A Novel Marker Demonstrating Endothelial Dysfunction in Behçet's Disease, YKL-40

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ABSTRACT

Background: To evaluate the relationship between disease activity and levels of YKL-40 in patients with Behcet's disease (BD).

Methods: Twenty five active and twenty six inactive BD patients and 24 age and gender matched healthy control subjects were included in the study. Serum YKL-40 levels were measured using Enzyme Linked Immuno Sorbent Assay (ng/mL). Disease activity was assessed using Behcet's Disease Current Activity Form (BDCAF).

Results: Serum YKL-40 levels were significantly higher in patients with active BD and inactive BD compared to the control group (respectively $p<0.001$, $p< 0.001$). There were no significant differences between active and inactive BD groups in terms of levels of YKL-40 ($p=0.9$). According to Pearson's analysis, YKL-40 levels were significantly positively correlated with Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and skin lesions of BDCAF scores.

Conclusion: Our study demonstrated that serumYKL-40 level was significantly higher in active and inactive BD compared to healthy controls. However, the groups with active and inactive disease had no significant difference with respect to YKL-40 level. In addition, there existed a significant correlation between serum YKL-40 level and ESR, CRP, and BDCAF's skin scores.

Keywords: Behcet's Disease, endothelial dysfunction, YKL-40

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INTRODUCTION

Behçet's disease (BD) is a chronic, inflammatory disorder of unknown origin that can involve many organ systems but is mostly characterized by ulcers involving oral cavity and genitalia as well as uveitis, which tend to occur in recurrent attacks intervening remission periods. The basic pathology in BD is vasculitis, which follows a chrnoic course in the form of recurrent attacks (1-3). Vasculitis is the hallmark of BD lesions and it is characterized by marked perivascular neutrophilic and monocytic infiltration, and sometimes mural fibrin deposition in blood vessels (4). Despite being still unclear, the pathogenesis of the vascular lesions of BD are possibly mediated by endothelial dysfunction. Vasculitis coupled with endothelial cell activation or injury are the leading histological findings in BD (5-6).

YKL-40 is member of 18 glycosyl hydrolase (mammalian chitinase) family and also known aschitinase 3-like protein 1 (CHI3L1) or cartilage glycoprotein-39 (gp-39). YKL-40 is among the major proteins secreted by human articular chondrocytes, synovial cells, endothelial cells and macrophages. It is also expressed by grown neutrophils (7). Despite its full-scale functions are largely unknown, YKL-40 is a proinflammatory protein believed to play a role in endothelial dysfunction (ED) and atherosclerosis. Both in vivo and in vitro studies have found that it is mainly located in activated macrophages and vascular smoth muscle cells that take part in inflammation and extracellular matrix remodeling, as in atherosclerotic plaques (8-11). Some cells of the immune system also express YKL-Its proinflammatory properties and role in vascular events has made it a suspected molecule in the etiology of endothelial dysfunction and atherosclerosis. Endothelial damage seems to be primary trigger of elevated serum YKL-40 level that involves in the processes of cell migration, reorganization, and tissue remodeling (12-13). We hypothesized that YKL-40 could have a role in the pathogenesis of BD. However, to our best knowledge, there is no study investigating the relationship between serum levels of YKL-40 and BD.

MATERIALS AND METHOD

Study population

Thirty-five patients with active disease (20 males and 15 females) and 36 patients with inactive disease (18 males and 18 females) were included. In addition, 34 age and gender-matched healthy control subjects (20 males, 14 females) were included in the study. Diagnoses were made according to the criteria of the International Study Group for Behçet's disease(14). Although some studies report using the clinical activity index(15) other studies report disease activity(16-17). In this study, clinical activity was assessed according to the methods used in previous studies. Active BD was defined by worsening clinical symptoms at clinical evaluation and ≥3 other major criteria (e.g. oral or genital ulcerations, anterior/posterior panuveitis, papulopustular or pseudofollicular skin lesions, positive pathergy test).The patients were selected from among patients presenting to Dicle University Faculty of Medicine, Department of Physical Medicine and Rehabilitation and Department of Ophtalmology between January 2015 and March 2015. The subjects were categorized into 3 groups as the active BD group, the inactive BD group, and the control group. The study was approved by the local ethics committee of the hospital. All subjects gave informed consent before participating in the study. Patients with additional systemic disorders (malignancy, coronary artery disease, asthma, collagen vascular disease, chronic kidney and hepatic failure, pulmonary embolism, and sepsis) were excluded, so were those under the age of 18 and the pregnant patients. An informed consent was obtained from each patient. Dicle University local ethics committee approved the study (2015/256).

