The effect of statin treatment on inflammation in patients with metabolic syndrome

Metabolik sendromlu hastalarda statin tedavisinin inflamasyon üzerine etkisi

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Objective: Inflammation plays an important role in the pathogenesis of metabolic syndrome (MS). We investigated the effect of fluvastatin treatment on inflammatory markers in patients with MS.

Study design: The study included 47 patients (36 female, 11 male; mean age 55±8) with MS. The diagnosis of MS was based on the presence of at least three criteria of the NCEP ATP III guidelines. All the patients received 80 mg fluvastatin treatment for six weeks. Laboratory parameters were measured before and after treatment, and flow cytometric analysis of peripheral blood leukocytes was performed. The results were compared with those of 47 age- and sex-matched healthy controls (33 female, 14 male; mean age 52±8).

Results: Fluvastatin treatment resulted in a significant decrease in total cholesterol, LDL cholesterol, triglyceride (p<0.005), and C-reactive protein (p<0.05) levels. Thirty-three patients (70.2%) had insulin resistance, which remained unchanged following treatment. Flow cytometric analysis after treatment showed a significant decrease in total lymphocytes, and in the surface antigens of CD16+56 and CD8+(CD28+) on leukocytes, CD11c on granulocytes, and a significant increase in the CD4/CD8 ratio (p<0.05). Compared to the control group, the mean baseline values of fluorescence density (FD) of CD14, CD11b, CD11c, and CD63 on monocytes, and CD11b and CD11c on granulocytes were significantly higher in patients with MS (p<0.05). Following fluvastatin treatment, there were significant decreases in the mean FD of CD3 on lymphocytes, and of CD11b and CD11c on both monocytes and granulocytes (p<0.05); of these, all FD values were similar to those in the control group (p>0.05).

Conclusion: Our data demonstrate that inflammation may have a significant role in the pathogenesis of MS and that this effect can be controlled with statin treatment.

Key words: Anticholesteremic agents/therapeutic use; antigens; biological markers; blood platelets; cell adhesion molecules; flow cytometry; leukocytes; metabolic syndrome X; monocytes.

Amaç: Metabolik sendromun (MS) patogenezinde inflamasyon önemli rol oynamaktadır. Bu çalışmada, MS'li hastalarda fluvastatin tedavisinin inflamatuvar belirteçler üzerine etkisi araştırıldı.

Çalışma planı: Çalışmaya MS tanılı 47 hasta (36 kadın, 11 erkek; ort. yaş 55±8) alındı. Metabolik sendrom tanısı NCEP ATP III ölçütlerinden en az üçünün varlığıyla kondu. Bütün hastalara altı hafta boyunca 80 mg fluvastatin tedavisi uygulandı. Tedavi öncesi ve sonrasında laboratuvar parametreleri ölçüldü; periferik kan lökositleri akım sitometri yöntemiyle değerlendirildi. Hasta grubunun verileri, yaş ve cinsiyet uyumlu 47 sağlıklı bireyden (33 kadın, 14 erkek; ort. yaş 52±8) oluşan kontrol grubuyla karşılaştırıldı.

Bulgular: Tedavi sonrasında total kolesterol, LDL-kolesterol ve trigliserid düzeylerinde (p<0.005) ve C-reaktif protein düzeyinde (p<0.05) anlamlı düşüş görüldü. Metabolik sendromlu grupta 33 hastada (%70.2) insülin direnci vardı. İnsülin direncinde tedavi sonrasında anlamlı değişiklik olmadı. Akım sitometrik incelemede, lökosit yüzey antijenlerinden CD16+56, CD8+(CD28+), granülosit CD11c ve total lenfositte tedavi öncesine göre anlamlı azalma (p<0.05), CD4/CD8 oranında ise anlamlı artış görüldü (p<0.05). Tedavi öncesinde MS'li hastalarda, monositlerdeki CD14, CD11b, CD11c ve CD63'ün, granülositlerdeki CD11b ve CD11c'nin ortalama floresan yoğunluğu kontrol grubuna göre anlamlı derecede yüksekti (p<0.05). Statin tedavisi sonrasında lenfositlerin yüzeyindeki CD3'ün, monositlerin ve granülositlerin yüzeyindeki CD11b ve CD11c'nin ortalama floresan yoğunluğunda anlamlı azalma sağlandı (p<0.05); bu değerlerin hepsi tedavi sonrasında kontrol grubu ile benzerlik içindeydi (p>0.05).

