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ORIGINAL ARTICLE

Determination of serum visfatin levels in patients with Behçet's disease: A case–control study



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KEYWORDS

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Abstract *Aim of the work:* Behçet's disease (BD) is an inflammatory, systemic and chronic disorder with unknown etiology affecting multiple systems. Visfatin is a new adipokine with insulin-mimetic properties and pro-inflammatory function. The serum visfatin levels were evaluated in BD patients to investigate its role in the pathogenesis and clinical manifestations of the disease.

Patients and methods: Forty BD patients were recruited from the Behçet's disease clinic at Shiraz University of Medical Sciences in southern Iran and 40 healthy control subjects of matching age, sex and body mass index (BMI) were also included. Serum visfatin level was measured using ELISA.

Results: The 40 BD patients included 16 males and 24 females. Seventeen had active clinical manifestations; 16 with oral ulcer, 5 with genital ulcer, 6 with arthritis and 2 with uveitis. The mean age of the BD patients was 34.95 ± 9.6 years and mean BMI was 23.98 ± 4.44 . There were no significant differences between cases (5.05 ± 3.05 ng/ml) and controls (4.72 ± 2.84 ng/ml) in the visfatin level ($p = 0.61$). The difference in the visfatin level between patients with active and inactive manifestations did not reach statistical significance (6.13 ± 3.20 and 4.25 ± 2.73 , respectively; $p = 0.07$). There was no significant difference according to the gender of the patients or the presence of clinical manifestations.

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Conclusion: In view of the results in this study, visfatin levels were not significantly different between cases and controls. There was a tendency to a higher level of serum visfatin in active BD patients compared to the inactive cases; however, the difference was unremarkable.

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1. Introduction

Behçet's disease (BD) is an inflammatory, systemic and chronic disorder of unknown etiology affecting multiple systems with an underlying immune-mediated vasculitis [1]. Central events have been involved in the pathogenesis of BD such as cytokine overproduction [2,3], oxidative stress [4] apoptosis [5] and genetic polymorphisms [6]. The cause is not clear but seems to be multifactorial, including humoral and cellular immune system dysfunction, endothelial cell dysfunction and genetic predisposition [7].

Classical form of Behçet's disease presents with recurrent oral and genital ulcers [7] and ocular involvement [8]. Other systems and organs are involved which may lead to mucocutaneous, articular, neurological [9], vascular [10] and pulmonary manifestations [11].

White adipose tissue produces a variety of proteins called adipocytokines, with important roles in body metabolism [12]. Many researchers have studied different adipocytokines in other rheumatic diseases; in a study on RA patients, the serum leptin level was significantly higher than in the control however, it did not significantly correlate with any of the clinical and laboratory parameters of disease activity [13]. There was a highly statistically significant increase in resistin levels in systemic lupus erythematosus (SLE) patients being more obvious in those with lupus nephritis and significantly correlated with the disease activity [14]. On studying different adipocytokines in ankylosing spondylitis patients, serum adiponectin was not elevated while the significantly elevated serum leptin level correlated with disease activity giving clue to its role in the inflammatory process of the disease. However the significantly elevated serum resistin level did not correlate with the disease activity [15].

One of the newly identified secreted adipocytokines is visfatin, which is secreted by the visceral fat and its plasma level increases during obesity. It has insulin-mimetic effects in metabolism of cultured cells and activates the insulin receptor [12]. Visfatin also stimulates inflammatory cells like monocytes and can induce increasing circulating levels of IL-6. It has been considered as a new proinflammatory adipocytokine [16]. Originally, the enzyme nicotinamide phosphoribosyltransferase (NAMPT) was identified as pre-B cell colony enhancing factor (PBEF) a presumptive cytokine-like protein involved in subclinical inflammation [17]. Later the protein was renamed visfatin, a visceral fat-derived adipocytokine believed to mimic insulin action [18].

A few investigations have been carried out on visfatin levels in Behçet's disease although other adipokines like resistin and leptin have been more thoroughly investigated and there are some suggestions for their increased levels in BD [19–22]. In a study on visfatin, it was shown to be higher in the rheumatoid arthritis (RA) and active Behçet's patients [23]. In another

study, it was lower in active and inactive BD patients compared to the normal control group [24].

