Detection of Acute and Chronic Toxoplasma gondii Infection among Women with History of Abortion in the Southwest of Iran

Jasem Saki,1 Maryam Zamanpour,2 Mahin Najafian,3 Niloofar Mohammadmour,1 and Masoud Foroutan4

1Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2Department of Medical Parasitology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3Department of Obstetrics and Gynecology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4Research Center for Environmental Contaminants (RCEC), Abadan University of Medical Sciences, Abadan, Iran

Correspondence should be addressed to Jasem Saki; jasem.saki@gmail.com

Received 15 December 2020; Revised 31 August 2021; Accepted 12 October 2021; Published 2 November 2021

Academic Editor: José F. Silveira

Copyright © 2021 Jasem Saki et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Toxoplasma gondii (T. gondii) is one of the most common intracellular protozoan parasites, which can infect humans and a wide range of mammals and birds. The current study is aimed at investigating the occurrence of T. gondii infection in women with a history of abortion in Khuzestan, Iran. Materials and Methods. A total of 480 women with an abortion history, as well as 200 pregnant women with a normal delivery, were examined in this study. The blood, placenta, and umbilical cord blood samples were assessed by the enzyme-linked immunosorbent assay (ELISA) and nested-polymerase chain reaction (PCR) assay. Results. Based on the results of ELISA assay, the prevalence of toxoplasmosis was 30.83% in women with a history of abortion (25.62% with T. gondii IgG and 5.20% with T. gondii IgM). According to the IgG avidity test, 60.16% of IgG-positive samples showed high avidity, while 27.64% showed low avidity. On the other hand, the prevalence of toxoplasmosis in women with a normal delivery was 23% (21.5% with T. gondii IgG and 1.5% with T. gondii IgM). According to the IgG avidity test, 81.39% of these women showed high avidity, while only 4.65% showed low avidity. Based on the nested-PCR method, T. gondii DNA was detected in 14.18% of blood samples, 4.69% of placental samples, and 1.34% of umbilical cord samples, collected from 148 seropositive women with a history of abortion. Besides, using this method, the parasite DNA was identified in 4.34% of blood samples, collected from 46 seropositive women with a normal delivery, but not in any of the umbilical cord or placenta samples. Conclusion. The present results showed that T. gondii infection contributes to abortion in Khuzestan Province, Iran. Therefore, it is essential to investigate toxoplasmosis in pregnant women, especially in those who are seronegative, using molecular and serological methods and inform them about their disease and the associated risks.

1. Introduction

Toxoplasma gondii (T. gondii) is one of the most common intracellular protozoan parasites, which can infect humans and a wide range of mammals and birds [1, 2]. Contamination with T. gondii primarily occurs due to the consumption of uncooked meat, infected with cysts, or the ingestion of oocysts through water, soil, and contaminated food. The incidence of this infection varies from one country to another, depending on the socioeconomic and health status [3–6]. Many cases of acquired toxoplasmosis are either asymptomatic or mildly symptomatic. However, in individuals with immunodeficiency, such as HIV patients and transplant recipients, it can be a life-threatening condition, causing eye and brain lesions. On the other hand, reactivation of a latent infection may cause severe complications with a poor prognosis [4, 7, 8]. Besides, infections during pregnancy can cause congenital toxoplasmosis in the fetus,
resulting in spontaneous abortion, stillbirth, hydrocephalus, microcephaly, and neurological symptoms that can be detected in the uterus or at birth [9, 10].

According to previous studies, maternal infection in the first or second trimester of pregnancy can be associated with stillbirth rates of 5% and 2%, respectively [11, 12]. The prevalence of congenital toxoplasmosis varies from 0.1% to 0.3% per 1000 live births. The risk of maternal transmission to the fetus increases from 15% to 70% from a gestational age of 13 weeks to 36 weeks, respectively [13]. In a previous study conducted in New York, USA, 6% of pregnant women acquired toxoplasmosis during pregnancy, and 13% of their newborns had congenital toxoplasmosis. Overall, the prevalence of congenital toxoplasmosis was 7 per 10,000 live births [14].

The diagnosis of congenital toxoplasmosis in the uterus or after birth is essential in preventing and reducing severe complications in the fetus or newborn and improving the prognosis of infection [14, 15]. Generally, maternal serological examination for toxoplasmosis is crucial, especially in seroconverting mothers during pregnancy, to prevent fatal injuries through medical treatment or prophylaxis [16]. To identify infections during pregnancy more accurately, DNA-based molecular methods, which have higher sensitivity and specificity than serological methods, have been employed in recent years, and different targets of T. gondii genome have been investigated. The B1 gene is one of the most valuable targets in identifying toxoplasmosis [17].

