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ORIGINAL ARTICLE

Roles of Dermcidin, Salusin-α, Salusin-β and TNF-α in the Pathogenesis of Human Brucellosis

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ABSTRACT

Background: *Brucella spp.* are facultative intracellular pathogens that can cause chronic infections in many tissues and organs. **Objectives:** To investigate serum dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels and their correlation with each other in patients with acute brucellosis. **Methods:** From 50 patients hospitalized upon diagnosis of acute brucellosis, blood samples were collected and dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels in serum samples were measured using an ELISA assay. The control group included 40 volunteers. **Results:** Brucellosis group had significantly lower plasma dermcidin, salusin- alpha, salusin-beta levels compared to the healthy control group (respectively p:0.008, p<0.001, p<0.001). Moreover, Brucellosis group had significantly higher plasma TNF-alpha levels comparisons with the controls (p=0.002). In the examination of the correlation between TNF-alpha and dermcidin, salusin-alpha and salusin-beta in the brucellosis group, only a negative correlation was found between salusin-beta and TNF-alpha. In the control group, there was a positive and statistically significant correlation between salusin-beta and TNF-alpha. **Conclusion:** Dermcidin, salusin-alpha, and, particularly salusin-beta levels are important in Brucella pathogenesis. The paradoxical correlation between TNF-alpha and salusin-beta in patients with brucellosis and control group is remarkable. However, there is a need for extensive studies conducted with more patients to further elucidate this topic.

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INTRODUCTION

Brucella spp. are facultative intracellular pathogens causing chronic infections in many tissues and organs (1). Although this pathogen primarily infects animals, over 500,000 cases of infection in humans are reported every year (2). Brucellosis is an endemic disease in many developing countries in The Middle East, Mediterranean region, Asia and Africa; all the same, this disease remains underestimated due to under-reporting and under-diagnosis (3). A remarkable feature of Brucella is the absence of classical pathogenic factors that can directly damage eukaryotic cells. Bacterial lipoproteins are potent inducers of innate immunity, a feature exhibited by Brucella (4). It has previously been shown that the main Brucella antigens that induce proinflammatory cytokine release are not LPS, but lipoproteins (5). Understanding the pathogenesis of Brucellosis is crucial because it not only clarifies the unique fundamental aspects of this disease, but also facilitates the understanding of the associated pathogeneses caused by other intracellular pathogens. All living species contain cationic antimicrobial peptides produced in large quantities in areas of infection and inflammation; these peptides may have broad-spectrum antibacterial, antifungal, antiviral, antiprotozoal and antisepsis properties. Another reported case involved transgenic mice expressing a cecropin B (silk-mite peptide) analogue which became resistant to Brucella abortus infections. Ability of a host to counteract the lethal effects of antimicrobial peptides (AMPs) is a crucial factor concerning the virulence of pathogens (6). In a study, resistance to AMPs was shown to play a key role in the *in vivo* survival of Brucella, and Brucella bacteria survived in the presence of antimicrobial peptide resistance (7). Human dermcidin (DCD), which is an anionic antimicrobial peptide, exhibits antimicrobial activity against gram-positive and fungus. Studies have further reported antimicrobial activity against gram-negative bactericides (8,9). Recently, Shichiri *et al.* have discovered the multifunctional endogenous bioactive peptides, namely salusin-alpha and salusin-beta (10) which are synthesised from preprosalusin. In humans, salusins are expressed and synthesised in the vessels and kidneys. In addition, monocytes and macrophages secrete salusins (11). Reportedly, salusin-alpha and salusin-beta are expressed by inflammatory cells. Lipopolysaccharides and TNF-alpha are known to induce the excretion of salusin-beta from monocytes/macrophages (11). To the best of our knowledge, there exist a limited number of studies in the literature regarding the correlation between cationic AMPs and Brucella, and there is no research on dermcidin, anionic antimicrobial peptides, and salusin levels. Therefore, the aim of this study was to investigate serum dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels and their correlation with each other in patients with acute Brucellosis.

MATERIALS AND METHODS

This study has been conducted in accordance with the principles of Helsinki Declaration and approved by Firat University local Institutional Review Board, dated 1.5.2018 and Decision number 1/ 4. All participants were informed and provided consent for the study.
Patients. Patients diagnosed with acute Brucellosis (duration of the disease: <8 week) in the infectious diseases clinic between 2014 and 2017 were included in the study. Patients aged <18 years and those with subacute and chronic Brucellosis (duration of the disease: 8-52 weeks, >52 weeks, respectively) were excluded from the study (12).

