Original paper

Serum calprotectin and ischemia modified albumin levels as markers of disease activity in Behçet's disease

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Abstract

Introduction: Behçet's disease (BD) is a complex multisystemic inflammatory disorder which is characterized by recurrent attacks of acute inflammation. As there is no universally recognized pathognomonic laboratory marker of BD, its diagnosis is still based on clinical findings.

Aim: To evaluate the role of calprotectin and ischemia modified albumin (IMA) as biomarkers in the assessment of disease activity of BD.

Material and methods: A total of 93 patients with BD and 62 age- and gender-matched healthy controls were included in the study. Disease activity was assessed with the BD Current Activity Form (BDCAF) score. Serum levels of calprotectin, high-sensitivity C-reactive protein (hsCRP) and IMA were measured in the patient and control groups.

Results: Serum levels of calprotectin, IMA and hsCRP in patients with BD were higher than those of the healthy control group ($p < 0.001$ for all). No correlations between calprotectin and IMA, hsCRP, erythrocyte sedimentation rate, CRP, or BDCAF score were found.

Conclusions: As the calprotectin level are increased in BD patients, it could be a candidate biomarker which plays a role in BD pathogenesis.

Key words: calprotectin, ischemia modified albumin, Behçet's disease, disease activity.

Introduction

Behçet's disease (BD) is a complex multisystemic inflammatory disorder which is characterized by recurrent attacks of acute inflammation [1]. Due to the lack of universally recognized pathognomonic laboratory, radiological, or histological findings of BD, diagnosis is still based on clinical findings [2]. The exact mechanism involved in the pathogenesis of BD remains unknown. Both innate and adaptive immune systems are activated in BD. Hyperactivity of neutrophils, and dysfunction and activation of endothelial cells play an important role in the pathogenesis of BD [3, 4]. Although many cytokines and biomarkers have been identified to diagnose and monitor disease activity in BD, they are not routinely used in daily practice [5–12].

Calprotectin, also known as MRP-8/MRP-14 or S100A8/A9, is a non-covalently associated heterocomplex of two S100 calcium binding proteins: myeloid-related protein 8 (MRP-8 or S100A8) and MRP-14 (or S100A9) [13]. After release of calprotectin from neutrophils or monocytes, it exerts pro-inflammatory effects mostly via binding to Toll-like receptor 4 (TLR4) and the receptor of advanced glycation endproducts (RAGE) [14]. Under specific conditions, calprotectin can be expressed and secreted from endothelial cells, keratinocytes, osteoclasts, chondrocytes, and fibroblast-like synoviocytes [15]. Although the involvement of S100A8 and S100A9 in the inflammatory process was demonstrated almost 20 years ago, its role in the pathogenesis of rheumatic diseases has only gained great attention in recent years [15]. It has been suggested that calprotectin has a significant correlation with disease activity in patients with rheumatoid arthritis, Still's disease, ankylosing spondylitis, psoriatic arthritis, primary Sjögren's syndrome, systemic lupus erythematosus and pediatric rheumatic disease [16–22]. Elevated serum levels of calprotectin have also been observed in patients with BD. However, no correlation has been found with C-reactive protein

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(CRP) levels, erythrocyte sedimentation rate (ESR), white blood cell count, disease activity scores, quality of life, depression or anxiety [23].

Ischemia modified albumin (IMA) is a relatively new biomarker of oxidative stress, and is generated as a result of changes in albumin capacity to bind heavy metals such as cobalt and nickel [24, 25]. It has been studied in different types of rheumatic diseases [26–29]. Increased serum levels of IMA have been observed in active periods of BD compared to inactive periods and healthy controls [30–32].

Aim

The objective of this study was to evaluate the potential use of calprotectin and IMA as biomarkers in assessing disease activity of BD.

Material and methods

Study population

A total of 93 patients with BD according to the International Study Group criteria [33] and 62 age- and gender-matched healthy control subjects were included.

Patients and control group subjects were excluded if they had one of the following combined diseases/situations: 1) concomitant autoimmune or autoinflammatory disease; 2) acute or chronic infection; 3) malignancy; 4) systemic disease such as diabetes mellitus or heart failure; 5) pregnancy or up to 6 months postpartum.

The demographic features and clinical characteristics were recorded. Disease activity was assessed with the Turkish version of the BD Current Activity Form (BDCAF) [34]. The BDCAF evaluates clinical features present during the last 4 weeks prior to the date of assessment, and has been shown to have good inter-observer reliability for the assessment of general disease activity [35]. The items of this activity form are as follows: headache, oral ulcers, genital ulcers, erythema, skin pustules, arthralgia, arthritis, intestinal involvement, new eye involvement, new nervous system involvement, new major vessel involvement. The BDCAF score was calculated by adding up the scores of each item, giving a total score in the range of 0–12. The study protocol was approved by the Local Ethics Committee. The research protocol complies with the 2000 Declaration of Helsinki and written informed consent was obtained from all participants.

