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ORIGINAL ARTICLE

Molecular Detection of T. Gondii DNA by Polymerase Chain Reaction in Blood Samples of Pregnant Women Attending Tertiary Care Unit in Islamabad, Pakistan

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ABSTRACT

Background and Purpose: Toxoplasmosis is caused by Toxoplasma gondii and is mainly diagnosed through serological methods, but the sensitivity is insufficient. When it fails, one must rely on direct detection of parasites or DNA testing via polymerase chain reaction (PCR). Our goal is to establish a molecular tool for domestic toxoplasmosis research through PCR technology targeting the B1 gene and compare it with ELISA results.

Design and Setting: This study was conducted at the Rawal Institute of Health Sciences, Islamabad between May 2023 and July 2023 on pregnant women attending Rawal General Hospital, Islamabad.

Patients and Methods: Peripheral blood samples were collected from patients (n=137). DNA was extracted and the B1 Toxoplasma gondii gene was amplified by PCR. Amplicons were visualized and sequenced, and the results analyzed. For comparison, anti-T. gondii IgG and IgM in serum were measured by enzyme-linked immunosorbent assay (ELISA).

Results: Among the 137 samples tested, 56 (41%) had B1 gene amplification detected by PCR. DNA sequencing confirmed these results. The IgM-ELISA test detected 9 of these cases (6.5%). Immunoglobulin G test results were positive in 53 patients (38.6%).

Conclusion: This study demonstrates the need for PCR as a confirmatory test in addition to serological testing to detect recent infection. We recommend nationwide implementation of these molecular diagnostic tools.

Keywords: T. Gondii DNA, Polymerase Chain Reaction, Pregnant Women, PCR.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite. It is a coccidioidal parasite that uses cats as a definitive host and affects all warm-blooded animals (including humans) except felines[3]. Although T. gondii can cause congenital toxoplasmosis in fetuses and newborns[5,6]. Infection may cause congenital toxoplasmosis, disease or subclinical infection. Globally, the prevalence and incidence of infections vary by population group and geographical location[7]. This may be related to various factors such as culture, dietary habits, age and hygiene practices. Some studies have shown differences in seroprevalence in different regions of the country. These studies showed that IgG prevalence ranged from as low as 25% to as high as 42.1%

Serological diagnosis is the most commonly used method to identify the stage of infection (current, new or previous infection).PCR-based techniques can be used when serological tests are unreliable or the clinical diagnosis is questionable. It saves time and manpower and has the advantages of high sensitivity and strong specificity. PCR has been used to demonstrate the presence of Toxoplasma gondii in a variety of clinical samples: brain[19], whole blood[20], amniotic fluid, cerebrospinal fluid[19], aqueous humor, and lymph nodes. PCR is necessary for the diagnosis of Toxoplasma infection in patients receiving immunosuppressive therapy. Various PCR-based methods have been developed and have significantly improved diagnosis, especially for congenital toxoplasmosis. Current PCR tests for Toxoplasma gondii target two main loci. The first one is B1 with 35 repetitions. Antigen P30.[21] Here again a different primer set was designed.

PATIENTS AND METHODS

The research was conducted at Rawal General Hospital, Islamabad. This study was approved by the institutional board review committee of Rawal Institute of Health Sciences (IBR), Islamabad. 137 pregnant women attending the hospital were selected for the study after written consent from women. 1-2 mL venous blood sample from each patient (n=137) was collected into EDTA tubes and transported to the microbiology laboratory. Positive controls were obtained from genomic DNA extracted from the brains of mice experimentally infected with RH strains. A negative control is a PCR reaction without a DNA template that is always included when performing the PCR reaction. Patients are considered to have acute infection when 2-fold dilutions of acute and convalescent sera are tested simultaneously by enzyme-linked immunosorbent assay (ELISA).

DNA isolation and PCR of Toxoplasma gondii B1 gene: DNA was isolated using a commercial purification system (Qiagen, Valencia, CA, USA) following the manufacturer's instructions for purifying DNA from blood. The resulting DNA pellet was resuspended in 30 µL of TBE buffer (10 mM Tris, 1 mM EDTA, pH 7.2). Primers were manufactured by Gibco BRL Life Technologies (Basel, Switzerland). The PCR method used here was adapted from Burg et al. Developed in 1989, he used the B1 gene as a target. PCR mix below. PCR mix below. In the first and second round of PCR, 0.5 µM primer, 200 µM dNTP, 1.5 mM MgCl2, 1.5 units of Amplitaq polymerase and 10 µL DNA template were used in a total volume of 50 µL. Use 10 µL of the first round DNA template and a total volume of 50 µL. The primer sequence, expected size of the PCR product, and PCR conditions for B1 gene amplification are shown in Table 1.

