Brucellosis impairs endothelial functions in chronic symptomatic patients without overt cardiac involvement

Bruselloz kronik semptomlu hastalarda belirgin kalp tutulumuna yol açmaksızın endotel fonksiyonlarını bozar

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ABSTRACT

Objective: Brucellosis is an important infectious disease, especially in developing countries, and may involve any organ, including the cardiovascular system. This study aimed to assess cardiac and endothelial functions in brucellosis patients.

Methods: Seventy-three patients with brucellosis and seventy-five healthy volunteers from Turkey were enrolled between 2011 and 2013 in this cross-sectional study. Diagnosis was established by the Rose-Bengal test, positive Brucella standart tube agglutination test, and Coombs STA and/or isolation Brucella species from blood. Cases were divided into three groups: Group I; acute brucellosis, Group II; subacute brucellosis, and Group III; chronic brucellosis. Healthy individuals comprised Group IV. All patients underwent transthoracic echocardiography and brachial artery flow-mediated dilatation (FMD) test.

Results: Groups I to IV comprised 35, 18, and 20 patients, and 75 controls respectively. The most frequent symptoms were arthralgia (82%), fever (92%), and fatigue (97%). Echocardiography revealed no difference among the 4 groups regarding left ventricular systolic and diastolic functions and valvular functions. Brachial artery diameter after hyperemia was significantly different among the groups (p=0.002). Post hoc test showed Group III to have significantly lower brachial artery diameter after hyperemia compared to other groups (p=0.02, p=0.004 and p=0.001, respectively). FMD was also significantly lower in Group III compared to Groups I, II and IV (p<0.001 for each).

Conclusion: Brucellosis impairs endothelial functions in chronic symptomatic patients without overt cardiac involvement.

ÖZET

Amaç: Bruselloz gelişmekte olan ülkelerde görülebilen önemli bir enfeksiyöz hastalıktır ve kardiyovasküler sistem dahil herhangi bir organı tutabilir. Bu çalışmada, brusellozlu hastalarda kalp ve endotel fonksiyonları incelendi.

Yöntemler: Türkiye'de 2011 ve 2013 yılları arasında gerçekleştirilen bu kesitsel çalışmaya brusellozlu 73 hasta ile sağlıklı 75 gönüllü alındı. Rose-Bengal testi, pozitif brusella standart tüp aglütinasyon testi, Coombs STA ve/veya kandan brusella türlerinin izolasyonu ile bruselloz tanısı konuldu. Hastalar üç gruba ayrıldı: Grup I akut brusellozlu, grup II subakut brusellozlu ve grup III kronik brusellozlu. Sağlıklı gönüllüler grup IV'ü oluşturdu. Tüm hastalara transtorasik ekokardiyografi ve brakiyal arter akım-ilişkili dilatasyon (FMD) testi uygulandı.

Bulgular: Grup I-IV sırasıyla 35, 18, 20 hasta ve 75 sağlıklı gönüllüden oluşmaktaydı. En sık bulgular, artralji (%82), ateş (%92) ve halsizlik (%97) idi. Ekokardiyografik incelemede sol ventrikül sistolik ve diyastolik fonksiyonları ve kapak fonksiyonları açısından dört grup arasında fark saptanmadı. Hiperemi sonrası ölçülen brakiyal arter çapları gruplar arasında anlamlı olarak farklı idi (p=0.002). Post-hoc testine göre kronik brusellozdan oluşan grup III'de hiperemi sonrası brakiyal arter çapı diğer gruplara göre anlamlı olarak daha düşük idi (sırasıyla, p=0.02, p=0.004 ve p=0.001). Benzer şekilde grup III'de FMD değerleri grup I, II ve IV'e göre (p<0.001 hepsi için) anlamlı olarak düşük idi.

Sonuç: Bruselloz kronik semptomlu hastalarda belirgin kalp tutulumuna yol açmaksızın endotel fonksiyonlarını bozar.

