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Investigation of ischemia-modified albumin levels and some atherosclerosis-related serum parameters in patients with diabetic foot

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Background/aim: To investigate serum ischemia-modified albumin (IMA), oxidized low-density lipoprotein (ox-LDL) levels, and paraoxonase 1 (PON1) activity in patients with diabetic foot.

Materials and methods: Thirty patients with diabetes mellitus (DM), 30 patients with diabetic foot (29 and 27 of these patients had type 2 DM, respectively), and 30 healthy volunteers as the control group were included in the study. The patients with diabetic foot were divided into 2 groups, as those who had or had not undergone lower extremity amputation. Serum PON1 activity, ox-LDL, and IMA levels were measured.

Results: Serum PON1 activity was lower ($P < 0.05$) and ox-LDL levels were higher ($P < 0.05$) in the diabetic foot group than in the control and diabetes groups. Albumin-adjusted IMA values were higher ($P < 0.001$) in the diabetic foot group compared to the diabetes group. The postamputation levels of IMA were decreased compared to the preamputation condition ($P < 0.05$).

Conclusion: The low activity of PON1 and the high levels of ox-LDL and IMA may play an important role in the pathogenesis of diabetic foot. The use of these parameters in the follow-up of patients with DM may prevent the development of diabetic foot. In order to reach a definitive judgment, further studies with a larger number of subjects are necessary.

Key words: Diabetic foot, ischemia-modified albumin, oxidized low-density lipoprotein, paraoxonase 1, peripheral arterial disease

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of DM is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (1). Diabetic foot ulcers are estimated to affect 15% of all patients with DM during their lifetime (2). Ulceration, infection, gangrene, and lower extremity amputation are complications often encountered in patients with DM. These complications frequently result in extensive morbidity, repeated hospitalizations, and high treatment costs. The etiology of diabetic foot ulcers is multifactorial. Risk factors identified include peripheral neuropathy, vascular disease, limited joint mobility, foot deformities, abnormal foot pressures, minor trauma, a history of ulceration or amputation, and impaired visual acuity. Despite considerable international efforts, DM continues to be the most common underlying cause of nontraumatic lower extremity amputations in the United States and Europe (3).

A prominent feature of type 2 DM is an atherogenic dyslipidemia, characterized by an increase in triglyceride-rich lipoproteins (very-low-density lipoproteins [VLDL] and their remnants), a decrease in plasma levels of high-density lipoprotein (HDL) cholesterol, and an increase in the number of small dense low-density lipoprotein particles (4). Oxidation of low-density lipoprotein (LDL) is a key process in the early progression of atherosclerotic diseases and DM complications (5). Paraoxonase 1 (PON1) has been recognized as an antioxidant enzyme, which plays an important role in preventing the accumulation of the lipid peroxides on LDL. Inhibition of HDL oxidation by PON1 could preserve the antiatherogenic functions of HDL in reverse cholesterol transport, as well as protecting LDL from oxidation (6,7).

The amino terminal end (N-terminal) of the albumin molecule, especially the aspartyl-alanyl-histidyl-lysine sequence, appears to be the primary binding site for transitional metals, such as cobalt, copper, and nickel. The N-terminal of albumin is modified during exposure

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to ischemic conditions, resulting in the formation of ischemia-modified albumin (IMA) with a low binding affinity to transitional metals. It has been reported that IMA levels rise in diseases associated with ischemia (8).

The number of studies evaluating PON1 activity and oxidized LDL (ox-LDL) levels in diabetic foot is very limited (9,10). To our knowledge there is no study concerning the IMA levels in patients with diabetic foot. The aim of this study was to investigate serum IMA, ox-LDL levels, and PON1 activity in patients with diabetic foot and to evaluate whether there was any difference between pre- and postamputation IMA values.

2. Materials and methods

2.1. Subjects

The study subjects consisted of 30 patients with DM who had never suffered from diabetic foot complications during their lifetime and 30 patients with DM who suffered from diabetic foot. The patients with diabetic foot were divided into 2 groups, according to who had or had not undergone lower extremity amputation. We also included 30 volunteers who had routine laboratory results within the reference interval, did not have any systemic disease, and did not use any medication in a control group. The study was conducted in the Department of Endocrinology, Faculty of Medicine, Erciyes University between July 2009 and January 2011. The case-control study protocol was approved by the Ethics Committee of Erciyes University and the study was conducted according to the Declaration of Helsinki. The participants were informed about the study and informed consent was obtained.

