

The Role of Brucella Infection Among Women with Spontaneous Abortion in an Endemic Region

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Abstract

Objective: Brucella infection in animals is associated with high incidence of abortion but in the human, there are controversies about the role of brucellosis in spontaneous abortion. Brucellosis is endemic in Iran. Since many causes of abortion are undiagnosed, this study has been designed for the evaluation of a possible association between the previous maternal brucella infection and spontaneous abortion.

Materials and Methods: In this study 81 women with spontaneous abortion were considered as the case group and 105 women with normal pregnancy outcome as the control group. IgG and IgM antibody were measured by enzyme-linked immunosorbent assay method in both groups. IgG or IgM levels equal to or greater than ten were considered to be positive.

Results: Both groups were matched on the basis of age, number of previous pregnancies and abortion, gestational age and consumption of unpasteurized cheese, 6.2% of the case group and 13.6% of the control group had positive IgG titer (IgG ≥ 10). In both groups IgM was negative (IgM < 10). There was no significant relationship between the brucella antibody and spontaneous abortion. There was a significant relationship between raw milk consumption and spontaneous abortion (OR=6.31).

Discussion: This study showed that the brucella seropositivity is no more common in women with spontaneous abortion than in normal pregnancy.

Keywords: brucellosis, spontaneous abortion, serology, pregnancy

Özet

Endemik Bir Bölgede Spontan Abortus Yapan Kadınlarda Brusella Enfeksiyonunun Rolü

Amaç: Hayvanlardaki brusella enfeksiyonu yüksek oranda abortus insidansı ile ilişkilidir, ancak insanlarda spontan abortuslardaki rolü tartışmalıdır. Brusellozis İran'da endemiktir. Çoğu abortus nedeni tanımlanamadığından, bu çalışma, önceden geçirilmiş brusella enfeksiyonu ve spontan abortus arasındaki ilişkiyi incelemek için tasarlanmıştır.

Materyal ve Metot: Bu çalışmada, 81 spontan abortusu olan kadın, olgu grubu olarak; normal gebeliği olan 105 kadın da kontrol grubu olarak belirlendi. Her iki grupta, brusella, IgG ve IgM antikorlara enzim-bağlı immünsorban metodu ile bakıldı. IgG ya da IgM seviyeleri 10 ve üzeri olanlar pozitif kabul edildi.

Bulgular: Her iki grup yaş, önceki gebelik sayısı, abortus sayısı, gestasyonel yaş ve pastörize edilmemiş süt tüketimi açısından değerlendirildi. Olguların %6.2'sinde ve kontrollerin %13.6'sında pozitif IgG titresi (IgG ≥ 10) izlendi. Her iki grupta IgM negatif (< 10) olarak bulundu. Bu sonuçlarla spontan abortus ve brusella enfeksiyonu arasında anlamlı bir ilişki görülemedi. Ancak çiğ süt alımı ve spontan abortus arasında anlamlı bir ilişki gözlemlendi (OR=6.31).

Tartışma: Bu çalışma, abortusu olan kadınlarda brusella seropozitivitesinin normal gebeliği olan kadınlardan daha fazla olmadığını gösterdi.

Anahtar sözcükler: bruselloz, spontan abortus, seroloji, gebelik

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Introduction

Brucellosis is a leading cause of zoonosis worldwide caused by the bacterial genus *brucella*. It can be transmitted to humans through contact with the infected animals or their products and consumption of the infected dairy products. Transmission can also be air born and through laboratory contact (1). The disease is endemic especially in countries of the Mediterranean basin, the Middle East, the Indian subcontinent and parts of Mexico and Central and South America. Human brucellosis is found to have significant presence in rural/nomadic communities where people live in close association with animals. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 per 100 000 population (2). Brucellosis remains a major public health problem in developing countries. Endemicity in this region results from the persistence of domestic animal reservoir. Persons in their third to fifth decades of life are most commonly affected (1). Iran is one of the countries where brucellosis remains a major endemic health problem. *Brucella mellitensis* is the most common species, 83% of the cases in the country are individuals under 40 years of age and women, especially in rural areas, make up high risk groups (3). Brucellosis is a systemic disease that can involve any organ or system of the body. In humans, the clinical spectrum of disease can be from asymptomatic to the severe forms. Disease is mostly asymptomatic, and is usually diagnosed by serological testing in the endemic areas and among the high-risk groups (4).

