Original Article

Toxoplasma gondii Infection in Neonates

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(Received 5 Apr 2007; accepted 26 Jun 2007)

Abstract

Background: To study toxoplasmosis in neonates using PCR and serological methods.
Methods: Sera and CSF of 104 neonates, hospitalized in infants' ward of Taleghani Hospital, Tehran, Iran were examined. The sera were examined for anti *Toxoplasma gondii* lgM and lgG specific antibodies with ELISA and IFA techniques, respectively. Meanwhile, obtained CSFs of the cases were evaluated for the genome of this parasite by PCR technique.
Results: Results showed positivity in 7 neonates (6.73%) which suggested congenital toxoplasmosis. Results of PCR were positive in 6 neonates (5.77%). The 1/100 titer of lgM specific antibodies was positive in 5(4.81%) of them by IFA technique and 6 neonates (5.77%) had positive results by ELISA technique for IgM specific. The rate of mortality was %0.96. Forty one neonates had 1/200 titer of specific lgG antibodies by IFA technique and 38 neonates had positive results by ELISA technique for IgG antibodies. The prevalence of chronic toxoplasmosis in mother was 32.7% and 30% by IFA and ELISA techniques, respectively.
Conclusion: Toxoplasmosis is still highly prevalent in neonates and should be considered due to the fact that suspected cases might be misdiagnosed and subsequently led to life– threatening or fatal condition.

Keywords: *Neonates, PCR, Serology,* Toxoplasma gondii*, Iran*

Introduction

Toxoplasmosis is a worldwide endemic disease. It is caused by the parasitic protozoan *Toxoplasma gondii*. In congenitally infected children and immunocompromised persons, it causes high rates of morbidity and mortality (1). Primary *T. gondii* infection in pregnant women can lead to parasite transmission to the fetus via the placenta. The risk of transmission increases during gestation (1). The clinical spectrum of fetal infection varies from fetal death or severe impairments of the central nervous system to sub clinical infection. The most severe consequences of fetal infection are most frequently observed in the rare cases of early maternal fetal transmission, whereas a large majority (85%) of infants appears normal at the birth as a result of late but more frequent transmission (2).
Serologic test are very important in the diagnosis of toxoplasmosis (3) and the standard toxoplasmosis diagnosis techniques are based on serology (1). Prenatal diagnosis of congenital toxoplasmosis may be made by detecting specific anti-*Toxoplasma* IgM antibodies in fetal blood (4) but congenital infections may be difficult to diagnose serologically because maternal IgG crosses the placental barrier and will appear and persist for several month in the circulation of the newborn. But since IgM antibodies do not cross the placenta, demonstration of anti-*Toxoplasma* IgM at birth or up to several months of age is presumptive evidence of congenital toxoplasmosis (3). Such antibodies are usually

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demonstrated by IFA (3) and ELISA tests, the most helpful techniques in the diagnosis of congenital toxoplasmosis (5) but their results are difficult to interpret for infants (1); additionally, the PCR technique has been used to detect a gene sequence specific for *Toxoplasma* in sample of amniotic fluid (4) and CSF (6).

As the morbidity and mortality of infants is very important in health of society, here we present the result of a study carried out on neonates hospitalized in Taleghani Hospital, Tehran, Iran during one year and estimated prevalence of *T. gondii* among them.

Materials and Methods

Sera and cerebrospinal fluid (CSF) of 104 neonates hospitalized in infants' ward of Taleghani Hospital, Tehran, Iran were tested by IFA, ELISA and PCR (CSF) techniques. The neonates' parents had given documented informed consent.

IFA test

Toxoplasma antigen (tachyzoite) was prepared in Pasteur Institute and sent to the Parasitology laboratory of Army University of Medical Sciences and fixed on slide (7). 1:100, 1:200 and 1:400 titers of sera were made to follow up IgG antibody and 1:100, 1:200 titers to follow up IgM antibody, then IFA test was done as described earlier (7).

ELISA test

This test was done on sera sample according to the 96 microelisa kit (Twinity Company). It contained the holder with a base covered by pure *T. gondii* antigen. The titers of sera used for ELISA/ IgG was 1:21 and for ELISA/IgM 1:41, subsequently. The ELISA reader was used and subsequently immune status ratio (ISR) was determined. If ISR was ≥ 1.1 meant positive, ≤ 0.0 was negative and from 0.91 to 1/09 the test had to be repeated.

PCR technique

The test was done on CSF samples. At first the DNA was extracted using proteinase K and lysis buffer, then materials of PCR was prepared as follows: PCR reaction contained 1 µg DNA, 20 pico mol each of forward and reverse primers, 3 mM MgCl2, 0.2 mM dNTP, 1X PCR buffer, 1.25 unit of Taq DNA polymerase (CinaGen, Iran) and dH2O up to 50 λ. The primers were corresponded to gene B1 nucleotides 694 to 714 and 887 to 868. PCR amplification was carried out with 30 cycles of denaturation at 94 °C for 1', annealing at 53 °C for 45"and extension at 72 °C for 45". PCR reaction was incubated at 94 °C and 72 °C for 5' before and after the PCR cycling, in that order. The product of PCR was electrophoresed in 2% agarose gel and visualized under UV light if revealed the 193 bp band, was positive.