PCR amplification of DNA from pregnant women usingT.gondiiB1 specific primer

Primer specificity was tested. External and nested B1 amplification reactions were performed using genomic DNA from pregnant women human samples .. The positive control consisted of Toxoplasma gondii genomic DNA. PCR was also performed on blood samples taken from study participants (n=137). Gel electrophoresis was performed on a 2% agarose gel containing 1X Trisborate-EDTA running buffer. After cooling, add ethidium bromide to a final concentration of 0.5 µg/mL. DNA was mixed with 6X DNA loading buffer and loaded onto the water-soaked gel. Electrophoresis was performed in TBE at 80 to 120 volts. Observe

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and photograph the fluorescence of ethidium bromide bound to DNA under short wavelength UV light.

Enzyme-linked immunosorbent assay: Anti-T. gondii IgM and IgG antibodies were detected in patient sera using commercially available ELISA kits (Human GMBH, Wiesbaden, Germany) according to the manufacturer's instructions. Optical density (OD) was measured using an ELISA plate reader (Humareader, Germany) at dual wavelengths of 450 nm and 630 nm. Amount of anti-T. gondii IgM and IgG in the patient's serum were calculated according to the standard curve using the built-in software of the ELISA plate reader in point-to-point mode.

Table1: Primers molecular weight and PCR condition for B1 gene.

Primers	Sequence (5'-3')	Size (bp)	PCR condition
First round		200	950 ^{0}C for 5 min followed by 40 cycles
	5-'GGAACTGCATCCGTTCATGAG-3'		
	5-'TCTTTAAAGCGTTCGTGGTC-3		
Second round		100	950 ^{0}c for 5 min followed by 30 cycles
	5-'TGCATAGGTTGCAGTCACTG-3'		
	5'-GGCGACCAATCTGCGAATACACC-3		

RESULTS

As shown in Figure 1A, after the first round of PCR, the positive control produced the expected band of approximately 200 bp, and the negative control lane had no band. The second round of PCR produced the expected band (approximately 100 bp) as shown in Figure 1B. Specificity testing showed that only genomic DNA isolated from T. gondii was amplified, whereas DNA from human, fungal, and bacterial sources was not amplified. Only the positive control (T. gondii DNA) was amplified (Figure 1C). The presence of human DNA in the sample does not prevent amplification of the 200 bp B1 PCR product of Toxoplasma gondii DNA.

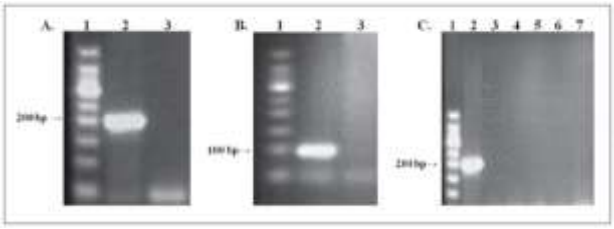

Figure 1: Amplification of the Toxoplasma gondii B1 gene by PCR.

A First round of PCR using an external primer pair targeting the B1 gene.
B second round of PCR using an internal primer pair targeting the B1 gene.
C The gel shows a positive control band but no band in the DNA sample. Lane 1, molecular size marker; lane 2, positive control with expected molecular weight; lane 3, human gDNA; lane 4, Candida albicans DNA; lane 5, M. fumigatus DNA; lane 6, Staphylococcus epidermidis DNA; lane 7, E. coli DNA.

