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ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 305–313
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Enhanced levels of soluble CD40 ligand and C-reactive protein in a total of 312 patients with metabolic syndrome

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Received 26 December 2008; accepted 23 April 2009

Abstract

The metabolic syndrome (MS) is associated with a systemic inflammatory response that plays an important pathogenetic role in atherothrombotic disease. Increasing evidence indicates that CD40-CD40 ligand interactions constitute an important mediator for vascular inflammation. The purpose of this study was to assess whether high-sensitivity C-reactive protein (hs-CRP) and soluble CD40 ligand (sCD40L) levels were increased in patients with MS. During the study period from January 2004 to August 2004, 312 patients with MS and 98 control subjects were included. Anthropometric measurements, blood pressure assessment, electrocardiography, and blood measurements including fasting blood glucose, postprandial blood glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, glycated hemoglobin, white blood cell (WBC), platelets, hs-CRP, and sCD40L were performed. Patients with MS were divided into 3 groups based upon their glucose tolerance (group 1, normal glucose tolerance; group 2, prediabetic group; and group 3, diabetes mellitus). Patients with MS showed a significant increase of WBC, hs-CRP, and sCD40L levels compared with control subjects. The levels of both hs-CRP and sCD40L were positively correlated with body mass index (BMI). High-sensitivity CRP levels were also positively correlated with waist circumferences, fasting blood glucose, postprandial blood glucose, and glycated hemoglobin, and negatively correlated with high-density lipoprotein cholesterol. In patients with MS, both hs-CRP and sCD40L levels were positively correlated with WBC count. We found a positive correlation between sCD40L and platelets. Among the subgroups of patients with MS, the mean levels of WBC, hs-CRP, and sCD40L did not show any significant differences. In conclusion, elevated levels of WBC, hs-CRP, and sCD40L in MS patients provide further insight into the relationship between MS and inflammation. In our study, positive correlations between BMI and both hs-CRP and sCD40L levels suggest that BMI is an important determinant of a chronic inflammatory state in patients with MS. Moreover, this study reports significantly increased levels of WBC, hs-CRP, and sCD40L not only in diabetic subjects with MS but also in prediabetic subjects and nondiabetic subjects with MS compared with control subjects. Our data suggest that MS patients have proinflammatory state independent of their glucose tolerance status. In our study, the positive correlation between the levels of sCD40L and platelets in patients with MS supports previous reports indicating that sCD40L are derived predominantly from platelets.

1. Introduction

The metabolic syndrome (MS), also known as the *insulin resistance syndrome*, is associated with increased risk for cardiovascular disease (CVD); and the risk is greater than the risk associated with any of the individual components [1-3]. The recently released "Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults" (NCEP Adult Treatment Panel III [ATP-III]) stresses the importance of targeting prevention strategies for such individuals. The ATP-III guideline also suggests a working

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0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.04.034

definition of the MS that includes the presence of at least 3 of the following characteristics: abdominal obesity, elevated triglycerides (TG), reduced levels of high-density lipoprotein cholesterol (HDL-C), high blood pressure, and high fasting glucose (FG) [4].

Insulin resistance is the underlying metabolic disturbance in MS [1-3]. Although both hereditary and environmental factors contribute to the development of the insulin resistance, little is known about the underlying pathogenetic mechanisms [5-7]. Insulin resistance is increasingly recognized as a chronic, low-level, inflammatory state [8]. Several mechanisms may explain the relation between chronic inflammation and insulin resistance. These include hypersecretion of proinflammatory cytokines from adipose tissue, which exert major stimulatory effects on the synthesis of acute-phase proteins. In addition, enhanced expression of inflammatory proteins may occur by counteracting the physiologic effect of insulin on hepatic acute-phase protein synthesis as a result of decreased insulin sensitivity [9]. Serum high-sensitivity C-reactive protein (hs-CRP) is the principal mediator of the acute-phase response. High-sensitivity CRP levels are elevated in many inflammatory disorders and have been used to predict clinical outcomes. High-sensitivity CRP is not only a marker of inflammation, but also an amplifier of it. A number of epidemiologic studies have shown that CRP is an important risk factor for atherosclerosis and coronary heart disease (CHD) [10-15]. Ridker et al [16] reported that measurement of CRP adds clinically important prognostic information concerning future vascular risk. In literature, a positive correlation between the level of CRP and all of the components of the MS was reported [16,17]. Besides the classic inflammatory markers, CD40 ligand (CD40L), being a transmembrane protein and member of tumor necrosis family, was introduced as a new inflammatory marker. It has been identified on T-helper cells, platelets, and vascular smooth muscle cells [18]. Studies on the cellular distribution of CD40L indicate that more than 95% of the circulating CD40L exist in platelets. Platelets express CD40L on their surface upon stimulation; CD40L is then cleaved and circulates as soluble CD40L (sCD40L). When expressed on the surface of platelets and exposed to CD40-bearing vascular cells, platelet-associated CD40L is capable of initiating various inflammatory responses [19-21]. Increasing evidence shows that CD40-CD40L interaction plays a crucial role in the pathogenesis of atherosclerosis and CHD [19,21,22]. Atherosclerosis and insulin resistance share a common inflammatory basis [8]. In literature, enhanced levels of CD40L were reported in patients with hypercholesterolemia, obesity, diabetes mellitus (DM) [23-30], and acute coronary syndromes [31-34]. There is evidence that the sCD40L level is a strong predictor of cardiovascular risk [35].