Laboratory analysis

Venous blood samples were collected from the participants after 12 h of fasting. Blood samples collected for analysis were centrifuged at 4000 rpm for 10 min. The separated sera were then aliquoted into Eppendorf tubes and stored at –80°C until the time of analysis.

The serum levels of calprotectin were detected with a commercial calprotectin ELISA (double antibody sandwich ELISA method) test kit (Hycult Biotech Inc, USA) according to the manufacturer's protocol. The high-sensitivity C-reactive protein (hsCRP) and albumin values were detected with the immunoturbidimetric method (values in mg/l and g/dl, respectively). The minimum detectable concentrations for hsCRP and calprotectin were 0.02 mg/l and 1.6 ng/ml, respectively. Ischemia modified albumin was measured with the albumin cobalt binding colorimetric assay as described by Bar-Or *et al.* [36] and the results were given in absorbance units (ABSU). To eliminate the effect of albumin and obtain corrected IMA values, the formula (individual serum albumin concentration/median serum albumin concentration of the population) × IMA ABSU value was used [37].

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) program version 11.0 for Windows (SPSS Inc., Chicago, IL). Conformity to normal distribution of the variables was investigated using visual and analytical methods. Normally distributed continuous values were expressed as mean ± standard deviation (SD) and categorical variables as number (*n*) and percentage (%). Parameters which were not normally distributed were stated as median values with interquartile range (IQR). Continuous parameters were analyzed using the Mann-Whitney *U* test or the Kruskal-Wallis test. Correlations between numerical data were evaluated with Spearman's correlation coefficient. A value of $p < 0.05$ was considered statistically significant.

Results

There was no statistically significant difference between the groups in respect of age and gender distributions (all $p > 0.05$). Of the patients with BD, 57 (61.3%) had active disease and 36 (38.7%) had inactive disease. The demographic characteristics and clinical features of the patients are presented in Table 1. The serum levels of calprotectin, IMA, and hsCRP in patients with BD were significantly higher than those in the healthy control group (Table 2). When disease activity was assessed with the BDCAF, both active and inactive patients were determined to have significantly higher levels of calprotectin, IMA and hsCRP than the healthy control group (Table 3). Although patients with active disease tended to have higher levels of calprotectin, IMA and hsCRP than those with inactive disease, the difference was statistically significant only for hsCRP (Table 3). No correlation was determined between the calprotectin levels and IMA, hsCRP, ESR, CRP, or BDCAF score. There was also no correlation between the BDCAF score and IMA or hsCRP.

Discussion

The results of this study demonstrated that serum levels of calprotectin, IMA and hsCRP were increased in patients with BD compared with the healthy control group. Although patients with active disease had higher average levels of calprotectin, IMA and hsCRP than those with inactive BD, only the difference in hsCRP level was statistically significant. Furthermore, there was no correlation between calprotectin level and IMA, hsCRP, ESR, CRP or the disease activity score.

Similar to the findings of the present study, Oktayoglu *et al.* observed increased serum concentrations of calprotectin in patients with BD and no correlation between serum levels of calprotectin, CRP, ESR or BDCAF score [23].

Calprotectin plays a crucial role as a significant marker of inflammation in various rheumatological diseases [13]. Viemann *et al.* reported expression of MRP8/MRP14 in affected vessels and deposition on the endothelial surface of the vessels. With treatment of vasculitis, the level of calprotectin decreases, so it is thought to play a functional role in systemic vasculitis [38]. Hirono *et al.* observed that elevated serum levels of MRP8/14 in patients with Kawasaki disease were correlated with disease activity and might be a predictor of response to IVIG treatment [39]. Behçet's disease is a complex inflammatory disorder and a well-known vasculitis syndrome. In the current study, serum levels of calprotectin were observed to be significantly higher in patients with BD than in the healthy control group.

Kim *et al.* analyzed the fecal calprotectin level of patients with intestinal BD, and found that the calprotectin level was significantly higher in BD patients with intestinal involvement than in those without intestinal involvement, and a higher fecal calprotectin level was significantly associated with intestinal BD with typical ulceration [40]. In the current study, increased levels of calprotectin were also observed.

