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Research Article

Use of Serum Levels of Matrix Metalloproteinases (MMP-1, MMP-9, MMP-13) in Predicting Development of Complications of Brucellosis

Maida Šiširak[1]* and Mirsada Hukić[2]

[1]*Department of Clinical Microbiology, Clinical Centre University of Sarajevo, Bosnia and Herzegovina*

[2]*International Burch University; Sarajevo, Bosnia and Herzegovina*

***Corresponding Author:** Maida Šiširak, Department of Clinical Microbiology, Clinical Centre University of Sarajevo, Bosnia and Herzegovina.

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Abstract

Introduction: Diagnosis of brucellosis is based on clinical picture, epidemiological and anamnestic data and laboratory analysis. Early diagnosis of brucellosis and including adequate therapy have crucial importance for patients, especially for prevention development of complications of brucellosis. Scientists tried to explain the role of matrix metalloproteinases (MMPs) in the immunopathogenesis of human brucellosis during last decade. MMPs are responsible for degradation and remodeling almost all of the components of the extracellular matrix. Exuberant expression of MMPs can cause a variety of destructive diseases. The aim of this study was to estimate the prognostic value of matrix metalloproteinases (MMP-1, MMP-9, MMP-13) as laboratory biomarkers in the assessment of disease activity and outcome of brucellosis.

Patients and methods: This study included 60 brucellosis patients treated at the Clinic for Infectious Diseases, Clinical Centre University of Sarajevo, in the period 2007 to 2014. Brucellosis was diagnosed by positive blood culture results and/or by positive relevant serological test results (ELISA, Rose-Bengal latex agglutination). Matrix metalloproteinases serum levels were quantified by sandwich ELISA (enzyme-linked immunosorbent assay) according to the manufacturer's instructions (R and D Systems, Inc., Minneapolis, USA).

Results: Investigation involved three groups: 30 patients with complications, 30 patients without complications of brucellosis and 30 control examinees. Matrix metalloproteinases were expressed in the serum samples of all brucellosis patients. There were statistically significant difference between serum levels of MMPs in the group of patients and control examinees ($p < 0.05$; $p = 0.001$). Pearson's correlation test showed statistically significant positive correlation between serum levels of MMPs (MMP-1, MMP-9, MMP-13) and development of complications of human brucellosis ($p < 0.05$; $p = 0.001$). We analyzed inflammatory biomarkers (CRP, SE, LE, AST, ALT, γGT) and correlation between tested MMPs and inflammatory biomarkers. Pearson's correlation test showed statistically significant positive correlation for MMP-1 toward CRP and SE ($p < 0.05$) and toward γGT ($p < 0.01$); statistically significant positive correlation for MMP-9 toward SE ($p < 0.05$) and statistically significant negative correlation toward CRP and LE ($p < 0.05$); statistically significant positive correlation for MMP-13 toward SE and γGT ($p < 0.01$) and statistically significant negative correlation toward CRP and LE ($p < 0.05$).

Conclusions: This study clearly showed that matrix metalloproteinases (MMP-1, MMP-9, MMP-13) are useful serum biomarker for assessment of disease activity and in predicting development of complications of brucellosis.

Keywords: *Brucellosis; Diagnosis; Matrix Metalloproteinase*

Introduction

Brucellosis is a worldwide zoonosis with a high degree of morbidity in humans. According to WHO data about 500.000 cases of this disease were registered in the world every year [1,2]. Brucellosis is one of the most frequent zoonoses in the Mediterranean region and, in spite of the measures undertaken to prevent and control it, the incidence in some countries is still too high [1-4]. *Brucella* spp. invade and proliferate within reticuloendothelial cells and cause acute, subacute and chronic disease.The acute form is characterized by weakness, irregular fever, sweat, malaise, anorexia, headache, back pain, splenomegaly and hepatomegaly. In the subacute form, the clinical patterns were presented with milder clinical symptoms, with the frequent presence of arthritis and an infrequent occurence of hepatomegaly. In the chronic form fever is rare and focal suppurative lesions in bones, joints, liver and spleen may be observed [1-6]. Diagnosis of brucellosis is based on clinical picture, epidemiological and anamnestic data and laboratory analysis. As the clinical picture in human brucellosis is fairly non-specific, a definitive diagnosis needs to be supported by laboratory tests [1,2,5].

