Brucellosis in pregnancy and its response to the changing immunoglobulin A: A prospective controlled study

Gebelikte bruselloz ve değişen immünoglobülin A yanıtı: Prospektif kontrolli çalışma

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Abstract

Objective: This study aimed to define the rare Brucella infection in pregnancy and its effects on immunoglobulins (Ig).

Materials and Methods: This prospective study has conducted Brucella screening using the Rose Bengal test on pregnant and non-pregnant outpatients who did not show any specific Brucella symptoms. The immunoglobulin levels were measured using the enzyme-linked immunosorbent assay. The study group consisted of pregnant women who were at 20 weeks or below gestation and applied to our hospital outpatient clinic for routine check-ups. The control group consisted of healthy patients who applied for routine controls.

Results: This study included a total of 584 participants, 293 of whom were controls and 291 were the study (pregnant) participants. The study revealed a 1.5% incidence of Brucella during pregnancy. In acute and chronic Brucella infection, lower levels of IgA response were observed in pregnant cases compared to the control group.

Conclusion: Brucella infection is a disease that can cause fetal problems, especially in endemic areas. The role of the altered IgA response in pathologies that are associated with Brucella infection stands out as a new target for disease pathophysiology.

Keywords: Brucella, immunoglobulin A, pregnancy

Öz

Amaç: Bu çalışma, gebelikte nadir görülen Brusella enfeksiyonunu ve immünoglobulinler (Ig) üzerine etkilerini belirlemek amacıyla planlandı.

Gereç ve Yöntemler: Bu çalışma prospektif olarak planlandı. Bu amaçla, hastanemiz polikliniğine başvuran 20. gebelik haftası ile altı gebeler spesifik Brusella semptomlarına bakılmaksızın Rose Bengal testi ile tanılandı. Immünoglobulin seviyeleri enzim bağlı immunosorbent deneyi ile ölçüldü. Kontrol grubu rutin kontroller için başvuran sağlıklı hastalardan oluşturuldu.

Bulgular: Bu çalışmaya 293’ü kontrol ve 291’i çalışma (gebe) olmak üzere toplam 584 katılımcı dahil edildi. Çalışmada gebelikte brusella görülme sıklığı %1,5 olarak bulunmuştur. Kontrol grubuna göre daha düşük seviyelerde IgA yanıt gözlemlendi. Akut ve kronik Brusella enfeksiyonunun gebe olgularında kontrol grubuna göre daha düşük seviyelerde IgA yanıt gözlendi.

Sonuç: Brusella enfeksiyonu özellikle endemic bölgede fetal problemlere neden olabildiği bir hastalık. Brusella enfeksiyonu ile ilişkili patolojilerde değişen IgA yanının rolü, hastalığın patofizyolojisi için yeni bir hedef olarak ön çıkıktadır.

Anahtar Kelimeler: Brucella, immunoglobulin A, gebelik

PRECIS: This study is a case-control study evaluating the relationship with brucellosis and IgA levels in pregnancy.

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Introduction

Brucellosis in pregnancy is a rare disease that is associated with various obstetric complications. Unpasteurized dairy product consumption, especially raw milk, soft cheese, butter, and ice cream, is the most common mode of transmission\(^1\). Human-to-human transmission due to blood transfusion, tissue transplantation, breastfeeding, sexual contact, congenital transmission, and hospital infection has also been reported in rare cases\(^2\). Brucellosis is an important health problem in endemic areas, such as South and Central America, India, the Mediterranean basin, the Balkans, and the Middle East. Turkey is among the endemic regions with an incidence of 25.7 cases per 100,000 population\(^3\). Brucellosis is rare in pregnancy, with an incidence in endemic areas from 1.3% to 12.2% 3-5\(^6\). Existing studies have examined brucellosis based on the collected data from the general population. Brucellosis in pregnancy is a rare condition, and most brucellosis cases are detected due to suspicions based on clinical findings. Brucellosis typically presents with an insidious onset of fever, malaise, night sweats (associated with a strong, peculiar, and musty odor), and arthralgia\(^11\). Pregnancy-specific findings are unclearly defined. However, the disease is stated to be associated with abortion, premature delivery, intrauterine fetal demise, congenital malformations, neonatal death, and low birth weight\(^12\).

