii* infection in women of childbearing age in KP, Pakistan, and requires preventive measures to reduce the abortion risks and congenital abnormalities in fetus.

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Authors' Contribution
KA designed the study. SB and SK performed the experiment. GN and SF advised on methods, experimentation, and interpretation of findings. SB, SK and KA conducted literature search, data analysis and manuscript preparation. KA and SF reviewed the manuscript. All authors participated in the study and concurred with the submission and subsequent revisions submitted by the corresponding author.

Key words
***Toxoplasma gondii*, LAT, ELISA, Pregnant women**

INTRODUCTION

Toxoplasma (T.) gondii, is an obligate intracellular parasite belonging to phylum Apicomplexa, is a zoonotic protozoan that infects warm-blooded animals including humans (Weiss and Dubey, 2009). People get the disease by the oral route through the utilization of half-cooked meat containing cysts, food items, or water polluted with oocysts (Tenter *et al*., 2000). The pregnant ladies infected may cause medical issues if the parasite is transmitted to the baby to cause congenital toxoplasmosis (Koneman *et al*., 2004). Congenital toxoplasmosis may cause abortion, neonatal death, or fetal anomalies with hindering ramifications for the fetus (Koneman *et al*., 2004).

Toxoplasma gondii accounts for a critical health hazard where the household cleanliness, dietary propensities, and disinfection are poor. *Toxoplasma gondii* is a cosmopolitan parasite with a variable recurrence rate around the globe (Tenter *et al*., 2000). Toxoplasmosis has infected one-third of the world's human populace (Dubey, 2008). The predominance of *T. gondii* varies from less than 10% to 80% (Robert-Gangneux and Dardé, 2012). Considering, seroepidemiological studies, a higher prevalence of 50% has been recorded in America, the Middle East, and sub-Sahara Africa (Agmas *et al*., 2015; Varella *et al*., 2009). However, in Pakistan so far recorded seroprevalence was 25.8% in Punjab (Shahzad *et al*., 2020), 40.6% in Mardan, KP (Rahman *et al*., 2021), and 20.37% in sub-tropical regions of Pakistan (Ahmad *et al*., 2019).

The serological detection methods used for *T. gondii* determine the phase of infection i.e., the chronic or acute stage (Hadi *et al*., 2016). Sabin-Feldman dye test was the most reliable test for *T. gondii* and viewed as the gold standard. However, the limitation of the assay was the utilization of live organisms and human serum from

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healthy people as a frill factor (Shin *et al*., 2009). The indirect fluorescent antibody test is profoundly specific but limited due to low sensitivity, requiring fluorescently marked conjugates, and special equipment. Enzyme-linked immunosorbent assay (ELISA) exhibits extraordinary sensitivity, is quantitative, requires minimal effort, and is considered an effective tool for epidemiological investigations of toxoplasmosis. Similarly, the latex agglutination test (LAT) is an effective technique for routine serologic evaluation of *T. gondii* antibodies (Shin *et al*., 2009). The accurate detection of toxoplasmosis is crucial to measure in epidemiological settings.

The serological assays are so far successful for large-scale epidemiological studies (Dubey, 2008) to counteraction and control of inherent toxoplasmosis (Jones *et al*., 2003). Unfortunately, serological screening during antenatal consideration isn't done regularly in most health centers of Khyber Pakhtunkhwa. For this purpose, public awareness is required to reduce the risk of zoonosis. Therefore, we performed a population-based study to ascertain the prevalence in pregnant females of Khyber Pakhtunkhwa, Pakistan. We also screened anti-*T. gondii* IgM and IgG antibodies level by using enzyme linked immunosorbent assay (ELISA) and with latex agglutination test.

MATERIALS AND METHODS

Study area, design, and period

The study participants belonged to different districts of Khyber Pakhtunkhwa i.e. Abbottabad, Mansehra, Battagram, Peshawar, and Haripur. The Ethical Committee of Quaid-I-Azam University Islamabad approved the study. Due to the COVID-19 restriction, the data collected from patients during sampling was their age, trimester, and profession. The study included a total of 425 participants comprised of 285 pregnant women and 140 non pregnant women. The study excluded 105 individuals who refused to provide blood samples. Blood sampling was carried out from January 2020 to February 2021 and subjected to LAT, IgG and IgM ELISA tests.