Early diagnosis and including adequate therapy have crucial importance for patients, especially for protection development complications in brucellosis. Complications in human brucellosis were registered among 16% patients. Unfortunately, in the large number of patients clinical finding is very serious and consequences are durable [6,7]. Pathogenesis of *brucella* complications is rather unknown. Despite the fact that clinical and imaging aspects of brucellosis have been described widely, the mechanisms involved in this process have not been completely explained. It is assumed that matrix metalloproteinase have very important role in the development of *brucella* complications [8,9].

Matrix metalloproteinases (MMPs) represent a family of matrix-degrading proteinases with structural similarities [10]. Normal tissues do not store MMPs, and constitutive expression is minimal. MMPs production is regulated by growth factors, cytokines, and extracellular matrix (ECM) components. MMPs are secreted as inactive proenzymes, and proteolytic activity is regulated within tissue by zymogen activation and enzyme inhibition. Cell surface localization (either via transmembrane domains or secretion and binding to surface molecules) represents another possible way to spatially control proteolysis. Given that MMPs have the capacity to catalyze the degradation of structural ECM proteins, it can be speculated that their main role is physiologic tissue remodeling during development, growth, uterine cycling, postpartum involutio, and wound repair [10,11]. MMPs are belived to play a role in the pathogenesis of acute and chronic destructive diseases through degradation of ECM. Specific MMPs are responsible for degradation and remodeling of almost all components of the extracellular matrix. Exuberant or anomalous expression of MMPs can cause tissue damage and has been associated with a variety of destructive diseases, including arthritis, atherosclerotic plaque rupture, aortic aneurysms, and tumor progression [12,13]. The role of matrix metalloproteinases in the immunopathogenesis autoimmune, malignant and infective diseases has been investigated over the last two decades [14-16].

The aim of this study was to estimate the prognostic value of matrix metalloproteinases (MMP-1, MMP-9, MMP-13) as clinical and laboratory markers of the human brucellosis outcome.

Patients and Methods

The study was coducted between January 2007 and January 2014. Investigation was performed at the Department of Clinical Microbiology and at the Department of Clinical Immunology, Clinical Centre University of Sarajevo, with an approval of the Ethical Committee of the Clinical Centre University of Sarajevo.

Patients

The study included 60 brucellosis patients of both genders, all ages, from different regions of the Federation B&H and 30 control examinees. Patients were divided in the two subgroups: 30 patients with complications and 30 patients without complications of brucellosis. The average age of patients was 49.9 ± 14.2 years (range 15 - 81). Among patients there were 39 (66%) males and 21 (34%) females. Clinical presentation of disease was mild up to very severe, in accordance with laboratory results on admission. All patients were treated

at the Clinic for Infectious Diseases, Clinical Centre University of Sarajevo. Control group represented age and gender-matched, apparently healthy individuals. Criteria for inclusion in the study was the etiological confirmation of diagnosis by using relevant laboratory tests: positive blood culture results and/or by positive relevant serological test results (ELISA, Rose-Bengal latex agglutination). Criteria for exclusion were autoimmune, malignant or others inflammatory diseases.

Methods

All subjects involved in the study went through detailed anamnestic questionnaire, physical examination and standard laboratory analyses. Standard laboratory analyses include blood erytrocyte, leukocyte and platelets, erythrocyte sedimentation rate, aspartat aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (γGT).

For serology, blood samples were centrifuged and the serum divided into refrigerator and stored at -80˚C until the analysis was performed. Matrix metalloproteinases serum levels were quantified by sandwich ELISA (enzyme-linked immunosorbent assay) according to the manufacturer's instructions (R and D Systems, Inc., Minneapolis, USA). The test result was read automaticaly by BIOTEK ELX50 ELISA processor on 450/620 nm. We converted receiving values into pg/ml by using programme MASTERPLEX 2010.

