Seroepidemiological study of toxoplasmosis in southern districts of Tamil Nadu

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Abstract The current study was conducted with the objectives of estimating the seroprevalence of *Toxoplasma gondii* and associated socio-demographic, clinical and behavioural characteristics in and around Tirunelveli. Serum samples from 175 immunodeficient and 175 immunocompetent patients were collected and were analyzed by in-house enzyme linked immunosorbent assay (ELISA). A questionnaire survey was administered for all study participants to gather information on risk factors. The present study revealed that anti- *T. gondii* IgG antibodies were detected in 13.14 % which constitutes 15.43 % in immunocompromised and 10.86 % in immunocompetent patients. There was a significant difference between sex of the study population and drinking water source. Seroprevalence was increased in the male (19.12 %, $p = 0.0075$, OR 2.316, 95 % CI 1.2362–4.3405) than the female and with river water consumption other than bore water (12.5 %, $p = 0.0483$, OR 0.857, 95 % CI 0.1853–3.4641). No significant relations were observed between anti-*Toxoplasma* IgG antibodies and age, residence, diet and animal contact in the study population. Toxoplasmosis will remain a problem, mainly in risk groups such as pregnant women and immunocompromised patients. Improvement can only be attained by increasing prevention and reducing the risk factors.

Keywords Seroprevalence · *Toxoplasma gondii* · Risk factors · Enzyme-linked Immunosorbent assay (ELISA) · Tamil Nadu

Introduction

Although toxoplasmosis is a cosmopolitan infection, the disease appears to be overshadowed in the tropics by other endemic diseases such as malaria and HIV (Fan et al. 2001). *Toxoplasma gondii* is a widespread protozoan parasite whose definitive host is the cat. Human infection mainly occurs via close contact with the soil or accidental ingestion of contaminated water or food by *Toxoplasma* oocysts excreted in cat faeces (Fayer et al, 2004). Upon acquiring *T. gondii* infection, immunocompetent adults and children are usually asymptomatic or have spontaneously resolved symptoms such as fever, malaise, and lymphadenopathy indicating thus, a symptomless latent infection (Montoya and Liesenfeld 2004).

Among immunocompromised individuals, e.g. people with acquired immunodeficiency syndrome (AIDS), *T. gondii* causes severe encephalitis via the acute infection or reactivation of latent infection if their CD4 + T cell count decreases below 200 cells/ μ l. (Hung et al. 2007; Innes 2010) Detection and monitoring of anti-*Toxoplasma* antibodies are of a great interest in HIV-infected patients. However, the toxoplasmosis serologic status and risk factors of infection among HIV-infected patients remain unknown. Therefore, this study aimed to determine the seroprevalence of anti-*T. gondii* IgG in HIV infected individuals and to identify the possible risk factors associated with toxoplasmosis.

Moreover, for pregnant women, newly acquired *T. gondii* infection can be transmitted to the fetus causing

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mental retardation, blindness, epilepsy, and death. Although congenital toxoplasmosis may be asymptomatic at birth, ocular problems can manifest later in life (Petersen 2007). There is scarce information about the epidemiology of *T. gondii* infection in pregnant women living in southern districts of Tamil Nadu. Therefore, we performed a cross-sectional study in order to determine the prevalence of *T. gondii* infection in pregnant women and identify the seroprevalence in different age, gender, residence and related risk factors including source of drinking water, obstetrical history, frequency and type of meat, vegetables, fruits and milk consumption, cooking preferences, owning cat, history of cleaning cat litter box or feeding raw meat scraps, history of blood transfusion, organ transplant etc. This provides valuable information about epidemiology of the infection and may be useful for implementing preventive measures in the community.

Materials and methods

Study population

After obtaining Institutional Ethical Committees approval and informed consent from the patients, a total of 350 peripheral blood samples for this study from 175 immunodeficient (HIV and malignant patients) and 175 immunocompetent patients (pregnant women, ocular chorioretinitis cases and patients with lymphadenopathy) were collected for the period of two years in and around Tirunelveli district of Tamil Nadu those admitted at Tirunelveli Medical College Hospital.