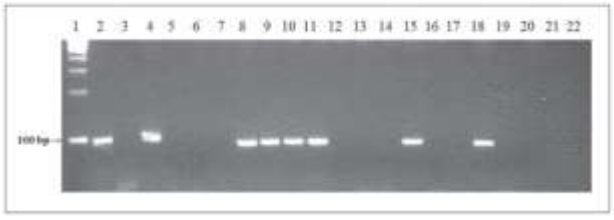

Figure 2: The gel shows a band for the positive control (lane 1) but no band for the negative control (lane 2). Lanes 3 to 22 show representative samples obtained from pregnant women in the study. After the second round of PCR, the positive sample produced a band of the expected molecular size (100 bp). PCR products (15 µL) were resolved on 2% agarose/TBE gels and visualized under UV illumination after ethidium bromide staining.

PCR method to detect Toxoplasma gondii DNA in pregnant women: After the second round of PCR, the positive sample gave a band of the expected molecular size (100 bp) (Figure 2). PCR results showed that Toxoplasma gondii DNA was found in 41% of patient samples (56 of 137 cases). When compared with the Toxoplasma gondii database (www.toxodb.org) and the National Center for Biotechnology Information (NCBI) database, all sequences were shown to be of Toxoplasma B1 gene origin.

PCR, IgM and IgG results: In the IgM ELISA test, 9 (6.5%) of the cases were determined to be immunoglobulin M positive and 128 (93.5%) were negative. The immunoglobulin G test result was positive in 53 cases (38.6%) and negative in 84 cases (61.4%). When PCR and ELISA tests were compared, it was seen that 8 and 32 blood samples were positive for PCR, IgM and IgG ELISA and there was agreement (91% and 58%, respectively). On the other hand, PCR detected 27 (49%) positives that were not detected by IgG ELISA (Table 2).

DISCUSSION

Various PCR-based tests have been formulated for the detection of T. gondii DNA with B1 repeats and have been shown to be more sensitive compared to other targets such as P30 and rDNA. It is a repetitive DNA sequence with a higher copy number than the single copy of the p30 gene. Recently, sequence AF146527 (a sequence with 200–300 copies in the T. gondii genome) was used. Other groups later used the same gene successfully. Many authors recommend the use of PCR over most serological techniques. and reported to colleagues that these primers could amplify parts of the human genome.

The high prevalence of Toxoplasma can be attributed to several factors. There is a high population of cats (the final host of the parasite) , so the risk of infection of intermediate hosts (including humans) is also high[35]. These cats live near human homes, workplaces, and restaurants. Living in close contact with animal hosts (cats and dogs) and means of oocyst transmission (flies and ants) are important risk factors for infection[36,3]. Other intermediate hosts of T gondii infections are sheep and chickens, which represent another important risk factor in the distribution of toxoplasmosis, as they are the main meat sources . A recent study in sheep reported that PCR testing using the B1 gene gave positive results in 50% of samples tested[38].Recent studies have identified water contaminated with sporulated oocysts as a potential source of infection. Consumption of contaminated shellfish can also lead to infection[5]. Circumstantial evidence suggests that oocyst-borne infections in humans are clinically more severe than tissue cyst-borne infections[37].

Other routes of transmission to humans include transplacental transmission and organ transplantation or blood transfusion. The results presented by Burg and colleagues showed that a single T.gondii parasite could be detected by PCR[22].Another explanation for the high rate of positive test results by PCR is that B1 amplification may represent samples containing parasite DNA but no viable pathogen. Since the PCR test does not rely on live parasites to show a positive result[38]. The results of this study suggest that PCR testing is useful as a confirmatory procedure in conjunction with routine serology testing in pregnant women. This study will help develop new strategies for therapeutic intervention for toxoplasmosis in Pakistan. Our current study is focused on and limited to the diagnosis of toxoplasmosis in pregnancy. This was the first commitment made when the protocol was proposed and

approved. Future directions of this research should be expanded to examine neonatal outcomes in PCR-positive pregnant women.

CONCLUSION

This study demonstrates the need for PCR as a confirmatory test in addition to serological testing to detect recent infection. We recommend nationwide implementation of these molecular diagnostic tools.