Few studies have reported the relationship between MS and sCD40L [36-39]. Therefore, this study was conducted to assess whether sCD40L levels were increased in patients with MS. To our knowledge, this study represents the investigation of hs-CRP and sCD40L levels in the largest series of patients with MS.

2. Methods

2.1. Patients and controls

Patients were recruited from the Department of Internal Medicine at the Dokuz Eylul University Faculty of Medicine Hospital. During the study period from January 2004 to August 2004, 312 patients with the diagnosis of MS were included. A total of 98 individuals participated in this study as control subjects. All subjects gave written informed consent, and the study protocol was approved by the Local Ethical Committee of Dokuz Eylul University. A standardized health questionnaire was completed by a physician covering the subjects' medical history and including current medication and information about other diseases (particularly hypertension, CHD, and myocardial infarction). *Coronary heart disease* was defined as using nitroglycerine, experiencing typical chest pain, or having a history of previous myocardial infarction. This information was validated against electrocardiogram changes compatible with ischemic heart disease. All subjects underwent anthropometric evaluation. Height, weight, and waist and hip circumferences were recorded with subjects wearing light clothing and without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Waist circumference was measured at the natural indentation between the 10th rib and the iliac crest (minimum waist). Hip circumference was measured over the widest part of the gluteal region, and the waist-to-hip ratio (WHR) was calculated as a measure of central obesity. Arterial blood pressure was measured on the right arm with the subjects in a sitting position and after a 5-minute rest, using a mercury sphygmomanometer.

2.2. Definition

Patients with 3 or more of the following attributes are typically defined as having the MS based on the NCEP ATP III guidelines [4]: (1) TG of at least 150 mg/dL, (2) HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women, (3) systolic blood pressure (SBP) of at least 130 mm Hg or diastolic blood pressure (DBP) of at least 85 mm Hg, (4) *abdominal obesity* as defined by a waist circumference greater than 88 cm (35 in) in women or greater than 102 cm (40 in) in men, and (5) *abnormal glucose metabolism* as defined by an FG of at least 110 mg/dL. Those without these features or patients who have only hypertension were designated as control subjects. Diabetes mellitus, impaired glucose tolerance (IGT), and impaired FG were diagnosed according to the World Health Organization criteria [40]. *Fasting* was defined as no caloric intake for at least