Study population, sample size calculation and blood sampling

The study participants belonged to the diverse background and comprised of females of reproductive age from 15 to 50 years. The sample size was determined by using the formula: $n=Z^2xp(1-p)/d^2$ (Daniel, 1995). Where, n is the minimum required sample size, Z is the confidence level at 95% (standard value of 1.96), p is the expected seroprevalence of *T. gondii*, d is precision or margin error of 5% (standard value 0.05). For each of the visiting patients, 4ml of cubital venous blood was collected using a sterile disposable syringe in a vacutainer tube, serum was extracted and stored at -20 °C.

Serological testing

LAT

Qualitative screening of *T. gondii* antibodies from serum samples was done by using a commercial kit (Wiener Laboratorios, Argentina) according to manufacturer instructions (Wiener Laboratorios, Argentina).

ELISA

The ELISA was performed to measure the *T. gondii* IgG and IgG antibodies titers by using commercial kits (Abcam Company, UK) according to manufacturer instruction.

Statistical analysis

Cross-tabulations of sero-status were done with socio-demographic characteristic as summary measures. Chi-square test and univariate logistic analysis were computed to measure the strength of association between toxoplasmosis and associated factors. Odds ratios (OR) were computed and variables resulting in $p\leq0.05$ was considered to be significantly associated with seroprevalence of toxoplasmosis. Statistical analysis was carried out in SPSS version 20.0 and Graph Pad Prism (V. 5) was used for graphical representation of OD values.

RESULTS

Study participants characteristics and overall seroprevalence of T. gondii

A total of 425 women enrolled in the study were divided into four age groups. Most of the women who participated in the study were housewives and the mean age of the participants was 29(±7.47). Of the total studying participants, 285 (67%) were pregnant and 140 (32.9%) were non-pregnant. The total seropositivity of *T. gondii* predicted by the LAT was found to be 60% (256), 52% (220) with *T. gondii* IgG ELISA, and 56.26% (238) with IgM ELISA. Table I presents the *T. gondii* IgG and IgM antibodies titers values (Mean ± SD (Min-Max)).

Latex agglutination test (LAT)

The seroprevalence of *T. gondii* across socio-demographic characteristics of studied participants is presented in Table II. The results obtained by LAT indicated that out of 256 positive patients maximum seropositivity was observed in Abbottabad district 15% (64), followed by Peshawar 12.9% (55), Mansehra 12.4% (53), and Haripur 11% (47) while the lowest seropositivity

Table I. *T. gondii* IgG and IgM antibodies titre in form of OD Mean ± SD (Min-Max) values among pregnancy status and stages.

Variables	n	IgG antibodies titre	IgM antibodies titre
		OD Mean ± SD (Min-Max)	OD Mean ± SD (Min-Max)
Pregnancy status			
Pregnant	285	1.22±0.56 (0.19-2.17)	1.06±0.49(0.21-2.17)
Non pregnant	140	1.01±0.59 (0.19-1.99)	0.47±0.50(0.21-1.99)
Stage of pregnancy			
First trimester		0.88±0.57 (0.19-2.17)	0.76±0.49(0.19-1.99)
Second trimester		1.23±0.61 (0.21-1.98)	1.01±0.50(0.21-1.99)
Third trimester		1.01±0.59 (0.19-1.91)	1.06±0.49(0.19-2.17)

Table II. Seroprevalence and univariate analysis showing association between *T. gondii* infection rate and sociodemographic factors by using IgG, IgM and LAT assays.

