PARAOXONASE-1 ACTIVITY IN DIFFERENT PATIENT GROUPS WITH HIGH RISK OF ATHEROSCLEROSIS

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ABSTRACT

Objective*: Brachial artery intima-media thickness (B-IMT), paraoxonase (PON) and arylesterase (ARE) are accepted as markers of atherosclerosis. In this study, we aimed to determine the importance of B-IMT, PON and ARE in four patient groups who are expected to have increased risk of atherosclerosis without vascular complications.*

Materials and method*: A total of 100 patients were enrolled in this study. They were divided into 4 groups: Group-1 (32 prediabetic patients), group-2 (13 prediabetic with subclinical hypothyroidism patients), group-3 (20 subclinical hypothyroidic patients), and group-4 (35 diabetic patients). Age, gender, body mass index (BMI), smoking, alcohol consumption and menopause status of all patients were recorded. Routine biochemical tests and glycated haemoglobin (HbA1c), lipid profiles, homocysteine, homeostasis model assessment of insulin resistance (HOMA-IR), PON and ARE were determined and also B-IMT was measured.*

Results*: There were no significant differences in age, BMI, homocysteine, mean platelet volume, PON, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and B-IMT levels between the groups. We found significant differences in ARE (p=0.0001), and triglyceride (p=0.005) levels between the groups. There was a correlation between thyroid stimulating hormone levels and ARE levels (but not PON levels) in only group-2 patients. On the other hand ARE levels were correlated with HOMA-IR and insulin levels in group-2, and with HbA1c, glucose, LDL-C levels in group-4. Regarding PON levels, they were correlated with HbA1c levels in both group-1 and group-4, and with glucose, HOMA-IR and HDL-C levels in group-4 patients.*

Conclusion*: According to our study results, paraoxonase-1 activity (especially ARE) may be superior to B-IMT in detecting early atherosclerosis in such high risky group of patients.*

Key words*: Paraoxonase, arylesterase, brachial artery intima-media thickness, prediabetes, subclinical hypothyroidism, prediabetes mellitus.*

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Introduction

Cardiovascular disease is still the leading cause of mortality in many countries[(1)]. Atherogenesis is the most common cause of mortality and morbidity in patients with prediabetes or overt diabetes. In the last thirty years there has been an increase in the prevalence of early onset type 2 diabetes mellitus. The prevalence of diabetes was 7.2% in 2000 and had increased to 13.7% by 2010 in Turkey[(2, 3)]. Prediabetes or overt diabetes are known as predisposing factors for cardiovascular complications[(4)]. Atherogenesis starts before diabetes is diagnosed, but the effect of prediabetes on micro- and macro-vascular risk remains poorly defined[(5)].

Subclinical hypothyroidism (SCH) is a disorder that is defined as a condition with elevated serum levels of thyroid stimulating hormone (TSH) and normal serum concentrations of thyroid hormones by the absence of clinical signs and symptoms[(6)]. Overt hypothyroidism has been found to be associated with atherosclerotic heart disease, but SCH and its association with cardiovascular morbidity and mortality are still controversial[(7)]. The effect of SCH on atherosclerosis must be clarified.

Paraoxonase-1 (PON-1) is a high-density lipoprotein associated enzyme that appears to contribute to the antioxidant and anti-atherosclerotic capabilities of high-density lipoprotein cholesterol (HDL-C)[8]. There are some evidence that, low PON-1 levels are an independent risk factor for atherosclerotic disease[9]. Shih et al. found that, the incidence of major adverse cardiac events was significantly lower in participants in the highest PON-1 activity quartile (7.3% and 7.7% for paraoxonase and arylesterase, respectively) compared with those in the lowest activity quartile (25.1% and 23.5%; $p<0.001$ for paraoxonase and arylesterase, respectively)[10].

Intima media thickness measures structural changes in the arterial wall and is a well-established marker of (subclinical) atherosclerosis[11, 12]. An increased brachial artery intima media thickness (B-IMT) has been shown to be of prognostic value[13]. In this study we aimed to determine the importance of B-IMT, paraoxonase (PON) and arylesterase (ARE) in four patient groups which are expected to have increased risk of atherosclerosis without vascular complications.

