Lower Circulating Levels of Soluble TNF-Like Weak Inducer of Apoptosis and Chromium in Treated Brucella Infection

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Abstract- Brucellosis is a common infectious disease around the globe. The aim of the present investigation was to evaluate the effect of brucellosis on the serum levels of chromium (Cr), lead (Pb), soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) as well as high-sensitivity C-reactive protein (hs-CRP) and homocysteine in patients suffering from brucellosis. The present case-control study was executed on 40 treated brucellosis patients (case) and 40 healthy individuals (control). Blood samples were obtained from all the participants for the measurement of the desired indices. The serum levels of chromium in the case group (0.109±0.025 µg/L) was significantly lower compared with that in the control group (0.121±0.027 µg/L, *P*=0.047). The levels of lead in serum were almost unchanged between the two groups (12.58±1.94 vs. 12.28±2.42 µg/dL, *P*=0.533). The sTWEAK levels were significantly lower in the case group as compared to the control subjects (235.20±48.45 pg/mL vs. 262.00±67.25 pg/mL, *P*=0.044). The levels of hs-CRP were significantly higher in the case group (2.23±0.34 mg/L) than that in the control group (2.05±0.32 mg/L, *P*=0.016); similarly, homocysteine levels were higher in the serum of patients in case group as compared to the control ones (16.18±4.47 µmol/L vs. 14.36±3.06 µmol/L, *P*=0.038). The current investigation underlined that brucellosis causes alterations in serum chromium and sTWEAK levels, the markers that are considered as the predictors of cardiovascular disease.

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Keywords: Brucellosis; Chromium; Lead; Soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK)

Introduction

Brucellosis is a zoonotic disease caused by Brucella species, a group of nonencapsulated, nonmotile, facultatively intracellular coccobacilli (1). In northwest Iran, brucellosis is a relatively prevalent disease with an annual incidence of 50 per 100000 population; however, in recent years, its incidence has been significantly decreased with rises in literacy rate and implementation of livestock vaccination programs (2). The main routes of infection are food-borne, typically through the ingestion of contaminated dairy products, and occupational exposure, as observed in veterinarians or abattoir employees. In farm animals, sexual transmission of brucella spp. is another source of infection, with bacteria being found both in semen and in vaginal fluids (3). Clinical presentation of brucellosis is often nonspecific, resembling other infectious and even noninfectious diseases. While osteoarticular complication is the most frequent sequela of the disease (4), the main clinical manifestations include fever, fatigue, arthralgia, and muscle pain (5).

Secreted principally by the macrophages, TNF–like weak inducer of apoptosis (TWEAK) is a cytokine belonging to the tumor necrosis factor (TNF) family (6). Fibroblast growth factor-inducible 14 (Fn14) functions as the sole receptor for TWEAK, and the contribution of TWEAK-Fn14 axis to the development of atherosclerotic plaques has been well documented in the experimental studies (7). Soluble TNF–like weak inducer of apoptosis (sTWEAK) is the product of proteolytic processing of TWEAK by furin (8). Low circulating sTWEAK levels are known to be associated with high carotid intima-media thickness (cIMT), the predictor of cardiovascular disease (9), and, therefore, is a suggested risk indicator for the development of

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cardiovascular disease (10). Low sTWEAK levels have been noted in patients with treated human immunodeficiency virus (HIV) infection, correlating with cIMT (11). Apart from sTWEAK and cIMT, homocysteine, and high-sensitivity C-reactive protein (hs-CRP) are other two important widely-accepted serum markers known to be increased in the individuals with CVD risk (12).

Chromium (Cr) is an essential trace element that participates in the modulation of insulin function, metabolic syndrome, and cardiovascular disease. The correlation between low serum chromium levels and the incidence of type 2 diabetes has previously been established. While existing evidence denotes a possible link between low circulating levels of chromium and the occurrence of cardiovascular disease (13), higher levels of lead (Pb) in blood have been reported to be associated with clinical cardiovascular outcomes (14). Fluctuations in the tissue levels of trace elements, including chromium and lead, have been observed in a variety of infectious diseases that affect the pathogenicity of the invading microorganisms and the responsiveness of the host immune cells (15).

