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ORIGINAL PAPER

Effects of one year simvastatin and atorvastatin treatments on acute phase reactants in uncontrolled type 2 diabetic patients

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Abstract Type 2 diabetes mellitus is the leading cause of macrovascular diseases and related death. Additionally, diabetes mellitus is frequently complicated by other cardiovascular risk factors, such as hypercholesterolemia, hypertension, obesity, hypercoagulability, and inflammation. We wanted to evaluate and compare the effects of treating with a one-year course of atorvastatin or simvastatin on inflammatory markers such as high sensitive C-reactive protein (hsCRP), fibrinogen, and ferritin in uncontrolled type 2 diabetic patients. Also, we planned to investigate the correlation between inflammatory markers and metabolic parameters. Fifty type 2 diabetic patients (30 women, 20 men; mean age: 49.9 ± 8.5 years) were enrolled into the study. Twenty healthy subjects, matched on body mass index and age, were also included in the study as a control group. Diabetic patients were divided into two groups and received simvastatin or atorvastatin (Group S and A, respectively). After 1 year of statin treatment (Group A), there were significant decreases in total cholesterol (217.3 ± 46.5–173.8 ± 37.2 mg/dl; $P < 0.0001$), LDL-cholesterol (146.7 ± 50.3–102.3 ± 31.1 mg/dl, $P < 0.0001$), hsCRP (0.88 ± 0.62–0.35 ± 0.18 mg/dl, $P < 0.0001$), fibrinogen (258.2 ± 16.9–215.5 ± 10.6 mg/l; $P < 0.0001$), and ferritin (118.2 ± 73.9–81.2 ± 72.5 ng/ml, $P < 0.0001$) levels compared to basal values. In the S group, there were significant decreases in total cholesterol (224.4 ± 61.2–175.0 ± 47.8 mg/dl; $P < 0.0001$), LDL-cholesterol (140.9 ± 56.7–110.9 ± 42.2 mg/dl, $P < 0.0001$), hsCRP (0.98 ± 1.3–0.46 ± 0.25 mg/dl, $P < 0.0001$), fibrinogen (265.7 ± 26.8–222.1 ± 20.6 mg/l; $P < 0.0001$), and ferritin (136.7 ± 101.1–85.6 ± 32.1 ng/ml, $P < 0.0001$) levels compared to basal values. At the end of the study, ΔhsCRP, Δfibrinogen, and Δferritin levels were correlated with ΔLDL ($r = 0.42$; $P = 0.005$, with ΔhsCRP), ($r = 0.40$; $P = 0.008$, with Δfibrinogen), ($r = 0.46$; $P = 0.002$, with Δferritin) and ΔHDL ($r = -0.50$; $P < 0.0001$, with ΔhsCRP), ($r = -0.32$; $p = 0.042$, with Δfibrinogen), ($r = -0.48$; $P < 0.0001$, with Δferritin) cholesterol levels. Atorvastatin and simvastatin treatments were found to be effective for the control of hypercholesterolemia and resulted in a significant decrease in acute phase reactants in uncontrolled type 2 diabetic patients.

Keywords Atorvastatin · Simvastatin · Type 2 diabetes · C-reactive protein · Fibrinogen · Ferritin

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Introduction

Type 2 diabetes is the leading cause of macrovascular diseases and related death. Additionally, diabetes mellitus is frequently complicated by other cardiovascular risk factors, such as hypercholesterolemia, hypertension, obesity, increased coagulation markers, and inflammation [1, 2]. Therefore, in addition to glucose regulation, other risk factors should be properly managed to prevent cardiovascular diseases in type 2 diabetic patients. It has been shown in many large scale studies that hyperlipidemia treatment that includes the hydroxymethylglutaryl-CoA reductase

molecule, known as statin, significantly reduces the risk of macrovascular diseases in type 2 diabetic patients [3, 4]. Statin treatment is beneficial, not only because of its lipid-lowering effect, but also due to regulation of endothelial function, inflammation, and coagulation parameters [5].

In a series of recent studies, plasma levels of inflammation and coagulation markers have been shown to be predictors of coronary heart disease [6–8]. High sensitive C-reactive protein (hsCRP) and fibrinogen are the best studied markers and both are available to practicing physicians. Both fibrinogen [6, 7] and CRP [9] predict fatal and/or non-fatal coronary events, stroke [10], and peripheral arterial disease (PAD) [11]. In primary and secondary prophylactic studies of statin administration, serum hsCRP levels are correlated with the risk of macrovascular disease [10, 12, 13]. However, data on the effects of statins on plasma fibrinogen levels are inconsistent, and different statin molecules used in the treatment of hyperlipidemia have resulted in different effects on plasma fibrinogen levels [12, 14–18].

In 1981, Sullivan [19] was the first to propose a relationship between body iron stores and coronary artery disease (CAD). In accordance with this hypothesis, evidence showed that iron deposition played a role in atherosclerosis by increasing lipid peroxidation induced by free radicals [20, 21], and by vascular smooth muscle proliferation [22]. However, some studies have reported paradoxical results on the relationship between biochemical markers of body iron (ferritin and/or transferrin saturation) and CAD [23].

