Concise report

High mobility group box 1 serum levels are increased in Behçet’s disease, but not associated with disease activity or disease manifestations

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Abstract

Objectives. High mobility group box 1 (HMGB1) is a nuclear protein that acts as an alarmin when released into the extracellular milieu. HMGB1 is a biomarker of active disease in several systemic autoimmune diseases. Behçet’s disease (BD) is a systemic inflammatory disorder with a waxing/waning course. The objective of this study is to evaluate serum HMGB1 levels as a possible biomarker for disease activity in BD.

Methods. A cross-sectional study measuring serum HMGB1 levels was performed in 26 BD patients and 20 healthy controls. The Brazilian version of the simplified BD Current Activity Form (BR-BDCAFs) was used to measure disease activity.

Results. Serum HMGB1 levels were higher in patients with active disease [3.82 (2.54–6.11) ng/ml], in patients with BD without active disease but still on therapy [2.76 (1.89–5.78) ng/ml] and in patients in remission without treatment [2.66 (1.86–4.70) ng/ml] than in healthy controls [0.96 (0.59–1.39) ng/ml], \( P < 0.001 \). Levels were comparable between BD patients with active disease, BD without active disease but still on therapy and those in remission without treatment (\( P = 0.432 \)). There was no correlation between serum HMGB1 levels and BR-BDCAF(s) (\( r = 0.195; P = 0.339 \)). No association could be found between serum HMGB1 levels and specific disease involvement or therapy. So serum HMGB1 levels cannot be used as a biomarker in BD.

Conclusion. Serum HMGB1 levels are increased in patients with BD as compared with healthy controls. However, no association was found with disease activity, specific organ involvement or therapy in BD.

Key words: Behçet syndrome, HMGB1, biomarkers, disease activity, acute phase reactants.

Rheumatology key messages

- Behçet’s disease is associated with increased serum high mobility group box 1 levels in comparison with healthy controls.
- No association was found between circulating high mobility group box 1 and disease activity or specific manifestations in Behçet’s disease.
- No association was observed between prednisone or AZA use and serum high mobility group box 1 levels in Behçet’s disease.
Introduction

Behçet’s disease (BD) is a chronic multisystemic inflammatory disease of unknown aetiology classified as a systemic vasculitis. It is characterized by recurrent attacks of oral ulcers, genital ulcers, cutaneous lesions, eye inflammation and the occurrence of articular, neurological, vascular and intestinal manifestations [1]. Although BD is reported to occur all over the world, its prevalence is higher in countries along the ancient Silk Road. The aetiology of BD is unknown, and it is believed that environmental factors play a role in genetically predisposed individuals. HLA-B51 is the most important genetic marker associated with BD, especially in Turkish and Asian populations from the Middle East to the Far East, whereas the association between BD and HLA-B51 is weaker in Caucasians [1, 2].

Innate immunity plays an important role in the pathophysiology of BD, with the participation of γδ T cells, NK cells and neutrophils. γδ T cells are involved in the protection of mucosa and skin against invading organisms. Increased numbers of γδ T cells are found in mucosal lesions and in peripheral blood of BD patients. These cells express activation markers (e.g. CD29 and CD69) and produce inflammatory cytokines such as IFN-γ, TNF-α and IL-8 [3, 4]. Activated NK cells have been shown to be increased in the peripheral blood of patients with active BD. Neutrophils are hyperactive and contribute to tissue injury in BD via increased chemotaxis, phagocytosis, MPO expression and production of reactive oxygen species [3]. Cytokines and chemokines secreted by antigen-presenting cells and T cells have been suggested to cause abnormal neutrophil activation in BD [4].

High mobility group box-1 (HMGB1) is a non-histone nuclear protein that contributes to chromatin architecture and regulates transcription. HMGB1 may be passively released by necrotic cells or actively secreted by activated cells [5]. Once outside the cell, HMGB1 acts as an alarmin or a danger-associated molecular pattern, binding to receptors and sensors of innate immunity such as the receptor for advanced glycation end-products and Toll-like receptor (TLR)-2, TLR-4 and TLR-9. Extracellular HMGB1 triggers inflammatory reactions, mainly by inducing cytokine secretion and chemotaxis [6].

