

Seroprevalence and Clinical Correlates of Toxoplasma Gondii Infection among Patients in Tertiary Care Hospital

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Abstract: Background: Toxoplasmosis is one of the most common parasitic infections seen in humans. Approximately one third of the population is exposed to this parasite. Infection in immunocompetent adult human is usually asymptomatic, while it is dangerous for immunocompromised patients. Toxoplasmosis is a common opportunistic infection among immunocompromised patients. Disease in immunocompromised patients is usually due to reactivation of latent infection but can result from acute infection as well. Toxoplasmosis in these persons may lead to lethal meningoencephalitis, focal lesions of CNS and less commonly myocarditis and pneumonitis. Determination of IgM antibodies against Toxoplasma encourages rapid diagnosis and treatment. Material and Methods: A total of 90 Immunocompromised patients were included in the study. The group consist of Patients with HIV reactivity, tuberculosis and on those who were receiving cancer chemotherapy were considered and clinical conditions suggestive of Toxoplasmosis were included. Blood sample collected from all these patients and were screened by ELISA for IgM antibodies. Results: Among immunocompromised patients, male were predominated with male to female ratio 2.91:1 was seen. Maximum cases were from the age group of 25-34 years. Ingestion of meat was most common risk factor. Fever, Headache, and fatigue were the most common clinical symptoms in immunocompromised and toxoplasma co-infected patients; Hypodensity was the common radiological feature. Conclusion: The prevalence of toxoplasmosis in immunocompromised patients was 26%. Hence, the high prevalence of co-infection definitely indicates that early diagnosis, high level of suspicion and effective and aggressive treatment of toxoplasmosis, strong commitment; a focused approach is the need so that toxoplasmosis can be diagnosed at an earliest stage.

Keywords: IgM ELISA; immunocompromised; Toxoplasmosis.

1. Introduction

Approximately one third of the population is exposed to this parasite. Infection in immunocompetent adult human is usually asymptomatic, while it is dangerous for immunocompromised patients¹. Seroprevalence studies vary according to the geographic locations. The global seroprevalence in general population is reported to be 46.1%². Seroprevalence of Toxoplasmosis in general Indian population ranges from 16.3% to 30.8%³. Toxoplasmosis is usually diagnosed by serological tests by detection of specific IgM and IgG antibodies. A positive IgM titre establishes recent infection where as negative IgM result virtually rules out recently acquired infection¹. Toxoplasmosis is a common opportunistic infection among immunocompromised patients. Disease in immunocompromised patients is usually due to reactivation of latent infection but can result from acute infection as well. Toxoplasmosis in these persons may lead to lethal meningoencephalitis, focal lesions of CNS and less commonly myocarditis and pneumonitis. Determination of IgM antibodies against Toxoplasma encourages rapid diagnosis and treatment⁴. Thus the present study was conducted to determine the seroprevalence and clinical correlates of Toxoplasma gondii infection in susceptible groups.

2. Material and Methods

After obtaining Institutional Ethical Committee approval the present clinical, prospective study of seroprevalence and clinical correlation of Toxoplasma gondii infection, was carried out in Department of Microbiology a Tertiary care Institute, during the period December 2011 to October 2013. Total of 90 immunocompromised indoor as well as outdoor patients referred from physician and surgeons, who were having strong index of clinical and/or radiological suspicion for Toxoplasmosis infection, were included in our study. The relevant history, clinical findings and investigations were noted. Exclusion criteria comprised of patients who were not willing to participate in the study.

1) Methodology

The study participants were explained about the study protocol and involved tests in the language of their understanding. After the informed consent they were enrolled for this study. Approximately 5 to 6 ml blood was collected under all aseptic precautions and it was then labelled correctly and was centrifuged at 3000 rotation per minute for ten minutes. Serum was transferred in the sterile labelled vials and these were stored at -20°C. Before performing the ELISA the samples and ELISA kit was brought to the room temperature.

2) Test Details⁵

Name of the test used: Enzyme linked immunosorbant assay (Enzywell Toxoplasma IgM - DIESSE – Italy)

3) Principle of the test

The test for the assay of Toxoplasma IgM is based on the principle of the capture of these immunoglobulins and the subsequent identification of those which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtiter wells). The antigen is composed of purified, inactivated and sonicated tachyzoite labeled with peroxidase bound to specific and anti-toxoplasma monoclonal antibodies.