Determination of disease activity

BDCAF

BDCAF was used for measuring the disease activity of BD. Duration of symptoms is the major factor on which BDCAF scoring for headache, fatigue, erythema nodosum (EN) or superficial thrombophlebitis, oral and genital ulceration, arthritis, arthralgia, pustuls, nausea or vomiting or abdominal pain and diarrhoea with altered/frank blood was based. Each domain can score a minimum of 0 point and maximum of 4 points depending on its duration (0= no symptoms, 1= symptoms for 1 week, 2= symptoms for 2 weeks, 3= symptoms for 3 weeks, 4= symptoms for 4 weeks). Behcet's oculopathy index evaluated eye activity and was assigned 0 to 3 points. BDCAF score was calculated by completing a scale from 0 to 6 that evaluates the overall disease activity within the previous 4 weeks (18). Hamuryudan et al. have verified its Turkish version for validity and reliability (19).

Determination of Levels of ESR, CRP and biochemical parameters

Venous blood samples were drawn into sterile tubes and serum and plasma samples were stored at -80 ºC until biochemical analysis. Erythrocyte sedimantation rate (ESR) was measured using Westergren method (mm/h), blood samples were collected between 8 am and 10 am, after an overnight fast of at least 12 h, for biochemical analyses. Glucose, Total cholesterol, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), and Triglycerides were measured with autoanalyser, using Standard enzymatic methods (Cobas 311; Roche Diagnostics, Mannheim, Germany). Serum C-reactive protein (CRP) levels were measured by standard nephelometry (Cobas 311).

Determination of Levels of YKL-40

The fasting blood samples were centrifuged immediately, and serum specimens for YKL-40 (myBiosource, San Diego, California, USA) were frozen immediately and preserved at -80 C until analysis. The serum levels were quantified by Uscn Life Science enzyme linked immunosorbent assays (ELISAs) as ng/ml.

Statistical analyses

The Kolmogorov-Smirnov test was used to confirm that data within the ranges of normal distribution in both groups. Descriptive statistics for continuous variables were expressed as the mean and standard deviation, and categorical variables were expressed as number and percentages. The Chi-square test was used to assess differences in categoric variables. The Student's t-test was applied evaluate statistical difference in continuous variables between the two groups. Correlations between different continuous variables were evaluated by Pearson correlation analysis. The level of statistical significance was set at a two-tailed P-value of 0.05 or less. All statistical analysis was performed using SPSS for Windows (Version 16.0).

RESULTS

There were no significant differences between the three groups with respect to mean age and gender distribution ($p= 0.59$, $p= 0.65$, respectively). The active BD group and inactive BD group had a significantly different mean serum YKL-40 level than the controls ($p<0.001$, $p< 0.001$, respectively). There was, however, no significant difference between the active and inactive BD groups ($p=0.9$). In addition, there was detected a significant difference between active and inactive BD in terms of all the average scores of the BDCAF and clinic's

impression of BD ($p<0.05$). The demographic, clinical, and laboratory data of three groups, and their statistical comparisons were shown in Table I.

Table II presents the clinical signs and their frequency in the active and inactive BD group. According to the Pearson correlation analysis, serumYKL-40 level was correlated to ESR, CRP, behcet's oculopaty index, and BDCAF's fatigue score and skin scores such as oral ulceration, genital ulceration, erythema nodosum, a pustula. Correlation between disease-related variables and domains of BDCAF scores are demonstrated in Table III.

DISCUSSION

Our study is the first to show the relationship between BD and YKL-40 level, with the mean serum YKL-40 levels of the active and inactive Behçet groups being significantly greater than the control group. Furthermore, YKL-40 level showed a parallel increase with ESR and CRP, which are considered as the activation criteria in BD.

BD is a generalized inflammatory disorder with recurrent attacks of oral and genital mucous ulcers, uveitis, and skin lesions. Neither its etiology nor its molecular mechanisms have been fully elucidated. Augmented and dysregulated immune responses has been proposed as the main derangement associated with the disease. Vasculitis is the feature of BD lesions and it is characterized by marked perivascular neutrophilic and monocytic infiltration, and sometimes mural fibrin deposition in blood vessels (20-21). ED has also been unequivocally shown by previous studies (22-24). BD injures tissues of human body through augmented inflammatory responses characterized by overactivation of neutrophils with exaggerated chemotaxis, phagocytosis, and reactive oxygen species production, as well as excess expression and release of proinflammatory cytokines including IL-1, IL-6, IL-8, IL-17, TNF-alfa, and IFN-alfa (25).