An association between increased serum HMGB1 levels and disease activity has been described in several systemic autoimmune diseases such as SLE, JIA, RA, primary SS, SSC and in ANCA-associated vasculitis [5–8]. Recently, a study performed in Korea found an association between higher serum HMGB1 levels and intestinal involvement in BD [9]. Gastrointestinal involvement of BD is commonly described in patients from the Far East, being observed in one-third of BD patients from Japan [10]. However, intestinal involvement in BD is extremely rare in BD patients from other countries, including Brazil where this manifestation is found in only 3.3% of cases [10, 11]. In this study we evaluated serum HMGB1 levels in patients with BD in order to assess associations with disease activity and specific disease manifestations in a Brazilian population of BD patients.

Patients and methods

Study population

Twenty-six consecutive patients with BD and 20 age-(39.0 ± 11.5 years vs 38.5 ± 13.5 years; $P = 0.888$) and sex- (65.4% vs 90.0% females; $P = 0.082$) matched healthy controls were included in this cross-sectional study. BD patients were under regular follow-up at the Vasculitis Outpatient Clinic at Universidade Federal de São Paulo (Unifesp). Inclusion criteria included age above 18 years and fulfilment of the International Study Group diagnostic criteria for BD [12]. Disease activity of BD was evaluated with the Brazilian version of the simplified Behçet’s Disease Current Activity Form (BR-BDCAF) [13]. Active disease was considered if BR-BDCAF(s) was ≥2. Patients with a BR-BDCAF(s) score equal to zero were divided into two groups: BD patients still on therapy and BD patients without treatment. Thirteen BD patients presented active disease, 10 BD patients did not present active disease but were still on therapy and 3 BD patients were in remission without treatment. All patients gave informed written consent according to the Declaration of Helsinki, and the study was approved by the institutional ethics committee (Comitê de Ética em Pesquisa da Unifesp-EPM).

Information about previous and current BD manifestations and about current therapy was retrieved from medical charts. The BR-BDCAF(s) was recorded at each visit, and comprehended scoring for oral ulcers, genital ulcers, cutaneous lesions (i.e. erythema nodosum-like and acne-like lesions), joint complaints, eye involvement (i.e. uveitis and/or retinal vasculitis), neuro-BD, vasculo-BD (i.e. venous thrombosis and/or arterial aneurysms) and intestinal involvement. Eye disease, neuro-BD and vasculo-BD were considered major organ involvement of BD.

Serum HMGB1

Serum HMGB1 levels were determined by ELISA using a commercial kit (Shino Test, Sagamihara, Kanagawa, Japan) according to the manufacturer’s instructions. Results were expressed in nanograms per millilitre.

Statistical analysis

Statistical analysis was performed using IBM SPSS software for Windows version 20.0 (IBM, Corp., Armonk, NY, USA) and graphs were created with Graph Pad Prism version 3.02. Mean ± s.d. or median and interquartile range were used to present continuous variables as appropriate. Categorical variables were presented as total number and percentage. Continuous parameters were analysed using Student’s t-test, the Mann–Whitney U-test or by Kruskal–Wallis test, and categorical variables were analysed using the Chi-square test or Fisher’s exact test. Correlations between numerical data were performed with Spearman’s correlation coefficient. The significance inference level was established at 5% ($P < 0.05$).
Results

Table 1 depicts the clinical features and current therapy of BD patients. Half of the patients were using immunosuppressive therapy and only one patient was treated with anti-TNFα agents. Five BD patients (19.2%) had only mucocutaneous involvement and joint complaints, whereas the remaining 21 BD patients had at least one actual or previous major involvement of BD (i.e. ocular, neurological or vascular).

Serum HMGB1 levels were significantly higher in BD patients as compared with healthy controls [2.86 (2.32–5.27) ng/ml vs 0.96 (0.59–1.39) ng/ml; \(P < 0.0001\)]. Also, no significant differences were found in serum HMGB1 levels between BD patients with active disease, BD patients without active disease but still on therapy and those in remission without treatment [3.82 (2.54–6.11) ng/ml vs 2.76 (1.89–5.78) ng/ml vs 2.64 (1.72–2.86) ng/ml; \(P = 0.432\)]. Serum HMGB1 levels were significantly higher in all BD subgroups compared with healthy controls (Fig. 1). There was no correlation between serum HMGB1 levels and BR-BDCAF(s) score (\(r = 0.195\); \(P = 0.339\)) or disease duration (\(r = 0.010; \ P = 0.962\)).

Within the group of 13 BD patients with active disease at the time of the study, no difference could be found in serum HMGB1 levels between the eight patients with major organ involvement (i.e. five presenting eye disease and three with neuro-BD) and the five patients with only mucocutaneous disease [3.82 (2.28–5.50) ng/ml vs 2.67 (2.54–7.48) ng/ml; \(P = 0.909\)].

