



**EVALUATION OF TOXOPLASMA GONDII IN ABORTION CASES BY PCR**

**TEQNIQUE IN ISFAHAN, IRAN**

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**ABSTRACT**

The PCR-based technique detects *Toxoplasma gondii* DNA in patient's samples. The aim of our study was to examine a probable association of Toxoplasmosis in Iranian samples of women undergone a recent abortion by the performance of PCR. Sixty five abortions were occurred from March 2008 to October 2009 in Isfahan, Iran. Specimens from their products of conception were screened for *Toxoplasma gondii* DNA by conventional PCR. The results of the conventional PCR were positive in 10 (15.48%) and negative in 55 (84.6%) out of 65 patients, respectively. A significant relationship was not observed between the presences of *Toxoplasma*, mother's age, time of abortion and illnesses such as Allergy, diabetes, Thalassemia and hypothyroidism.

**Keywords: *Toxoplasma gondii*, PCR, Abortion, Products of Conception**

**INTRODUCTION**

*Toxoplasma gondii* is an intracellular protozoan that causes an infection named toxoplasmosis. This organism is the third cause for the infections transmitted through food in USA [1].

This infection usually is with no signs or limited signs such as skin rashes or ocular complications in adults and is characterized with formation of recurrent hidden cysts with no clinical signs in many organs of the

body. Parasite is protected against immune system of the body at this stage. Tissue cysts are more placed in brain, eye, heart and muscular system and may remain in their infected host lifelong [2, 3, 4]. This problem endangers individuals, especially the pregnant women who infect their fetus or infant as well as a host with defective immune system such as HIV/AIDS patients [5]. Congenital toxoplasmosis as a result of

infection transmission through placenta during mothers' primary infection can lead to miscarriage, fetal death or acute congenital defects such as hydrocephalus, microcephaly, mental retardation and coriotionite. Among those with suppressed immunity like HIV/AIDS patients, transplantation recipients and patients with cancers such as Hodgkin etc, toxoplasma can be reactivated and cause toxoplasmic encephalitis which is a mortality cause among these patients [6,7]. The spread of the disease has been reported from 11% in USA to over 70% in Brasillia [8, 9]. With regard to the irrecoverable complications and injuries among those with suppressed immunity as well as the new born, diagnosis of toxoplasmosis and its treatment are essential. Although the methods based on serological tests in laboratories are conventional, these methods are not adequately efficient. At the early stage of infection with parasite, its diagnosis through serological tests is impossible due to delayed appearance of antibody in the serum. Direct method of parasite existence test and its observation in tissue sections is not also so precise [10]. PCR method has been proved to be very precise and sensitive in direct detection and study of genomic toxoplasma gondii DNA due to its high sensitivity.

In this method, toxoplasma gondii B1 gene which is found in any organism is used [11]. The first toxoplasma gondii DNA was separated from ocular tissue through PCR technique in 1990 [12].

Diagnosis of infecting factors is specifically important in pregnancy as they not only threaten mother's health but also may lead to fetal death and / or infants' congenital defects. Therefore, diagnosis of toxoplasma in the host through PCR method is of great value when its epidemiology and association with miscarriage can be defined.

#### **MATERIALS AND METHODS**

Total of 65 paraffinized sample blocks from delivery products of the patients with miscarriage referring to hospitals in Isfahan were collected and investigated. Patients' pathology files were also investigated concerning mothers' age, gestational age of miscarriage, history of any diseases, residential address and mothers' job.

#### **DNA Extraction**

Firstly, 5-10  $\mu\text{m}$  cuts of paraffinized block samples were put in a 5.1 ml vial and were deparaffinized by Xylol. Then, DNA was extracted based on DNA extraction kit protocol according to manufacturer's instructions (Roche, Germany). At this stage, 200  $\mu\text{l}$  cell lysing buffer together with 20  $\mu\text{l}$  proteinase K (20 mg/ml) was added to each tube which were incubated for 24 hr at 55°C.

After the samples were cooled down to room temperature, 200µl bounding buffer was added and were transferred to filtrated centrifuge tubes and centrifuged at 1400 g. The filter was removed from the tube and was inserted into another vial where 500 µl of inhibitors deletion buffer was added ,and then , the solution was centrifuged .Next, the filter was again removed and inserted into another vial and rinsed by 500 µl buffer twice. Finally, DNA was extracted. The amount of DNA was measured by photo spectrometry in 260 nm through UV spectrometry and the level of DNA purity was measured through photo spectrometry in 260-280 nm [13].

#### PCR Method

The primer pairs of B1 (B22 and B23) which amplified a115bp sequence were used based on what was reported by Bretagne et al in 1993.

Each 25 µl of PCR solution contains 5 mg DNA, 50 pm primers and 2.0 mM desocynucleotide triphosphate, 5.1 mM mgcl<sub>2</sub> and 5.2 µ DNA tag polymerase (US Hanover MD, Fermentase Inc).