Characteristics	IgG ELISA			IgM ELISA			LAT		
	Positive n (%)	OR 95% CI (Range)	p value	Positive n(%)	OR 95% CI (Range)	p value	Positive n (%)	OR 95% CI (Range)	p value
Districts									
Abbottabad	60(14.1)	1.36(0.75-2.44)	0.30	51 (12.0)	1.09(0.59-2.00)	0.21	64(15)	1.01(0.55-1.85)	0.95
Mansehra	42(9.8)	0.88(0.48-1.63)	0.70	55 (13)	1.19(0.65-2.15)	0.56	53(12.4)	0.99(0.53-1.85)	0.98
Battagram	31(7.2)	0.89(0.46-1.70)	0.72	43 (10.1)	2.00(1.07-3.73)	0.02*	37(8.7)	0.81(0.42-1.59)	0.55
Peshawar	46(10.8)	1.02(0.56-1.85)	0.94	43 (10.1)	2.18(1.11-4.28)	0.02*	55(12.9)	1.12(0.60-2.09)	0.71
Haripur	41(9.6)	Reference		35 (8.27)	Reference		47(11.0)	Reference	
Age in years									
15-20	11(2.5)	1.1(0.56-1.85)	0.83	13 (3.07)	1.008(0.56-1.79)	0.97	16(3.76)	1.65(0.66-4.13)	0.28
21-30	130(30.5)	1.85(1.06-3.25)	0.30	60 (14.18)	1.88(0.64-5.50)	0.24	155(36.4)	2.02(1.15-3.56)	0.14
31-40	55(12.9)	2.56(1.33-4.92)	0.00 ***	71 (16.78)	1.10(0.60-2.01)	0.74	56(13.17)	1.75(0.91-3.35)	0.9
41-50	24(5.64)	Reference		25(5.91)	Reference		29(6.82)	Reference	
Pregnancy status									
Pregnant	164(38.5)	0.49(0.32-0.74)	0.001 ***	147 (34.7)	0.32(0.29- 0.41)	0.001 ***	188(44.2)	0.48(0.32-0.73)	0.001 ***
Non pregnant	56(13.17)	Reference		79(18.6)	Reference		68(16)	Reference	
Stage of pregnancy									
First trimester	103(36.1)	1.86 (0.85-4.03)	0.11	13.4(4.90)	1.43(0.81-2.53)	0.20	115(40.3)	1.57(0.74-3.33)	0.23
Second trimester	46(16.14)	1.58(0.68-3.66)	0.28	47(17.7)	0.65(0.29-1.45)	0.30	55(12.9)	2.18(0.93-5.10)	0.07
Third trimester	15(5.26)	Reference		88(33.20)	Reference		18(6.31)	Reference	
Profession									
Housewives	137(32.2)	2.13(1.07-4.25)	0.03*	122 (28.8)	0.45(0.17-1.21)	0.11	159(37.4)	3.74(1.85-7.56)	0.00 ***
Teacher	27(6.3)	1.12 (0.52-2.41)	0.76	35 (8.27)	0.49(0.20-1.19)	0.11	32(7.5)	1.9(0.88-4.10)	0.87
Student	40(9.4)	0.56 (0.30-1.44)	0.29	33 (7.80)	0.58(0.21-1.54)	0.27	49(11.5)	1.06(0.48-2.31)	0.1
Others	16(3.7)	Reference		10 (2.36)	Reference		16(3.7)	Reference	

was observed in district Battagram of 8.7% (37). However, statistically, no significant (p=0.92) association was observed between infection and districts. The seropositivity was significantly (p=0.001, OR=0.48) higher 44% (188) in pregnant women as compared to non-pregnant women. The women of age group 21-30 years showed higher *T. gondii* seropositivity of 36.4% (155) and the lowest seropositivity was 3.76% (16) between the age group 12- 20 years. The seropositivity among pregnant women according to trimesters was 40.3% (115) in 1st trimester followed by 12.9% (55) in the 2nd trimester and 6.31% (33) in 3rd trimester. However, the strength of association was not significantly (p>0.05) related to age groups and trimesters. Significantly (p=0.000, χ^2=30.28) higher seropositivity of 37.4% was observed in housewives as compared to other professions.

Enzyme-linked immunosorbent assay

Anti-T. gondii *IgG ELISA*

Table II shows the results of anti-*T. gondii* IgG ELISA and their association with socio-demographic characteristics. The anti-*T. gondii* IgG level among different districts of KP indicated higher prevalence in Abbottabad district 14.1%, followed by Peshawar 10.8%, Mansehra 9.8%, Haripur 9.6% (n=41), and Battagram 7.2%, while the difference was not significant (p=0.59). Scatter plot depicting the ranges of OD values obtained among different districts (Fig. 1a). The prevalence of anti-*T. gondii* IgG was significantly (p=0.001, OR=0.49) higher 38.5% in pregnant women than non-pregnant women. The mean (±SD) values of antibodies titers were 1.59±0.26 among pregnant women (Fig. 1b). Anti-*T. gondii* IgG level varied significantly (χ^2=9.743, p=0.02) among age groups and the highest 30.5% prevalence was found in the 21–30 year group, however, the higher OD values of 1.62±0.21 were obtained among 41-50 years age group (Fig. 1c). High seroprevalence of 36.1% followed by 16.14% and 5.26 % was observed in 1st, 2nd, and 3rd trimesters of pregnancy, while the association was not significant (p=0.27) with OD values of 1.58±0.27 in 1st trimester (Fig. 1d). Significantly (p=0.000; OR=2.13) higher anti-*T. gondii* IgG prevalence of 32.2% and OD values of 1.56±0.26 were observed in housewives compared to other professions (Fig. 1e).