Previous studies have evaluated the visfatin level in immunologic disorders like RA and showed it was significantly higher compared to the control subjects [16,25–27]. It is also considered as a responsive marker to treatment [28]. In patients with ankylosing spondylitis, baseline levels of adipocytokines did not predict the change of disease activity or functional ability [29]. In patients with systemic sclerosis, visfatin has a potential role in the disease process; it may exert a direct anti-fibrotic effect on dermal fibroblasts and an indirect effect by promoting Th1 immune polarization [30]. Serum visfatin was strongly associated with lupus nephritis in a study on SLE patients and was considered a promising biomarker for prediction of renal involvement in those patients [31].

In this study, we have evaluated the serum visfatin level in BD patients to detect if it plays a role as a proinflammatory marker in the pathogenesis and clinical manifestations of this disorder.

2. Patients and methods

In the present study, we included 40 Behçet's disease patients who were 17–55 years old and were selected by a convenient and sequential method from patients referred to the Behçet's clinic of Shiraz University of Medical Sciences and 40 healthy subjects aged 19–58 years during 23 Oct. 2010 and 21 Apr 2011. All patients fulfilled the international diagnostic (ISG) criteria for Behçet's disease [32]. The patients with sepsis or acute lung injury, patients using more than 7.5 mg of prednisolone, those with renal or liver insufficiency, metabolic diseases and smoking ones (including current smokers or ex-smokers) were excluded from the study. Patients with Behçet's disease and healthy subjects were matched for age, body mass index (BMI) and sex.

Screening, demographic measurements and clinical assessment were performed by a rheumatologist who was involved in this research protocol. The study group received a checklist with inclusion and exclusion criteria. A written informed consent was signed by the patients and the data were registered chronologically. The study was approved by the local ethics committee of Shiraz University of Medical Sciences.

In patients included in this study, clinical activities were assessed at the time of venipuncture, on the basis of active signs and symptoms (oral ulcers, genital ulcers, eye lesions, skin lesions, vascular lesions, active arthritis, and neurological manifestations) during the preceding 2 weeks. Patients whose clinical signs and symptoms worsened during 2 weeks before sampling were considered to have active manifestations. All samples were centrifugated to receive serum. Then samples were kept in the refrigerator (–80 °C) in the autoimmune research center of Shiraz University of Medical Sciences. The

Nampt (PBEF/Visfatin) ELISA Kit was used for determination of visfatin in the serum of the patients and controls. This assay was a sandwich Enzyme Linked-Immunesorbent Assay (ELISA).

The statistical analysis was performed using the statistical Package for Social Sciences, version 16.0 (SPSS-16). The results were expressed as mean value \pm standard deviation. The Mann-Whitney *U*-test, Kruskal-Wallis test and Pearson correlation test were applied. *p*-values < 0.05 were considered statistically significant.

3. Results

The 40 BD patients had a mean age of 34.95 ± 9.6 years (ranging from 17 to 55 years), they were 16 males and 24 females (M:F 2:3) with a mean BMI of 23.98 ± 4.44 . The 40 healthy control group had a mean age of 35.68 ± 8.3 years (ranging from 19 to 58 years), and were 17 males and 23 females (M:F 1:1.4) with a BMI of 23.28 ± 3.32 . Both patients and controls had a matching age, gender ratio and BMI.

In the BD patients, the mean visfatin level was 5.05 ± 3.05 ng/ml (1.5–13.07 ng/ml) and in the healthy control group was 4.72 ± 2.84 ng/ml and the difference between them was insignificant (*p* = 0.61). The mean visfatin levels in the BD patients were comparable between males (5.03 ± 3.8 ng/ml) and females (5.06 ± 5 ng/ml).

There were 17 patients with the active clinical manifestations during blood sampling. In patients with active disease, there were 16 with oral ulcer, 5 with genital ulcer, 6 with arthritis, and 2 with uveitis. Differences in the serum visfatin levels between patients with active and inactive clinical manifestations approximated to the significance levels (6.13 ± 3.2 ng/ml and 4.25 ± 2.73 ng/ml, respectively; *p* = 0.07) (Table 1).

