Research Article [Araştırma Makalesi]

Increased asymmetric dimethylarginine (ADMA) levels and decreased homocysteine thiolactonase/paraoxonase (HTLase/PONase) activities are related to the risk of cardiovascular disease in prediabetic/diabetic patients

[Prediyabetik/diyabetik hastalarda artmış asimetrik dimetilarjinin (ADMA) ve azalmış homosistein tiyolaktonaz/paraoksonaz (HTLase/PONase) aktiviteleri kardiyovasküler hastalık riski ile ilişkilidir]

Tülin Bayrak[1],
Ahmet Bayrak[2],
Mehlika Işıldak[3],
Erdinç Çakır[4],
Alper Gürlek[3],
Filiz Akbıyık[1]

[1]Hacettepe Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Ankara
[2]Ordu Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Ordu
[3]Hacettepe Üniversitesi Tıp Fakültesi, Endokrinoloji Bölümü, Ankara
[4]Gülhane Askeri Tıp Akademisi, Tıbbi Biyokimya Anabilim Dalı, Ankara

Correspondence Address
[Yazışma Adresi]

Tülin Bayrak

Ordu Üniversitesi Tıp Fakültesi
Tıbbi Biyokimya Anabilim Dalı, Ordu, Turkey.
Phone: +90 0452 2265200
E-mail: bayrakt09@gmail.com

ABSTRACT

Objective: Individuals with impaired glucose tolerance and/or impaired fasting glucose are at high risk, not only to develop diabetes mellitus, but also to experience cardiovascular disease later in their life. The aim of this study was to evaluate the cardiovascular risk markers (such as serum ADMA, homocysteine, high-sensitivity C-reactive protein, small dense LDL levels, paraoxonase/homocysteine thiolactonase and platelet-activating factor acetylhydrolase activities between patients with diabetes, prediabetes, and control group.

Methods: Subjects were grouped as Type 2 diabetes mellitus (n=20), IGT (n=16), IFG (n=19) and control group (n=23). Enzyme activities were measured spectrophotometrically. ADMA levels were measured by HPLC. Serum concentrations of homocysteine were measured by fluorescence polarization immunoassay. sLDL was measured by using heparin-magnesium precipitation method. h-CRP was measured by using immuno nephelometric method.

Results: Plasma ADMA levels were significantly lower in the control group than the IFG, IGT, DM groups (p<0.05, p<0.01, p<0.01 respectively). Serum HTLase activity was significantly higher in the control group than in the IFG, IGT, DM groups (p<0.01, p<0.01, p<0.001 respectively). No difference was found in plasma Hcy, sLDL, h-CRP concentrations and PAF-AH acivity between groups.

Conclusion: We concluded that increased ADMA levels and decreased HTLase/PONase activities in prediabetic patients can indicate that the risk of atherosclerosis in prediabetic stage.

Key Words: Paraoxonase, homocysteine thiolactonase, platelet-activating factor acetylhydrolase, ADMA, atherosclerosis, prediabetes, diabetes

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Bozulmuş glukoz toleransı ve/veya bozulmuş açlık glukozu olan bireyler hayatlarının ilerleyen safhalarında sadece diyabet gelişmesi değil aynı zamanda kardivasküler hastalık riski de taşırlar. Bu çalışmanın amacı diyabet, prediyabet ve sağlıklı kontrol grupları arasında kardiyovasküler risk belirteçlerinin (ADMA, homosistein, HsCRP, sLDL, paraoksonaz/ homosistein tiyolaktonaz ve trombosit aktive edicifaktör hidrolaz (PAFAH) gibi) düzeylerinin nasıl değiştiğini araştırmaktır.

Metod: Çalışma grubu Tip 2 diyabet (n=20), bozulmuş glukoz tolerans (IGT) (n=16), bozulmuş açlık glukozu (IFG) (n=19) ve kontrol group (n=23) olarak 4 gruba ayrıldı. Enzim aktiviteleri spektrofotometrik olarak ölçüldü. ADMA düzeyleri HPLC metodu ile tayin edildi. Serum homosistein düzeyleri floresans immuassay ile ölçüldü. sLDL heparin-magnezyum çöktürme yöntemi kullanılarak ölçüldü. h-CRP düzeyleri nefelometrik yöntemle ölçüldü.