Table 1
Characteristics of study participants

	No MS	MS	*P*
	n = 98	n = 312	
Sex (M/F)	21/77	118/194	**.003**[a]
Age (y)	52.04 ± 10.31	55.13 ± 9.93	**.008**
Smoking	27	57	.226[a]
CVD	0	63	**.000**[a]
HT, n (%)	36 (36.7)	286 (92)	**.000**[a]
BMI (kg/m^2)	25.45 ± 3.16	30.86 ± 5.47	**.000**
Female	25.34 ± 3.35	32.05 ± 5.45	**.000**
Male	25.85 ± 2.33	29.13 ± 4.17	**.000**[b]
Waist (cm)			
Female	79.14 ± 6.50	96.67 ± 10.13	**.000**
Male	91.57 ± 6.35	101.43 ± 9.32	**.000**
WHR	0.82 ± 0.08	0.90 ± 0.07	**.000**
SBP (mm Hg)	120.40 ± 13.29	129.34 ± 15.49	**.000**
DBP (mm Hg)	77.19 ± 9.16	80.38 ± 8.81	**.002**
FG (mg/dL)	90.05 ± 6.81	122.58 ± 39.27	**.000**
PPG (mg/dL)	98.56 ± 20.86	156.86 ± 68.96	**.000**
TC (mg/dL)	193.73 ± 31.34	196.06 ± 38.77	.590
TG (mg/dL)	90.77 ± 24.34	176.20 ± 102.06	**.000**
LDL-C (mg/dL)	112.94 ± 25.55	114.82 ± 32.87	.556
HDL-C (mg/dL)	64.43 ± 14.00	47.56 ± 11.80	**.000**
Female	66.68 ± 11.81	49.85 ± 12.04	**.000**
Male	56.19 ± 18.14	43.80 ± 10.41	**.000**[b]
HbA_{1c} (%)	5.50 ± 0.49	6.79 ± 1.60	**.000**
WBC (×1000/mm^3)	6.54 ± 1.51	7.90 ± 2.22	**.000**
Platelets	262.88 ± 60.30	270.13 ± 77.64	.364
Use of ACEIs/ARBs	19	172	**.000**[a]
Use of ASA	11	129	**.000**[a]
hs-CRP (mg/L)	2.03 ± 1.88	4.48 ± 4.92	**.000**
sCD40L (ng/mL)	0.44 ± 0.42	0.86 ± 0.73	**.000**

Data are mean ± SD. HT indicates hypertension.
[a] χ^2.
[b] Mann-Whitney *U*.

8 hours. *Postprandial glucose* (PPG) was defined as blood glucose measurement 2 hours after a standard meal. Subjects taking insulin or oral antidiabetic drugs were considered to have diabetes. The major exclusion criteria were as follows: asthma/chronic obstructive pulmonary disease, chronic congestive heart failure, rheumatologic disease, renal or hepatic dysfunction, cancer, and use of antiinflammatory therapy or immunosuppressants.

2.3. Blood sampling and assay

Subjects underwent complete blood count and routine biochemical evaluations including FG, PPG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, TG, and glycated hemoglobin (HbA_{1c}). All blood analyses were performed in a central laboratory. Triglycerides, TC, and HDL-C were measured on Roche Diagnostics Modular Analytics-DP analyzer (Roche Diagnostics, Tokyo, Japan) with the dedicated kits (Roche Diagnostics, Mannheim, Germany). The LDL-C concentrations were estimated according to Friedewald formula at concentrations of TG less than 400 mg/dL. The direct LDL-C analysis was performed on a Roche Diagnostics Modular Analytics-DP analyzer at concentrations of TG of at least 400 mg/dL. Glycated hemoglobin was measured on a Cobas Integra 400 Plus analyzer with a dedicated kit (Tina-quant Hemoglobin A_{1c} Gen 2; Roche Diagnostics, Mannheim, Germany). Fasting venous blood samples (10 mL) were drawn from the antecubital vein into pyrogen-free blood collection tubes without any additives. All blood samples were collected under minimal tourniquet pressure. Blood samples were allowed to clot for 15 to 30 minutes and were centrifuged at 1500*g* and 4°C for 10 minutes. The plasma was then separated and stored at under −20°C until analysis. Samples were thawed only once. Serum sCD40L concentrations were determined using an enzyme-linked immunosorbent assay kit from Biosource (Bender MedSystems Human sCD40L Instant ELISA). The intraassay and interassay coefficients of variation for sCD40L were 5.00% and 6.20%, respectively, with a sensitivity of 0.062 ng/mL, according to the manufacturer. The hs-CRP analysis was performed on a Cobas Integra 400 Plus analyzer (Roche Diagnostics, Rotkreuz, Switzerland) based on particle-enhanced turbidimetry (CRPLX; Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.085 mg/L and an extended measuring range of 0.085 to 1600 mg/L (with auto rerun) according to the manufacturer.

2.4. Statistical analysis

All statistical analyses were performed with an SPSS program for Windows (version 10.0; SPSS, Chicago, IL). Means and proportions for baseline variables were compared between cases and controls using Student *t* test, correlation test, analysis of variance for continuous variables, and nonparametric Kruskall-Wallis, Mann-Whitney *U* test when appropriate. Differences in categorical variables were measured by χ^2 test.