Materials and methods

The study protocol was approved by local ethics committee of Bakirkoy Dr. Sadi Konuk Education and Research Hospital and was conducted in accordance with the Decleration of Helsinki. A total of 100 patients who were evaluated in our institution between February 2013 and September 2013 were included in the study. They were divided into 4 groups: Group-1 (32 prediabetic patients), group-2 (13 prediabetic with subclinical hypothyroidism patients), group-3 (20 subclinical hypothyroidic patients), and group-4 (35 diabetic patients). After full history and physical examination, their age, gender and body mass index (BMI) were recorded. Routine biochemical tests, lipid profiles, glycated haemoglobin (HbA1c), insulin, homocysteine, PON, ARE were performed and B-IMT was measured. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) as described by Matthews et al[14].

The prediabetic condition is defined as having either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). The former is determined when fasting blood glucose levels are between 100 mg/dl and 125 mg/dl, the latter is determined when blood glucose levels are between 140 mg/dl and 199 mg/dl-after the oral glucose tolerance test (OGTT)[15].

SCH was diagnosed by elevated TSH (>4 to 10 μU/mL) levels with normal free thyroxin (fT4) and free triiodothyronine (fT3) levels[16]. Exclusion criteria were as follow: Previous history of thyroid disease and its treatment, medications that could change thyroid hormone levels including amiodarone and corticosteroids, hypertension, diabetes mellitus, high serum creatinine, known atherosclerotic disease, presence of arrhythmias, psychiatric conditions and previous pregnancy in the past two years.

Type 2 diabetic patients who underwent oral antidiabetic therapy and diagnosed within the past five years were included in this study. Subjects who have microvascular and macrovascular complications of diabetes were excluded.

Levels of serum TSH, fT3 and fT4 were measured by immunochemiluminescence (Cobalt 6000, E601). Reference ranges for TSH, fT3 and fT4 were 0.27-4.2 μIU/m L, 2.5-3.9pg/ml and 0.933-1.7 ng/dL, respectively. Serum glucose, urea, creatinine, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglyceride, iron and total iron binding capacity (TIBC), ferritin, TSH, fT3, fT4 and other biochemical parameters were determined by Abbott Architect C16200 Integrated System and using commercial kits (Abbott Laboratories, IL, USA). Complete blood count was determined in a Coulter LH 750 auto analyzer (Beckman Coulter, CA, USA).

PON and ARE activities were determined using a novel automated measurement method developed by Erel (Relassay®, Turkey)[17]. Briefly, the rate of paraoxon hydrolysis was measured by the increased absorbance at 412 nm at 25°C. The PON activity is expressed as U/L serum. The coefficient of variation (CV) for individual samples was 1.8%. ARE activity was measured spectrophotometrically using phenyl acetate. The reaction was started by the addition of the serum; the increase in absorbance was read at 270 nm. Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol. One unit of ARE activity was defined as 1 μmol phenol generated/min under the defined assay conditions and expressed as U/L serum. The CV for individual serum samples was 3.3%.

Measurement of brachial artery intima-media thickness

The same radiologist has performed all ultrasonographic examinations of B-IMT measurements. After a 5 minute rest in supine position the brachial artery was examined in a longitudinal plane between antecubital fossa and axilla by continuous grey scale imaging with a linear, high resolution Dynamic Micro Slice (7-18 MHz) transducer. Ultrasonographic examinations were performed by Toshiba Aplio 500 (Toshiba Medical Systems Corporation, Nasu, Japan). Measurement of intima media thickness in the brachial artery was performed at the proper site where the intima media thickness was thought to be the thickest and where the clearest B mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained above the antecubital fossa. Intima media thickness was measured three times and B-IMT was defined as the mean of these three measurements.

Statistical analysis

In this study, statistical analysis was performed with NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA). Besides descriptive statistical methods (mean, standard deviation) for the evaluation of data, the one-way analysis of variance was used for comparisons between groups, Tukey's multiple comparison test for sub-group comparisons, chi-square test for qualitative comparisons of data, and Pearson's correlation test for determining the correlation of data. P value of<0.05 was considered as significance.

