Endocr (2009) 36:425–432
DOI 10.1007/s12020-009-9236-5

ORIGINAL ARTICLE

A novel indicator of widespread endothelial damage and ischemia in diabetic patients: ischemia-modified albumin

Kubilay Ukinc · Selcuk Eminagaoglu · Halil Onder Ersoz · Cihangir Erem · Caner Karahan · Arif Bayram Hacihasanoglu · Mustafa Kocak

Received: 23 April 2009 / Accepted: 4 August 2009 / Published online: 26 September 2009

Abstract Ischemia-modified albumin (IMA) is a novel marker of tissue ischemia. Nowadays, IMA is accepted as a marker of oxidative stress. In this study, we aimed at establishing an association between IMA and hyperglycemia, blood pressure, lipid parameters, microvascular complications, hsCRP, and microalbuminuria in type 2 diabetes patients without overt macrovascular disease and acute ischemia. Fifty type 2 diabetes mellitus patients without a history of macrovascular disease or end-stage renal disease were enrolled into the study. Age-matched 30 healthy individuals were also included in the study as a control group. Plasma IMA (0.329 ± 0.046 and 0.265 ± 0.045 AbsU; $P < 0.0001$) and hsCRP levels (0.51 ± 0.36 and 0.32 ± 0.17 mg/dl; $P < 0.0001$) were significantly higher in the diabetic group compared to healthy controls. IMA level was significantly correlated with hsCRP ($r = 0.76$; $P < 0.0001$), HbA1c ($r = 0.72$; $P < 0.0001$), microalbuminuria ($r = 0.40$; $P = 0.004$), systolic blood pressure ($r = 0.28$; $P = 0.049$), diastolic blood pressure ($r = 0.44$; $P = 0.005$), and HOMA-IR ($r = 0.42$; $P = 0.005$) levels in the entire diabetic subjects. In the diabetic patients group, presence of microalbuminuria was associated with a higher plasma IMA level (0.355 ± 0.035 and 0.265 ± 0.0045 AbsU; $P < 0.0001$, patients with microalbuminuria and control subjects, respectively). In the type 2 diabetes patients with nephropathy, IMA level (0.355 ± 0.035 and 0.311 ± 0.046 AbsU; $P = 0.002$) was determined higher compared to the diabetes patients without nephropathy. Diabetic patients without an overt cardiovascular disease still have a higher serum IMA level compared to healthy controls. The correlation of high plasma IMA levels with high hsCRP and microalbuminuria levels in diabetic subjects indicates the presence of a chronic ischemic process. Therefore, elevated IMA levels may indicate an underlying subclinical vascular disease in type 2 diabetes mellitus patients.

Keywords Ischemia-modified albumin · Type 2 diabetes · hsCRP · Microvascular complications · Diabetic nephropathy

K. Ukinc (✉)
Canakkale Onsekiz Mart Universitesi, Tip Fakultesi, Endokrinoloji ve Metabolizma Hastaliklari BD, 17100 Çanakkale, Turkey
e-mail: kukinc@comu.edu.tr

S. Eminagaoglu · C. Karahan
Department of Biochemistry, Karadeniz Technical University, Trabzon, Turkey

H. O. Ersoz · C. Erem · A. B. Hacihasanoglu · M. Kocak
Department of Endocrinology and Metabolism, Karadeniz Technical University, Trabzon, Turkey

Introduction

The most frequent cause of death in the world is macrovascular diseases such as myocardial infarction, stroke, sudden death, and peripheral artery disease. The most important underlying etiology of macrovascular diseases is atherosclerosis. As shown by the evidence of laboratory and clinical studies, atherosclerosis is not merely a simple lipid accumulation disease. Endothelial dysfunction and low grade systemic inflammation are complex situations which play a starter role in atherosclerosis development and also cause its progress [1–3]. Macrovascular diseases are the most frequent complications of type 2 diabetes and this process starts before the diagnosis of overt diabetes [4]. The most important reason for risk increase in diabetic patients, as shown in Hoorn study [5], is endothelial dysfunction and

subendothelial low grade systemic inflammation. The information obtained as a result of studies shown that type 2 diabetes and atherosclerosis share common pathogenic mechanisms [4].

Coexistence of insulin resistance, vascular inflammation, and high blood pressure risk factors causes endothelial dysfunction and damage, and this forms the first step of atherosclerosis. Lots of markers such as high sensitivity C-reactive protein (hsCRP), microalbuminuria, and hyperhomosysteinemia which clinically show the start of endothelial dysfunction, its intensity and extensity and also are powerful and predictive factors for macrovascular diseases that may develop have been determined [6–8].

