Research Article

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Peroxisome proliferator-activated receptor gamma and osteoprotegerin levels as an indicator and diagnostic predictor of endothelial dysfunction

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Abstract

Objectives: Peroxisome proliferator-activated receptor gamma (PPAR-γ) modifies many cellular processes that contribute to atherosclerosis. The increased concentrations of osteoprotegerin (OPG) are related with coronary artery disease, calcification in vascular tissue, advanced atherosclerosis, and diabetic complications has been informed. The aim of our study was to define the relation among PPAR-γ Pro12Ala and, OPG and PPAR-γ in Peripheral Vascular Disease (PVD) and hypertension (HT). Also, it was aim to investigate the relationship between flow-mediated dilatation (FMD) in HT and ankle brachial index (ABI) in PVD in terms of endothelial dysfunction (ED).

Methods: Fifty-four patients with HT, 47 with PVD, and 52 healthy for the controls were included. Blood samples were used for analyzing PPAR-γ and OPG by Enzyme-Linked Immunosorbent Assay (ELISA), and biochemical assays. The PPAR-γ Pro12Ala was examined using TaqMan with PrimerProbMix. p value less than 0.05 was accepted as the limit of significance.

Results: The PPAR-γ was significantly decreased in both HT and PVD ($p<0.001$). The serum concentrations of OPG were higher in HT ($p<0.001$) and increased in diabetic ones ($p<0.05$). CG genotype of PPAR-γ Pro12Ala was more frequent in HT patients ($p<0.001$). In the HT patients, increased OPG and decreased PPAR-γ were found in CC ($p<0.001$). In the PVD patients, PPAR-γ levels decreased in carrying with CC ($p<0.05$).

Conclusions: It may be significant that increased OPG, as a marker of endothelial dysfunction, is found in HT. Moreover, decreased PPAR-γ in those who have to carry CC may be protective in both HT and PVD.

Keywords: osteoprotegerin; peroxisome proliferator-activated receptor gamma; hypertension; peripheral vascular disease; flow mediated dilatation; ankle brachial index

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Introduction

Peripheral vascular disease (PVD) is a chronic progressive atherosclerotic disease causing to peripheral vascular occlusion. It affects lower extremities [1]. The progression of vascular disease is associated with multiple genetic factors and environmental influences. The impairment of endothelial wall structure is a first occurring process in atherosclerosis and a predictor of cardiovascular events [2–4]. Endothelial dysfunction (ED) is associated with many diseases. Early detection of atherosclerosis is critical in the decrement of mortality and morbidity associated with cardiovascular events [5, 6].

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One of the molecules associated with endothelial dysfunction is nitric oxide (NO); its level is affected under oxidative stress conditions. In many chronic conditions such as diabetes, hypertension (HT), and hyperlipidemia, it is observed that the vascular endothelial structure is damaged by the emergence of oxidative stress.

The vasodilator or flow-mediated dilatation (FMD) test is used clinically to measure the NO release capacity of peripheral vascular endothelial cells in response to vasoconstrictive stimuli [7, 8]. FMD is a valuable index for many diseases, including hypertension. It is thought that biochemical markers such as osteoprotegerin (OPG) and peroxisome proliferator-activated receptor gamma (PPAR-γ), which are suggested to be related with atherosclerosis and cardiovascular events, may also be useful in determining endothelial dysfunction [9, 10].

In coronary artery disease (CAD), advanced atherosclerosis, and diabetic complications, increased OPG has been determined [11]. In addition to inflammation, OPG has been reported to increase risk factors such as hyperlipidemia, ED, diabetes and hypertension [9, 12]. OPG was thought to be an indicator of endothelial dysfunction, which is an early pathophysiological process in atherosclerosis [13]. Increased OPG concentrations are related to coronary artery disease, frequently accompanied by atherosclerosis and stroke [14, 15].

Regulation of vascular inflammation is important to prevent cerebrovascular and coronary artery diseases. PPAR-γ modifies cellular processes that contribute to atherosclerosis. The PPAR-γ activation on improving endothelial function may occur by suppressing inflammatory gene expression and inhibiting endothelial inflammation. PPAR-γ receptors are expressed by PPAR genes: PPARα, PPARβ/δ and PPAR-γ. The regulation of gene expression of related metabolism and inflammation in fatty tissue and macrophages is very critical [16, 17].

