A multicentric study on Clinical, microbiological and psychological aspects of human toxoplasmosis in pregnant women of South-East regions of India

[1]Dr Smrutilata Sahoo, [2]Dr. Rajnikant Shukla, [3]Dr. Snehalata Mallick,

[4]Dr. SwayankaSamantray, [5]Dr. Ashrumochan Sahoo, [6] Dr Roshni Dandapat,

[1]Assistant Professor, Department of Microbiology, KIMS, BBSR

[2]Associate Professor, Department of Psychiatry, SJ, MCH, PURI

[3]Assistant Professor, Department of OBGY, PRMMCH, Baripada.

[4]Senior Resident, Department of Psychiatry, KIMS, BBSR

[5]Assistant Professor, Department of Psychiatry, PRMMCH, Baripada.

[6]3rd year PG student, Department of Microbiology, KIMS, BBSR

Correspondent Author:

Dr.Ashrumochan Sahoo,
Assistant Professor,
Department of Psychiatry,
PRMMCH, aripada.

Abstract

Introduction: Serological tests based on the detection of specific IgG and IgM antibodies are the primary diagnostic methods for Toxoplasma infection. Prompt diagnosis of maternal seroconversion during pregnancy is necessary to prevent transmission to the foetus, by means of specific treatment, and also to permit close monitoring of at-risk foetuses and neonates.

Material and Methods: This prospective multicentric study was conducted to evaluate the seroprevalence of T. gondii in humans and to determine various risk factors and psychiatry comorbidity associated with the disease. During the study, samples were collected from randomly selected pregnant women at various centres.Sera were sent to laboratory for antibody detection by ELISA. Proper history was recorded and psychiatry evaluation was done.

Results: Most of patients belonged to 31-40 years (28.3%) followed by 21-30 years (21.6%), 41-50 years (18.3%) and least were belongs to 15-20 years (15%). Most of the patients showedFever, Lymphadenopathy, Abdominal pain as presenting symptoms. In our study, women reported higher scores of somatization, depression and phobic anxiety.Eating of raw

or undercooked meat and Inappropriately washed vegetables were identified as two important risk factors for maternal toxoplasmosis.

Conclusion: Although most immunocompetent individuals infected with toxoplasmosis remain asymptomatic throughout life, this parasite can cause many complications in immunocompromised patients and in pregnant women. Newborns presenting with congenital toxoplasmosis can be asymptomatic but can also develop retinochoroiditis and serious CNS complications . So, if women conceive, they should be educated regarding their eating habits and the value of good hygiene at the first visit for antenatal care. Health education for women of childbearing age is extremely important to prevent maternal toxoplasmosis.

Keywords: Human toxoplasmosis, ELISA, Lymphadenopathy, Retinochoroiditis

Introduction

Toxoplasma gondii is an obligate intracellular parasite that parasitizes members of the cat family as definitive hosts and has a wide range of intermediate hosts like man, goat, sheep, pig, cattle etc. Infection is common in many warm-blooded animals, including humans. In most cases infection is asymptomatic, but devastating disease can occur.The seroprevalence of *Toxoplasma gondii* ranges widely in different populations of different areas of the world. One-third of the human population is assumed to be infected with *T. gondii* [1,2]. Few studies have been done on human population in Australia and New Zealand showing prevalence of upto 50%. Extensive epidemiological studies have been conducted in China and India revealing high prevalent rates in human populations.

Although toxoplasmosis is typically benign and asymptomatic, it induces major complications in immunocompromised individuals and during pregnancy. In immunocompetent individuals, clinical symptoms of toxoplasmosis present as mild self-limiting illness presenting with fever, malaise and lymphadenopathy [3]. However, infection is usually more severe in immunocompromised people and in pregnant women. In these groups, the infection is accompanied by more complications, such as encephalitis, retinochoroiditis, foetal abortion, splenomegaly and pneumonitis[4].Prevention of maternal primary infection constitutes the major tool for avoiding congenital *T. gondii* infections and toxoplasmosis complications. The preventive measures depend on the women's knowledge about toxoplasmosis [5,6].

Disease in humans caused by *T. gondii* was first recognized in the late 1930s.Improved diagnostic techniques continue to enable seroepidemiological studies in humans as well as a broad range of animal species which provides evidence for a wide distribution and high prevalence of *T. gondii* in many areas of the world [5,6,7]. Diagnosis of toxoplasmosis depend on direct microscopy, serological tests(antibody or antigen detection) and molecular method(PCR). Serological diagnosis entails detection of specific anti-*Toxoplasma* immunoglobulins, i.e., IgM, IgG, or IgA through application of immunology-based techniques, e.g., enzyme-linked immunosorbent assays (ELISA), Sabin-Feldman dye test, immunofluorescent assay (IFA), or modified agglutination test (MAT) [8,9,10].