Bulgular: Plasma ADMA düzeyleri kontrol grubunda IFG, IGT, DM grubuna göre anlamlı olarak daha düşüktü (sırasıyla p<0.05, p<0.01, p<0.01). Serum HTLase düzeyleri kontrol grubunda IFG, IGT, DM grubuna göre daha yüksekti (sırasıyla, p<0.01, p<0.01, p<0.001). Hcy, sLDL, h-CRP düzeyleri ve PAFAH aktivitesi gruplar arasında istatistik olarak anlamlı değildi.

Sonuç: Çalışmanın sonuçlarına göre prediyabetik hastalarda artmış ADMA düzeyleri ve azalmış HTLase/PONase aktivitesi prediyabetik evrede kardiyovasküler riski göstermektedir.

Anahtar Kelimeler: Paraoksonaz, homosistein tiyolaktonaz, trombosit aktive edici faktör asetil hidrolaz, ADMA, ateroskleroz, prediyabet, diyabet

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Diabetes mellitus (DM) is associated with an increased risk of cardiovascular disease (CVD) and mortality [1]. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are well characterized prediabetic conditions and about half of those proceed with the development of type 2 diabetes [2]. During the pre-diabetic state, the risk of a CVD is modestly increased [3-5], even in healthy people, increased blood glucose levels may lead to an increased risk of CVD [6]. It has been proposed that high glucose level is a cardiovascular risk factor, similar to hypercholesterolemia and hypertension [6]. Thus, vascular complications may already start in prediabetic stage and accelerated atherosclerosis has been documented in patients with DM [2,6].

Oxidative stress is a key factor in atherogenesis which is closely associated with the inflammatory response and bioactive lipid formation. Hyperglycemia, implicated in the pathogenesis of atherosclerosis, may promote oxidative modification of low-density lipoprotein (LDL) into oxidized LDL (OxLDL) [3,4]. Small dense LDL (sLDL) is considered highly atherogenic due to its higher penetration into the arterial wall and its susceptibility to oxidation [7]. Therefore, any mechanism that would decrease OxLDL should be antiatherogenic. The high density lipoprotein (HDL) particle(s) is known to prevent the formation of OxLDL by means of the HDL-associated enzymes paraoxonase (PONase) and platelet-activating factor acetylhydrolase (PAF-AH); their antioxidant properties prevent the accumulation of lipid peroxides on LDL [8,9].

Paraoxonase is a multifunctional antioxidant enzyme that not only can destroy OxLDL but also can detoxify the homocysteine thiolactone [8,10]. Jakubowski [10] suggested that PON1 possesses a thiolactonase activity; hydrolyzing Hcy thiolactone (HTL) to Hcy. excess of Hcy generates HTL, a chemically reactive metabolite that acylates free amino groups of lysine residues, causing protein alterations and cellular damage [10]. It has been shown that HTL can homocysteinylate LDL leading to its aggregation and enhanced uptake by endothelial cells in vitro [11]. HTL has been implicated as the molecular basis of Hcy-induced vascular damage [10]. In addition, hyperhomocysteinemia reduces the activity of enzyme dimethylarginine dimethylaminohydrolase (DDAH) which metabolizes asymmetrical dimethyl- L-arginine (ADMA) [12]. ADMA is an endogenous inhibitor of nitric oxide (NO) synthesis causing endothelial dysfunction. Recent studies indicated that ADMA may be a novel risk factor for endothelial dysfunction in humans [12].

Oxidation of LDL results in the formation of oxidized phospholipids and PAF-like molecules, which are potent bioactive lipids contributing to inflammation [9]. C-reactive protein (CRP) is a clinical marker of the state of inflammation and has been shown to be an independent predictor of atherosclerosis [13]. PAF-AH is another an-tioxidant enzyme associated with HDL. This enzyme is considered to play an important role in inflammatory diseases and atherosclerosis [9]. PAFAH degrades platelet-activating factor (PAF) and PAF-like molecules produced on inflammation and LDL oxidation, and is proposed to exert an antiatherogenic role.

The ADMA, Hcy, CRP, sLDL levels, PONase/HTLase and PAF-AH activities in an early DM state has not been evaluated yet. Several studies have examined mechanisms and potential etiological factors leading to the development of prediabetes [2-6]. Growing body of evidence suggests a strong relationship between prediabetes and CVD. The aim of the present study is to evaluate serum ADMA, Hcy, CRP, sLDL levels, PONase/HTLase and PAF-AH activities in IGT, IFG subjects and in diabetic patients.

