Research Article

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Serum adipokines and gene polymorphisms in peripheral vascular disease

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Abstract

Objectives: Peripheral vascular disease (PVD) is an atherosclerotic disease by the occlusion of arteries in the lower extremities. Buerger's disease is autoimmune due to inflammation of small and medium-sized vessels. The aim was to investigate the relations among adiponectin, leptin, resistin, and variants of *ADIPOQ -11,377 C/G, LEP2548G/A, RETN -420 G/C* in PVD and Buerger's disease.

Methods: The study group consisted of 151 patients (127 PVD, 24 Buerger's disease). Adiponectin, leptin, and resistin were analyzed by ELISA. *ADIPOQ –11377 C/G, LEP2548G/A, and RETN -420 G/C* were genotyped with designed primers, and probes in LightCycler 480.

Results: Adiponectin was found to be insignificantly higher in the PVD than that Buerger's disease (p=0.17). Leptin was found to be significantly lower in the Buerger's disease than in the PVD (p=0.018). The relationships were among age and leptin (r= −0.264; p=0.047), ankle brachial index (ABI) (r= −0.206; p=0.045), adiponectin (r=0.210; p=0.026) in PVD. In Buerger's disease, resistin and leptin had a significant relationship (r= −0.508; p=0.019).

Conclusions: It is thought that the high serum leptin levels observed in the Buerger group carrying the *ADIPOQ GG* genotype and the PVD group carrying the *RETN GG* genotype may be related to the etiopathogenesis of both diseases. Our study is the first to examine the proteins and genetic variations of adipokines in the PVD and Buerger's disease in Turkish population, and will provide direction for future studies to be conducted with the variants of leptin, adiponectin, resistin, and related proteins.

Keywords: leptin; resistin; adiponectin; peripheral vascular disease; buerger's disease

Introduction

Peripheral vascular disease (PVD) is a progressive atherosclerotic disease characterized by occlusion of arteries in the lower extremities [1, 2]. Inflammation is important for the initiation and progression of PVD.

Buerger's disease or thromboangiitis obliterans (TAO) is a disorder characterized by inflammation of small and medium-sized arteries and veins in the upper and lower extremities. It is a non-atherosclerotic disease and accounts for 40–60 % of peripheral vascular diseases [3].

Adiponectin has anti-inflammatory and anti-atherogenic effects. Low serum adiponectin levels are an independent risk factor for endothelial dysfunction and are associated with cardiovascular disease [4]. Symptomatic atherosclerotic peripheral arterial disease (PAD) has been associated with decreased adiponectin levels and impaired vascular reactivity [5]. One of the variants identified in the regulatory region of the adiponectin gene (*ADIPOQ*), the *–11377 C/G* variant, has been shown to affect serum adiponectin and is associated with risk factors such as hypertension, dyslipidemia, obesity, and type 2 diabetes mellitus (T2DM) [6–9]. The rs266729 and rs182052 SNPs in the *ADIPOQ* gene are associated with adiponectin levels, and the minor alleles of these regions have been associated with the prevalence of coronary artery disease (CAD) and cardiovascular and metabolic diseases, including unstable angina and T2DM [9–11].

Leptin is a pro-inflammatory adipokine that stimulates vascular inflammation and causes vascular smooth muscle

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hypertrophy, which may contribute to the pathogenesis of T2DM, hypertension, atherosclerosis, and coronary heart disease (CHD) [11]. –2,548 G>A (rs7799039) is a common variant of the leptin promoter, resulting in a guanine to adenine change at –2,548 upstream of the ATG start site [12]. Furthermore, the –2,548 G>A LEP polymorphism has been reported to have a strong role in the transcriptional activation of the LEP promoter by insulin-mediated glucose metabolism [13].

The Resistin (*RETN*) *-420 G>C rs1862513* polymorphism in the 5′ promoter region of the resistin-encoding gene *(RETN)* has been associated with cardiovascular risk factors [14]. In particular, the *-420 C>G* polymorphism has been reported to have a potential role in vascular inflammation and the development and progression of abdominal aort aneurysm (AAA) [15].

Our study aimed to investigate relationship between serum adipokine (adiponectin, leptin and resistin) levels and their gene variants *ADIPOQ -11,377 C/G rs266729, LEP2548G/A rs7799039 and RETN -420 G/C rs1862513* polymorphisms with PVD and Buerger's disease.

Materials and methods

Subject selection

The study group consisted of 151 patients with 127 PVD (female/male: 35/92) and 24 with Buerger's disease (female/male: 1/23). All patients were admitted to the Peripheral Vascular Surgery Unit, Istanbul University, Istanbul Medical Faculty. Our study was conducted with the approval of Istanbul University Istanbul Medical Faculty Clinical Research Ethics Committee (2015/615).

