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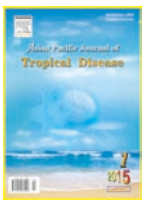

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Seroprevalence of *Toxoplasma* among HIV infected and HIV non-infected individuals in North India

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ABSTRACT

Objective: To determine the seroprevalence of *Toxoplasma* infection in the HIV infected and HIV non-infected individuals in our region, including antenatal women.
Methods: Five mL of blood sample was collected from a total of 1 181 individuals aged 12 years and above. These included 661 (55.9%) from HIV positive patients and 520 (44.1%) from HIV negative individuals. A total of 238 samples out of the 520 HIV negative patients were collected from the antenatal women. Demographic profile of the subjects was recorded. Immunoglobulin G ELISA was performed for all the samples, while only the samples received from antenatal women were tested by immunoglobulin M capture ELISA.
Results: Seroprevalence among HIV infected and non-infected was found to be 21.3% (95% confidence interval = 18.4%-24.6%) and 14.2% (95% confidence interval = 11.5%-17.5%), respectively. The difference was statistically significant (P = 0.003). No significant gender differences were found. Seroprevalence increased from 9.1% to 30% with increasing age in the HIV infected patients. Only 2 (0.84%) samples of antenatal women were positive for immunoglobulin M capture ELSIA, while one sample was equivocally reactive.
Conclusions: Seroprevalence of latent toxoplasmosis in our region is moderately high, particularly in the HIV infected patients, exposing them to the risk of reactivation. This suggests that serologic testing of all HIV infected patients is essential to initiate *Toxoplasma* prophylaxis. Similarly, screening for active *Toxoplasma gondii* infection during antenatal care and preventive education is essential to prevent and minimize congenital toxoplasmosis.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a protozoan parasite, infecting most of the warm-blooded animals, including humans who are the intermediate host. Members of cat family Felidae are the definitive hosts. *T. gondii* infection is acquired by humans by accidental ingestion of oocysts in water, food or soil contaminated with cat feces, or by eating raw or undercooked meat containing cysts. Studies conducted worldwide indicate that toxoplasmosis is one of the most common infections of human beings[1].

Infection in immunocompetent individuals is generally asymptomatic and self-limited. However, it acquires clinical significance in immunocompromised patients, antenatal women and children born to mothers with active *Toxoplasma* infection. Immunocompromised patients are likely to develop *Toxoplasma* encephalitis, myocarditis or pneumonitis due to reactivation of the latent infection. *Toxoplasma*-HIV co-infected patients have a risk as high as 30% to 40% of developing *Toxoplasma* encephalitis, especially those with significant immunosuppression (CD4 cell count < 200 cells/μL)[2,3]. Thus, identification of latently infected immunocompromised patients by determining anti-*Toxoplasma* IgG (immunoglobulin G) antibodies becomes essential.

The severity and incidence of congenital infection depends on the duration of pregnancy at the time when acute infection is acquired. Infection acquired earlier during the pregnancy can lead to severe malformations in fetus such as the classical triad of retinochoroiditis, cerebral calcification and convulsions, and still birth in some cases. Detection of IgM (immunoglobulin M)

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and low avidity IgG antibodies in antenatal women can indicate active infection and thus direct management to avert transmission of infection to the fetus is necessary.

Despite several worldwide studies, the geographical variability mandates surveillance studies to estimate the regional seroprevalence. Very few studies have been conducted in India to compare seroprevalence in HIV positive and HIV negative individuals. Thus, this study was designed not only to describe the seroprevalence of toxoplasmosis in HIV infected and non-infected individuals, but also across various age groups which would help in better understanding the disease epidemiology and improved outcome control.

2. Materials and Methods

A cross sectional observational study was conducted over a period of three years from 1st January 2011 to 31st December 2013 in the Department of Microbiology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India. This is a centrally located at 1 676 bedded tertiary care hospital with thickly populated catchment areas. Most of the patients fall in the low to middle socio-economic groups.