Table 2
Pearson correlation test between hs-CRP/sCD40L and all other parameters evaluated in MS patients

Variable	hs-CRP		sCD40L	
	r	*P*	*r*	*P*
Age (y)	0.00	.932	−0.04	.414
BMI (kg/m^2)	0.23	**.000**	0.14	**.017**
Waist (cm)	0.19	**.001**	0.06	.296
WHR	−0.03	.599	−0.05	.415
SBP (mm Hg)	0.03	.658	0.02	.737
DBP (mm Hg)	0.005	.402	−0.05	.345
FG (mg/dL)	0.13	**.026**	0.04	.523
PPG (mg/dL)	0.14	**.018**	0.05	.362
TC (mg/dL)	0.02	.688	0.04	.529
TG (mg/dL)	0.09	.127	−0.30	.641
LDL-C (mg/dL)	0.03	.573	0.04	.462
HDL-C (mg/dL)	−0.14	**.016**	0.00	.963
HbA_{1c} (%)	0.20	**.001**	0.02	.695
WBC (×1000/mm^3)	0.15	**.011**	0.13	**.034**
Platelets	0.09	.099	0.19	**.001**

Table 3
Stepwise multiple regression analysis with hs-CRP and sCD40L as the dependent variable in patients with MS

Variable	*B*	SE	Coefficient β	*P*
hs-CRP				
Constant	−7.95	2.26		.001
BMI	0.23	0.05	0.24	.000
HbA_{1c}	0.78	0.18	0.25	.000
sCD40L				
Constant	0.37	0.15		.016
Platelets	1.847E-0.3	0.00	0.19	.001

All data of continuous variables were expressed as mean ± SD; *P* values of .05 or less were considered to be statistically significant.

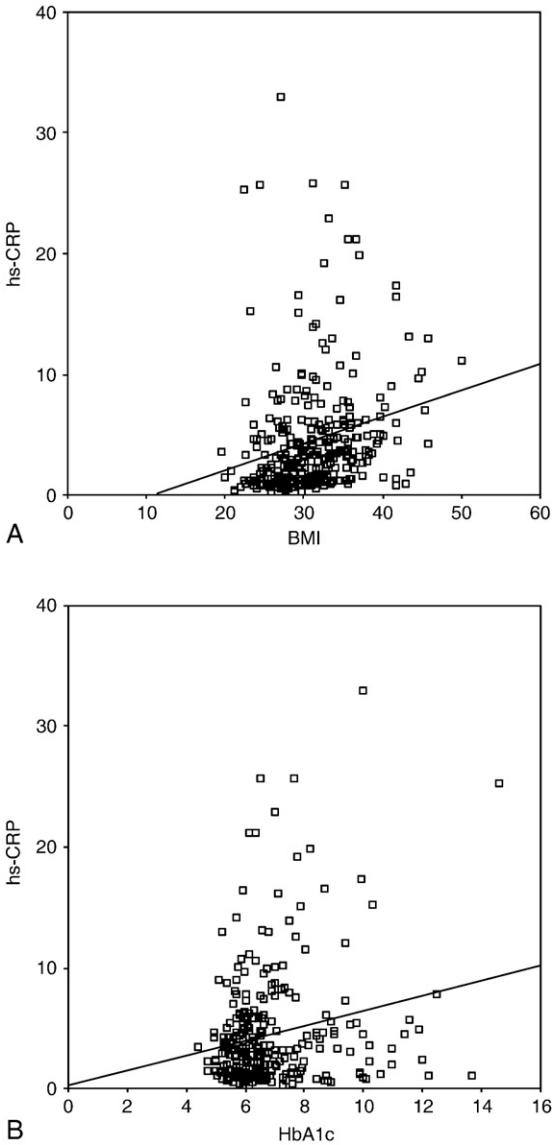

Fig. 1. A, Positive correlation between hs-CRP and BMI in MS patients. B, Positive correlation between hs-CRP and HbA_{1c} in MS patients.