Socio-demographic, clinical and behavioural data

The history of illness and patients information and socio-demographic data like epidemiological risk, antenatal risk, HIV risk and clinical risk assessment factors were collected in the specially designed data sheet for this purpose. Socio-demographic data including age, education, occupation, parity, residency and related risk factors including source of drinking water, obstetrical history, frequency and type of meat, vegetables, fruits and milk consumption, cooking preferences, owning cat, history of cleaning cat litter box or feeding raw meat scraps, history of blood transfusion, organ transplant etc., were collected for further analysis.

Ethical aspects

This study was approved by the Institutional Ethical Committee. The purpose and procedures of the study were explained to all participants and an informed consent was obtained from all of them.

Laboratory tests

Sera of 350 were analyzed by in-house enzyme linked immunosorbent assay (ELISA) in which tachyzoite soluble antigen (TSA) derived from solubilized whole organisms was used as per Sucilathangam et al. (2013).

Statistical analysis

The odds ratio (OR) and 95 % confidence interval (CI) range was calculated for each risk factors of different category of case control study using David Hutchon's odds ratio calculating software.

The Chi squared test was calculated for the analytic assessment by SPSS 11.5 version software and the *p* value was calculated using Vassar Stat software. The differences were considered to be statistically significant when the *p* value obtained was less than 0.05 ($p < 0.05$).

Results

Out of 350 patients tested by in-house IgG ELISA, 46 patients (13.14 %) had antibodies for toxoplasmosis. Among the immunocompetent group of 175 patients, 19 patients (10.86 %) had antibodies to toxoplasmosis whereas, in immunodeficient group of 175 patients, 27 patients (15.43 %) had antibodies for toxoplasmosis. There was no statistical difference between the immunocompetent and immunodeficient group.

Various socio demographic risk factors like age, gender, residence, water source, diet and animal contact, associated with the seroprevalence of toxoplasmosis in and Tirunelveli is presented in Table 1. The statistical analysis was worked out by Odds ratio (OR) with 95 % Confidence interval (CI) range and the *p* value was arrived by Chi squared test for significance is also depicted.

The seroprevalence of toxoplasmosis according to age and sex of the study group is depicted in the Table 2. Among the different age groups (<20–80 years) studied, there was significant difference between 21 and 30 years group than the other ($p = 0.02$, OR 3.46, 95 % CI 1.08–11). In this age group, there was 26.32 and 9.33 % of seroprevalence in male and females respectively.

There was a significant difference between sex of the study population and drinking water source. Seroprevalence was increased in the male (19.12 %, $p = 0.0075$, OR 2.316, 95 % CI 1.2362–4.3405) than the female and with river water consumption other than bore water (12.5 %, $p = 0.0483$, OR 0.857, 95 % CI 0.1853–3.4641). No significant relations were observed between anti-*Toxoplasma* IgG antibodies and age, residence, diet and animal contact in the study population.

Table 1 Analysis of socio demographic risk factors associated with seroprevalence of toxoplasmosis

Sl. no.	Risk factors	No. of patients tested (<i>n</i> = 350)	No. of patients positive for IgG	Percentage (%)	Odds ratio	95 % CI	<i>p</i> value (χ^2 test)#
1	Age						
	a. <20 years	19	3	15.79	1.255	0.3512–4.4901	0.7255
	b. >20 years	331	43	12.99			
2	Gender						
	a. Male	136	26	19.12	2.316	1.2362–4.3405	0.0075*
	b. Female	214	20	9.35			
3	Residence						
	a. Rural	278	39	14.02	1.515	0.6476–3.5450	0.3351
	b. Urban	72	7	9.72			
4	Water source						
	a. River water	336	42	12.5	0.857	0.1853–3.4641	0.0483*
	b. Bore water	14	2	14.29			
5	Diet						
	a. Vegetarian	2	1	50.0	6.73	0.4138–109.569	0.1218
	b. Non-vegetarian	348	45	12.93			
6	Animal contact						
	a. Yes	40	8	20.0	1.789	0.768–4.1695	0.1726
	b. No	310	38	12.26			