The N-terminal end of human serum albumin is a temporarily binding site for divalent forms of metals like cobalt, copper, and nickel [9]. Ischemia-modified albumin (IMA) is the variant form of human serum albumin of which N-terminal end has been altered after it was exposed to oxidative stress and/or ischemia [10, 11]. Metal binding ability of N-terminal end is decreased after this molecular alteration [10, 11]. In clinical and experimental studies conducted so far, it has been demonstrated that IMA elevates depending on oxidative stress after acute ischemia and returns to normal levels in hours after reperfusion [12–15]. In the view of these studies, IMA is accepted as a marker of oxidative stress and it was determined to be associated with other oxidative stress markers [16, 17].

In this study, we aimed at establishing an association between IMA and endothelial damage and low grade systemic inflammation which are risk factors for the development of macrovascular disease in type 2 diabetes patients without overt macrovascular disease and evaluating whether it may be a risk factor for macrovascular diseases that may appear in the future.

Materials and methods

Patient selection

Type 2 diabetic patients who had been admitted to our endocrinology outpatient clinics were carefully evaluated for macrovascular diseases, 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. Fifty type 2 diabetic patients (22 women, 28 men; mean age: 51.84 ± 9.4 years) with or without microvascular diabetic complications were enrolled into the study. Thirty non-diabetic healthy subjects (15 men, 15 women; mean age: 51.1 ± 12.4 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.

Anthropometric measurements

Body weight and height were measured in the morning with light clothing and without shoes. Body mass index 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 microvascular complications

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 30–299 mg/day in a 24-h collected urine specimen on at least two consecutive occasions. Renal functions of the patients were determined by volume, creatinine levels from urine collected during 24 h. Urine creatinine was calculated by using creatinine clearance formula with urine volume and serum creatinine values [Creatinin Clearance = (Urine creatinin mg/Dl × urine volume ml)/(serum creatinin mg/dL × 1,440 min)].

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%, respectively. Peripheral insulin resistance (PIR) was calculated with the Homeostasis Model Assessment (HOMA-R) method [18]. hsCRP level was 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. For IMA measurement, blood samples were taken from the brachial vein immediately placed in ice and centrifuged at 3,000 rpm for 15 min and pipetted into Eppendorf tubes in the ED and stored at −80°C until analyzed (maximum 6 months). Reduced cobalt to albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method developed by Bar-Or et al. [19]. This is based on the principle of quantitative scanning of the free cobalts present after cobalt binding has taken place. The results were reported as absorbance units (ABSU). This means that high absorbance levels as a result of increased mounts of free cobalt in the environment can be determined. In our laboratory, the within-run % coefficient of variation for IMA assay is averaged to be 3.1% [20].

Statistical analysis

Statistical calculations were performed using SPSS 10.0. Basal values for relevant variables were compared with χ^2, 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.

Results

All diabetic and control group comparisons

Studied and measured parameters were compared between all diabetes patients and control groups at first. No difference was observed in terms of sex, age, smoking status, presence

Table 1 General characteristics and biochemical parameters of diabetic patients in comparison to healthy control subjects

	Diabetic group ($n = 50$)	Control subjects ($n = 30$)	*P*
Women (*n*) (%)	22 (44%)	15 (50%)	N.S
Men (*n*) (%)	28 (56%)	15 (50%)	N.S
Hypertension treatment (*n*) (%)	31 (62%)	15 (50%)	N.S
Smoking (*n*) (%)	16 (31%)	10 (30%)	N.S
Age (Year)	51.84 ± 9.4	51.1 ± 12.4	N.S
BMI (kg/m^2)	31.2 ± 5.2	32.9 ± 10.8	N.S
Total cholesterol (mg/dl)	173.7 ± 44.8	171.9 ± 39.3	N.S
HDL-cholesterol (mg/dl)	48.6 ± 11.4	48.40 ± 10.24	N.S
LDL-cholesterol (mg/dl)	123.4 ± 34.1	105,96 ± 33,20	N.S
Triglyceride (mg/dl)	118.4 ± 55.0	105.9 ± 33,2	N.S
ApoA1 (mg/dl)	134.3 ± 22.0	136.6 ± 24.5	N.S
ApoB100 (mg/dl)	90.4 ± 21.5	85.50 ± 25.91	N.S
Creatinine clearance (ml/min)	118,7 ± 52,6	120,52 ± 29,33	N.S
Fasting plasma Glucose (mg/dl)	149.1 ± 49.1	87.5 ± 10.1	$P < 0{,}0001$
HOMA-IR	5.75 ± 3.657	1.73 ± 1.21	$P < 0.0001$
IMA (AbsU)	0,329 ± 0,046	0.265 ± 0.045	$P < 0.0001$
hsCRP (mg/dl)	0.51 ± 0.36	0.32 ± 0.17	$P < 0.0001$
Systolic blood pressure (mmHg)	132.2 ± 18.9	119.2 ± 8.8	$P = 0.001$
Diastolic blood pressure (mmHg)	83.5 ± 11.1	73.9 ± 6.8	$P < 0.0001$
Microalbuminuria (mg/24 h) hours)	43,54 ± 73,085	3.23 ± 2.303	$P < 0{,}0001$
Duration of diabetes (years)	7.08 ± 5.1	-	-
HbA1c (%)	7.5 ± 1.9	-	-
Microvascular complications (n) (%)	30 (60%)	-	-
Retinopathy (*n*) (%)	27 (54%)	-	-
Nephropathy (*n*) (%)	21 (42%)	-	-
Neuropathy (*n*) (%)	36 (72%)	-	-