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Abbreviations:

A	*Late diastole*
CRP	*C-reactive protein*
DT	*Deceleration time*
E	*Early*
ED	*Endothelial dysfunction*
ESR	*Erythrocyte sedimentation rate*
FMD	*Flow-mediated dilatation*
LV	*Left ventricular*
LVEF	*Left ventricular ejection fraction*
PW	*Pulsed wave*
STA	*Standard tube agglutination*
TDI	*Tissue Doppler imaging*

Brucellosis is a common zoonosis, with an annual occurrence of more than 500,000 cases, and is mostly seen in Asia and Mediterranean regions.[1] Brucellosis is caused by Gram-negative bacteria, *Brucella spp*. The main modes of transmission include occupational exposure to animals, ingestion of raw dairy products, and consumption of infected meat from domestic animals.[2] Brucellosis has a broad range of clinical presentations, which can overlap with many other infectious and non-infectious diseases.[3] It is a systemic disease which can involve any organ system, including the musculoskeletal, genitourinary, gastrointestinal, hematologic, nervous, skin and mucous membranes. Respiratory complications can also be observed.[2,4-8] Although cardiovascular involvement is rare, accounting for only 1-2% of all cases, mortality risk is high due to cardiovascular complications.[9-11] Prevalence of cardiac involvement in brucellosis may be underestimated because thorough echocardiographic studies in patients with brucellosis are lacking. Brucellosis may also be associated with vascular complications, including vasculitis, thrombosis of deep arteries and deep veins,[12-13] but there is a lack of data on the underlying mechanisms of vascular injury. Clinically, brucellosis may progress in several forms, which are defined according to their history, symptoms, and clinical presentation time: acute brucellosis (0-2 months), subacute brucellosis (2-12 months), and chronic brucellosis (>12 months).[2]

Flow-mediated dilatation (FMD) is defined as vasodilation induced in response to a sudden increase in shear stress during reactive hyperemia.[14] It indicates the capacity of the endothelium to cause smooth muscle cell relaxation and vasodilation. Previous studies have shown that impaired FMD is associated with endothelial dysfunction (ED), which is considered to be the precursor of several systemic diseases.[15,16] FMD may permit early detection of ED. Recent data have shown that several chronic infections may be associated with ED.[17,18] The risk of atherosclerotic process may be higher in subjects with a prominent inflammatory response. Brucellosis may also be complicated by multiple organ damage, but no previous study has evaluated endothelial functions in patients with brucellosis.

In this study, we aimed to assess cardiac and endothelial functions in brucellosis patients.

METHODS

Study population

Brucellosis was diagnosed in 124 patients from September 2011 to November 2013 at three referral hospitals in eastern Turkey; the Department of Infectious Diseases of Iğdır State Hospital, the leading research center, Doğubeyazıt State Hospital, and Kars Kafkas University Medical Faculty. These patients also underwent cardiovascular evaluation in the Cardiology Departments of the same centers, following which 73 were included in the study group. Exclusion criteria were: age >45 years, history of coronary artery disease (CAD), hypertension, diabetes mellitus, dyslipidemia, and cigarette smoking (>one cigarette/d currently or within the previous five years). Seventy-five healthy volunteers admitted to Iğdır State Hospital comprised the control subjects. FMD measurements of all brucellosis patients and healthy volunteers were performed in Iğdır State Hospital. Approval was granted by the local Ethics Committee for this cross-sectional study, and all participants provided written informed consent prior to the study. The study was conducted in accordance with the principles of the Helsinki Declaration.

Diagnosis and classification of brucellosis

Diagnosis of brucellosis was based on the following criteria: (1) isolation of *Brucella spp.* in blood culture; (2) a compatible clinical picture, such as arthralgia, fever, sweating, chills, headache, and malaise, and detection of specific antibodies at significant titers and/or demonstration of a fourfold rise, at least, in antibody titer in serum specimens taken over two or three weeks (Titers ≥1/160 in the standard tube agglutination test (STA) were determined to be significant). The slide agglutination or Rose Bengal plate agglutination test was performed for screening; (3) determination of positive Coombs STA titers ≥1/320 despite negative STA. Each criterion was considered adequate for initial diagnosis of brucellosis, and with all three study centers located in endemic regions, low STA level was also considered an indication of brucellosis on the basis of high clinical suspicion.

Patients with acute brucellosis were reported as Group I, those with subacute brucellosis as Group II, and those with chronic brucellosis as group III. Healthy individuals comprised Group IV.

Demographic, epidemiological, clinical and laboratory data were collected from all patients in accordance with the study protocol. Standard laboratory measurements including complete blood count, electrolytes, creatinine, fasting glucose, fasting lipid profile, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were obtained. Blood cultures were taken from patients depending on their clinical findings.