Detection of novel markers to monitor the development of macrovascular disease in type 2 diabetic patients and increasing knowledge regarding disease control are essential in preventing complications. The first aim of the present study was to evaluate and compare the effects of a one-year atorvastatin and simvastatin treatment on hsCRP, fibrinogen, and ferritin molecule in uncontrolled type 2 diabetic patients. The second aim was to find the relation of possible changes in the levels of these molecules by statin treatment with metabolic parameters.

Materials and methods

Patient selection

Type 2 diabetic patients who had been admitted to our endocrinology outpatient clinics were carefully evaluated for secondary inflammatory conditions, such as chronic respiratory diseases, end-stage renal disease, hepatic dysfunction, heart failure, acute febrile illness, asymptomatic infection, malignant or chronic inflammatory diseases, and heart valve disorders. These conditions were considered to be exclusion criteria. Another factor that was considered grounds for exclusion was the use of medications that could affect the coagulation system and lipid metabolism, such as anticoagulants, oral contraceptives, hormonal replacement therapy, and hypolipidemic drugs. Also, smoking and anemia were considered as exclusion criteria, regardless of anemia etiology. Fifty poorly-controlled type 2 diabetic patients (30 women, 20 men; mean age: 49.9 $\pm$ 8.5 years) with or without macro- and micro-vascular diabetic complications were enrolled into the study. Twenty non-diabetic healthy subjects (10 men, 10 women; mean age: 46.8 $\pm$ 6.0 years), matched on body mass index (BMI) and age, were included in the study as a control group. The study protocol was approved by the local Ethics Committee, and all patients gave written informed consent before randomization. An open-label, randomized study design was used.

Anthropometric measurements

Body weight and height were measured in the morning with light clothing and without shoes. BMI was calculated as body weight in kilograms divided by height in meters squared. Waist circumference was measured with a flexible plastic tape midway between the lower rib margin and iliac crest, and hip girth was measured at the widest part of the hip. Both circumferences were measured in the standing position after normal expiration.

Evaluation of macro- and micro-vascular complications

Presence of CAD was defined as previously documented acute myocardial infarction, coronary artery by-pass surgery, or coronary angioplasty. Those individuals who met Minnesota coding [24], based on resting ECG Q-wave or clinical history of angina pectoris, were also included in the total clinical CAD. A history of documented stroke defined cerebrovascular accident. Peripheral arterial disease (PAD) was established by the presence of at least two of the criteria for intermittent claudication, an ankle-brachial pressure index of less than 0.9, and the absence of peripheral pulses. Retinopathy was diagnosed as none, background or proliferative by an ophthalmology specialist. Nephropathy was established by the presence of at least two criteria of micro-/macroalbuminuria, decreased creatinine clearance, and hypertension. Neuropathy was established by examining symptoms and completing neurologic evaluations of the patients. Microalbuminuria was defined as an albumin excretion rate of >30 mg/day in a 24-hour collected urine specimen on at least two consecutive occasions.

Biochemical analysis

Blood was collected in the morning, between 8:00 and 9:00 a.m., after an overnight fast. Serum glucose, total

cholesterol, triglycerides, and HDL- and LDL-cholesterol levels were measured with a Hitachi 917 auto analyzer using Roche Diagnostics kits (Roche Diagnostics, Mannheim, Germany). Intraassay and interassay coefficients of variation were 0.9 and 1.8% (serum glucose), 0.8 and 1.7% (total cholesterol), 1.5 and 1.8% (triglycerides), 1.3 and 2.6% (HDL-cholesterol levels), and 0.71 and 1.2% (LDL-cholesterol levels). Serum apoprotein A1 and B100 levels were measured with a Dade Behring auto analyzer using original kits (Dade Behring, Marburg, Germany); intraassay and interassay coefficients of variation were 2.2 and 5.7% and 1.9 and 2.4%, respectively. Serum insulin levels were measured with an Immulite One auto analyzer and DPC kit (DPC, Los Angeles, USA); intraassay and interassay coefficients of variation were 3.8 and 4.2%. Peripheral insulin resistance (PIR) was calculated with the Homeostasis Model Assessment (HOMA-R) method [25]. For fibrinogen measurement, a venous blood sample (9 ml) was collected into Vacutainer tubes (Becton Dickinson, Mountain View, Calif., USA) containing 0.129 mol/l trisodium citrate (1 vol). Platelet-poor plasma was obtained by centrifugation at 3,500*g* at 10°C for 20 min. Fibrinogen measurement was performed with a Dade Behring BN II auto analyzer using the Nephelometric High sensitivity method with original kits (Dade Behring. Marburg. Germany); intraassay and interassay coefficients of variation were 1.4 and 2.0%. hsCRP and ferritin levels were measured with a Dade Behring BN II auto analyzer by using Nephelometric High sensitivity method and original kits (Dade Behring. Marburg. Germany); intraassay and interassay coefficients of variation were 2.3 and 2.5%, 1.8% and 2.9%, respectively.