There are studies showing the relation between the PPAR-γ polymorphisms and complications in microvascular and macrovascular processes of coronary and carotid arteries in Type 2 Diabetes Mellitus (T2DM). Genetic polymorphisms of PPAR-γ have so far been reported to be related with metabolic and cardiovascular disorders [18, 19].

Recently, several polymorphisms of PPAR-γ have been identified. The most common variant of PPAR-γ is Pro12Ala (rs1805192) [20]. Few studies in the literature have associated the PPAR-γ Pro12Ala variant with elevated systolic blood pressure and diastolic blood pressure [21–23]. In a study with peripheral arterial disease, it was reported that the PPAR-γ2 Ala12 allele was associated with an increased risk of disease [24].

In light of this knowledge, we aimed to determine the relationship among PPAR-γ Pro12Ala variation and OPG and PPAR-γ levels in PVD and HT. In addition, it was aimed to investigate the relationships of these parameters with FMD in hypertension and ankle brachial index (ABI) in PVD in terms of ED.

Materials and methods

Subject selection

Our study was carried out with the Istanbul University Istanbul Medical Faculty Clinical Research Ethics Committee approval (2015/615).

Fifty-four patients with hypertension (40–74 years), 47 patients with PVD (41–82 years) as patients' group and 46 healthy adults (30–52 years) for control group were included to the study.

Hypertension group selected from patients admitted to Istanbul Medical Faculty, Department of Internal Medicine, Internal Medicine Outpatient Clinic. Existence of hypertension was described by blood pressure higher or equal to 130/80 mmHg or who were on hypertensive medication and also had additional factors such as hyperlipidemia, diabetes, obesity and smoking. Patients who had acute infection, coronary artery disease, myocardial infarction, cerebrovascular disease and pregnant were excluded.

Forty-seven patients with PVD who applied to the Peripheral Vascular Surgery Unit of Istanbul Medical Faculty General Surgery Department and newly diagnosed with atherosclerosis were included in the study. ABI was estimated by measuring the systolic blood pressure in the ankles and arms using a hand-held doppler and then calculating a ratio in patients with PVD. Hypothyroidism, pernicious anemia, concomitant renal failure, malignancy or severe psoriasis, and thiazide diuretics medication were taken as exclusion criteria. Salicylate was started at 100 mg/day postoperatively and general health examinations were performed at six-month intervals. In the last 10 years, any patient with a smoking habit has been identified as a smoker.

Forty-six adults who applied to the General Surgery Department and had no signs of vascular disease were included in the study as controls. Obese individuals, smokers or ex-smokers, pregnant, drug users, and hypertension were exclusion criteria for control group. Signed consent was obtained from all volunteers participating in the study.

Biochemical measurements

Venous blood samples collected in vacuum tubes after a 12 h fasting and were centrifuged. Serum was separated immediately. Biochemical parameters were analyzed by modular system (Roche/Cobas 8000 c702, Hitachi, Japan) in the same day. Total cholesterol, low density lipoprotein cholesterol (LDL-chol) and high density lipoprotein cholesterol (HDL-chol), triglyceride, fasting glucose, glycated haemoglobin A1c (HbA_1c), high sensitive C-reactive protein (hs-CRP) levels were analyzed and also body mass index (BMI) were calculated.

Blood samples were taken to second vacuum tubes for analyzing of PPAR-γ and OPG. Serum samples were removed immediately and stored at −80 °C until studied. PPAR-γ (detection range: 10–160 ng/L, minimum detection range: <1.0 ng/L, intra-assay precision: <9 %), and

OPG (detection range: 125–2,000 pg/mL, minimum detection range: <10 pg/mL, intra-assay precision: <9 %) were determined by ELISA (Abbkine, China, for both determination).

