ORIGINAL ARTICLE

Serum YKL-40 and MDA levels in Behcet disease

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Abstract

Objective: To measure plasma levels of chitinase-3-like 1 protein and its association with malondialdehyde in Behcet's disease patients.

Methods: The case-control study was conducted at Faculty of Medicine, Ataturk University Erzurum, Turkey, from October 2012 to March 2014, and comprised patients with Behcet's disease and healthy subjects. The patients were divided into two groups, as active and inactive, based on the classification of phases of activity in Behcet's disease. Differences between groups were analysed. SPSS 20 was used for data analysis.

Results: Of the 79 participants, 51(64.56%) were patients and 28(35.44%) were controls. The mean age of the first group was 29.45±7.82 years and the second group was 32.21±9.61 years. Among patients, 37(72.55%) were categorised as "active" and 14(27.45%) as "inactive". Median serum Chitinase-3-like 1 protein and malondialdehyde levels were 37.57 ng/mL (interquartilerange: 13.7-293.0 ng/mL) in patients and 26.25 ng/mL (interquartile range: 17.0-44.7 ng/mL) in controls. There was no significant correlation between Chitinase-3-like 1 protein and malondialdehyde ($p>0.05$).

Conclusion: Chitinase-3-like 1 protein might be associated with Behcet's disease. Elevated malondialdehyde levels were not only the cause of inflammation but also indicator of oxidative stress in Behcet's disease.

Keywords: Chitinase-3-like 1 protein (YKL-40), Behcet's disease, Malondialdehyde (MDA). (JPMA 66: 1299; 2016)

Introduction

Chitinase-3-like 1 protein (YKL-40) is a newly discovered acute-phase protein is secreted by a variety of cells, including activated macrophages and neutrophils in different tissues with inflammation. Serum YKL-40 has been investigated in patients with cancer, osteoarthritis, cardiovascular diseases, rheumatoid arthritis and inflammatory bowel disease.[1] Behcet's Disease (BD) is a complex inflammatory multisystem disorder characterised by recurrent episodes of acute inflammation. The International Study Group Criteria (ISGC) for diagnosis of BD are recurrent oral ulcers, mandatory finding, along with two of the four: recurrent genital ulcers, eye involvement, skin lesions and pathergy test positivity.[2] Although aetiology of BD is still unknown, the main factors are genetic background and the activation of innate and adaptive immunity by several pathogens as well as impairment of antioxidant defence system.[3] Tissue injury occurs as a result of an increased amount of superoxides and an excess of lysosomal enzymes produced by neutrophils in BD.[4] In an inflammatory situation like BD, abnormal production of inflammatory molecules and reactive oxygen species (ROS) increases by the effect of activated neutrophils. Increased ROS cause lipid peroxidation in the cells, leading to increase in the levels of malondialdehyde (MDA), which is the end product of lipid peroxidation, and serves as an indicator of oxidative damage. The current study was planned to determine the alterations in the levels of serum YKL-40, the new biomarker of inflammation, and MDA, a marker of oxidative stress, in BD patients. Also, we investigated the relationship between YKL-40 and MDA levels with BD activity.

Patients and Methods

The case-control study was conducted at Faculty of Medicine, Ataturk University, Erzurum, Turkey, from October 2012 to March 2014, and comprised patients with BD and their age- and gender-matched healthy controls. Patients fulfilling the criteria defined by the International Study Group for diagnosis of BD[2] were included. The patients were divided into two groups, as active and inactive, based on the criteria of BD Research Committee of Japan prepared in 2003.[5] According to BDRCJ clinical activity index, the existence of one or more of the following clinical characteristics was classified as active disease at medical examination: subcutaneous venous thrombosis, uveitis, skin lesion such as erythema nodosum, arthralgia, genital ulcers (those relating to the female reproductive cycle were excluded), intestinal ulceration, progressive vasculitis,

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epididymitis and progressive central nervous system lesions. Additionally for skin/genital ulcers, oral aphthous ulcers, and ocular symptoms, cases with a score of 2 or above were defined as BD in the active phase.

The study was approved by the Institutional Ethics Committees, and informed consent was obtained from each participant.

All patients were treated with colchicine. Serum levels of YKL-40 were measured using a commercial Chitinase 3-like 1 Quantikine enzyme-linked immunosorbent assay (ELISA) Kit Cat DC3L10 (R&D Systems, Inc. Minneapolis, Minnesota, United States) in serum samples stored at -80°Celsius. Serum samples were diluted 1:50 with assay buffer. Limit of detection was 3.5pg/mL. MDA was determined spectrophotometrically according to the method described by Ohkawa.[6]

All data was tested for normality using the Kolmogorov-Smirnov test. As the data was non-parametric, it was represented as medians (ranges, minimum to maximum). Differences between groups were analysed using the Mann-Whitney U test. Correlations were assessed using Spearman's rank correlation. P<0.05 was considered significant. Data was analysed using SPSS 20.

Results

Of the 79 participants, 51(64.56%) were patients and 28(35.44%) were controls. The mean age of the first group was 29.45±7.82 years and the second group was 32.21±9.61 years. Among patients, 37(72.55%) were categorised as "active" and 14(27.45%) as "inactive". The median serum YKL-40 level was 37.57 ng/mL (interquartile range [IQR]: 13.7-293.0 ng/mL) in BD patients and 26.25 ng/mL (IQR: 17.0-44.7 ng/mL) in controls (p=0.002). In addition, median serum MDA level of BD patients was 61.59µmol/L (IQR: 22.9-188.4) compared to32.27µmol/L (IQR: 22.5-66.1) among controls (p=0.0001). In active patients, median serum MDA levelwas70.22µmol/L (IQR: 32.0-188.4) compared to 30.00 (IQR: 22.9-111.1) in inactive ones (p=0.002). Besides, median serum YKL-40 level was 38.76 ng/mL IQR: 13.7-293.0) in active patients and 40.36 (IQR: 18.9-88.8) in inactive patients (Table).