BMI body mass index, *HOMA-IR* homeostasis model assessment-insulin resistance, *IMA* ıschemia-modified albumin, *hsCRP* high sensitive C-reactive protein

of hypertension, BMI, lipid parameters, and creatinin clearence in comparison done between diabetes patients and control group (Table 1). Average duration of diabetes of diabetes patients was 7 year and their glycemic controls were at medium level (HbA1c = 7.5%). Microvascular complications were observed in 30 (60%) of diabetic group patients. Fasting blood glucose and HOMA-R measurements were found significantly high as expected in diabetic patient group compared to healthy group. Even though frequency of hypertension was similar in both groups, systolic blood pressure (132.2 ± 18.9 and 119.10 ± 8.80 mmHg; $P = 0.001$) and diastolic blood pressure (83.5 ± 11.1 and 73.80 ± 6.79 mmHg; $P < 0.0001$) were determined to be statistically significantly high in diabetic group patients compared to control group (Table 1). When both groups were compared in terms of parameters showing extensive subendothelial inflammation and endothelium damage, hsCRP (0.51 ± 0.36 and 0.32 ± 0.17 mg/dl; $P < 0.0001$) and microalbuminuria (43.54 ± 73.085 and 3.23 ± 2.303 mg/day; $P < 0.0001$) levels were significantly high in diabetic patient group compared to control group. Ischemia-modified albumin (IMA) levels (0.329 ± 0.046 and 0.265 ± 0.045 AbsU; $P < 0.0001$) were determined to be significantly high in entire diabetic patient group compared to control group (Table 1 and Fig. 1). Afterwards, correlation analyses were done within the entire diabetic group to put forth the association between IMA level and the parameters that cause endothelium dysfunction (metabolic control and blood pressure) and show subendothelial inflammation (hsCRP) and extensive endothelial damage (microalbuminuria). Positive correlations were determined between IMA level and hsCRP ($r = 0.76$; $P < 0.0001$), HbA1c ($r = 0.72$; $P < 0.0001$), microalbuminuria ($r = 0.40$; $P = 0.004$), systolic blood pressure ($r = 0.28$; $P = 0.049$), diastolic blood pressure ($r = 0.44$; $P = 0.005$),

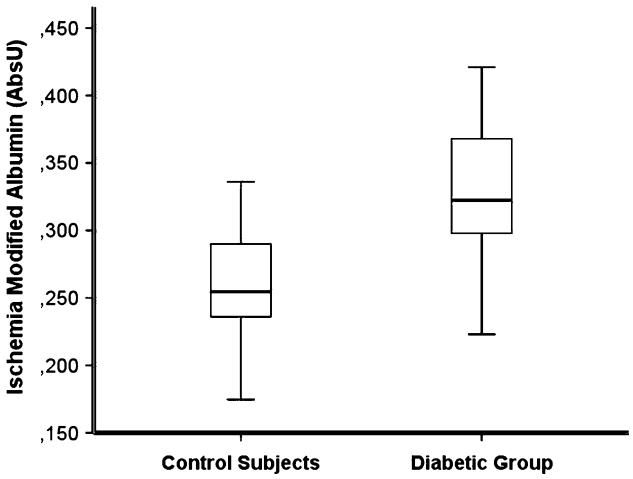

Fig. 1 Ischemia-modified albumin (IMA) levels of diabetic patients in comparison to healthy control subjects ($P < 0.0001$)

Table 2 Correlation analyses of ischemia-modified albumin level with HbA1C, hsCRP, microalbuminuria, systolic, and diastolic blood pressure levels in whole diabetic patients group

	r	*P*
HbA1C (%)	0.72	<0.0001
hsCRP (mg/dl)	0.76	<0.0001
Microalbuminuria (mg/24 h)	0.40	0.004
Systolic blood pressure (mmHg)	0.28	0.049
Diastolic blood pressure (mmHg)	0.44	0.001
HOMA-IR	0.42	0.005

hsCRP high sensitive C-reactive protein, *HOMA-IR* homeostasis model assessment-insulin resistance

and HOMA-IR ($r = 0.42$; $P = 0.005$) levels and values during correlation analyses done within the entire diabetic group (Table 2 and Fig. 2).