Demonstrating different specific classes or subclasses of antibody response in brucellosis has been suggested as useful in disease diagnosis and prognosis, as well as elucidating the differences between acute and chronic stages of brucellosis\(^13\)\(^14\). Patients with acute brucellosis have elevated Brucella-specific immunoglobulin (Ig) M alone. Patients with chronic brucellosis have elevated IgG and IgA antibodies only\(^15\)\(^16\). Changes in maternal immune regulation are observed during pregnancy. These changes do not constitute a case of generalized immunosuppression; however, they may include selective suppression and modulations. The process radically affects progesterone and estrogen, of which the levels change during pregnancy. Progesterone inhibits the synthesis of nitric oxide and tumor necrosis factor \(\alpha\) by macrophages by causing Th2 polarization. At the beginning of pregnancy, a relatively strong Th1 response occurs, which provides the required inflammatory environment for implantation. These interactions determine the effect of some developing infections during this period in the fetus. However, the relationship between Brucella infection and pregnancy is unclearly known. This study attempted to evaluate specific IgM, IgG, and total IgA levels and obstetric results by performing Brucella screening in the first and second trimester in pregnant women who did not have any specific symptoms and applied to the hospital for routine control. This study aimed to evaluate the changing immune parameters, especially IgA levels, in brucellosis and contribute to the literature on this subject.

Materials and Methods

This study was prospectively conducted at Yozgat Bozok University Faculty of Medicine, Department of Obstetrics and Gynecology. The study group consisted of pregnant women who were at 20 weeks or below gestation and applied to our hospital outpatient clinic for routine check-ups. Patients under 20 weeks of age were included in the study to detect complications that are associated with early pregnancy. The control group consisted of non-pregnant patients between the ages of 18 and 40 years. Individuals with hypertension, diabetes, cancer with hematological and rheumatological diseases, and acquired immunodeficiency syndrome that may cause immunosuppression, as well as those who used drugs, such as glucocorticoids, were excluded from the study. Data on potential risk factors for brucellosis, including age, rural area residence, socioeconomic status, contact with animals, consumption of raw and pasteurized milk, and previous intrauterine fetal death, were collected through individual interviews before the study. Additionally, patients’ body mass index (BMI), gravidity, gestational age, systemic diseases, and used medications were recorded. Pregnancy loss that occur at \(\leq 20\) gestational weeks was defined as abortion, whereas \(>20\) gestational week as in utero fetal death. Following that, blood samples were collected from the patients. All epidemiological, obstetric, clinical data, pregnancy outcome and newborn evaluations, and all laboratory results were recorded in a standardized manner. Patients with brucellosis were treated with cotrimoxazole or rifampin for at least 4 weeks.

Ethics committee approval 2017-KAEK-189_2018.02.27_01 and written consent from all participants were obtained.

Laboratory Evaluation

Blood samples are taken from the patients for serological tests and their sera were separated. The Rose Bengal tests and Coombs tests, which are used in the serological diagnosis of brucellosis from the obtained serum samples, were immediately studied, and the serum samples were stored at -20 °C until enzyme-linked immunosorbent assay (ELISA) tests were used to conduct ELISA IgG, IgA, and IgM studies. The Rose Bengal test (Biomedica, Turkey), Brucella Agglutination test with Coombs (Red Cell Biotechnology, Turkey), and Brucella IgM, IgA, and IgG (Novatec, Germany) tests by ELISA were studied with patient sera. Agglutination formation in the Rose Bengal test, titrations of 1/160 and above in the Coombs test, and Brucella IgM and IgG values of >11 Nova Tec Units (NTU) in the ELISA method were evaluated as positive.

Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences program (version 20, SPSS, Chicago, IL). Data were expressed as mean ± standard deviation and percentages. Data distribution was assessed using the Kolmogorov-Smirnov test. With non-parametric numerical data, the Mann-Whitney U test was conducted, whereas the
Student t-test for parametric numerical data. Triple comparisons were made via the employment of the Kolmogorov-Smirnov test. Categorical data were compared using the chi-square test. P-values of <0.05 were accepted as statistically significant.

**Results**

This study included a total of 584 participants, of whom 293 were controls and 291 were the study (pregnant) participants. The mean age of the pregnant women was 27.3±6 years, and the mean BMI was 26.4±5.1. The mean abortion was 0.2±0.6. The mean age of the control group was 27.5±5.6 years and the mean BMI was 26.2±4.8. The mean abortion was 0.2±0.7, without statistically significant differences between the groups’ mean values (p>0.05). Additionally, 48.5% of the pregnant women and 50.2% of the control group lived in rural areas, without significant differences between the groups (p>0.05). Moreover, no significant differences were found in the use of pasteurized milk between the groups (p>0.05). The mean week of gestation at the time of examination of the pregnant women was 11±3.7. Table 1 shows the demographic characteristics of the participants.

IgM results were positive in 16 (2.7%) participants, of whom 10 (3.6%) were pregnant and 6 (2.1%) were from the control group, without statistically significant differences between the groups (p=0.412). Additionally, 25 (4.3%) participants had positive IgG, of whom 10 (3.6%) were pregnant and 15 (5.3%) were from the control group, without significant differences in the results (p=0.148). The IgA result was 1.42±0.56 in pregnant women and 1.52±0.9 in the control group, without significant differences between the groups (p=0.612). The IgA concentration result was 8.46±4.77 in pregnant women and 10.47±6.27 in the control group, with significant differences between the groups (p<0.001) (Table 2).

IgA results and concentrations are shown in Table 3. Among the patients who had never encountered the disease, with negative IgM and IgG, a significantly lower IgA concentration was found

### Table 1. Demographic characteristics

|                        | Control group | Study group | OR   | 95% CI           | p-value |
|------------------------|---------------|-------------|------|------------------|---------|
|                        |               |             |      | Lower              | Upper              |         |
| Age (years)            | 27.5±5.6      | 27.3±6      | 0.49 | -0.82             | 1.11               | 0.559   |
| BMI (kg/m²)            | 26.2±4.8      | 26.4±5.1    | 0.42 | -0.95             | 0.71               | 0.954   |
| Gestational weeks      | -             | 11±3.7      | -    | -                 | -                  |         |
| Parity                 | 0.9±1         | 0.9±1       | 0.08 | -0.14             | 0.19               | 0.642   |
| Abortion               | 0.2±0.7       | 0.2±0.6     | 0.06 | -0.12             | 0.10               | 0.625   |
| Region of residence, n (%) | -             | -          | 1.06 | 0.76              | 1.48               | 0.678   |
| Rural                  | 147 (50.2)    | 141 (48.5)  | -    | -                 | -                  | -       |
| Urban                  | 146 (49.8)    | 150 (51.5)  | -    | -                 | -                  | -       |
| Use of pasteurized milk, n (%) | -             | -          | 0.84 | 0.60              | 1.18               | 0.364   |
| No                     | 122 (41.6)    | 132 (45.4)  | -    | -                 | -                  | -       |
| Yes                    | 171 (58.4)    | 159 (54.6)  | -    | -                 | -                  | -       |

Unless otherwise specified, results are presented as mean ± SD. CI: Confidence interval, BMI: Body mass index, OR: Odds ratio, SD: Standard deviation