Statistical analysis

Statistical calculations were performed using SPSS 10.0. Basal and final values for relevant variables were compared with chi-square, ANOVA, multiple regression analysis, paired *t*-tests or Wilcoxon rank tests within groups, and Student *t*-tests or Mann–Whitney *U*-tests between groups, where appropriate. A *P*-value of less than 0.05 was accepted as significant. Data are presented as mean ± standard deviation. After 1 year of statin treatment, variation in all of the metabolic parameters and acute phase reactants levels were calculated by subtracting the end values from the beginning values. Differences of values before and after treatment are referred to as delta-values (Δ).

Treatment protocol

Initially, diabetic patients were divided into two subgroups, labeled group S and group A. The two subgroups were similar to each other regarding patient use of sulfonylurea, metformin, and insulin treatments. At the start of the study, appropriate medical nutrition therapy and exercise education were given to all diabetic patients. Patients were controlled at three-month intervals. Patients received their anti-diabetic medications according to their metabolic control levels. An LDL-cholesterol level higher than 100 mg/dl was accepted as indication for statin treatment in diabetic patients. Simvastatin or Atorvastatin were randomly administered to group S and A, respectively, in a 10 mg dosage. After 6 months, if LDL-cholesterol levels did not decrease to target levels (<100 mg/dl), doses of statins were doubled to 20 mg. Although atorvastatin is considered to be more potent in comparison to other statins, the results of meta-analysis of studies with lower doses of atorvastatin, such as 10–20 mg, have revealed that atorvastatin is not superior to other statins with respect to its effect on LDL-cholesterol and hsCRP levels in secondary prevention groups and diabetic patients [26]. We initially administered the same doses of atorvastatin and simvastatin (10 mg) to group A and S, respectively. Twenty patients in group S and 18 patients in group A received a final dose of 20 mg of statin. All patients were followed for 1 year. In addition to statin treatment, all diabetic patients received 100 mg/day amino-salicylic acid as an antithrombotic agent, and ramipril 10 mg/day as cardio-protective therapy, as recommended in the HOPE study [27]. Although none of the individuals had a history of hypertension, a total number of 20 patients (40%) in the diabetic group and eight cases (40%) in the control group were diagnosed with stage 1 hypertension following the initial evaluation. Final lipid parameters and inflammatory marker evaluation were performed at the end of the study.

Results

Entire diabetic group

The diabetic patient group exhibited micro-(n: 40; 80%) and macro-vascular (n: 23; 46%) complications. At baseline (pre-treatment), type 2 diabetic patients and control groups were compared with each other (Table 1). There were no significant differences between the groups regarding age, anthropometric measurements, or plasma levels of total cholesterol, Apo A1, Apo B100, and LDL-cholesterol. Also there were no differences between the two groups regarding hypertension, coronary artery disease, or cerebrovascular accident incidence. Plasma levels of triglyceride, fibrinogen, CRP, ferritin, and HOMA-R were found to be significantly higher in the type 2 diabetic patients as compared to the healthy subjects, while HDL-cholesterol levels were found to be significantly lower (Table 1). At baseline, there were positive correlations

Table 1 Features of diabetic and control subjects in the beginning of the study

	Type 2 diabetic patients ($n = 50$)	Control group ($n = 20$)	*P*-value
Age (years)	49.9 ± 8.5	46.8 ± 6.0	N·S
Body mass index (kg/m^2)	34.0 ± 8.0	34.5 ± 4.7	N·S
Waist/hip ratio	0.91 ± 0.07	0.89 ± 0.065	N·S
Fasting plasma glucose (mg/dl)	164.2 ± 41.6	88.8 ± 7.6	<0.0001
Total cholesterol (mg/dl)	220.8 ± 53.9	196.7 ± 32.2	N·S
Triglyceride (mg/dl)	195.90 ± 110.59	121.80 ± 21.60	0.007
HDL-cholesterol (mg/dl)	42.65 ± 9.20	48.83 ± 9.69	0.021
LDL-cholesterol (mg/dl)	143.80 ± 53.10	117.90 ± 60.67	N·S
Apo A1 (mg/dl)	136.81 ± 31.11	136.20 ± 29.60	N·S
Apo B100 (mg/dl)	118.66 ± 30.621	108.4 ± 20.5	N·S
HOMA-R	3.55 ± 2.77	1.49 ± 0.38	<0.0001
Fibrinogen (mg/l)	261.99 ± 22.61	241.81 ± 11.27	<0.0001
hsCRP (mg/dl)	0.93 ± 1.01	0.28 ± 0.09	<0.0001
Ferritin (ng/ml)	127.48 ± 88.12	78.52 ± 9.59	<0.0001
Coronary artery disease (n) (%)	14 (%28)	4 (%20)	N·S
Cerebrovascular accident (n) (%)	12 (%24)	2 (%10)	N·S
Peripheral arterial disease (n) (%)	2 (%4)	–	*
Hypertension (n) (%)	20 (%40)	8 (%40)	N·S