Molecular diagnosis entails the detection of whole or fractions of parasite, e.g., nucleic acids or proteins by polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) and hybridisation[11].

Material and Methods

This prospective and multicentric study was conducted between October 2020 and January 2022 in different tertiary care hospitals of southern-east region of India. The study was approved by the Institutional Review Board and by the administrations of the healthcare institutions participating in the study. All data were anonymously collected and safely stored. The aim of the study was to evaluate the seroprevalence of T. gondii in humans and to analyse various clinical and psychiatric manifestations associated with the disease. During the study, samples were collected from randomly selected pregnant women at various centres.

The inclusion criteria were the following: not having chronic diseases (e.g., diabetes or cancer), and not being subjected to hormonal therapy or psychiatric medication. Three to five ml of blood was drawn from a superficial vein of upper limb (fore arm) with the help of disposable syringe. It was then transferred into sterile screw capped tube slowly and carefully to avoid haemolysis. It was allowed to clot then centrifuged at 3000 rpm for 15 minutes. Serum was separated and transferred with the help of micropipette to another sterile vial and stored at -200°C until processed for further analysis.

Serological tests

All the sera were sent to the laboratory for detection of antibody against Toxoplasma by IgG ELISA and by IgM ELISA method (ENZY-WELL DIESSE Diagnostics Senese Spa ITALY kit). Diagnosis of acute infection with *T.gondii* can be established by simultaneous detection of IgG and IgM antibodies to *Toxoplasma* in serum. The test was performed according to the manufacturer's instructions. If the adsorbance of the sample is higher than that of the Cut-off, the sample is taken positive for the presence of specific IgM. The ratio between the O.D. value of the sample and Cut-off is calculated. If the ratio is >1.2, the result is positive and if less it is taken negative.

To measure symptoms of general psychopathology, we used the Spanish version of the Symptom Checklist-90-Revised (SCL-90-R). Previous 30 Adaptive Human Behavior and Physiology (2021) 7:28–42 studies have shown a moderate-to-high validity and reliability of the Spanish version in Mexican samples (Cronbach's $\alpha = 0.80$, Cruz Fuentes et al. 2005; Lara et al. 2015). SCL-90-R is a 90-item self-report symptom inventory that assesses psychological distress (e.g., headaches, trouble remembering things, trembling, suddenly scared for no reason, feeling blue, trouble falling asleep, etc.) in relation to nine primary symptom dimensions and three summary scores (termed 'global scores'), using a five-point Likert-type scale (1 = not at all; 5 = extremely). In this research, we used the nine primary dimensions labeled as Somatization ($\alpha = 0.81$), Obsessive-Compulsive ($\alpha = 0.80$),

Interpersonal Sensitivity (α = 0.83), Depression (α = 0.79), Anxiety (α = 0.81), Hostility (α = 0.74), Phobic Anxiety (α = 0.79), Paranoid Ideation (α = 0.80), and Psychoticism (α = 0.81).

Results

Total 120 number of samples were processed. Among them 80 samples(66.6%) gave positive result and 40 samples(33.4%) gave negative result.

Table 1: Distribution of Age group

Age group Years	**Frequency**	**Percentage**
15-20	18	15
21-30	26	21.6
31-40	34	28.3
41-50	22	18.3
51-60	20	16.6
Total	120	100

In table 1, most of patients were belong to 31-40 years (28.3%) followed by 21-30 years (21.6%), 41-50 years (18.3%) and least were belongs to 15-20 years (15%).

Table 2: Distribution of Symptoms

Symptoms	**Frequency**	**Percentage**
Fever	53	88.3
Lymphadenopathy	51	85.0
Headache	46	76.6
Myalgia	28	46.6
Anorexia	31	51.6
Sore throat	11	18.3
Arthralgia	43	71.6
Confusion	12	20.0
Nausea/ vomiting	48	80.0
Abdominal pain	52	86.6

Most of the patients showedFever, Lymphadenopathy, Abdominal pain as presenting symptoms.

Table 3: Results of ELISA tests (IgG, IgM) for toxoplasmosis in 120 persons in accordance with the observed symptoms.

