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Trends of seropositivity and clinical spectra of Toxoplasmosis- A two-year observational study from a tertiary care centre in North India

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Abstract

Background: Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is highly prevalent in man and other warm-blooded animals. Most of the affected adults do not manifest any serious illness, but it can cause mental retardation and blindness in congenitally infected children and serious illness in immunocompromised individuals. We aimed to study the disease burden and clinical manifestations of Toxoplasma seropositive patients in the hilly region of North India.

Methods: This is a hospital based retrospective cross-sectional study conducted between January-2018 and December-2019.Sera of suspected cases of toxoplasmosis were examined for presence of anti-Toxoplasma antibodies (IgG and IgM) by enzyme-linked immunosorbent assay. Antibody

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index of >1.1 was interpreted as positive. Clinical data were retrieved from medical records maintained in the department.

Result: Out of 174 included cases, 70 (40.22%) cases showed positivity to both IgG and IgM antibodies. Most of the suspected cases were sent from the Pediatrics department followed by Departments of Obstetrics and Gynecology, Ophthalmology and General Medicine. Majority of the patients (45.7%) presented with mild non-specific symptoms like fever, malaise and generalized weakness and lymphadenopathy in 25.7%. Ocular and neurological manifestations were observed in 21.4% and 20% respectively. Other rare presentations were hepatic, cutaneous, pulmonary and endocrine diseases. More than one clinical feature could be found in one patient. Co-infection with HIV and Tuberculosis was found in 8.6% and 5.7% respectively. 20% cases were associated with congenital anomalies; while 18.6% cases were detected in routine anti-natal screening.

Conclusion: Toxoplasmosis is highly prevalent in this hilly region showing wide variety of clinical manifestations. Therefore, prevention and control policies mainly targeting the anti-natal cases are the need of the hour.

Keywords: *Toxoplasma gondii,* seroprevalence, Clinical manifestation, Toxoplasmosis, North India

Introduction: Toxoplasmosis is a disease caused by *Toxoplasma gondii (T. gondii)*, an intracellular protozoan parasite. It affects nearly one-third of the world population [1]. In India, sero-prevalence was recorded to be as high as 24.3% [2]. However, it varies from place to place depending upon climatic conditions of the place. In North India, toxoplasmosis seroprevalence ranged from 4.7% to 51.8% and in South India it was found to be 37.3% [2-6]. The diagnosis of toxoplasmosis is by serological screening i.e., detecting anti-*T. gondii* antibodies IgM & IgG. Presence of IgM indicates recent infection and IgG indicates past exposure.

Toxoplasmosis is a zoonotic disease and ingestion of *T. gondii* oocyst present on raw and unwashed vegetables or raw or undercooked meat causes human infection [7,8]. Infection with *Toxoplasma gondii* is usually asymptomatic in immuno-competent persons, whereas it can have serious consequences in person with compromised immunity. This disease has baffled the medical fraternity due to its ability to cause a variety of symptoms ranging from mild lymphadenopathy to fatal neurological complications. There can be acute infection or reactivation of past infection which can lead to severe lethal diseases involving cerebral, pulmonary or disseminated toxoplasmosis [9-11]. Particularly in pregnancy, primary infection or reactivation under condition of compromised immune system, cause congenital toxoplasmosis with serious fetal outcome like hydrocephalus, retinitis pigmentosa or even death in-utero [12,13]. Prevention of this infection is an important aspect to reduce its disease burden. The diagnosis of toxoplasmosis relies on detection of Toxoplasma specific IgM and IgG. The seroprevalence of this disease varies according to geographical location, clinical presentation, age, gender and immune status of individual [14,15].

Toxoplasmosis in antenatal cases shown by maternal seroconversion incidence rate is around0.2%-1% in many countries [16]. Mortality due to toxoplasmosis is 10-30% of patients with human immunodeficiency virus infection in USA and 20-50% in Europe [17]. In transplant recipients, the prevalence is up to 50% [18,19]. There are also literature showing increase in neurological disorders related to Toxoplasmosis [20]. So, from all these factors, it is important to know the spectra of clinical presentationand burden of toxoplasmosis in a particular geographic region; so that appropriate measures can be taken for its prevention and control. It is to be noted that currently, there is no health program in India directing screening of toxoplasmosis.