YKL-40, a newly discovered 40 kDa chitin-binding glycoprotein without chitinase activity, is involved in ED and atherosclerosis in conjunction with cell migration, reorganization, and tissue modeling that result from endothelial injury (26). YKL-40 is an acute phase protein expressed by various cells one of whic is macrophage. It has been shown to involve in the regulation and modification of acute and chronic inflammation and tissue remodeling (27-29). Some reports have indicated that YKL-40, among other molecules, activates innate immune system and its expression is particularly elevated in some specific macrophages included by atherosclerotic plaques (30). It also reportedly boosts chemotaxis, cellular binding, migration of vascular endothelial cells in atherosclerotic plaque formation. It has been detected in high amounts is various diseases such as cancer, diabetes, infections, and inflammatory disorders (31-33). Despite extensive research and a relatively large body of knowledge, its role and mechanism of action are still unclear and warrants further research.

Recently, Lee et al. demonstrated a significant association between microvascular damage and serum YKL-40 levels in type 2 diabetic patients (34). Erfan et al. reported that the elevation of plasma YKL-40 in psoriasis can be associated both with inflammation of the disease, and also with endothelium dysfunction and serum YKL-40 can be used as a new biological marker for angiogenesis and disease activity in psoriasis with or without psoriatic arthritis(35). Jafari et al. found endothelial dysfunction to be correlated with higher serum YKL-40 level in obstructive sleep apnea (36). Aziz et al. failed to demonstrate a YKL-40 increase in women with polycystic ovarian disease with low-grade inflammation (37).

In a very large study in Danish population Kjaergaard et al. demonstrated that elevated serum YKL-40 level was asssociated with a thirty-four percent increased triglyceride level and a two-fold increased stroke risk, although no genetic YKL-40 elevation were found in these individuals(38). Bouvet et al. reported a correlation between elevated serum YKL-40 level and inflammation-induced dysglycemia in a group of patients with

cystic fibrosis (39). We excluded subjects with systemic disorders such as hyperlipidemia or diabetes that are regarded as risk factors for coronary artery disease. Additionally, there was no significant differences between the study groups with respect to mean glucose and lipid parameters. Matsumoto et al. in a patient population with rheumatoid arthritis, found a higher YKL-40 level in the patient group compared to the controls and reported that serum YKL-40 level was significantly correlated to serum IL-6 and CRP levels (40). Our study also pointed a significant correlation between serum YKL-40 level and serum ESR and CRP levels. Serum YKL-40 level also showed significant correlations with ophtalmological involvement, oral ulceration, genital ulceration, or BDCA's mean scores such as erythema nodosum or superficial involvement. These results suggest that serum YKL-40 level may be a valuable marker for evaluation of activation of skin lesions in BD.

Melikeoglu et al. in a domestic study demonstrated a significant correlation between newly-developed erythema nodosum, superficial thrombophlebitis, or joint involvement and elevated levels of ESR and CRP (41). El Menyawi et al. detected a significant difference between active and inactive BD groups with respect to TNF-alpha level whereas serum levels of ESR and CRP were similar in both groups (42). In our study, on the other hand, the patients with active disease had a significantly higher levels of ESR and CRP compared to the inactive disease group. Moreover, there was a significant correlation between all clinical BDCAF scores and ESR and CRP. As for the serum YKL-40 score, the active and inactive disease groups had significantly higher levels of serum YKL-40 compared with the control group although they lacked any significant difference between each other with regard to the same parameter. This result suggests that YKL-40 level may be more linked to the presence of the disease than to its activity. We believe that further studies should be conducted to clarify this subject.

Mesquida et al. reported a significant increase in the the level of the proinflammatory cytokines (interferon-gamma, TNF-alpha, and IL-17A) and CRP in active BD with uveitis compared to inactive disease and healthy controls (43). We found that the mean Behçet's oculopathy index score was significantly greater in the active disease group than the control group, and a significant correlation existed between the oculopathy index and serum levels of ESR, CRP, and YKL-40.

CONCLUSION

Our study demonstrated that serum YKL-40 level was significantly higher in patients with active and inactive BD compared to the healthy individuals. This difference was, however, not the case between the active and inactive disease groups. In addition, a significant correlation was found between serum YKL-40 level and ESR, CRP, and BDCAF'significantly skin scores.

Limitations

Our study had some limitations. First, we did not evaluate the role of cytokines such as IL-1, IL-6, IL-8, IL-17, TNF-alpha. Second, our sample volume was relatively small. Lastly, more efficient markers are needed to diagnose Behçet disease and determine its activity and prognosis. We do not know YKL-40's sensitivity and specifity for clinical use. This subject should be addressed by future studies.