HOMA Homeostasis model assessment, *hsCRP* High sensitive C-reactive protein

* Not calculated

Table 2 At the beginning of the study, correlations between hsCRP, fibrinogen, ferritin, microalbuminuria, LDL and HDL-cholesterol levels in the entire diabetic group

	hsCRP (mg/dl)	Fibrinogen (mg/L)	Ferritin (ng/ml)
Fibrinogen (mg/L)	$r = 0.73$; $p < 0.0001$	–	–
Ferritin (ng/ml)	$r = 0.69$; $p < 0.0001$	$r = 0.65$; $p < 0.0001$	–
Microalbuminuria (mg/24 hour)	$r = 0.47$; $p = 0.001$	$r = 0.33$; $p = 0.022$	$r = 0.30$; $p = 0.042$
LDL-Cholesterol (mg/dl)	$r = 0.25$; $p = 0.047$	$r = 0.32$; $p = 0.035$	$r = 0.30$; $p = 0.028$
HDL-Cholesterol (mg/dl)	$r = -0.23$; $p = 0.023$	$r = -0.26$; $p = 0.035$	$r = -0.25$; $p = 0.043$

* *hsCRP: High sensitive C-Reactive Protein*

between hsCRP, fibrinogen, and ferritin levels with microalbuminuria and LDL-cholesterol. Conversely, while a negative correlation was observed between these parameters and HDL-cholesterol levels (Table 2).

Treatment groups' analysis

The diabetic patients were divided into two groups, group S and A. There were no baseline differences in relevant variables between the two treatment groups (Table 3). Further, the groups were similar regarding their use of sulfonylurea ($n = 15/25$; 60% and 14/25; 56%, Group S and A, respectively), metformin (20/25; 80% and 21/25; 84%, respectively), insulin (12/25; 48% and 13/25; 52%, respectively), amino-salicylic acid (25/25; %100 and 25/25; %100, Group S and A, respectively), and ramipril (25/25; %100 and 25/25; %100, Group S and A, respectively) treatments.

When each group was individually compared with the control group, there were no differences noted in age, BMI, waist and hip circumference, waist/hip ratio, and levels of total cholesterol, LDL-cholesterol, Apo A1, and Apo B100 between clinical and control groups. In group A, triglyceride (178.5 ± 77.8 and 121.8 ± 21.6 mg/dl, $P = 0.002$, Group A and Control Subjects, respectively), HOMA-R (4.09 ± 2.76 and 1.49 ± 0.38, $P < 0.0001$, respectively), hsCRP (0.88 ± 0.62 and 0.28 ± 0.09 mg/dl, $P < 0.0001$, respectively), fibrinogen (258.2 ± 16.9 and 241.81 ± 11.27 mg/l, $P = 0.001$, respectively) and ferritin (118.2 ± 73.9 and 78.52 ± 9.59 ng/ml, $P < 0.0001$, respectively) levels were significantly higher and HDL-cholesterol (42.3 ± 8.1 and 48.83 ± 9.69 mg/dl, $P = 0.026$, respectively) levels were significantly lower than the control group. In group S, triglyceride (213.3 ± 135.3 and 121.8 ± 21.6 mg/dl, $P = 0.003$, Group S and Control Subjects, respectively), HOMA-R (3.02 ± 2.72 and 1.49 ± 0.38, $P < 0.0001$,

Table 3 Comparison of atorvastatin (Group A) and simvastatin (Group S) groups in the beginning of the study

	Group A ($n = 25$)	Group S ($n = 25$)
Age (years)	49.8 ± 9.4	49.8 ± 7.8
Body mass index (kg/m^2)	34.3 ± 7.8	33.7 ± 8.3
Waist/hip ratio	0.91 ± 0.08	0.89 ± 0.06
Duration of diabetes (years)	9.0 ± 7.7	9.3 ± 1.6
Fasting plasma glucose (mg/dl)	166.7 ± 28.5	161.7 ± 52.0
HbA1c (%)	9.6 ± 3.1	9.3 ± 2.8
Total cholesterol (mg/dl)	217.3 ± 46.5	224.4 ± 61.2
Triglyceride (mg/dl)	178.5 ± 77.8	213.3 ± 135.3
HDL-cholesterol (mg/dl)	42.3 ± 8.1	43.0 ± 10.4
LDL-cholesterol (mg/dl)	146.7 ± 50.3	140.9 ± 56.7
Apo-A1 (mg/dl)	137.2 ± 25.3	136.5 ± 36.5
Apo-B100 (mg/dl)	120.9 ± 30.8	116.4 ± 30.9
HOMA-R	4.09 ± 2.76	3.02 ± 2.72
Fibrinogen (mg/L)	258.2 ± 16.9	265.7 ± 26.8
hsCRP (mg/dl)	0.88 ± 0.62	0.98 ± 1.3
Ferritin (ng/ml)	118.2 ± 73.9	136.7 ± 101.1
Microalbuminuria (mg/24 h)	88.6 ± 120.0	84.2 ± 116.3