The aim of the current investigation was to assess the effects of brucellosis on the serum markers that are considered as the predictors of cardiovascular disease, including serum chromium and lead levels, as well as homocysteine, hs-CRP, and sTWEAK levels in patients with brucellosis.

Materials and Methods

Participants

In the present investigation, 40 brucellosis patients (29 men, 11 women, age range 27-68 years and mean±SD=44.72±13.28) were included. The patients received the gold standard treatment of brucellosis, i.e., streptomycin (0.75-1 g daily for 14-21 days) along with doxycycline (100 mg twice daily for 6 weeks) (16). The participants were admitted from October 2017 to December 2018 in an infectious disease ward at the Imam Khomeini Hospital of Urmia County, West Azerbaijan Province, Iran. Brucellosis was diagnosed based on a history of potential exposure, a presentation consistent with the disease (fever, sweating, appetite, and weight loss, myalgia, headache, chills, acute monoarthritis), positive serological tests, i.e., Wright titer ≥1/160 together with 2-mercaptoethanol test (2 ME) ≥1/80 or Coombs Wright test ≥1/320, detection of bacteremia via blood cultures or peripheral blood-based polymerase chain reaction (17). Selected from the same geographical region, the control group consisted of 40 healthy participants (30 men and 10 women, age range 25-62, mean±SD=35.40±6.85) who did not have previous exposure to livestock, clinical signs and symptoms associated with brucellosis, and positive laboratory findings on serologic examinations. This study has been officially confirmed by the Ethics Committee in Biomedical Research, Urmia Branch of Islamic Azad University (No.: IR.IAU.URMIA.REC.1397.09), and throughout the research, an informed consent was obtained from all the participants.

Sample collection

Collected in anticoagulant-free tubes, fasting blood samples (10 ml) were obtained from all the participants, and the separated serum samples were stored at −70° C.

Measurement of the serum levels of chromium and lead

To eliminate the matrix interference, 300 µL of serum was mixed with 15 µL ultrapure concentrated nitric acid in a 500 µL microtube and vortexed for 1 min. The microtubes were then heated for 5 min in a 70°C water bath, vortex-mixed for 10 s, and centrifuged for 10 min at 900 g. The supernatant was then transferred into a Teflon sampling cup for analysis. Finally, the concentrations of the trace elements were determined using an atomic absorption spectrometer (Varian Spectrum 220, Australia). The wavelengths of the hollow-cathode lamp for chromium and lead were 357.9 nm and 283.3 nm, respectively.

Immunochemical analyses

Serum hs-CRP levels were quantitated with an immunoturbidimetric assay (Pars Azmoon, Tehran, Iran) using an automated clinical chemistry analyzer (Dirui CS-400, Changchun, China). Serum TWEAK (RayBiotech, Norcross, GA; Cat# IQH-TWEAK-O43508) and homocysteine (Cusabio, Wuhan, China; Cat# CSB-E13814h) levels were determined by commercial kits based on enzyme-linked immunosorbent assay.

Data analysis

The sample size was calculated to be 40 individuals per each group. To achieve a power of 80%, type one (α) and type two errors (β) were set to be 0.05 and 0.20, respectively. Based on a previously published investigation 18, a mean difference (d) of 27.76 mg/L and a standard deviation (SD) of 48.31 mg/L for hs-CRP

were implemented to calculate the sample size.

An independent sample *t*-test using SPSS version 18 was carried out to analyze data. Results were expressed as mean±SD; $P<0.05$ was considered a significant difference.

Results

Chromium and lead

No significant differences were observed in the serum levels of lead between the case and control groups (Table 1). By contrast, the serum levels of chromium in brucellosis patients were significantly lower than that in the healthy individuals, as indicated in table 1.

The serum levels of homocysteine in brucellosis patients (16.18±4.47 µmol/L) were significantly higher than those in the control subjects (14.36±3.06) (P=0.038). Similarly, the levels of hs-CRP in the brucellosis group were significantly higher than its levels in the control group [2.23±0.34 mg/L vs. 2.05±0.32 mg/L; P=0.016]. By contrast, the levels of sTWEAK were significantly lower in brucellosis patients as compared to the subjects in the control group [235.20±48.45 pg/mL vs. 265.00±67.25 pg/mL; P=0.044] (Table 2).