Evaluation of endothelial function

Endothelial function was assessed at the start of the study by brachial artery FMD during reactive hyperemia. The test was performed according to Coretti et al. guidelines.[19] Patients were instructed to lie quietly in a supine position for ten minutes before the study. The left brachial artery was used in all subjects. All tests were conducted by a trained research technician, and reviewed by an experienced cardiologist. Arterial diameter was assessed by high-resolution ultrasound (Philips HD11 XE, Philips Medical Systems, Andover, Massachusetts) with a standard 7.5-MHz linear-array transducer. The brachial artery segment just above the antecubital fossa was scanned in longitudinal direction. The pneumatic blood pressure cuff was positioned above the elbow, and inflated to 50-60 mmHg above systolic pressure for approximately five minutes. The cuff was then released, and the brachial artery scanned continuously for five minutes. Mean diameter of the brachial artery segment was measured with simultaneous electrocardiography (ECG) recordings, connected to ultrasonography. Frames from three consecutive cycles were obtained at the R-wave peak, and the average of the results was noted. FMD was measured one minute after cuff deflation. FMD value was accepted as the percent change of vessel width compared to basal value. FMD was calculated using the formula '([maximum diameter-basal diamete]/basal diameter) x 100'. Mean FMD values were also compared among the groups.

Echocardiographic evaluation

Echocardiographic recordings were obtained using standard ultrasonography (Philips HD11 XE, Philips Medical Systems, Andover, Massachusetts) with a 2.5-3.5 Hz transducer at the parasternal and apical windows. Septal wall thickness, left ventricular (LV) posterior wall thickness, and LV end-diastolic and end-systolic diameters were measured using two-dimensional images and M-mode cross-sections targeting the papillary muscle level. Left ventricular ejection fraction (LVEF) was measured using the biplane modified Simpson's method, and long-axis function by tissue Doppler imaging (TDI) S' wave.[20] LV diastolic function was estimated by pulsed wave (PW) Doppler on transmitral flow, assessing peak velocities in early (E) and late diastole (A), E/A ratio, deceleration time of E wave (DT) and TDI-septal E' (early) and A' (late) waves. The E/E' ratio was calculated and used as an index of LV filling pressures.

Statistical analysis

Continuous variables are presented as medians and interquartile ranges or as mean ± SD, as appropriate. Categorical variables are presented as observed frequencies and percentages. Continuous variables were compared using Student's t test or the Mann–Whitney U-test, as appropriate. Categorical variables were compared using the Chi square test. Inter-rater agreement between the cardiologists, and intra-rater agreement in determining FMD values were investigated using the Kendall tau c test. Analysis of variance (ANOVA) was used to compare the four groups for continuous variables after testing for normal distribution. The Bonferroni correction was applied for post hoc analysis. P values <0.05 were considered significant. Statistical analyses were performed using the SPSS, version 17 (SPSS, Chicago, Illinois).

RESULTS

The study was composed of patients (Groups I to III) with brucellosis (n=73) and healthy individuals (n=75, group IV). With regard to clinical findings, 35 (48.0%) cases were evaluated as having acute (group I), 18 (24.6%) subacute (group II), and 20 (27.4%) chronic disease (group III). In terms of gender distribution, 20 acute cases (57.1%), 9 subacute cases (50.0%), and 14 chronic cases (70.0%) were female. Forty-four (58.7%) healthy individuals were female. Demographic characteristics of all study subjects are presented in Table 1. There was no significant difference among the four study groups in terms of age, gender, height, weight, body mass index, systolic and diastolic blood pressure and heart rate.

Table 1. Demographic characteristics of subjects

Variables	Group I (n=35)	Group II (n=18)	Group III (n=20)	Group IV (n=75)	*p*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Gender (Female / Male)	20/15	9/9	14/6	44/31	0.72
Age (years)	34.9±12.6	35.4±14.7	34.7±12.8	35.5±12.2	0.92
Height (cm)	166.3±7.1	170.2±8.5	166.3±5.8	167.6±7.1	0.24
Weight (kg)	60.8±4.9	60.5±5.5	59.3±5.3	60.3±5.0	0.75
Body mass index (kg/m^2)	21.9±3.4	21.2±4.2	21.4±4.8	20.8±3.7	0.84
Systolic blood pressure (mmHg)	122.6±15.9	127.3±11.2	124.6±10.7	124.2±13.6	0.69
Diastolic blood pressure (mmHg)	74.1±9.4	72.2±7.2	74.2±6.5	73.5±8.05	0.85
Heart rate (beat/min)	81.4±11.8	78.5±10.8	82.7±13.9	80.6±12.0	0.73

Most frequent symptoms were arthralgia (82%), fever (92%), and fatigue (97%). Other symptoms were myalgia (45%), loss of appetite (60%), weight loss (36%), abdominal pain (19%), nausea (16%), vomiting (10%), and headache (10%). Physical examination also revealed hepatomegaly (30%), splenomegaly (25%), and peripheral arthritis (8%).

