Levels of F_2 isoprostane in Behcet's disease: Correlation with cardiometabolic risk factors

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Objective: Behcet's disease (BD) is a chronic inflammatory disease and recent findings suggest a role of oxidative stress in the pathogenesis of BD. Free radical-induced oxidative stress is also involved in the pathogenesis of cardiovascular and other rheumatic diseases. Oxidative stress may be detected *in vivo* by measuring F_2 isoprostanes. Here, we measured plasma levels of F_2 isoprostane in patients with BD and evaluated the correlation of F_2 isoprostane with cardiometabolic risk factors.

Methods: Forty-three patients with BD in remission and 37 age- and sex-matched controls were recruited for the study. Blood samples were obtained to determine F_2 isoprostane, C-reactive protein levels, erythrocyte sedimentation rate, and other biochemical parameters. Homeostasis model assessment insulin resistance and body mass index were calculated. Systolic blood pressure, diastolic blood pressure, and waist circumference were measured.

Results: Plasma F_2 isoprostane, fasting plasma glucose, triglyceride, and C-reactive protein levels were significantly higher in patients with BD compared with healthy controls, whereas high-density lipoprotein cholesterol levels were significantly lower in patients with BD. F_2 isoprostane levels did not correlate with cardiometabolic risk factors, C-reactive protein levels, or erythrocyte sedimentation rate.

Conclusion: High levels of F_2 isoprostane in patients with BD indicate oxidative stress. Antioxidant therapeutic approaches could potentially affect the course of this disease.

Keywords: Behcet's disease, F_2 isoprostane, Cardiometabolic risk factors

Introduction

Behçet's disease (BD) is a relapsing, multisystemic, chronic inflammatory disease characterized by recurrent oral and genital aphthous ulcers, together with ocular, arthritic, and neurologic involvement and non-specific vasculitis. Although the etiology of the disease is not certain, autoimmune, genetic, microbiologic, and environmental factors are implicated.[1] In BD, the generation of reactive oxygen species by neutrophils is increased, leading to the tissue injury associated with BD.[2–5] Free radical-induced oxidative stress results from the imbalance between high free radical exposure and antioxidants. Free radicals cause critical and direct damage to various components, e.g., lipids, proteins, and DNA.

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In 1990, Morrow *et al.*[6] discovered the *in vivo* formation of a family of prostaglandin derivatives, called F_2 isoprostanes, by non-enzymatic free radical-induced oxidation of arachidonic acid. Indeed, measurement of F_2 isoprostanes in plasma and various biologic fluids provides a reliable quantitative index of oxidative stress *in vivo*.[7] Furthermore, products of isoprostane pathways play an important role and have potent biologic actions representing the pathophysiologic aspects of BD. The involvement of F_2 isoprostanes has been evaluated in various arthritic and rheumatic diseases.[8–10] Various oxidative stress markers and oxidative states have also been studied in patients with BD with inconsistent results.[11–13]

Oxidative stress and inflammatory processes are the main features of many acute and chronic diseases, such as cancer, lung disease, cardiovascular disease, and rheumatic disease, as well as normal aging processes.

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Oxidant stress and the production of intracellular reactive oxygen species are implicated in the pathogenesis of a variety of cardiovascular diseases.[14] Kim *et al.*[15] reported a two-fold increase in the odds ratio for the highest F_2 isoprostane quartile compared to the lowest quartile for cardiovascular disease. In a case–control study, Gross *et al.*[16] compared F_2 isoprostane concentrations and the extent of coronary artery calcification in a healthy population cohort and found that a greater percentage of women in the highest F_2 isoprostane quartile manifest calcification compared with the lowest F_2 isoprostane quartile in both sexes, although the prevalence of calcification is lower in women overall. Obesity and associated cardiometabolic risk factors, such as C-reactive protein (CRP) and increased blood pressure, are also associated with F_2 isoprostane.[17]

Several studies have investigated the interaction between BD and cardiometabolic risk factors, such as carotid intima media thickness, endothelial dysfunction, and microvascular involvement, whereas other studies have not found atherosclerotic features in BD.[18–21] A few researchers have investigated the interaction between isoprostane, rheumatic disease, and cardiometabolic risk factors and reported inconsistent results. Rho *et al.*[22] concluded that F_2 isoprostane excretion is higher and that F_2 isoprostane excretion is positively associated with the severity of coronary calcification in patients with rheumatoid arthritis than in control subjects, whereas Soep *et al.*[23] found normal isoprostane levels despite the high burden of atherosclerotic risk factors in children and young adult patients with systemic lupus erythematosus.