Sonuç: Bulgularımız metabolik sendrom patogenezinde inflamasyonun önemli rol oynayabileceğini ve statin tedavisi ile bu etkinin kontrol altına alınabileceğini göstermektedir.

Anahtar sözcükler: Antikolesteremik ajan/terapötik kullanım; antijen; biyolojik belirteç; trombosit; hücre bağlanma molekülü; akım sitometri; lökosit; metabolik sendrom X; monosit.

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INTRODUCTION

Metabolic syndrome (MS) is an endocrinopathy with a high mortality and morbidity rate which starts with insulin resistance and continues with the combination of systemic disorders including abdominal obesity, glucose intolerance or diabetes mellitus, dyslipidemia, hypertension and coronary artery disease.[1] Metabolic syndrome is also defined as a prothrombotic and proinflammatory process.[2,3] The relationship between inflammation and cardiovascular disease has been shown in many studies so far.[4-6] However the relationship between metabolic syndrome and inflammation has not been studied sufficiently. In addition, changes in inflammation markers with statin treatment is still unknown. In this study, the effects of statin treatment on peripheral blood leukocyte surface antigens, C-reactive protein, insulin and homocystein have been investigated.

PATIENTS AND METHODS

The study included 47 patients (36 female, 11 male; mean age 55±8) who were consulted at the internal medicine and cardiology outpatient clinics and are diagnosed with MS. The diagnosis of metabolic syndrome was based on the presence of at least three criteria of the NCEP ATP III guidelines including abdominal obesity (waist circumference >102 cm for men, >88 cm for women), serum triglyceride levels ≥150 mg/dl, HDL cholesterol (<40 mg/dl for male, <50 mg/dl for female), blood pressure ≥130/85 mmHg, fasting plasma glucose ≥110 mg/dl.[1] A control group consisting of 47 age- and sex-matched healthy individuals (33 female, 14 male; mean age 52±8) was formed to make a comparison with the baseline data.

Patients with immune system disorders, hepatic and renal dysfunctions, malignancies, pregnancy, any acute diseases, LDL cholesterol <100 mg/dl, triglyceride >400 mg/dl and having received statin treatment in the last 3 months were excluded from the study.

The study was approved by the local ethics committee. In addition, all patients and controls were informed about the objective and scope of the study and their consents for participation were obtained.

A thorough medical history was taken and a complete physical examination was performed for all participating subjects at the baseline. Age, gender, disease and follow-up history, smoking and alcohol consumption habits, concomitant medication, medical history and data for cardiovascular diseases in the family history were recorded. The cardiac functions of all patients were monitored using 12-lead electrocardiography and echocardiography.

In the anthropometric evaluation; weight, height, waist and hip circumference were measured with clothings on an empty stomach and in standing position by using standard measurement tools and by the same staff. The body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters (kg/m^2). The waist circumference was considered as the shortest diameter between arcus costarium and spina iliaca anterior superior, whereas the hip circumference was considered as the longest diameter between gluteus maxima posteriorly and symphysis pubis anteriorly. The waist/hip ratio was calculated in centimeters measured by dividing the waist circumference to the hip circumference.

Patients with metabolic syndrome received fluvastatin 80 mg tablet once in the evenings for six weeks, irrespective of lipid levels. Six weeks later, medical history was taken and physical examination was performed for each patient who came for the second visit. The anthropometric measurements were repeated.

12-hour fasting blood samples from the antecubital vein were collected before and after treatment. And fasting blood glucose, urea, AST, ALT, creatine kinase, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, erythrocyte sedimentation rate, C-reactive protein, insulin, homocystein and complete blood count tests were performed. The leukocyte surface antigens were determined via peripheral blood flow cytometry.

The blood samples collected in a dry plain tube for biochemical parameters were centrifuged at 3000 cycle/min following the coagulation period.

Insulin resistance was measured by HOMA equation [HOMA: Fasting insulin (μu/ml) x fasting plasma glucose (mg/dl)/405].[7,8] HOMA level below 2.7 was accepted as normal, whereas above 2.7 was accepted as insulin resistance.

