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Full Length Research Paper

Toxoplasma gondii Infection Among Pregnant Women: A Seroprevalence Study

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The present work aimed to evaluate the seroprevalence of *Toxoplasma gondii* and their associated risk factors among the pregnant women. This study was carried out from 2009 to 2010 in 232 pregnant women attending the private hospitals in and around Salem, Tamil Nadu. Anti-toxoplasma specific IgG and IgM were assessed using Enzyme-linked immunosorbent assay (ELISA) test kits. Of these 232 samples, anti-toxoplasma specific immunoglobulin G (IgG) antibodies were detected in 23 (9.9%) cases while 9 (3.9%) had positive anti-toxoplasma specific immunoglobulin M (IgM) suggestive of acute infection during or just before pregnancy. A structured questionnaire interview for pregnant women was performed to investigate the risk factors associated with the *T. gondii* infection. The higher infection rate of 7.9% (for anti-toxoplasma IgG antibodies) was found with pregnant women living in rural area (15 subjects) than in urban area (8 subjects) and it was statistically significant (P<0.03). A few risk factors such as stillbirth (P<0.02), miscarriage (P<0.009), rearing cat as pet animal (P<0.005) and outdoor gardening (P<0.03) were statistically associated with the seroprevalence rate. Even with low seroprevalence rate of 9.9% latent infection and 3.9% acute infection there is a need for the pregnant women to be educated on the ways to minimize the exposure to *T. gondii* infection and to reduce the risk of congenital transmission.

Key words: *Toxoplasma gondii*, seroprevalence, pregnancy, anti-toxoplasma immunoglobulin G (IgG), immunoglobulin M (IgM).

INTRODUCTION

Toxoplasma gondii is a protozoan parasite widely distributed around the world (Liesenfeld and Janitschke., 2005; Hill et al., 2005) and it is the major opportunistic pathogen in immune-compromised hosts, which affects one third of the world population. Infection is mainly acquired by ingestion of food, water or soil contaminated with oocysts shed by the cat, or by eating under cooked meat containing oocysts and meat from animals infected with *T. gondii* (Dubey et al., 2005). Primary infection is usually subclinical, but in severely immune-compromised patients it may be life threatening (Montoya et al., 2004) and may even leads to some neurological damage like mental retardness, blindness and fetal death. The chances of fetal infection by *T. gondii* increase with the stage of pregnancy, from 5 to 15% in the first half to 60 to 80% in the second half of gestation. Conversely the chances of serious lesions and death decrease from 70 to 80% in the first half to less than 10% in the second half of gestation (Couto et al., 2003). Anti-parasitic treatment during pregnancy may reduce the risk of transmission to the fetus if it is identified early (Remington et al., 2001). Though the demonstration and isolation of *T. gondii* is confirmative it was found to be very difficult (Hung et al., 2007). Hence acute and latent infections are mostly diagnosed by serological tests including the detection of antibodies by Latex agglutination test, indirect fluorescent antibody test or the specific IgM and IgG-ELISAs (Montoya and Liesenfeld, 2004).

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Typically the organism causes only asymptomatic or mild infection in pregnant women but it can cause much more serious clinical complications to the fetus. Identification of these susceptible women is essential sothat early treatment can be offered to minimize the congenital transmission. Epidemiological studies indicate that prevalence of *T. gondii* infection in pregnant women varies substantially among countries. For instance in European countries, prevalence varies from 9 to 67% (Nash et al., 2005). In studies conducted in Sudan 34.1% and in New Zealand 33% anti-toxoplasma antibodies frequencies were found, respectively, (Elnahas et al., 2003; Morris and Croxson, 2004). A study performed at South Brazil in America, showed a higher prevalence, 74.5% and in Cuba it was 70.9% (Spalding et al., 2005; Gonzalez-Morales et al., 1995). In Asian countries, low prevalence values of *T. gondii* infection were found, that is 0.8% in Korea and 11.2% in Vietnam (Song et al., 2005; Buchy et al., 2003). In contrast, high prevalence was reported for Indian, Malaysian and Nepalese populations (Singh and Pandit, 2004; Akoijam et al., 2002; Nissapatorn et al., 2003; Rai et al., 1998). There was only little information available about the epidemiology of *T. gondii* infection in pregnant women living in South India, especially in the rural area of Tamil Nadu. Therefore the present study aimed to explore this and to analyze demographic factors, symptoms and risk factors associated to positive serology.