3. Results

The clinical and biochemical characteristics of the 410 subjects, aged 27 to 81 years, with and without MS, are summarized in Table 1. The hs-CRP and sCD40L concentrations were statistically significantly higher in patients with MS compared with control subjects ($P = .000$ and $P = .000$, respectively). The analysis of leukocyte count disclosed a statistically significant difference between patients with MS and controls ($P = .000$). The rate of male patients was significantly higher in the MS group than the control group, and patients with MS were older than controls. The number of patients with CVD was statistically significantly higher in the MS group than the control group ($P = .000$). As expected, the values of BMI, waist circumferences, WHR, SBP, DBP, FG, PPG, TG, and HbA_{1c} were significantly higher and the levels of HDL-C were significantly lower in MS patients compared with controls (Table 1).

We observed no association of hs-CRP ($P = .890$) and sCD40L ($P = .440$) with sex in controls. However, women with MS had statistically significantly higher values of hs-CRP (5.08 ± 4.95 vs 3.50 ± 4.74, $P = .006$) than men with MS. In addition, women with MS had higher values of sCD40L than men; but the difference did not reach statistical significance (0.92 ± 0.79 vs 0.77 ± 0.60, $P = .101$). In the MS group, both hs-CRP and sCD40L concentrations were not significantly different ($P > .05$) in patients with CVD compared with patients without CVD (4.35 ± 5.9 vs 4.51 ± 4.65 mg/L and 0.82 ± 0.70 vs 0.87 ± 0.73 ng/mL, respectively). Similarly, both hs-CRP and sCD40L concentrations were not significantly different ($P > .05$) in smoking patients with MS compared with nonsmoking patients with MS (4.56 ± 4.07 vs 4.01 ± 4.58 mg/L and 0.82 ± 0.55 vs 0.86 ± 0.78 ng/mL, respectively).

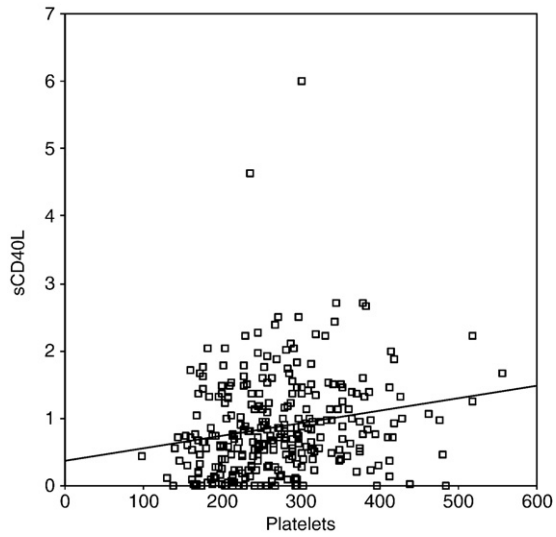

Fig. 2. Positive correlation between sCD40L and platelets in MS patients.

Table 4
The mean levels of hs-CRP and sCD40L according to medications

Medications	hs-CRP	*P*	sCD40L	*P*
ASA				
Yes	4.51 ± 5.70		0.85 ± 0.66	
No	4.48 ± 4.31	.955	0.87 ± 0.77	.880
Lipid-lowering therapy				
Yes	4.52 ± 5.35		0.84 ± 0.66	
No	4.44 ± 4.46	.886	0.88 ± 0.79	.641
ACEIs/ARBs				
Yes	4.59 ± 4.56		0.96 ± 0.82	
No	4.08 ± 4.65	.590	0.77 ± 0.50	.245
Antidiabetics				
Yes	4.71 ± 5.49		0.89 ± 0.66	
No	4.31 ± 4.46	.474	0.84 ± 0.77	.621

Data are mean ± SD.

We observed no association of hs-CRP (r = 0.09, P = .346) and sCD40L (r = −0.06, P = .65) with age in controls. Similar findings were observed in subjects with MS (Table 2). In MS patients, hs-CRP levels were positively correlated with BMI, waist circumferences, FG, PPG, HbA_{1c}, and WBC, and negatively correlated with HDL-C. Similarly, in MS patients, sCD40L levels were positively correlated with BMI, WBC, and platelets (Table 2). To further define the relationship between MS and both hs-CRP and sCD40L, stepwise multiple linear regression analysis was determined. In this analysis, hs-CRP was correlated with BMI and HbA_{1c} (Table 3; Fig. 1A, B); and sCD40L was correlated with platelets (Table 3, Fig. 2). However, we observed no association of sCD40L (r = 0.05, P = .687) with platelets in the control group. We investigated the influence of acetylsalicylic acid (ASA), angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor–I blocking agents (ARBs), lipid-lowering therapy, and antidiabetics on hs-CRP and sCD40L concentration. No significant difference was found in the levels of hs-CRP and sCD40L among MS patients with or without these medications (Table 4).