Blood sample analysis. 2 ml venous blood samples from the antecubital vein were collected using sterile injectors into standard tubes with K3 EDTA and lymphocyte subgroups were analyzed by Coulter Epic XL flow cytometry, whereas the total leukocyte counts were analyzed by Coulter STKS. The same procedure was performed following the 6-week statin treatment and the results were compared.

Flow cytometric analysis. The blood samples in standard tubes with K3 EDTA were at first analyzed

by the leukocyte preparation system (Coulter Immunoprep Leukocyte Preparation System; Beckman Coulter, Florida, USA) for flow cytometric analysis, and then 20 μL monoclonal antibodies were added to the 100 μL cell preparation for lymphocyte subgroup analysis. The incubation was performed in the dark, and at room temperature for 15 minutes. Monoclonal antibodies were then conjugated with FITC (fluorescein isothiocyanate) and PE (phycoerythrin). Cells labeled with the antibodies were analyzed by Coulter Epics XL II flow cytometry and the absolute number of the cells was determined.

Statistical analysis. Data were analyzed by SPSS for Windows (version 11.0) and were evaluated in numbers and percentages, as mean±standard deviation (SD). Kolmogorov-Smirnov test was used to determine whether the variables were in normal distribution. Significance test for the difference between two mean values of normal distributed variables was performed to compare the control group with the treatment group before and after treatment. In variables with normal distribution, significance test for the difference between two means was used to compare the control group with the treatment group before and after treatment and the significance test for difference of two identicals before and after treatment was performed. In variables with abnormal distribution, Wilcoxon signed-rank test was performed to compare before and after treatment. $p<0.05$ value was deemed statistically significant.

RESULTS

The anthropometric measurements of the subjects before and after treatment are shown in Table 1.

The laboratory data of the patients with metabolic syndrome are shown in Table 2. There were no significant differences between groups in terms of fasting blood glucose, HDL cholesterol, creatine kinase, AST and ALT before and after treatment ($p>0.05$). Significant reductions were observed in total cholesterol, LDL cholesterol and triglyceride levels compared to pre-treatment levels ($p<0.005$).

In patients with metabolic syndrome, 33 patients (70.2%) had insulin resistance. There was no significant difference following treatment in insulin resistance. Homocystein levels also did not change significantly after treatment. However, C-reactive protein decreased significantly after statin treatment ($p<0.05$).

Peripheral blood flow cytometric analysis showed a significant decrease in total lymphocytes, and in leukocyte surface antigens of CD16+56 and CD8+(CD28+) and in granulocyte surface antigen of

Table 1. A comparison of pre-/post-treatment anthropometric measurements (Mean±SD)

	Pre-treatment	Post-treatment	*p*
Weight (kg)	88.7±14.7	88.4±14.5	0.5
Body mass index (kg/m^2)	35.2±5.9	35±6.1	0.1
Waist circumference (cm)	107.7±9.5	105±10.0	<0.001
Hip circumference (cm)	118.6±12.8	117.4±13.0	0.2

Table 2. A comparison of pre-/post-treatment laboratory results

	Pre-treatment (Mean±SD)	Pre-treatment (Mean±SD)	*p*
Fasting blood glucose (mg/dl)	131.8±68.2	124.7±45.8	0.8
Total cholesterol (mg/dl)	216.0±28.3	165.7±34.0	<0.001
HDL cholesterol (mg/dl)	43.5±9.2	42.3±10.7	0.2
LDL cholesterol (mg/dl)	133.3±24.0	87.9±27.7	<0.001
Triglyceride (mg/dl)	199.0±75.1	170.4±54.2	0.003
Aspartate transaminase (u/l)	26.5±11.8	30.0±12.2	0.09
Alanine aminotransferase (u/l)	26.5±11.8	32.0±26.7	0.8
Creatine kinase (u/l)	99.5±83.5	105.0±77.5	0.4
C-reactive protein (mg/l)	8.0±7.2	5.8±4.6	0.03
HOMA	5.5±4.0	4.6±3.6	0.17
Homocystein (μmol/l)	9.9±3.7	10.1±3.2	0.5

CD11c compared to pre-treatment levels ($p<0.05$; Table 3). A significant increase in the CD4/CD8 ratio was seen after treatment ($p<0.05$). There were no significant differences among other parameters (Table 3).