The aim of this study was to study the disease burden and trend of sero-positivity of Toxoplasmosis in the state of Uttarakhand and its spectrum of clinical presentations.

Material & Methods

Study setting: The study was conducted at All India Institute of Medical Sciences, Rishikesh, a major referral centre which is located in the foothills of Himalayas. We retrospectively reviewed the medical records of suspected cases of Toxoplasmosis, of whom sera were examined for presence of anti-Toxoplasma antibodies, from January 2018 to December 2019. The study was approved by Institutional Ethics Committee of our institute (AIIMS/IEC/20/835 **Date:** 12/12/2020)

Inclusion criteria: Patients with clinical suspicion of toxoplasmosis and those undergoing routine screening were included in the study. Clinical presentations and demographic details like age, gender and location of patients who had undergone testing for the presence of anti-toxoplasmosis antibodies IgM & IgG were extracted from medical records. Individual case histories were recorded to illustrate important and/or unusual presentations that may accompany seropositive patients.

Exclusion criteria: The samples that were inadequate, unlabelled or damaged were not tested and were excluded from the study. Serum samples showing equivocal results were excluded from the study. We also excluded the cases where medical data were unavailable.

Laboratory procedure: Serological tests were performed within 48 hours of receipt or if delayed stored at -20^0C. The samples and testing reagents were brought to room temperature before the tests were performed. Anti-*T. gondii* IgM and IgG Enzyme-linked immunosorbent assay (ELISA) (CALBIOTECH Toxoplasma IgM and IgG ELISA)was performed on all the samples according to the kit literature. The kit had 95.8% sensitivity and 99.3 % specificity with 98% agreement. Antibody index (AI) of >1.1 was interpreted as detectible Toxoplasma IgM and IgG. equivocal results (AI of 0.9-1.1 for both IgM and IgG)

Results:

General characteristics of study population: The study conducted in a tertiary care hospital, Rishikesh collected data of 204 patients who were clinically suspected of toxoplasmosis or underwent ante-natal screening from January 2018 to December 2019. Analysis of *T. gondii*

serology (IgM/IgG ELISA) was done for the patients. A total of 174 patients with complete data examined for anti-Toxoplasma IgM/IgG in serology laboratory were finally enrolled in the study. 97(55.74%) were from male and 77 (44.25%) were from female patients. 102 (58.6%) patients were from Uttarakhand, 58 (33.3%) from Uttar Pradesh and 14 (8.05%) from other states (one each from Maharashtra, Rajasthan, Madhya Pradesh and Kerala). [Fig No.1]. Maximum patients of suspected toxoplasmosis were from the age group of 0-10years (49.4%), followed by 21-30years 45 (25.9%) and 31-40 years 22 (12.6%) respectively [Table No.1]. Different wards from which patients were advised for testing for toxoplasmosis shown in [Fig no. 2]. Maximum samples received for toxoplasma serology were in month of April, November and December same trend was seen in both year i.e., 2018 and 2019.