Materials and Methods

Subjects with diabetes, IGT, IFG and normal glucose tolerance (control group) participated in this study. All participants underwent a standard oral glucose tolerance test (OGTT) except DM patients. Subjects with CVD, kidney or liver dysfunction or any inflammatory condition are not included in the study. The study was approved by the Hacettepe University Faculty of Medicine Ethics Committee (04.11.2004, FON 04/26-14) and all subjects had given written informed consent.

Clinical and demographic features of the subjects were recorded. Blood samples were drawn in the morning after an overnight fasting. Type 2 diabetes, impaired fasting glucose and IGT were defined according to the American Diabetes Association criteria (2003) using a 75 g oral glucose tolerance test [14]. The degree of insulin resistance was determined using Homeostasis Model Assessment Insulin Resistance (HOMA IR) index [15].

The body weight and height of the subjects were measured with subjects in light clothing and without shoes. Fasting venous blood sample was collected in tubes containing EDTA or in plain tubes. All blood samples were centrifugated at 1500xg for 5 min at 4 ºC.. For HTLase activity and ADMA, the serum aliquot was stored at −700C. Lipid profile was evaluated at the Laboratory of Clinical Pathology in Hacettepe University Hospital. Triglycerides, LDL-C and HDL-cholesterol (HDL) levels were measured spectrophotometrically using Roche Modular Systems. Plasma glucose concentrations were measured with the glucose oxidase method (Human Gesellschaft Wiesbaden Germany). Plasma insulin levels were measured using radioimmunoassays (Immunotech IRMA, Czech Republic). Serum homocysteine concentrations were measured by fluorescence polarization immunoassay (Abbott, Germany). hs-CRP levels were measured by using immune nephelometric method (IMMAGE Immunochemistry Systems; Beckman Coulter, California, USA).

Homocysteine thiolactonase activity

HTLase activity was measured spectrophotometrically

Table 1. Demographic characteristics in IFT, IGT, DM and controls.

	Type II DM (n=20)	IGT (n=16)	IFG (n=19)	Control (n=23)
Age	50.9±4.7	48.1±5.0	50.6±2.1	45.4±4.9
BMI (kg/m^2)	32.1±3.7	32.8±4.9	31.5±5.1	27.1±3.6
Waist circumference (cm)	102(90-119)	102(82-124)	102(82-127)	92(74-110)

using a modification of the method described by Billecke [16]. Homocysteine thiolactone was used as substrate at a final concentration of 2 mM in 50 mM Hepes buffer (pH 7.4). Thiolactone hydrolysis was measured at 37 °C, using Ellman's procedure [17] for monitoring at 412 nm the accumulation of free sulfhydryl groups reacting with DTNB. Enzymatic activity was calculated from the molar extinction coefficient of DTNB (€412= 13600 M-1cm-1) and corrected for the non-enzymatic hydrolysis. One unit of HTLase activity is defined as 1 µmol of substrate hydrolyzed per min, under the defined assay conditions.

Paraoxonase activity

PONase activity towards phenyl acetate was measured spectrophotometrically at 270 nm [17]. Reaction mixtures contained 50 mM Tris/HCl (pH 8.0), 1 mM CaCl$_2$, 1 mM phenyl acetate and 5-20 µl enzyme in a total volume of 1 ml. Assays were measured for up to 60 sec at 27ºC. The reaction was linear during this period. All rates were determined in triplicate and corrected for non-enzymatic hydrolysis. One unit of PONase activity is equal to 1 µmol of phenyl acetate hydrolyzed/min/mg protein.[18]

PAF-AH activity

PAF-AH activity was determined spectrophotometrically using a modification of the method described by Stafforini et al [19]. 2-thio-PAF (Cayman Chemical) was used as substrate at a final concentration of 1 mM in 100 mM Tris-HCl buffer (pH 7.4). Hydrolysis of thioester substrate was measured at 37 °C, using Ellman's procedure [17] for monitoring at 412 nm the accumulation of free sulfhydryl groups reacting with DTNB. Enzymatic activity was calculated from the molar extinction coefficient of DTNB (€412= 13600 M-1cm-1). One unit of PAF-AH activity is defined as 1 µmol of substrate hydrolyzed per min, under the defined assay conditions.