When patients were grouped as diabetics and non-diabetics, a difference was observed on average before and after treatment (Table 4). However, this difference did not gain statistical significance. A significant decrease was seen on CD54 on monocytes (ICAM-1) in diabetics after treatment ($p<0.05$). The mean duration of diabetes was 9 years. Three patients were newly diagnosed with diabetes. A relationship between the disease duration and findings was not established in diabetic patients.

Peripheral blood cells of patients with metabolic syndrome and healthy individuals were assessed by fluorescence density in the cell membranes (Table 5). The mean fluorescence density of CD3, CD28 and CD11b on lymphocytes was not different from the control group ($p>0.05$), while fluorescence density of CD14, CD11b, CD11c and CD63 on monocytes was significantly higher than controls ($p<0.05$). There was no difference between the groups in terms of CD54 on monocytes. Mean fluorescence density of CD11b and CD11c on granulocytes was significantly higher than the controls ($p<0.05$).

Table 3. A comparison of pre-/post-treatment leukocyte surface antigens (%)

	Pre-treatment (Mean±SD)	Pre-treatment (Mean±SD)	*p*
Lymphocytes			
CD3	70.7±8.0	71.5±7.9	0.3
CD19	11.6±3.9	11.1±3.8	0.18
CD16+56	20.1±7.8	18.6±8.0	0.002
CD3+16+56	6.0±4.2	5.3±4.0	0.189
CD3+HLA-DR	4.2±2.1	4.1±2.5	0.82
CD4/CD8	2.0±0.8	2.2±1.1	0.001
CD4+CD8	1.1±1.3	1.0±1.2	0.5
CD8+(CD28+)	9.7±3.5	9.0±3.7	0.04
CD8+(CD28-)	18.2±6.4	18.1±6.6	0.8
TCRgamadelta	3.9±2.7	3.5±2.2	0.19
CD11b	37.1±9.1	37.3±11.1	0.9
CD11c	15.0±6.5	14.3±6.8	0.4
Monocytes			
CD11b	98.0±2.0	98.0±2.0	0.6
CD11c	97.0±3.4	97.0±3.6	0.9
CD63	56.8±24.4	48.6±29.5	0.4
CD54	87.4±8.4	87.0±5.6	0.7
Granulocytes			
CD11b	99.7±0.2	99.7±0.3	0.3
CD11c	96.5±4.2	95.1±4.2	0.029
CD63	3.2±2.1	2.7±1.9	0.4
Total lymphocytes	34.6±7.8	32.5±7.5	0.034

Table 4. A comparison of pre-/post-treatment leukocyte surface antigens in diabetics and non-diabetics (%)

	Diabetics (n=21)			Non-diabetics (n=26)		
	Pre-treatment (Mean±SD)	Post-treatment (Mean±SD)	*p*	Pre-treatment (Mean±SD)	Post-treatment (Mean±SD)	*p*
Lymphocyte CD3	69.9±9.2	72.2±7.8	0.069	71.4±7	71±8.2	0.7
Lymphocyte CD19	12.5±3.6	11.5±3.8	0.07	10.9±4	10.8±3.9	0.7
Monocyte CD63	62.2±24	49.1±29.7	0.08	51.6±25.6	51.3±32	0.9
Monocyte CD54	89.3±5.7	86.3±5.5	0.02	85.9±10	87.7±5.7	0.3
Granulocyte CD63	3.6±2.7	2.5±1.9	0.09	2.96±1.8	3±2.2	0.8

Table 5. Distribution of pre-/post-treatment mean fluorescence density of leukocyte surface antigens in healthy controls and patients with metabolic syndrome

	Control (1) (n=47)	Pre-treatment (2) (n=47)	Post-treatment (3) (n=47)	*p* (1-2)	*p* (2-3)	*p* (1-3)
Lymphocytes						
CD3	10.0±2.3	10.1±2.4	9.3±2.0	0.8	0.03	0.14
CD28	3.3±0.7	3.5±0.7	3.4±0.7	0.2	0.4	0.5
CD11b	6.8±3.5	7.0±2.1	6.8±2.2	0.7	0.5	0.9
Monocytes						
CD14	8.5±4.9	12.9±7.3	12.2±6.7	0.001	0.6	0.003
CD11b	39.4±10.9	48.3±16.9	42.9±11.0	0.003	0.02	0.1
CD11c	11.8±4.2	14.0±5.4	12.4±3.2	0.03	0.04	0.4
CD63	1.4±0.1	2.3±0.7	2.1±0.6	0.000	0.08	0.000
CD54	2.7±0.6	2.7±0.7	2.6±0.4	0.5	0.5	0.8
Granulocytes						
CD11b	31.4±12.8	43.5±15.5	33.4±13.3	0.000	0.000	0.4
CD11c	3.4±0.9	4.3±1.2	3.6±1.0	0.000	0.000	0.2