Results

Demographic features of patients were shown in Table-1.

There were no statistically significant differences between the groups in terms of the mean age, gender, BMI, menopause status and hypertension (p≥0.05).

There were no significant difference in right B-IMT and left B-IMT measurements between four groups (p≥0.05) (Table 2). Statistical differences were shown between other biochemical parameters in Table 3. While there were no statistically significant differences in the levels of PON between the groups (p=0.761), there were significant differences in the levels of ARE (p=0.0001) (Table 3).

		Group-1 n=32	Group-2 n=13	Group-3 n=18	Group-4 n=35	p value
Age (year)		54.39±12.58	49.62±13.21	44.85±10,67	51,77±12,87	0,064
Gender	Female	30 93.75%	11 84.62%	17 94.44%	34 97.14%	
	Male	2 6.25%	2 15.38%	1 5.56%	1 2.86%	0,457
BMI (kg/m^2)		33.19±8.41	32.62±5.08	29.8±4.26	29.54±6.72	0,105
Hypertension		14 43.75%	3 23.08%	7 35.00%	17 48.57%	0,399
Menopause status		14 43.75%	5 38.46%	6 30.00%	13 37.14%	0,8

Table 1: Evaluation of descriptive characteristics according to the groups.
Data are mean ± standard deviation. BMI: body mass index

	Group-1 n=32	Group-2 n=13	Group-3 n=18	Group-4 n=35	p value
Rigt B-IMT (mm)	0.36±0.12	0.28±0	0.29±0.08	0.35±0.1	0.425
Rigt B-Diameter (mm)	4.49±0.57	4.85±1.2	3.96±0.52	4.57±0.72	0.148
Left B-IMT (mm)	0.33±0.08	0.29±0.05	0.3±0.08	0.35±0.15	0.735
Left B-Diameter (mm)	4.29±0.66	5.15±1.2	3.86±0.26	4.34±0.63	0.061

Table 2: Evaluation of brachial artery intima-media thickness and brachial artery diameter results according to the groups.
Data are mean ± standard deviation. B-IMT: Brachial artery intima-media thickness, B-Diameter: Brachial artery diameter

	Group-1 n=32	Group-2 n=13	Group-3 n=18	Group-4 n=35	p value
HbA1c (%)	6.62±0.86	6.38±0.8	5.83±0.55	6.9±1.53	**0.009**
Glucose (mg/dl)	112.19±10.7	116.31±16.41	105.7±15.88	149.94±55.35	**0.0001**
Insulin (µIU/mL)	16.05±11.72	12.9±5.84	9.46±9.16	21.38±8.33	**0.0001**
HOMA-IR	4.41±3.3	3.61±1.28	2.31±1.96	8.1±4.43	**0.0001**
ALT (U/L)	19.19±6.26	19.69±4.84	17.1±5.93	25.17±11.57	**0.003**
TSH (µIU/mL)	1.39±0.5	6.62±1.26	6,53±1.42	2.3±1.57	**0.0001**
MPV (fL)	8.99±0.59	9.11±0.95	9.52±1.09	9±0.83	0.119
Homocysteine (mmol/L)	11.36±2.02	15.43±8.33	11.35±1.83	11.6±3.7	**0.012**
TG (mg/dl)	121.41±49.28	161.69±74.93	134.65±71.45	184.09±86.09	**0.004**
UMA (mg/dl)	15.41±19.55	7.33±8.78	29.41±34.02	55.91±56.14	**0.0001**
PON (U/L)	132.03±40.82	120.98±33.07	124.23±50.94	132.11±35.78	0.761
ARE (U/L)	191.38±40.49	141.73±38.1	164.71±41,42	149.9±37.27	**0.0001**

Table 3: Evaluation of laboratory results according to the groups.
Data are mean ± standard deviation. HOMA-IR: Homeostatic model assessment-insulin resistance, ALT: Alanine aminotransferase, TSH: Thyroid stimulating hormone, MPV: Mean platelet volume, TG: Trigliseride, UMA: Urinary microalbumin, PON: Paraoxonase, ARE: Arylesterase.

