

## Seroprevalence of *Toxoplasma Gondii* in Immunocompetent and Immunocompromised Patients Using IgG - Modified Direct Agglutination Test (IgG MAT)

G Sucilathangam<sup>1\*</sup>, N Palaniappan<sup>1</sup>, C Sreekumar<sup>2</sup> and T Anna<sup>2</sup>

<sup>1</sup>Department of Microbiology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India

<sup>2</sup>Department of Veterinary Parasitology, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

### Abstract

**Background/Objective:** The present study was conducted to assess the seroprevalence of *Toxoplasma gondii* in and around Tirunelveli, Tamil Nadu, India by in-house IgG assay, using MAT.

**Methods:** Serum samples from 175 immunodeficient and 175 immunocompetent patients were collected at Tirunelveli District, Tamil Nadu from May 2006 to October 2007. They were subjected to in-house IgG assay using Modified Direct Agglutination test (MAT).

**Results:** Out of 350 patients tested by MAT, 39 patients (11.41%) had antibodies for toxoplasmosis with mean MAT titre of  $53.85 \pm 55.28$  and the titre ranged from 1:20 to 1:160. Among the immunocompetent group of 175 patients, 18 patients (10.29%) had antibodies to toxoplasmosis whereas in immunodeficient group of 175 patients, 21 patients (12%) had antibodies for toxoplasmosis. The sensitivity, specificity of IgG MAT in detecting toxoplasmosis was 80 and 90 % respectively.

**Statistical analysis:** The results were analysed by using the statistical software SPSS.

**Conclusions:** The overall seroprevalence of toxoplasmosis in and around Tirunelveli District of Tamil Nadu was 11.41% based on IgG MAT. The study has proved that MAT is recommended as the most convenient method for serodiagnosis of toxoplasmosis and has the additional advantage of not requiring species-specific conjugates.

**Keywords:** Seroprevalence; *Toxoplasma gondii*; Modified Agglutination Test; Tamil Nadu; India

### Introduction

*Toxoplasma gondii* (*T. gondii*) is a ubiquitous parasite of warm-blooded animals that causes one of the most common parasitic infections in humans [1]. Although Toxoplasmosis infects large proportion of world's human populations, it is thought to be an uncommon cause of typical disease in early days. Nowadays, certain individuals however are at high risk for severe or life threatening disease due to this parasite. Although usually asymptomatic in immunocompetent adults, it can cause severe disease manifestations and even death in immunocompromised patients. If acquired during pregnancy it can cause various congenital anomalies in the child.

At present, the diagnosis of acute acquired infection with *Toxoplasma gondii* depends on serological test results. A number of problems associated with available methods for serodiagnosis of this infection have been an impetus to search for alternative methods. The routine methods presently available to physicians in practice are often expensive to perform, time consuming, not readily adaptable to screening programs, or not sensitive enough to be useful in early diagnosis of the infection [2].

The toxoplasma agglutination (AG) test was first described by Fulton et al. [3-6]. This method uses whole killed organisms and is now routinely performed in France with an antigen that has been prepared as described by Ardoin et al. [7] and that is commercially available (BioMerieux, France). The method is very simple and useful but as presently used has two drawbacks. First, it lacks sensitivity; the titer in the AG test is usually much lower than that in the dye test (DT) or the conventional immunofluorescent-antibody (IFA) test. As

a consequence, some sera that are positive in the latter two tests are reported as negative in the AG test. Second, it lacks specificity; some sera that are negative in the DT and IFA test are reported as positive in the AG test [8]. This has been shown to be due to the binding of normal immunoglobulin M (IgM) ("natural IgM antibody") to the surface of the parasite [9].

We describe here a method for preparing antigen which increases the sensitivity of the AG test and a method for suppressing nonspecific agglutination by the use of a buffer containing 2-mercaptoethanol (2ME). When the AG test is modified by these two methods, its specificity and sensitivity parallel those of the DT. The technique and the reading are so simple and accurate that, if the antigen is made available, the modified AG test method would be convenient for laboratories that perform serology only occasionally as well as for those that perform large-scale surveys.