Results of χ^2 test and Fisher's Exact test by *p* value of < 0.05 as significant difference*

Table 2 Seroprevalence of toxoplasmosis according to age and sex

Sl. no.	Age	No. of patients		No. of patients positive for IgG		Percentage (%) of positivity		Odds ratio	95 % CI	<i>p</i> value (χ^2 test)#
		Male	Female	Male	Female	Male	Female			
1	<20 years	6	7	2	1	33.34	14.29	3	0.19–45.2	0.41
2	21–30 years	19	150	5	14	26.32	9.33	3.46	1.08–11.0	0.02*
3	31–40 years	54	38	10	4	18.19	10.52	1.93	0.55–6.69	0.29
4	41–50 years	29	6	4	1	13.79	16.67	0.8	0.07–8.75	0.85
5	51–60 years	14	8	3	–	21.43	–	5.67	0.47–6.73	0.15
6	61–70 years	12	3	2	–	16.67	–	3.84	0.01–1.40	0.44
7	71–80 years	2	2	–	–	–	–	–	–	–
	Total	136	214	26	20	19.12	9.34	2.31	1.2–4.3	0.007

Results of χ^2 test and Fisher's Exact test by *p* value of < 0.05 as significant difference*

Seroprevalence of toxoplasmosis in different regions in Southern Districts of Tamil Nadu is presented in Table 3. Out of 208 patients belonging to rural area and 56 cases of urban area of Tirunelveli District tested, 33 (15.71 %) and 9 (10.71 %) were had anti-*Toxoplasma* antibodies, respectively. There was a significant difference between the urban and rural areas of Tuticorin District with respect to the seroprevalence of toxoplasmosis, at *p* = 0.0161, OR 1.09, 95 % CI 0.116–10.26. Out of 65 patients belonging to rural area and 12 cases of urban area of Tuticorin District tested, 5 (7.69 %) and 1 (8.33 %) were had anti-*Toxoplasma* antibodies, respectively. Among the 5 patients

belonging to rural area of Kanyakumari District tested, only 1 (20 %) had anti-*Toxoplasma* antibodies.

The various risk factors like sexual behaviour, vertical transmission, blood transfusion, I/V drug abuser, Needle injury/surgery and different stages of HIV infection and their influence in toxoplasmosis seropositive group is depicted in Table 4. Male HIV patients with multiple heterosexual behaviour were observed to be having higher seropositivity for toxoplasmosis (25.58 %) than single sexual behaviour. Among the different stages of HIV infections, seroprevalence of toxoplasmosis was found in 22.91 % of males and 9.09 % of females in Stage III group.

Table 3 Seroprevalence of toxoplasmosis in different regions in Southern Districts of Tamil Nadu

Sl. no.	Region	No. of patients tested	No. of patients positive for IgG	Percentage (%)	Odds ratio	95 % CI	<i>p</i> value (χ^2 test)#
1	Tirunelveli						
	a. Urban	56	6	10.71	0.636	0.252–1.6048	0.3349
	b. Rural	208	33	15.87			
2	Tuticorin						
	a. Urban	12	1	8.33	1.09	0.116–10.26	0.0161*
	b. Rural	65	5	7.69			
3	Kanyakumari						
	a. Urban	4	0	0	0.165	0.003–8.5371	0.3248
	b. Rural	5	1	20			
	Total	350	46	13.14			

Results of χ^2 test and Fisher's Exact test by *p* value of < 0.05 as significant difference *

Table 4 Risk factors in HIV patients with seropositive and seronegative group of toxoplasmosis