HOMA Homeostasis Model Assessment, *hsCRP* High sensitive C-reactive protein

There was no difference for both groups in the studied parameters

respectively), hsCRP (0.98 ± 1.3 and 0.28 ± 0.09 mg/dl, $P < 0.0001$, respectively), fibrinogen (265.7 ± 26.8 and 241.81 ± 11.27 mg/l, $P = 0.001$, respectively), and ferritin (136.7 ± 101.1 and 78.52 ± 9.59 ng/ml, $P < 0.0001$, respectively), levels were significantly higher than the control group in at baseline.

During the study course, we did not observe any new incidences of coronary artery disease, cerebrovascular accident, or PAD as a macrovascular complication. Moreover, we did not observe any worsening of microvascular complications or any patient death. Side effects of statins were not observed in doses of 10 or 20 mg/day in either group.

After 1 year of statin treatment, the changes in metabolic parameters and acute phase reactants in group A and S were compared with each other. There were significant reductions in total cholesterol, LDL-cholesterol, triglyceride, and Apo B100 levels in both groups compared to their basal values, but these reductions were similar between the two groups. There was also a significant increase in HDL-cholesterol levels in both groups, as compared to basal values. There was no significant difference between the two groups regarding the metabolic parameters at the end of the study (Table 4).

Significant decreases in hsCRP, fibrinogen, and ferritin were noted in group A patients at study completion, compared with basal values (Table 4). Also, in group S there were significant decreases in CRP, fibrinogen, and ferritin levels over the course of the study (Table 4). There were no significant differences between the two groups regarding the changes in acute phase reactants at the end of the study (Table 4).

At study completion, delta CRP (ΔCRP), delta fibrinogen (Δfibrinogen), and delta ferritin (Δferritin) levels were found to be positively correlated with each other (Fig. 1). Also ΔhsCRP, Δfibrinogen, and Δferritin levels were positively correlated with ΔLDL (Fig. 2) and negatively with ΔHDL-cholesterol levels (Fig. 3).

When the possible effects of sulfonylurea, metformin, and insulin treatments used by type 2 diabetic patients were

Table 4 Comparison of metabolic parameters and acute phase reactants of Group A and S in the beginning and at the end of the study

	Group A0 ($n = 25$)	Group A1 ($n = 25$)	Group S0 ($n = 25$)	Group S1 ($n = 25$)	P values	
					A0 vs. A1	S0 vs. S1
Fasting plasma glucose (mg/dl)	166.7 ± 28.5	158.6 ± 31.7	161.7 ± 52.0	159.4 ± 53.4	NS	NS
HbA1c (%)	9.6 ± 3.1	9.5 ± 3.8	9.3 ± 2.8	9.2 ± 2.7	NS	NS
Total cholesterol (mg/dl)	217.3 ± 46.5	173.8 ± 37.2	224.4 ± 61.2	175.0 ± 47.8	$P < 0.0001$	$P < 0.0001$
Triglyceride (mg/dl)	178.5 ± 77.8	141.0 ± 61.5	213.3 ± 135.3	174.9 ± 110.0	$P < 0.0001$	$P < 0.0001$
HDL-cholesterol (mg/dl)	42.3 ± 8.1	43.0 ± 8.3	43.0 ± 10.4	44.5 ± 10.7	$P < 0.0001$	$P < 0.0001$
LDL-cholesterol (mg/dl)	146.7 ± 50.3	102.3 ± 31.1	140.9 ± 56.7	110.9 ± 42.2	$P < 0.0001$	$P < 0.0001$
Apo-A1 (mg/dl)	137.2 ± 25.3	138.5 ± 25.6	136.5 ± 36.5	136.3 ± 35.7	NS	NS
Apo-B100 (mg/dl)	120.9 ± 30.8	99.2 ± 25.3	116.4 ± 30.9	97.8 ± 17.3	$P < 0.0001$	$P < 0.0001$
Fibrinogen (mg/L)	258.2 ± 16.9	215.5 ± 10.6	265.7 ± 26.8	222.1 ± 20.6	$P < 0.0001$	$P < 0.0001$
hsCRP (mg/dl)	0.88 ± 0.62	0.35 ± 0.18	0.98 ± 1.3	0.46 ± 0.25	$P < 0.0001$	$P < 0.0001$
Ferritin (ng/ml)	118.2 ± 73.9	81.2 ± 72.5	136.7 ± 101.1	85.6 ± 32.1	$P < 0.0001$	$P < 0.0001$