The seroprevalence varies widely in different regions of the globe, measuring between 30 % and 60 % in most countries [10]. The

**\*Corresponding author:** Sucilathangam Ganesan, Department of Microbiology, Tirunelveli Medical College, Tirunelveli, Tamil Nadu, India, E-mail: [drgsucila@rediffmail.com](mailto:drgsucila@rediffmail.com)

**Received** November 08, 2011; **Accepted** January 05, 2012; **Published** January 09, 2012

**Citation:** Sucilathangam G, Palaniappan N, Sreekumar C, Anna T (2012) Seroprevalence of *Toxoplasma Gondii* in Immunocompetent and Immunocompromised Patients Using IgG - Modified Direct Agglutination Test (IgG MAT). J Medical Microbiol Diagnosis 1:102. doi:10.4172/2161-0703.1000102

**Copyright:** © 2012 Sucilathangam G, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

prevalence changes according to social and cultural habits, geographic factors, climate, and transmission route, and typically increases with age [11,12]. It has been reported that the prevalence is higher in warm and humid areas [11,13]. Toxoplasmosis is not a reportable disease and disease prevalence is based on regional studies.

In India, the exact seroprevalence of this infection is not known. There is a lack of awareness and knowledge about this zoonotic infection, diagnosis and interpretation of the test results in the Indian context. Since testing for toxoplasmosis is not a routine activity in our country. Further complicating the situation, there are several commercial organizations that are promoting their products without proper background knowledge and baseline data from India. In Tamil Nadu, various studies have done in northern districts [14,15] but so far not reported in southern districts except our earlier recent report [16].

Under this situation, it is highly imperative to assess the seroprevalence of toxoplasmosis in and around Tirunelveli district of Tamil Nadu by using in-house IgG MAT and the IgG MAT test results were compared with our previous report on seroprevalence of toxoplasmosis using same set of samples by IgG IFAT.

## Materials and Methods

### Study population

After obtaining an approval from the institutional ethical committee and an informed consent from the patients, a total of 350 peripheral blood samples were collected from 175 immunodeficient patients (HIV and patients with malignancy) and 175 immunocompetent patients including pregnant women (135), ocular chorioretinitis cases (20), and patients with lymphadenopathy (20), in and around Tirunelveli district of Tamil Nadu.

Inclusion criteria for immunodeficient group were of HIV-infected patients with CD<sub>4</sub> T-cell counts < 200/ $\mu$ l and proved cancer patients. Exclusion criteria for immunodeficient group were of transplant recipients under immunosuppressive therapy or haemodialysis patients with chronic renal failure. Inclusion criteria for immunocompetent group were of asymptomatic pregnant women, pregnant women with bad obstetrics history (BOH), ocular chorioretinitis cases, and patients with non malignant causes of lymphadenopathy. Exclusion criteria for immunocompetent group were of myocarditis and pericarditis.

### Preparation of MAT antigen

Direct agglutination method described by Desmots and Remington [2] was followed with minor modification. Adult male and female Swiss albino mice (*Mus musculus*) were procured from the Madras Veterinary College, Chennai. The animals were approximately 8 weeks old and weighed 25-30g. They were maintained on concentrate feed, fully soaked White Bengal gram and water ad lib. Litter from mouse cages and floors of sheds were collected daily and disposed off after adding concentrated ammonia. All cages were thoroughly washed with 30 per cent ammonia solution between various batches of animals. Dead laboratory animals were immersed in 10 per cent formalin before disposal. Due consideration for animal ethical concern was made before implication of this study for rearing experimental animal, mice.