REFERENCES

1 Ajioka JW, Soldati d. toxoplasma- molecularand cellular biology. norfolk,UK. Horizon biosci-ence. 2007; 37- 58.

2 Kim K, Weiss LM. toxoplasma: the next 100years. Microbes. infect. 2008; 10(9): 978- 984.

3 dubey Jp, Lindsay dS, Speer cA. Structures oftoxoplasma gondii tachyzoites, bradyzoites, andsporozoites and biology and development of tissuecysts. clin Microbiol rev. 1998; 11: 267-299

4 Hany eM, Azab MS, Abousamra nK, rahbarMH, elghannam M, raafat d. Seroprevalence ofand risk factors for toxoplasma gondii antibodiesamong asymptomatic blood donors. egypt. parasi-tology research. 2009; (104):1471–147. 4

5 Jones JL, dubey Jp. Waterborne toxoplasmosis--recent developments. exp parasitol. Jan; 2010:124(1):10-25.

6 Varella, i. S., canti, i.,c.,t, Santos, b.r.,coppini,A. Z., Argondizzo, L. c., tonin, Wagner, M.b. preva-lence of acute toxoplasmosis infection among41,112 pregnant women and the mother to childtransmission rate in a public hospital in South bra-zil. Mem inst Oswaldo cruz, rio de Janeiro. 2009:104(2): 383-388.

7 Giannoulis c, Zournatzi b, Giomisi A, diza e,tzafettas i. toxoplasmosis during pregnancy: acase report and review of literature. Hippokratia.2008;12 (3):139-143.

8 Jones JL, Lopez A, Wilson M, Schulkin J, Gibbsr. congenital toxoplasmosis: a review. Obstet Gy-necol Surv. 2001; 56:296-305.

9 Montoya JG, Liesenfeld O. 2004. toxoplasmosis.Lancet. 363, 1965-1976.

10 Abbas, S.A., basalamah, A., Serebour, F., Al-fonso, M. the prevalence of toxoplasma gondiiantibodies in Sauwomen and the outcome ofcongenital infection among newborns in SaudiArabia. Saudi. Med. J. 1986: (7) 346-54.

11 Al-Mulhim, A. A. and Al Qurashi, M.A. Serp-revalence of toxoplasmosis in pregnant mothersand new born infants in eastern province. SaudiArabia. Saudi Society of Family and communityMedicine. 2001: 8 (1): 45-53.

12 Tonkal, A. pcr versus eLiSA in diagnosis ofhuman toxoplasmosis in Jeddah, Saudi Arabia.Journal of egyptian Society of parasitology. 2008:38 (3): 707–714.

13 Al Mohammad, H.i., balaha, M.H., Amin, t.t.,el damarany, e.e., dwedar A. the accuracy ofigG avidity for detection of acute toxoplasmosisamong pregnant Saudi women. tAF preventivemedicine bulletin. 2010: 9 (1): 7–14.

14 Al-Harthi, S.A., Jamaoom, M.b., Ghazi H. O.pregnant women in Makkah , Saudi Arabia. UmmAl- Qura Univ J Sci Med eng . 2006: 18 (2): 217-227.

15 el- Hady, H. M. toxoplasmosis among pregnantmothers in Abha, Saudi Arabia. J. egyptian. Soc.parasit. 1991: 21: 811-816.

16 nagaty, i.M., ibrahim, K.M., Abdel-tawab, A.H.,Hassan, A.e. diagnosis of toxoplasma gondii byeLiSA and pcr mothers and their infants. J egyptSoc parasitol . 2009: Aug;39(2):625-32.

17 Derouin, F., Mazeron, M.c., Garin, y.J. compar-ative study of tissue culture and mouse inoculationmethods for demonstration of toxoplasma gondii.J. clin. Microbiol . 1987: 25:1597–600.

18 Bastien, p. Molecular diagnosis of toxoplasmo-sis. trans. r. Soc. trop. Med. Hyg. 2002: 96 (Sup1):205- 215.

19 Savva, d., Morris, J. c., Johnson, J. d. & Hol-liman, r. e. polymerase chain reaction for detec-tion of toxoplasma gondii. J. Med. Microbiol. 1990:32,25-31.

20 Johnson, J. d., p. d. butcher, d. Savva and r.e. Holliman. Application of the polymerase chainreaction to the diagnosis of human toxoplasmosis.Journal of infection. 1993: 26(2): 147-158.

21 O'driscoll, J. c. and r. e. Holliman. toxoplas-mosis and bone marrow transplantation. rev MedMicrobiol. 1991: 2: 215-22.

22 Burg, J.L., Grover, c.M., pouletty, p., boothroyd,J.c. direct and sensitive detection of a pathogenicprotozoan, toxoplasma gondii, by polymerasechain reaction. J. clin. Microbiol. 1989: 27, 1787-1792.

