Return to contents page

DETECTION OF TOXOPLASMA GONDII IN DEAD FETUSES BY POLYMERASE CHAIN REACTION (PCR)

M. Assmar, A. Terhovanessian, H. Fajrak, S.R. Naddaf

Department Of Parasitology, Pasteur Institute of Iran, Tehran

- **ABSTRACT**

Background: Mouse inoculation and tissue culture employed in the earlier studies of toxoplasmosis, although specific and sensitive, are rather time-consuming and may require up to six weeks to reach a diagnosis.
Objective: To use polymerase chain reaction (PCR) as a reliable alternative method in the detection of *Toxoplasma gondii* organisms in tissue samples.
Methods: This method relies on the detection of B1 gene which is unique for *T. gondii*. After preparing the samples, DNA was separated using centrifugation, then, by application of primers and DNA polymerase, amplification was performed.
Results: PCR detected the presence of *T. gondii* in about 20% of formalin-fixed tissue samples from dead fetuses aborted within four months of gestation.
Conclusion: PCR can be considered the method of choice, particularly when the parasites are already destroyed by preservatives or their viability is adversely affected by suboptimal storage and transportation conditions.

Irn J Med Sci 2000; 25(1&2): 59-61

Key Words • Toxoplasmosis, congenital• fetus • polymerase chain reaction

Introduction

Toxoplasmosis, an infectious disease of worldwide distribution, is caused by the protozoan *Toxoplasma gondii*.

In Iran, about 51.8% of the population are estimated to have antibodies against the infection.[1] One-third of primary toxoplasmosis cases occurring during pregnancy lead to transplacental transmission and involvement of the fetus with devastating sequelae such as microcephaly, hydrocephaly, blindness, calcification of brain cells and even death in utero.[2] Former attempts to detect *T. gondii* in tissue samples including aborted fetuses were based on mouse inoculation and tissue culture techniques.[3-5] These methods though sensitive and specific are time-consuming, and require

up to six weeks to obtain a diagnosis.[6] PCR by means of primers selected either from B1 or P30 genes of *T. gondii* provides a highly sensitive and specific method for the detection of the infection.[7,8] This method provides a sensitivity of 10 genome equivalent in the presence of 10^5 human leukocytes.[7]

In this study, the 35-fold repetitive B1 gene (coding function unknown) of *T. gondii* was targeted to detect the infection in dead fetuses. So far, this gene has been detected by PCR in all 21 *Toxoplasma* strains tested, yet it is undetectable in DNA of closely related organisms and humans.[7]

Materials and Methods

Preparation of samples for PCR

Formalin-fixed specimens were cut and washed in NaCl several times. The specimens, were then homogenized in lysis buffer (200 mM NaCl, 10 mM Tris-HCl [pH 8], 10 mM EDTA and 10% sodium

dodecyl sulfate [SDS] with 100 µ g/ml proteinase K). Incubation took place at 55 ?C for one hour or at 37 ?C overnight, followed by extraction with phenol/chloroform and chloroform (Applied Biosystems, CA, USA) successively (with centrifugation at3000 rpm for 3 min at each stage). To precipitate the DNA, the supernatant was recovered and after adding 3 volumes cold 95% ethanol was kept overnight at –20?C and centrifuged at 14000 rpm for 10 min. The pellet was resuspended in 20 µ l of TE buffer (1 mM EDTA and 10 mM Tris-HCl [pH 8]) and volumes of 1 µ l were tested for the presence of *T. gondii* (V. Bakayev personal communication).