In some European countries, the monthly serological survey of sensitive pregnant women is a routine program, while in many countries, there is no information regarding congenital toxoplasmosis. Therefore, maternal and fetal lives can be threatened, especially in areas with a high prevalence of toxoplasmosis. In Khuzestan Province, with a population of approximately four million people, there is not enough information about congenital toxoplasmosis or the role of T. gondii in abortion. The present study is aimed at investigating anti-T. gondii antibodies and identifying parasite DNA in the maternal blood, placenta, and umbilical cord samples collected from two groups of women with a history of abortion and women with a normal delivery in hospitals, affiliated to Jundishapur University of Medical Sciences, Ahvaz, Iran, during 2012-2017.

2. Materials and Methods

Khuzestan Province is situated in the southwest of Iran (Figure 1). This study was carried out at the educational hospitals of Jundishapur University of Medical Sciences in Ahvaz, capital of Khuzestan Province, Iran. This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. A written consent form was obtained from all women. Informed consent was also obtained from the participants’ legal representatives.

From each patient (480 women with an abortion history and 200 with a normal delivery), peripheral blood samples, placenta, and umbilical cord samples (1-2 mL) were collected and transferred to the Parasitology Department of the School of Medicine. Next, serological enzyme-linked immunosorbent assay (ELISA) and molecular polymerase chain reaction (PCR) assays were carried out on the samples. A positive control was obtained from genomic DNA, extracted from the RH strain of T. gondii. The negative control for the PCR reaction was distilled water rather than DNA template, which is commonly used in PCR reactions.

2.1. ELISA Assay. A total of 480 peripheral blood samples were collected from women with a spontaneous abortion at <20 weeks of gestation, and 200 peripheral blood samples were collected from women with a normal delivery at a gestational age of 38-39 weeks. The participants in this study were in the age range of 14-53 years. The serological evidence of toxoplasmosis was investigated by detecting T. gondii IgM and IgG antibodies. The serum samples were separated and stored in aliquots at −20°C until further analysis. Next, they were tested for the presence of IgM and IgG antibodies, using an ELISA-based NovaLisa test kit. Moreover, a Toxoplasma-specific IgG avidity assay was performed using an avidity T. gondii IgG ELISA kit (NovaTec GmbH, Germany) to differentiate between acute and chronic infections. All samples were tested according to the manufacturer’s instructions. The results were then read by an ELISA microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) and compared with the calibrator and the controls.

2.2. DNA Isolation and PCR of T. gondii B1 Gene. DNA was extracted from each blood sample, placenta, and umbilical cord sample (1-2 mL) (480 samples from women with an abortion history and 200 samples from women with a normal delivery), using a DNA extraction kit, according to the manufacturer’s instructions (Bioneer, Korea). PCR was performed to amplify 194 bp segments of the T. gondii B1 gene by nested PCR [18]. For the first and second rounds of PCR, 0.5 μM primers, 200 μM dNTPs, 1.5 mM MgCl₂, 1.5 units of AmpliTaq gold DNA polymerase, and 4 μL of DNA template were used in a total volume of 25 μL. The PCR product was electrophoresed on 2% agarose gel. A 196 bp band was considered as the positive PCR control. Data were analyzed in SPSS version 13.5, using Chi-square and Fisher’s exact tests.

This study was performed on 480 placenta, 480 umbilical cord, and 480 peripheral blood samples collected from women with an abortion, as well as 200 placenta, 200 umbilical cord, and 200 peripheral blood samples collected from women with a normal delivery, at university-affiliated hospitals in Ahvaz, Iran, during 2013-2017.

3. Results

3.1. Serological Results. Out of 480 blood samples collected from women with an abortion history by the ELISA method, 148 (30.83%) were positive, including 25 (5.20%) samples with IgM antibodies and 123 (25.62%) samples with IgG antibodies. According to the IgG avidity test, 74 out of 123 samples with IgG positivity (60.16%) showed high avidity; 34 (27.64%) samples showed low avidity, and 15 (12.19%)
samples showed borderline avidity. In the control group, 46 out of 200 blood samples (23%) were positive in the ELISA assay. Three of these samples contained IgM antibodies (1.5%), while 43 (21.5%) contained IgG antibodies; only one sample included both IgG and IgM. The IgG avidity test showed that 35 (81.39%) and 2 (4.65%) samples had high and low avidity in this group, respectively (Table 1).

### Table 1: The serological results of toxoplasmosis in two groups of women with an abortion history and a normal delivery.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>IgM⁺ No. (%)</th>
<th>IgG⁺ No. (%)</th>
<th>IgG avidity⁺ No. (%)</th>
<th>Total⁺ No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with an abortion history</td>
<td>480</td>
<td>25 (5.20%)</td>
<td>123 (25.62%)</td>
<td>74 (60.16%)</td>
<td>148 (30.83%)</td>
</tr>
<tr>
<td>Women with a normal delivery</td>
<td>200</td>
<td>3 (1.5%)</td>
<td>43 (21.5%)</td>
<td>35 (81.39%)</td>
<td>46 (23%)</td>
</tr>
</tbody>
</table>

3.2. Molecular Test Results. According to the results of nested-PCR assay in 148 seropositive women, *T. gondii* DNA was detected in 30 (20.27%) samples, which were collected from the group of women with an abortion history, including 21 (14.18%) blood samples, 7 (4.72%) placenta samples, and 2 (1.35%) umbilical cord samples. In this group, two women were positive in all samples (blood, placenta, and umbilical cord). Among other positive cases, 19 were only found in blood samples and five in only placental samples. On the other hand, in women with a normal delivery, only two women had serum-positive blood samples infected with *T. gondii*, while no infection was found in any of the placenta or umbilical cord samples (Figure 2 and Table 2).