23 Jenum, p. A., Holberg-petersen, M., Melby, K.K. & Stray- pedersen, b. diagnosis of congenitaltoxoplasma gondii infection by polymerase chainreaction (pcr) on amniotic fluid samples. Ai'MiS.1998: 106, 680-686.

24 pujol-rique, M., deruin, F., Garcia-Quintanilla,A., Valls, M. e., Miro, J. M. & Jimenez de Anta, M.t. design of a one tube hemi-nested pcr for thedetection of toxoplasma gondii and comparisonof three dnA purification methods. J. Med. Micro-biol. 1999: 48 (1 999), 857-862.

25 James, G. S., Sintchenko, .G.,dickeson, d. J.& Gilbert, G. L. comparison of cell culture, mouseinoculation and pcr for detection of toxoplasmagondii: effects of storage conditions on sensitivity.J. clin. Microbiol. 1996: 34, 1572-1575.

26 Pappas, G., roussos, n., Falagas, M.e. toxo-plasmosis snapshots: Global status of toxoplasmagondii seroprevalence and implications for preg-nancy and congenital toxoplasmosis. int. J. para-sitol. 2009: 39; 1

27 Eida, O.M., eida, M.M., Ahmed, A.b. evaluationof polymerase chain reaction on amniotic fluid fordiagnosis of congenital toxoplasmosis. J egyptSoc parasitol. 2009: 39(2):541-50.

28 Su c, Shwab eK, Zhou p, Zhu XQ, dubey Jp.Moving towards an integrated approach to mo-lecular detection and identification of toxoplasmagondii. parasitology. 2010: 137(1):1-11.

29 Chabbert, e., Lachaud, L., crobu, L., bastien,p. comparison of two widely used pcr primersystems for detection of toxoplasma in amnioticfluid, blood and tissues. J. clin. Microbiol. 2004: 42,1719-1722.

30 Slawska, H., czuba, b., Gola ,J., Mazurek, U.,Wloch, A., Wilczok, t., and Kaminski, K . diagnos-tic difficulties of toxoplasma gondii infection inpregnant women. is it possible to explain doubtsby polymerase chain reaction?. Ginekol pol . 2005:76, 536-542.

31 Guy, e.c. and Joynson, d.H.M. potential of thepolymerase chain reaction in the diagnosis of ac-tive toxoplasma infection by detection of parasitein blood. J. infect. dis. 1995: 172: 319–322.

32 Jones, c. d., n. Okhravi, p. Adamson, S. taskerand S. Lightman. comparison of pcr detectionmethods for b1, p30, and 18S rdnA genes of tgondii in aqueous humor. investigative Ophthal-mology & Visual Science. 2000: 41(3): 634.

33 Wahab, t., b. edvinsson, d. palm and J. Lindh.comparison of the AF146527 and b1 repeatedelements, two real-time pcr targets used for de-tection of toxoplasma gondii. Journal of clinicalMicrobiology. 2010: 48(2): 591.

34 Kompalic-cristo, A., nogueira, S.A., Guedes,A.L., Frota, c. , González, c.F., brandão, A., Amen-doeira, M.r., britto, c., Fernandes, O. Lack oftechnical specicity in the Molecular diagnosis oftoxoplasmosis. trans. r. Soc. trop. Med. Hyg. 2004:98 :92-95.

35 Dubey, J. p. History of the discovery of the lifecycle of toxoplasma gondii. international Journalfor parasitology. 2009: 39(8): 877-882

36 Mason, S., Quinnell, r.J., Smith, J.e. detectionof toxoplasma gondii in lambs via pcr screeningand serological follow-up. Vet. parasitol. 2010: 11;258-63.

37 Vaillant, V., de Valk, H., baron, e., Ancelle, t.,colin, p., delmas, M.c., dufour, b., pouillot, r., LeStrat, y., Weinbreck, p., Jougla, e., desenclos, J.c.,Foodborne infections in France. Foodborne pat-hog. dis. 2005: 2, 221–232.39.. Wastling J.M., nicoll S, buxton d. compari-son of two gene amplification methods for thedetection of toxoplasma gondii in experimentallyinfected sheep. J Med Microbiol. 1993: 38: 360-365.reFerences

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