Following statin treatment a significant decrease was obtained in the mean fluorescence density of CD3 on lymphocytes ($p<0.05$) and there was no difference between the post-treatment and control groups ($p>0.05$). In addition, considerable decrease was observed in the mean fluorescence density of CD11b and CD11c on monocytes after treatment ($p<0.05$) and there was no difference between the post-treatment and control groups ($p>0.05$). No significant decrease was observed in the mean fluorescence density of CD28 and CD11b on lymphocytes and CD63, CD54 and CD14 on monocytes ($p>0.05$), whereas a significant decrease was seen in the mean fluorescence density of CD11b and CD11c on granulocytes after treatment ($p<0.05$). There was also no difference between the post-treatment values and control group values ($p>0.05$).

DISCUSSION

Hyperlipidemia is known to be one of the major components of metabolic syndrome. Currently, hyperlipidemia can be effectively treated with statins.[9,10] It has been demonstrated in primary and secondary prevention trials that statins could reduce cardiovascular mortality and morbidity.[9-14] However, they are thought to exert these benefits on cardiovascular events not only by lowering cholesterol but also by their pleiotropic effects. Stabilization of atheromatous plaque is the leading pleiotropic effect.[15-21] With the effect on the foam cells condensed in the intima, statins reduce the amount of LDL cholesterol in the lipid core, thus any cracking or splitting in the plaque can be prevented. Statins reduce the amount of oxidized LDL cholesterol via these antioxidant effects and affect the proliferation of smooth muscle cells in the plaque as well.[16] Recently, there are increasing number of studies investigating the inflammatory process related to these pleiotropic effects and which has an impact on the whole system. In our study, we investigated the relationship between inflammation and fluvastatin, a member of the statin family.

We observed a significant decrease in CRP with fluvastatin 80 mg given for 6 weeks ($p<0.05$). This might be considered as an expected result since many large-scale studies also demonstrated similar results and the antiinflammatory characteristics of statins are proposed to be associated with the CRP lowering effects of statins.[22]

In the light of current data, it can be suggested that there is a relationship between MS and inflammation. Ridker *et al*[23] demonstrated that there was a strong relationship between MS and inflammatory activity. In addition, many studies suggested that statins decreased low-level inflammation as well as CRP, thereby reduced the cardiovascular event risk.[13,24-30] It was also shown that the LDL cholesterol lowering effect of statins delayed the progression of vascular lesions, and together with the lowering of hs-CRP, this resulted in remission of atherosclerotic plaques.[27,28] In our study, we obtained similar results to current data. We determined significant reductions in LDL cholesterol level next to remarkable reductions in many inflammatory markers. Given the fact that metabolic syndrome increases the risk of coronary artery disease, the beneficial effects of this reduction cannot be ignored.

With regard to diabetes, many patients with metabolic syndrome were diabetic in our study. The mean insulin level was 16.9±9 mU/ml in the whole patient group. Following a 6-week statin treatment, we did not observe a significant reduction in insulin level ($p>0.05$). Forst *et al*[31] also did not achieve a significant reduction in fasting insulin level following a 3-month simvastatin treatment in Piostat trial, and they even observed an increase. On the other hand, they obtained a significant reduction in post-prandial insulin level with the combination of pioglitazone and simvastatin ($p<0.05$). However, we did not investigate post-prandial insulin level in our study. We suggested a decrease in inflammation with statin treatment in patients with metabolic syndrome, as such a significant reduction in CRP level supported our hypothesis. In addition, we also suggested that a decrease in inflammation would accompany a decrease in insulin resistance and thus fasting insulin level would be reduced, yet we could not confirm this. This result strengthens the hypothesis that insulin resistance is not solely associated with inflammation. Waist circumference above normal is one of the major causes of insulin resistance. Many of our patients had waist circumferences which were much above normal. A significant reduction was observed in waist circumferences following statin treatment, but it could not reach normal values. Unchanged insulin resistance might be due to this insufficient reduction. On the other hand, only two patients had impaired fasting glucose and the findings for these two patients did not differ much from the whole group. Further large-scale studies using long-term statin treatment along with a well-prepared diet programme in non-diabetic patients with metabolic syndrome are needed.