MATERIALS AND METHODS

In this study samples were collected from the private hospitals in and around Salem, Tamil Nadu. In this region many villages are there, most study subjects were farmers and could be taken as a model for the general population of Tamil Nadu with its socio-economic, cultural and ethnic diversity.

Study population

A descriptive cross-sectional study was conducted from 2009 to 2010. A total of 232 study subjects (pregnant women) participated in this study. They answered a structured questionnaire and signed an informed consent after they were advertised about symptoms and risks of toxoplasmosis infection. Pregnant women above 15 years of age were included. They were considered of low (normal pregnancy) or high risk (those having bad obstetric history, including repeated miscarriages, still-births and births of malformed products). Women undergoing an *in vitro* fertilization (IVF) were excluded from this study.

Ethical approval

Ethical approval for the study was obtained from the Institutional ethical committee, Vivekananda college of Pharmacy, Elayampalayam, Namakkal. (IEC/July/10/12).

Socio-demographic, clinical and behavioural data

Socio-demographic data including age, residential place, gravid status, educational level and gestation trimester were obtained from all study subjects, as well as clinical data including previous history of still-births and miscarriages, babies with eye infections or hydrocephalus; also, data about risk factors like pet cat owning, cleaning up cat excrement/sandbox, outdoor gardening, farm work and some associated symptoms like cough, headache and fever were also gathered.

Table 1. Prevalence of Toxo – IgM and Toxo – IgG.

Result	Prevalence of Toxo – IgM		Prevalence of Toxo – IgG	
	No.	%	No.	%
Positive	009	03.9	23	9.9
Negative	223	96.1	209	90.1
Total	232	100.0	232	100.0

Table 2. Prevalence of Toxo – IgM and IgG in different age groups.

Age	Total	Toxo – IgM Positive		Toxo - IgG Positive	
		No.	%	No.	%
Below 20	7	0	0.0	0	0.0
20-30	122	4	3.3	15	12.4
30-40	101	5	5.0	08	7.9
Above 40	002	0	0.0	0	0.0
Total	232	9	3.9	23	9.9

Serological detection

Sera or plasma obtained from the pregnant women were analyzed for anti-toxoplasma specific IgM and IgG antibodies by Euroimmun Kits (manufactured by Medizinische Labordiagnostika AG, Deutschland), following the instructions details in the products' insert.

Statistical analysis

Descriptive statistics of social-demographic variable and other characteristics of sampled population were computed. Percentage with 95% confidential interval (CI) was used to describe the prevalence. Odd ratio (OR) and 95% confidence interval (CI) was calculated for each association. A P-value less than 0.05 ($P < 0.05$) was calculated to be statistically significant. The statistical difference was also evaluated by applying the Chi-square test. All the Statistical analysis was done using the SPSS software package version 10 (SPSS Inc. Chicago, Illnois, USAT).

RESULTS

A total of 232 pregnant women were enrolled and screened for the presence of anti-toxoplasma IgM and IgG antibodies. The seroprevalence of *T. gondii* IgG and IgM antibodies are given in Table 1. The results revealed that 23 (9.9%) out of 232 pregnant women were positive for anti-Toxoplasma specific IgG and nine (3.9%) for IgM. All positive study subjects were in the 20 to 40 years age

group (Table 2). Various socio-demographic factors and their associated risk factors were statistically assessed and are shown in Table 3 and Table 4 separately for anti-toxoplasma IgM and IgG. Seven members from the nine anti-toxoplasma IgM pregnant women reside in rural areas and only two were from urban zones (Table 3). Similarly, from 23 positive anti-toxoplasma IgG subjects, eight belonged to urban areas, while the majority (n=15) were living in rural places (Table 4) (OR-0.38; C95%: 0.148-0.957; P<0.034). A higher seroprevalence was observed in first and second trimesters for both anti-toxoplasma IgM (9 subjects) and IgG antibodies (22 subjects) as compared to third trimester. Of 232 pregnant women enrolled, only 4 reported to have previous history of stillborns; 3 of them were positive for IgG and one for IgM antibodies (OR-9.17; C95%: P<0.027). One of the risk factor miscarriage was statistically associated with the positivity (eight IgM positive subjects and 12 IgG positive subjects - OR-3.05; 95% CI: 1.27-7.32 and P<0.0029.

