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Original Article

Early detection of Toxoplasma gondii by real-time polymerase chain reaction methods in patients with recurrent spontaneous abortions

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Abstract

Introduction: One of the causes of recurrent spontaneous abortions (RSA) is an infection by the toxoplasmosis Protozoa. In comparison, we present detailed results using real-time polymerase chain reaction (PCR) methods of detection. In this study, it was tried to detect Toxoplasma gondii (T. gondii) by real-time PCR methods in patients with RSA.

Methods: Amniotic fluid sampling was performed in the 16-20th weeks of gestation in 50 pregnant women with a history of RSA. The extracted deoxyribonucleic acid (DNA) samples were analyzed using quantitative real-time PCR.

Results: In all the cases, the detection of T. gondii was negative in the peripheral blood, and amniotic fluid samples by using the molecular methods (real-time PCR). Using the serological detection methods, 6% of patients were diagnosed as positive for the immunoglobulin M (IgM) antibody. In addition, the IgG antibody was positive in 46% of the patients.

Conclusion: It can be concluded that the serological methods lack specificity.

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Introduction

Recurrent spontaneous abortion (RSA) is of the most prevalent problems of pregnancy period. In some societies incidence rate of RSA has been reported up to more than 12-15% in pregnant women that lead to the fetus loss.[1] One of the reasons for spontaneous abortions has been mentioned as pathogenic infectious agents.[2] Toxoplasmosis is one of the most prevalent human infections all around the world.[3] Prevalence rate of this infection has been reported very differently, and it is not limited to geographical borders and covers Alaska to Australia.[4]

Toxoplasmosis is created by a protozoan parasite as Toxoplasma gondii (T. gondii) and remains in people's body for a long time and in average one-third of people become infected by this parasite.[5] In terms of general health, transmission risk of this parasite is

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important during pregnancy.[3]

Although Toxoplasmosis in women is often benign but its transmission through placenta could lead to severe dangers such as stillbirth or birth of infants with mental and physical retardations.[3,6-8] In recent techniques of diagnosing infection to this parasite, which are generally based on serological methods alongside with measuring immunoglobulin G (IgG) and IgM antibody levels against infectious agents, after years of use, it has been reported that they lack a high sensitivity and accuracy and are useless in some cases,[9,10] and in some cases, due to interfering reactions with other infectious or non-infectious agents they make it too difficult to interpret results.[8,11,12]

Nowadays possibility of very exact and sensitive and early diagnosis of infectious agents has been provided using molecular methods. In this methods possibility of existence of interfering factors and acquiring false positive or negative results has approximately reached zero and on the other hand using these methods do not need to investigate or immune response of body and due to high sensitivity it is possible to trace the minimum genome and even in therapeutic stages to be sure about its complete removal. Therefore considering anxiety of pregnant women suspected of infection to one of infectious agents such as T. gondii and also vitality of decision about abortion or keeping fetus, necessity for using very accurate and exact daily methods is increasing.

Therefore in this parallel study for the first time in Iran results of using exact molecular methods and those of old methods were compared so that after being sure about precision and accuracy of molecular test, they may be used as credible alternative for routine and early diagnosis. In the present study, it was tried to detect T. gondii by real-time polymerase chain reaction (PCR) methods in patients with RSA.

Methods

During present descriptive cross-sectional study, which was carried on educational-medical centers of Tabriz, Iran, from June 2011 up to June 2013, 50 pregnant women with RSA history and suspected of infection by Toxoplasmosis, who had referred to referral educational-medical centers during the mentioned time interval were referred to medical laboratory to conduct serological diagnostic tests after filling informed consent (in accordance with declaration of Helsinki). Also for amniotic fluid sampling they were referred to the gynecologist. Amniotic fluid sampling was conducted in 16-20th weeks. After getting, amniotic fluid sample was referred to single genetic diagnosis center which was the center library of Tabriz University of Medical Sciences for conducting molecular tests of patients.