Subgroup analyses

Subgroups were constituted in diabetic patient group in order to evaluate that if IMA levels were different between groups with and without risk factors which may develop endothelial damage and progress it. Evaluations between subgroups, and between subgroups and control groups were done.

Metabolic parameters and blood pressure

Diabetic patients were divided into two groups as well (HbA1c < 7%) or poorly (HbA1c ≥ 7%) controlled according to metabolic control. Once we compared IMA levels of well and poorly metabolically controlled diabetic patients with control group separately, we determined that IMA levels in both diabetic groups were statistically significantly high compared to control group (Table 3 and Fig. 3). Also, in the evaluation between well and poorly metabolically controlled diabetic patients, IMA levels were significantly high in badly metabolically controlled group (Table 3 and Fig. 3). When diabetic patients with hypercholesterolemia ((LDL-Cholesterol ≥ 100 mg/dl)) and without hypercholesterolemia (LDL-Cholesterol < 100 mg/dl) according to lipid parameters were compared separately with control group, IMA levels of both diabetic groups were observed higher compared to control group. However, there was no difference in terms of IMA levels between diabetic patients with hypercholesterolemia or without hypercholesterolemia (Table 3 and Fig. 3). IMA levels of diabetic patients with and without hypertension diagnosis were determined significantly high compared to control group (Table 3 and Fig. 3). When hypertensive and normotensive diabetic patients were compared similarly, IMA concentration in

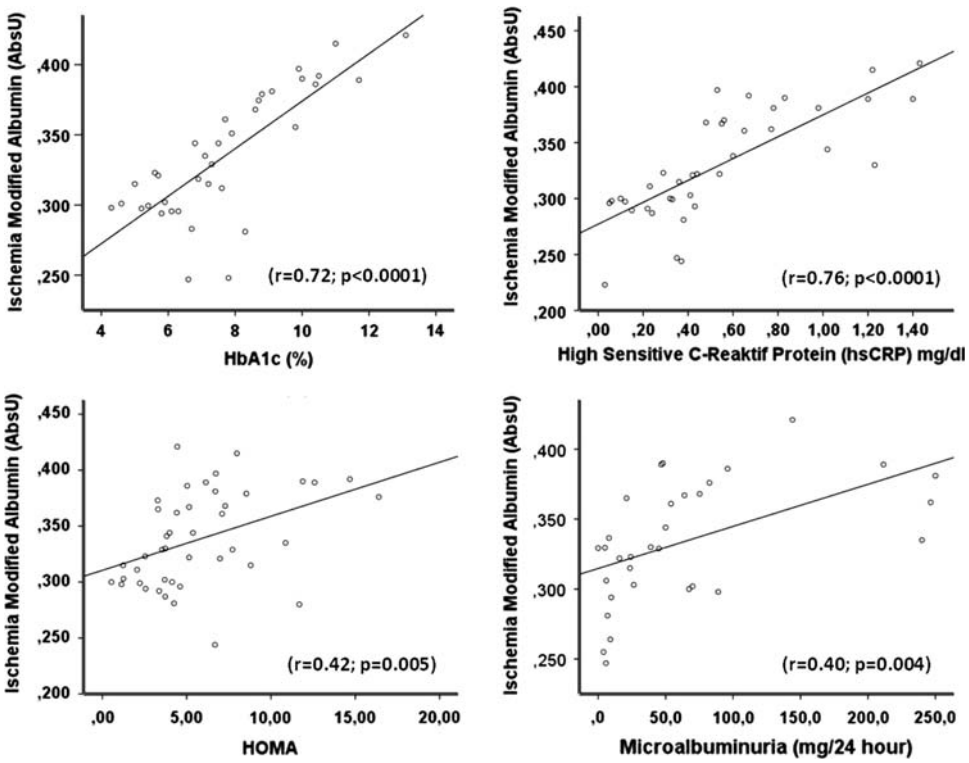

Fig. 2 Correlation analyses of ischemia-modified albumin level with HbA1C, hsCRP, microalbuminuria, systolic, and diastolic blood pressure levels in whole diabetic patients group

patients with hypertension diagnosis was significantly high compared to normotensive diabetic group.

Microvascular complications

In order to evaluate the IMA levels in presence and absence of microvascular complication, diabetic patients were divided into two groups as patients having or not having microvascular complications. When both groups were compared with control group separately, IMA levels of both diabetic groups were determined significantly high compared to control group (Table 3 and Fig. 3). Also, when patients with and without microvascular complications were compared, IMA concentration in patients with complication was statistically significantly high compared to patients without complication. When diabetic patients with and without diabetic nephropathy, neuropathy, and retinopathy were compared with control group separately, IMA levels in diabetic patient groups with complication was significantly high compared to control group (Table 3). As diabetic patients with and without neuropathy and retinopathy were compared, no difference was determined between IMA levels in both groups. However, IMA levels in diabetic group with nephropathy were significantly high compared to the group without nephropathy (Table 3).