In active patients, there were no significant difference between visfatin levels in patients with oral ulcer (*p* = 0.13), genital ulcer (*p* = 0.88), arthritis (*p* = 0.73), and uveitis (*p* = 0.79) compared to the patients without these manifestations.

There was no significant correlation between the BMI and visfatin levels in the Behçet's patients and control groups (*p* = 0.88).

4. Discussion

Visfatin is a newly discovered adipokine, with insulin-mimetic properties and pro-inflammatory function. Along with other adipokines, there are suggestions for their role in the pathogenesis of inflammatory diseases whereas visfatin serum concentration was not associated with insulin resistance and carotid atherosclerosis in selected rheumatic diseases, it was higher in the RA and active BD patients [23].

There are other studies conducted on other adipokines like resistin and leptin in Behçet's disease. Yalçındağ and coworkers have evaluated serum leptin levels in 57 BD patients compared to 20 healthy subjects. They found a significantly higher serum leptin level and CRP in the patients. Interestingly, serum leptin level was higher in patients with nonocular BD compared to the ocular ones [33]. Kavuncu and coworkers in their study included 28 male BD patients with ocular involvement and 15 healthy subjects and measured their serum leptin level. Even though the ESR was higher in the ocular Behçet's patients, the serum leptin level was insignificantly different [20]. In the study of Pehlivan et al. the serum resistin, CRP, and ESR levels were significantly elevated in BD patients compared with healthy controls. Patients with active BD also had significantly higher levels of these parameters than did patients with inactive disease [22].

A previous study on visfatin and its role as a pro-inflammatory marker in Behçet's revealed that patients' serum visfatin levels in 58 BD patients were lower compared to healthy controls and the levels in active patients were lower in comparison with inactive ones, but this difference was not significant [24]. However, in another study on 30 BD patients conducted by Ozgen et al. it was determined that serum visfatin levels were higher in RA and active BD which are Th1-mediated diseases, but not in the SLE and SSc which are Th2-mediated diseases [23].

In our study, serum visfatin level was not significantly different between patients with Behçet's disease and healthy control group. Although, a tendency toward a significant difference was achieved when the patients with active and inactive forms of Behçet's disease were compared for visfatin level (6.13 ± 3.2 ng/ml and 4.25 ± 2.73 ng/ml, respectively; *p* = 0.07).

Table 1 Serum visfatin level in patients with and without active clinical manifestations of Behçet's disease at the time of sampling.

Clinical manifestation in Behçet's disease patients (<i>n</i> = 40)	<i>N</i> (%)	Visfatin level (ng/ml) mean \pm SD	<i>p</i> -Value
Active oral ulcers			
Yes	16 (40)	5.97 ± 3.2	0.12
No	24 (60)	4.4 ± 2.8	
Active genital ulcer			
Yes	5 (12.5)	5.78 ± 4.02	0.57
No	35 (87.5)	4.94 ± 2.95	
Active arthritis			
Yes	6 (15)	5.48 ± 3.03	0.71
No	34 (85)	4.97 ± 3.90	
Active uveitis			
Yes	2 (5)	4.64 ± 3.76	0.85
No	38 (95)	5.07 ± 3.07	
Total active manifestations			
Yes	17 (42.5)	6.13 ± 3.20	0.07
No	23 (57.5)	4.25 ± 2.73	

It is worth mentioning that replication of this study with a higher number of cases is recommended and considering to recruit more active cases. Also there was no significant difference between different clinical manifestations including ocular or non-ocular manifestations and levels of visfatin. Probably, further studies on more patients with different active manifestations will help to confirm the results of this study.

As to the present work, it could be concluded that visfatin levels were not different between cases and control groups. Although there was no significant difference between visfatin levels in BD patients with active disease comparing to non active ones, it should be considered to have a role in the pathogenesis, clinical manifestations and disease activity.

Conflict of interest

None.

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