Although the involvement of F_2 isoprostane in various rheumatologic diseases has been investigated, we found no studies in the literature reporting the plasma levels of F_2 isoprostane in BD. The main objective of this study was to determine the plasma levels of F_2 isoprostane as well as standard measures of inflammation (CRP and erythrocyte sedimentation rate (ESR)) in patients with BD and to compare them with healthy controls. We also assessed whether F_2-isoprostane concentrations were correlated with cardiometabolic risk factors, despite the inconsistent results between BD and cardiometabolic risk factors.

Materials and method

We conducted a cross-sectional study on 43 patients diagnosed with BD according to the Study Group of Behcet's Disease who were admitted to Istanbul Medeniyet University Goztepe Training and Research Hospital BD outpatient clinic between June 2011 and December 2011.[24] All patients were in remission with mucocutaneous involvement and treated with colchicine. Thirty-seven healthy age- and sex-matched subjects were included in the study as controls. Patients and controls with diabetes mellitus, dyslipidemia, cardiovascular disease, malignancy, smoking, alcohol consumption, any other rheumatologic or inflammatory disorders, or acute and chronic infective diseases were excluded from the study. The Institutional Review Board of Istanbul Medeniyet University Goztepe Training and Research Hospital approved the study (12/A; 17 May 2011), and written informed consent was obtained from each subject.

Biochemical methods

In both the patient and control groups, morning blood samples were obtained after overnight fasting. Blood samples were centrifuged at 1500 g for 10 minutes at room temperature within 2 hours of collection. Serum CRP levels were measured using nephelometry (Immage® Immunochemistry System, Beckman Coulter, USA) as a part of daily clinical practice. Plasma (EDTA-anticoagulated) samples were stored at −20°C until analysis. ESR levels were determined by the Western Green method (Berkhun SDM-100, Turkey).

To evaluate oxidative stress, we analyzed plasma levels of 8-iso-prostaglandin F_2alpha (also known as 8-epi-PGF_2alpha, 8-isoprostane, or 15-isoprostane F_2t), a biomarker of lipid peroxidation, using the 8-iso-prostaglandin F_2alpha enzyme-linked immunosorbent assay kit (Cell Biolabs, Inc., San Diego, CA, USA), according to the manufacturer's guidelines. The intra- and inter-assay coefficients of variability of the test were 4.5 and 7.8%, respectively.

For biochemical analysis, blood specimens were collected after 12–16 hours of fasting. Fasting plasma glucose (FPG), total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and fasting insulin were measured by standard assays. The homeostasis model was used to assess insulin resistance homeostasis model assessment-insulin resistance (HOMA-IR) according to the following formula: insulin resistance (HOMA-IR) = (fasting insulin (μU/ml) × fasting glucose (mg/dl))/405.[25]

Anthropometric measurements

Height (centimeters) and weight (kilograms) of the subjects were measured in light clothing and without shoes. Body mass index (BMI; kilograms per square meter) was calculated. Waist circumference (WC) was assessed at the midpoint between the 12th rib and the iliac crest. Hip circumference (HC) was measured at the level of the greater trochanter.

Statistical analysis

The statistical analyses were performed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA).

The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov–Smirnov) to determine whether they were normally distributed. For the data analysis, descriptive methods (mean, standard deviation, median) were applied. Categorical data are represented as numbers and percentages. The descriptive statistics are given as means ± SD for normally distributed variables, whereas medians are used for non-normally distributed variables. For comparison of parameters between two groups with a normal distribution, Student's t-test was used. For comparison of parameters between two groups without a normal distribution, the Mann–Whitney *U* test was used. Pearson's and Spearman correlation coefficients were used on continuous variables to assess the relationship between variables. A value of $P < 0.05$ indicated statistical significance.

Results

All BD patients had recurrent oral aphthous lesions ($n = 43$, 100%), 28 (65%) had genital ulcers, 21 (49%) had acneiform lesions, 10 (23%) had erythema nodosum, 19 (44%) had uveitis, and 7 (16%) had thrombosis. Five patients were treated with azathioprine, four with cyclosporine, nine with pentoxifylline, and four with acetylsalicylic acid. Plasma F_2 isoprostane was higher in patients with BD than in healthy controls (250.4 ± 292.1 versus 79.84 ± 89.2 ng/ml; $P < 0.001$). FPG, TG, and CRP levels were significantly higher, whereas HDL-C was significantly lower in patients with BD than in healthy controls. BMI, WC, HC, systolic blood pressure (SBP), diastolic blood pressure (DBP), uric acid, total cholesterol, LDL-C, HbA1c, HOMA-IR, and ESR levels did not differ significantly between groups (Table 1).