Anti-T. gondii *IgM ELISA*

The anti-*T. gondii* IgM ELISA results across socio-demographic characteristics of studied participants are provided in Table II. The results indicated that significantly (χ^2=9.98, p=0.045) highest 13% of anti-*T. gondii* IgM seroprevalence was obtained in the Mansehra district, followed by Abbottabad, Battagram, Peshawar, and Haripur districts. Mean (±SD) values of anti-*T. gondii* IgM antibodies titer is given in (Fig. 2a). The significantly (p=0.001, OR=2.18) higher seroprevalence of 34.7% with an absorbance value of 1.06±0.49 was observed in pregnant women as compared to non-pregnant women (Fig. 2b). Anti- *T. gondii* IgM prevalence was found 16.78 % highest among 31-40 years group, while high mean OD values of 1.01±0.50 were obtained among 21-30 years age group (Fig. 2c). The anti-*T. gondii* IgM level was found 33.20% highest in the 3rd trimester of pregnancy with OD values of 1.06±0.49 (Fig. 2d). Among housewives, the seropositivity was 29.53% with an OD of 0.97±0.49 highest compared to other professions (Fig. 2e). The results showed that the strength of association between anti-*T. gondii* IgM level and age, pregnancy stages, and profession were not significant (p>0.05).

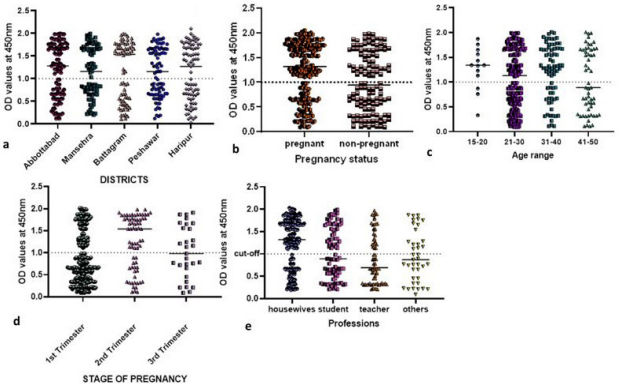

Fig. 1. Scatter plots depicting the ranges of OD values obtained for test sera by using IgG ELISA with respect to (a) study districts, (b) pregnancy status, (c) age groups (d) stages of pregnancy and (e) profession. A serum is considered positive when its absorbance value is above cut-off value OD≥1.00. Dotted line represents cut-off point.

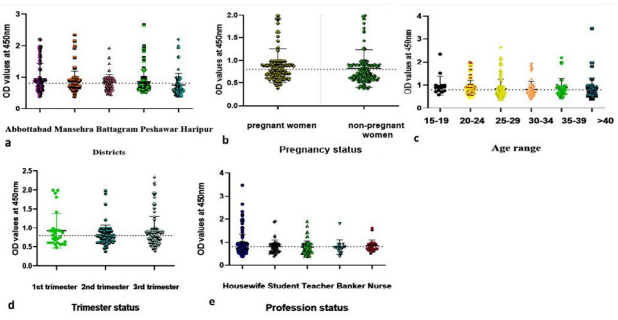

Fig. 2. Scatter plots depicting the ranges of OD values obtained for test sera by using IgM ELISA with respect to (a) study districts, (b) pregnancy status, (c) age groups (d) stages of pregnancy and (e) profession. A serum is considered positive when its absorbance value is above cut-off value OD≥0.7. Dotted line represents cut-off point.

DISCUSSION

In present study, we investigated prevalence rate of 52-60% *T. gondii* antibodies in pregnant and childbearing age women from Khyber Pakhtunkhwa, Pakistan. This prevalence is much higher to older studies that found a prevalence of 17.6% from Multan (Nazir *et al*., 2017), 20.37% in the Pothwar region (Ahmad *et al*., 2019), and 48% in Kashmir (Aleem *et al*., 2018). The prevalence rate varies inside the country; these differences could be related to the geographic condition and living standard of participants (Pozio *et al*., 2003). The geographical location might be an impression of the lifestyle of individuals that makes them more prone to the infection and climate may favor the *T. gondii* oocysts to sporulate (Gebremedhin *et al*., 2013). Studies recorded lower toxoplasmosis prevalence among people living in a cold or dry climate (Dubey, 2008). The high prevalence in KP could be related to eating half-cooked or crude meat, raising of the feline is much high, and numerous individuals have high exposure to animal excreta while cultivation and water administration offices are also not well established (Khan *et al*., 2011).