Genotyping of PPAR-γ Pro12Ala polymorphism

Genomic DNA was isolated from all groups by using DNA isolation kit (Jena Bioscience, Germany, PP213), and then stored at −80 °C until for assay of PPAR-γ variation. The genotyping of PPAR-γ (rs1801282) Pro12Ala was examined by using TaqMan System (Applied Biosystems, Life Technologies) with PrimerProbMix and qPCR ProbesMaster (Jena Bioscience, France, PCR-360). The isolated DNA was done to real-time polymerase chain reaction (RT-PCR) using VIC/FAM fluorescent dye in qPCR ProbesMaster following primers sequence AACTCTGGGAGATTCTCCTATTGAC[C/G]CAGAAAGCGATTCCTT-CACTGATAC in Real-Time PCR instrument (Biorad CFX). The implemented steps as follows; 2 min at 95 °C 1 cycle, 15 s at 95 °C 40 cycles, and 1 min at 60 °C 40 cycles.

Flow-mediated dilatation

In 27 HT subjects' endothelial functions were assessed by a single ultra-sonographer. FMD assays were executed on the right brachial artery with Vivid 7 Dimension ultrasonography (GE Vingmed, N-3190 Horten, Norwey) after eight to 12 h fasting. FMD is calculated as both the maximum absolute change and maximum percentage change in vessel diameter during reactive hyperemia [FMD=100×(diameter of after reactive hyperemia − diameter of baseline)/diameter of baseline] [25].

Statistical analysis

Statistical analysis was done using SPSS 21.00 Statistical Package Program. The distribution of numeric variables was done Kolmogorov–Smirnov test. While Student-t test was applied for parameters as normally distributed, Mann Whitney U test was used for non-normally distributed ones. Categorical parameters were evaluated Chi-square test. Correlation analyses were used Spearman and Pearson correlation tests (respectively for normal and non-normal ones). Multivariable logistic regression analysis was performed on the confounding factor of age, glucose, hs-CRP, OPG, PPAR-γ on hypertension and PVD. Sensitivity, specificity and area under curve (AUC) for OPG and PPAR-γ tests in HT and PVD groups were calculated with Receiver Operational Characteristics (ROC) analysis. p<0.05 was accepted statistically significant.

Results

Tables 1 and 2 show the distributions of demographic and biochemical characteristics for control, HT and PVD groups. There were no differences in gender distributions between HT patients and controls.

Table 1: Demographic and biochemical characteristics of the controls, HT, and PVD groups.

	Controls (n=46)	HT (n=54)	PVD (n=47)	p-Value[b,c]
Age	39±6	57±9	63±10	<0.001, <0.001
Gender, female/male	23/23	28/26	15/32	NS, NS
BMI, kg/m^2	23.9±3.3	30.0±5.1	–	<0.001
DM, %	–	42.6	97.7	–
Hypertension, %	–	100	46.8	–
Fasting blood glucose, mg/dL	88±14	114±36	176±101	<0.001, <0.001
Total cholesterol, mg/dL	191±37	219±46	170±43	<0.01, <0.05
LDL-chol, mg/dL	115±29	138±41	101±37	<0.01, NS
HDL-chol, mg/dL	52±15	53±14	30±11	NS, <0.001
Triglyceride, mg/dL	96±43	152±66	161±70	<0.001, <0.001
Hs-CRP[a]	0.80 (0.30–1.51)	2.55 (1.21–5.79)	88.4 (16.8–165.3)	<0.001, <0.001
Creatinine, mg/dL	0.77±0.15	0.79±0.20	1.87±1.84	NS, <0.001
PPAR-γ, ng/L[a]	59.8 (30.5–147.8)	32.6 (19.2–51.1)	41.2 (24.7–71.1)	<0.001, <0.05
OPG, pg/mL[a]	268.7 (177.5–576.8)	1,345.6 (538.8–3,526.7)	216.6 (131.3–693.3)	<0.001, NS

DM, diabetes mellitus; PVD, peripheral vascular disease; HT, hypertension; NS, non-significant; LDL-chol, low density lipoprotein cholesterol; HDL-chol, high density lipoprotein cholesterol; hs-CRP, high sensitive c-reactive protein; PPAR-γ, peroxisome proliferator-activated receptor gamma; OPG, osteoprotegerin. [a]Median (25–75 %); [b]HT group vs. controls; [c]PVD group vs. controls.

Table 2: Comparisons of serum PPAR-γ and OPG levels with DM presence in HT patients.