sLDL assay

We measured the sLDL-C using a modified version of the heparin-magnesium precipitation method described previously [20]. In brief, the precipitation reagent (0.2 mL) containing 150 U/mL of heparin sodium salt and 90 mmol/L MgCl2 was added to a serum sample (0.2 mL) and incubated for 10 min at 37 °C. After centrifugation at 5000×g for 1 min, sLDL and HDL were collected by filtering off the more buoyant lipoproteins. Then the penetrate solution containing sLDL-C and HDL-C was removed for the measurement of LDL-C by a direct and selective homogeneous assay method (LDL direct, Olympus Diagnostics, Hamburg, Germany). This direct LDL-C assay was performed with an Olympus AU-2700 autoanalyzer (Hamburg, Germany).

ADMA assay

Measurement of ADMA was accomplished by HPLC, using the method described by Chen et al. [21]. Briefly, to 1 ml plasma, 20 mg of 5-sulfosalisilic acid was added and the mixture was left in an ice-bath for 10 min. The precipitated protein was removed by centrifugation at 2000 for 10 min. Ten micro liters of the supernatant, which was filtered through a 0.2 µm filter was mixed with 100 µl of derivatization reagent (prepared by dissolving 10 mg o-phtaldialdehyde in 0.5 ml of methanol, and 2 ml of 0.4 M borate buffer (pH 10.0) and 30 µl of 2-mercaptoethanol were added) and then injected into the chromatographic system. Separation of ADMA was achieved with a 150×4 mm I.D. Nova-pak C18 column with a particle size of 5 µm (Waters, Millipore, Milford, MA, USA) using 50 mM sodium acetate (pH 6.8), methanol and tetrahydrofurane as mobile phase (A, 82:17:1; B, 22:77:1) at a flow-rate of 1.0 ml/min. The areas of peaks detected by fluorescent detector (Ex: 338 nm; Em: 425 nm) were used for quantification. The variability of the method was less than 7%, and the detection limit of the assay was 0.1µM.

Statistical analysis

SPSS software, version 11.0 was used for statistical analysis. P values less than 0.05 were judged as significant. Continuous variables with normal distribution were expressed as mean±SD, variables with skew distribution are expressed as median (minimum-maximum). For parametrically distributed data, comparisons between the groups were made using analysis of variance (ANOVA) followed by the Tukey honestly significant difference post hoc test. For nonparametrically distributed data, the Kruskal-Wallis and Man-Whitney U tests with Bonferroni correction were used where appropriate. Correlations between changes in variables were tested using the Pearson correlation test.

Results

A total of 78 subjects participated in our study. Twenty of them were previously diagnosed diabetics. Other subjects are grouped as IGT and IFG according to the American Diabetes Association criteria [14] following a 75 g oral
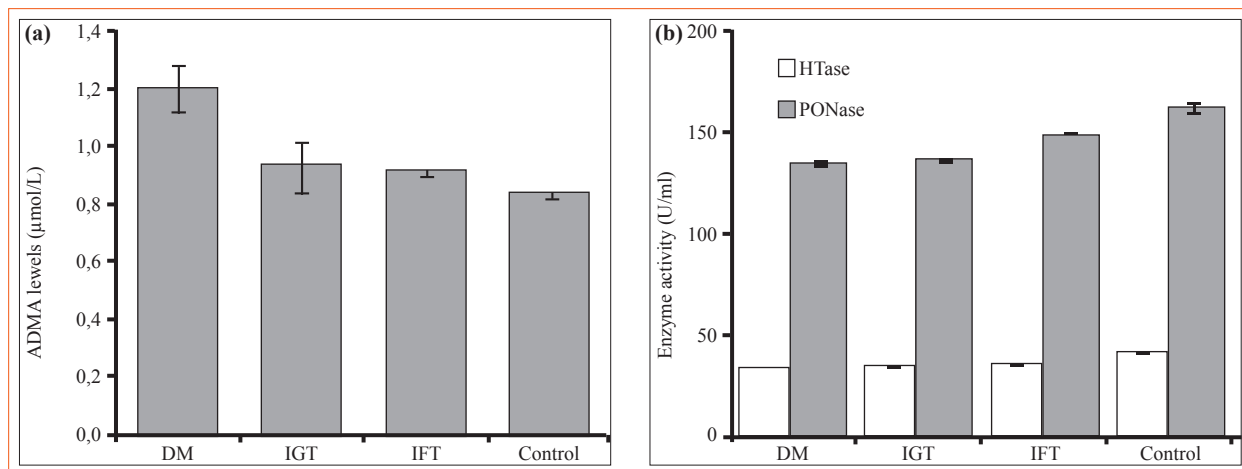
Figure 1. ADMA levels **(a)** and PONase, HTase activities **(b)** in patient with diabetes, IFG, IGT and control group.