	Presenting suggestive symptoms of toxoplasmosis		**Presenting unspecific symptoms**	
Serological Profile	**Frequency**	**Percentage**	**Frequency**	**Percentage**
IgG + / IgM +	**58**	**54.7**	**6**	**42.8**
IgG + / IgM -	**26**	**24.5**	**4**	**28.5**
IgG - / IgM -	**22**	**20.7**	**4**	**28.5**
Total	**106**	**100**	**14**	**100**

Table 4: Relationship between different variables and results of anti-Toxoplasma IgM Ab, and IgG Ab

Duration of pregnancy (month)	**IgG**		**IgM**	
	Positive	**Negative**	**Positive**	**Negative**
1	4	2	6	2
2	6	4	6	2
3	8	2	6	2
4	4	2	4	4
5	6	2	4	2
6	4	4	6	2
7	4	2	6	2
8	2	2	4	4

Table 5: Distribution of Psychopathological symptoms among pregnant women

	Mean±SD
Somatization	1.03±0.34
Obsessive-Compulsive	1.64±0.34
Interpersonal Sensitivity	1.12±0.73
Depression	1.31±0.83
Anxiety	1.13±0.64
Hostility	0.83±0.34
Phobic Anxiety	0.84±0.21
Paranoid Ideation	0.94±0.31
Psychoticism	0.79±0.36

Table 6: Distribution of Trimester among pregnant women

Trimester	Frequency	Percentage
First	41	34.16
Second	59	49.16
Third	20	16.66
Total	120	100

Table 7: Risk factors and their relationship with toxoplasmosis

Risk factors	Frequency	Percentage
Cats	2	1.66
Dogs	3	2.5
Flies	7	5.83
Cockroaches	11	9.16
Rats	13	10.83
Lack of water treatment	19	15.83
Lack of sewage treatment	21	17.5
Geophagy	17	14.16
Soil handling	23	19.16
Eating of raw or undercooked meat	43	35.83
Inappropriately washed vegetables	41	34.16
Raw or underdone eggs	33	27.5
Unpasteurized goat's milk	9	7.5

Discussion

In our study, we got seroprevalence for *T. gondii* about 66.6%. The first nationwide representative seroprevalence study for *T. gondii* antibodies was conducted in Germany, they found a high seroprevalence (59%) compared to other countries. Similarly, in France, where comprehensive serosurveys reported a higher overall seropositivity in women of childbearing age (36.7%; n = 15,130; age 15–49), it decreased from 54.2% in 1995 to 43.8% in 2003 and to 36.7% in 2010. In The Netherlands in 2006–2007, seroprevalence was significantly lower than in our study (26%; n = 5,541; age 0–79), decreasing from 41% in 1995/1996. In the USA, seroprevalence declined from 14.1%; in 1988–1994 to 9.0% (n = 10,477; age 12–49) in 1999–2004 [12].

The high seropositivity rate was also found in Eastern region of African countries. In Madagascar (Lelong et al. 1995) seropositivity rate was highest followed by Ethiopia (Gebremedhin et al. 2013), Zambia (Watts et al. 1984), Zimbabwe (Watts et al. 1984), 85, 81.4, 54, and 18.7 %, respectively. The seroprevalence of *Toxoplasma gondii* in southern part of Africa is one of the lowest reported rates worldwide. The rate among pregnant women is

similar to the rate in general population, ranging from 15 to 23 % (Capretti et al. 2014; Zumla et al. 1991; Nabias et al. 1998; Lindstrom et al. 2006) with an overall seropositivity of 20 % (Lindstrom et al. 2006)[12,13].

The majority of the immunocompetent subjects will remain asymptomatic lifelong, but both competent and immunocompromised subjects can develop the disease, especially retinochoroiditis. Infected individuals may present with asymptomatic cervical lymphadenopathy during the acute systemic infection; as well as signs and symptoms that mimic mononucleosis infection, like myalgia, sore throat, fever, maculopapular rash and, infrequently, polymyositis and myocarditis.[12] Newborns presenting with congenital toxoplasmosis can be asymptomatic but can also develop retinochoroiditis and/ or CNS involvement. Ocular toxoplasmosis (OT) generally causes characteristic looking retinal lesions that are focal and white, which are usually smaller than 1,000 microns in size, with a vigorous vitreous inflammatory reaction resulting in a typical 'headlight in the fog' appearance. These lesions are both due to direct parasitic tissue invasion and the ensuing immune response directed against the parasite;[12]

Detection of prior infection can be documented by serology, with changes in serology used to infer acute infection. The IgG response generally appears within 7 to 14 days of infection, has a peak within 30 to 60 days and usually persists lifelong.IgM appears prior to IgG after primary infection but typically do not persist, and if present, indicate acute infection; but confirmatory tests should be executed, because its specificity is not always satisfactory. By testing the avidity for *T. gondii* IgG, one can discriminate whether the infection was acquired recently or in the past,as in a small percentage of the cases *T. gondii* IgM can remain positive for up to 24 months.