Laboratory findings of the study patients and control groups are summarized in Table 2. ANOVA analysis revealed significant difference among the four groups in terms of ESR and CRP ($p<0.001$ for each). Post hoc analysis showed Group II (subacute brucellosis) to have significantly increased ESR compared to Groups I, III and IV ($p=0.005$, $p=0.044$ and $p<0.001$ respectively), while Group IV (control group) had significantly decreased ESR compared to Groups I to III ($p<0.001$ for each). Group IV had decreased CRP levels compared to Groups I to III ($p<0.001$ for each).

The most common species was Brucella melitensis. Rose-Bengal test was positive in all patients except one (98.6%). Sixty patients (82.2%) had titers >1/160 in STA test. Four (5.5%) cases who had at least a fourfold rise in antibody titer received the diagnosis of brucellosis. Seven cases (9.6%) who had a negative STA were found to be positive by Coombs STA. Two patients (2.7%) showed *Brucella spp.*

Table 2. Laboratory findings of study patients

Variables	Group I	Group II	Group III	Group IV	*p*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
White blood cell count (x10^9/μL)	9.3±10.0	6.9±1.9	7.7±2.2	9.5±9.6	0.62
Hemoglobin (g/dl)	13.9±1.4	13.9±0.9	14.2±0.9	14.1±1.2	0.82
Platelet count (10^3 μL)	239.7±66.1	217.4±41.9	244.2±76.7	221.3±46.6	0.21
Fasting glucose (mg/dL)	85.1±13.3	82.0±12.8	83.8±13.2	82.3±10.3	0.66
BUN (mg/dl)	15.8±3.2	16.6±4.1	15.5±4.0	16.1±3.8	0.79
Creatinine (mg/dl)	0.7±0.2	0.7±0.1	0.7±0.2	0.7±0.2	0.90
SGOT (U/L)	35.6±8.5	35.0±10.0	35.7±9.6	35.0±8.8	0.90
SGPT (U/L)	47.5±11.1	48.1±12.8	53.2±10.6	49.4±11.4	0.34
TSH (μIU/mL)	2.5±0.9	2.6±0.9	2.3±0.8	2.2±0.8	0.19
Sedimentation rate (mm/hr)	16.6±6.3	20.6±4.3	16.0±6.6	7.9±2.9	<0.001*
C-reactive protein (mg/L)	0.8±0.4	1.2±1.3	0.8±0.5	0.4±0.2	<0.001*

BUN: Blood urea nitrogen; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate pyruvate transaminase; TSH: thyroid-stimulating hormone. *Post hoc test (Bonferroni) was performed to compare the groups (see text).

growth in their blood cultures.

Brachial artery basal diameter, brachial artery diameter after hyperemia, and FMD values are given in Table 3. There was no significant difference among the groups regarding basal brachial artery diameter (p=0.54). Brachial artery diameter values after hyperemia were significantly different among the groups (p=0.002). Post hoc analysis showed Group III had significantly lower brachial artery diameter after hyperemia compared to Groups I, II and IV (p=0.02, p=0.004 and p=0.001 respectively). Similarly, FMD value was significantly lower in Group III compared to Groups I, II and IV (p<0.001 for each).

Magnitude of FMD of the brachial artery did not correlate with serum levels of inflammatory markers such as ESR and CRP (p=0.119 and p=0.585). Moreover, there was no correlation between FMD and antibody levels (p=0.112).

Left ventricular systolic and diastolic diameters and wall thicknesses were similar among the groups. LVEF was within normal range in all groups (p<0.30). Evaluation of diastolic parameters revealed a similar mitral E and A velocity, E/E' ratio, and DT among the groups. Aortic and pulmonary velocities were also similar. Of two patients (3.1%) having mild valve disease, one had mild mitral regurgitation and the other

Table 3. Flow-mediated dilatation test findings

Flow-mediated dilatation parameters	Group I	Group II	Group III	Group IV	*p*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Basal diameter (mm)	28.2±2.2	28.6±1.5	28.5±2.1	28.7±1.6	0.54
Hyperemic diameter (mm)	33.0±2.2	33.4±1.9	31.2±2.2	33.0±1.8	0.002
Flow-mediated dilatation ratio (%)	15.0±3.8	14.4±3.8	9.1±1.8	14.5±2.0	<0.001*

*Post hoc test (Bonferroni) was performed to compare the groups (see text).