#### Forward Primer:

**TOXO-F (5-TCCCCTCTGCTGGCGAAAAGT-3)**

#### Reverse Primer:

**TOXO-R (5-AGCGTTCGTGGTCAACTATCGATTG-3)**

#### PCR was Done Under the Following Conditions

T(°C)	Time (Minute)	Cycles Number
94	4	1
94	45	35
58	45	
72	1	
72	10	1

Then PCR products were taken to agars gel 2% [14].After conducting the above tests, the obtained resultswere analyzed by chi-square and t- test statistical tests in SPSS.

#### RESULTS

Based on **Table 1**, out of 65 miscarriage products samples in mothers with mean age of 27 years, 12 mothers (18.5%) had frequent previous miscarriages. Miscarriages occurred at 2-18 weeks of gestational age (with mean of 9 weeks). As observed in **Table 2**, two cases (31%) had history of hypothyroidism, one case (1.5%) talacemy, two cases (4.6%) diabetes, three cases allergy and 47 cases had history of no diseases except miscarriage. Chi- square test showed a significant difference between the group with history of diseases and the group with history of no diseases ( $p \leq 0.05$ ) (**Figure 1**).

Based on **Table 2**, 10 cases (15.48%) out of 65 were toxoplasma positive. There was a significant difference between the groups of toxoplasma positive and negative ( $p \leq 0.05$ ).

Table 1: Frequency Distribution, SD, Median, Mode, Min and Max of Subjects' Age and Gestational Week

	Age	Gestational Age
Mean	27.43	8.95
Median	27	8
Mode	25	8
Std. Deviation	6.26	3.96
Variance	39.21	15.70
Minimum	17	0
Maximum	45	19

Table 2: Frequency Distribution and Percentage of Disease History Among all Subjects

History of Disease	Frequency	Percent
No history of diseases	47	72.3
History of appendicitis	10	15.4
History of hypothyroidism	2	3.1
History of talacemy	1	1.5
History of diabetes	2	3.1
History of allergy	3	4.6
Total	65	100

Table 3: Table of Toxoplasma Positive and Negative Cases Distribution Among the Subjects

	Frequency	Percent
Positive	10	15.4
Negative	55	84.6
Total	65	100

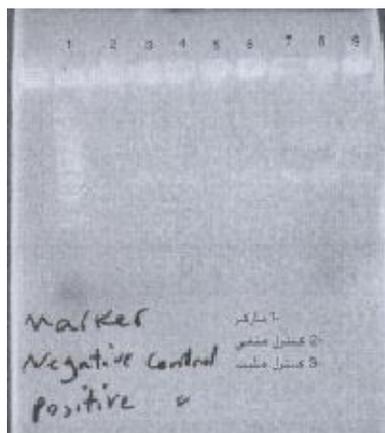


Figure1: PCR Products Electrophoresis Results

NOTE: 1: Marker; 2: Negative Control; 3: Positive Control; 4, 6, 7, 8, 9: Positive Samples

## DISCUSSION

Serological method has been the conventional laboratory method for diagnosis of toxoplasmosis from previous decades until now. With onset of HIV and an increase in organ transplantation and consumption of immunosuppressant drugs, various clinical manifestation of

toxoplasmosis infection appeared and made the infection as a life threatening disease. On the other hand, irrecoverable complications of congenital toxoplasmosis infection made the researchers use sensitive and specific methods other than serological ones [15, 16]. Existence of toxoplasma gondii among pregnant women

and incidence of miscarriage in different countries has been variously reported (it has been reported about 44% in Spain in 2008 [17], about 6.5% in Morocco in 2007 [18], about 51-72% in Latin America in 2000 [19] and 33% in Denmark in 1990 [12]. In the present study, we observed frequent miscarriages in about 4-15% of pregnant women which concords with the resultset obtained in Spain reporting that 4.14% of Spanish women were toxoplasmosis positive [17]. It is estimated that 47.49% of the studied women with frequent miscarriage (285 subjects) were toxoplasma positive concerning immunoglobulin M (IgM) [20].

There was no significant association between existence of toxoplasma and hypothyroidism, talacemy, diabetes and allergy. There was no significant association between mothers' age, miscarriage gestational age and existence of toxoplasma .The conventional laboratory method to diagnose toxoplsmosis is serologicalmethod, but with regard to high prevalence of various immune-defective diseases and increase of organ transplantation and the effect of toxoplasma on frequent miscarriages, these methods face problems and non- serological and more sensitive methods such as PCR are considered more [21].

It has been proved that every disease factor, especially latent factors, can be more efficiently detected and separated by PCR technique [22].

This method is also very sensitive and precise in patients infected by toxoplasma with immune system defect and is used as an optional method in early diagnosis of infection [23, 24]. When toxoplsma gondii was separated from ocular samples for the first time in 1990 [12], Aouizerate et al reported ocular infection as a result of this parasite as 8.33% among 59 cases through PCR technique [25]. In a study on level of specification in serological methods, compared to PCR, it was reported 89% for ELISA (Enzyme –Linked Immunosorbent Assay) and 100% for PCR [26].

The results obtained from study population of the women with miscarriages show that toxoplasmosis acts as a warning and main risk factor for pregnant women's health and their fetuses in these areas of Iran. This problem necessitates a development in educational programs for a population with immune system defect. PCR is a used tool for epidemiologic investigations and is potential to affect the advances in innovative programs.

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