Informed consent was taken from the patients or the guardians (if the subject was a minor) prior to inclusion into the study. Five mL blood sample was collected in plain vial from a total of 1 181 individuals (12 years old and above), presenting to various departments of the hospital. These included 661 (55.9%) samples from HIV positive patients and 520 (44.1%) samples from HIV negative individuals. Two hundred and thirty eight samples of the 520 HIV negative patients were collected from the antenatal women. The blood samples were allowed to clot and centrifuged at 3 000 r/min for 10 min to separate the sera, which were then stored at -20 ℃ till further testing.

All the samples from both HIV positive and HIV negative individuals were tested for anti-*Toxoplasma* IgG antibodies by ELISA (Euroimmun, Medizinische Labordiagnostika AG) as per manufacturer's instructions. Values < 8 IU/mL were taken as negative and ⩾ 11 IU/mL were considered positive. Intermediate values were interpreted as equivocal reaction and repeat samples were requested. Out of 520 samples from HIV negative individuals, 238 samples from the antenatal women were tested for anti-*Toxoplasma* IgM antibodies by IgM capture ELISA (Dia. Pro, Italy), strictly according to the manufacturer's instructions. The results were interpreted qualitatively as positive, negative or equivocal based on the O.D. of the reaction obtained.

2.1. Statistical analysis

Data were entered and analyzed using SPSS version 20 statistical package. Results were presented in terms of median, range and percentages as appropriate. 95% confidence intervals (*CI*) were calculated using SE of proportions. Differences in proportions were evaluated by Pearson's *Chi*-square test. In cases where more than 20% of the expected values were less than 5, Fisher's exact test was used instead of the *Chi*-square test. Age-wise analysis was done using the One-way ANOVA test. Statistical test result was considered significant whenever *P* value was < 0.05.

3. Results

3.1. Study population and demographic characteristics

A sample load of 1 181 samples, including 661 samples from HIV positive patients and 520 from HIV negative individuals above 12 years of age, was received over a period of 3 years. In the HIV positive group, male:female ratio was 1.38, while for the HIV negative group it was 0.72. Serum samples were also received from 3 trans-gender HIV positive patients. Out of the 520 HIV negative individuals, 238 were antenatal women, whose serum samples were collected as a part of routine TORCH screening. Median age was 31 years old (range = 12 to 85 years old) for HIV positive group, and 33 years (range = 14 to 61 years old) for the HIV negative group. In both groups, maximum numbers of samples were received from individuals between 19 and 40 years old. Majority of the patients were Hindus (69.1%), followed by Muslims (28.8%), Sikhs and Christians (1% each approximately).

3.2. Seroprevalence of Toxoplasma infection

The distribution of *Toxoplasma* IgG seropositivity among HIV positive and HIV negative serum samples is shown in Table 1. Overall, 141 (21.3%, 95% *CI* = 18.4%-24.6%) samples out of 661 HIV positive samples tested reactive for anti-*Toxoplasma* IgG antibodies. Prevalence among male and female HIV infected individuals was very similar to each other (22.3% and 20.2%, respectively), without any statistically significant difference (*P* = 0.5). There was a gradual and regular increase in positivity rate from 9.1% to 30.0% as the age of the individuals increased (Figure 1). Eight samples (1.2%) were tested equivocal by the ELSIA with antibody levels ranging between 8 and 11 IU/mL.

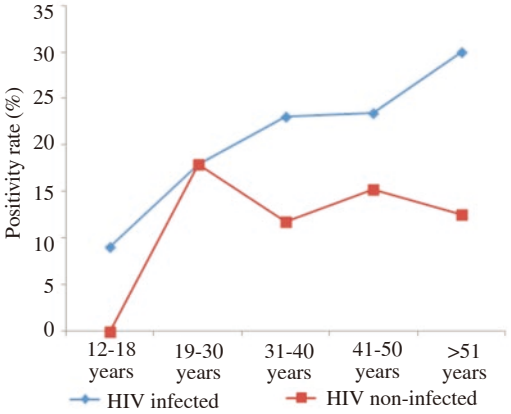

Figure 1. Age-wise distribution (%) of IgG seroprevalence in HIV infected and non-infected.