### Figure 1: The map of Iran and Khuzestan Province in the southwest of Iran. The study region is shown with red asterisk.

### Figure 2: Amplification of a 194 bp band of *T. gondii* DNA in the blood, placenta, and umbilical cord samples. Lane M: molecular weight marker; Lane 1: positive control; Lanes 2-9 and 11-13: positive samples; Lane 10: negative control (2% agarose gel electrophoresis).

4. Discussion

In this study, the prevalence of toxoplasmosis, based on serological methods, was estimated at 30.83% and 23% in women with a history of abortion and those with a normal delivery, respectively; the difference between the two groups was significant (*P* < 0.05). Based on the IgG avidity test, the
During pregnancy, infection with Toxoplasma gondii can cause congenital toxoplasmosis, which is one of the causes of spontaneous abortion in humans and animals. During pregnancy, infection with T. gondii can cause congenital toxoplasmosis [24–26]. The rate of toxoplasmosis is relatively high among pregnant women in Iran. This rate has been estimated at 27% in Zahedan in the southeast of Iran [27], 60.6% in the north of Iran [28], 34.09% in Abadan (southwest of Iran) [29], 27.8% in Hormozgan Province [30], and 77.2% in Shiraz (south of Iran) [31]; overall, the rate was lower among high-school girls [32].

Moreover, in a study conducted in New York, USA, 0.6% of pregnant women acquired toxoplasmosis during pregnancy, and 13% of the newborns had congenital toxoplasmosis. Also, the rate of toxoplasmosis was 7 per 10,000 live births [14]. In another study in Colombia, 61 out of 15,333 cord blood samples contained IgM anti-T. gondii antibodies. Today, the prevalence of congenital toxoplasmosis is 39 per 10,000 live births [33]. A study on the seroprevalence of toxoplasmosis in pregnant women in Kosovo indicated that 1.2% of women had become infected with toxoplasmosis during pregnancy [34]. In the present study, the rate of T. gondii infection was 4.27% in the aborted placenta, according to the PCR method, which is 14.4% lower than the rate reported in Shiraz (south of Iran) [31]. It seems that the sample type, sampling method, and other parameters, such as climatic conditions and eating habits, can affect the prevalence of toxoplasmosis in different regions.

In 2017, Matin et al., in a comparison of placental PCR with maternal serology, showed that 53.5% of women with a history of abortion had anti-T. gondii antibodies; overall, 4% of these women had IgM, and 43% had IgG. A nested-PCR assay for identifying T. gondii in the placenta indicated that 10.5% of the samples were infected [35]. In the present study, infection with T. gondii was found in none of the placenta or umbilical cord samples of women with a normal delivery. The infection rate of the umbilical cord with T. gondii was 1.35% in women with an abortion history. Based on the results, the rate of umbilical cord contamination was lower than the placenta. These results are consistent with the results reported by Bessieres et al., detecting T. gondii in 60% of placenta samples and 43% of umbilical cord samples [36]. Some studies have also reported that detection of T. gondii in the placenta does not confirm congenital toxoplasmosis. In this regard, Sardarian et al., in 2018, tested ten placenta samples of women with a normal delivery and found that 6 (60%) samples were positive for T. gondii [37].

In conclusion, considering the significant differences in the prevalence of toxoplasmosis between the two groups of women with an abortion history and a normal delivery, T. gondii seems to contribute to the occurrence of abortion in Khuzestan Province. Therefore, it is essential to consider the risk of spontaneous abortion and stillbirth before and during pregnancy.

**Abbreviations**

ELISA: Enzyme-linked immunosorbent assay
IgG: Immunoglobulin G
IgM: Immunoglobulin M
T. gondii: Toxoplasma gondii.

**Data Availability**

All relevant data are within the manuscript.

**Ethical Approval**

This study was approved by Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) (Ethics Committee approval ID: OG 1817202862).

**Conflicts of Interest**

The authors declare that there are no conflicts of interest related to this manuscript.
Authors’ Contributions

J. Saki, M. Zamanpour, N. Mohammadpour, and M. Foroutan conceived, designed, and drafted the manuscript; J. Saki, M. Foroutan, and M. Najafian contributed to data acquisition; J. Saki and M. Zamanpour contributed to statistical analysis and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

This study was financially supported by the Research and Technology Deputy of Ahvaz Jundishapur University of Medical Sciences (No: OG-93110).

References