Patients with MS were divided into 3 groups according to their glucose tolerance (group 1, normal glucose tolerance; group 2, prediabetic group; and group 3: type 2 DM). The prediabetic group was composed of patients with IGT and impaired FG. Among the subgroups of patients with MS, the mean levels of hs-CRP and sCD40L were not statistically significantly different (Table 5). As shown in Table 5, the mean age was significantly higher in the diabetic group than the controls and group 1 (P < .05). The patients in groups 1, 2, and 3 were significantly heavier than the controls (P < .05). Furthermore, we found that waist circumferences, SBP, DBP, TG, and HDL-C were not significantly different among the subgroups of MS; and these variables were statistically significantly higher compared with controls except for HDL-C, which was statistically significantly lower compared with controls (P < .05). Waist-to-hip ratio of the diabetic patients was statistically significantly higher than that of the patients in group 1 and the controls (P < .05); however, WHR of the diabetic patients was similar with that of the patients in group 2. As expected, the diabetic group showed higher FG, PPG, and HbA_{1c} compared with the other 3 groups (P = .000). In addition, the patients in group 2 had significantly higher levels of FG and PPG compared with patients in group 1 (P = .002 and P = .001, respectively) and controls (P = .000 and P = .000, respectively). In the subgroups of MS and the control group, platelet levels were similar, whereas leukocyte count was significantly lower (P < .05) in the control group compared with subgroups of the MS. Leukocyte count did

Table 5
Clinical and biochemical characteristics of the patients according to glucose tolerance status

	Controls (n = 98)	Subgroups of MS			*P*
		Group 1 (NGT; n = 89)	Group 2 (PDG; n = 61)	Group 3 (DM; n = 162)	
Age (y)	52.04 ± 10.31	51.44 ± 9.10	55.67 ± 9.88	56.89 ± 9.95	**.000**
BMI (kg/m^2)	25.45 ± 3.16	32.05 ± 5.43	31.40 ± 4.26	30.14 ± 5.28	**.000**
Waist (cm)					
Female	79.14 ± 6.50	96.12 ± 9.41	96.67 ± 10.34	96.99 ± 10.71	**.000**
Male	91.57 ± 6.35	104.76 ± 9.38	103.38 ± 7.92	100.09 ± 9.46	**.000**[a]
WHR	0.82 ± 0.08	0.87 ± 0.08	0.88 ± 0.06	0.91 ± 0.07	**.000**
SBP (mm Hg)	120.40 ± 13.29	127.02 ± 13.56	130.81 ± 12.32	129.87 ± 17.48	**.000**
DBP (mm Hg)	77.19 ± 9.16	81.62 ± 8.14	80.00 ± 8.26	79.72 ± 9.43	**.009**
FG (mg/dL)	90.05 ± 6.81	93.10 ± 7.59	110.27 ± 10.75	143.37 ± 43.80	**.000**
PPG (mg/dL)	98.56 ± 20.86	106.01 ± 18.31	139.19 ± 30.47	189.98 ± 76.95	**.000**
HbA_{1c} (%)	5.50 ± 0.49	5.68 ± 0.45	6.06 ± 0.49	7.55 ± 1.76	**.000**
TG (mg/dL)	90.77 ± 24.34	187.42 ± 117.88	180.14 ± 107.27	168.0 ± 90.02	**.000**
HDL	64.43 ± 14.00	49.03 ± 13.19	47.86 ± 12.76	46.60 ± 10.54	**.000**
WBC (×1000/mm^3)	6.54 ± 1.51	7.68 ± 2.07	7.60 ± 1.85	8.15 ± 2.41	**.000**
Platelets	262.88 ± 60.30	278.60 ± 73.25	271.18 ± 80.00	264.94 ± 78.84	.487
hs-CRP (mg/L)	2.03 ± 1.88	3,94 ± 3.51	3.67 ± 4.00	5,10 ± 5.76	**.000**
sCD40 (ng/mL)	0.44 ± 0.42	0,86 ± 0,90	0.86 ± 0.66	0,86 ± 0,65	**.000**

Data are mean ± SD. NGT indicates normal glucose tolerance; PDG, prediabetic group.
[a] Kruskal-Wallis.