Table 4. Echocardiographic parameters

Variables	Group I	Group II	Group III	Group IV	*p*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
LVEDD (cm)	4.8±0.4	4.6±0.3	4.9±0.4	4.8±0.3	0.45
LVESD (cm)	2.8±0.4	2.7±0.4	2.9±0.5	2.8±0.4	0.82
IVS (cm)	0.9±0.9	0.9±1.0	0.9±0.9	0.9±1.0	0.92
Posterior wall (cm)	0.9±1.0	0.9±1.0	0.9±0.8	0.9±0.9	0.80
Aortic diameter (cm)	2.4±0.2	2.5±0.3	2.4±0.3	2.4±0.3	0.88
Left atrium diameter (cm)	2.8±0.4	2.9±0.4	2.8±0.5	2.9±0.5	0.60
EF (%)	63.6±3.0	63.2±3.3	63.0±3.3	64.2±2.8	0.30
PW and CW Doppler echocardiographic measurements					
Aortic velocity (m/sec)	1.2±0.2	1.1±0.1	1.1±0.2	1.1±0.1	0.52
Pulmonary velocity (m/sec)	0.9±0.2	0.9±0.2	0.8±0.1	0.9±0.1	0.73
E-wave (m/sec)	0.7±0.1	0.7±0.2	0.8±0.1	0.8±0.2	0.77
A-wave (m/sec)	0.4±0.2	0.4±0.3	0.5±0.2	0.4±0.1	0.33
E/E' ratio	0.6±0.2	0.8±0.6	0.7±0.2	0.7±0.1	0.1
DT (msec)	180.8±13	179.1±13.7	179.0±11.3	183.1±12.6	0.46

CW: Continuous Wave; DT: Deceleration time; EF: Ejection fraction; IVS: Interventricular septum; PW: Pulse Wave; LVEDD: Left ventricular end-diastolic diameter; LVESD: Left ventricular end-systolic diameter.

mild-to-moderate aortic regurgitation without any sign of endocarditis. Pericarditis or myocarditis was not detected in any patient.

M-mode, 2D, PW, and CW Doppler echocardiographic measurements of the four study groups are summarized in Table 4.

Intra-rater agreement in determining FMD values was almost perfect (Kendall tau-c= 0.98, p<0.001). Inter-rater agreement in determining FMD values was substantial (Kendall tau-c= 0.73, p<0.001).

DISCUSSION

This study revealed decreased FMD values in patients with chronic brucellosis when compared with age- and gender-matched healthy controls. All four groups were comparable regarding gender, age, blood pressure, lipid profile, family medical history, and smoking status. Therefore, decreased FMD values cannot be associated with traditional atherosclerotic risk factors, and may be attributed to the inflammatory nature of brucellosis. Furthermore, there was no significant cardiac involvement in the current study; only mild valvular insufficiency was observed, and this was not associated with infective endocarditis.

Brucellosis is a chronic infection which causes morbidity in animal and human populations. It is an extremely important disease worldwide, but especially in developing countries.[2,21] Brucellosis has not been yet eradicated in Turkey, and is particularly endemic in eastern regions due to lack of a coordinated national approach and low socioeconomic status.[22] Our study included patients admitted to three health centers located in eastern Turkey (Iğdır, Ağrı, Kars) with the most common species, Brucella melitensis.

Clinical diagnosis of brucellosis is challenging, and may take months and even years.[23] Clinical presentation includes fever, fatigue, sweating, weight loss, and arthralgia. Subacute cases show a variable clinical picture, and usually present less severe symptoms compared to the acute form. Patients with chronic brucellosis usually present with complaints of generalized musculoskeletal pain, malaise and psychiatric abnormalities.[4-9]

Cardiac complications of brucellosis (endocarditis, myocarditis and pericarditis) are rare. It is reported to be as low as 1% of all cases.[10] The most common cardiac involvement is endocarditis, in which the aortic valve, and less frequently the mitral valve, is affected.[9,24,25] It is the most serious complication, and the principal cause of death. Unfortunately, it requires a high level of suspicion. Early recognition and echocardiographic assessment may guide the clinician toward appropriate diagnosis and treatment. However, there is much controversy regarding appropriate treatment (medical versus surgery), and duration of antibiotherapy,[26] necessitating further studies. Prevalence of myocarditis and pericarditis in the absence of endocarditis is much lower, but survival rates are higher in cases of pure pericarditis or myocarditis.[10] In our study, two patients (3.1%) had minimal valve disease without any sign of endocarditis. No patient had pericarditis or myocarditis. There was no significant difference among the four groups in terms of systolic and diastolic echocardiographic parameters.