glucose tolerance test; (1) IFG: fasting plasma glucose 100-125 mg /dl (2) IGT: 2h plasma glucose in the 75g OGTT 140-199 mg/dl. Subjects with normal glucose tolerance are taken as the control group.

Demographic features of the subjects are shown in Table 1. All four groups were similar regarding age and BMI. There was no significant difference in age, BMI and waist circumference between groups.

Plasma ADMA levels were significantly lower in the control group than the IFG, IGT, DM groups (p<0.05, p<0.01, p<0.01 respectively) (Figure 1a). Plasma ADMA levels were significantly lower in the IFG group than in the IGT, DM groups (p<0.05, p<0.05, respectively). Serum HTLase and PONase activities were significantly higher in the control group than in the IFG, IGT, DM groups (p<0.01, p<0.01, p<0.001 and p<0.01, p<0.01, p<0.01, respectively) (Figure 1b). Serum HTLase and PONase activities were significantly higher in the IFG group than the DM group (p<0.05, p<0.05, respectively). No difference was found in plasma Hcy, sLDL, Lp (a), hCRP concentrations and PAF-AH activity between groups. As shown in Table 2, HDL levels were significantly lower in the DM group compared to the control group (p<0.05). Serum triglycerides were significantly higher in the DM group compared to the control group (p<0.05) and IGT group (p<0.05). HOMA-IR was significantly higher in IFG, IGT, and DM groups than the control group (p<0.05, p<0.01, p<0.01, respectively). Insulin levels were significantly higher in IGT and DM subjects than the control group (p<0.05, p<0.01, respectively). In diabetic patients, HOMA-IR negatively correlated with HTLase activity (r=-0.488, p=0.029), ADMA levels negatively correlated with PONase activity (r=-0.455, p=0.022), PONase activity positively correlated with PAF-AH activity (r=0.615, p=0.020). In IGT group, HDL levels negatively correlated with homocysteine levels (r=-0.513, p=0.042) and BMI (r=-0.632, p=0.009), PAF-AH activity negatively correlated with CRP levels (r=-0.687, p=0.003). In IFG

groups, PONase activity negatively correlated with CRP levels (r=-0.939, p=0.017), ADMA levels negatively correlated with CRP levels (r=-0.512, p=0.025) and PONase activity (r=-0.699, p=0.01), sLDL levels negatively correlated with BMI (r=-0.463, p=0.046), Insülin levels positively correlated with BMI (r=0.654, p=0.002). In control groups, PAF-AH activity negatively correlated with sLDL(r=-0.451, p=0.031).

Discussion

Individuals with IFG and IGT are at high risk for DM 1-5). Studies evaluated the association between CVD and blood glucose levels even in non-diabetic people, but the results are inconclusive [3]. The mechanism of the association between prediabetes and CVD is not well known. In this study, serum HTLase activity, ADMA, and Hcy levels were investigated in the subjects with IGT and IFG. The data obtained were compared with healthy subjects.

PON1 hydrolyzes a wide range of substrates, such as esters, thioesters, phosphotriesters, carbonates, lactones, and thiolactones [8,10,11]. It is now well established that PON1 possesses HTLase activity that degrades an endogenous substrate, HTL, involved in the oxidative modification of proteins [10]. Thus, we evaluated HTLase activity in addition to PONase activity in prediabetic stage. Several studies have shown that HTLase and/or PONase activities were decreased in CVD and type 2 DM patients [22,24]. HTLase and PONase activities were found significantly lower in IFG, IGT and DM groups. We didn't find a significant difference in plasma homocysteine levels between the groups. It has been suggested that elevation of Hcy can be due to demographic, genetic, nutritional or metabolic factors [10]. HTLase activity was suggested to be inversely correlated with oxidative stress [22]. Research has shown that oxidized LDL concentration is negatively correlated with serum HTLase/PON1 activity [8]. Although no difference in Hcy levels, the decrease in HTLase activity could be due to the increased

Table 2. Biochemical characteristics of the DM, IGT, IFT, and control subjects.