Discussion

When a literature review is performed, it is seen that ischemia-modified albumin (IMA) results from oxidative stress which develops after acute ischemic events and endothelial hypoxia and correspondingly produced reactive oxygen types have been shown in many studies [12–15, 17]. In the view of present day evidence, IMA is accepted as the marker of oxidative stress and it has been determined to be associated with other oxidative stress markers [16, 17]. In this study, we principally determined that IMA levels elevated significantly in patients with type 2 diabetes without overt macrovascular disease and acute ischemic disease when compared to healthy control group. Furthermore, within the entire diabetics patient group, we found that IMA level was positively correlated with the parameters which cause endothelial dysfunction (metabolic control and blood pressure) and demonstrate subendothelial inflammation (hsCRP) and extensive endothelial damage (microalbuminuria). These results make us think that normal human serum albumin may change secondary not only to reactive oxygen radicals emerged as a result of acute ischemia but also to free oxygen types produced as a result of endothelial dysfunction. As reactive oxygen types are such molecules that are known to be responsible for damage on many biological molecules. Oxidative stress on endothelium elevates both physiological and pathological

Table 3 IMA levels in diabetes patients according to metabolic control status and presence of microvascular complications in comparison to healthy control subjects

Groups	Parameters	Number of subjects (*n*)	IMA (AbsU)	Analysis	*p*
Metabolic control	HbA1c ≥ 7%	23	0.355 ± 0.045	HbA1c ≥ 7% versus HbA1c < 7%	<0.0001
	HbA1c < 7%	27	0.299 ± 0.026	HbA1c ≥ 7% versus control subjects	<0.0001
	Control subjects	30	0.265 ± 0.045	HbA1c < 7% versus control subjects	0.009
Blood pressure	Hypertensive	32	0.346 ± 0.037	Hypertensive versus normotensive	0.011
	Normotensive	18	0.307 ± 0.049	Hypertensive versus control subjects	<0.0001
	Control subjects	30	0.265 ± 0.045	Normotensive versus control subjects	0.002
Lipid levels	LDL-C ≥ 100 mg/dl	36	0.334 ± 0.049	LDL-C ≥ 100 versus LDL-C < 100	N.S
	LDL-C < 100 mg/dl	14	0.327 ± 0.046	LDL-C ≥ 100 versus control subjects	<0.0001
	Control Subjects	30	0.265 ± 0.045	LDL-C < 100 versus control subjects	<0.0001
Microvascular complications (MC)	MC (+)	30	0.340 ± 0.040	MC (+) versus MC (−)	0.041
	MC (−)	20	0.312 ± 0.044	MC (+) versus control subjects	<0.0001
	Control subjects	30	0.265 ± 0.045	MC (−) versus control subjects	0.002
Nephropathy	Nephropathy (+)	21	0.355 ± 0.035	Nephropathy (+) versus nephropathy (−)	0.002
	Nephropathy (−)	29	0.311 ± 0.046	Nephropathy (+) versus control subjects	<0.0001
	Control Subjects	30	0.265 ± 0.045	Nephropathy (−) versus control subjects	<0.0001
Retinopathy	Retinopathy (+)	27	0.339 ± 0.047	Retinopathy (+) versus retinopathy (−)	N.S
	Retinopathy (−)	23	0.318 ± 0.044	Retinopathy (+) versus control subjects	<0.0001
	Control Subjects	30	0.265 ± 0.045	Retinopathy (−) versus control subjects	<0.0001
Neuropathy	Neuropathy (+)	30	0.355 ± 0.039	Neuropathy (+) vs.Neuropathy (−)	*P*:N.S
	Neuropathy (−)	18	0.322 ± 0.045	Neuropathy (+) versus control Subjects	<0.0001
	Control subjects	30	0.265 ± 0.045	Neuropathy (−) versus control subjects	0.002

P values are results of parametric Bonferroni Post Hoc tests

IMA ischemia-modified albumin, *LDL-C* LDL-Cholesterol, *MC* Microvascular complication