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Table 1: Demographic and clinical data and mean laboratory results of the overall study population

	ABD (n:35)	**IBD (n:36)**	**Control (n:34)**
Age (years)	31.8±8.2	33.0±4.4	33.3±3.1
Sex (n, %)			
Female	20 (57.1%)	18 (50%)	20 (58.8%)
Male	15 (42.9%)	18 (50%)	14 (41.2%)
Disease duration (month)	106.12±84.36	103.55±95.22	
YKL-40 (g/L)	98.76±62.08[c]	92.54±56.35[c]	51.74±17.01[ab]
CRP (mg/dL)	18.48±4.87[bc]	7.65±1.72[ac]	1.80±1.12[ab]
ESR (mm/h)	32.63±10.04[bc]	16.08±3.93[ac]	6.26±2.15[ab]
Glucose (mg/dL)	101.25±13.6	102.02±7.33	95.45±20.28
Total cholesterol(mg/dL)	180.33±27.55	173.62±26.30	181.65±35.61
HDL (mg/dL)	46.65±7.07	46.55±7.56	45.49±4.09
LDL (mg/dL)	126.44±21.84	135.54±21.31	129.75±23.08
Triglycerides (mg/dL)	142.15±15.42	138.85±25.96	135.05±19.47
BDCAF fatigue	2.63±1.01[b]	1.62±0.78	
BDCAF headache	0.76±0.98[b]	0.34±0.56	
BDCAF oral ulceration	1.38±1.45[b]	0.98±0.71	
BDCAF erythema nodosum or superficial thrombophlebitis	23.34±0.91[b]	0.46±1.72	
BDCAF pustule	2.78±0.75[b]	0.97±0.88	
BDCAF arthralgia	2.26±0.85[b]	0.79±0.66	
BDCAF arthritis	2.24±0.85[b]	0.98±0.80	
BDCAF nausea or vomiting or abdominal pain	2.21±0.78[b]	0.34±0.63	
BDCAF diarrhoea with altered/frank blood	2.44±0.89[b]	0.76±0.81	
Behcet's oculopathy index	2.15±1.12[b]	1.85±0.96	
Clinican's impression of DA	3.78±1.15[b]	2.26±1.45	
Patient's impression of DA	3.21±1.34	2.06±1.18	

ABD:Active Behcet Disease, IBD:Inactive Behcet disease, CRP:C-reactive protein, ESR: Erythrocyte sedimantation rate, HDL: High-density lipoprotein, DA: disease Activity, LDL: low-density lipoprotein, BDCAF: Behcet Disease Current Activity Form a; significant compared to ABD group, b; significant compared to IBD c; significant compared to control group

Table 2: Clinical findings of the patients with active and inactive Behcet's Disease

	ABD (n:35) Positive (%)	IBD (n:36) Positive (%)
Oral ulcer	60	27
Genital ulcer	16	4
Pathergy test	64	19
Pustule	18	11
Erythema nodosum or Superficialthrombophlebitis	24	15
Eye problems	36	11
Arthritis	16	4
Arthralgia	56	19
Arterial involvement	0	0
Vein involvement	4	0
Neurobehcet	8	0

ABD:Active Behcet Disease, IBD:Inactive Behcet disease

Table 3. Correlation between the domains of BDCAF, disease duration and laboratory variables in patients with active Behcet's disease

Active BD Group	ESR	CRP	YKL-40
ESR (mm/h)		r:0.842**	r:0.3**
CRP (mg/dL)			r:0.290*
Behcet's oculopathy index	r:0.385**	r:0.422**	r:0.275*
Clinican's impression of DA	r:0.614**	r:0.661**	r:0.192
Patient's impression of DA	r:0.696	r:0.707**	r:0.168
Disease duration	r:0.420**	r:0.472**	r:0.230*
BDCAF fatigue	r:0.720**	r:0.421**	r:0.275*
BDCAF oral ulceration	r:0.237*	r:0.373**	r:0.272*
BDCAF genital ulceration	r:0.550**	r:0.667**	r:0.266*
BDCAF erythema nodosum	r:0.751**	r:0.780**	r:0.255*
BDCAF pustule	r:0.747**	r:0.803**	r:0.257*
BDCAF arthralgia	r:0.728**	r:0.746**	r: 0.213
BDCAF arthritis	r:0.721**	r:0.735**	r:0.235
BDCAF nause or vomiting or abdominal pain	r:0.736**	r:0.796**	r:0.185
BDCAF diarrhoea with altered/frank blood	r:0.755**	r:0.759**	r:0.204

BDCAF: Behcet's disease current activity form; BD: Behcet's disease; DA: disease activity; ESR: Erythrocyte sedimentation rate; CRP:C-reactive protein; r:correlation coefficient. *: $p<0.05$, **:$p<0.01$