	DM (+) (n=23)	DM (−) (n=31)	p-Value
PPAR-γ, ng/L	23.6 (7.89–215.7)	38.4 (11.0–114.6)	0.167
OPG, pg/mL	3,331.9 (162.0–6,846.6)	880.2 (178.2–5,365.5)	0.033

Median (minimum − maximum) values are used. DM, diabetes mellitus; PPAR-γ, peroxisome proliferator-activated receptor gamma; OPG, osteoprotegerin.

Biochemical test results

Significant differences were recorded in the fasting blood glucose, total cholesterol, LDL-cholesterol, triglyceride, hs-CRP in hypertension (p<0.001, p<0.01, p<0.01, p<0.001, p<0.001, respectively) compared to controls. In HT group, there were no differences in HDL-cholesterol and creatinine compared to the control group (Table 1).

In the PVD group, blood glucose, total cholesterol, HDL-cholesterol, triglyceride, hs-CRP, and creatinine were significantly different compared to controls (p<0.001, p<0.05, p<0.001, p<0.001, p<0.001, p<0.001, respectively). There were no significant differences in LDL-cholesterol between PVD and controls (Table 1).

Biochemical tests related with endothelial functions

PPAR-γ and OPG levels

When serum PPAR-γ and OPG levels were determined as determinants of endothelial function, the levels of OPG were increased in HT patients significantly (p<0.001), but there were no differences between PVD and controls (p>0.05) (Table 1). Controversially, PPAR-γ levels were decreased in both HT and PVD groups compared with those of controls (p<0.001, p<0.05, respectively) (Table 1).

When we classified hypertensive group as diabetics and non-diabetics, the OPG levels were significantly increased (p<0.05) in diabetic hypertensive group compared with non-diabetic ones. There were lower levels of PPAR-γ in diabetic hypertensive group than non-diabetic ones but the difference was not significant (p>0.05) (Table 2).

PPAR-γ Pro12Ala

Table 3 shows the distributions of genotypes and alleles frequencies of PPAR-γ Pro12Ala in all study groups. The PPAR-γ Pro12Ala genotype distributions in both control (X^2=38.12, p=0.00) and PVD group (X^2=37.27, p=0.00) were found not to fit in Hardy–Weinberg equilibrium. In the HT group, it fit in Hardy–Weinberg equilibrium (X^2=1.18, p=0.275).

Table 3: Genotype and allele distributions of PPAR-γ Pro12Ala polymorphism in controls, HT and PVD groups.

	Controls, n (%)	HT, n (%)	PVD, n (%)
CC	39 (84.8)	41 (75.9)	38 (84.4)
CG	1 (2.2)	11 (20.4)	1 (2.2)
GG	6 (13.0)	2 (3.7)	6 (13.3)
p-Value		0.007[a], 0.99[b]	
C allele	79 (85.8)	93 (86.1)	77 (85.5)
G allele	13 (14.1)	15 (13.8)	13 (14.4)
p-Value		0.96[a], 0.95[b]	

PVD, peripheral vascular disease; HT, hypertension. [a]HT group vs. controls; [b]PVD group vs. controls.

Statistically significant differences were observed in frequencies between HT and controls in the PPAR-γ Pro12Ala genotype (p<0.001). Carriage of the Pro12Ala CG genotype was more frequent in HT than in the control group (p<0.001). There were no differences in C/G alleles compared to controls and HT groups (p>0.05). There were found similar distributions of genotype and alleles frequencies of PPAR-γ Pro12Ala between PVD and controls (p>0.05) (Table 3).

OPG and PPAR-γ levels in Pro12Ala C/G genotypes

There was a significant difference in serum PPAR-γ levels in terms of carrying the CC genotype among the control, HT and PVD groups (p<0.001). When we compared OPG levels with Pro12Ala genotypes, it was observed increased levels in whom have CC genotype (p<0.001) compared to controls (Table 4).

In PVD patients, PPAR-γ were significantly lower in those carrying the CC genotype than controls (p<0.05) (Table 4).