One of the purposes of running such a study was to determine the role of the cells in MS, and the response of these cells to statin treatment, since it is known that the inflammatory cells have a role in certain steps of inflammation and in the pathogenesis of atherosclerosis. Within this scope, inflammatory cells were investigated in the peripheral blood. In the metabolic syndrome group, the mean fluorescence density of CD14, CD11b and CD11c on monocytes and of CD11b and CD11c on granulocytes were significantly higher than in the control group ($p<0.05$). This result suggests an increased monocyte activation in patients with MS. As it is well known, CD11b and CD11c that are referred to as integrin have a role in binding CB3 molecules, in the phagocytosis of particules defined as foreign bodies, and holding of neutrophils and monocytes to endothelium and extracellular matrix.[32] These surface molecules suggest an induced inflammation process in these patients. Particularly the increase in monocyte and granulocyte series are more likely associated with the pathogenesis of atherosclerosis. The major role of integrins is to bind leukocytes to extracellular matrix and endothelium.[33] CD14 is specifically present on monocytes, macrophages and granulocytes, binds to lipopolysaccharide protein complex and is essential for lipopolysaccharide-induced macrophage activation. Observing high levels of integrins in patients with MS is not surprising since they play an important role in the pathogenesis of inflammation.

A study including 33 patients with MS by Arteaga *et al*[34] showed a significant increase in mean fluorescence density of CD54 on lymphocytes compared to healthy individiuals. There was also an increase in mean fluorescence density of CD11b on all leukocytes compared to control group, while no difference was observed in the mean fluorescence density of CD54 on monocytes and granulocytes in the same study. Only the increase in mean fluorescence density of CD54 on lymphocytes was considered as an unexpected finding and suggested that it was due to CD54's tight binding to neutrophils and loose binding to lymphocytes.[35] However, we could not observe any significant change in CD54 level on monocytes in our study which might be due to the sample size and characteristics of the patient group.

Considering CD11b, only an increase in mean fluorescence density of CD11b on monocytes and granulocytes was observed, while no significant difference was seen on lymphocytes. However, Arteaga *et al*[34] observed a significant increase, since they have made a global evaluation on leukocytes without any differentiation. Differing from our study, Blanco-Colio *et al*[36] found CD54 levels higher in patients at high risk of cardiovascular events than healthy individuals. The primary causes of this divergence were the small numbers of patients that we had and the inclusion of low risk patients in our study. On the contrary, the sample size was 2117 in the study of Blanco-Colio *et al*[36] and patients with coronary artery disease, diabetes mellitus, peripheral vascular disease and cerebrovascular disease were included. In our study group only three cases had coronary artery disease, 21 cases had diabetes and one had peripheral artery disease, whereas there was no case with cerebrovascular disease. It is obvious that all diseases mentioned are associated with increased endothelial

dysfunction and leukocyte adhesion. Given the fact that leukocyte adhesion is one of the most important factors following the endothelial dysfunction in the pathogenesis of atherosclerosis and that the adhesion molecules play the major role in this process, observing higher levels of CD54 in these patient groups is an inevitable expectation.

We found significant changes in peripheral blood cells following the 6-week fluvastatin treatment. We initially analyzed the mean fluorescence densities and found a significant reduction in the mean fluorescence density of CD3 molecule on lymphocytes compared to baseline ($p<0.05$). The mean fluorescence density of CD3 molecule was not much different from the control group after treatment ($p>0.05$). CD3 molecule is responsible for maintaining the structural integrity of T-cell receptor and intracellular transfer of the signal. A reduction in this molecule may have an important role in the reduction of cellular immunity. T-lymphocytes play a regulatory and cytotoxic role at all stages of inflammation. We did not find any publication showing a decrease in this molecule with statin treatment. It may be suggested that, decreasing the density of T-lymphocytes carrying CD3 molecules in the inflammatory site with statin treatment can lead to weakening of cytokine-mediated regulatory function of these cells and thus there may be a decrease in the severity of inflammation; however further large-scale clinical studies as well as laboratory studies are required to test this hypothesis.