Table 6
The mean hs-CRP and sCD40L levels of the patients according to their total number of components of the MS

	Controls (n = 98)	MS			P
		3 Components (n = 196)	4 Components (n = 74)	5 Components (n = 42)	
hs-CRP (mg/L)	2.03 ± 1.88	3.80 ± 4.46†	5.64 ± 5.60*, §	5.55 ± 5.25*	.000
sCD40 (ng/mL)	0.44 ± 0.42	0.84 ± 0.77*	0.82 ± 0.64‡	1.02 ± 0.65*	.000

* $P = .000$ compared with controls.
† $P = .006$ compared with controls.
‡ $P = .008$ compared with controls.
§ $P = .012$ compared with subjects with 3 components.

not show any significant differences between groups 1, 2, and 3 (Table 5).

Table 6 displays the distribution of mean hs-CRP and sCD40L levels after patients were classified according to their total number of components of the MS. As shown, mean hs-CRP and sCD40L levels of those with 3, 4, and 5 characteristics of the MS were statistically significantly higher than the control subjects. Soluble CD40L levels were highest in subjects with 5 components of MS followed by subjects with 3 and 4 components of MS, but the differences did not reach statistical significance (Table 6).

Correlation between hs-CRP and sCD40L was determined by Pearson correlation test, and no correlation was found ($r = 0.02$, $P = .705$).

4. Discussion

Recent guidelines stress the importance of identifying individuals with the MS as a high-risk group for the development of CVD [4]. It has been reported that obesity, insulin resistance, and atherosclerosis are closely related phenomena in which low-grade inflammatory state and prothrombotic condition have pivotal roles [41]. In the present study, we have confirmed significantly higher levels of hs-CRP and sCD40L in patients with MS compared with controls. In addition, we have demonstrated positive correlations between BMI and both hs-CRP and sCD40L levels in MS patients. This finding, suggesting that obesity may promote CD40L overexpression, is consistent with the previous study demonstrating that sCD40L plasma values are elevated in obese men and decrease concomitantly with BMI reduction [23]. In addition, Schernthaner et al [29] have shown a marked decrease in circulating sCD40L after weight loss in morbidly obese patients. Previous studies have shown increased levels of CRP in patients with insulin resistance, atherosclerosis, and obesity [41,42]. In addition, decrease in the levels of CRP after weight loss was reported in these patients [9,15,43,44]. In our study, positive correlations between BMI and both hs-CRP and sCD40L levels suggest that BMI is an important determinant of a chronic inflammatory state in patients with MS.

We observed statistically significantly higher levels of WBC, which is another marker of inflammation, in MS patients compared with controls ($P = .000$). In patients with MS, both hs-CRP and sCD40L levels were closely related to WBC ($P = .011$ and $P = .034$; respectively) (Table 2). Our data support the opinion that MS is a chronic subclinical inflammatory disease as reflected by levels of WBC, hs-CRP, and sCD40L [8,36,41].

Although both men and women with MS had elevated hs-CRP and sCD40L in our study, women had higher values than men. This finding is in accordance with a recent study by Varo et al [28] who found that women with type 2 DM had higher values of sCD40L than men. Furthermore, Angelico et al [36] reported that women with MS had higher values of sCD40L than men with MS. The mechanism through which women with MS have higher values of hs-CRP and sCD40L than men is unknown and deserves further investigation. We thought that the sex difference in the plasma levels of hs-CRP and sCD40L in this study arises from the sex difference in the levels of BMI. Indeed, women were more obese than men in our study (Table 1, $P = .000$).

In our study, there was a statistically significant positive correlation between hs-CRP and BMI, waist circumference, FG, PPG, and HbA_{1c}, and negative correlation with HDL-C (Tables 2 and 3; Fig. 1A, B). In literature, CRP levels have been found to be positively correlated with BMI, TG, TC, LDL-C, insulin, FG, history of diabetes, age, smoking, blood pressure, waist and hip circumferences, and WHR, and negatively correlated with HDL-C [9,10,15,17,30,41-47]. In this study, our results were in accordance to previous reports. Herein, the positive correlation between the waist circumferences and hs-CRP shows the association between visceral abdominal obesity and chronic inflammation [46,48,49]. In literature, it is reported that visceral adipose tissue alone is a strong correlate of insulin resistance; and it is associated with increased cardiovascular morbidity and mortality [46,48-51].