DNA Amplification

Amplification of *T. gondii* was carried out in 30 µ l of reaction mixture containing 10X buffer [160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8), and 0.1% Tween 20], 3 mM $MgCl_2$, 200 µ M of each dNTP, 20 pmol of each primer and 1 U of taq DNA polymerase (Pharmasia Biotek, Department of Biotechnology, Pasteur Institute of Iran). The primers were5'-AAAAGAGGAAGAGACGCTGCCGCTG-3' (position 572 to 596, primer 3), 5'-GCCATTTTCTGAGCATCCCTTCCG-3' (position 1137 to 1160, primer 4), 5'-GGAACTGCATCCGTTCATGAG-3' position 694 to 714, primer T1) and 5'-GGCGACCAATCTGCGAATACACC-3' position 853 to 931, primer T Oligo). All primers were located on B1 gene and amplification was performed during two stages. Primers 3 and 4 were added at the first stage and the reaction mixture was overlaid with two drops of mineral oil to prevent evaporation. Thirty cycles of amplification were performed in an automated PCR thermocycler (Techne, NJ, USA). Each cycle consisted of 1 min of denaturation at 94? C, 45 sec of annealing at 53? C and 45 sec of extension at 72 ? C. The first denaturation, and the final extension steps, continued for an additional 4 and 5 mins, respectively. The PCR products were then diluted to 1:10 in double distilled water. The second primer sets (T1 and T oligo) added and re-amplification was performed with the same program. Each thermocycler was run with two controls; a positive control containing *T. gondii* DNA and a negative one with distilled water only.

PCR Optimization

To optimize PCR technique three aborted fetuses, one belonging to a woman whose serum proved to be positive for toxoplasmosis both by IFA and ELISA an increasing IgM titer, and the other two belonging to women whose sera showed no sign of sero-conversion against toxoplasmosis, were tested for *T. gondii* using both PCR and mouse inoculation techniques. The former sample turned positive with both methods and the parasite was isolated, while the latter two were negative with both methods. According to these results, inoculation of laboratory animals and isolation of the parasite was considered the gold standard, and given the fact that the PCR technique is of 100% sensitivity and specificity, the samples were tested using only PCR technique.

Detection of PCR products

Twenty microliter volume of PCR products were analyzed by electrophoresis on a 1.5% agarose gel (containing 0.5 µ g/ml ethidium bromide) in 1X TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA) and photographed under an ultraviolet transluminator. PCR product sizes were compared with a PBR322-*Hin*fI size marker (Pharmacia Biotech) and products with the exact same size, as those in the positive controls (about 160-base pairs), were regarded positive for *T. gondii.*

Results and Discussion

Seven out of 35 dead fetuses turned out to be positive for *T. gondii* by PCR method (Fig. 1), and the method appeared to be quite successful in detection of parasites in formalin-fixed tissue samples. Considering the poor viability of *T. gondii* outside the host, and the fact that the suboptimal conditions may adversely affect the mouse inoculation and cell culture results, PCR can be considered as a valuable tool for diagnosis of *Toxoplasma* infection when the specimen is of dubious condition or subjected to preservative materials usually causing destruction of viable *T.gondii* organisms.

Acknowledgement

We gratefully thank Valery V. Bakayev for his valuable advice in the initial stage of this work. We also thank all our colleagues from the Parasitology Department for their collaboration

References

1. Assmar M, et al: Toxoplasmosis in Iran, results of a seroepidemiological investigation. *Bull Soc Path Ex 1997;***90:**19-21.
2. Desmont G,Couvreur J : Congenital toxoplasmosis: a prospective study of 378 pregnancies. *N Engl J Med 1974;***290:** 1110-6.
3. Ghorbani M, Farzaneh I, Vashtani H, Rezaian H: Congenital toxoplasmosis in Iran. *Irn J Publ Hlth 1977;***8(1):**52.
4. Ghorbani M, Hafizi A, Sanati A, Modares Musavi H: Isolation of *Toxoplasma gondii* from human tissues. *Irn J Publ Hlth 1979;***6(1):**51-2.
5. Change CH, et al: Isolation of *Toxoplasma gondii* in tissue culture. *J Pediatr 1972;***81:**790-1.
6. Desmonts G, et al: Prenatal diagnosis of congenital toxoplasmosis. *Lancet 1985;***i:**500-4.
7. Burg JL, Grover CM, Pouletty P, Boothroyd JC: Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii,* by polymerase chain reaction. *J Clin Microbiol 1989*;**27:**1787-92.
8. Burg JL, et al: Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii.J Immunol 1988;***141:**3584-91.

Return to contents page