Sl. no.	Risk factor	No. of patients tested		No. of patients positive for IgG		Percentage (%)	
		Male	Female	Male	Female	Male	Female
1	Sexual behaviour						
	a. Single	54	55	8	3	14.81	5.45
	b. Multiple	43	2	11	1	25.58	50.0
2	Vertical transmission	2	4	1	–	50.0	–
3	Blood transfusion	4	–	–	–	–	–
4	I/V drug abuser	1	–	–	–	–	–
5	Needle injury/surgery	4	6	–	–	–	–
6	Stages of HIV infection						
	1. Stage I	35	42	7	3	20.0	7.14
	2. Stage II	10	1	2	–	10.0	–
	3. Stage III	48	15	11	1	22.91	9.09
	4. Stage IV	6	3	–	–	0	–

The seroprevalence of toxoplasmosis in pregnant women in and around Tirunelveli based on gravid, trimester of pregnancy and bad obstetric cases is presented in Table 5. Among the 135 pregnant women tested, 13 (9.63 %) were seropositive for toxoplasmosis. Out of 52 primi tested, 5 (9.62 %), 56 Gravida-2 (G2) tested 4 (7.14 %), 21 Gravida-3 (G3) tested 2 (9.52 %) and 3 Gravida-3 (G4) tested 2 (66.67 %) were seropositive for toxoplasmosis. Seroprevalence of toxoplasmosis during I, II and III Trimester of pregnancy was noticed in 9.1, 13.6 and 7.5 % cases respectively. Out of 19 cases with bad obstetric history (like abortion/premature delivery), only 3 (15.79 %) were seropositive for toxoplasmosis.

Discussion

In the present study, the overall seroprevalence of toxoplasmosis in and around Tirunelveli District of

Tamil Nadu was found to be 13.14 % based on IgG ELISA in contrast with lower results obtained in a study conducted in Chandigarh, in which they have reported the overall prevalence of 5.4 and 4.66 %, IgM and IgG anti-*Toxoplasma* antibodies respectively (Mohan et al. 2002).

On the other hand, there are studies which have shown a higher prevalence than results in our study. A high prevalence (75–77 %) of *T. gondii* was reported from North India (Singh 2002). A study carried out in Jodhpur on seroprevalence of toxoplasmosis reported that *T. gondii* specific IgG and IgM antibodies assayed by ELISA were observed in 17.2 % cases (Joshi et al. 1998). Another study performed by ELISA on the seroprevalence of *T. gondii* in Karnataka in 1,000 healthy adult population of voluntary blood donors, and reported the overall prevalence of 20.3 % were positive for *T. gondii* IgG antibody, of which, 63 % had high and 7 % low avidity and 3.6 % IgM positive (Sundar et al. 2007).

Table 5 Seroprevalence of toxoplasmosis in pregnant women

a. Based on gravid				
Sl. no.	Gravida	No. of patients tested	No. of patients positive for IgG	Percentage (%)
1	Primi	52	5	9.62
2	G ₂	56	4	7.14
3	G ₃	21	2	9.52
4	G ₄	3	2	66.67
5	G ₅	3	0	0
	Total	135	13	9.63
b. Based on trimester of pregnancy				
Sl. no.	Trimester of pregnancy	No. of patients tested	No. of patients positive for IgG	Percentage (%)
1	I Trimester	11	1	9.1
2	II Trimester	44	6	13.6
3	III Trimester	80	6	7.5
	Total	135	13	9.63
c. Based on bad obstetric history (Abortion/premature delivery)				
Sl. no.	Trimester of pregnancy	No. of patients with bad obstetric history (Abortion/PMD)	No. of patients positive for IgG	Percentage (%)
1	I Trimester	3	1	33.34
2	II Trimester	8	–	–
3	III Trimester	8	2	25.0
	Total	19	3	15.79

In this study, there was a significant difference between sex of the study population and drinking water source. Seroprevalence was increased in the male than the female and with river water consumption other than bore water. There was significant difference between 21 and 30 years group than the other. About 15 % of patients belonging to rural area of Tirunelveli District had anti-*Toxoplasma* antibodies.