2.2. Data collection

The duration of DM was accepted as the time from the diagnosis of DM, according to American Diabetes Association (ADA) criteria, to the time of the study. The patients were evaluated for diabetic neuropathy and retinopathy based on neurology and ophthalmology findings, and for coronary artery disease based on their history of myocardial infarction or electrocardiographic findings. Patients with end-stage renal failure, a history of thyroid or liver disease, or any malignancy were excluded from the study. Body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2).

The ulcers in patients with diabetic foot were graded from 1 to 5 according to the presence of infection and/or gangrene by using the Wagner (11) Classification (Grade 1: Presence of superficial ulcer confined to the epidermis; Grade 2: Infection extending to the dermis, muscle, tendon, and ligaments but no signs of osteomyelitis; Grade 3: Presence of deep soft tissue infection and osteomyelitis; Grade 4: Gangrene localized to the distal foot; Grade 5: Extensive gangrene). Examination findings and culture and radiology results were used to classify the foot ulcers.

2.3. Laboratory analysis

All blood samples were collected into plain tubes and anticoagulated (EDTA) tubes, as appropriate for biochemical tests in the morning after an overnight fast. Blood samples were taken twice from patients with DM undergoing amputation for diabetic foot, once before amputation and once at the end of the first week after amputation. Hemoglobin A1c (HbA1c) was detected with an HPLC system (Agilent 1100, Germany). After centrifuging the blood samples for 10 min at 2000 × *g*, serum glucose, creatinine, albumin, triglyceride (TG), total cholesterol, LDL cholesterol (direct LDL cholesterol measurement kit for patients, TG level was >400 mg/dL), and HDL cholesterol were analyzed on an automatic analyzer (Architect c8000, Abbott, USA), and C-reactive protein (CRP) was measured by using immunonephelometry (Dade Behring, Marburg, Germany).

Serum samples that were separated for PON1, ox-LDL, and IMA measurements were stored at –70 °C until analysis. PON1 activity in serum samples was determined using the method described by Eckerson et al. (12). Serum ox-LDL levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Immündiagnostik, Bensheim, Germany). LDL cholesterol values were estimated by using Friedewald's equation (13). Serum IMA levels were measured by the method described by Bar-Or et al. (14). Albumin-adjusted IMA values were used to prevent the effect of low albumin level on IMA. This value was obtained by multiplying the IMA value with the coefficient determined by dividing the subject's serum albumin level to the mean serum albumin level of the population (15).

2.4. Statistical analysis

IBM SPSS Statistics 20 was used to evaluate the data. The Shapiro–Wilk test was used to determine whether data distribution was normal. The summary statistics of numerical variables with a normal distribution were presented as mean ± standard deviation. The statistics of numerical variables without a normal distribution were presented as medians (25th–75th percentile). Study groups with a normal distribution were compared with one-way analysis of variance (ANOVA) and those without a normal distribution were compared with Kruskal–Wallis analysis. Parametric and nonparametric Tukey tests were used for multiple comparisons. The independent t-test or the Mann–Whitney U test was used respectively for variables with and without a normal distribution when comparing two groups. Summary statistics of categorical variables were presented as percent (%), and the chi-square exact method was used to compare these variables. Comparisons of two repeated measurements were performed using the Wilcoxon t-test. The Spearman test

was used for correlation analysis. P < 0.05 was considered statistically significant.

3. Results

The descriptive characteristics of both the patient groups and the control group are presented in Table 1. There was no statistically significant difference in the comparison of mean age between the control and diabetes groups or between the diabetes and diabetic foot groups. BMI values were higher in both patient groups than in the control group. There was no significant difference in the comparison of sex between the study groups. The duration of DM and type 2 DM did not differ significantly between the patient groups. The coronary artery disease, diabetic retinopathy, and peripheral sensory neuropathy rates were significantly higher in the diabetic foot group than the diabetes group.

Biochemical parameters of study groups are given in Table 2. Serum glucose, HbA1c, and TG levels were significantly higher in both patient groups than in the control group. Serum CRP and ox-LDL levels were found to be significantly higher; PON1 activity and albumin levels were found to be significantly lower in the diabetic foot group compared to the control and diabetes groups. Serum creatinine and albumin-adjusted IMA levels were significantly higher in the diabetic foot group than the diabetes group. Total cholesterol, LDL cholesterol, and HDL cholesterol were significantly reduced in the diabetic foot group compared to the diabetes group.

When foot ulcers were graded according to the Wagner Classification there were 3 (10%) patients with Grade 1, 13 (43.3%) patients with Grade 2, 10 (33.4%) patients with Grade 3, 1 (3.3%) patients with Grade 4, and 3 (10%) patients with Grade 5. Lower extremity amputation was performed on 9 (30%) of the patients with diabetic foot and the foot ulcers were Grade 3 in 5 (55.6%), Grade 4 in 1 (11.1%), and Grade 5 in 3 (33.3%) of these patients.