Parameters	Typell DM n=20	IGT n=16	IFG n=19	Control n=23
Glucose (mg/dl)	114(82-257) [a]	98(75-139) [a]	108(99-124) [a]	84 (72-99)
LDL-C (mg/dl)	104(55-169)	127(80-179)	112(61-166)	114(61-159)
HDL-C (mg/dl)	46.3±8.1 [d]	46.8±9.8	49.7±9.4	56.6±14.3
Triglyceride (mg/dl)	146(62-278) [e]	196(55-385) [e]	123(57-230)	115(220-184)
sLDL (mg/dl)	13.1±0.9	11.2±1.2	10.5±0.9	9.4±0.81
Lp(a) (mg/dl)	18.7±10.2	23.5±15.2	20.3±10.8	27.7±10.4
Homocysteine(µmol/L)	12.3±0.8	12.4±0.9	12.7±0.6	11.3±0.5
İnsülin(µIU/ml)	16.2±8 [b]	14.2±7.9 [d]	12.9±5.1	9.6±4.4
HOMA-IR	3.1±0.5 [c]	2.8±0.3 [c]	2.5±0.4 [d]	1.6±0.3
h-CRP(mg/dl)	0.55±0.2	0.41±0.4	0.39±0.29	0.35±0.4
PAF-AH (U/ml)	0.129±0.01	0.130±0.02	0.132±0.02	0.134±0.01
ADMA (µmol/L)	1.2±0.08 [c,f]	0.93±0.04 [c,f]	0.91±0.06 [d]	0.83±0.05
HTase (U/mL)	33.7±0.8 [b,g]	34.1±2.1 [c]	35.3±1.0 [c]	41.3±4.7
PONase(U/mL)	134.7±6.7 [c,g]	136.1±3.7 [c]	148.5±5.1 [c]	161.8±10.1

[a]: p<0.001 vs control (p values obtained by Kruskal Wallis and Man-Whitney U test); [b]: p<0.001 vs control (p values obtained by ANOVA and Tukey post hoc test); [c]: p<0.01 vs control (p values obtained by ANOVA and Tukey post hoc test); [d]: p<0.05 vs control (p values obtained by ANOVA and Tukey post hoc test); [e]: p<0.05 vs control (p values obtained by Kruskal Wallis and Man-Whitney U test); [f]: p<0.05 vs IFG (p values obtained by ANOVA and Tukey post hoc test); [g]: p<0.05 vs IFG (p values obtained by ANOVA and Tukey post hoc test).

oxidative stress. Thus, decreased HTLase activity in pre-diabetic stage and diabetic patients could accounts for the increased homocysteinylation of proteins, which in turn leads to atherosclerosis. Moreover, reduction of HTLase activity might play central role in the atherosclerotic process, because HTLase activity modulate the susceptibility of LDL to atherogenic modifications such as glycation, oxidation and homocysteinylation.

ADMA is the major inhibitor of nitric oxide biosynthesis in humans. Clinical studies have been shown that high ADMA level is associated with endothelial dysfunction and CVD [12]. Elevated ADMA levels is considered as an early marker of the initial stage of atherosclerosis [12, 25]. Recent studies have been shown that in type 2 DM, increased ADMA level is the powerful indicator for MI and stroke [25, 26]. Hyperglycemia probably may increase ADMA concentration. ADMA is mainly metabolized by dimethylarginine dimethylaminohydrolase (DDAH) Lin et al. [26] hypothesized that hyperglycemia impairs the ADMA metabolizing enzyme activity (DDAH). In other study demonstrated that elevated glucose levels are capable of inhibiting DDAH activity in cultured endothelial cells [26]. Hyperglycaemia is associated with endothelial dysfunction both *in vivo* [27] and *in vitro*[28], therefore, endothelial dysfunction is an early feature in the development of vascular complications in people with diabetes These findings suggest that the elevated ADMA in diabetes could contribute to accelerated atherosclerosis in this population [29].