Vascular complications due to Brucella infection have been reported only rarely in the literature. Some complications include arteritis,[11] DVT,[12] thrombophlebitis[13] and cerebral vein thrombosis.[27] The impact of brucella infection on endothelial integrity is not clear. Several mechanisms have been proposed for the development of vascular complications: direct endothelial damage, indirect effect of toxins or cytokines, immune reaction in the vessel wall to a Brucellosis antigen.[12,28] Development of a hypercoaguable state may be responsible for thrombotic complications.[12] According to some previous reports, infectious disease may cause transient ED (evaluated by FMD). It increases inflammatory markers and generates an atherogenic lipid profile.[29] For instance, several microorganisms, such as *Chlamydia pneumoniae*, *Human cytomegalovirus*, etc. may cause endothelial dysfunction by having a direct effect on arterial walls. This may lead to smooth muscle cell proliferation, platelet aggregation and release of cytokines.[18] The inflammatory response may be associated with high virulence of the underlying pathogen or atherogenic host-pathogen interactions.[17]

ED is defined as the impairment of endothelial cell dilation.[30] Nitric oxide is one of the most important vasodilating substances released by the endothelium. Diminished nitric oxide levels and the activation of vasoconstrictors such as endothelin-1 and angiotensin II play a key role in ED development. ED is not only associated with cardiovascular disease, but may also

precede its development.[31] The current gold standard technique for noninvasive assessment of ED is FMD, which facilitates the relaxation of an artery in response to increased shear stress. Reactive hyperemia occurring after ischemia is evaluated and vasodilatation due to reactive hyperemia is visualized in superficial arteries in ultrasonographic scan.[32]

Since our study was based on the hypothesis that brucellosis could also be associated with ED, we evaluated endothelial functions using FMD. FMD values did not differ between acute/subacute brucellosis and control subjects. However, patients with chronic brucellosis had significantly reduced FMD. The underlying mechanism is not clear, but the microorganism might be interacting with genetical and environmental predisposing factors. On the other hand, there was no correlation between antibody levels and FMD, and both ESR and CRP levels did not correlate with FMD. Patients with subacute brucellosis (Group II) had increased inflammatory parameters compared to chronic brucellosis patients (Group III), but FMD was found to be more impaired in chronic brucellosis patients (Group III). This shows that ED due to brucellosis is associated with duration and chronicity of the infection. Future large-scale studies are needed to evaluate endothelial functions in patients with brucellosis.

In routine practice, brucellosis may be misdiagnosed, and therefore the physicians in both endemic and non-endemic areas should consider brucellosis in differential diagnosis in cases of febrile disease with arthralgia and other focal findings. A coordinated national approach is necessary for recognition and reporting of this endemic disease.

Limitations

Several limitations are present in our study. The first was the relatively small sample size, which limited the power of our statistical analysis. The disease is endemic in our country, but the true incidence can not be estimated as most cases remain unrecorded. Although the preliminary findings are interesting, their clinical relevance could be better understood if endothelial functions could be reassessed after administration of specific antibiotherapy and during long-term follow-up. The study sample included younger subjects with minimal risk factors, so the rate of cardiac complications may not reflect the real incidence. Although FMD is frequently used for ED evaluation, the measurement has physiological variations which should be taken into consideration during analysis of the findings. Furthermore, FMD permits assessment of macrovascular endothelial function and is less sensitive in the determination of early changes of ED.

In conclusion, we have shown that FMD values were significantly decreased in patients with chronic brucellosis without overt cardiac involvement. This raises the possibility that the chronicity of brucellosis may contribute to mechanisms relevant to the development of ED. Larger studies are required to evaluate the impact of brucellosis and the association with genetic and environmental factors.

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Key words: Brucellosis; echocardiography; endothelial dysfunction; flow-mediated dilatation.

Anahtar sözcükler: Bruselloz; ekokardiyografi; endotel disfonksiyonu; akım ilişkili dilatasyon.