As shown in Table 3 comparison of the subgroups of the diabetic foot group as amputated or not amputated revealed no significant difference in terms of HbA1c, creatinine, TG, total cholesterol, LDL cholesterol, albumin-adjusted IMA, and ox-LDL, while CRP levels were higher (P = 0.022) and HDL cholesterol and albumin levels were lower (P = 0.035, P = 0.049, respectively) in the amputated group.

As shown in the Figure postamputation IMA values were found to be lower compared with preamputation IMA values (0.672 ± 0.108 vs. 0.773 ± 0.144, P < 0.05). In contrast, preamputation albumin levels showed no significant difference when compared to postamputation albumin values (3.27 ± 0.51 vs. 3.41 ± 0.50, P > 0.05).

There was a negative correlation between ox-LDL and PON1 in the diabetes group (P = 0.020, rho = –0.422). The level of serum HDL cholesterol was negatively correlated with the level of serum CRP in the diabetic foot group (P = 0.023, rho = –0.415).

4. Discussion

Lifestyle changes have led to an increase in type 2 DM and its chronic complications (16). In the literature so far, studies have reported on concerns regarding antioxidant status, lipid peroxidation, serum asymmetric dimethylarginine, nitric oxide, and immune mediator levels in patients with diabetic foot ulcers (17–21).

Table 1. Comparison of descriptive characteristics of both patient groups and control group.

	Patient groups			
	Control (n = 30)	Diabetes (n = 30)	Diabetic foot (n = 30)	P
Age (year)	52.60 ± 8.76[a]	56.53 ± 8.91[ab]	58.67 ± 9.23[b]	0.033
Sex				
(male/female)	16 (53.3)/14 (46.7)	15 (50)/15 (50)	17 (56.7)/13 (43.3)	0.964
BMI (kg/m^2)	25.42 ± 2.71[a]	30.19 ± 4.24[b]	29.14 ± 5.21[b]	<0.001
Duration of DM (years)	-	11.73 ±5.53	13.27 ± 6.93	0.347
Type 2 DM	-	29 (96.6)	27 (90)	0.347
Coronary artery disease	-	5 (16.7)	13 (43.3)	0.047
Diabetic retinopathy	-	9 (30)	23 (76.6)	<0.001
Peripheral sensory neuropathy	-	16 (53.3)	28 (93.3)	<0.001

Data are presented as mean ± standard deviation or n (%). [a,b]: Significant findings between groups (P < 0.05) are marked with different letters. BMI: Body mass index, DM: diabetes mellitus.

Table 2. Biochemical parameters of study groups.

Parameters	Control (n = 30)	Diabetes (n = 30)	Diabetic foot (n = 30)	P
Glucose (mg/dL)	90.5 (84–98)[a]	200 (153–231)[b]	175.5 (142–216)[b]	<0.001
HbA1c (%)	5.2 (4.6–5.8)[a]	8.9 (7.9–10.7)[b]	7.8 (7.1–9.1)[b]	<0.001
Creatinine (mg/dL)	0.8 (0.7–0.9)[a]	0.92 (0.82–1.0)[b]	1.2 (0.97–1.43)[c]	<0.001
CRP (mg/L)	3.41 (3.27–3.41)[a]	3.34 (3.27–4.13)[a]	23 (7.14–73.6)[b]	<0.001
TG (mg/dL)	83 (73–106)[a]	136 (96–190)[b]	155 (116–224)[b]	<0.001
Total cholesterol (mg/dL)	183.5 (175–192)[a]	203 (184–223)[b]	183 (160–196)[a]	0.002
LDL cholesterol (mg/dL)	114.3 ± 12.9[a]	137.8 ± 34.0[b]	112.1± 22.9[a]	<0.001
HDL cholesterol (mg/dL)	48.99 ± 11.43[a]	42.93 ± 8.60[b]	33.37 ± 8.47[c]	<0.001
Albumin (g/dL)	4.16 ± 0.27[a]	4.13 ± 0.37[a]	3.56 ± 0.54[b]	<0.001
PON1 (U/L)	97.7 (56.4–149.1)[a]	74.8 (46.3–147.1)[a]	71.5 (36.2–90.7)[b]	0.025
ox-LDL (ng/mL)	123 (100–135)[a]	132 (109–170)[a]	175 (150–215)[b]	<0.001
IMA (ABS U)	0.395 ± 0.054[a]	0.478 ± 0.095[b]	0.721 ± 0.123[c]	<0.001
IMA♣ (ABS U)	-	0.475 ± 0.093[a]	0.660 ± 0.122[b]	<0.001

Data are presented as mean ± standard deviation or median (25th–75th percentile).
[a,b,c]: Significant findings between groups (P < 0.05) are marked with different letters. PON1: paraoxonase 1, ox-LDL: oxidized low-density lipoprotein, IMA: ischemia-modified albumin, IMA♣: albumin-adjusted ischemia-modified albumin.