Mouse adapted RH strain of *Toxoplasma gondii* maintained at the Department of Veterinary Parasitology, Madras Veterinary College, Chennai, which was originally obtained from All India Institute of

Medical Sciences, New Delhi was used in this study. Ten mice were infected with RH strain of *T. gondii* maintained in the small laboratory animal facility and examined for signs of infection. On 5 days post infection the mice were euthanized by chloroform anesthesia and the peritoneal cavity was opened aseptically. Using an insulin syringe, the ascitic fluid (peritoneal exudates) was aspirated and diluted with sterile phosphate buffered saline (PBS, pH 7.2). A drop of this fluid was examined microscopically for the presence of tachyzoites. Repeated washing of the peritoneal cavity with sterile PBS was carried out to collect maximum tachyzoites. The tachyzoites thus harvested were counted and the Formalin killed freshly prepared antigen was reconstituted in sterile PBS so as to reach the final concentration of  $1 \times 10^7$  tachyzoites per ml, which was used for the MAT [2,17].

### Preparation of MAT antigen mixture

Antigen diluting Borate buffer was prepared by dissolving 7.01g of Sodium chloride, 3.09g Boric acid, 2g Sodium azide in 900ml deionised water. 24ml of 1N NaOH was added to this and the pH was adjusted to 8.95. The volume was made up to 1 litre and used as stock solution which was stored at room temperature.

For the preparation of working antigen diluting buffer, 0.4g of Bovine Serum Albumin was dissolved in 100ml of Borate buffer and stored at 4°C. For each plate, 2.5 ml of antigen diluting buffer was mixed with 35 $\mu$ l of 2-mercaptoethanol, 50 $\mu$ l of Evans blue dye solution as tachyzoite colouring agent and 0.15 ml of Formalin killed antigen, which was used as antigen mixture for the MAT.

### Standardization and test procedure of MAT

The known positive and known negative *Toxoplasma gondii* reference sera were gifted by Dr. C. Sreekumar, Toxo Laboratory, USDA, Beltsville, Maryland, U.S.A. The sensitivity, specificity and positive and negative predictive value of the assay were calculated using 10 known positive and negative reference sera. End titer of positive sera control was standardized. Positive and negative sera were used to titrate each lot of antigen to determine the working dilution and were also used as controls in each test. An antigen control (consisting of PBS, 2-ME, and diluted antigen) was included in each test to check for autoagglutination of antigen. The serum samples were diluted with sterile serum diluting buffer [Serum diluting buffer: Phosphate buffered saline (PBS) without Ca<sup>++</sup> and Mg<sup>++</sup> (1x, pH 7.2) Sodium chloride - 8.00g, Potassium chloride - 0.20g, Disodium hydrogen orthophosphate dehydrate - 1.41g, Potassium dihydrogen orthophosphate - 0.20g, Distilled water to make (adjust pH to 7.2) - 1000 ml] in microtitre plates. The initial dilution was started at 1:20. 25 $\mu$ l of antigen mixture was added to each well on U bottom microtitre plate (Tarson products Pvt. Ltd., Kolkata, India) immediately after mixing. 25  $\mu$ l of serum of all dilutions prepared as above were added. The antigen and sera were mixed by repeated pipetting action and the plate was covered with sealing tape. The positive and negative controls were maintained in each plate. The plate was read after overnight incubation at 37°C. The negative reaction was indicated by formation of blue button at the bottom of the well whereas; the positive reaction had matted appearance. Antibody titers of 1:20 or more were taken as significant for reporting positive results. Those test samples positive at 1:20 were further serially two fold diluted until the end titre was reached.

### Comparison of MAT with IFAT

The efficiency of the IgG MAT was statistically compared for its

test of significance by McNemar's test with our previous report on seroprevalence of toxoplasmosis using same set of samples by IgG IFAT (16).

## Results

### Evaluation of MAT

The sensitivity, specificity of MAT in detecting toxoplasmosis in the present study was 80 and 90% respectively.