The risk factors such as owning the cat, handling the cat litter, working in soil such as outdoor gardening and farm work were analyzed for the association with the toxoplasma infection. The cat was reared as pet animal by 35 women out of 232, among these 5 of them positive with anti-toxoplasma IgM and 8 subjects positively asso-ciated with anti-toxoplasma IgG and this variable was statistically significant (OR-3.60; 95% CI : 1.39-4.28 and P<0.005). But handling the cat litter does not show any association with the prevalence. Based on their occupational exposure most of the pregnant women were routinely work with the soil by doing the outdoor gardening (67 subjects) and went to farm work (113). Among the two variables analyzed, outdoor gardening was found to have significant association with the presence of anti-toxo IgG antibodies (OR – 2.50; 95% CI: 1.04 to 5.99 and P<0.034). The symptoms of the toxoplasmosis were analyzed, such as fever, cough and headache were positively associated and the symptom of cough alone is statistically significant with anti-toxoplasma IgG antibodies (OR – 8.05; (95% CI: 1.06 to 1.14 and P<0.017) and other signs were not significant for both IgG and IgM. The relevant signs and symptoms for congenital toxoplasmosis were the baby with eye infection and Hydrocephalus. Of the 232 women studied, 19 members reported that their babies suffered from eye infection and of these 3 were found tested to be recent infection and 16 had a latent infection. The specific symptom of the congenital toxoplasmosis was Hydrocephalus. Only 4 out of the 232 pregnant women delivered their previous baby with hydrocephalus and 3 of them were positive for IgG antibodies and 2 were positive with IgM antibodies.

DISCUSSION

Toxoplasmosis infection caused by the parasite *T. gondii* leads to many serious health complications. It has been estimated that one third of the world population is infected by *T. gondii* (Montoya and Liesenfeld, 2004). Primary infection may be mild and asymptomatic, but when transmitted transplacentally, can cause congenital toxoplasmosis. Congenital toxoplasmosis leads to wide range of manifestations including mild chorioretinitis to mental retardation, microcephaly, hydrocephalus and seizures. It can also cause some repeated abortions, still birth and fetal loss in infected pregnant women. During pregnancy the clinical implications of these infections are tremendously dangerous which necessitates the importance of evaluating the immunological status of the pregnant women regarding toxoplasmosis.

Traditionally, screening for toxoplasmosis has been carried out in France (Jeannel et al., 1988) and Austria (Aspock and Pollak, 1992) as a mandatory part of the prenatal care. Prenatal screening have also been carried out as pilot projects in Finland (Lappalainen et al., 1992), Norway (Stray-Pedersen and Lorentzen-Styr, 1979), some parts of Sweden (Anlfors et al., 1989) and Germany (Krausse et al., 1993) but it is not mandatory in Italy (Valeria and Francesca, 2010). Women should be encouraged to perform tests for Toxoplasmosis before and during pregnancy. This screening should be based on the detection of specific IgG and IgM antibodies for the differentiation of recent and latent infections and the positive women should be referred to the reference laboratory for further confirmation and follow up studies.In North India seroprevalence of toxoplasmosis were reported to vary from 5 to 46.7% (Dhumne et al., 2007; Akojam et al., 2002; Yasodhara et al., 2001). In the present study seroprevalence of anti-IgG and IgM anti-bodies in pregnant women are 9.9 and 3.9% res-pectively, which is lower than rates previously reported from other regions of India (Mohan et al., 2002; Singh and Pandit, 2004; Singh et al., 1994). A slightly higher prevalence of 15.33% was reported Khurana et al. (2010) in Chandigarh. In a study conducted by Munoz *et al.,* (2004) the prevalence of toxoplasmosis was low (28.6%) but acute toxoplasmosis was detected mainly by sero-conversion during pregnancy. Nine women out of 12 with an acute toxoplasma infection became sero-converted during their pregnancies and five of them had infants with congenital toxoplasmosis (vertical transmission: 41.6%). All four children born alive had no symptoms during their follow-up. The frequency of maternal-fetal transmission was near half of cases. This study states the importance of detection of toxoplasmosis. The prevalence report of a Democratic Republic of Sao Tome and Principe is very high of 75.2% (Chia-Kwung Fan et al., 2007). Similarly 75.4% in Nigeria (Onadeko et al., 1996), 60% in Yopougon (Adou-Bryn et al., 2004), 58.4% in Tunisia (Bouratbine et al., 2001) and 34.1% from pregnant women in Sudan (Elnahas et al., 2003). As stated by Montoya and Liesenfeld, (2004) the reasons for the low