hsCRP High sensitive C-reactive protein, *NS* Non significant

Group A0 and group S0: Values in the beginning of the study

Group A1 and group S1: Values at the end of study

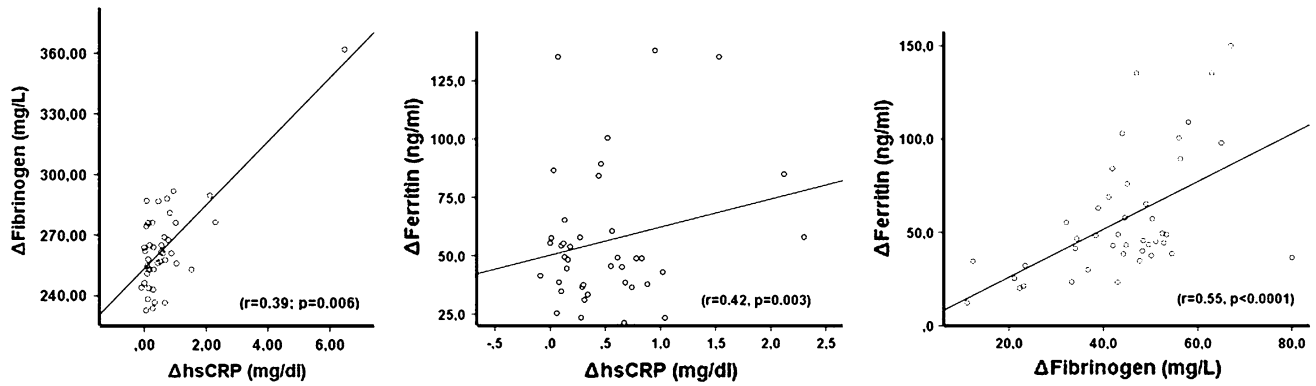

Fig. 1 At the end of the study, there were positive correlations between ΔhsCRP, Δfibrinogen and Δferritin across the entire diabetic group

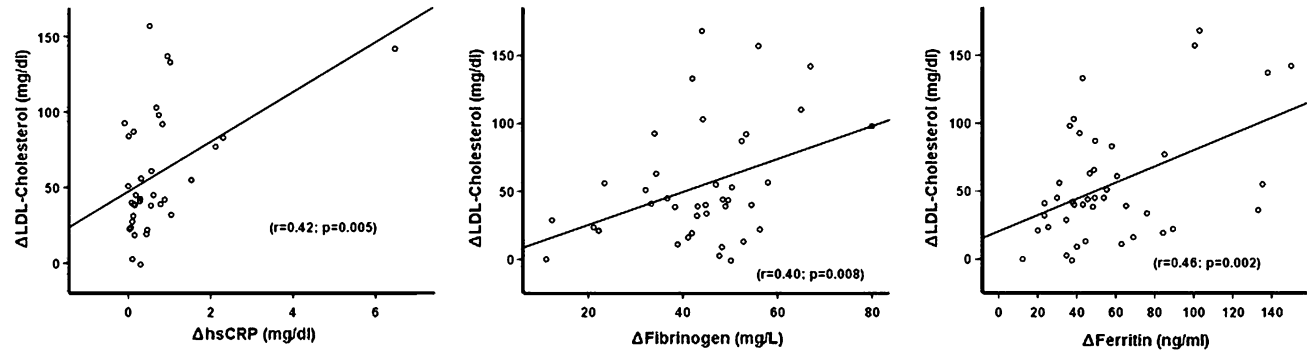

Fig. 2 At the end of the study, ΔLDL-cholesterol levels were positively correlated with ΔhsCRP ($r = 0.42$; $P = 0.005$), Δfibrinogen ($r = 0.40$; $P = 0.008$), and Δferritin ($r = 0.46$; $P = 0.002$) levels across the entire diabetic group

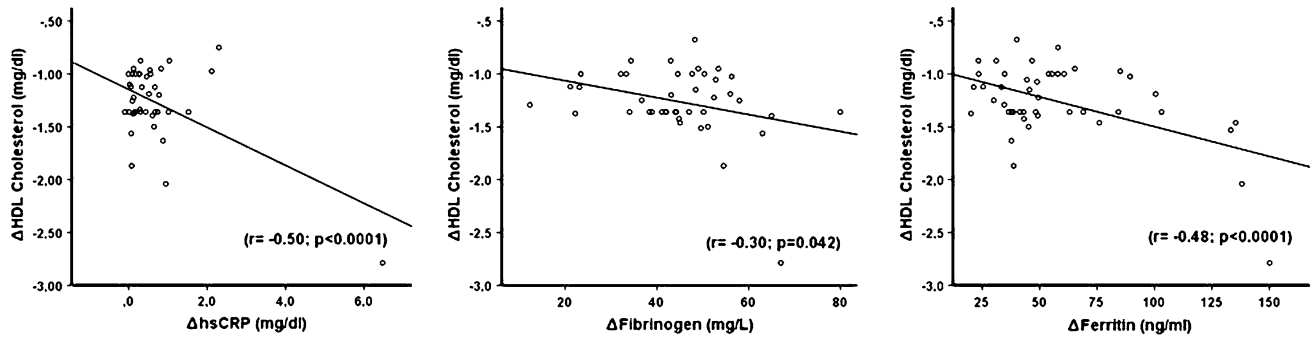

Fig. 3 At the end of the study, ΔHDL-cholesterol levels were negatively correlated with ΔhsCRP ($r = -0.50$; $P < 0.0001$), Δfibrinogen ($r = -0.32$; $P = 0.042$), and Δferritin ($r = -0.48$; $P < 0.0001$) levels across the entire diabetic group

evaluated separately, no differences between treatments on hsCRP, ferritin, and fibrinogen levels were found.

