

Seroepidemiological Survey of *Toxoplasma* Infections of Mentally Retarded Children

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Abstract

Toxoplasmosis is a widespread infection in the world. Although the infection by *Toxoplasma gondii* is widely prevalent in humans and animals, the disease is uncommon and most of the acquired infections are asymptomatic. The important aspect of this parasitic infection is the probable danger of congenital transmission and its severe effects on the fetus. In the present descriptive study, a total of 353 samples from mentally retarded children and adolescents in rehabilitation centers in Tehran, were examined from 2001 to 2002. In order to determine the *Toxoplasma* antibodies, the samples were tested using an IFA technique and in some cases ELISA technique was applied as well. Fourteen percent of the examined samples were positive. In this research *Toxoplasma* antibody titer in 36 cases were reported as 1:100 and it was found to be 1:200, 1:400 and 1:800 in 9, 1 and 3 cases, respectively. In three cases for which the titer was reported 1:800, samples were tested applying Ig-M ELISA technique in order to determine if there was an acute infection but all tests were negative. In the current study, we did not find any significant differences in the prevalence rate between the genders, but the prevalence significantly increases with age. We used standard questionnaires for causative relation in congenital toxoplasmosis within sero-positive children and 3 cases of infection were documented during the mother's pregnancy period.

Keywords: *Toxoplasmosis, Mentally retarded children, Seroepidemiology, Iran*

Introduction

As an obligate intracellular parasitic protozoan, *Toxoplasma gondii* can infect birds and a large proportion of the world's human population, and mammals, yet it is an uncommon cause of disease (1). Cats are the final host and variety of birds and mammals are intermediate host (2). *T.gondii* infection is acquired by ingestion of either oocysts extracted by felines or tissue cysts in raw or undercooked meat (2).

Toxoplasmosis is usually asymptomatic in adults, but the infection acquired during pregnancy may cross the placenta and cause irreparable harm and injuries to fetus and infant. In some cases, abortion is observed and some-

times the infection may lead to severe sequelae which are observed during infancy (3).

Mental retardation is seen in 16% of cases (1) and in some regions, 9% of mental retardation is attributed to toxoplasmosis (4). In infected infants the most observed symptoms are chorioretinitis, blindness and low IQ (5).

Materials and Methods

This descriptive study was designed to determine toxoplasmosis in mentally retarded children in rehabilitation centers of Tehran, in which, seven centers were selected as the study centers. These centers were as follows: Rofaydeh, Vardavard, Narmak, Tehran, Novin, Ar-

maghan and Farkhondeh. Rofaydeh, Vardavard and Narmak are governmental centers. The studied samples comprised 353 mentally retarded cases and blood sampling was performed. In the study population there was an age limit and 34.56%, 63.45%, and 1.99% of the study population were in the age group of 0-9, 10-19 and 20-29, respectively. In our study, 90.65% of the population was under 16; about half of the population (47.87%) was children under 11 years. Serum samples were tested for anti-*Toxoplasma* antibody by IFA technique, and in some cases ELISA technique was applied as well. In this study, for IFA technique Fluotox Kit and Fluorescein conjugated anti-human globulin produced by Baharafshan company and for ELISA technique a *Toxoplasma* IgM ELISA Kit (for 96 tests) with serial number GD81 produced by an English company called Genesis Diagnosis were used. Routine IFA technique was applied as follows: 10 ml of diluted serum samples (1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200) was added to *Toxoplasma* antigen. After being incubated at 37°C for 30 min, using PBS the slides were washed. Then Conjugated anti-human globulin diluted by PBS (1:20) was added to the complex. After being incubated at 37°C for 30 min and being washed, the slides were examined under a fluorescent microscope. Positive samples were seen as fluorescent green.

Using the kit brochure ELISA technique applied in the study is as follows:

Serum samples were diluted (1:100), then negative control, cut-off calibrator, positive control and diluted serum were all poured into wells (100µl each). Strips were then incubated for 20 min in an incubating bag in room temperature.

Using diluted buffer, wells were washed three times and HRP (Horse Radish Peroxidase) conjugated anti-human IgM was added to each well (100µl), incubation and washing process was repeated then 100µl of TMB (tetramethyl benzidine) was applied as enzyme substrate. After being incubated for 10 min in room temperature positive samples turned into blue.

OD < cut-off OD → Negative samples

OD > cut-off OD → Positive samples

We used a questionnaire in order to obtain some information regarding the mothers of sero-positive children and to detect the congenital cases. All the important pregnancy events such as: consumption of raw or undercooked meat or vegetables, having contact with cats, birds and other domestic animals were recorded and information about the familial pattern of the mental retardation and its background were also gathered using this questionnaire.

Results

According to the study results, 13.89% of the cases were seropositive. Table 1 shows the distribution of *Toxoplasma* antibody titer in mentally retarded cases using total/IFA.