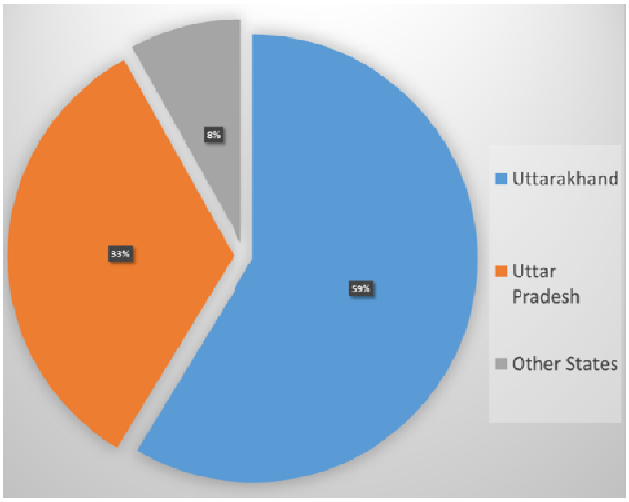

Fig No.1 Distribution of patients from different states

Table No.1 Gender-wise distribution of patients among different age group

Age group (in years)	Female n (%)	Male n (%)	Total n (%)
0-10	21 (12.0)	65 (37.4)	86 (49.4)
11-20	3 (1.7)	5 (2.9)	8 (4.6)
21-30	38 (21.8)	7 (4.1)	45 (25.9)
31-40	8 (4.6)	14 (8.0)	22 (12.6)
41-50	3 (1.7)	1 (0.6)	4 (2.3)
51-60	2 (1.15)	2 (1.15)	4 (2.3)
>60	4 (2.3)	1 (0.6)	5 (2.9)
Total	79 (45.4)	95 (54.6)	174 (100.0)

Data expressed as n, (%)