Discussion

Vascular endothelial cells are exposed to the damaging effects of hyperglycemia, hypertension, and hyperlipidemia, which are risk factors for the development of macrovascular diseases in type 2 diabetic patients. The presence of these risk factors in diabetic patients causes endothelial cells to be exposed to continuous oxidative stress and consequent high atherogenic oxide and increased modified lipoprotein fractions [28, 29]. On the initial evaluation at the beginning of the study, all of the type 2 diabetic patients were found to have significantly elevated levels of hsCRP, fibrinogen, and ferritin as compared to the control group. These elevations were independent from blood

pressure, BMI, and history of macrovascular disease. At baseline, all diabetic patients showed a positive correlation between hsCRP, fibrinogen, ferritin, and LDL-cholesterol levels; a negative correlation was observed between these parameters and HDL-cholesterol levels. The first step in the development of macro and microvascular complications in diabetic patients is the diffuse endothelial injury triggered by exposure to risk factors. In the type 2 diabetic patient group, due to the presence of high hsCRP levels and a positive correlation between hsCRP and microalbuminuria in particular, diffuse endothelial dysfunction and subendothelial systemic inflammation were observed.

Ferritin is a protein—binding and storing iron with high capacity; it is also the best clinical marker of body iron quantity as it is directly proportional to the intracellular iron concentration [30]. Free superoxide radicals, produced as a result of endothelial injury and dysfunction were shown to cause iron chelation from ferritin molecules in vitro [31, 32]. Free iron (ferrous +2) produced in in vivo conditions accelerates the process with a catalyst function in Fenton and Haber–Weiss reactions [33]. Increased free radicals cause lipid peroxidation, which in turn, results in oxidized-LDL-cholesterol formation and deposition at the subendothelial level. This process plays a role in the development of atherosclerosis, and initiates atherosclerotic plaque formation [29]. Subendothelial accumulation of oxidized-LDL-cholesterol causes an increase in inflammatory cells and systemic low grade inflammation. hsCRP, which demonstrates the presence of inflammation, contribute to its persistence and serves as a strong marker of macrovascular disorders that may occur in the future, increased [8, 13]. In the light of the extant research, the elevations in ferritin and hsCRP levels and their relations with other metabolic parameters found in all diabetic patients at baseline were considered to be increased dependently as a result of endothelial dysfunction and subsequent events. Fibrinogen is an important molecule leading to increased blood viscosity and to platelet-thrombus formation through direct and indirect effects. Many epidemiological studies have shown a clinically significant relationship between elevated plasma fibrinogen levels and coronary artery disease [34–37]. Plasma fibrinogen levels were relatively elevated, especially in type 2 diabetic patients with poor glycemic control, irrespective of the presence or absence of microvascular complications [38–41]. Increased plasma fibrinogen in diabetes patients shown in the literature was found to be related with serum glucose and HDL levels. Our results support this finding. While plasma fibrinogen levels were positively correlated with LDL-cholesterol in diabetic patients, their negative correlation with HDL-cholesterol levels showed that impaired metabolic parameters create a procoagulant medium. Given the positive relation of plasma fibrinogen levels with microalbuminuria, we think that this procoagulant medium emerges from diffuse systemic endothelial injury. Examining analyses on all the patients in the diabetic group and combining this with findings from the literature, we propose that increased hsCRP and ferritin levels are markers in endothelial dysfunction and serve as process markers while fibrinogen elevation serves as a disease marker in established endothelial dysfunction.

One-year atorvastatin and simvastatin treatments were relatively effective for the control of dyslipidemia in diabetic patients. Both statin molecules were observed to be of similar strength, according to their lipid-lowering potential at the end of 1 year. Parallel to post-treatment findings with respect to lipid parameters, significant decreases in hsCRP, fibrinogen, and ferritin levels were found. We also propose that the differences in the values of hsCRP, ferritin, and fibrinogen (Δ values) post-treatment, which showed a significant positive correlation with ΔLDL-cholesterol and a negative correlation with ΔHDL-cholesterol, are an important result of this study.