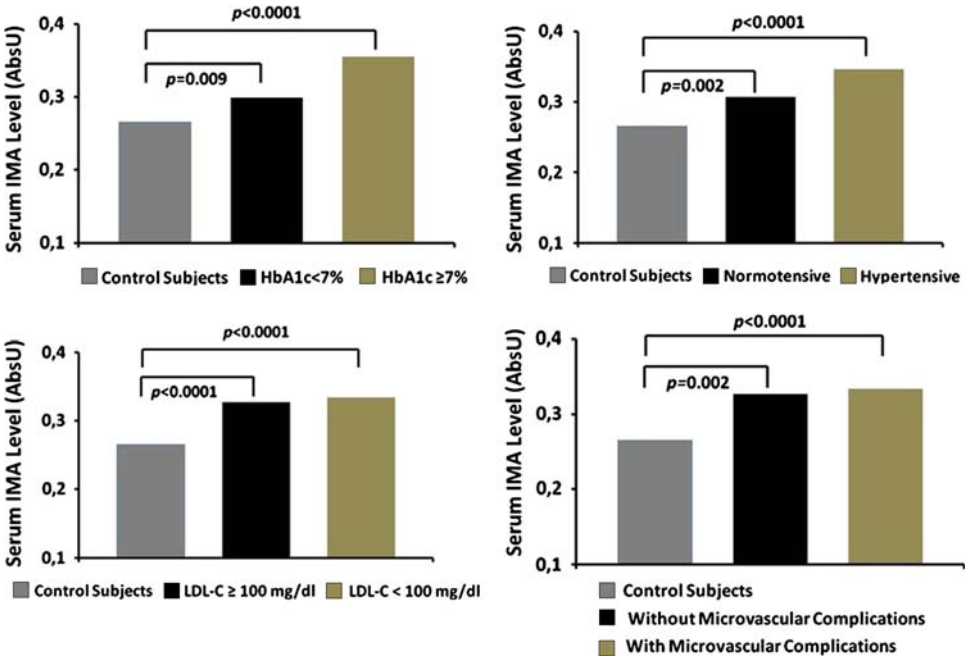

Fig. 3 IMA levels in diabetes patients according to metabolic control status (HbA1c, LDL-Cholesterol levels), presence of microvascular complications and hypertension in comparison to healthy control subjects

cases [21, 22]. As a consequence of increased oxidative stress, free oxygen radicals occur at non-compensated ratios. In diabetic patients, hyperglycemia is a major risk factor for starting diabetic endothelial stress and dysfunction [23]. During pathogenesis of complications seen on diabetes mellitus, many of biochemical disorders caused by

hyperglycemia result in oxidative stress. However, endothelial dysfunction developing on type 2 diabetes disease may occur with the effect of additional factors such as increased blood pressure, hypercholesterolemia, smoking, microalbuminuria as well as hyperglycemia. Although the underlying mechanisms of endothelial dysfunction development are multifactorial, substantially evidence suggests that increased reactive oxygen types cause this [24]. As the risk factors and oxidative stress in patients increase, along with biochemical changes, endothelial dysfunction develops and deteriorates. In all sub-group analysis of our study done with metabolic control, hypertension, and lipid levels, IMA levels in diabetic patient groups were noticeably high compared to control group. Increased oxygen types occur in patients in consequence of endothelial damage which develops in presence of all of these major risk factors that cause macro and microvascular complications. Although in vivo mechanism of IMA formation is not known precisely, reactive oxygen types seem to cause molecular changes on metal binding sites of albumin molecule [25, 26]. By endothelial dysfunction and free radical formation, albumin molecules of our diabetic patients may be structurally modified. High IMA levels of diabetic patients on our study compared to control group may be explained by this pathomechanism.

It has been shown that hsCRP levels are markers of systemic low grade inflammation and macrovascular diseases which may develop in the future [3, 8, 27]. In our study, we determined strong and significant positive correlation between IMA and hsCRP levels in the diabetic patient group. In the view of such a result, we think that increased IMA molecule in diabetic patients may be a novel marker for systemic low grade inflammation and even macrovascular diseases that may develop in diabetic patients as well as extensive endothelial dysfunction.

Microalbuminuria is the first and the most important marker that shows the presence of extensive endothelial damage and progression to macrovascular disease in diabetic and non-diabetic individuals [6]. In epidemiological studies, it has been set forth that presence of microalbuminuria is strongly associated with systemic endothelial dysfunction and macrovascular disease development [6]. First, there was a noticeable positive correlation between microalbuminuria levels and IMA levels in the entire type 2 diabetic patient group. In addition to this finding, IMA levels in type 2 diabetic patients with microalbuminuria were noticeably high compared to normoalbuminuric diabetic individuals in our study. Both of these two important results show that IMA levels elevate with the presence of extensive endothelial damage without macrovascular disease and/or acute ischemia.