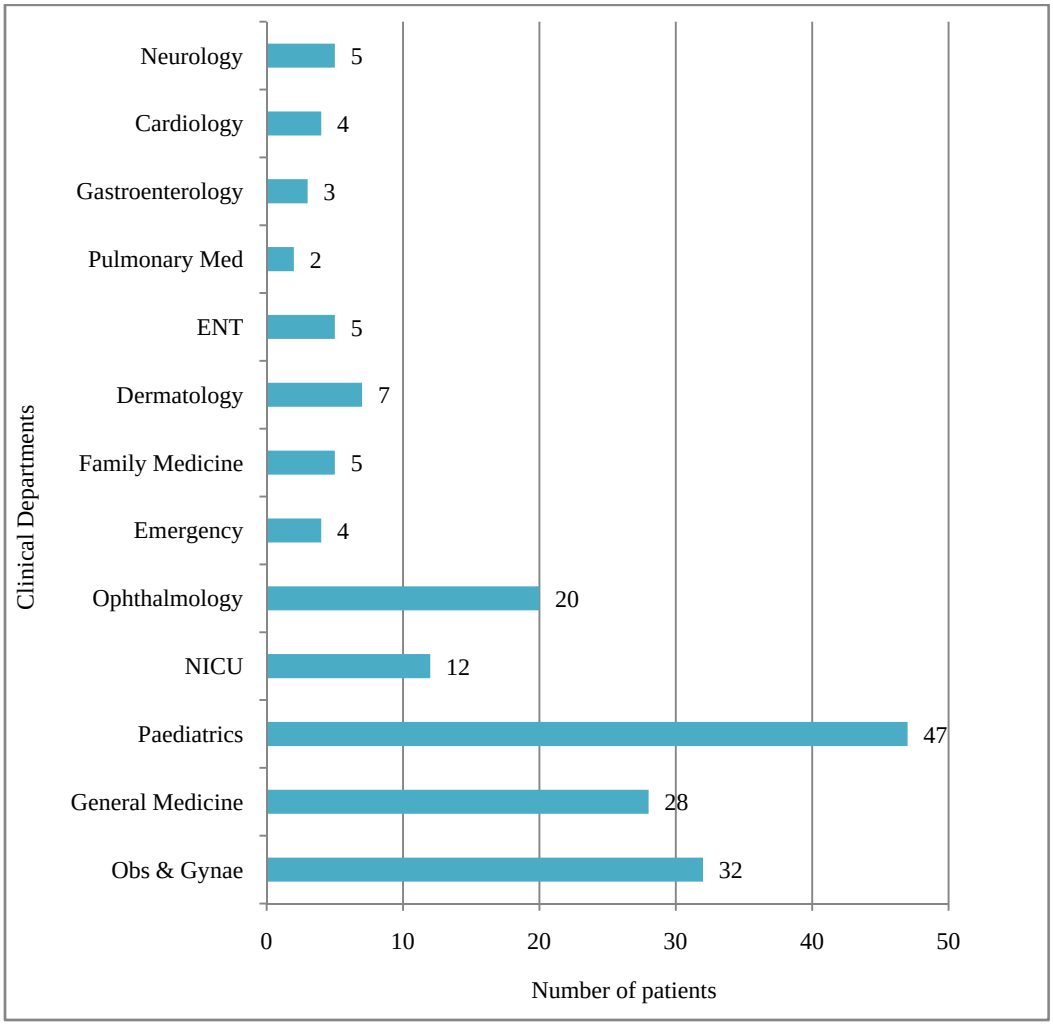

Fig No.2 Distribution of patients from different wards

Serological findings: A total seropositivity was 70 (40.22%), IgM positivity is 9 (5.17%) (Table no 2), IgG positivity is 67(38.5%) and in 6 (3.4%) both IgM & IgG is positive. Mean serum IgM value was 2.38 IU/ml and it ranged from 1.1IU/ml to 3.8 IU/ml and Mean serum IgG level was 6.41 IU/ml with a range of 1.1 IU/ml to 9.27 IU/ml. Of 70 seropositive cases, 53/70 (67.14%) were from Uttarakhand, 21/53(39.6%) were from hilly areas and 32/53(60.3%) from plain areas of Uttarakhand. For the 67 IgG positive cases most of the samples were from paediatric age group i.e. 0-10years, 31/67 (46.26%), followed by 21-30years, 14/67 (20.89%).

Table No. 2 Toxoplasma seropositivity in the patients

	Number of positives, n	%
IgM Positive	9	5.2
IgG Positive	67	38.5
Both Positive	6	3.4
Total positive	70	40.2

Spectrum of clinical presentation: Table No-3 tabulates the different clinical presentations by the *Toxoplasma* seropositive patients. Commonest clinical presentations were nonspecific symptoms like fever, headache and generalized weakness (45.7%), followed by ocular manifestations (30%), and lymphadenopathy (25.7%). Altogether 25.7% showed neurological manifestations including inflammation of brain/meninges in 10% and other neurological disorders like focal neurological signs (Giddiness, tingling and numbness in the limbs), seizures, neuro-psychiatric signs in 15.7%. Involuntary movement like chorea was exhibited by 8.6%. Skin manifestations like erythematous nodules, macules or itchy papular eruptions, hepatic diseases, panhypopituitarism were some of the rarer manifestations. Co-infection with HIV infection was noted in 8.6% patients who mainly comprised of neurological and ocular manifestations. Congenital anomalies were observed in (20%) cases of congenital toxoplasmosis that included blindness, mental retardation and microcephaly.13(%) cases were found in routine screening of pregnant women of whom 11 were asymptomatic and 2 had symptoms (cervical non-tender lymphadenopathy and mild fever with malaise).

Table-3 Clinical manifestations and outcomes of the *Toxoplasma gondii* seropositive cases

Clinical Observations*	**Number of patients**	**Percentage †**
1. Clinical presentations*		
Asymptomatic	7	10
Mild non-specific symptoms (Fever, headache, malaise, myalgia)	32	45.7
Lymphadenopathy	18	25.7
Ocular symptoms (Uveitis / Choreo-retinitis/decreased vision)	15	21.4
Meningoencephalitis / Encephalitis/Meningitis	7	10
Focal-neurological symptoms, chorea, neuro-psychiatric manifestations/personality changes, altered sensorium	7	10
Hepatitis/Acute on chronic liver failure	4	5.7

Respiratory symptoms(acute cough/haemoptysis/dyspnea)	2	2.9
Cutaneous manifestations	4	5.7
Hypopituitarism	1	1.4
2. Co-infection		
As an opportunistic infection with HIV	6	8.6
Co-infection with Pulmonary Tuberculosis	4	5.7
Acute bacterial infection like sepsis	1	1.4
3. Congenital syphilis		
Congenital anomalies	14	20
Asymptomatic/mild symptoms	3	4.3
4. Routine Screening		
Ante-natal cases	13	18.6