Toxoplasma antibody titer was reported as 1:100 in 36 cases and it was detected 1:200, 1:400 and 1:800 in 9, 1 and 3 cases, respectively. In three cases in which the *Toxoplasma* antibody titer was reported 1:800, serum samples were further tested for specific IgM, applying ELISA technique and these cases were IgM negative. The negative results were confirmed using a second test performed after three weeks.

Table 2 shows both the infection percentage in different age groups undergone the survey and the distribution of the infection according to sex. In this study, 22 cases of the test positive patients (44.9% of the positive cases) comprised female and 27 of the positive cases (55.1% of the positive cases) were men. We did not find significant difference in the prevalence rate between the sexes, but the prevalence significantly increased with age since the possibility of having contact with the parasite increases with age. According to the result and as it is observed in Table 3, Narmak is considered the center with the highest infection rate while the lowest infection rate is attributed to Tehran center.

Table 1: Distribution of *Toxoplasma* antibody titre by T/IFA in mentally retarded cases in rehabilitation centers in Tehran.

Percentage	Frequency	Titer
10.20	36	1:100
2.55	9	1:200
0.29	1	1:400
0.85	3	1:800
13.89	49	Total of Positive
86.11	304	Total of Negative
100.00	353	Total

Table 2: Positive cases in different age groups and genders

Age group	Positive cases	Positive cases in men	Positive cases in women	Total cases
0-9	14	9	5	122
10-19	32	16	16	224
20-29	3	2	1	7
Total	49	27	22	353

Table 3: Distribution of prevalence rate of *Toxoplasma*-infection according to different rehabilitation centers in Tehran

Center	Total Cases	Positive Cases	
		Number	%
Rofaydeh	80	11	13.50
Vardavard	80	11	13.50
Narmak	40	9	22.5
Tehran	40	3	7.5
Novin	23	5	21.6
Armaghan	37	5	13.51
Farkhondeh	53	5	8.43
Total	353	49	100

Discussion

As the results indicate, the infection rate was reported 13.89% which was the prevalence rate in the study population. In this survey, in three cases that *Toxoplasma* antibody titer was reported 1:800 using IFA technique, we performed IgM ELISA technique to detect the acute cases, although IgM was negative both in the first and the second samplings performed in a 3 week interval. Thus, there has not been any acute infection (recent acquisition of the infection in rehabilitation centers) in this study. Therefore in three mentioned cases the high *Toxoplasma* antibody titer does not prove the acute infection and the samples should be tested for specific IgM. Narmak and Tehran were reported as the centers with the highest and lowest infection rate respectively. Because of the high rate of cat exposure in Narmak center's children dormitories, high infection rate in the center is explicable. In Rofaydeh and Vardavard centers (with the infection rate of 37.5%) cat exposure was reported as well.

Since congenital toxoplasmosis is one of the most important teratogenic factors which may cause mental retardation, in this study we decided to look for a cause and effect relation between congenital toxoplasmosis and mental retardation. Therefore we selected younger children as the study population because the younger children are easier to be followed up retrospective pregnancy of their mothers. But unfortunately we faced the problem of age limitation and in many cases, because of convulsion drug consumption and illness blood sampling was inhibited, so we had to conduct the survey on older groups. Although the only possible way to confirm a congenital case is to prove that mother acquired the infection during the pregnancy. In this study most children were orphan and in some cases mothers were not willing to take part in the interviews and just in few cases we succeeded in gathering the information from the mothers.

Considering the previous statement, we interviewed mothers of 15 seropositive children and three of them remembered the infection during pregnancy, and in each the children titer was less than 1: 200.

As there is no history and evidence for serological tests of the mothers, we can not conclude that these 3 children had congenital toxoplasmosis.

In China, Wen et al evaluated 95 pregnant women with positive IgM antibodies and followed up their children for 12 months and concluded that congenital *Toxoplasma* infection is closely correlated with abnormal pregnancy outcomes and infantile mental retardation. Early intervention can improve intelligence development (6).

In Brazil, Caiaffa et al compared the prevalence of *T.gondii* infection between mentally retarded children and normal controls by designing a case-control study, and concluded that the congenital toxoplasmosis, in its sub clinical form, appears to be an important component in the etiology of mental retardation, especially in high risk (lower socio-economic) groups (7).

Ameri et al evaluated the prenatal infections in Egyptian children with unknown cause of mental retardation, based on their study results, specific toxoplasmosis IgG antibodies were 43.75% for MR cases and 37.5% for their mothers. Six (18.75%) mothers gave history of eating raw meat and canned food, while 8 (25%) had pet cats (8).

According to this study and considering the international knowledge information, we recommend further studies with a Cohort or Case-Control design to be able to establish a significant causative relation and in order to obtain more details of this rare but important health problem.

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