SEROLOGICAL DIAGNOSIS OF *TOXOPLASMA GONDII* INFECTION IN VARIOUS PATIENT POPULATION IN THE ARMED FORCES

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Abstract

The study included 200 healthy age and sex matched controls (120 healthy volunteers and 80 healthy antenatal cases without any bad obstetric history) and 300 cases comprising patients with bad obstetric history (BOH) 80 cases, seizure or encephalitis 50 cases, cervical lymphadenopathy 30 cases, congenital deformity 30 cases, HIV infected 80 cases and patients on long term immunosuppression 30 cases. Anti-toxoplasma IgG & IgM were measured by ELISA and individuals found positive for anti *Toxoplasma gondii* IgM or with high titre of IgG were subjected to repeat testing after 3 weeks for rise in titre. True sero-prevalence of *T gondii* infection was found to be 19% and it increased with increasing age in the population. 2.33% patients were found to have acute toxoplasma infection. Statistical significance was seen only in patients with BOH and children born with congenital abnormality.

MJAFI 2001; 57 : 298-301

KEY WORDS : Bad obstetric history; Congenital abnormality; Serodiagnosis; Toxoplasmosis.

Introduction

T *gondii* is a ubiquitous intracellular protozoan parasite belonging to class Soprozoalida. The parasite's definitive host is cat and other feline species. Humans are intermediate hosts and it rarely causes disease in an immunocompetent host. Toxoplasmosis usually occurs due to reactivation of chronic infection seen mostly in immunocompromised host and can lead to various clinical illness in the form of acute toxoplasmosis, i.e. pneumonitis, encephalitis, generalised lymphadenopathy, chronic nephritis, recurrent abortions, stillbirth and various congenital abnormalities [1].

Among various diagnostic modalities available, serological tests are by far the most suitable and are commonly employed for diagnosis. Infection elicits primarily IgM and IgG class of antibodies, IgM indicating recent infection and IgG latent/past infection. More than a single high titre of IgG, a four fold rise in the titre at 3-4 weeks interval is suggestive of acute infection. Therefore, estimation of IgM and IgG isotypes are essential for the diagnosis [1]. This study has been undertaken to find out the seroprevalence of *T gondii* infection and to ascertain the incidence of toxoplasmosis in various patient populations in Armed Forces and their families.

Material and Methods

The study population included 200 age and sex matched controls (120 healthy volunteers and 80 antenatal cases without any BOH). The patient population comprised 300 cases from various

disease groups as given below :-

BOH	- 80
Asymptomatic HIV positive cases	- 80
Seizure/encephalitis	- 50
Cervical Lymphadenopathy	- 30
Patient on long term immuno suppressant	- 30
Congenital abnormality	- 18
Chorioretinitis	- 12

5 ml. of blood was collected and serum after separation was stored at -70°C for testing. Serum was assayed for IgG and IgM antibody to *T gondii* by using commercial ELISA kits (United Biotech U.S.A). All the samples were tested in duplicate along-with kit controls and the mean value of each sample was taken into consideration for calculating results. Manufacturer's instruction was followed for ELISA procedure. Serum samples from patients were re-tested for anti-toxoplasma IgG antibody after 3 weeks if found positive for IgM isotype or with high titre of IgG antibody in the first testing. Statistical analysis was carried out by Chi-square test and odds ratio.

Results

The present study showed no IgM positivity in the control group. In the control group 38/200 (19%) showed positivity for anti toxoplasma IgG. In the healthy volunteer group 23/120 (19.1%) and in the control antenatal cases 15/80 (18.7%) were seropositive (Table 1). Table-2 shows the seropositivity for toxoplasma IgG antibodies in various patient populations. In patients with BOH 26/80 (32.5%), with CNS manifestation 12/50 (24%), with lymphadenopathy 8/30 (26.7%), in congenital deformity group 6/18 (33.3%) and in chorioretinitis cases 3/12 (25%) showed positivity for anti-toxoplasma IgG antibody. In asymptomatic HIV cases and in-patients with immunosuppression 18/80 (22.5%) and 5/30 (16.6%) showed seropositivity for anti-toxoplasma IgG antibody respectively. The percentage of sero prevalence increased along with increasing age in the control

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group (Table 1). Only 7 cases showed antitoxoplasma IgM antibody positivity (Table 3) out of which 5 cases showed a four fold rise in IgG level after 3 week when re-tested. 2 cases of congenital deformity which were positive for IgM antibody did not show significant rise in IgG levels when re-tested after 3 weeks (Table 3).