4) Procedure

Bring the kit and sample at room temperature before start of the procedure. Prepare the required number of strip. Prepare the washing buffer by diluting the wash buffer 10x (100ml + 900ml H₂O). Prepare the immunocomplex by adding the conjugate to the antigen (volume shown on the label).

Dilute samples 1:101 distributing 10microlitre of serum into 1 ml of diluents. Dispense 100microlitre of each diluted sample per well. Place UNDILUTED control in a strip (100microlitre in each well). The minimum requisite is 1 negative control, 2 cut off, 1 positive control. Leave one well for blank, performed using 100microlitre of substrate mixture. Wells are covered with protective film and incubated for 45minutes at 37°C. After washing four times for 30 seconds (300microlitre), add 100 microlitre of immunocomplex (Antigen- anti Toxoplasma gondii monoclonal antibodies labeled with peroxidase) to each well and incubate again for 45 minutes at 37°C, covering the well with the protective film. The plate is washed again 4 times as described above. Finally, substrate is distributed 100microlitre/well and incubated for 15 minutes at room temperature. After 15 minutes at room temperature the enzymatic reaction is stopped by adding 100microlitre of stop solution. The adsorbance (O.D.) is read at 450nm or 450/620nm within 30 minutes.

Scheme of test procedure:

Step 1: Place 100 micro litre of diluted sample/controls in the wells of the strips.
 Incubate for 45min at 37° C
 Wash 4 times (300 micro litre)

Step 2: Add 100 micro litre of immunocomplex to each well
 Incubate for 45min. at 37°C
 Wash 4 times (300 micro litre)

Step 3: Add 100 micro litre of substrate to each well
 Incubate for 15 min. at room temperature.

Step 4: Add 100 micro litre of stop solution
 Read absorbance at 450 nm within 30min.

5) Test validation

Subtract the value of the blank (≤ 0.150) from all other readings. The O.D. values of the control Cut-off serum tested in triplicate must be within 25% of the mean value. Disregard any abnormal value and recalculate the mean. The Positive control must have an O.D. at least 1.5 times that of cut-off serum. The ratio between Negative control and cut-off must be ≤ 0.6 . The O.D. Cut-off must be ≥ 0.2 at 450nm and 0.16 at $\geq 450/620$ nm.

6) Interpretation of Results

If the absorbance of the sample is higher than that of cut-off, the sample is positive for the presence of specific IgM. Calculate the ratio between the O.D. value of the sample and that of the cut-off.

The sample is considered,

Positive, if the ratio is > 1.2

Negative, if the ratio is < 0.8

Doubtful, $\pm 20\%$ of cut-off.

ELISA test was put for detection of IgM antibodies and its level for Toxoplasma gondii infection. Presence of IgM antibodies does indicate ongoing current infection which may range from last 7 to 10 days. Positive and Negative findings of ELISA were correlated with clinical findings and/or radiological findings. Data were recorded in the proforma and analysed statistically by using student 't' test, standard error of difference between two means and Chi-square test. The SPSS version model was used. $p < 0.05$ was considered as statistically significant and $p < 0.01$ was considered as highly significant.

3. Results

In this study 90 serum samples from immunocompromised patients who were suspected to be having toxoplasmosis were evaluated for presence of anti-Toxoplasma IgM antibodies by using ELISA method.

Table 1: Age- wise distribution of patients in immunocompromised group

Age (Years)	Gender		Number of cases(n=90)
	Male	Female	
1 to 15	6	2	8 (8.88%)
16 to 24	7	3	10 (11.11%)
25 to 34	21	9	30 (33.33%)
35 to 44	22	6	28 (31.11%)
>45	11	3	14 (15.55%)
Total	67 (74.44%)	23 (25.55%)	90 (100%)

Maximum number of patients suspected with toxoplasmosis i.e. $n=30$ (33.33%) were from the age group of 25 to 34 years followed by $n=28$ (31.11%) in the age group of 35 to 44 years

Table 2: Risk factors in immunocompromised patients with Toxoplasmosis

Risk Factors	No. of immunocompromised patients with Toxoplasmosis(n=24) (%)
Contact with pet animals	12(50%)
Mixed diet	20(83.33%)
Past history of Toxoplasmosis	2(8.3%)
Contact with soil	14(58.33%)

Out of 24 IgM positive patients, 20 (83.33%) patient consumed mixed diet.