Table 1

Seroprevalence of IgG antibodies against *Toxoplasma* in HIV positive and HIV negative individuals [n (%)].

Samples		HIV positive				HIV negative			
		Total number	Positive	Positive percent (95% *CI*)	Equivocal	Total number	Positive	Positive percent (95% *CI*)	Equivocal
Male		381 (57.6)	85 (22.3)	18.4–26.7	6 (1.6)	218 (41.9)	28 (12.8)	9.0–18.0	8 (3.7)
Female		277 (41.9)	56 (20.2)	15.9–25.4	2 (0.7)	302 (58.1)	46 (15.2)	11.6–19.7	10 (3.3)
Trans-gender		3 (0.5)	0 (0.0)	-	0 (0.0)	0	0	0	
Age (years)	12-18	11 (1.7)	1 (9.1)	0.0–39.9	0 (0.0)	11 (2.1)	0 (0)	0.0–30.0	1 (9.1)
	19-30	274 (41.5)	49 (17.9)	13.8–22.9	5 (1.8)	162 (31.2)	29 (17.9)	12.7–24.6	6 (3.7)
	31-40	190 (28.7)	44 (23.1)	17.7–29.7	3 (1.6)	227 (43.7)	27 (11.8)	8.3–16.8	7 (3.1)
	41-50	136 (20.6)	32 (23.5)	17.1–31.4	0 (0.0)	112 (21.5)	17 (15.2)	9.6–23.1	2 (1.8)
	> 51	50 (7.6)	15 (30.0)	19.0–43.9	0 (0.0)	8 (1.5)	1 (12.5)	0.1–39.2	2 (25.0)
Total		661 (100.0)	141 (21.3)	18.4–24.6	8 (1.2)	520 (100.0)	74 (14.2)	11.5–17.5	18 (3.5)

Seventy four (14.2%, 95% *CI* = 11.5%-17.5%) of 520 HIV negative samples were positive for IgG antibodies. Seropositivity was not influenced by sex of the individual as it was again similar for both males and females (12.8% and 15.2%, respectively, $P = 0.52$) (Table 1). IgG seroprevalence was similar with all the age groups, ranging from 11.8% to 17.9%, except for the 12 to 18 years age group in which no sample was reactive (Figure 1). A total of 3.5% (18 out of 520) samples showed equivocal reactivity. There was statistically significant difference between anti-*Toxoplasma* IgG seroprevalence in HIV positive and HIV negative groups ($P = 0.003$) as determined by two-tailed Pearson's *Chi*-square test.

Only the serum samples received from antenatal women ($n = 238$) were tested for IgM antibodies to *Toxoplasma*. Out of them, only 2 (0.84%) samples were positive for IgM capture ELSIA, while one sample was equivocally reactive.

4. Discussion

Toxoplasmosis, a disease caused by *T. gondii* has varied presentations depending upon the immune status of the individual and duration of pregnancy at the time of active infection. In the present study, *Toxoplasma* specific IgG and IgM antibody levels were analyzed using ELISA which is one of the standard methods for detection of anti-*Toxoplasma* antibodies[4]. Simultaneous detection of both IgG and IgM help to determine the chronological status of such exposure.

Being a centrally located tertiary care centre with associated anti-retroviral treatment centre and ante-natal clinic, we received a large sample load of 661 HIV positive patients and 520 HIV negative individuals (including 238 antenatal women). The sex ratio was in favour of males in the HIV positive group, while in the HIV negative group, the females outnumbered the males owing to large number of antenatal women. In both the groups, most of the samples were received from individuals between 19 years to 50 years, representing the sexually active age group who are more likely to be HIV infected individuals or antenatal women.