Table 3. Comparison of amputated and not amputated diabetic foot groups.

Parameters	Diabetic foot (n = 30)		P
	Not amputated diabetic foot (n = 21)	Amputated diabetic foot (n = 9)	
HbA1c (%)	7.8 ± 1.6	8.1 ± 1.9	0.711
Creatinine (mg/dL)	1.19 ± 0.33	1.44 ± 0.44	0.096
CRP (mg/L)	18.0 (6.34–43.25)	74.30 (27.30–101.90)	0.022
TG (mg/dL)	156 (105.5–250)	142 (115–224)	0.894
Total cholesterol (mg/dL)	186.05 ± 27.04	167.44 ± 31.86	0.113
LDL cholesterol (mg/dL)	114.4 ± 19.8	106.5 ± 29.5	0.394
HDL cholesterol (mg/dL)	35.48 ± 7.5	28.44 ± 8.96	0.035
Albumin (g/dL)	3.69 ± 0.51	3.27 ± 0.51	0.049
PON1 (U/L)	72.6 ± 35.7	58.9 ± 30.3	0.324
ox-LDL (ng/mL)	170 (147–210)	182 (149–258)	0.449
IMA (ABS U)	0.699 ± 0.109	0.773 ± 0.144	0.133
IMA♣ (ABS U)	0.652 (0.587–0.726)	0.666 (0.529–0.690)	0.790

Data are presented as mean ± standard deviation or median (25th–75th percentile). PON1: paraoxonase 1, ox-LDL: oxidized low-density lipoprotein, IMA: ischemia-modified albumin, IMA♣: albumin-adjusted ischemia-modified albumin.

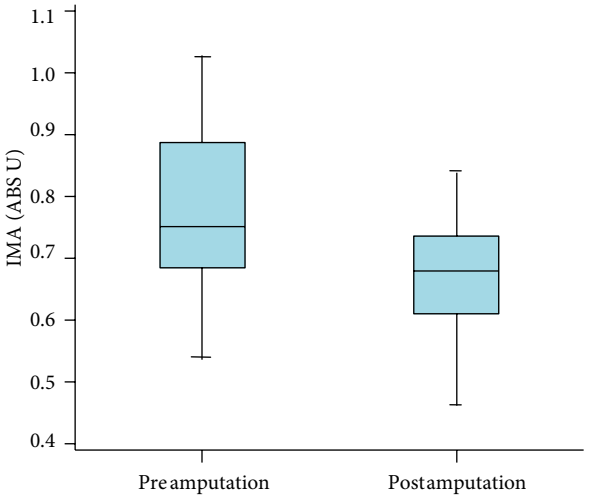

Figure. Comparison of preamputation and postamputation IMA values.

However, there have not been enough studies regarding atherosclerosis-related parameters except routine lipid tests such as TG, LDL cholesterol, HDL cholesterol, and total cholesterol in patients with diabetic foot.

It has been reported that PON1 activity decreases in patients with symptomatic peripheral arterial disease, and low PON1 activity is associated with endothelial dysfunction (22). Kasprzak et al. (23) reported low PON1 activity in patients with lower extremity necrosis due to peripheral arterial disease. Lixandru et al. (9) also reported decreased HDL cholesterol levels and PON1 activity in patients with diabetic foot ulcers. We compared PON1 activity in the diabetic foot group not only with a control group but also with a diabetes group. We found a low activity of PON1 and a decreased level of HDL cholesterol in the diabetic foot group, as reported in the literature.

The increase in inflammatory markers such as CRP and serum amyloid A (SAA) in type 2 DM leads to changes in HDL structure (24). These acute phase reactants replace antioxidant enzymes of HDL, PON1, and platelet-activating factor acetyl hydrolase (PAF-AH). HDL becomes inadequate in protecting LDL from oxidation and becomes a proinflammatory molecule (24,25). The acute decrease in PON1 activity due to the acute inflammatory event exacerbates LDL oxidation (6).

In this study, based on the negative correlation between HDL cholesterol and CRP in patients with diabetic foot, we suggest that high CRP levels may accompany the decrease in HDL cholesterol values and PON1 activity.