Correlation analysis revealed no relationship between F_2 isoprostane and obesity indices and cardiometabolic risk factors. Further, inflammatory markers, such as CRP and ESR, were not correlated with F_2 isoprostane (Table 2). None of the clinical features (oral apthous lesions, genital ulcers, acneiform lesions, erythema nodosum, uveitis, or thrombosis)

Table 2 Correlation analysis of cardiovascular risk factors and inflammatory markers with F_2 isoprostane in patients with Behcet's disease

	R	*P*-value
Age[#]	0.072	0.646
BMI[#]	0.166	0.286
WC[#]	0.028	0.861
HC	0.102	0.516
SBP[#]	0.031	0.853
DBP[#]	0.151	0.366
FPG[#]	0.102	0.548
Uric acid[#]	−0.194	0.257
Cholesterol[#]	−0.163	0.334
HDL-C[#]	0.059	0.728
LDL-C[#]	−0.161	0.341
TG	−0.072	0.673
HOMA-IR	0.080	0.608
CRP	−0.045	0.799
ESR	−0.050	0.770

Spearman correlation coefficient and [#]Pearson's correlation coefficient were used. BMI, body mass index; WC, waist circumference; HC, hip circumference, SBP, systolic blood pressure; DBP, diastolic blood pressure, FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol, TG, triglyceride; HOMA-IR, homeostasis model assessment-insulin resistance; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 1 Descriptive characteristics, anthropometric indices, cardiometabolic risk factors, inflammatory markers and F_2 isoprostane levels of the study population with BD and controls

Number of subjects *n* (%) Sex (M/F)	Behcet's disease 43 15/28	Control 37 (12/25)	*P*-value
Age (years)	39.5 ± 8.8	40.4 ± 8.1	0.7
BMI (kg/m^2)	27.1 ± 4	26.7 ± 5.3	0.7
WC (cm)	99.2 ± 10.5	95.1 ± 10.9	0.09
HC (cm)	107 ± 6.8	107.7 ± 9.8	0.1
SBP (mmHg)	113.1 ± 11.8	113.1 ± 14	0.8
DBP (mmHg)	73.1 ± 8	70.2 ± 8.2	0.1
FPG (mg/dl)	96.3 ± 9.1	91.7 ± 8.5	0.03
Uric acid (mg/dl)	4.4 ± 1.6	4.7 ± 1.4	0.4
Cholesterol mg/dl)	192.1 ± 41.5	203.65 ± 39.3	0.2
LDL-C (mg/dl)	123.9 ± 3	125.6 ± 4	0.9
HDL-C (mg/dl)	46.9 ± 9.8	57.8 ± 12.3	<0.001
TG (mg/dl)	138.6 ± 7	101.2 ± 5	0.008
HbA1c (%)	5.6 ± 0.3	5.5 ± 0.3	0.3
HOMA-IR	1.8 ± 1.5	1.5 ± 1.1	0.5
ESR (mm/hour)	24.5 ± 17.3	20.4 ± 11.5	0.5
CRP (mg/dl)	0.7 ± 0.9	0.3 ± 0.2	0.004
F_2 isoprostane (ng/ml)	250.4 ± 292.1	79.84 ± 89.2	<0.001

(*n* (%), mean±SD). M, male; F, female; BMI, body mass index; WC, Weight circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol, TG, triglyceride; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment-insulin resistance; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

of BD were associated with plasma F_2 isoprostane (data not shown).

Discussion

The results of the present study indicate that F_2 isoprostane concentrations are high in patients with BD compared to healthy controls, and this finding confirms the presence of marked oxidative stress with enhanced lipid peroxidation induced by chronic inflammatory processes in BD. To our knowledge, this is the first study to report a high concentration of F_2 isoprostane in patients with BD. Findings from Crocowski *et al.*[26] with a larger group of scleroderma spectrum patients that urinary F_2 isoprostane levels are twice as high in scleroderma patients as in controls support the present findings. Though the patients included in this study were in a different spectrum of scleroderma, urinary F_2 isoprostane did not significantly differ among patients. A study with a limited number of patients representing a wide spectrum of scleroderma and controls showed that concentrations of a metabolite of F_2 isoprostane in the urine are significantly higher in patients with scleroderma.[27] Volpe *et al.*[9] not only concluded that these findings support the role of oxidant stress in the pathogenesis of scleroderma but also concluded that oxidative stress is strongly correlated with the severity of lung involvement and nailfold videocapillaroscopic patterns. Some other studies evaluated serum F_2 isoprostane concentrations in different rheumatologic diseases, such as systemic lupus erythematosus, rheumatoid arthritis, psoriatic arthritis, reactive arthritis, and osteoarthritis and found them to be higher in patients with arthritic diseases than in healthy control subjects.[8,10] All of these studies evaluated different rheumatologic diseases with different severity and stages, but all concluded that F_2 isoprostane concentrations are significantly higher in patients than in healthy control groups.

