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

Gebelikte bruselloz ve değişen immünoglobülin A yanıtı: Prospektif kontrollü 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 ve altı gebeler spesifik Brusella semptomlarına bakılmaksızın Rose Bengal testi ile tarandı. İmmünoglobulin seviyeleri enzim bağlı immünosorbent 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 edilmiştir. Çalışmada gebelikte Brusella görülme sıklığı %1,5 olarak bulunmuştur. Akut ve kronik Brusella enfeksiyonunda gebe olgularda kontrol grubuna göre daha düşük seviyelerde IgA yanıtı gözlendi.

Sonuç: Brusella enfeksiyonu özellikle endemik bölgelerde fetal problemlere neden olabilen bir hastalıktır. Brusella enfeksiyonu ile ilişkili patolojilerde değişen IgA yanıtının rolü, hastalığın patofizyolojisi için yeni bir hedef olarak öne çıkmaktadır.

Anahtar Kelimeler: Brucella, immünoglobulin 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[1,2]. 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[3-6]. 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[7]. Brucellosis is rare in pregnancy, with an incidence in endemic areas from 1.3% to 12.2% 3-5[8-10]. 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 α 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), gravida parity, gestational age, systemic diseases, and used medications were recorded. Pregnancy loss that occur at ≤20 gestational weeks was defined as abortion, whereas >20th 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 serums 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,

Table 3. IgA levels in acute, chronic, and previous Brucella infection, and those without infection

		Control group		Study group		p-value
		Mean	SD	Mean	SD	
IgG (-)	**IgA**	1.50	0.85	1.42	0.56	0.606
	IgA concentration	10.32	5.98	8.49	4.83	0.000
IgG (-) IgM (-)	**IgA**	1.50	0.85	1.43	0.56	0.310
	IgA concentration	10.32	5.95	8.59	4.86	<0.001
IgG (+)	**IgA**	1.91	1.44	1.31	0.51	0.956
	IgA concentration	12.81	10.00	7.59	3.05	0.618
IG (+) IgM (-)	**IgA**	2.01	1.44	1.43	0.45	0.283
	IgA concentration	13.47	10.03	8.24	2.82	0.086
IgM (-)	**IgA**	1.52	0.89	1.43	0.55	0.758
	IgA concentration	10.49	6.24	8.58	4.81	<0.001
IgM (+)	**IgA**	1.34	1.20	0.92	0.33	0.588
	IgA concentration	9.16	7.82	5.25	1.87	0.233

Results are presented as mean ± SD. SD: Standard deviation, Ig: Immunoglobulin

Table 4. The relationship between Coombs titer and IgA levels in acute infection

	Coombs titer						p-value
	80		160		640		
	Mean	SD	Mean	SD	Mean	SD	
IgA	0.93	0.26	0.88	0.78	1.36	1.09	0.717
IgA concentration	5.66	1.97	5.85	4.75	8.34	7.51	0.943

SD: Standard deviation, Ig: Immunoglobulin

evidence on the relationship between Brucella and abortion in the literature remains insufficient[19], and brucellosis is estimated to cause fewer spontaneous abortions in humans than in animals due to the absence of Erythritol in the human placenta and fetus[20]. However, most studies lack a control group in their research designs. The current study revealed that total IgA levels and concentrations in the study group were lower than in the control group. Previous studies on Ig levels in pregnancy have revealed varying findings. Amino et al.[21] revealed that IgG, IgA, and IgM concentrations significantly decreases in the second and third trimesters, and the average values decrease in the second trimester as 18%, 13%, and 9%, respectively, and these findings were independent of maternal age, hyperemesis, ABO incompatibility, and the sex and weight of the baby at birth. Recent studies revealed that the change in IgA levels during pregnancy is "dynamic"[22,23]. When the changes in brucellosis cases were examined in the current study, the IgA levels in individuals who had active Brucella infections during pregnancy were at lower levels than those who did not have Brucella infections, although these changes were not statistically significant. Similarly, lower IgA levels were found in pregnant women who had the infection compared to those who did not. Results revealed that the IgA levels significantly decreased in individuals who had Brucella infections during pregnancy. This decrease continued after the patients recovered from brucellosis, which indicates that Brucella infections affect mucosal immunity-related IgA levels. Additionally, IgA concentrations were found to increase with increasing Coombs titers. This may be related to the transmission of the infection through the gastrointestinal tract or to the altered immune response during pregnancy. A conducted study on rats to elucidate the response of Brucella infection in the human body revealed low-level and short-term IgA antibodies in rats infected with Brucella abortus. Additionally, the study revealed that IgA antibody production is induced in infected rats, but its role in providing protection is unknown[24]. In a study evaluated all Ig changes in acute and chronic brucella infection in the normal population, anti-brucellosis IgG, IgM, IgA, IgE, IgG1 and IgG3 antibodies were increased in patients with acute brucellosis, while an increase in IgG, IgA, IgE and IgG4 was observed in patients with chronic brucellosis[25].

Brucellosis in pregnancy is described as a condition that should be primarily considered in areas where it is endemic and in cases with continuous fever and vaginal bleeding; however, the current study revealed that the disease can also occur in completely asymptomatic individuals. The biggest limitation of this study is that functional changes in antibody concentrations could not be completely excluded, which was also a limitation for many similar studies on different populations using different immunoassays. However, this is the first study to include a control group to better understand brucellosis and changes in IgA levels during pregnancy.

Conclusion

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 Bozok 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.Ş., M.D.Ç., N.Y., Analysis or Interpretation: M.K., M.D.Ç., T.O., E.S.Y., Literature Search: D.A.K., Writing: D.A.K., E.B.

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