Studies comparing ADMA levels of prediabetic subjects with healthy people are inconclusive. Although some studies report increased ADMA levels in prediabetics [3, 30-32]. Eliana et al. [33] report no significant difference between groups. Associations between increased levels of ADMA and many cardiovascular risk factors such as age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, smoking and BMI have been documented [26]. In our study we found that ADMA levels were a little above normal value. We considered that this increase may be the result of insulin resistance, increased BMI and smoking.

In our study, plasma ADMA levels were significantly increased in the IFG, IGT, DM groups compared the control group (respectively, p<0.05, p<0.01, p<0.01). We suggest that increased ADMA levels could contribute to impaired endothelial function in prediabetic stage.

Recent studies have indicated that LDL particle concentrations, and specifically sLDL levels are predictive for coronary events and it is an independent coronary disease risk factor [7]. Studies show that subjects with the metabolic syndrome and/or DM have higher sLDL levels than controls [7,34,35]. In our study, we didn't find any significant difference in serum sLDL levels between the groups.

Since plasma PAF-AH activity provides a key defense against the actions of PAF and oxidized phospholipids, its decreased activity could lead to such pathological conditions as atherosclerosis and thrombosis [9]. Some studies demonstrate that patients with DM have decreased activity of PAF-AH [9,36]. Cavallo-Perin et al [37] did not find any difference in PAF-AH activity between groups of patients with DM1 and control subjects. In our study, we did

not find any significant difference between groups. Recently, studies have focused on the inflammatory risk factors of prediabetes and type 2 DM [35,38,39]. These studies indicate that higher hs-CRP levels is associated with DM, metabolic syndrome or prediabetes [13,35,38,39]. In the present study, we could not found a significant difference in plasma CRP levels between the groups.

In conclusion, our data showing increased ADMA levels and decreased HTLase/PONase activities in prediabetic patients can indicate the risk of atherosclerosis in prediabetic stage. Prediabetic people are under risk not only for DM but also for atherogenesis. The evaluation of the HTLase/PONase activities and ADMA levels may improve the early diagnosis of CVD in prediabetic patients.

Conflict of Interest

The authors declared no competing interest with any group.

References

[1] Decode Study Group, European Diabetes Epidemiology Group. Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? Diabetes Care 2003; 26(3):688-96.

[2] Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, et al. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract 2007; 78(3):305-12.

[3] Surdacki A, Stochmal E, Szurkowska M, Bode-Böger SM, Martens-Lobenhoffer J, et al. Nontraditional atherosclerotic risk factors and extent of coronary atherosclerosis in patients with combined impaired fasting glucose and impaired glucose tolerance. Metabolism 2007; 56(1):77-86.

[4] Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Arch Intern Med 2004; 164(19):2147-55.

[5] DeFronzo RA, Abdul-Ghani M. Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. Am J Cardiol 2011; 108(3 Suppl):3B-24B.

[6] Gerstein HC. Glucose: a continuous risk factor for cardiovascular disease. Diabet Med 1997; 14 Suppl 3:S25-31.

[7] Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation 2006; 113(1):20-9.

[8] Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, et al. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101(8):1581-90.

[9] Wegner M, Araszkiewicz A, Pioruńska-Mikołajczak A, Zozulińska-Ziółkiewicz D, Wierusz-Wysocka B, et al. The evaluation of IL-12 concentration, PAF-AH, and PLA(2) activity in patients with type 1 diabetes treated with intensive insulin therapy. Clin Biochem 2009; 42(16-17):1621-7.

[10] Jakubowski H. Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation. J Biol Chem 2000; 275(6):3957-62.

[11] Bełtowski J. Protein homocysteinylation: a new mechanism of atherogenesis? Postepy Hig Med Dosw (Online) 2005;59:392-404.

[12] Tousoulis D, Siasos G, Oikonomou E, Stougianos P, Papageorgiou N, et al. Asymmetric dimethylarginine (ADMA): is really a biomarker for cardiovascular prognosis? Int J Cardiol 2011; 153(2):123-5.

[13] Sabanayagam C, Shankar A, Lim SC, Lee J, Tai ES, et al. Serum C-reactive protein level and prediabetes in two Asian populations. Diabetologia 2011; 54(4):767-75.

[14] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2004; 27 Suppl 1:S5-S10.

[15] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7):412-9.