The synthesis of ferritin molecules is increased in a manner similar to the increase of acute phase reactants. Ferritin acts to collect free iron molecules and decrease the oxidative stress experienced by endothelial the cells [42]. Clinically, Juckett et al. [43] discovered ferritin and evidence of inflammation in all samples obtained from the coronary lesions of patients who had died due to atherosclerotic heart disease; normal coronary arteries did not show this pattern. Inflammation plays an important role in atherosclerosis and ferritin synthesis is up-regulated by pro-inflammatory cytokines [44]. Serum levels of ferritin molecules may be an additional marker for endothelial dysfunction and chronic low-grade inflammation in type 2 diabetes patients who carry a high risk for atherosclerotic disease. In our diabetic patients, the decrease in hsCRP and ferritin levels following statin treatment combined with a parallel improvement in lipid parameters, suggests that oxidized lipid accumulation decreased at subendothelial level and subsequent low grade systemic vascular inflammation was somewhat controlled. We think that the stimulating effects on ferritin synthesis decreased during treatment, and that the significant decreases in the ferritin levels occurred by reducing low grade inflammatory stimuli and somewhat reducing endothelial injury and/or dysfunction. However, although hsCRP and ferritin levels had significantly decreased at the end of the study, these values were still higher than those of the control group at this time. This result may be attributed to two reasons: the failure to reach target lipid values recommended by the guidelines and, the failure to provide glycemic control at the end of 1 year. The first part of our study showed that even with partial control of lipid parameters independent of blood glucose, there was partial resolution in subendothelial low grade systemic inflammation and endothelial dysfunction.

An additional aim of this study was to investigate the effects of hyperlipidemia treatment on plasma fibrinogen levels in diabetes patients using two different statin molecules. In contrast to previously demonstrated beneficial pleiotropic effects of statin treatment, controversial results have been reported with respect to elevated plasma fibrinogen levels [14–16]. In studies using atorvastatin treatment, fibrinogen levels at the end of the study were usually higher than initial values. This increase in fibrinogen levels was statistically significant during the treatment and was between 22.2 and 48% higher than initial values at the completion of the study [15]. Significant findings were not obtained, however, in other studies [14, 16]. Studies evaluating simvastatin either did not find any changes in fibrinogen levels [12] or reported statistically insignificant decreases [17, 18]. However, the effect of statin treatment on plasma fibrinogen level should not be neglected. Indeed, Meade et al. [35] reported that an increase of serum fibrinogen of 0.71 g/l increased the 5-year heart disorder ratio by 84%. As shown in the Rotterdam study [45] with a large patient population, high fibrinogen levels can be associated with poor glycemic control in type 2 diabetic patients. In the current study, treatment for 1 year with atorvastatin or simvastatin caused a significant decrease in plasma fibrinogen levels in both groups (by 19.8 and 18%, respectively). This decrease in fibrinogen levels was observed to be independent from glycemia in each group. Additionally, decreases in fibrinogen levels displayed a positive relationship with LDL-cholesterol and a negative relationship with HDL-cholesterol. As well as finding that one-year atorvastatin and simvastatin treatments decreased fibrinogen levels parallel to serum lipid parameters, we found that the effects of these two molecules on fibrinogen levels were similar. This finding in our study may be added to the body of literature detailing controversial relations between statins on fibrinogen levels.

All diabetic patients in our study had been using aminosalicylic acid (100 mg) and ramipril (10 mg). However, medications used for glycemic control varied. When the possible effects of oral anti-diabetics, insulin, and combination treatments used by type 2 diabetic patients were evaluated separately, no differences between treatments on hsCRP, ferritin, and fibrinogen levels were found.

There are some limitations on the current study. The most important study weakness is the failure to obtain glycemic control in our diabetes patients at the end of 1 year. However, this provided the opportunity to evaluate the results independent from glycemic recovery. The second weakness is that we could not lower the LDL-cholesterol level to those recommended by the guidelines despite the use of statin treatment. This may be the reason why hsCRP, ferritin, and fibrinogen levels remained high compared to the control group at the end of 1 year. The follow-up period was short (1 year) and no atherosclerotic event was observed throughout the study. We could not assess the relationship between changes in hsCRP, ferritin, and fibrinogen levels and acute macrovascular complications. Finally, detecting hsCRP, ferritin, and fibrinogen levels at the third, sixth, and ninth months throughout the study would have been helpful to assess the changes in the early period. However, we could not perform this due to the insufficiency of the study's budget.

Conclusion

hsCRP, ferritin and fibrinogen levels were elevated in uncontrolled type 2 diabetic patients. We think that hsCRP and ferritin molecules are process markers in the development of endothelial injury and fibrinogen is a disease marker in developed endothelial injury. Atorvastatin and simvastatin treatments decreased hsCRP, ferritin, and fibrinogen levels effectively by achieving lipid control in type 2 diabetic patients, independent from glycemic control. Particularly, both statin molecules similarly decreased plasma fibrinogen levels. In our study, dyslipidemia treatment in uncontrolled type 2 diabetes patients partially controlled systemic low grade inflammation and procoagulant states. In addition, no differences in beneficial pleiotropic effects were detected between the statin molecules used for the treatment of dyslipidemia.

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