### Seroprevalence of toxoplasmosis by MAT

Out of 350 patients tested by MAT, 39 patients (11.41%) had antibodies for toxoplasmosis with mean MAT titre of  $53.85 \pm 55.28$  and the titre ranged from 1:20 to 1:160. Among the immunocompetent group of 175 patients, 18 patients (10.29%) had antibodies to toxoplasmosis whereas in immunodeficient group of 175 patients, 21 patients (12.0%) had antibodies for toxoplasmosis (Table 1).

Out of 135 pregnant women tested, 12 (8.89%) were seropositive with mean MAT titre of  $55 \pm 63.32$  (titre ranged from 1:20 to 1:160). Among the 20 cases of lymphadenopathy, only 3 (15%) were seropositive with mean titre of  $33.33 \pm 11.54$  (titre ranged from 1:20 to 1:40). Of the 20 ocular cases tested, only 3 (15%) were seropositive with mean titre of  $73.33 \pm 75.71$  (titre ranged from 1:20 to 1:160).

Among the 160 HIV positive individuals tested by MAT, 18 (11.25%) were seropositive with mean titre of  $50 \pm 51.44$  (titre ranged from 1:20 to 1:160). Out of 15 cases of malignancy, only 3 (20%) were seropositive with mean titre of  $33.33 \pm 11.54$  (titre ranged from 1:20 to 1:40).

### Comparison of IgG MAT with IgG IFAT

The IgG MAT test results were compared with our previous report on seroprevalence of toxoplasmosis using same set of samples by IgG IFAT (16). The overall seroprevalence of *T. gondii* in and around Tirunelveli district of Tamil Nadu was 11.71% based on IgG IFAT. Out of 350 patients tested by IgG IFAT, 41 patients (11.71%) had antibodies for *T. gondii* with mean IFAT titre of  $43.32 \pm 58.7$  and the titre ranged from 1:16 to 1:256. Among the immunocompetent group of 175 patients, 19 patients (10.86%) had antibodies to *T. gondii*, whereas in immunodeficient group of 175 patients, 22 patients (12.57%) had antibodies for *T. gondii*.

Sl. No.	Category of patients	Number of patients examined	Number of patients positive	Percent positive (%)	Mean titre with $\pm$ SE and Range of positive patients
1.	Immunocompetant	135	12	8.89	$55 \pm 63.32$ (1:20 - 1:160)
	Pregnant women				
2.	Lymphadenopathy	20	3	15.0	$33.33 \pm 11.54$ (1:20-1:40)
3.	Ocular	20	3	15.0	$73.33 \pm 75.71$ (1:20-1:160)
4.	Immunodeficient	160	18	11.25	$50 \pm 51.44$ (1:20-1:160)
	HIV positive individuals				
5.	Malignancy	15	3	20.0	$33.33 \pm 11.54$ (1:20-1:40)
	<b>TOTAL</b>	<b>350</b>	<b>39</b>	<b>11.14</b>	<b><math>53.85 \pm 55.28</math> (1:20-1:160)</b>

Table 1: Seroprevalence of Toxoplasmosis by Mat.

The testing efficiency of the IgG MAT was statistically compared for its test of significance by McNemar's test with IgG IFAT for detection of *T. gondii* antibodies. Statistically there was no significant difference between IgG IFAT and MAT in detecting toxoplasmosis ( $p > 0.05$ ).

## Discussion

In the present study, we assessed the seroprevalence of antibodies to *Toxoplasma gondii* by MAT test and the MAT was compared and discussed with our previous report on seroprevalence of toxoplasmosis using same set of samples by IgG IFAT.

The original direct agglutination test, described by Fulton and Turk [5], entailed the reaction of specific antibody with formalin-fixed, *Toxoplasma* tachyzoites. The method lacked sensitivity, producing lower titres than those recorded by the dye test, and gave false positive reactions due to the binding of non-*Toxoplasma* specific IgM immunoglobulin to the parasite. Subsequent modifications of the technique suppressed non-specific reactions by the use of 2-mercaptoethanol buffer and increased its specificity and sensitivity parallel those of the DT. The technique of modified agglutination test (MAT/MAGT) and the reading were so simple and accurate that, if the MAT test antigen using 2ME was made available, the MAT method would be convenient for laboratories that perform serology only occasionally as well as for those that perform large-scale surveys [2].