Though Seyahi *et al.*[18] concluded that the traditional overall cardiometabolic risk factors are not a prominent feature in BD, in the present study, patients with BD had significantly higher FPG and TG and lower HDL-C. Various conflicting results are reported for cardiometabolic risk factors among BD patients with different disease features. Messedi *et al.*[19] concluded that morphologic evidence of subclinical atherosclerosis is present in BD patients. In one study, researchers found that, during an active disease period, microvascular functional impairment is more evident in BD patients than in patients with inactive disease.[21] Many cardiometabolic risk factors, such as hypertension, dyslipidemia, smoking, and obesity, are associated with increased oxidative stress.[28] Despite the presence of some of these risk factors, such as dyslipidemia and hyperglycemia, we observed no correlation between F_2 isoprostane concentrations and cardiometabolic risk factors in BD patients. For different rheumatologic diseases, F_2 isoprostane levels and the association of F_2 isoprostane with cardiovascular diseases have also been investigated. A cross-sectional study concluded that F_2 isoprostane excretion is higher in patients with rheumatoid arthritis than in control subjects and that, among patients with rheumatoid arthritis, higher F_2 isoprostane excretion is positively associated with the severity of coronary calcification.[22] In a group of patients comprising children and young adults with systemic lupus erythematosus, isoprostane levels did not differ from those of normal controls, despite the abundance of atherosclerotic risk factors.[23]

Oxidative stress, based on plasma F_2 isoprostane level, is increased in patients with BD compared to healthy control subjects. F_2 isoprostane levels are not correlated with any cardiometabolic risk factors or obesity indices in patients with BD. Therefore, the oxidative burden in patients with BD may not be related to cardiometabolic risk factors. The main role of F_2 isoprostane in the pathogenesis and therapeutic approach to BD should be considered in further studies.

The present study has several limitations. First, although 43 patients is a respectable size for a disease as rare as BD, greater statistical power could be obtained with a larger number of patients. The cross-sectional design of the study is also a limitation. In addition, active Behcet's disease patients were not included in the study and therefore these results cannot be extended to patients with active disease. Further, urinary F_2 isoprostane levels should be measured concomitantly with serum F_2 isoprostane or alone. Some authors state that urinary F_2 isoprostane indicates endogenous lipid peroxidation, whereas others use serum and urinary F_2 isoprostane to evaluate oxidative injury.[8,29] Finally, other markers of oxidative stress were not measured.