TABLE 1

Distribution of age and toxoplasma specific IgG seroprevalence in control group*

Controls	No Studied	0-15 Yrs	16-30 Yrs	31-45 Yrs	> 45 Yrs	Total
Healthy Volunteers	120	2/20 (10)	7/52 (13.5)	8/30 (26.7)	6/18 (33.3)	23/120 (19.1)
Antenatal cases without BOH	80	—	10/63 (15.9)	5/17 (29.4)	—	15/80 (18.7)
Total control	200	2/20 (10)	17/115 (14.8)	13/47 (28.5)	6/18 (33.3)	38/200 (19)

*Figures in parenthesis indicate percentage.

TABLE 2

Age wise distribution of toxoplasma specific IgG antibodies in various patient groups

Patient group	Total cases	0-15 yrs	16-30 yrs	31-45 yrs	> 45 yrs	Total
Bad obstetric history	80	0	19/63 (30.1)	7/17 (41.2)	—	26/80 (32.5)
Seizure/encephalitis	50	0/5	4/24 (16.7)	3/10 (30)	5/11 (45.5)	12/50 (24)
Lymphadenopathy	30	2/7 (28.6)	5/12 (41.6)	1/5 (20)	0/6	8/30 (26.7)
Congenital deformity	18	6 (33.3)	—	—	—	6/18 (33.3)
Chorioretinitis	12	0	1/5 (20)	2/5 (40)	0/2	3/12 (25)
HIV +ve	80	0	9/38 (23.7)	7/34 (20.6)	2/8 (25)	18/80 (22.5)
Immuno suppressed	30	1/3 (33.3)	2/15 (13.2)	0/5	2/7 (28.6)	5/30 (16.6)

*Figures in parenthesis indicate percentage.

TABLE 3

Antitoxoplasma IgM positivity in various patient groups

Patient group	No	Age (yrs)	IgM value (EU/ml)	IgG Value (EU/ml)	IgG levels (after 03 weeks)
Bad obstetric history	2/80	32	189.6	32.6	297.6
Seizure disorder	1/50	52	189.4	48.6	391.4
Cervical lymphadenopathy	1/30	18	165.2	59.8	315.6
Chorioretinitis	1/12	62	256.0	66.9	428.0
Children with congenital malformation	2/18	01	146.0	200.6	236.1
		0.5	122.2	157.0	206.2

Discussion

Toxoplasmosis being a treatable condition is not a major public health problem. However, for an individual it can have deleterious and sometimes disastrous consequences as in pregnant women and in immuno-compromised host. In the present study toxoplasma specific IgM and IgG antibody levels were assayed by ELISA, which is one of the standard procedures for detection of antibodies [1]. Detection of both IgG and IgM simultaneously helps in establishing exposure to *T gondii* and the chronological status of such exposure. In the present study the control groups of 200 individuals were tested for both IgG and IgM antibody. None of them showed IgM positivity. 38 out of 200 (19%) from the control groups tested positive for antitoxoplasma IgG antibody. There was no significant difference in the prevalence rate of the healthy antenatal group and that of healthy volunteers (18.7% and 19.1% respectively). This prevalence rate is in agreement with that of various workers in India as well as abroad [2-5]. Highest seroprevalence rate in India was 57% as reported by Nautyal from the Kumaon Region [6]. This may be attributed to geographical distribution, variance in type of population studied and different dietary habits. This study showed rise in trend of sero positivity with rise in the age (10% in < 15 years and 33.3% in > 45 years of age) except in one case of lymphadenopathy. The rise in sero-prevalence with age is attributed mainly to increased exposure to the parasite. Similar trend has been noted in USA [7]. Bad obstetric outcome (patients with history of repeated abortions, still births and giving birth to babies with congenital abnormalities) is one of the commonest presentations of toxoplasma infection during pregnancy [2]. In this group 26 cases out of 80 (32.5%) were found to be positive for anti-toxoplasma IgG antibody which was significantly higher than that seen in the control group ($P < 0.05$). This finding is in agreement with various workers from India and abroad [1,8-11]. In all studies the sero-prevalence was higher than the age matched controls. 2 cases of BOH tested positive for IgM (Table-3) and also had a four-fold rise in titre of IgG when tested after 3 weeks interval, suggesting that they had acute infection. 1 of the 2 patients had a history of giving birth to a child with congenital malformation earlier. Both were treated for toxoplasmosis but the outcome of the pregnancy could not be ascertained as the patients were lost to follow up. Anti toxoplasma IgM positivity has been reported to be as high as 20% in mothers giving birth to babies with congenital abnormality [11]. But in this study it was