In the present study, overall IgG seroprevalence of *Toxoplasma* was 18.2 % (215 out of 1 181 samples). Serological studies conducted in humans indicate that the seroprevalence of latent *Toxoplasma* infection in highly variable. Studies conducted within the same country can show variable results based on the temporal variation and population studied. Worldwide, the lowest seroprevalence (about 1%) was found in some countries in the Far East and the highest (> 90%) in some parts of European and South American countries[5]. In India, owing to great geographical and cultural differences, great variation has been reported by authors from different states. Mittal *et al.* have revealed an overall seropositivity of only 1% from Delhi[6]. On the other hand, one of the highest *Toxoplasma* seroprevalence in India (57%) has been reported in the villagers of Kumaon region of Uttar Pradesh[7]. Dhumne *et al*. reported a national *Toxoplasma* IgG seroprevalence of 24.3%, with higher rates in the South India as compared to North India[8].

In the present study, the prevalence of latent (IgG) *Toxoplasma* infection in HIV negative individuals was 14.2% (95% *CI* = 18.4%-24.6%), while in the HIV positive group, it was much higher at 21.3% (95% *CI* = 17.5%-25.7%). This difference was statistically significant as may be expected because of immunocompromised status of HIV positive patients ($P = 0.003$). Unfortunately, very few Indian authors have compared the seroprevalence in HIV infected and HIV non-infected. A study done in Mumbai, Indian compared the two groups and found the seroprevalence in HIV positive to be more than double of that in HIV negative (67.8% *vs.* 30.9%)[9]. Significantly higher prevalence in HIV positive group has also been reported by international studies from Nigeria[10], Mali[11] and Ethopia[12]. However, few studies have denied no significant differences in the latent *Toxoplasma* seroprevalence between HIV infected and HIV non-infected individuals. In a recent study from Nigeria, Ogoina *et al.* reported 32.4% in HIV negative healthy adults and 38.7% in HIV-infected adults ($P > 0.05$)[13].

Though not statistically significant by ANOVA, age-wise analysis of IgG *Toxoplasma* seroprevalence in the HIV positive revealed a gradual and regular increase from 9.1% to 30% as the age of the study subjects increased. This can be explained as increasing age increases the possibility of exposure at some point of time in life, and once a person becomes seropositive for IgG against *Toxoplasma*, he is likely to remain latently infected and

IgG positive for long. The trend was however, not regular in case of the HIV negative individuals, probably because they did not truly represent the general population, but only those with certain indications such as antenatal women and other symptomatic patients. Similar trend of increasing seroprevalence with increase in age has also been demonstrated in few other studies[14,15].

In case of antenatal women (n = 238), the IgG prevalence was 16.8%. In this group, IgM capture ELISA was also performed as it indicates a recent or active infection, and only women with recent or active infection are at risk of transmitting it to their children. Thus, early and prompt identification of such cases is essential and should be managed appropriately and with urgency. In the present study, only two women tested positive out of 238 (0.8%). One woman tested equivocal who was later tested to be negative on repeat testing. Previous study from India has reported a much higher IgG and IgM seroprevalence among pregnant women of 45% and 3.3%, respectively. This may be due to the fact that they used multiple tests like direct agglutination test, IgG and IgM-ELISA and IgM-immunosorbent agglutination assay[16]. While another study from India reported 22.4% prevalence of anti-*Toxoplasma* IgG antibodies among women of child-bearing age[17].

In conclusion, it can be said that the seroprevalence of latent toxoplasmosis in our region is moderately high, particularly in the HIV infected patients, exposing them to the risk of reactivation. This suggests that serologic testing and cautious follow up of all HIV infected patients is essential to initiate chemoprophylaxis against *Toxoplasma* when the CD4 + T cell count falls. Likewise, screening of *T. gondii* infections during antenatal care and preventive education should be considered to prevent and minimize congenital toxoplasmosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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