The following diagnostic criteria were considered: (1) *Brucella* spp. isolation from blood cultures and (2) detection of an antibody titre of ≥1:160 for *Brucella* using the STA method in the presence of a well-matched clinical picture involving the symptoms of acute or deceptive onset of fever, undue fatigue, anorexia, night sweats, weight loss, arthralgia and headache or of a clinically well-matched case epidemiologically linked to a confirmed case or supportive serology. From the 50 patients hospitalized upon diagnosis of acute Brucellosis, a blood culture and a 5 cc venous blood sample were collected. The plasma portion was separated and stored at -20^{o}C until the day of analysis. Blood samples were obtained prior to initiating the anti-biotherapy for a complete blood count, biochemical parameters, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels . The control group consisted of 40 healthy volunteers whowere STA negative, non-smoker and non-pregnant, and had ESR, renal and liver function tests within normal limits.

Sandwich ELISA. Dermcidin (Human DCD; Catalog no: SEC896Hu Cloud - Clone Corp., USA), Salusin-alpha (Human Salusin-α; Catalog no: 201-12-1269 Sunred Biological Technology Co., Ltd, Shanghai, China), Salusin-beta (Human Salusin-β; Catalog no: 201-12-1273 Sunred Biological Technology Co., Ltd, Shanghai, China) and TNF-alpha (TNF-*a*; Catalog no:KAP1751 DIAsourceImmunoAssays S.A.Belgium) levels in serum samples were measured using an ELISA kits according to manufacturer's instructions. The measurement range of the human DCD ELISA kit was 0.8-50 ng/mL and minimum detection level was 0.29 ng/mL. Measurement range of human salusin-alpha ELISA kit was 7.5-2000 pg/mL and analytical sensitivity was 7.152 pg/mL. Measurement range of human salusin-beta ELISA kit was 10-3000 pg/mL and analytical sensitivity was 8.756 pg/mL. Measurement range of DIA source TNF-alpha ELISA kit was 4.6-12.4 pg/mL and minimum detection level was 0.7 pg/mL. Plates were washed with automatic washer Bio-Tek ELX50 (BioTek Instruments, USA), and absorbance readings were performed with ChroMate, Microplate Reader P4300 device (Awareness Technology Instruments, USA). Test results for Salusin-alpha, Salusin-beta, TNF-alpha were expressed as pg/mL, and those for DCD were expressed as ng/mL.

Statistical Analysis. Data were analyzed using IBM Statistical Package for Social Sciences v22 (SPSS, Inc., Chicago, IL, USA). Baseline demographic data and clinical characteristics were summarized using descriptive statistics; categorical variables were expressed as frequencies and percentages and continuous variables were expressed as mean (± standard deviation) or median (interquartile range IQR). The Mann-Whitney U test was applied to compare continuous variables. To determine the correlation between two continuous variables, Spearman's rank correlation analysis was used for asymmetric variables. p values <0.05 were considered as statistically significant for all analyses.

RESULTS

Median age (IQR) was 38.5 years (28.75–50.50) in the Brucellosis group, while 43 years (35.25–53) in the controls. There was no significant difference between the groups in terms of age (p=0.179). The patient cohort comprised 29 (58 %) male and 21 (42 %) female patients, while the control group included 15 (37.5%) female and 25 (62.5%) male participants subjects. There was no significant difference in terms of sex-based distribution between the two groups (p=0.665). The various laboratory parameters recorded on the day of admission are summarized in Table 1.

Table 1. Various laboratory results measured at the time of initial presentation in brucellosis.

Parameter	Median (interquartile range)	Reference ranges
WBC (mm^3)	5790 (5112-7440)	3800- 8600
Neutrophil %	55 (44.3-65.5)	40- 77
Lymphocyte	33 (24.7-43)	16- 44
Monocyte (%)	7 (5.1-8)	0- 12
Hb (g/dL)	13.2 (11.4-14.4)	11.1- 17.1
Plt (mm^3)	255 (202-287)	140- 360
AST (U/L)	50 (27.5-67.5)	5- 40
ALT (U/L)	51.5 (24.5-82)	5- 40
Urea (mg/dl)	33.5 (25-41)	10- 50
Creatinine (mg/dL)	0.7 (0.6-09)	0.6- 1.2
Albumin (g/dL)	4 (3.5-4.3)	3.5- 5.3
Total protein (g/dL)	7.2 (6.4-7.8)	6.6- 8.7
C-reactive protein (mg/L)	14.0 (3.1-45.9)	0-5
ESR (mm/h)	24.5 (18.0-46.0)	0-20

WBC; White blood cell, Hb; Hemoglobin, Plt; platelet, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, ESR; Erythrocyte sedimentation rate.