Statistical Analysis

For evaluation of the results, standard statistical methods were used. An analysis of the normality of the continuous variables was performed with the Kolmogorov-Smirnov test. The test showed that all variables satisfied the characteristics of normal distribution. A comparison of the categorical and continuous variables between the groups was performed using the chi-square test and one-way variance analysis (ANOVA). Correlation between investigated variable was found using Pearson's coefficient linear correlation. Statistical significance was defined at $p < 0.05$. Statistical analysis was performed using the statistical package IBM Statistics SPSS V19.0.

Ethical Principles

Ethical principles outlined in the World Medical Association Declaration of Helsinki were applied in this study.

Results

Investigation involved three groups: 30 patients with complications, 30 patients without complications of brucellosis and 30 control examinees.

Different forms of complications of brucellosis were registered. Osteoarticular complications dominated and presented 70% (21/30). Cardiovascular complications presented 13.30% (4/30), but these were the most serious (Figure 1).

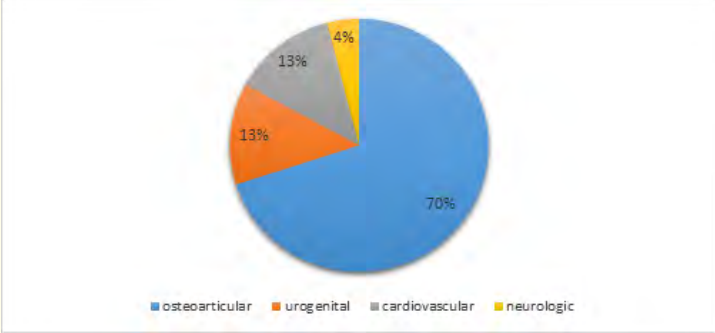

Figure 1: *Different forms of complications of brucellosis.*

The serum level of MMP-1 in patients with complications was the highest at 9.55; in patients without complications it was 3.75 and in the control examinees it was the lowest at 3.62 ($p < 0.05$; $p = 0.001$). The serum level of MMP-9 in patients with complications was the highest at 105.74; in patients without complications it was 64.62, and in the control examinees it was the lowest at 36.92 ($p < 0.05$; $p = 0.001$). The serum level of MMP-13 in patients with complications was the highest at 138.95; in patients without complications it was 64.85; and in the control examinees it was the lowest at 29.64 ($p < 0.05$; $p = 0.001$) (Figure 2).

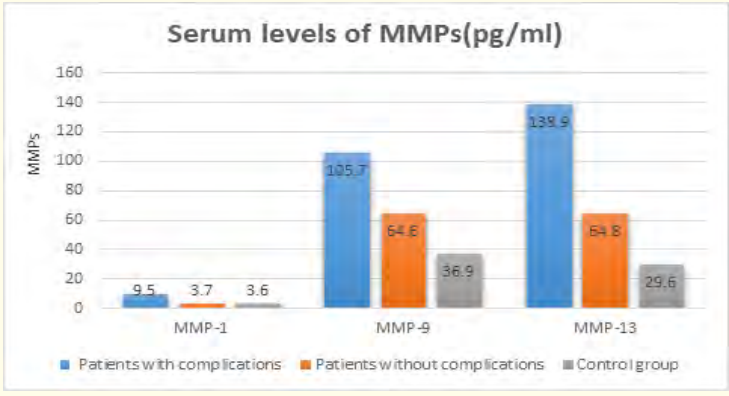

Figure 2: *Review of evaluation of serum levels MMPs.*

Based on the results obtained in our study, our hypothesis was that matrix metalloproteinases may harm osteoblast function, contributing to bone and joint destruction. Accordingly, we analyzed MMPs particularly in the group of patients with osteoarticular complications: MMP-13 expressed very high level and was dominant (Figure 3).