prevalence may be due to the dry climate and high temperature which reduces the infectivity of the *T. gondii*

Table 3. Association of IgM anti-*Toxoplasma* antibodies and different variables.

Variables	Total (n)	Positive	Odds ratio	*P* value
Age				
<20	7	0		
20-30	121	4	-	
30-40	101	5		0.935
>40	2	0		
Rural /urban				
Rural	189	7	0.79	0.771
Urban	43	2		
Literacy				
Illiterate	102	4		
School level	78	3	1.02	0.999
Graduate	52	2		
No. of children				
None	138	5		
One	86	3	-	0.437
More than one	8	1		
Trimester				
T- I	93	4		
T- II	117	5	1.000	0.627
T- III	22	0		
Experience of miscarriage				
Yes	67	8	22.24	5.08
No	165	1		
Experience of still birth				
Yes	4	1	9.17	0.027**
No	228	8		
Pet animal as cat				
Yes	35	5	8.04	0.000***
No	197	4		
Handling of cat litre				
Yes	6	2	15.64	0.000***
No	226	7		
Outdoor gardening				
Yes	67	5	3.25	0.071
No	165	4		
Farmwork				
Yes	113	6	2.17	0.271
No	119	3		
Fever				
Yes	184	8	2.14	0.469
No	48	1		
Cough				
Yes	175	9	-	0.080
No	57	0		
Headache				
Yes	176	8	2.62	0.351
No	56	1		
Previous baby with eye infection				
Yes	19	3	6.47	0.005***
No	213	6		
Baby with Hydrocephalus				

Table 3. contd.

Yes	4	2	31.57	1.45
No	228	7		

Table 4. Association of IgG anti-*Toxoplasma* antibodies and different variables.

Variables	Total(n)	Positive	Odds ratio	*P* value
Age				
<20	7	0		
20 to 30	121	15	0.00	0.665
30 to 40	101	8		
>40	2	0		
Rural /urban				
Rural	189	15	0.38	0.034**
Urban	43	8		
Literacy				
Illiterate	102	12		
School level	78	7	-	0.685
Graduate	52	4		
No. of children				
None	138	13		
One	86	9	0.89	0.938
More than one	8	1		
Trimester				
T- I	93	14		
T- II	117	8	-	0.095
T- III	22	1		
Experience of miscarriage				
Yes	67	12	3.05	0.009***
No	165	11		
Experience of still birth				
Yes	4	3	31.20	1.11
No	228	20		
Pet animal as cat				
Yes	35	8	3.60	0.005***
No	197	15		
Handling of cat liter				
Yes	6	3	10.30	0.00***
No	226	20		
Outdoor gardening				
Yes	67	11	2.50	0.034**
No	165	12		
Farmwork				
Yes	113	11	0.96	0.929
No	119	12		
Fever				
Yes	184	20	1.83	0.34
No	48	3		
Cough				
Yes	175	22	8.05	0.017**
No	57	1		

Table 4. contd.