In 45 (90%) patients, STA test results were ≥1/160, while 5 (10%) patients were diagnosed with Brucellosis based on a positive *Brucella* Coombs' test. *Brucella spp*. was isolated from the blood cultures of 15 (30%) patients collected prior to the treatment. Dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels specified in the Brucellosis and control groups are summarized in Table 2.

Table 2. Dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels determined in the brucellosis and control groups.

	Brucellosis	Control	p-values
Dermcidin*	3.70 (2.06-6.44)	5.85 (3.05-10.44)	0.008
Salusin-alpha*	479.5 (354.5- 893.5)	1153.5 (665.75-1942.25)	<0.001
Salusin-beta*	1421.5 (857.5- 2808)	2955 (2590-3145)	<0.001
TNF-alpha*	11.42 (9.38- 16.54)	8.25 (4.82-14.05)	0.002

*Median (interquartile range).

In the examination of the correlation between TNF-alpha and dermcidin, salusin-alpha and salusin-beta in the Brucellosis group, only a negative correlation was observed between salusin-beta and TNF-α (Rho: −0.514, p<0.001; Figure 1). In the control group,

there was a poor, positive and statistically significant correlation between salusin-beta and TNF-α (Rho: 0.363, p=0.021; Figure 2). Furthermore, no correlation was found between TNF-alpha and dermcidin and salusin-alpha (Rho: −0.006, p=0.969 and Rho: 0.115, p=0.480, respectively).

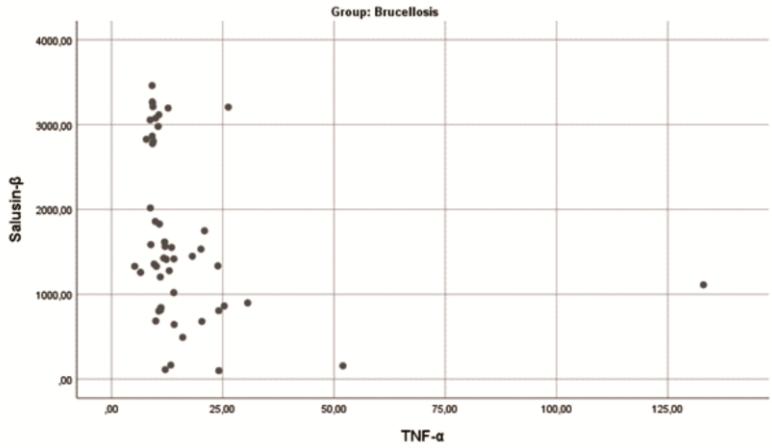

Figure 1. Correlation between TNF-alpha and salusin-beta in the patients with brucellosis.

Neither was there a significant correlation between CRP levels and dermcidin, salusin-alpha, salusin-beta and TNF-alpha levels (Rho: 0.118, p=0.414; Rho: −0.06, p=0.648; Rho: −0.026, p=0.860 and Rho: 0.092, p=0.524, respectively).

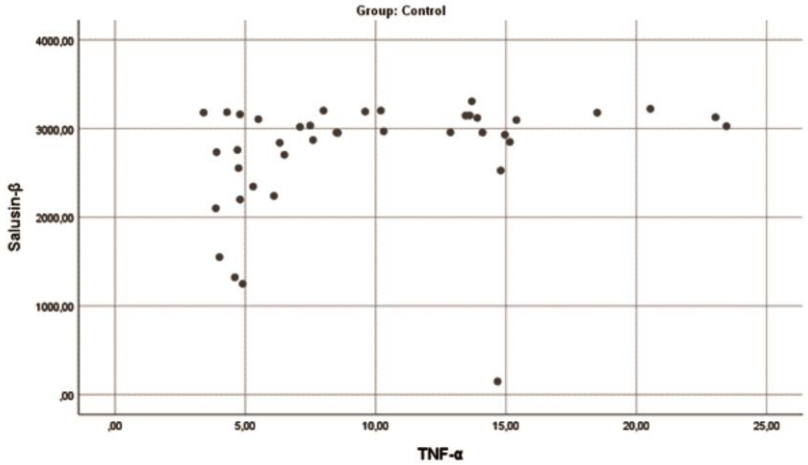

Figure 2. Correlation between TNF-alpha and salusin-beta in control group.