Table 3: Clinical features in immunocompromised Patients

Clinical presentation	IgM Positive(%)	IgM Negative(%)	No. of cases(%)	'P' value
Headache	8(33.33)	42(63.33)	50(55.5)	0.0159
Fever	11(45.83)	49(74.24)	60(66.6)	0.0214
Increased intracranial	00	00	00	1.000

tension				
Seizures	5(20.83)	8(12.12)	10(11.1)	0.3204
Altered sensorium	4(16.66)	10(15.15)	11(12.2)	1.000
Fatigue	10(41.66)	42(63.63)	52(57.7)	0.0906

Of the 24 immunocompromised – Toxoplasma co-infected patients, most common presentation was fever i.e., 11(45.83%) followed by fatigue and headache.

Table 4 : Radiological findings in immunocompromised patients suspected with Toxoplasmosis

Radiological features	IgM Positive(%)	IgM Negative(%)	No. of cases(%)	'P' value
Only hyperdensity	4(20)	3(20)	7(20)	0.0785
Only hypodensity	13(65)	5(33.33)	18(51.43)	0.0001
Only Ring-enhancement	1(5)	2(13.33)	3(8.57)	1.000
Hyperdensity and Ring-enhancement	1(5)	3(20)	4(11.42)	1.000
Hypodensity and Ring-enhancement	1(5)	2(13.33)	3(8.57)	1.000
Total	20(22.22)	15(16.66)	35(38.88)	--

35(38.55%) cases showed radiological features suggestive of Toxoplasmosis in this study. Out of these, 24 patients were Toxoplasma IgM positive, 20 had positive radiological findings. Among these maximum cases were showing hypodensity on CT scan, which was found highly significant in our study.

Table 5: Age and gender wise distribution of IgM seropositive immunocompromised patients

Age(years)	Gender		IgM positive(%)
	Male	Female	
1-15	1	1	2
16-24	1	0	1
25-34	9	2	11
35-44	5	3	8
>45	1	1	2
Total	17(18.88%)	7(7.77%)	24(26.66%)

In our study, IgM positivity in immunocompromised patients was 24(26.66%). Maximum patients with seropositivity belonged to age group 25-34 years, followed by the age group 35-44 years.

4. Discussion

In our study out of 90 immunocompromised subjects maximum cases were from the age group range of 25-34 years 30 (33.33%) followed by the age group range of 35-44 years 28(31.11%) (Table 1). Males were more as compared to females. Similarly the study conducted by Veeranoot Nissapatorn *et al*⁶⁸ and MA Davarpanah *et al*⁶ reported maximum cases falling in the age group range of 25-34 years followed by the age group range of 35-44 year. V. Nissapatorn *et al*⁷ noticed that the preponderant age group of 35-44 years followed by 25-34 years and males were more commonly affected than females.

Toxoplasmosis mainly spreads through ingestion of undercooked meat and meat products, contact with pet animals like cats whose faeces are infected with oocysts of toxoplasma, mother to child transmission (Congenital toxoplasmosis) if mother is infected during pregnancy and may also occur due to organ transplant procedures, blood transfusion and other immunocompromised states such as patients receiving cancer chemotherapy, diabetics and patients with tuberculosis, HIV infections. However in immunocompromised patients, common risk factor in our study was ingestion of mixed diet in 20 cases (83.33%) and history of contact with pets like cats and dogs i.e., 12 (50%). There were only 2 (8.3%) cases that had past history of toxoplasmosis (Table 2). Similarly in pregnant women the

most common risk factor was ingestion of mixed diet i.e., 13(72.22%) in our study.

Study conducted by Eileen M Proctor *et al* 1994⁸ found positive correlation between ingestion of meat, consumption of unpasteurized milk and detection of antibodies to *T gondii*. They concluded that consumption of undercooked meat and unpasteurized milk may result in the acquisition of toxoplasmosis. However it also suggests that acquisition of toxoplasmosis occurs more likely via environmental oocysts or cysts in food than by direct contact with cats. While study conducted by Eileen MP *et al* 1994⁸ concluded that seropositivity did not differ between cat owners and non-cat owners. W Buffolano 2008⁹ stated that 30-63% of seroconversion was due the consumption of undercooked meat and meat products, 16-17% were result of soil contact. Another study by Wallace MR *et al* 1993¹⁰, concluded that antibody seroconversion in an adult HIV-infected population is unusual and appears unrelated to cat ownership or exposure. As per statistical evaluation this association is not significant.