Table: Serum YKL-40 (median) and MDA (median) levels in active and inactive patients with BD.

Study Groups	Serum YKL-40 (ng/mL)	Serum MDA (µmol/L)
Active patients with BD (n=37)	38.76 (13.7-293.0)	70.22 (32.0-188.4)*
Inactive patients with BD (n=14)	40.36 (18.9-88.8)	30.00 (22.9-111.1)
Total patients with BD (n=51)	37.57 (13.7-293.0)***	61.59 (22.9-188.4)**
Healthy controls (n=28)	26.25 (17.0-44.7)	32.27 (22.5-66.1)

*: p=0.002 compared with inactive patients.
**: p=0.002 compared with healthy controls.
***: p<0.0001 compared with healthy controls.
YKL: Chitinase-3-like protein 1.
MDA: Malondialdehyde.
BD: Behcet's disease.

Discussion

To the best of our knowledge, the present study is the first to investigate both serum YKL-40, a marker of inflammation and remodelling, and MDA, an indicator of oxidative damage, levels in BD patients.

The etiopathogenesis of BD is unknown. It has been postulated that infectious agents, immune mechanism, genetic and environmental factors are accepted to be the main factors in its pathogenesis. The most important feature of BD is systemic vasculitis with endothelial dysfunction. Inflammation and thrombosis of the small arteries and veins result in vasculitis. Direskeneli[7] debated the etiopathogenesis of BD on the basis of autoimmunity versus auto inflammatory.

YKL-40 is a heparin- and chitin-binding lectin without chitinase activity and a member of the mammalian chitinase-like protein family and mainly produced by macrophages, neutrophils and cancer cells.[8] YKL-40 plays a role in the differentiation of monocytes to activated macrophages in tissues characterised by inflammation.[9] Serum levels of YKL-40 increase in patients with acute infections.[10] High serum YKL-40 levels have also been documented in some conditions with inflammation and/or tissue remodelling, like rheumatoid arthritis, cardiovascular diseases, Crohn's disease, bronchial asthma, diabetes mellitus, liver fibrosis and cancer.[11] Serum YKL-40 levels have been investigated in different inflammatory dermatological diseases such as psoriasis, psoriatic arthritis, hidradenitis suppurativa.[11,12] Imaiet al.[13] found that serum levels of YKL-40 increased in psoriasis vulgaris and in generalised pustular psoriasis, characterised by neutrophil infiltration into the epidermis leading to Kogoj's spongiotic pustule. They demonstrated that serum levels of YKL-40 were about 3 times higher in psoriasis vulgaris cases than in healthy subjects. Another study showed that in patients with psoriatic arthritis, both serum levels of YKL-40 and the Psoriasis Area and Severity Index scores were improved only after 6 weeks of treatment with infliximab as an anti-tumour necrosis factor alpha (anti-TNF-α) agent. Therefore, serum levels of YKL-40 might be a useful biomarker to reflect the severity of skin lesions in

patients with psoriatic arthritis.[14] Another study demonstrated that plasma YKL-40 may be useful as a biomarker showing disease activity and effectiveness of treatment in psoriatic arthritis patients, but not in patients with psoriasis vulgaris alone.[11] Although serum YKL-40 levels have been investigated in different dermatological diseases like psoriasis and psoriatic arthritis, only one study has been reported in literature as far as BD is concerned. Seo et al. showed increased serum YKL-40 levels in patients with BD and a positive correlation of YKL-40 levels with disease activity. According to their results, they hypothesised that YKL-40 may play a role in the pathophysiology of inflammation in BD.[15] Our results are consistent with that study about high serum levels of YKL-40 in BD patients but not correlated with disease activity.

Abnormalities of neutrophil functions and ROS production have been suggested as the important factors triggering various findings of BD. Cellular oxidative damage results in the oxidation of deoxyribonucleic acid (DNA), proteins and membrane lipids. Measurement of MDA is commonly used to determine the degree of lipid peroxidation induced by ROS.[16] In practical application, clinicians use inflammatory tests such as C-reactive protein (CRP), erythrocyte sedimentation rate and haemogram as activation markers, although they are nonspecific tests in BD.[17] Bekpinar et al. reported CRP and MDA increased in BD patients than those of controls.[18] Elevated MDA levels reflect increased oxidative stress, which is one of the suspected factors in the etiopathogenesis of BD.[19] Buldanlioglu et al.[20] demonstrated increased oxidative stress and decreased antioxidant capacity in patients with BD. They reported higher MDA levels in BD patients as compared to those of control subjects. Furthermore, in patients with active disease, MDA levels were higher than in inactive ones. The results of the current study concurred with those of above-mentioned studies in terms of higher serum MDA levels in active BD patients than in inactive ones. In other words, there was an association between disease activity and MDA levels.

In our study, although YKL-40 and MDA levels were elevated, there was no significant correlation between YKL-40 and MDA in BD group. This result seems paradoxal but it may suggest that the disease activity is related with increased oxidative stress. However, to support this hypothesis a large-scale study is necessary.

Our results showed that serum YKL-40, a marker of inflammation and remodelling, was higher in BD patients compared with healthy controls, but increase in serum YKL-40 levels was independent of the clinical activity for BD patients. This result may be associated with limited number patients in our study.

Conclusion

Serum MDA and YKL-40 levels were higher in BD patients.MDA levels may reflect the disease activity while YKL-40 did not show an increase in disease activity.

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Conflict of Interest: None.

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