Headache				
Yes	176	18	1.16	0.776
No	56	5		
Previous baby with eye infection				
Yes	19	9	12.79	1.19
No	213	14		
Baby with hydrocephalus				
Yes	4	3	31.20	1.11
No	228	20		

*** Significant at 1% level, ** Significant at 5% level.

oocysts. Controversially a very low prevalence was reported from a state in Southern Brazil (0.1%) (Marah et al., 2006) and 0.6 to 0.85% in other regions of the same country (Mozzato and Soibelmann-Procianoy, 2003; Segundo et al., 2004). In the present study seropositivity was observed more in the age group of 20 to 40 years, where as it was lower for those below 20 years and above 40 years which is similar to those found with the study of Hung et al. (2007). But in few studies it was found that prevalence increases as the age increases, the reason might be increasing risk of exposure with age (Hill and Dubey, 2002; Morris and Croxson, 2004; Peterson et al., 2000).

In this study most of the pregnant women belonging to the first and second trimester showed the higher seropositivity than in the third trimester. Those women infected during the first trimester could be very harmful to the fetus because after maternal acquisition of *T. gondii* for the first time during gestation the parasite will enter the fetal circulation by the infection through the placenta. This may result in severe congenital toxoplasmosis and can result in death of the fetus *in vitro* and can also result in spontaneous abortion (Montoya and Liesenfeld, 2004). In the prevalence of Toxo-IgG (7 among 9) and IgM (15 among 23) were found to be higher in rural area than in urban people and statistical significance was found with toxo-IgG positivity (OR-0.38; 95% CI: 0.14 to 0.95 and P<0.034), but controversially no statistical significance was observed between urban and rural areas (Baril et al., 1999) and in a study with (Ades et al., 1993) higher seroprevalence was found in urban areas. The present study showed that the rate of seropositivity of *T. gondii* among women who had cats as pet animal was significantly higher (22.9%) than those without any cat in their house (7.6%). This finding is in accordance with those reported by other workers (AI-Omar et al., 1993; Muna and Nadham, 1997).The risk of developing toxoplasmosis among those who had cats was three times greater than those not living with cats and a statistical significance was found with the present study (OR-3.60; 95% CI: 1.39 to 4.28 and P<0.005) for positivity owning cat.

The lack of an association with cat was also previous in a study conducted by Kapperud et al. (1996). This is because cats excrete oocysts for 2 weeks during infection and within 5 days they become infective and can survive for more than a year. Hence through soil, surface water and cat the infection may survive; therefore it is advised to thoroughly wash all fruits and vegetables before consumption. In the current analysis, a statistical significance was observed with the seropositivity of women working in outdoor garden (OR-2.50; 95% CI: 1.04 to 5.99 and P<0.034). This may be due to that neighbourhood or feral cats that defecate in garden or sand boxes which may pose the greatest risk for *T. gondii* infection in some people regardless of whether they own a cat. In congenital toxoplasmosis, miscarriage and still birth are controversial subjects. But a study in India reports high pregnancy wastage in those positive for IgG (Mookherjee et al., 1995) while in another study 34.5% of women with recurrent pregnancy wastage tested positive toxoplasma specific IgM (Zargar et al., 1999) and it was similar to the present study (12 subjects-17.9%) with a significant association of miscarriage (OR-3.05; 95% CI: 1.27 to 7.32 and P<0.009) and also with still birth 1 subject (25%) was positive for anti-toxoplasma IgM (OR-9.17; 95% CI: 0.85 to 98.11 and P<0.027). Some reports have suggested that pregnant women diagnosed with the acute toxoplasmosis should be treated as soon as possible to reduce the risk and severity of congenital infection (Foulon et al., 1999; Gilbert et al., 2001; Gras et al., 2005). Others have argued that there is still no treatment capable of reducing vertical transmission. Neverthless, a recent meta-analysis reported that a maternal treatment started within three weeks of sero-conversion had a small effect in the reduction of vertical transmission when compared to the treatment that were started eight or more weeks after sero-conversion (Thiebaut et al., 2007).

These results pointed out the need for antenatal screening before pregnancy and treatment for those infected mother. There must be an educational programme for the general public as they are given to the pregnant women, because the health information and

health protection strategies should be relevant to the general people also. Based on the results of the present study, it is recommended and requested for the government to educate the people about the source, transmission and preventive measures of toxoplasmosis. The knowledge regarding the risk factors should first be given to the health care workers, pregnant women and women at child bearing age. All these data emphasize the need for developing a general screening programme for toxoplasmosis in India.

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