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Disclaimer statements

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References

1 Onder M, Gürer MA. The multiple faces of Behçet's disease and its aetiological factors. J Eur Acad Dermatol Venereol 2001;15: 126–36.
2 Niwa Y, Miyake S, Sakane T, Shingu M, Yokoyama M. Auto-oxidative damage in Behçet's disease–endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. Clin Exp Immunol 1982;49:247–55.
3 Niwa Y, Mizushima Y. Neutrophil-potentiating factors released from stimulated lymphocytes; special reference to the increase in neutrophil-potentiating factors from streptococcus-stimulated lymphocytes of patients with Behçet's disease. Clin Exp Immunol 1990;79:353–60.
4 Pronai L, Ichikawa Y, Nakazawa H, Arimori S. Enhanced superoxide generation and the decreased superoxide scavenging activity of peripheral blood leukocytes in Behçet's disease–effects of colchicine. Clin Exp Rheumatol 1991;9:227–33.
5 Bozkurt M, Yüksel H, Em S, Oktayoglu P, Yildiz M, Akdeniz D, *et al.* Serum prolidase enzyme activity and oxidative status in patients with Behçet's disease. Redox Rep 2014;19(2):59–64.
6 Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJII. A series of prostaglandin F_2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87:9383–7.
7 Moore K, Roberts LJII. Measurement of lipid peroxidation. Free Radic Res 1998;28:659–71.
8 Basu S, Whiteman M, Mattey DL, Halliwell B. Raised levels of F(2)-isoprostanes and prostaglandin F(2alpha) in different rheumatic diseases. Ann Rheum Dis 2001;60:627–31.
9 Volpe A, Biasi D, Caramaschi P, Mantovani W, Bambara LM, Canestrini S. Levels of F_2-isoprostanes in systemic sclerosis: correlation with clinical features. Rheumatology (Oxford) 2006;45: 314–20.
10 Abou-Raya A, el-Hallous D, Fayed H. 8-Isoprostaglandin F_2 alpha: a potential index of lipid peroxidation in systemic lupus erythematosus. Clin Invest Med 2004;27:306–11.
11 Isik A, Koca SS, Ustundag B, Selek S. Decreased total antioxidant response and increased oxidative stress in Behcet's disease. Tohoku J Exp Med 2007;212:133–4.
12 Yazici C, Köse K, Caliş M, Demir M, Kirnap M, Ateş F. Increased advanced oxidation protein products in Behçet's disease: a new activity marker? Br J Dermatol 2004;151:105–11.
13 Akcay YD, Sagin FG, Aksu K, Keser G, Taylor E, Knight I, *et al.* A panel of oxidative stress assays does not provide supplementary diagnostic information in Behcet's disease patients. J Inflamm (Lond) 2012;9:13.
14 Hamilton CA, Miller WH, Al-Benna S, Brosnan MJ, Drummond RD, McBride MW, *et al.* Strategies to reduce oxidative stress in cardiovascular disease. Clin Sci 2004;106:219–34.
15 Kim JY, Hyun YJ, Jang Y, Lee BK, Chae JS, Kim SE, *et al.* Lipoprotein-associated phospholipase A2 activity is associated with coronary artery disease and markers of oxidative stress: a case-control study. Am J Clin Nutr 2008;88:630–7.
16 Gross M, Steffes M, Jacobs DRJr. Yu X, Lewis L, Lewis CE, *et al.* Plasma F_2-isoprostanes and coronary artery calcification: the CARDIA Study. Clin Chem 2005;51:125–31.
17 Alkazemi D, Egeland GM, Roberts LJII, Kubow S. Isoprostanes and isofurans as non-traditional risk factors for cardiovascular disease among Canadian Inuit. Free Radic Res 2012;46: 1258–66.
18 Seyahi E, Ugurlu S, Cumali R, Balci H, Ozdemir O, Melikoglu M, *et al.* Atherosclerosis in Behçet's Syndrome. Semin Arthritis Rheum 2008;38:1–12.
19 Messedi M, Frigui M, Ben Mahfoudh K, Feki H, Ben Mahfoudh ST, Mnif J, *et al.* Intima-media thickness of carotid artery in patients with Behçet's disease. Arch Med Res 2011; 42:398–404.
20 Acikgoz N, Ermiş N, Yağmur J, Cansel M, Karincaoğlu Y, Ataş H, *et al.* Elevated oxidative stress markers and its relationship with endothelial dysfunction in Behçet disease. Angiology 2011;62:296–300.
21 Gullu H, Caliskan M, Erdogan D, Yilmaz S, Dursun R, Ciftci O, *et al.* Patients with Behcet's disease carry a higher risk for microvascular involvement in active disease period. Ann Med 2007;39:154–9.
22 Rho YH, Chung CP, Oeser A, Solus JF, Gebretsadik T, Shintani A, *et al.* Interaction between oxidative stress and high-density lipoprotein cholesterol is associated with severity of coronary artery calcification in rheumatoid arthritis. Arthritis Care Res (Hoboken) 2010;62:1473–80.
23 Soep JB, Mietus-Snyder M, Malloy MJ, Witztum JL, von Scheven E. Assessment of atherosclerotic risk factors and endothelial function in children and young adults with pediatric-onset systemic lupus erythematosus. Arthritis Rheum 2004;51(3): 451–7.
24 International Study for Behcet's Disease. Criteria for diagnosis of Behcet's disease. Lancet 1990;335:1078–80.
25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC, *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
26 Cracowski JL, Marpeau C, Carpentier PH, Imbert B, Hunt M, Stanke-Labesque F, *et al.* Enhanced in vivo lipid peroxidation in scleroderma spectrum disorders. Arthritis Rheum 2001;44: 1143–8.
27 Stein CM, Tanner SB, Awad JA, Roberts LJII, Morrow JD. Evidence of free radical-mediated injury (isoprostane overproduction) in scleroderma. Arthritis Rheum 1996;39:1146–50.
28 Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J, *et al.* Urinary 8-isoprostaglandin F_2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. Circulation 2004;109:843–8.
29 Richelle M, Turini ME, Guidoux R, Tavazzi I, Métairon S, Fay LB. Urinary isoprostane excretion is not confounded by the lipid content of the diet. FEBS Lett 1999;459:259–62.