The mean fluorescence density of integrins CD11b and CD11c on monocytes and granulocytes were significantly higher in patients with metabolic syndrome compared to pre-treatment control group. These values significantly decreased following a 6-week fluvastatin treatment ($p<0.05$). There was no significant difference between post-treatment level and control group. Surface expressions of CD11b and CD11c have increased in patients with metabolic syndrome; lipid lowering effect of fluvastatin demonstrated beneficial effects on adhesion expressions of monocytes and granulocytes and reversed the effects of MS. Monocytes' binding to endothelium and subsequent migration to intima is the initial and the vital step in forming atherosclerotic lesions. These cells with high level of activity in subendothelial space are converted into mature macrophages, and thus synthesize proinflammatory and prothrombic molecules. CD11b and CD11c have a major role during this monocyte adhesion. The pathophysiological value of increase in monocyte and granulocyte adhesion molecules in patients with metabolic syndrome is still unknown. In a study including 13 cases with hypercholesterolemia and stable coronary disease, Serrano *et al*[37] demonstrated a significant increase in mean fluorescence density of adhesion molecules CD11b and CD14 on monocytes compared to controls ($p<0.0001$) and they provided a significant reduction following a 10-week statin treatment ($p<0.0001$). In this study it was shown that increasing expression of the adhesion molecules could be reversed by reducing the high cholesterol level with simvastatin treatment. Similarly, Weber *et al*[38] also found a significant decrease in CD11b expression on monocytes ($p<0.05$) *in vitro*. However, the authors were not able to explain whether this result was due to the direct effect of simvastatin or normalized hypercholesterolemia. In our study, we could not fully explain the mechanism of this effect and differing from the above mentioned study, we could not find a significant difference in CD14 level following treatment. This may be due to the differences in sample size and patient groups.

When we reviewed the data, we could not find any publication suggesting that, in metabolic syndrome, the mean fluorescence density of integrins CD11b and CD11c on granulocytes could be reduced by statin treatment. This is the first study showing that these integrins can be reduced by statins in patients with statin treatment. Based on these results, it can be suggested that not only monocytes but also granulocytes have an important role in the inflammation in MS pathogenesis and statin treatment can reduce the severity of this stage of inflammation. Further large-scale and long-term studies are needed to determine the extent of the reduction on cardiovascular mortality and morbidity, achieved by the decrease in these mediators.

Following statin treatment, we did not find a significant reduction in mean or in percentage fluorescence density of CD54 on monocytes ($p>0.05$). However, we observed a significant reduction in cell percentage in 21 diabetic patients following statin treatment versus non-diabetics. It was not one of the expected results, since different results have been achieved in previous studies but nothing similar to this. In a study of 2117 patients who had coronary artery disease or who had >20% risk of 10-year coronary artery disease, Blanco-Colio *et al*[36] showed that 12-week-statin treatment in different doses significantly reduced CD54 (s-ICAM-1) level ($p<0.0001$).

Authors reported that circulating ICAM-1 level was considerably higher in patients with MS compared to non-MS. Atorvastatin 10 mg was the most effective among different dose regimens in this study (p=0.005). Though atorvastatin 20, 40, and 80 mg also reduced the level of ICAM-1, the difference with 10 mg dose was not significant (p<0.05). We used high dose fluvastatin (80 mg) in our study. Maybe that's why we had similar results with high dose results in the statin group of Blanco-Colio *et al*[36]. If we had also used fluvastatin 10 mg, we could observe a reduction in the whole group. However, Blanco-Colio *et al*[36] did not evaluate the diabetic group separately. The data we found about diabetic patients suggest that in the pathogenesis of MS overt diabetes may be prevalent in the inflammation, not the prediabetic stage, and high dose statins can be preferred in this patient group.

Consequently, there are significant increases in the inflammatory markers in patients with MS compared to healthy controls and 6-week-fluvastatin treatment both reduced total cholesterol and LDL cholesterol levels significantly and established significant improvements in the inflammatory markers. In the light of these data, statin treatment may be considered to reduce the severity of the inflammation which plays a major role in coronary atherosclerosis. However, large-scale and long-term studies are required to obtain definite conclusions.

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