[16] Billecke S, Draganov D, Counsell R, Stetson P, Watson C, et al. Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. Drug Metab Dispos 2000; 28(11):1335-42.

[17] Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7:88-95.

[18] Gan KN, Smolen A, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 1991; 19(1):100-6.

[19] Stafforini DM, McIntyre TM, Prescott SM. Platelet-activating factor acetylhydrolase from human plasma. Methods Enzymol 1990; 187:344-57.

[20] Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. J Lipid Res 2003; 44(11):2193-201.

[21] Chen BM, Xia LW, Zhao RQ. Determination of N(G),N(G)-dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997; 692(2):467-71.

[22] Lakshman MR, Gottipati CS, Narasimhan SJ, Munoz J, Marmillot P, et al. Inverse correlation of serum paraoxonase and homocysteine thiolactonase activities and antioxidant capacity of high-density lipoprotein with the severity of cardiovascular disease in persons with type 2 diabetes mellitus. Metabolism 2006; 55:1201-6.

[23] Bayrak A, Bayrak T, Volkan-Salancı B, Deniz A, Yavuz B, et al. Serum Poni Activity but not Q192R Polymorphism is related to the Extent of Atherosclerosis. J Atheroscler Thromb 2012; 19(4):376-84.

[24] Connelly PW, Zinman B, Maguire GF, Mamakeesick M, Harris SB, et al. Association of the novel cardiovascular risk factors paraoxonase 1 and cystatin C in type 2 diabetes. J Lipid Res 2009; 50(6):1216-22.

[25] Krzyzanowska K, Mittermayer F, Wolzt M, Schernthaner G. Asymmetric dimethylarginine predicts cardiovascular events in patients with type 2 diabetes. Diabetes Care 2007; 30(7):1834-9.

[26] Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, et al. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 2002; 106(8):987-92.

[27] Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, et al. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. J Am Coll Cardiol 1999; 34(1):146-54.

[28] Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997;

96(1):25-8.

[29] Jawalekar SL, Karnik A, Bhutey A. Risk of cardiovascular diseases in diabetes mellitus and serum concentration of asymmetrical dimethylarginine. Biochem Res Int 2013; 2013:189430.

[30] Anderson JL, Carlquist JF, Roberts WL, Horne BD, May HT, et al. Asymmetric dimethylarginine, cortisol/cortisone ratio, and C-peptide: markers for diabetes and cardiovascular risk? Am Heart J 2007; 153(1):67-73.

[31] Konukoglu D, Firtina S, Serin O. The relationship between plasma asymmetrical dimethyl-L-arginine and inflammation and adhesion molecule levels in subjects with normal, impaired, and diabetic glucose tolerance. Metabolism 2008; 57(1):110-5.

[32] Surdacki A, Kruszelnicka O, Rakowski T, Jaźwińska-Kozuba A, Dubiel JS. Asymmetric dimethylarginine predicts decline of glucose tolerance in men with stable coronary artery disease: a 4.5-year follow-up study. Cardiovasc Diabetol 2013;12:64.

[33] Eliana F, Suwondo P, Makmun LH, Harbuwono DS. AADMA as a marker of endothelial dysfunction in prediabetic women. Acta Med Indones 2011; 43(2):92-8.

[34] Yoshino G, Hirano T, Kazumi T. Atherogenic lipoproteins and diabetes mellitus. J Diabetes Complications 2002; 16(1):29-34.

[35] Nakano S, Kuboki K, Matsumoto T, Nishimura C, Yoshino G. Small, dense LDL and high-sensitivity C-reactive protein (hs-CRP) in metabolic syndrome with type 2 diabetes mellitus. J Atheroscler Thromb 2010; 17(4):410-5.

[36] Gomes MB, Cobas RA, Nunes E, Castro-Faria-Neto HC, da Matta MF, et al. Plasma PAF-acetylhydrolase activity, inflammatory markers and susceptibility of LDL to in vitro oxidation in patients with type 1 diabetes mellitus. Diabetes Res Clin Pract 2009; 85(1):61-8.

[37] Cavallo-Perin P, Lupia E, Gruden G, Olivetti C, De Martino A, et al. Increased blood levels of platelet-activating factor in insulin-dependent diabetic patients with microalbuminuria. Nephrol Dial Transplant 2000; 15(7):994-9.

[38] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286(3):327-34.

[39] Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004; 53(3):693-700.