The principle manifestation of brucellosis in animals is spontaneous abortion and the presence of erythritol in placenta of these animals which is believed to play an important role in the localization of brucella. This is true only about brucella abortus (5).

It is believed that brucellosis causes fewer spontaneous abortions in humans than it does in animals because of the absence of erythritol in the human placenta and fetus. An additional reason for the less significant role of brucella infection in human abortion is the presence of anti-brucella activity in the human amniotic fluid (6).

The gold standard for the diagnosis of brucellosis is the isolation and identification of brucella species from clinical specimens by culture. However, it is time-consuming and hazardous to the laboratory personnel. Therefore, most cases are diagnosed by serological testing. The most frequently used method is the standard tube agglutination test (4). Enzyme-linked immunosorbent assay (ELISA) typically uses the cytoplasmic proteins as antigens and measures IgM, IgG, and IgA, which allow for better interpretation. It has been reported to be superior to others serology due to its higher sensitivity and specificity (5,7,8).

Spontaneous abortion, which is the loss of a pregnancy without outside intervention before 20 weeks of gestation, is the most common complication of pregnancy. Up to 20% of

the recognized pregnancies will end in spontaneous abortion (9). Causes of many abortions are unknown. One of the causes of the abortion is infection (10). Although brucellosis can also result in human abortion, it has been debated whether it is any more frequent than with the other bacterial infections (5). Reports from the areas where *Brucella melitensis* infection is endemic, suggest that there is an increased rate of abortion in asymptomatic pregnant women (1).

Because there are still controversies about the role of brucellosis and brucella infection in spontaneous abortion, this study was designed to evaluate this probable relationship and ascertain whether screening for brucellosis by serology is needed in all the pregnant women in the endemic areas.

Materials and Methods

In this case-control study, women who were admitted to the university affiliated Amir hospital with spontaneous abortion of no obvious cause were enrolled as cases. Women with normal pregnancy outcome that came for antenatal care in the obstetrics clinic were considered as the control group. The control group were followed and if any fetal problem developed, the mother was excluded from the study. Women with previous history of brucellosis or showing symptoms and signs compatible with brucellosis such as prolonged fever, skeletal pain, night sweating, anorexia and weight loss were excluded. The presence of previous brucellosis was based on the patient's history and if they used treatment for brucellosis. Women with known causes of abortion were excluded. Two groups were matched for age, gestational age and number of previous pregnancies. Both patients and controls were from the same epidemiological area. Abortion was defined as pregnancy loss before 20 weeks of gestation (10). Information on the gestational age at the time of abortion, history of consumption of raw milk and unpasteurized cheeses was also collected.

In both groups, anti-brucella IgM and IgG was measured in blood samples by ELISA and titer ≥ 10 defined as positive for both antibodies (IBL-Hamburg, Germany).

The χ^2 test was used for the comparison of qualitative variables; and *t*-test and Mann-Whitney test were used for quantitative variables. Finally, because the two groups were not matched for history of raw milk consumption, logistic regression analysis was performed to estimate the effect of suspected risk factors on the spontaneous abortion. We used backward stepwise regression to determine which factors were most strongly related to the outcome of interest.

Tests were considered significant when the two tailed *p*-value was less than 0.05. All statistical analyses were performed with SPSS 9.0 for windows (SPSS, IL, USA).

This research was approved by committee of research in the vice-chancellor for research in Semnan University of Medical Sciences.

Results

A total of 186 subjects (81 cases, 105 controls) were enrolled in this study. The mean age of women was 26.5±8.6 years in the case group with spontaneous abortion and 25.7±4.8 years in the control group, and the difference was not significant ($p=0.354$). The mean number of previous pregnancy was 1.2±1.6 in the cases and 0.75±1.2 in the controls which was not significantly different ($p=0.151$). Data on gestational age, number of previous abortions, consumption of raw milk and unpasteurized cheese are shown in Table 1; 80.2% of the cases and 83.5% of the control had no history of abortion.