When we evaluate IMA levels by dividing our diabetic patients as badly controlled (HbA1c $\geq$ %7) and well controlled (HbA1c $<$ %7), we determined that IMA levels elevate when glycemic control got worse. We also evaluate IMA levels with the presence of other conventional risk factors which cause and increase extensive endothelial damage on diabetic patients. With the presence of hypertension, IMA levels determined as higher compared to normotensive diabetics. In addition to that, an elevation on IMA levels by an increased microalbuminuria and hsCRP levels rise clearly exhibits the relation of IMA with other conventional risk factors and parameters that cause and/or show endothelial damage. We think this information demonstrates that IMA occurs as a result of endothelial damage and when the damage progress IMA levels increase. However, despite all of these outcomes, although IMA level of group whose LDL-cholesterol level seemed to be numerically high ($\geq$100 mg/dl) compared to the diabetic group whose LDL-cholesterol level is normal, it did not reach statistical significance. It was not actually an expected outcome. There may be several reasons for this outcome. First, fewness of patient number ($n = 14$) whose LDL-cholesterol level is $<$100 mg/dl might have prevented IMA levels from reaching statistical significance. Another reason is that instead of LDL-cholesterol level, if we have been examined small dense LDL-cholesterol level which is more atherogenic in diabetes patients, we might have obtained more significant results in this patient group that does not have macrovascular complication.

IMA levels were significantly high especially in diabetic patient group in which microvascular complications developed compared to those in which complications did not develop. However, this significance did reach to statistical significance only in patients with diabetic nephropathy. Although, we determined that IMA levels were high in diabetic patient groups with diabetic retinopathy and neuropathy compared to those who had not these complications, this difference was not statistically significant. In our study, we diagnosed diabetic nephropathy with the presence of at least two of these factors; increased blood pressure, microalbuminuria presence, and decreased creatinine clearance. While the patient whom we enrolled the study was forming type 2 diabetic group, since overt nephropathy and renal failure were our exclusion criteria, all of our diabetic patients in diabetic nephropathy group were hypertensive and had microalbuminuria. As it has been shown previously in the literature, presence of microalbuminuria is along with longer diabetes age, more distinctive dyslipidemia, and higher arterial blood pressure [28]. Features of our patients with diabetic nephropathy were supporting this literature knowledge. We may think that more distinctive and extensive endothelial damage occur in diabetic patients with the presence of these factors compared to the presence of retinopathy and neuropathy. Therefore, this more extensive endothelial dysfunction

might have caused that we found IMA levels significantly high in patients with diabetic nephropathy, although high in our patients with diabetic retinopathy and neuropathy it might have caused not to reach significance.

There is several weakness of the study. The majority of the examinations was positive and showed significant correlation not only with the factors that reflect endothelial damage or inflammation such as hsCRP but also with metabolic parameters such as HbA1c, blood pressures, and HOMA-IR, IMA may not be specific applicable to be a marker for the detection of endothelial ischemic damage in diabetic patients who are already at a high risk for cardiovascular disease. Significance of IMA to those metabolic parameters might reflect a chronic production of ROS, but on the other hand, there is a concern that the increased IMA was a response to other factors such as glycation of albumin by chronic hyperglycemia. This study demonstrates an elevation of IMA relative to the level in nondiabetic subjects. Previous studies have demonstrated elevated IMA in coronary heart disease and end-stage renal disease, so the event is not necessarily predictive of diabetes per se but rather the degree of cardiovascular disease accompanying diabetes. While it is a small study, we found it potentially important to clinical evaluation of diabetic macrovascular complications. This, of course, would need further verification in larger patient populations and by independent investigators but it is an intriguing initial observation.

Consequently, endothelial dysfunction is a characteristic marker of atherosclerotic vessel disease; also it is the most important predictive value showing that cardiovascular event ratio increase in long term [29]. In our study, IMA levels were found high in diabetic patients with no overt ischemic stress compared to healthy control group. We think that elevated IMA in diabetic patients is because of uncontrolled oxidative stress occurs on endothelium due to hyperglycemia and presence of other conventional risk factors and the effects of subsequently released reactive oxygen types on albumin. Since IMA levels were high in patients with microalbuminuria and it was positively associated with hsCRP and microalbuminuria levels, we think that we may accept IMA as a novel risk factor and a marker in diabetic patients for extensive endothelial dysfunction, low grade inflammation, and macrovascular disease that may develop in the future. In the view of all of these outcomes, IMA can be an additional parameter to show sub-clinic vascular disease.

Acknowledgments Part of this study was presented in 41st Annual Meeting of EASD (Athens–2005) printed as an abstract in Diabetologia 48(S1): A409;2005.