Also in the mentioned center 10 cc of blood samples in Ethylenediaminetetraacetic acid (EDTA) solution were obtained from patients and deoxyribonucleic acid (DNA) was extracted from both amniotic fluid sample and blood sample using molecular kits and special methods. Extracted DNAs were studied by quantitative real-time PCR (ABI, USA) method and using the kit of Genesig company. In this method toxoplasma primer and probe have been designed for a part of DNA that specifically reproduce target piece. During the performance of PCR positive and negative control samples were used to approve the validity of PCR.

Main pathogenic factors which could lead to the fetus loss in pregnant women or congenital defects in the fetus could be diagnosed by this method. Statistical analysis was performed by SPSS for Windows (version 16, SPSS Inc., Chicago, IL, USA). Quantitative data are presented as mean ± standard deviation while qualitative data are demonstrated as frequency and percent (%). The study protocol was approved by the ethics committee of Tabriz University of Medical Sciences, which was in compliance with Helsinki declaration.

Results

In this study, 50 women with RSA history in age range of 16-40 years were studied. Mean

age of patients was 26 years. Most of the patients were living in rural districts (68%) and the others were urban citizens. Most of the studied patients were housewives (84%) and often had elementary educational level (58%).

In all studied patients diagnosis of T. gondii by peripheral blood and amniotic fluid via molecular method (real-time PCR) were negative. Serological frequency of IgG and IgM antibodies against T. gondii in women with RSA history was investigated; positive, equivocal, and negative IgG were detected in 23 (46%), 6 (12%), and 21 (42%) patients, respectively. Moreover, positive, equivocal, and negative IgM were detected in 3 (6%), 9 (18%), and 38 (76%) patients, respectively.

Discussion

In this study, positive cases of IgG anti-toxoplasma were obtained 46%, and IgM anti-toxoplasma cases were 6% by serological method. In a study conducted in 2010 in Iran on women with and without abortion history, women with a history have been reported 55.6% positive in terms of IgM antibody titration against toxoplasma and 44.4% negative. While in women without a history 2 persons (3.4%) were positive, and 57 persons (96.6%) were negative[13] which does not correspond with current study. In this study in terms of IgG titration also in women with history 42 persons (66.7%) were positive and 21 persons (73.3%) were negative. In a study conducted in Kashmir a significant relationship was observed between abortion and toxoplasma infection in a way that of 285 women with abortion history 49.47% had positive IgM, while among 160 persons of control group there were only 8.8% positive.[14] Also in a study by Acici et al. in Turkey similar results were obtained.[15]

Other study in India showed the role of toxoplasma infection in abortion and existence of antibody titration against toxoplasma in women with abortion history.[16] It is while in this study all cases were diagnosed negative by molecular method and also by serological method only 6.0% were diagnosed positive so a significant relationship was not found between abortion history and toxoplasma infection, which is in accordance with other studies such as Ebadi et al. in Jahrom, Iran,[17] Ertug et al. in Turkey[18] and Qublan et al. in Jordan.[19] Ebadi et al. have also reported frequency of IgG and IgM against toxoplasma as 34.2% and 7.9% respectively[17] which is in complete accordance with current study in terms of amount of positive titration of both IgG and IgM antibodies.

Conclusion

In this study, according to negativity of all diagnosed cases by molecular method and positivity of 3 cases of samples by serological method, it seems that the reason lies in lack of required specificity of serological methods comparing with molecular method. Of mentioned problems it is possible to point to the effect of rheumatoid factor on most of Iranian women and production of IgM and creation of false positive in diagnosis procedure and problems in serological methods, but PCR method in which a piece of toxoplasma genome DNA is identifiable has better results than those of other current diagnostic methods due to specificity and proper sensitivity and also rapid obtaining of result.[20] Therefore, it is better to use also sensitive molecular methods and amniotic fluid sample to make careful decisions on orders about fetus abortion.

Conflict of Interests

Authors have no conflict of interest.

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