A study by Magnerou AM *et al* 2012¹¹, evaluate clinical diagnosis, CT-scan findings, therapy and evolution of AIDS patients with toxoplasmic encephalitis the main presenting complaints were motor deficits (65%), seizures (40%), headaches (31.7%), language and the speech disturbances (35%). Signs of meningeal irritation and raised intracranial pressure were found in 21% and 10% of the cases respectively. Another study by Venugopal A¹², noted most common clinical presentations were fever (58%) and headache (52%). While Nissapatorn V¹³ reported headache as the most common presenting symptom i.e. 56%. Vidal *et al*^{14,15} reported headache, hemiparesis and seizure as the most common neurological presentations that are found in cerebral toxoplasmosis patients. Uneke CJ *et al*¹⁶ evaluated the clinical findings of patients with concomitant toxoplasmosis and HIV infection which greatly implicated fever (63.5%), headache (44.7%), rashes (41.2%) and anorexia (34.1%). Chaddha DS *et al*¹⁷ found that the common presenting symptoms were fever (80%), seizures (45%), headache (45%) and altered sensorium (25%). Focal neurological deficit was present in 80% of cases.

Similarly in our study out of 24 Immunocompromised/toxoplasmosis co-infected patients, fever was the most common symptom 45.83%, followed by fatigue and headache 41.66% and 33.33% respectively (Table 3). Most of the patients presented with symptoms in

the combination of two to three. Headache and fever were found to be statistically significant in our study. ($p= 0.0159$ and 0.0214 respectively). While rash, lymphadenopathy and raised intracranial tension were not found in our study. Our results were concordant with other studies.

Radiological investigations, using either contrast enhanced computed tomography or Magnetic Resonance Imaging (MRI), both are useful tools for presumptive or empirical diagnosis of toxoplasma. In immunocompromised patients, hyperdensity was the only finding seen in 4 (20%) patients, and only hypodensity was present in 13 (65%) patients whereas 1 (5%) case showed only ring enhancement as the radiological feature in our study. (Table 4). Hypodensity was found to be statistically significant in our study. Similar study done by Vidal *et al*^{14,15}, found that hypodense lesions with ring enhancing and perilesional edema were present in nearly 80% of toxoplasma patients and in nearly 20% cases a typical pattern of hypodense lesions without contrast enhancement was seen. Another study by Veeranoot Nissapatorn *et al*¹⁸ done in HIV/AIDS patients inferred that CT scan findings were, lesions which were multiple (96.4%), Hypodense (66.7%), and in the parietal region (39.3%).

In our study seropositivity of toxoplasmosis in immunocompromised cases was 26.66% with maximum age group of 25-34 years followed by 35-44 years as shown above (Table 5). Similarly study conducted by V Nissapatorn *et al*¹⁹ noticed that the preponderant age was 35-44 years followed by 25-34 years. Holliman²⁰ showed the seropositivity of toxoplasmosis to be 26.06%. Sykora *et al* 1992²¹ reported 29.8% seropositivity of toxoplasmosis. Brindle *et al*²² found seropositivity of toxoplasmosis to be 22%.

5. Conclusion

- The prevalence of toxoplasmosis in immunocompromised patients was 26%. Hence, the high prevalence of co-infection definitely indicates that early diagnosis, high level of suspicion and effective and aggressive treatment of toxoplasmosis, strong commitment; a focused approach is the need so that toxoplasmosis can be diagnosed at an earliest stage. The initiation of antitoxoplasma treatment and HAART at the proper time reduces the morbidity and mortality due to toxoplasmosis and its complications like toxoplasma encephalitis, convulsions etc can be prevented.
- It should be mandatory to screen every immunocompromised patient for toxoplasmosis, and initiation of judicious treatment on time can, thus be provided to prevent morbidity and mortality due to toxoplasmosis.

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