References

1. P. Libby, Circulation **91**, 2844–2850 (1995)
2. P. Libby, P.M. Ridker, Circulation **100**, 1148–1150 (1999)
3. R. Ross, N. Engl. J. Med. **340**, 115–126 (1999)
4. F.B. Hu, M.J. Stampfer, S.M. Haffner, C.G. Solomon, W.C. Willett, J.E. Manson, Diabetes Care **25**, 1129–1134 (2002)
5. J.J. de, J.M. Dekker, A. Kooy, P.J. Kostense, G. Nijpels, R.J. Heine, L.M. Bouter, C.D. Stehouwer, Arterioscler. Thromb. Vasc. Biol. **26**, 1086–1093 (2006)
6. C.E. Mogensen, P.L. Poulsen, Curr. Opin. Nephrol. Hypertens. **3**, 248–256 (1994)
7. F. Perticone, R. Ceravolo, A. Pujia, G. Ventura, S. Iacopino, A. Scozzafava, A. Ferraro, M. Chello, P. Mastroroberto, P. Verdecchia, G. Schillaci, Circulation **104**, 191–196 (2001)
8. N. Rifai, P.M. Ridker, Clin. Chem. **47**, 403–411 (2001)
9. P.J. Sadler, A. Tucker, J.H. Viles, Eur J. Biochem. **220**, 193–200 (1994)
10. P.O. Collinson, D.C. Gaze, J. Clin. Pathol. **61**, 1025–1028 (2008)
11. J. Dusek, J. St'asek, M. Tichy, J. Bis, J. Gregor, J. Vojacek, V. Masin, P. Polansky, M. Brtko, D. Cernohorsky, Clin. Chim. Acta. **367**, 77–80 (2006)
12. H. Abboud, J. Labreuche, E. Meseguer, P.C. Lavallee, O. Simon, J.M. Olivot, M. Mazighi, M. Dehoux, J. Benessiano, P.G. Steg, P. Amarenco, Cerebrovasc. Dis. **23**, 216–220 (2007)
13. R. Sharma, D.C. Gaze, D. Pellerin, R.L. Mehta, H. Gregson, C.P. Streather, P.O. Collinson, S.J. Brecker, Am. J. Kidney Dis. **47**, 493–502 (2006)
14. R. Sharma, D.C. Gaze, D. Pellerin, R.L. Mehta, H. Gregson, C.P. Streather, P.O. Collinson, S.J. Brecker, Clin. Sci. (Lond.) **113**, 25–32 (2007)
15. S. Turedi, A. Gunduz, A. Mentese, S.C. Karahan, S.E. Yilmaz, O. Eroglu, I. Nuhoglu, I. Turan, M. Topbas, Am. J. Emerg. Med. **25**, 770–773 (2007)
16. D. Borderie, Y. Allanore, C. Meune, J.Y. Devaux, O.G. Ekindjian, A. Kahan, Clin. Chem. **50**, 2190–2193 (2004)
17. M. Senes, N. Kazan, O. Coskun, O. Zengi, L. Inan, D. Yucel, Ann. Clin. Biochem. **44**, 43–47 (2007)
18. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Diabetologia **28**, 412–419 (1985)
19. D. Bar-Or, E. Lau, J.V. Winkler, J. Emerg. Med. **19**, 311–315 (2000)
20. S. Turedi, A. Gunduz, A. Mentese, B. Dasdibi, S.C. Karahan, A. Sahin, G. Tuten, M. Kopuz, A. Alver, J. Resusc. doi:10.1016/j.resuscitation.2009.06.007
21. M. Kalousova, A. Zak, J. Soukupova, S. Stipek, I.M. Malbohan, T. Zima, Cas. Lek. Cesk. **144**, 385–389 (2005)
22. E.R. Stadtman, B.S. Berlett, Drug Metab. Rev. **30**, 225–243 (1998)
23. S.C. Satchell, J.E. Tooke, Diabetologia **51**, 714–725 (2008)
24. T. Munzel, C. Sinning, F. Post, A. Warnholtz, E. Schulz, Ann. Med. **40**, 180–196 (2008)
25. G. Lippi, M. Montagnana, G.C. Guidi, Int. J. Cardiol. **108**, 410–411 (2006)
26. A. Piwowar, M. Knapik-Kordecka, M. Warwas, Dis. Markers **24**, 311–317 (2008)
27. P.M. Ridker, E. Danielson, F.A. Fonseca, J. Genest, A.M. Gotto Jr., J.J. Kastelein, W. Koenig, P. Libby, A.J. Lorenzatti, J.G. Macfadyen, B.G. Nordestgaard, J. Shepherd, J.T. Willerson, R.J. Glynn, N. Engl. J. Med. **359**, 2195–2207 (2008)
28. A.S. Ametov, T.I. Demidova, S.A. Kosykh, Klin. Med. (Mosk) **83**, 62–68 (2005)
29. J.A. Suwaidi, S. Hamasaki, S.T. Higano, R.A. Nishimura, D.R. Holmes Jr., A. Lerman, Circulation **101**, 948–954 (2000)