Table 1. Levels of chromium and lead in the case and control groups

	Chromium (µg/L)	Lead (µg/dL)
Control	0.121±0.027	12.28±2.42
Brucellosis	0.109±0.025*	12.58±1.94

Results are represented as mean±SD
*$P < 0.05$, significantly compared to the control group
Homocysteine, hs-CRP, and sTWEAK

Table 2. The levels of homocysteine, hs-CRP, and sTWEAK in the case and control groups

	Homocysteine (µmol/L)	Hs-CRP (mg/L)	sTWEAK (pg/mL)
Control	14.36±3.06	2.05±0.32	262.00±67.25
Brucellosis	16.18±4.47*	2.23±0.34*	235.20±48.45*

Results are represented as mean ± SD
*$P < 0.05$, significantly compared to the control group

Discussion

Trace elements are essential for the optimal functioning of the body organs. Apart from playing salient roles in the immune system, trace elements function as catalysts in a variety of biochemical reactions. Nevertheless, only a small quantity of trace elements is needed for proper functioning, and alterations in their serum levels pose the body at risk for various disorders, especially cardiovascular diseases (19,20). Therefore, in the current investigation, we aimed to examine the serum levels of the two essential trace elements, i.e., chromium and lead as well as risk markers of atherosclerotic cardiovascular disease in brucellosis patients.

Chromium is an essential element that primarily affects glucose metabolism by improving the biological activity of insulin (21). We found reduced levels of chromium in patients with brucellosis compared with the subjects in the control group; the levels of serum lead, however, were almost unchanged between the two groups. Alterations in trace elements' levels have been documented in previous studies conducted on brucellosis patients; Mobaien *et al.*, (22) reported elevated concentrations of copper and reduced levels of zinc in the serum of brucellosis patients. Zanganeh *et al.*, (23) also assessed serum levels of some trace elements, including copper, zinc, manganese, and magnesium in patients suffering from brucellosis, reporting elevated copper, and reduced zinc levels. Significantly reduced levels of chromium in the sera of tuberculosis patients and patients with HIV infection have also been reported (24,25). The higher prevalence of atherosclerotic cardiovascular disease in chromium deficient individuals was documented in a study conducted several years ago; however, it has recently been shown that chromium inhibits the expression of thrombospondin-1 (TSP-1), a potent proatherogenic protein that controls cell-cell and cell-matrix interactions (26). It should, however, be underlined that Davies *et al.*, reported significant age-related reductions in chromium levels in 51665 hair, sweat, and serum samples obtained from 40872 patients (r= -.598 to -.762, P< .0001 for all correlations) (27). Among those data, serum chromium levels of 35-40-year-old reduced by 8% compared to that of 45-50-year-old. In the current study, serum levels of chromium in case and control

groups were 0.109±0.025 µg/L and 0.121±0.027 µg/L, respectively; therefore, the contribution of age to the difference observed should also be accounted.

Our findings revealed that brucellosis patients had higher levels of homocysteine and hs-CRP and lower concentrations of sTWEAK in their sera. Togan *et al*., (18) reported increased levels of hs-CRP in brucellosis patients during the first-year follow-up period. In addition to increased amounts of hs-CRP, elevated values of intima-media thickness (IMT) and flow-mediated dilatation (FMD) have been shown in subjects who had been treated for brucellosis two years before, denoting a possible relation between chronic brucellosis and cardiovascular disease (28). Among these markers, sTWEAK has a unique feature as it independently predicts the presence and the severity of atherosclerotic cardiovascular disease (29). A similar schema has been noted in HIV patients who are known to be at increased risk of cardiovascular disease, with elevated hs-CRP and decreased sTWEAK levels, probably due to the chronic inflammatory state (30). It has, however, been reported that in a total of 6060 healthy people, serum levels of hs-CRP were significantly increased with aging, particularly in those with age over 45-year-old; correlation analysis also revealed that serum levels of hs-CRP positively correlated with age in that study (31). Here again, the contribution of aging to the observed difference should also be taken into account.

In summary, brucellosis probably causes alterations in serum markers known to predict cardiovascular disease. In addition to decreased levels of chromium observed in the serum of brucellosis patients, higher homocysteine and hs-CRP levels, together with lower sTWEAK concentrations, were noticed in the present study. Moreover, according to these findings, the age of the subjects should be considered; and the data cannot identify whether the effects were from brucellosis only.

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