positive in 2.5% of cases. No method was adopted to find out other causes like Cytomegalovirus, Rubella or Herpes virus infection. The incidence of foetal infection depends upon the time of infection in the mother during pregnancy, i.e. 10-25% in first, 30-54% in second and 60-65% in third trimester. Rarely transmission may occur in immunocompetent women who acquire acute infection 6-8 weeks before conception [12]. It is important to detect acute infection in pregnant women as the outcome of the pregnancy depends on the time of infection. A woman acquiring infection during pregnancy has to be treated preferably with spiramycin till term to prevent a bad obstetric outcome. Once acquired, maternal infection is confirmed by serological tests. It is also advisable to detect foetal infection by polymerase chain reaction (PCR) carried out on amniotic fluid obtained by amniocentesis after 18 weeks of pregnancy. Once foetal infection is confirmed then decision regarding drug therapy with (pyrimethamine and sulpha) or termination of pregnancy can be taken [1]. Various foetal abnormalities such as microcephaly, hydrocephalus, cerebral calcification and growth retardation are likely to occur in babies born to infected women. In this study out of 18 children with congenital deformity (cerebral calcification, hydrocephalus, microcephaly and mental retardation), 6 (33.3%) were positive for IgG and 2 (11.1%) were positive for IgM antibody to *T gondii*. All the IgG positive cases were more than two years of age. In such cases, detection for IgM antibody need not signify congenital infection as a child during postnatal life can get infected by *T gondii*. However, since all the 6 cases were born to IgG positive mothers, it was reasonable to assume that they had acquired infection congenitally as, by that age, maternal IgG would have disappeared. 2 cases positive for IgM antibody were probably harbouring persistent infection. When compared to age matched controls odds ratio was very high (< 0.05). In India, congenital malformation has been noted in 13.4% babies born to infected mothers [13].

3 out of 12 cases of chorio-retinitis tested positive for IgG antibody to *T gondii*. 1 was positive by IgM ELISA and repeat serum testing showed more than four-fold rise in IgG levels suggesting an acute infection. Acute infection in an immunocompetent individual can manifest as chorioretinitis and is usually seen after 40 years of age whereas congenitally acquired chorioretinitis manifests before 40 years [1]. Our patient was an elderly man of 62 years and had ophthalmoscopic findings suggestive of toxoplasma chorioretinitis. Serological study confirmed acute ac-

quired infection. High serum IgG values are not usually observed following ocular toxoplasmosis and demonstration of high antibody titre in aqueous humor has been found to be of more value [14]. However in this study no aqueous humor has been tested.

The high incidence of toxoplasma infection as a cause of encephalitis / seizure disorder is of concern in HIV positive and immunosuppressed cases. In this study, 12 out of 50 patients (24%) with CNS involvement (all HIV negative) showed positivity for IgG which is higher than the control population. The incidence of toxoplasma encephalitis has been found to be low (3.6%) in immunocompetent population [15]. However, since in most of the adult population toxoplasmosis represents reactivation of the latent infection, toxoplasmosis especially in immuno-compromised individuals assumes greater significance. In this study only 1 patient out of 50 (2%) was diagnosed to have toxoplasma encephalitis. The low prevalence is mostly attributed to immunocompetence of the host.

Our study also included asymptomatic HIV infected cases and immunocompromised patients (post renal transplant cases on immunosuppression) as a part of descriptive study. The two conditions did not represent the outcome of *T gondii* infection. Such conditions are known to lead to reactivation of infection [1]. In this study 18 out of 80 HIV infected patients (22.5%), showed positivity for IgG antibody and none for IgM class. Among the immunosuppressed group, only 5 out of 30 (16.6%) cases were positive for IgG antibody with no IgM positivity. The present study indicates sero-prevalence to be lower than healthy volunteer control group. Similar findings have been published by other workers [16,17]. The prevalence rate in HIV positive cases was not significantly higher than in the control groups (22.5% versus 19%). This was due to inclusion of only asymptomatic HIV infected cases suggesting early stage of infections. They had seroprevalence comparable to the control population. Such patients would not have developed immunosuppression in the early stage and therefore showed seroprevalence comparable to control group.

The serum value of parasite specific IgG, IgM and IgA may remain low in immunocompromised patients. Therefore, a panel of tests with overlapping sensitivity may be required to identify *T gondii* infection accurately. Though, anti-toxoplasma IgG is elevated and tends to be stable in acute toxoplasmosis, IgM antibody may be rarely present [18]. Hence a panel of tests (*T gondii* serologic profile) consisting of the Sabin- Feldman dye test, IgM, IgA and IgE ELISAs, IgE immunosorbent agglutination assay and the dif-

ferential agglutination tests should be carried out to determine infection in these patients [1]. Toxoplasma antigen detection by double sandwich EIA and parasite isolation by animal inoculation can also be employed for diagnosis of acute toxoplasmosis in immunocompromised patients [18]. Those HIV infected patients who are seronegative for toxoplasma infection have to be educated regarding prevention of toxoplasma infection. Periodic follow up for anti-toxoplasma IgG to detect seroconversion in these patients has to be carried out on annual basis. It has been shown that > 50% of HIV infected cases showing positivity for IgG are likely to develop toxoplasma encephalitis and these cases are to be monitored with CD4 count. Prophylaxis should be started when the CD4 count falls below 200/cmm [1].

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