The IgG MAT was standardized and the cut-off titre for positive was arrived at 1: 20 and above. The sensitivity and specificity of IgG MAT in the present study in detecting *T. gondii* was 80 and 90 %, respectively. Most of the recent authors taken dilutions of 1:20 and above were positive for MAT. Singh et al. [17] modified the direct agglutination test described by Desmonts and Remington [2], 1980 and reported that antibody titers of 1:20 or more were taken as positive. Sema et al. [18] considered titers of  $\geq 20$  were positive for the direct agglutination test using formalin fixed tachyzoites as antigen. The formalin killed antigen mixture with 2-mercaptoethanol and Evans blue was prepared and used for MAT whereas Johnson et al. [19] stated that AG test titers were usually markedly lower than those obtained with the DT or IFA test and that "negative" results were often obtained in DT-positive sera. For AG test doubling dilutions started from 1:20 and a positive direct agglutination test result was defined as that with titre of  $\geq 40$ .

The overall seroprevalence of *T. gondii* in and around Tirunelveli district of Tamil Nadu was 11.14 % based on IgG MAT. In India, the exact seroprevalence of *T. gondii* is not known. However, using various diagnostic tests, the prevalence has been reported to be as low as 1% and as high as 80% in adults [20]. Until recently, the prevalence of *T. gondii* in the general population of India was considered to be low when compared with Western countries [21,22]. This may be partially true, however, the data are based on samples obtained from patients admitted to hospitals in big cities. Because most of the population of India lives in rural villages with little means to get to city hospitals, the data are not reflective of the population as a whole. The type of serological tests used and the titres taken as evidence of infection are also important factors. However, only studies during 1990 indicated that the prevalence may be a lot higher than previously considered [23,24].

On the other hand, there are studies which have shown a higher prevalence than results in our study. In earlier studies, only 12 %

seropositivity for *T. gondii* was reported from South India, whereas, of late, a high prevalence (75-77%) of *T. gondii* was reported from North India [14,25].

Meisheri et al. [26] conducted a study of seroprevalence of toxoplasmosis in general population and in HIV/AIDS patients in Bombay and reported that the overall seroprevalence was 30.9% (51/165) in the immunocompetent adult (34% in men and 26.2% in women). In HIV infected hosts the seroprevalence was 67.8%.

In the present study, among the immunocompetent group of 175 patients, 18 patients (10.29%) had antibodies to toxoplasmosis whereas in immunodeficient group of 175 patients, 21 patients (12.0%) had antibodies for toxoplasmosis. In our study, 12 (8.89%) were seropositive for *Toxoplasma gondii* in 135 pregnant women. High prevalence rate of anti-*Toxoplasma* antibody in pregnant women have been reported in recent studies. Akoijam et al. [27] performed a study among primigravid women attending a secondary level hospital in a district of North India using IgG ELISA and reported 41.75% were seropositive for *Toxoplasma gondii* infection. A prospective study conducted by Singh and Pandit [28] on incidence and prevalence of toxoplasmosis in Indian pregnant women indicated that the overall IgG seroprevalence rate of toxoplasmosis was 45 per cent by using IgG Avidity ELISA test.

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. In this present study also, only 3 out of 19 (15.79%) cases with bad obstetric history (BOH) were seropositive for toxoplasmosis. Yasodhara et al. [29] from Hyderabad, South India reported toxoplasmosis seroprevalence of 33% (IgG) in lower socioeconomic group compared to higher socioeconomic group (22%) in 236 women with bad obstetric history.