*More than one abnormality could be found in one patient

† Percentage is calculated out of the total seropositive cases (both IgG and IgM)

Discussion

The most common clinical presentations of toxoplasmosis in our study were nonspecific symptoms like fever, generalized weakness malaise etc. thus baffling the clinicians as PUO (pyrexia of unknown origin); which is in conformity with other studies conducted elsewhere [21,22]. Lymphadenopathy, which was the second most clinical finding of our study, is another common presentation of toxoplasmosis [1,15]. But, it was usually associated with other symptoms and organ/system involvement. Although a vast majority of seropositive cases of toxoplasmosis are asymptomatic (80-90%) due to latent infection [1,22], but in our study, 10% cases were asymptomatic. This may be because it was a hospital-based study, and majority of the cases were clinically ill.

Cerebral and orbital toxoplasmosis are the most serious forms with usually high disability or fatal outcome [13,14,23,24]. It is usually associated with immunocompromised conditions like HIV infection but can be observed in immunocompetent persons too, as was observed in our study [11,23,24]. The prevalence of ocular and neurological manifestation in our study was found to be high as it is a hospital-based study and being a tertiary care center, patients with serious manifestations usually visit this hospital.

Toxoplasmosis is a common opportunistic infection in HIV-infected adults [10,11,17]. Serious complications like Toxoplasma encephalitis can be life threatening inspite of the availability of

highly active anti-retroviral therapy (ART), especially in resource-scant countries like India [10,11,21]. It often results from the reactivation of endogenous infections in patients who are infected with HIV. In our study we observed toxoplasma seropositivity in 8.6 % of cases with HIV. Their clinical presentation were neurological manifestations like vomiting, focal neurological signs, altered sensorium, seizures, and involuntary movements like chorea; and ocular manifestations like chorioretinitis, decreased vision, uveitis. Interestingly, we also observed a co-infection of toxoplasmosis with Tuberculosis. Although there is no evidence of any significance of its association, the decrease immunity and other factors leading to reactivation of dormant tuberculosis, may be responsible for reactivation of latent *T. gondii* infection.

Transplacental infection occurs when an uninfected mother acquires infection during pregnancy which may have consequences like abortion, intra-uterine growth restriction, jaundice, hepatosplenomegaly or even intra-uterine death [6,8,25]. Congenital toxoplasmosis may also result in neurological or ocular manifestations like intracranial calcifications, hydrocephalus or retinochoroiditis [12,13,18,25].We observed congenital anomalies in 20% cases that included blindness or decreased vision, mental retardation and microcephaly. Also, a vast majority of requisition for test had come from Paediatrics department (47/204, 23.1%) with high degree of suspect for congenital toxoplasmosis. Therefore, routine antenatal screening for *T. gondii* infection is of paramount importance. In some countries like Austria and France, it is compulsory by law to test all pregnant women for *T. gondii* which has resulted in dramatic reduction of congenital toxoplasmosis [26]. The overall seroprevalence of toxoplasmosis in Indian women of reproductive age amounted to 22.4% [27]; while our study recorded 18.6% seroprevalence in pregnant women. Preventive therapy of seropositive cases during ante-natal period can lead to reduction in prevalence of congenital toxoplasmosis.

The study had certain limitations. Being a retrospective study, we could not follow up the cases prospectively to observe clinical outcomes after treatment or secondary prophylaxis. Following up the seropositive cases would have helped immensely in knowing the clinical and serological outcome of different regimen and thereby help in formulation of health policies.

Conclusion

T. gondii is a coccidian parasite and most widely prevalent in warm-blooded animals including humans. Toxoplasma seropositivity is present in a substantial proportion of people from Uttarakhand. Although serious involvement of eyes, nervous system, liver, lungs were observed in our study, milder presentations like fever, malaise and lymphadenopathy dominated most of the sero-positive cases. Infection during pregnancy was also common (mostly asymptomatic) and it lead to congenital toxoplasmosis, which led to mild to severe congenital abnormalities.

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Competing interests: The author(s) declare that they have no competing interests.

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