Studies reported that *T. gondii* causes moderate infections in non-pregnant ladies, while pregnant women have drastic impacts, especially on the fetus (Liu *et al*., 2015). Congenital defects, deficits in intrauterine development, and fetal death contribute to both economic and social burdens (Wam *et al*., 2016). Identification of these infections in both the mother and fetus is the most crucial component of prenatal care. Routine prenatal diagnosis of *T. gondii* is advised during the first trimester (Zhang *et al*., 2016). In current study we used most sensitive qualitative and quantitative serological assays for the early detection of toxoplasmosis.

The higher seroprevalence of *T. gondii* 60% with LAT, 52% and 56% with IgG and IgM ELISA was observed, the results were higher with previous findings reported in in the general population 29.5% by latex agglutination test from Southern Punjab (Tasawar et al., 2012) and 19.3% in pregnant women by Toxo-latex test of District Swabi (Alvi et al., 2014). However, the present results were lower than seroprevalence of *T. gondii* reported in Ghana, Ethiopia, Europe and the USA (Ayi *et al*., 2009; Agmas *et al*., 2015; Pappas *et al*., 2009). The possible reasons for variations in the prevalence could be due to differences in climatic conditions, mothers' characteristics i.e. management of cats, educational level, hygienic and feeding practices and type of serological methods used (Agmas *et al*., 2015; Abamecha and Awel, 2016).

The significantly higher seroprevalence of 34.7%-44.2% was recorded in pregnant women in the current study, consistent with a comparative study from Brazil (Barbosa *et al*., 2009). *T. gondii* infections in women of childbearing age vary among countries of the world, India 45% (Sigh and Pandit, 2004), Turkey 43-85% (Tamer *et al*., 2009), 18.8% in Spain (Gutierrez-Zufiaurre *et al*., 2004), and 14-25.7% in Sweden (Peterson *et al*., 2000). This could be attributed due to weak immunity during early pregnancy possess a high risk of getting an infection, lack of awareness about the parasite, its transmission, prevention, and control (Gebremedhin *et al*., 2013). However, lifestyle such as frequent contact with the soil, eating habits such as consumption of undercooked meat and raw vegetables could be the possible risk factors for getting an infection (Sakikawa *et al*., 2012; Majid *et al*., 2016).

The present study recorded 14.18%-36.4% seroprevalence of *T. gondii* in the 20-30 years age group with ELISA and LAT, agrees with previous investigations (Jittapalapong *et al*., 2010; Wu *et al*., 2009; Alvi *et al*., 2014; Ally and Idris, 2004). The possible reason could be that females of these age groups get married and have more active lifestyles which increase their exposure to the environment with a high density of contamination. During pregnancy might be due to the consumption of poorly washed contaminated fresh fruits and vegetables (Tamer *et al*., 2009; Abdullah and Mahmood, 2017).

According to trimesters during pregnancy 33.2%-40% seroprevalence was obtained during the 3rd and 1st trimesters, which agrees with previous studies (Wong and Remington, 1994; Ertug *et al*., 2005; Giannoulis *et al*., 2008; Abdullah and Mahmood, 2017). Similar to present study, a significantly higher prevalence of 30.5% and 30.7% was recorded in the first and third trimester's respectively (Alayande *et al*., 2013). The possible foetal complications during the first trimester due to toxoplasmosis are deformation and even death of the foetus. The present ongoing disease rate in pregnant ladies requires the need of creating antenatal care programs for toxoplasmosis.

The result of the present study predicted high seroprevalence among housewives, showing they have more contact with the parasite than the other professions. A similar finding was recorded from previous studies (Qurashi *et al*., 2001; Kadhim and Mohammed, 2013). Traditionally, housewives handle and chop meat without gloves, take care of pets, taste uncooked meat while cooking, clean vegetables and fruits, remain engaged in gardening. Oocysts can easily be carried via wind and rain in gardens and exposure to contaminated soil may cause transmission of toxoplasmosis.

In summary high seroprevalence of toxoplasmosis was recorded in pregnant women belonging to 21-30

years age groups during 1st and 3rd trimesters with LAT and ELISA. These findings indicated that toxoplasmosis is a major public health concern in all studied districts of Khyber Pakhtunkhwa, Pakistan. Further studies are required to promote awareness and health education programs to reduce the transmission risk of *T. gondii* infections.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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