Circulating sCD40L was believed to derive predominantly from platelets [20]. Consistent with this study, we found a significant correlation between the levels of sCD40L and platelets in patients with MS. In addition, Cipollone et al [25] showed an association between enhanced sCD40L and platelet activation in patients with hypercholesterolemia. In our study, the positive correlation between sCD40L and BMI supports the previous study by Davi et al [46] who demonstrated increased lipid peroxidation and platelet activation in obese women. The mechanism accounting for

CD40L expression by activated platelets is still unclear, but a recent study provided the first evidence that platelet O^{-}_{2} production plays a key role in CD40L expression [52].

Consistent with previous reports, no significant association was found between the degree of diabetic control (HbA_{1c}) and the levels of sCD40L [28,53]. However, in a study by Jinchuan et al [27], a positive correlation was found between sCD40L and HbA_{1c}.

Although both sCD40L and CRP are inflammatory markers, we did not detect any association between the 2 proinflammatory markers in this study. Azar et al [54] found no correlation between hs-CRP and sCD40L levels in patients with coronary artery disease. Furthermore, Guldiken et al [30] found no correlation between hs-CRP and sCD40L levels in patients with different degrees of BMI.

In a study by Gokulakrishnan et al [37], diabetic subjects with MS had statistically significantly higher levels of sCD40L compared with nondiabetic subjects with MS. In contrast to this study, we found no statistically significant difference in the mean levels of WBC, hs-CRP, and sCD40L among the subgroups of MS according to glucose tolerance status. The atherosclerotic risk factors start operating even at the stage of prediabetes [9]. Choi et al [55] documented higher serum hs-CRP and WBC concentrations in subjects with IGT, indicating altered inflammatory markers in prediabetic stages. In this regard, this study is of interest because it reports increased levels of WBC, hs-CRP, and sCD40L not only in diabetic subjects with MS but also in prediabetic subjects and nondiabetic subjects with MS. This study suggests that MS patients have proinflammatory state independent of their glucose tolerance status.

In literature, a positive and statistically highly significant trend in CRP levels was observed with increasing number of components of the MS [16,17]. In this study, we could not find a gradual increase of the hs-CRP and sCD40L levels with the number of components of the MS probably because of the effect of medications in these patients. However, the present data demonstrate that, at all levels of severity of MS, patients have higher levels of inflammatory markers than the healthy controls.

The results of the previous studies indicated that blood pressure was directly related to both insulin resistance and insulin concentration [56,57]. Renin-angiotensin system may contribute to inflammatory process within the vascular wall and to the development of acute coronary syndromes [58]. In this study, we found no association between the levels of blood pressure and hs-CRP/sCD40L probably because of antihypertensive therapy of these patients. Because some medications have demonstrated to down-regulate hs-CRP and sCD40L levels, we investigated the effect of ASA, lipid-lowering therapy, ACEIs/ARBs, and antidiabetics on hs-CRP and sCD40L concentration in patients with MS. Recent studies have demonstrated that ACEIs and ARBs have anti-inflammatory properties [59,60] and that they cause a significant reduction in the levels of CRP and sCD40L [61-64]. Acetylsalicylic acid has been attributed to reducing levels of the CRP and sCD40L, although the evidence relating to the 2 markers is conflicting [31,65-67]. Beside the lipid-lowering effect, statins and fenofibrates seem to slow the progression of atherosclerosis through a series of anti-inflammatory effects, including a reduction of sCD40L and CRP [26,61-63,68-71]. In this study, we observed no significant association of hs-CRP and sCD40L with the use of ASA, lipid-lowering therapy, ACEIs/ARBs, or antidiabetics. However, the cross-sectional nature of our study did not allow us any interpretation about a drug effect.

5. Conclusion

We have demonstrated a significant increase of WBC, hs-CRP, and sCD40L levels in patients with MS compared with healthy controls, supporting the underlying inflammatory state in these patients. Identification of the MS is very important in the risk assessment and treatment of the patients. The effective administration of anti-inflammatory agents is only the beginning of a promising approach in the management of this syndrome. Therapeutic modalities that down-regulate CD40-CD40L interaction may represent a new therapeutic approach in these patients.

Acknowledgment

Project supported by the Dokuz Eylul University Research Foundation Accountancy (no. 04.KB.SAĞ.095).

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