Correlation analysis

When we analyze the correlation between demographic and biochemical parameters in hypertensive patients, negative correlation was observed between PPAR-γ concentrations and systolic blood pressure (r: –0.283, p=0.038). Also, positive correlations were found between OPG and triglyceride (r: 0.470, p=0.001), hs-CRP (r: 0.348, p=0.016), HbA1c levels (r: 0.455, p=0.001), BMI (r: 0.328, p=0.016).

In the PVD group, between OPG levels and glucose (r: 0.398, p=0.01), hs-CRP levels (r: 0.322, p=0.040) were determined as positive correlations.

Endothelial dysfunction related parameters

Twenty-seven patients diagnosed with HT were stratified with the existence of ED assessed by the FMD assay [20]. Twelve subjects had a value of lower than 7.1 % of FMD was grouped ED (+), and the remaining 15 subjects had a higher 7.1 % of FMD, indicating ED (–). When we stratified the HT group according to FMD values, there were no significances in terms of age, systolic and diastolic blood pressures, glucose, total cholesterol, LDL-chol, HDL-chol, hs-CRP, HbA1c, PPAR-γ, and OPG values (p>0.05). In terms of gender distribution among the subgroups we created according to FMD, the number of females was found to be significantly lower in group with FMD <7.1 % (p=0.031).

Table 4: The serum levels of PPAR-γ and OPG in C/G genotypes of PPAR-γ Pro12Ala in controls, HT, and PVD groups.

	Controls (n=46)			HT (n=54)			PVD (n=47)		
	CC	CG[d]	GG	CC	CG	GG	CC	CG	GG
PPAR-γ, ng/L	59.8[a,e] (30.5–161.1)	35.0 (21.5–48.5)	61.3 (45.4–79)	28.9[c] (17.3–42.7)	48.4 (34.7–81.0)	119.7 (23.6–215.7)	38.4 (25.7–59.8)	–	41.4 (14.2–66.7)
OPG, pg/mL	244.4[a,b] (177.5–576.8)	388.3 (74.0–702.6)	277.5[b,d] (217.7–365.2)	909.6[c] (533.6–3,343.5)	2,483.2 (926.5–3,638.9)	3,054.6 (754.2–5,355)	208.8 (126.1–479.9)	–	169.9[d] (108.8–716.3)

PVD, peripheral vascular disease; HT, hypertension; PPAR-γ, peroxisome proliferator-activated receptor gamma; OPG, osteoprotegerin. [a]p<0.001, among controls, HT, and PVD groups; [b]p<0.001, vs. HT group; [c]p<0.001, vs. PVD group; [d]p<0.05, vs. HT group; [e]p<0.05, vs. PVD group.

The serum creatinine was significantly higher in the FMD <7.1 % subgroup (p=0.009) (Table 5).

In PVD and HT groups, ROC results corresponding to PPAR-γ (AUC: 0.615, 95 % confidence interval (CI): 0.512–0.711, sensitivity: 60.9 %, specificity: 58.5 %, p: 0.042 for PVD; AUC: 0.711, 95 % CI: 0.615–0.794, sensitivity: 35.2 %, specificity: 94.3 %, p<0.001 for HT) (Figure 1A and B), and to OPG (AUC: 0.562, 95 % CI: 0.458–0.661, sensitivity: 10.87 %, specificity: 98.11 %, p: 0.298 for PVD; AUC: 0.817, 95 % CI: 0.731–0.885, sensitivity: 85.2 %, specificity: 66.0 %, p<0.001 for HT) (Figure 2A and B) are presented.

Table 5: Demographic and biochemical characteristics in HT group according to classification of FMD >7.1 % and FMD <7.1 %.

	FMD >7.1 %	FMD <7.1 %	p-Value
Age	56.46±5.96	54.58±9.09	0.52
Gender, female/male	10/5	3/9	0.031[b]
Systolic blood pressure, mm Hg	135±13	134±9	0.69
Diastolic blood pressure, mm Hg	82±6	86±11	0.35
Fasting blood glucose, mg/dL	125±47	108±24	0.28
Total cholesterol, mg/dL	205±22	212±44	0.60
Triglyceride, mg/dL	136±63	142±29	0.78
LDL-chol, mg/dL	131±24	133±37	0.89
HDL-chol, mg/dL	49±11	50±11	0.77
Creatinine, mg/dL	0.70±0.13	0.89±0.19	0.009[c]
Hs-CRP, mg/dL[a]	2.97 (0.48–181.4)	1.97 (0.51–7.58)	0.394
HbA1c, %	6.5±1.1	6.3±1.0	0.54
PPAR-γ, ng/L[a]	18.4 (7.89–215.7)	41.0 (16.3–80.1)	0.172
OPG, pg/mL[a]	829.8 (162.0–6,846.6)	1,148.1 (178.2–5,355.0)	0.714