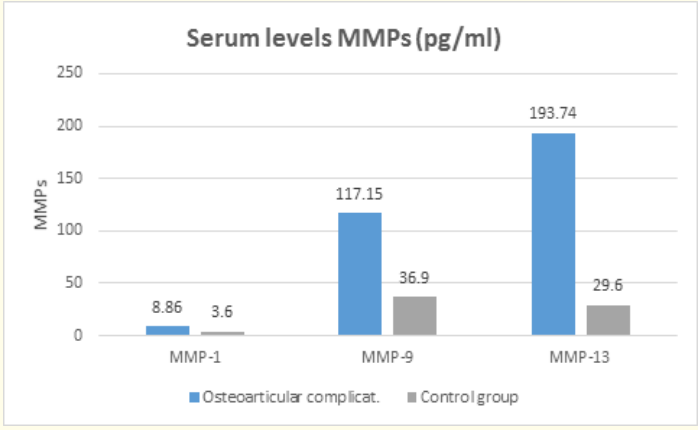

Figure 3: *MMPs in the group of patients with osteoarticular complications.*

Although, osteoarticular complications were the most common, cardiovascular complications were the most serious. Clinical manifestations in acute form was endocarditis and in chronic form aortal/mitral vegetations with regurgitation and already presented heart

decompensation. We analyzed MMPs particularly in the group of patients with cardiovascular complications: MMP-9 expressed very high level and was dominant (Figure 4).

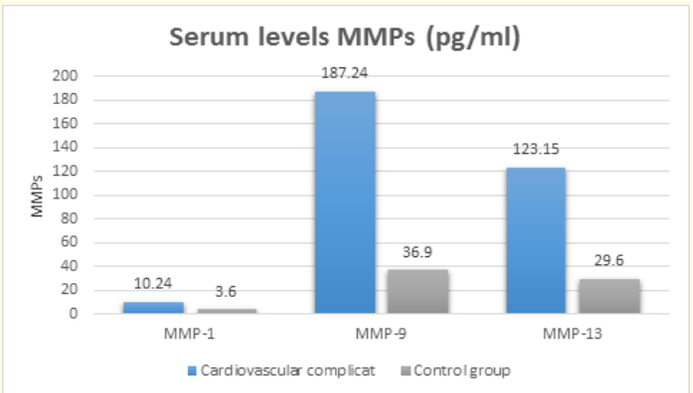

Figure 4: *MMPs in the group of patients with cardiovascular complications.*

We analyzed inflammatory biomarkers (CRP, SE, LE, AST, ALT, γGT) and correlation between tested MMPs and inflammatory biomarkers. Pearson's correlation test showed statistically significant positive correlation for MMP-1 toward CRP and SE ($p < 0.05$) and toward γGT ($p < 0.01$); statistically significant positive correlation for MMP-9 toward SE ($p < 0.05$) and statistically significant negative correlation toward CRP and LE ($p < 0.05$); statistically significant positive correlation for MMP-13 toward SE and γGT ($p < 0.01$) and statistically significant negative correlation toward CRP and LE ($p < 0.05$) (Table 1).

MMP-1 MMP-9 MMP-13			
Pearson's rho	CRP	Ro	0.042* -0.305* -0.055*
		p	0.751 0.018 0.675
		N	60 60 60
	LE	Ro	0.095 -0.259* -0.105*
		p	0.469 0.046 0.425
		N	60 60 60
	SE	Ro	0.150* 0.053* 0.346**
		p	0.254 0.689 0.265
		N	60 60 60
	AST	Ro	0.209 -0.041 -0.039
		p	0.109 0.754 0.768
		N	60 60 60
	ALT	Ro	0.182 0.006 -0.044
		p	0.164 0.965 0.740
		N	60 60 60
	γGT	Ro	0.349** 0.009 0.139**
		p	0.006 0.947 0.289
		N	60 60 60

Table 1: *Correlation between matrix metalloproteinases and inflammatory biomarkers.*

**Statistically significant correlation $p < 0.05$*

***Statistically significant correlation $p < 0.01$*

Discussion

Despite the fact that clinical and imaging aspects of brucellosis have been described widely, the immunopathological mechanisms have not been completely explained. It is assumed that MMPs have very important role in the development of *brucella* complications. Scientists tried to explain the role of matrix metalloproteinases in the immunopathogenesis of human brucellosis during last several years [8,9].

The results obtained in this study showed that matrix metalloproteinases were expressed in the serum samples of all brucellosis patients. There were statistically significant difference between serum levels of MMPs in the group of patients and control examinees ($p < 0.05$; $p = 0.001$). Statistically significant positive correlation was detected between serum levels of MMPs (MMP-1, MMP-9, MMP-13) and development of complications of human brucellosis ($p < 0.05$; $p = 0.001$).