Oxidative stress is a key factor in vascular injury. Ischemia modified albumin is a systemic biomarker of oxidative stress and has recently been extensively investigated [41]. Elevated serum levels of IMA in patients with BD have been demonstrated in previous studies [28, 29]. Bekpinar *et al.* observed that oxidative stress may occur during the active stage of BD caused by inflammatory conditions [42]. Buldanlioglu *et al.* demonstrated that in BD, the antioxi-

Table 1. Demographic characteristics and clinical features of patients with BD

Parameter	Value
Age, mean ± SD [years]	38.3 ±8.4
Age at diagnosis, mean ± SD [years]	30.4 ±7.6
Disease duration, median (IQR) [months]	96.3 (74.1)
Female/male, *n* (%)	36 (38.7)/ 57 (61.3)
Active disease, *n* (%)	57 (61.3)
BDCAF, median (IQR)	2.0 (0.0–3.0)
Disease manifestations ever presented by BD patients, *n* (%):	
Oral ulcers	93 (100)
Genital ulcers	72 (77.4)
Erythema nodosum	42 (45.2)
Papulopustular lesions	55 (59.1)
Positivity of pathergy test	20 (21.5)
Arthritis	37 (39.8)
Uveitis	35 (37.6)
Vascular involvement	31 (33.3)
Gastrointestinal system involvement	1 (1.1)
Central nervous system involvement	Parenchymal 7 (7.5) Cerebral venous sinus thrombosis (1.1)
Pulmonary aneurysm	2 (2.2)
Medical therapy, *n* (%):	
Colchicine	71 (66)
Azathioprine	47 (50.5)
Cyclophosphamide	3 (3.2)
Corticosteroids	50 (53.8)
Interferon	1 (1.1)
Anti-TNF	1 (1.1)
No therapy	4 (3.7)

Table 2. Serum levels of calprotectin, IMA and hsCRP of patients with BD and healthy control subjects

Parameter	Patients with BD	Healthy control group	*P*-value
IMA	0.55 (0.49–0.60)	0.46 (0.40–0.51)	< 0.0001
hsCRP	6.33 (3.10–12.80)	1.45 (0.80–2.12)	< 0.0001
Calprotectin	4807.50 (4062.0–5861.75)	3079.50 (2255.25–3861.50)	< 0.0001

All values are stated as median (IQR). IMA – ischemia modified albumin, hsCRP – high-sensitivity C-reactive protein.

Table 3. Serum levels of calprotectin, IMA and hsCRP according to disease activity in patients with BD

Parameter	Patients with inactive disease (*n* = 36)	Patients with active disease (*n* = 57)	Healthy control group (*n* = 62)	*P*-value	*P*-value*	*P*-value**	*P*-value***
CRP	2 (1–5.25)	7 (2–13.5)	–	–	< 0.0001	–	–
ESR	6 (3–12.25)	8 (3–14.50)	–	–	0.481	–	–
IMA	0.55 (0.49–0.59)	0.55 (0.49–0.61)	0.46 (0.40–0.51)	< 0.0001	0.586	< 0.0001	< 0.0001
hsCRP	4.03 (2.14–7.27)	7.60 (4.75–15.86)	1.45 (0.80–2.12)	< 0.0001	0.001	< 0.0001	< 0.0001
Calprotectin	4764.00 (3782.25–5586.75)	4890.50 (4065.75–6133.00)	3079.50 (2255.25–3861.50)	< 0.0001	0.268	< 0.0001	< 0.0001

*All values are stated as median (IQR). *Difference between active and inactive patients, **difference between inactive patients and healthy control group, ***difference between active patients and healthy control group. CRP – C-reactive protein, ESR – erythrocyte sedimentation rate, IMA – ischemia modified albumin, hsCRP – high-sensitivity C-reactive protein.*

dant system is deficient and inadequate, especially in patients in the active stage of the disease [43].

In contrast to the current study, Ozyazgan *et al*. found that IMA levels were significantly increased in patients with active periods of BD compared with the healthy control group and patients in remission periods of BD [30]. Çapkın *et al*. observed that BD patients with vascular involvement had significantly higher IMA levels than BD patients without vascular involvement [31].

Behçet's disease is characterized by recurrent attacks and remission. Currently there are no laboratory markers that correlate well with the clinical activity of BD [44].

This study can be accepted as a pioneer study as it focuses on IMA, calprotectin and hsCRP as different biomarkers of inflammation and oxidative stress in patients with BD.

The major limitation of this study was the cross-sectional design. No definitive conclusion could be drawn about the actual relationship between the changes in serum calprotectin levels and changes in disease activity or specific organ involvement in BD. The data were obtained from a single center only, and therefore patient selection bias was not completely avoided. At the time of the sample collection, all the patients were already taking corticosteroids and immunosuppressive agents, which might have affected the calprotectin levels.

Conclusions

Increased serum levels of calprotectin in patients with BD strengthen the possibility that calprotectin plays a role in the pathogenesis of BD. There is a need for further, prospective, large scale, longitudinal studies to investigate serum calprotectin levels in conjunction with disease activity, specific organ involvement and treatment response.

Conflict of interest

The authors declare no conflict of interest.

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