PVD, peripheral vascular disease; HT, hypertension; FMD, flow-mediated dilatation; LDL-chol, low density lipoprotein cholesterol; HDL-chol, high density lipoprotein cholesterol; hs-CRP, high sensitive c-reactive protein; PPAR-γ, peroxisome proliferator-activated receptor gamma; OPG, osteoprotegerin. [a]Median (minimum – maximum) values are used. [b]p=0.031, the number of female gender was significantly lower in the FMD <7.1 % subgroup; [c]p=0.009, the serum creatinine was significantly higher in the FMD <7.1 % subgroup.

Multiple logistic regression analysis was used to evaluate of age, glucose, hs-CRP, OPG, and PPAR-γ as independent variables, and PVD and HT diseases groups as dependent variables as separate models.

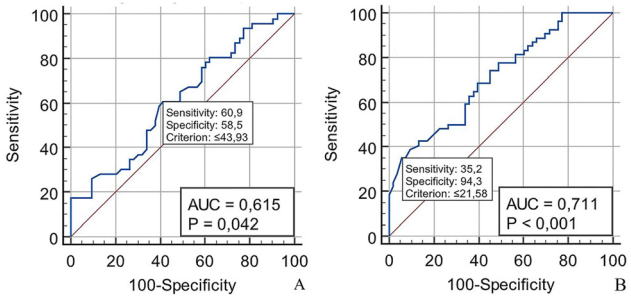

Figure 1: The ROC curves for PPAR-γ in the PVD and HT group.
(A) Area under the ROC curve (AUC) in the PVD group was 0.615 (95 % CI: 0.512–0.711), p: 0.042, sensitivity: 60.9 % specificity: 58.5 %.
(B) Area under the ROC curve (AUC) in the HT group was 0.711 (95 % CI: 0.615–0.794), p<0.001, sensitivity: 35.2 %, specificity: 94.3 %. Criterion: the smaller or larger size of this value than the specified number allows it to be evaluated as a criterion or indicator for the disease.

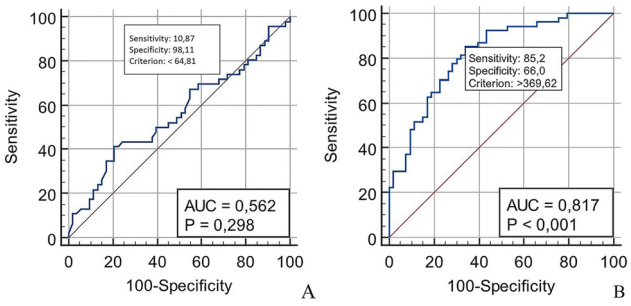

Figure 2: The ROC curves for OPG in the PVD and HT group.
(A) Area under the ROC curve (AUC) in the PVD group was 0.562 (95 % CI: 0.458–0.661), p: 0.298, sensitivity: 10.87 %, specificity: 98.11 %.
(B) Area under the ROC curve (AUC) in the HT group was 0.817 (95 % CI: 0.731–0.885), p<0.001, sensitivity: 85.2 %, specificity: 66.0 %. Criterion: the smaller or larger size of this value than the specified number allows it to be evaluated as a criterion or indicator for the disease.

When we set PVD as the independent variable, we found that hs-CRP, and age were significant in relation to vascular disease (p=0.005, Odds Ratio (OR): 0.014, 95 % CI: 0.001–0.269; p=0.000, OR: 0.001, 95 % CI: 0.000–0.032 respectively). We found that the independent variables of serum glucose and PPAR-γ were insignificant for PVD disease (p=0.097, OR: 0.116, 95 % CI: 0.009–1.478; p=0.404, OR: 2.889, 95 % CI: 0.239–43.961 respectively).