Finally, serum HMGB1 levels did not differ between BD patients with and without prednisone [2.86 (1.65–4.94) ng/ml vs 2.92 (2.45–6.09) ng/ml; \(P = 0.598\)] or between BD patients with and without AZA [3.18 (2.57–5.10) ng/ml vs 2.67 (2.01–5.79) ng/ml; \(P = 0.815\)]. Among BD patients on prednisone, serum HMGB1 levels were similar between patients with a high daily dose (i.e. >20 mg/day) and those with a low daily dose (i.e. <10 mg/day) [3.83 (2.28–5.22) ng/ml vs 2.64 (1.22–6.41) ng/ml; \(P = 0.251\)].

Discussion

In this study, we observed higher serum HMGB1 levels in BD patients than in healthy controls. All BD subgroups (patients with active disease, patients without active disease but still on therapy and patients in remission without treatment) presented significantly higher serum HMGB1 levels than healthy controls. Although serum HMGB1 levels were higher in patients with active disease than in patients without active disease (with or without therapy), the difference was not significant. No correlation could be found between serum HMGB1 levels and disease activity measured by BR-BDCAF(s). No association could be found with individual BD manifestations or with major disease involvement such as neuro-BD, eye inflammation or vascular-BD. Furthermore, there was no association between prednisone or immunosuppressant use and serum HMGB1 level.

Neutrophils are important effector cells in the pathogenesis of BD, contributing to tissue damage and disease manifestations [3, 4]. There is evidence that extracellular HMGB1 exerts several effects on neutrophils. In vitro studies have shown that binding of HMGB1 to TLR4 activates neutrophils, leading to nuclear translocation of NFkB. In addition, HMGB1 induces the production of cytokines such as TNFα and IL-8, and the activation of NADPH oxidase, resulting in reactive oxygen species production and increase in Mac-1-mediated adhesive and migratory functions of phagocytes [14–17]. Moreover, HMGB1 also induces the formation of neutrophil extracellular traps through interactions with TLR4 [18]. Thus, the observation of elevated serum HMGB1 levels in BD patients might indicate a possible role of extracellular HMGB1 as a mediator of neutrophil activation in BD.

A previous study evaluated HMGB1 levels in Korean patients with BD. As in our study, BD patients had significantly higher serum HMGB1 levels than healthy controls, and no difference could be found between patients with active disease and remission. Patients with gastrointestinal involvement presented the highest HMGB1 serum levels, whereas no association could be found with any
other manifestation of BD [9]. The association between higher serum HMGB1 levels and intestinal involvement in BD seems reasonable, since gastrointestinal involvement may be extensive and is characterized by deep ulceration involving any segment of the gastrointestinal tract [1, 2].

The lack of association between serum HMGB1 levels and specific disease manifestations in the present study may be due to the relatively low disease activity in BD patients evaluated in this study and to the absence of BD patients with active gastrointestinal involvement. Vascular and neurological involvement in BD represents severe disease manifestations that have an impact on the prognosis of BD patients [1, 2]. Among the 13 BD patients with active disease enrolled in this study, only three had active neuro-BD at the time of evaluation; the remaining patients presented either ocular or mucocutaneous disease, which may not be associated with intense systemic inflammatory reactions. Therefore, evaluation of a higher number of patients with severe BD manifestations would help to clarify this issue. Although neurological involvement may be found in up to one-third of BD patients in Brazil, it might be difficult to enrol patients at the onset of neuro-BD [11]. We could not find any association between serum HMGB1 levels and specific disease manifestations in BD including severe disease involvement (i.e. neuro-BD, eye disease or vasculo-BD), since BD has a relapsing–remitting course and most of these patients were in remission at the time of the study.

We also could not find associations between therapy and serum HMGB1 levels. Patients on prednisone or AZA presented similar HMGB1 levels compared with those without those agents. Reduced serum HMGB1 levels have been described in patients on statins or prednisone [19]. However, this effect has not been observed with immunosuppressive agents [20].

Limitations of the present study include the relatively small number of BD patients and the cross-sectional nature of this survey. The lack of longitudinal data makes it difficult to draw definite conclusions about associations between serum HMGB1 levels and disease activity or expression of particular manifestations of BD.

In conclusion, serum HMGB1 levels were shown to be increased in patients with BD. However, no association could be found between serum HMGB1 and disease activity or specific organ involvement in BD.

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