In the group of patients with complications, patients with osteoarticular complications dominated and presented 70.00% (21/30). Recent surveilance studies in the hospitals in the various parts of the world reported similar results [6,7,17,18]. Scientists explain that osteoarticular complications in human brucellosis manifested as cartilage degradation and the bone loss [8,9,14]. The results of presented study suggested that MMPs have important role in the development osteoarticular complications. Namely, our results indicated statistically significant positive correlation between serum levels of MMPs and development osteoarticular complications ($p < 0.05$; $p = 0.001$). Serum levels of MMP-13 dominated in all patients with osteoarticular complications. Recent studies concluded that MMP-13 serum levels are useful in predicting joint destruction [15,16]. Based on the results obtained in the present study, we hypothesize that MMP-13 is useful serum biomarker in predicting development of osteoarticular complications of brucellosis.

Cardiovascular complications of brucellosis are infrequency, but these were very serious. In our study among patients with complications, cardiovascular complications were presented at 13.30% (4/30). Diagnosis was confirmed by classical and transesophageal heart ultrasonography. Clinical manifestations in acute form was endocarditis and in chronic form aortal/mitral vegetations with regurgitation and already presented heart decompensation.The role of matrix metalloproteinases in the immunopathogenesis of cardivascular diseases has been investigated during last decade. Many studies explained that MMPs have very important role in the development of cardiovascular diseases [19,20,21]. However, investigations about the role of MMPs in the *brucella* cardiovascular complications are very scanty. The results obtained in the presented study suggested that MMPs have important role in the development these complications. Namely, our results indicated statistically significant positive correlation between serum levels of MMPs and clinical disease development and progression cardiovascular complications. Serum levels of MMP-9 dominated in all patients with cardiovascular complications. So, we hypothesize that MMP-9 is useful serum biomarker in predicting development of cardiovascular complications of brucellosis. Baldi., *et al.* [22] in his study explained the cellular and molecular bases of immunopathological phenomena probably involved in the pathogenesis of brucellosis. *Brucella* spp. infected and replicated in human endothelial cells, inducing the production of chemokines and IL-6, and increased expression of MMPs. The sustained inflammatory process may be important for the development of endocarditis.

The medical histories of brucellosis patients were analyzed. Patients data indicated that matrix metalloproteinases were detected in the serum of all patients in the first week of hospitalization. This fact suggests that MMPs are useful biomarker in the early stage of illness. Early diagnosis of brucellosis and including adequate antibiotic therapy have crucial importance for patients, especially for protection development of complications. MMP-9 and especially MMP-13 expressed correlation with the length of diagnostic time. In the patients with very long period of diagnostic time MMP-13 was the highest. All of these patients had complications of brucellosis. Patients data demonstrated that in order to assess morphological changes in the osteoartcular system, conventional radiological methods had been used (standard radiography-X-ray, a radionuclide bone scan, computerized tomography-CT and magnetic resonance imaging-MRI). MMPs degree of expression in the serum samples correlate with results of conventional radiological methods. Also, our results showed that MMPs degree of expression in the serum samples correlate with inflammatory markers, especially erytrocyte sedimentation rate (ESR) and C-reactive protein (CRP). It is known that CRP and ESR, increase in acute brucellosis and decrease to normal level after treatment [23].

Conclusion

Measurement of serum levels of MMPs in human brucellosis is a simple and relatively rapid laboratory test. Although increased MMP-1, MMP-9 and MMP-13 levels are not specific and do not provide definite clinical diagnosis, they can be used as a biochemical indicator of development complications, when evaluated in combination with imaging techniques, such as standard radiography-x-ray, computerized tomography-CT, magnetic resonance imaging or echocardiography. Accordingly, we concluded that matrix metalloproteinases are useful serum biomarker in predicting development of complications of brucellosis. In addition, serum levels of MMPs decrease very quickly after adequate antibiotic therapy, thus demonstrating that it might be promissing procedure for monitoring efficiency of therapies.

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