In the model we created for HT, independent variables PPAR-γ, OPG, and age were found to be significant risk factors (p=0.010, OR: 8.779, 95 % CI: 1.700–45.348; p=0.004, OR: 0.24, 95 % CI: 0.002–0.304; p=0.000, OR: 0.006, 95 % CI: 0.001–0.064 respectively). Serum hs-CRP was not found to be significant for the HT group (p=0.828, OR: 0.837, 95 % CI: 0.169–4.141).

Discussion

PVD affects about 10 million people in the world. PVD, an atherosclerotic disease, is usually associated with hypertension, diabetes and dyslipidemia [26, 27].

Endothelial dysfunction, which can be detected before structural changes in the arterial wall, is an early biomarker for atherosclerosis. In recent years, studies associated with endothelial dysfunction has an importance in assessment of the changes in the early phase of atherosclerosis which is important in determining of coronary vascular diseases (CVD) at early stage [28]. Therefore, the determinations of the serum levels of various markers such as PPAR-γ and OPG have gained importance.

PPAR-γ is a ligand-activated transcription factor and expressed in various cell types such as vascular smooth muscle cells (VSMC), endothelial cells (EC), monocytes and macrophages. PPAR-γ which regulates the transcription of various genes is associated with regulation of glucose and lipid metabolism. PPAR-γ has also anti-inflammatory and anti-atherogenic effects [14]. Therefore, PPAR-γ plays a protective role against many atherosclerotic diseases such as hypertension, diabetes and dyslipidemia.

Besides its known strong regulatory role in energy metabolism, there are studies showing that PPAR-γ is associated with the regulation of vascular function [29, 30]. It is also proposed that an association between PPAR-γ Pro12Ala and the progression of atherosclerosis [31]. It has been found a relationship between PPAR-γ Pro12Ala polymorphism and hypertension in a meta-analysis, and it has been thought that Ala allele may have a protective effect against hypertension [32]. Moreover, it has been suggested that PPAR-γ Pro12Ala polymorphism may be a locus leading to risk for progression of CAD [33]. Wei et al. informed that there was a close relationship between the levels of PPAR-γ and gene variation of PPAR-γ in atherosclerotic cerebral infarction [34]. In contrary, it has been suggested that there was no statistical relationship PPAR-γ gene polymorphism and the risk of atherosclerosis by Wang P et al. [31]. The studies on this issue are still controversial.

Osteoprotegerin is a soluble glycoprotein that is a member of tumor necrosis factor superfamily. It is expressed by various cell types and tissues such as osteoblasts, endothelial cells, vascular smooth muscle cells and heart. It is related to atherosclerosis and bone calcification. It plays a role in endothelial dysfunction and plaque development in atherosclerosis [6]. It is also an important indicator of inflammation [9]. In recent studies, it has been shown that OPG is an important regulatory molecule in various inflammatory diseases including cerebral atherosclerosis [35].

In our study, we determined the relationship between endothelial function and PPAR-γ and osteoprotegerin levels in both hypertension and PVD group. Also, we investigated whether Pro12Ala polymorphism was related to hypertension and PVD. We observed that serum PPAR-γ were found to be significantly lower in hypertension than controls. Also, PPAR-γ was lower in PVD than those of the controls. There was also negative correlation between PPAR-γ and systolic blood pressure in hypertensive group.

Kulkarni et al. informed statistically significant increment in PPAR-γ levels in patient with hypertensive type 2 diabetic patients compared with the control group [36]. It has been shown that PPARs have blood pressure lowering effects in another study [37]. In our study, decreased serum PPAR-γ in the hypertensive group and the negative correlation between systolic blood pressure and PPAR-γ were found to be consistent with the study of Akyurek et al. [38].

OPG levels were higher in hypertensive patients than controls in our study. Also, OPG was higher in patients with DM than those of the non-diabetic hypertensive patients. In the study performed by Blazquez-Medela et al., they reported that there was a relationship between OPG and cardiovascular risk in hypertension and/or diabetics [12]. These results show that increased OPG levels are associated with both endothelial dysfunction and vascular damage pathogenesis in patients with micro- and macrovascular complications of diabetes [39].