Posthoc analysis showed that this difference in ARE levels was between groups-1&2 and groups-1&4 (p=0.001, and 0.0001 respectively) (Table 4).

There was a correlation between ARE and TSH levels (but not PON) (r=0.594, p=0.032) in only group-2 patients. On the other hand, ARE levels were correlated with HOMA-IR (r=0.644, p=0.045),

and insulin levels (r=0.727, p=0.017) in group-2, and with HbA1c (r= -0.553, p=0.001), glucose(r=0.-652, p=0.0001), HOMA-IR (r=-0.680, p=0.0001), HDL-C levels (r=0.485, p=0.003) in group-4. Regarding PON levels, they were correlated with HbA1c levels in both group-1 and group-4 [(r=0.455, p=0.010) and (r=-0.567, p=0.0001) respectively], HOMA-IR (r=-0.707, p=0.0001), and HDL-C levels (r=0.373, p=0.027) in group-4 patients only (Table 5).

Tukey's multiple comparison test	Group 1&2	Group 1&3	Group 1&4	Group 2&3	Group 2&4	Group 3&4
HbA1c (%)	0.92	0.066	0.724	0.495	0.483	**0.005**
Glucose (mg/dl)	0.984	0.913	**0.0001**	0.827	0.019	**0.0001**
Insulin (µIU/mL)	0.819	0.141	0.208	0.781	0.066	**0.0001**
HOMA-IR	0.932	0.248	**0.002**	0.767	0.003	**0.0001**
BUN (mg/dl)	0.076	0.935	0.698	**0.037**	0.353	0.412
ALT (U/L)	0.998	0.817	**0.022**	0.82	**0.049**	**0.005**
TSH (µIU/mL)	**0.0001**	**0.0001**	0.089	0.998	**0.0001**	**0.0001**
Homocysteine (Nmol/L)	**0.012**	0.998	0.994	**0.023**	**0.018**	0.996
TG (mg/dl)	0.323	0.916	**0.003**	0.714	0.77	0.072
UMA (mg/dl)	0.919	0.58	**0.0001**	0.377	**0.001**	0.073
ARE (U/L)	**0.001**	0.09	**0.0001**	0.36	0.919	0.536

Table 4: Evaluation of laboratory results between the groups.
Data are mean ± standard deviation. HbA1c: Glycated haemoglobin, ALT: Alanine aminotransferase, TSH: Thyroid stimulating hormone, TG: Trigliseride, UMA: Urinary microalbumin, ARE: Arylesterase.

Group-1	Group-2	Group-3	Group-4
Right B-IMT and Hct*	ARE and insulin*	Right B-IMT and Glucose*	Rigt B-IMT and ALT*
Left B-IMT and c peptit*	ARE and TSH*	Right B-IMT and PLT**	Left B-IMT and ALT*,
PON and BMI*	ARE and HOMA-IR*	Left B-IMT and age*	Left B-IMT and HS*
PON and HbA1c*		Left B-IMT and PLT**	Left B-IMT and UMA*
PON and uric acid*			PON and ARE*
			PON and HbA1c **
			PON and Glucose**
			PON and HDL-C*
			PON and HOMA**
			ARE and HbA1c**
			ARE and Glucose**
			ARE and HDL-C*
			ARE and HOMA**

Table 5: Correlations between results of biochemical marker and brachial artery intima media thickness.
**Positive correlation, ** Negative correlation. B-IMT: Brachial artery intima-media thickness, PON: Paraoxonase, ARE: Arylesterase, Hct: Hematocrit, HbA1c: Glycated haemoglobin, TSH: Thyroid stimulating hormone, HOMA-IR: Homeostatic model assessment-insulin resistance, PLT: Platelet, ALT: Alanine aminotransferase, HS: Homocysteine, UMA: Urinary microalbumin.*

The correlation analysis in all patients revealed that there was positive correlation between right B-IMT and hematocrit (r=0.377, p=0.018). There were positive correlations between left B-IMT and age (r=0.411, p=0.009), and alanine aminotransferase (ALT) (r=0.501, p=0.001), and aspartate aminotransferase (AST) (r=0.322, p=0,046), and c-peptide (r=0.370, p=0,020), and homocysteine (r=0.349, p=0,029). Also, the findings revealed that there were negative correlations between ARE and HOMA-IR (r=-0.314, p=0,006), and glucose (r=-0.360, p=0.0001).