Maintaining LDL cholesterol at less than 100 mg/dL is widely encouraged in patients with DM; however, serum ox-LDL levels have been found to increase in patients with DM even when LDL cholesterol values are below 100 mg/dL (26). It has been found that low HDL cholesterol levels and high ox-LDL/LDL cholesterol ratios could affect vascular calcification on the plain radiography in the feet of hemodialysis patients (10). In another study, high serum lipoprotein (a) levels in patients with gangrenous diabetic foot lesions have been reported (27).

In this study, there was no significant difference in the ox-LDL levels of the diabetes and control groups. This result may be due to the negative correlation between the ox-LDL levels and PON1 activity and may be interpreted as LDL oxidation being prevented by PON1 in these patients. We found high levels of ox-LDL in the diabetic foot group. This finding might suggest that decreased PON1 activity is responsible for the high ox-LDL values in patients with diabetic foot. The low HDL cholesterol, increased ox-LDL levels, and low PON1 activity may be mediators of advanced atherosclerosis. Our study results suggest that while LDL cholesterol is related to the development of atherosclerosis in patients with DM, it is not as important of a determinant as ox-LDL in the patients with diabetic foot.

The first data regarding IMA levels in patients with DM were reported by Piwovar et al. (28). The same study found that chronic oxidative stress triggered by hyperglycemia could increase IMA production by decreasing the cobalt-binding characteristic of albumin. Their study determined higher IMA levels in the patients with DM compared with the healthy control subjects, but no significant difference in terms of IMA levels was determined among patients with DM with various levels of vascular complication. It was also reported that IMA values in patients with good glycemic control (HbA1c of ≤7%) were lower than in those with poor glycemic control (28).

IMA levels have been shown to increase in patients with DM and even further in patients with diabetic nephropathy (29) or diabetic retinopathy (30). It was also reported that detecting microvascular hypoxia at an early stage is important in preventing potential retinal complications and IMA can be beneficial as an indicator to determine diabetic retinopathy risk (30).

Another study found high IMA levels in patients with lower extremity ischemia, along with serum albumin levels within the reference range but no acute coronary syndrome, and it was stated that IMA could be useful for the diagnosis of peripheral ischemia (31). IMA levels have been reported to increase in patients with type 2 DM with peripheral arterial disease compared to those without peripheral arterial disease. IMA has been shown to be an independent risk factor for peripheral arterial disease (32).

Gaze et al. (33) reported that IMA levels are influenced by changes in serum albumin levels and there is a strong negative correlation between IMA and low albumin levels (≤3.4 g/dL), although this is much less marked

across the reference interval for albumin. Albumin levels should be taken into account when interpreting IMA levels. Low albumin can also be the cause of high IMA levels in hypoalbuminemic patients besides the ischemia. Therefore, the use of an albumin-adjusted correction has been proposed.

In another study, IMA was shown to have high sensitivity and a negative predictive value in excluding acute coronary syndrome (34). In contrast to troponins that are released when necrosis occurs in cardiac tissue, IMA is regarded as a marker of myocardial ischemia (35). IMA is not specific for cardiac ischemia and also increases noncardiac ischemic disorders such as peripheral vascular disease, end-stage renal disease, cerebrovascular disease, and acute infections (8).

In this study, high IMA levels may be related to the oxidative stress caused by hyperglycemia, as reported in the literature. The fact that IMA levels are even higher in patients with diabetic foot than the diabetes group may be interpreted as a result of infection and peripheral ischemia. The significant decrease in IMA levels following amputation might be related to tissue reperfusion and the removal of necrotic and infected tissue.

Nather et al. (36) reported that significant univariate predictive factors for limb loss were age above 60 years, stroke, ischemic heart disease, nephropathy, peripheral vascular disease, peripheral sensorial neuropathy, HbA1c level, gangrene, and infection. The results of another study demonstrated that nephropathy, ischemic diabetic foot, and admission fasting blood glucose of >200 mg/dL, as well as history of previous amputation, are independent risk factors for amputation (37). In our study, statistically significant differences in CRP, HDL cholesterol, and albumin levels were seen in the amputated group, and foot ulcers in this group were graded from 3 to 5. Although serum creatinine levels were higher and PON1 activity was lower in the amputated group, there were no statistically significant differences. The limitation of our study was small sample size.

In conclusion, we suggest that low PON1 activity, high ox-LDL, and IMA values may play an important role in diabetic foot pathogenesis. The increased CRP levels and decreased HDL cholesterol and albumin levels in patients with diabetic foot may increase the risk of amputation. The use of these parameters in the follow-up of patients with DM may prevent the development of diabetic foot and lower extremity amputations. In order to reach a definitive judgment, further studies with a larger number of subjects are necessary.

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