We observed positive correlations among OPG and BMI, triglyceride, hs-CRP and HbA_1c in hypertensive patients. In a study showing the relationship between OPG and atherosclerosis and inflammatory processes in patients with metabolic syndrome, it has been reported that positive correlations between OPG and CRP, and BMI were observed [40]. In another study performed in patients with peritoneal

dialysis, it has been suggested that OPG was significantly correlated with markers of systemic inflammation [41]. These results are consistent with the results of our study. Also, hs-CRP and triglyceride levels were significantly higher than those of the control group in patients with PVD. There was a positive correlation between OPG and hs-CRP in PVD group.

In our study, no significant differences in OPG levels between PVD and controls. In a study performed by Pennisi P et al., in atherosclerosis of peripheral vessels, there was no difference in serum OPG levels compared to controls [42].

At the same time, we aimed to investigate whether PPAR-γ Pro12Ala polymorphism was related to hypertension and PVD. It is well known both hypertension and PVD lead to endothelial dysfunction so that it functions a role in the pathogenesis of atherosclerosis.

The PPAR-γ Pro12Ala variation has also been shown to play a role in hypertension in many studies. On the other hand, there is research that PPAR-γ Pro12Ala causes oxidative stress in patients with T2DM [18, 19]. It has been suggested that polymorphisms of PPAR-γ may be related to the risk of T2DM, obesity and cardiovascular diseases [33]. But there are conflicting findings on this issue. In our study, there was significantly more frequent CG genotype of Pro12Ala in hypertensive patients. When we compare control group and PVD group, there were no differences both in terms of distributions of genotype and allele.

When we compare control group and hypertension group in terms of genotype distribution and allele frequency, there was no difference in allele frequency, while there was statistically a significant difference in genotype distribution.

When we compare genotype distribution with PPAR-γ and OPG in both hypertensive and PVD groups, significantly decreased serum PPAR-γ levels and increased OPG levels were found in patients with CC genotype than those of controls. In the PVD group, significantly decreased serum PPAR-γ levels were determined in those carrying the CC genotype of Pro12Ala.

FMD is an important determinant of endothelial function and it is valuable for evaluating the future risk of cardiovascular disease in hypertensive patients. In the literature, the risk of developing cardiovascular disease has been found in patients with low brachial artery FMD. In our study, we found that creatinine concentrations were higher significantly in the HT with FMD below 7.1 % compared to the group with FMD above 7.1 %, suggesting that endothelial dysfunction may be associated with impaired renal function [43].

In the results obtained from OPG and PPAR-γ ROC curves of HT group, the diagnostic values of these tests were found to be statistically significant for HT. In the PVD group, the OPG test did not show significance for the disease, however, it was found to be at the limit of significance for PPAR-γ and could have a diagnostic value as a test.

Evaluation of the ABI value in patients with PVD is very important in addition to other risk factors in the development of myocardial infarction, stroke and cerebrovascular events. Dyslipidemia, diabetes mellitus, smoking, age, gender and high blood pressure are risk factors in people with PVD, accelerating atherosclerosis and are associated with the development of cardiovascular and cerebrovascular diseases.

In our study, we found higher HbA_1c and cholesterol values in the group with an ABI value less than 0.9; it suggests that diabetes mellitus and serum cholesterol levels are significantly important in PVD patients with low ABI values, along with other risk factors.

It was found that age was a risk factor for both PVD and HT groups in the logistic regression models. In the logistic regression model, we found that hs-CRP continued to be significant for the PVD group, while high OPG and low PPAR-γ were risk factors for the HT group.

The limitation is the small number of participants in our patient and control groups. In future studies, with larger groups to be formed, the effects of PPAR-γ and osteoprotegerin on the disease etiopathology in both hypertension and peripheral arterial disease in larger subgroups.

Conclusions

It may be important that increased OPG levels, as an indicator of endothelial function, are found in patients with hypertension. Moreover, the decrements in PPAR-γ levels in those carrying the CC genotype may be a protective effect in both hypertensive and PVD.

Research ethics: This study was carried out with the approval of Istanbul University Istanbul Medical Faculty Clinical Research Ethics Committee (2015/615).
Informed consent: Informed consent was obtained from all individuals included in this study.
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