Other methods that may also be used to assess the disease are the histology of infected tissues, polymerase chain reaction (PCR) of body fluids (like cerebrospinal fluid), or culture of the parasite. For prenatal diagnosis, PCR should be done to detect parasite DNA on amniotic fluid.PCR of aqueous humor and/ or vitreous can be an important tool to diagnose OT with atypical presentation in AIDS patients.[14,15,16]

The most characteristic psychiatric symptom associated with *Toxoplasma*-seropositivity was increased anxiety. Typical toxoplasmosis is usually associated with autism, schizophrenia, attention deficit hyperactivity disorder, obsessive compulsive disorder, antisocial personality disorder, learning disabilities, and anxiety disorder etc. The association of toxoplasmosis with schizophrenia has been confirmed by many studies and a similar association has been documented for obsessive compulsive disorder and anxiety disorder. The association between toxoplasmosis and autism has been suggested on the basis of three case-control studies and also on various indirect evidence [17,18,19].

The influence of *T. gondii* on the development of psychiatric disorders is most probably mediated both by an immune reaction of the brain to *T. gondii* and by the biochemical activity of the parasite itself. Interferon-gamma secreted in response to toxoplasmosis maintains this infection in a latent form because it induces astrocytes to synthetize

indoleamine-2, 3-dioxygenase (IDO), the enzyme responsible for tryptophan degradation *via* the kynurenine metabolic pathway . It results in both a lack of tryptophan, an amino acid essential for *T. gondii* replication, and increased levels of the final products of kynurenine pathway. Tryptophan is degraded by IDO into kynurenine which is either metabolized to kynurenic acid, an antagonist of the glutamate NMDA (N -methyl- D - aspartate) receptor or hydroxylated into quinolinate, a potent NMDA neurotoxic agent . These metabolites exert both neurotoxic (quinolinate) and pro-psychotic (kynurenic acid) effects and can also influence the neurotransmitter balance [20].

Toxoplasma gondii seropositivity among pregnant African women was found to be strongly associated with eating undercooked or raw meat (adjusted OR 5.73, 95 % CI 1.35–24.39; $p = 0.02$) (Walle et al. 2013). This finding is in consistent with findings from other African and Arab countries (Cook et al. 2000; Jones et al. 2009). Similar results were obtained from Sudan where authors concluded that, eating raw meat, undercooked meat, and living in the Southern region are predictors of seropositivity of *T. gondii* infections in pregnancy (Elnahas et al. 2003). Daily consumption of raw meat in many part of Africa is very common. This may explain the high seropositivity rate seen in many parts of Africa.

Many studies have found that food animals are potentials source of infection. In Saudi Arabia, sheep and goats as potentials source of infections were examined for IgG antibodies.A recent study, showing high prevalence of infection in dairy goats, suggests a potential risk of the transmission route of toxoplasma via unpasteurized milk (Zhao et al. 2012). This high seropositivity in animals observed in both Arab and African countries may be potential sources of infection for pregnant women by either consumption of raw or undercooked meat or unpasteurized milk from these animals. These results are consistent with studies from other countries showing meat as a source of infection in China, Norway, and the United States (Li and Wu 2002; Jones et al. 2009; Cook et al. 2000)[21,22,23]

Consumption of unwashed raw vegetables or fruits was reported as a significant factor for *T. gondii* infection. There many infectious oocysts shed from cats in vegetables which are capable of producing infection to human. Contact with animals and in particular cats, or cleaning the cat litter boxwas found to be significantly associated with seropositivity for toxoplasmosis (Fakhfakh et al. 2013). In one study in Ethiopia it was found that living with domestic cats increase the rate infection by *T. gondii* by fivefold (OR 5.82, 95 % CI 1.61–20.99; $p < 0.05$) (Zemene et al. 2012). Cats can infect other domestic animals leading to an increased chance of infectivity and creating another potential source of infection. The risk of contracting toxoplasma is higher when there is frequent exposure to feline feces or neglect of preventive measures like washing hands.

Living in a rural area and high chance of developing *T. gondii* in pregnancy is also consistent with global literature by Pappas et al. (2009). High prevalence in rural areas is an accepted finding in settings where there is poor sanitary facilities, contact with soil or animals, and drinking of unpasteurized or unboiled water and milk[21,22,23]

Conclusions

The observed clinical, microbiological, and psychological evidence indicates that the protozoan *T. gondii* is a major pathogen responsible for the outbreak in the South-East regions of India. The transmission of the infection possibly occurred through the ingestion of food containing the parasite's oocyst, direct contact with dirty hands after gardening activities or even the inhalation of oocysts suspended in the air. A history of raw meat intake was also identified to be a risk factor related to toxoplasmosis. Therefore, to lower the risk of toxoplasmosis, pregnant women should refrain from eating raw and undercooked meat and maintain personal hygiene. Health education for women of childbearing age is extremely important to prevent maternal toxoplasmosis. If women conceive, they should be educated regarding their eating habits and the value of good hygiene at the first visit for antenatal care. Therefore, counseling pregnant women about the risk factors for toxoplasmosis can reduce the risk of congenital toxoplasmosis.

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