IgM titer in both groups was negative. Mean of IgM titer was 2.42±1.17 in the cases and 2.41±1.14 in the controls, the difference showing no significance ($p=0.957$), 6.2% of cases and 13.6% of control group were positive for IgG (mean titer of 27.8±15.2 and 19.4±9.15, respectively).

Mean of the IgG titer was 5.30±7.06 in the cases and 5.95±6.57 in the control group ($p=0.518$). Because the two groups were unmatched for history of raw milk consumption, data was analyzed by the logistic regression (abortion as a dependent variable and other variables as independent) and a

significant relationship only for raw milk consumption was found (OR=6.31) (Table 2).

Discussion

The association of infection with abortion is a very controversial issue and has only been partly explored as a potential cause of abortion. Maternal infections caused by some pathogens are proposed as causes of some of the spontaneous abortions; either symptomatic or asymptomatic (11); and infection with the *Brucella* species is one of them. A few studies have shown that the symptomatic and asymptomatic brucellosis can cause spontaneous abortion (12,15).

In our study, there was no significant relationship between IgM and IgG titers and spontaneous abortion. IgM titer that usually rises in acute infection was negative in both the case and the control groups. IgG that usually shows chronic or relapsing infection (4), was positive in 6.2% of the cases and 13.6% of the controls, but the results were not significantly different ($p=0.518$).

Our findings were contrary to some of the earlier reports. In a study in Kuwait utilizing ELISA and microagglutination, IgM and IgG titers were significantly higher in the abortion group

Table 1. Characteristics of women with (n=81) and without (n=105) spontaneous abortion

Factor	Control		Spontaneous abortion		p
	n	%	n	%	
Number of previous abortion					
0	86	83.5	65	80.2	0.989
1	13	12.6	14	17.3	
2	2	1.9	2	2.5	
3	2	1.9	0	0.0	
Gestational age (Week)					
<12	86	83.5	66	81.5	0.721
≥12	17	16.5	15	18.5	
Consumption of unpasteurized cheese					
+	30	28.2	30	37.0	0.200
-	75	71.8	51	63.0	
Consumption of raw milk					
+	3	1.9	9	11.1	0.009
-	102	98.1	72	88.9	

* $p<0.05$ significant.

Table 2. Spontaneous abortion risk-assessment model

Variables	β Coefficient	SE*	p	R partial correlation	Odds ratio (OR)	95% CI † for OR
Consumption of raw milk	1.84	0.80	0.0208	0.12	6.31	(1.3-30.1)
Constant	-0.34	0.15	0.0282	-	-	-

*Standard Error.
†CI: Confidence Interval.

than in the control group (13). In another study involving antibody detection by many methods except ELISA 6.46% of women with spontaneous abortion had positive serology (14). In a study in Saudi Arabia, utilizing the standard tube agglutination test, the rate of abortion in women with antibody titers ≥ 160 and ≤ 106 were 17.6% and 7.7%, respectively, showing a significant difference ($p < 0.04$) (15).

In accordance with our results, 4% of women with spontaneous abortion have been shown in another study to have serological data consistent with chronic brucellosis but without acute infection and an increased incidence of abortion could not be documented (16).

In most of the mentioned studies, except in that by Makhseed et al. (13), antibody detection was by methods other than ELISA. We have used ELISA as it is the most sensitive and specific serological assay (5). It is probable that some positive serological results in other studies are false positives, due to the cross-reaction of *Brucella* spp antigens with other gram negative bacilli sharing common antigens.

One striking finding in our study was the significant relationship between raw milk consumption as an independent variable and spontaneous abortion ($p = 0.009$). To find out any relationship between the consumption of raw milk and spontaneous abortion requires more research.

Therefore, we have concluded that brucella seropositivity was not more common in the women with spontaneous abortion than in women with normal pregnancy outcome in our region. More studies with ELISA in other regions are recommended.

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