The present study has highlighted an overall 11.41% of seroprevalence of *T. gondii*, which constitutes 12% in immunocompromised and 10.29% in immunocompetent patients in and around Tirunelveli District of Tamil Nadu. The study underlines the importance of screening of this parasite especially in the immunocompromised patients.

The seroprevalence of *T. gondii* in pregnant women was about 8.89%, which necessitates initial screening for IgG antibodies and then a paired sera sample after 3 weeks should be tested for rise in titre. Thereafter, the IgM antibodies should be tested to exclude the recent infection. All seropositive women who seroconvert during pregnancy should be monitored and their amniotic fluid / foetus should be screened by PCR and Ultrasound, respectively to assess the infection.

Our recent previous report [16] on seroprevalence toxoplasmosis in same districts of Tamil Nadu using same set of samples by in-house IgG IFAT revealed 11.71% which was in close agreement with the present study of 11.41% prevalence with in-house IgG MAT. The sensitivity and specificity of IG IFAT in detecting toxoplasmosis was 80 and 90%, respectively which is in very close agreement with the present assay IgG MAT. Moreover statistically there was no significant difference between IgG IFAT and MAT in detecting toxoplasmosis ( $p > 0.05$ ).

The MAT required the least amount of time to perform. All reagents were added to the wells during the initial set-up and allowed to react. The MAT results were evaluated visually and subjectively

and depended on the interpretive skills of the technician. The MAT required no special instrumentation other than the microtiter mirror for determining the results. The antigen for the IFA is whole, killed tachyzoites affixed to microscope slides. The IFA requires use of a fluorescence microscope. Also, live organisms are not used in the 2 tests, thus all are relatively safe for laboratory personnel. Hence the MAT was preferred over the IFAT because: (1) it was the least labour-intensive, (2) it was convenient for single samples, (3) the results were evaluated visually and required no special microscope (4) the MAT could be used to test sera from any species, because species-specific conjugates were not used, and MAT reading allows a greater number of sera to be analyzed in a certain period of time compared to IFAT.

## References

1. Tenter AM, Heckeroth AR, Weiss LM (2000) *Toxoplasma gondii*: from animals to humans. Int J Parasitol 30: 1217-1258.
2. Desmonts G, Remington JS (1980) Direct Agglutination Test for diagnosis of *Toxoplasma* infection: Method for increasing sensitivity and specificity. J Clin Microbiol 11: 562-568.
3. Fulton JD (1965) Micro-agglutination test for *Toxoplasma* antibodies. Immunology 9: 491-495.
4. Fulton JD (1965) Studies on agglutination of *Toxoplasma gondii*. Trans R Soc Trop Med Hyg 59: 694-704.
5. Fulton JD, Turk JL (1959) Direct agglutination test for *Toxoplasma gondii*. Lancet 2: 1068-1069.
6. Fulton JD, Voller A (1964) Evaluation of immunofluorescent and direct agglutination methods for detection of specific *Toxoplasma* antibodies. Br Med J 2: 1173-1175.
7. Ardoin P, Couzineau P, Baufine-Ducrocq H (1967) On the use of T.G. 180 sarcoma for obtaining a suspension rich in extracellular *Toxoplasma gondii*. C R Seances Soc Biol Fil 161: 117-119.
8. Couzineau P, Baufine-Ducrocq H, Peloux Y, Desmonts G (1973) Toxoplasmosis serodiagnosis by direct agglutination. Nouv Presse Med 2: 1604-1606.
9. Desmonts G, Baufine-Ducrocq H, Couzineau P, Peloux Y (1974) Natural antibodies against *Toxoplasma*. Nouv Presse Med 15: 1547-1549.
10. Flegel J, Preiss M, Klose J, Havlicek J, Vitakova M, et al. (2000) Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii* dopamine, a missing link between schizophrenia and toxoplasmosis? Biol Psychol 63: 253-268.
11. Garcia JL, Navarro IT, Ogawa L, de Oliveira RC, Kobilka E (1999) Seroprevalence, epidemiology and ocular evaluation of human toxoplasmosis in the rural zone Jauguapita (Parana) Brazil. Rev Panam Salud Publica 6: 157-163.
12. Cantos GA, Prando MD, Siqueira MV, Teixeira RM (2000) Toxoplasmosis: occurrence of antibodies anti-*Toxoplasma gondii* and diagnosis. Rev Assoc Med Bras 46: 335-341.
13. Coelho RA, Kobayashi M, Carvalho LB Jr (2003) Prevalence of IgG antibodies specific to *Toxoplasma gondii* among blood donors in Recife, Northeast Brazil. Rev Inst Med Trop Sao Paulo 45: 229-231.
14. Bhatia VN, Meenakshi K, Agrawal SC (1974) Toxoplasmosis in South India - a serological study. Indian J Med Res 62: 1818-1820.
15. Palanisamy M, Madhavan B, Balasundaram MB, Andavar R, Venkatapathy N (2006) Outbreak of ocular toxoplasmosis in Coimbatore, India. Indian J Ophthalmol 54: 129-131.
16. Sucilathangam G, Palaniappan N, Sreekumar C, Anna T (2010) IgG - Indirect fluorescent antibody technique to detect seroprevalence of *Toxoplasma gondii* in immunocompetent and immunodeficient patients in southern districts of Tamil Nadu. Indian J Med Microbiol 28: 354-357.
17. Singh S, Singh N, Maniar JK (1996) AIDS Associated Toxoplasmosis in India