If the PCR test was positive or the level of specific IgM antibody in sera was revealed high by the ELISA or IFA technique, it would be positive (6, 8) and if only the level of specific IgG antibody in sera was revealed high, after deletion positive cases from it, meant chronic toxoplasmosis between the mothers whose neonates was confirmed in hospital (9).

Results

The results of all three tests (PCR, IFA, ELISA) showed that 7 neonates were positive (%6.73) (Table 1).

Table 1: Prevalence of toxoplasmosis in neonate by the three techniques (PCR, IFA, ELISA)

	+		-	
	n	**(%)**	**n**	**(%)**
PCR	6	(5.77)	98	(94.23)
IFA	5	(4.81)	99	(95.19)
ELISA	6	(5.77)	98	(94.23)
Total	7	(6.73)	97	(93.27)

According to the result of IFA test 1:100 titer of specific IgM antibody had more value in detection of acute toxoplasmosis and 1:200 titer of specific IgG antibody had more coordination with the result of ELISA test in detection of toxoplasmosis infection (%39.42 ~ %36.54) (Table 2).The result of IgM antibody in ELISA test was the same as PCR test (%5.77) (Table 3).

Table 2: Prevalence of IgG and IgM antibody in neonates by IFA test

Antibody	**IgG**						**IgM**			
	1:100 titer		1:200 titer		1:400 titer		1:100 titer		1:200 titer	
Test	+	-	+	-	+	-	+	-	+	-
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
IFA	60	44	41	63	17	87	5	99	2	102
	(57.69)	(42.31)	(39.42)	(60.58)	(60.58)	(83.65)	(4.81)	(95.19)	(1.92)	(98.08)
Total	104		104		104		104		104	

Table 3: Prevalence of IgG and IgM antibody in neonates by ELISA test

Antibody	**IgG**		**IgM**	
	+	-	+	-
Test	n (%)	n (%)	n (%)	n (%)
ELISA	38 (36.54)	66 (63.46)	6 (5.77)	98 (94.23)
Total	104		104	

Discussion

Toxoplasmosis is definitely a subject of challenge in the field of infectious diseases that still need more efforts in its initial elimination and further steps should also be taken in eradicating this disease especially from Southeast Asian people (10). As a few studies have been reported on *Toxoplasma* seroprevalance in children, newborn or still births (10) this study was designed. The sera and cerebraospinal fluid of 104 neonates hospitalized in infants' ward of Taleghani Hospital, Tehran, Iran were tested by IFA, ELISA and PCR (CSF) techniques. Anyhow the seven neonates (%6.73) with congenital toxoplasmosis reported in this study and showed high rate of *Toxoplasma* infection in neonate.

Compared with other studies (1, 12) the higher number diagnosed in the present study may be due to using three techniques which increased the exactitude of the assay. Normally the diagnosis of congenital toxoplasmosis is based on serological demonstration of IgM antibody but may be specific IgM antibodies not present and antibody synthesis is delayed in infants, therefore we added PCR technique to increase the validity of results as was suggested earlier (1). It is mentioned that PCR for detection of *T. gondii* had high sensitivity and specificity (11).

Overall, one neonate died (%0.96) and in addition the evidence of congenital toxoplasmosis is not well documented in this group (10) therefore it should be more importantly given an attention due to the fact that suspected cases might be misdiagnosed and subsequently led to life- threatening or fatal condition.

No neonates show the characteristic of severe clinical damage as mentioned in other studies (1, 11, 12); most infected newborn babies do not have overt signs of disease at birth.

Forty one neonates had 1:200 titer of specific lgG antibodies by IFA technique and 38 neonates had positive results by ELISA technique for IgG antibodies (Table 2, 3) and if the number of infected neonates were eliminated the prevalence of chronic toxoplasmosis in mother was respectively %32.7 and %30 by IFA and ELISA techniques.

To evaluate the different titer of antibodies in IFA technique in the present study and according to the results the 1:100 titer of IgM antibody in IFA technique is more valid than 1/200 titer in diagnosis of acute toxoplasmosis and the result of 1:200 titer of IgG antibody in IFA technique is closer to the result of IgG antibody in ELISA technique for detection of chronic toxoplasmosis but as whole,

the results of IFA technique show less validity in detection of acute toxoplasmosis in comparison with the other techniques and it is confirmed by other studies (5).

Conclusively, to prevent primary *Toxoplasma* infection during pregnancy, women should be tested for *Toxoplasma* serological status, which can reduce the possibility of congenital toxoplasmosis.

Acknowledgements

This study was supported by and performed in Army University of Medical Sciences and the infant ward of Taleghani Hospital, Tehran, Iran.

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