DISCUSSION

Brucellosis is a zoonosis disease prevalent in developing countries such as Turkey. It is considered as a serious public health concern because it causes economic loss and directly affects food safety. Brucellosis is more common among males in countries with low

incidence of the disease, primarily due to occupational risk, whereas no sex-based difference is observed in endemic countries (13). However, although our region is endemic for Brucellosis, the ratio of male patients is still higher. The disease is observed in almost all age groups, but it commonly affects young adults and the middle-aged. Moreover, its incidence is lower among children and the elderly (13). Median age (interquartile range) of the patients in our study was 38.5 years (28.75–50.50). Antimicrobial peptides are significant effector molecules of the innate immune defence protecting epithelial barriers. In addition to their antimicrobial activity, AMPs possess other important cellular functions including immunomodulation (14). Brucella speedily translocate across the mucosal epithelium layer and are endocytosed by mucosal macrophages and dendritic cells (15). Brucella exhibit potent tissue tropism for lymphoreticular system with an intracellular lifestyle that limits exposure to immune responses. AMPs exhibit broad-spectrum antimicrobial activity, an advantage of which is that microbes do not easily gain resistance against them owing to their non-specific binding to the cell membrane. Therefore, they can be used in treating infections caused by antibiotic-resistant microorganisms. Dermcidin is an anionic AMP encoded by the DCD gene in humans (16). This molecule is constitutively secreted in human sweat and is not inducible by skin injury or inflammation. It has a broad spectrum of antimicrobial activity against pathogenic microorganisms, and its antimicrobial activity against intracellularly located *Mycobacterium tuberculosis* has been previously demonstrated (17). The same study concluded that AMPs may be novel therapies to treating the bacteria less susceptible to the existing antibiotics. Similarly, Brucella species show intracellular localization, sometimes complicating the treatment process. In our study, dermcidin level was found to be statistically lower in the Brucellosis group compared with that in the control group ($p=0.008$). In particular, in chronic patients and patients with inadequate response to treatment, dermcidin may be a novel treatment alternative or may guide human vaccination studies. In our study, TNF-alpha levels were significantly higher in the Brucellosis group compared with those in the control group ($p=0.002$). Similar to our study, Akbulut *et al.* reported that TNF-alpha levels in brucellosis group were significantly higher than those in the controls (12). Previous studies have indicated that B. abortus induces human monocytes to secrete proinflammatory cytokines (18,19). On the contrary, Refik *et al.* (20) observed that cases of brucellosis did not have significantly elevated TNF- alpha serum levels compared to healthy controls. Reportedly, Brucella spp. strains may not induce TNF-alpha in human macrophages (21). Salusin-alpha and salusin-beta are synthesized from a preprosalusin protein. The correlation between TNF- alpha and salusins has been shown many times, previuslly. A study indicated salusin-α suppressed TNF-alpha-induced inflammatory responses in human umbilical vein endothelial cells and anti-inflammatory effects of salusin-alpha (22). It was reported that among the TNF-alpha raised with the ethanol application, TNF- alpha levels were reduced in groups treated with salusins; TNF-alpha levels showed more changes in the group receiving salusin-beta compared with the group that received salusin-α treatment (23). Another study demonstrated that lipopolysaccharide and TNF-alpha released salusin-beta from human monocytes/macrophages, while the release of preprosalusin was not augmented (11). In our study, TNF-alpha levels were significantly higher in the Brucellosis group than in the control group, and a significant negative moderate correlation was observed with salusin-beta (Rho: −0.514, $p<0.001$). In the control group, there was a positive statistically significant correlation between TNF-alpha and salusin-beta (Rho: 0.363, $p=0.021$). This may be associated with an inadequate response to TNF-

alpha for salusin-beta release by monocytes/macrophages in individuals with Brucellosis and may highlight an important aspect in the pathogenesis of Brucellosis previously unexplained. Likewise, it was demonstrated that Kupffer and hepatocellular cells in the liver synthesized both salusin-alpha and salusin-beta. Liver is a major organ of the mononuclear phagocytic system, and likely involved in all cases of Brucellosis. In the reticuloendothelial system of the respective natural hosts, all *Brucella spp*. remarkably established persistent infection. In patients with Brucellosis, salusin-alpha and salusin-beta levels were significantly lower comparisons with those in the control group. A previous study demonstrated the anti-apoptotic effects of salusins (24). Whereas it is partially clear how *Brucella* bacteria maintain their intracellular life cycle, it has been understood that they have developed diverse strategies to escape the immune system by altering normal host functions. These strategies include surviving in an acidic vesicle, inhibiting apoptosis in macrophages, preventing phagosomal–lysosomal fusion, suppressing cellular immune response and expressing virulence genes. Via these mechanisms, bacteria maintain their survival by suppressing bactericidal response in the cell (25). To the best of our knowledge, this study is the first to show that dermcidin, salusin-alpha and particularly salusin-beta levels are important in Brucella pathogenesis. We believe that our study can create new horizons for the treatment of Brucellosis and act as a guide for future human vaccination studies. The paradoxical correlation between TNF-alpha and salusin-beta in patients with Brucellosis and control group is yet another important result of our study. However, there is a need for expansive studies conducted with more patients to further elucidate this topic.

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