### Table 2. Participants' immunoglobulin levels and results

|                        | All patients | Control group | Study group | p-value |
|------------------------|--------------|---------------|-------------|---------|
| IgG result, n (%)      | -            | -             | -           | 0.148   |
| Negative               | 559 (95.7)   | 268 (94.7)    | 270 (96.4)  | -       |
| Positive               | 25 (4.3)     | 15 (5.3)      | 10 (3.6)    | -       |
| IgM result, n (%)      | -            | -             | -           | 0.412   |
| Negative               | 568 (97.3)   | 277 (97.9)    | 270 (96.4)  | -       |
| Positive               | 16 (2.7)     | 6 (2.1)       | 10 (3.6)    | -       |
| IgA                    | 1.47±0.75    | 1.52±0.9      | 1.42±0.56   | 0.612   |
| IgA Concentration      | 9.43±5.63    | 10.47±6.27    | 8.46±4.77   | <0.001  |

Unless otherwise specified, results are presented as mean ± SD. Ig: Immunoglobulin, SD: Standard deviation
in pregnant women than in the control group (p<0.001). The IgA concentration of the pregnant women who had the disease, with positive IgG and negative IgM, was lower than the control group, but without statistically significant differences between the groups (p=0.086). IgA concentrations in patients with positive acute infections (IgM positive) were lower in pregnant women than in the control group; however, no significant differences were found between the groups (p=0.233). Three patients were found to be both IgG and IgM positive, of whom two were pregnant and one was from the control group (data not shown). Table 4 shows the IgA results of patients with positive Coombs test. Accordingly, no statistically significant differences were found between the groups; however, IgA concentrations were increased as the titer increased.

Discussion

This study revealed that Brucella infection can be observed in pregnancy without causing any specific symptoms. Study results revealed a 1.5% incidence of brucellosis during pregnancy. This rate was 2% in the study population. No brucellosis-related maternal/fetal death and fetal anomaly were observed in this study. In acute and chronic Brucella infection, lower levels of IgA response were observed in pregnant cases compared to the control group. The evaluation of cases without infection revealed low IgA levels in pregnant women.

The main immunoregulatory effect in pregnancy is to protect the developing fetus, which is an allograft for the mother, from maternal immune responses. Th2 polarization develops as a useful aberration for fetal protection. The question remains as to how this results for Brucella, for which there is a primarily cellular immune response. The Th1/Th2 shift is important for successful pregnancy continuation and changes throughout the pregnancy. The required inflammatory environment for implantation is provided by the relatively dominant effect of Th1 in the first trimester, a sufficient combat environment for intracellular sample Toxoplasma gondii is created during this period, and this mechanism works in the prevention of disease-related abortion. Additionally, until the end of the second and third trimesters, the Th2 response remains dominant, and this parasite becomes difficult to eradicate, which makes it easier for the fetus to become infected. Previous studies revealed different findings on maternal and fetal outcomes of brucellosis in pregnancy. Some studies revealed an increased risk of abortion and congenital anomalies\(^{17,18}\). However,

| Coombs titer | 80 | 160 | 640 |
|-------------|----|----|----|
| Mean        |    |    |    |
| SD          |    |    |    |

SD: Standard deviation, Ig: Immunoglobulin
The changing immune response during pregnancy and the course of Brucella infection is unclearly known. The current study revealed lower levels of IgA response in pregnant cases with acute and chronic Brucella infection compared to the control group. This might be due to the relatively dominant effect of the inflammatory environment that Th1 is required for implantation in the first trimester. However, further studies with control groups are needed to examine the changing immune response and immunoglobulin levels in pregnancy. These studies can guide the evaluation and treatment of pregnancy-related outcomes, especially in regions where Brucella is endemic.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethics Committee in Yozgat Bozkent University Faculty of Medicine, with approval number 2017- KAEK-189_2018.02.27_01.

Informed Consent: Written consent from all participants were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.B., Design: D.A.K., E.B., Data Collection or Processing: E.Y.S., M.D.C., N.Y., Analysis or Interpretation: M.K., M.D.C., T.O., E.S.Y., Literature Search: D.A.K., Writing: D.A.K., E.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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