Discussion

Serum PON-1 is an HDL-C dependent enzyme that shows anti-atherosclerotic functions by inhibiting LDL oxidation. There is strong evidence that serum PON-1 activity is determinant for cardiac risk[18]. It has been shown that PON-1 activity decreases in patients with Type 2 diabetes. The reduction in PON-1 activity in diabetics is probably dependent on glycosylation of PON-1 enzyme. Reduction in PON-1 activity has also been suggested to play a role in endothelial dysfunction in diabetic patients and the pathogenesis of macrovascular complications in the long term[10]. In studies, low level of PON-1 activity in diseases like diabetes and metabolic syndrome is explained by the low level of HDL-C. Although there was no significant difference between HDL-C levels of our groups of patients, there was a positive correlation between PON-1 activity and HDL-C levels in diabetic patients. The emergence of this relationship despite the same HDL-C levels suggests that PON-1 activity may be an early indicator of atherosclerosis in diabetic patients. In addition, there was no any correlation between B-IMT and PON-1 levels in our patients, we should mention that the B-IMT measures were higher in diabetic group but not reached statistically significance (p≥0.05) (Table 2).

Paraoxonase acts as an antioxidant enzyme because of its oxidation protective feature for LDL and neutralization capacity for radicals, including hydrogen peroxide. ARE is considered as an indicator of the native protein which is not affected by the changes in PON. Even though the effect of subclinical hypothyroidism on atherosclerosis had not been clarified, possible atherosclerosis enhancing effect has been shown because of its effect on lipid profile, vascular endothelium and coagulability[19-21]. In our study, the level of PON was not different in all patient groups but ARE level was significantly lower in patients with diabetic and prediabetic with SCH groups com-

pared to the groups only with SCH and only with prediabetic (Table 3).

In addition, in group-2 patients, ARE was correlated with only insulin, TSH, and HOMA-IR levels (Table 5). These may be explained by the accompanied subclinical hypothyroidism and its atherosclerotic effect.

B-IMT increases proportionally with the increase in Framingham risk score. Other factors that may lead to an increase in B-IMT measures are smoking, high BMI, increase in systolic and diastolic blood pressure, age, heart rate, and glucose levels[22]. Iwamato et al. found the relationships between B-IMT and diabetes, hypertension and dyslipidemia. B-IMT could be used in determining the risk of atherosclerosis and the risk score used in determination of 10-year mortality[22]. In an autopsy study it has been shown that often atherosclerosis in the brachial artery could be available in young people[23]. Carotid intima-media thickness and B-IMT have been shown to be correlated[22]. But in our study, there were no differences in B-IMT values of four different patient groups and no correlation was found between PON-1 activity and B-IMT values as well. This may be attributed partially to the low sample size of the patients in our study. Although there was no significant difference in paraoxonase levels and B-IMT measures as well between the groups, there was a correlation between a relationship between B-IMT measures and hematocrit and c-peptide in group-1 and also there was a relation between it and glucose, platelet count, and age in group-3. On the other hand, B-IMT measurements were correlated with urinary albumin excretion amount, ALT, and homocysteine levels in group-4 patients (Table 5). This may be explained by the difference in the mechanism and pathophysiology of atherosclerosis in these different patients groups[24, 25].We should also mention that the group-4 consisted of uncomplicated and of less than five years duration of DM patients.

Limitations

As we mentioned above, one of the important limitations of our study was the small sample sizes in all patients groups, and selecting of uncomplicated and less than five years old DM patients. Another limiting point was the absence of healthy control group subjects in our pilot study.

Conclusions

Although further studies are needed in this field, our study results show that PON-1 activity (especially ARE) may be superior to B-IMT in detecting early atherosclerosis in such high risky group of patients.

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