As in the study of Bobic et al. (1998) and Sema et al. (2005) that have found that prevalence increases as the age increases, as the age effect was observed in the current study. The reason might be increasing risk of exposure with age.

In the current study, no statistical meaningful difference was observed between urban and rural areas for toxoplasmosis seroprevalence in Tirunelveli District. Baril et al. (1999) have found similar results with the current study; however, Ades et al. (1993) have found higher seroprevalence of toxoplasmosis in urban areas.

Sema et al. (2005) also reported higher prevalence of toxoplasmosis in municipal network water users which was well correlated with the current study. Frequent consumption and type of meat in non-vegetarian group were identified as the principle risk factor in several recent studies of *T. gondii* infections in humans (Bobic et al. 1998 and Sema et al. 2005). No relation was observed between

seroprevalence of vegetarian and non-vegetarian in the current study and most of the study population (99.5 %) was belonging to non-vegetarian.

The association of cats and human toxoplasmosis was difficult to assess by epidemiological surveys because soil, not the cats, is the main culprit. In the current study, the number of cat owners was just a few and no relation was detected which was similar to the findings of Sema et al. (2005).

In the present study, the seroprevalence of toxoplasmosis in HIV patients was found to be 15 % and male HIV patients with multiple heterosexual behaviour were observed to be having higher seropositivity for toxoplasmosis (25.58 %) than single sexual behaviour. Several explanations for the interaction between both infections resulting in high *Toxoplasma* infection in HIV patients have been previously proposed (Lin and Bowman 1992). One possibility is an increase in risky behaviour in *Toxoplasma* infected individuals that leads to increased exposure to HIV infection. Such a change in behaviour could be due to parasite-driven personality changes, as described in *Toxoplasma*-infected individuals by others (Fleger et al. 1996). Another plausible explanation is that *Toxoplasma* infection is a marker of exposure to risky social contacts or habits, which correlates with early HIV infection.

HIV Patients with IgG antibody to *Toxoplasma* should receive prophylaxis for toxoplasmosis with trimethoprim/

sulfamethoxazole. Those who are seronegative should be counselled about ways to minimize the risk of primary infection including avoiding the consumption of undercooked meat and careful hand washing after contact with soil or changing the cat litter box.

In this study, 13 (9.63 %) were seropositive for *T. gondii* in 135 pregnant women. Only 3 out of 19 (15.79 %) cases with bad obstetric history (BOH) were positive for toxoplasmosis. Though, *Toxoplasma* infection does not cause repeated foetal losses, this is the most common indication for investigation of toxoplasmosis in India. There are some reports on the high prevalence rate of anti-*Toxoplasma* antibody in pregnant women with bad obstetric history. A study from Hyderabad, South India reported seroprevalence of toxoplasmosis was 33 % (IgG) in lower socio-economic group compared to higher socio-economic group (22 %) in 236 women with bad obstetric history (Yashodhara et al. 2004).

High prevalence rate of anti-*Toxoplasma* antibody in pregnant women have been reported in recent studies. A study among primigravid women attending a secondary level hospital in a district of North India using IgG ELISA revealed 41.75 % were seropositive for *T. gondii* infection (Akoijam et al. 2002). A prospective study (Singh and Pandit 2004) on incidence and prevalence of toxoplasmosis in Indian pregnant women indicated that the overall IgG seroprevalence rate of toxoplasmosis was 45 % by using IgG Avidity ELISA test. The prevalence of toxoplasmosis in pregnant women from Calcutta was reported as 7.1 % whereas; it was 45 % in pregnant women from New Delhi by using IgG Avidity ELISA test (Mohan et al. 2002).

In conclusion, the present findings showed a high seroprevalence of anti-*T. gondii* antibodies in HIV infected patients which necessitate active preventive programs in these high risk groups to control the burden of active and chronic infection in the community.

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