- and its correlation with serum tumor necrosis factor-alpha. J Parasit Dis 20: 49-52.
18. Ertug S, Okyay P, Turkmen M, Yuksel H (2005) Seroprevalence and risk factors for *Toxoplasma* infection among pregnant women in Aydin province, Turkey. BMC Public Health 5: 66.
19. Johnson J, Duffy K, New L, Holliman RE, Chessum BS, et al. (1989) Direct agglutination test and other assays for measuring antibodies to *Toxoplasma gondii*. J Clin Pathol 42: 536-541.
20. Singh S (2003) Mother to child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. Indian J Med Microbiol 21: 69-76.
21. Bowerman RJ (1991) Seroprevalence of *Toxoplasma gondii* in rural India: a preliminary study. Trans R Soc Trop Med Hyg 85: 622.
22. Mittal V, Bhatia R, Singh VK, Sehgal S (1995) Prevalence of toxoplasmosis in Indian women of child bearing age. Indian J Pathol Microbiol 38: 143-145.
23. Singh S, Nautiyal BL (1991) Seroprevalence of Toxoplasmosis in Kumaon region of India. Indian J Med Res 93: 247-249.
24. Singh S, Ahlawat S, Singh N, Chaudhary VP (1995) High Torch seroprevalence rate in multiply transfused beta-Thalassemic children in India. Eur J Haematol 54: 64-66.
25. Singh S (2002) Prevalence of torch infections in Indian pregnant women. Indian J Med Microbiol 20: 57-58.
26. Meisheri YV, Mehta S, Patel U (1997) A prospective study of seroprevalence of Toxoplasmosis in general population, and in HIV/AIDS patients in Bombay, India. J Postgrad Med 43: 93-97.
27. Akoijam BS, Shashikant, Singh S, Kapoor SK (2002) Seroprevalence of *Toxoplasma* infection among primigravid women attending antenatal clinics at a secondary level hospital in North India. J Indian Med Assoc 100: 591-602.
28. Singh S, Pandit AJ (2004) Incidence and Prevalence of Toxoplasmosis in Indian Pregnant Women: a Prospective Study. Am J Reprod Immunol 52: 276-283.
29. Yashodhara P, Ramalakshmi BA, Lakshmi V, Krishna TP (2004) Socioeconomic status and prevalence of toxoplasmosis during pregnancy. Indian J Med Microbiol 22: 241-243.