The ages of PVD were between 60 and 82 years (71.6 ± 11), and Buerger's disease were between 49 and 66 years (57.6 ± 8.6). Aneurysmatic diseases, Behcet's disease, connective tissue disease, and chronic renal disease were considered as exclusion criteria for both groups. The atherosclerotic patient group with below-knee involvement was accepted as inclusion criteria for PVD. For Buerger's group, patients with segmental inflammatory occlusive disease in the lower and upper extremities, frequently affecting medium-sized muscles and small-sized arteries and veins of the extremities, were included in the group.

Ankle-brachial index (ABI) was estimated by measuring the systolic blood pressure at the ankles and arms using a hand-held Doppler, and then calculating a ratio in patients with both PVD and Buerger's disease. In our study, those with an ABI classification below 0.6 were considered serious or severely pathological, those with an ABI classification between 0.9 and 0.6 were considered moderately pathological, and those with an ABI greater than 0.9 were considered normal [16]. ABI index less than 0.6 was taken as borderline diagnostic criterion for PVD.

A positive history of hypertension was defined as the need for antihypertensive medications or systolic blood pressure of 160 mmHg or greater, or diastolic blood pressure of 95 mmHg or greater. A positive history of hypercholesterolemia was defined as the need for cholesterol-lowering medications or serum cholesterol concentrations of 5.0 mmol/L or above. Cardiovascular risk factors (diabetes mellitus, hypertension, dyslipidemia, smoking) were also evaluated as clinical features in patients with PVD and Buerger's disease.

The operations and applications that PVD and Buerger's disease underwent together with their accompanying diseases are shown in Table 1. After surgery, 100 mg/day salicylate was started and general health examinations were performed at 6-month intervals. Any patient who had a smoking habit in the last 10 years was defined as a smoker.

Written informed consent was taken from all subjects for the study.

Biochemical determination

After 12 h of fasting, venous blood samples were collected between 08:00 and 09:00 a.m. into heparin-free vacuum tubes and centrifuged at 400 g for 20 min at ambient temperature to obtain serum samples. Separated serum samples were aliquoted and stored at –20 °C until adipokine analyses. Serum hs-CRP levels were determined immediately.

Determinations of serum adipokines

Serum adipokines were determined by the sandwich enzyme-linked immunosorbent assay (ELISA) technique using plates coated with antibodies specific for human leptin, human adiponectin, and human resistin (Elabscience, USA). A biotinylated detection antibody specific for human adipokines and avidin-horseradish peroxidase (HRP) conjugate were added to each well. Optical density (OD) was measured spectrophotometrically at 450 nm in proportion to the concentrations of adipokines.

The sensitivity reported for serum adiponectin in the kit was 0.10 ng/mL and the detection range was 0.16–10 ng/mL [17]. The sensitivity and detection range of the kit used for serum resistin were 18.75 pg/mL and 31.25–2,000 pg/mL, respectively [18], and the sensitivity and detection range of the kit used for serum leptin were 9.38 pg/mL and 15.63–1,000 pg/mL, respectively [19]. The % coefficient of variation (%CV) for each adipokine is<10 %.

Table 1: Demographic, clinical, and biochemical evaluations of PVD and Buerger's disease groups.

	All patient groups (n=151)	PVD (n=127)	Buerger's disease (n=24)	p-Value[b] 95 % CI
Gender (male/female)	(115/36)	(92/35)	(23/1)	**0.014**
(% male)	(76.1)	(72.4)	(95.8)	0.66–0.86
Age, year[a]	69.2 ± 11.7	71.6 ± 11	57.6 ± 8.6	**<0.001**
				8.9–18.6
Diabetes mellitus, n (%)	102 (70.83)	98 (81)	4 (17.4)	**<0.001**
				1.90–11.39
Hypertension, n (%)	74 (52.48)	69 (58.5)	5 (21.7)	**0.001**
				1.22–5.93
Hyperlipidemia, n (%)	14 (11.57)	13 (13.0)	1 (4.8)	0.256
				0.37–19.75
Coronary heart disease, n (%)	45 (32)	42 (35.6)	3 (14.3)	0.108
				1.03–8.66
Passed cerebrovascular disease n (%)	8 (6.89)	8 (8.2)	–	0.212
				0.86–0.97
Smoking, %	31.25	27.5	52.2	0.053
Smoker	54.86	58.3	34.8	0.34–0.83
Non-smoker	13.88	14.2	13.0	
Quit smoker				
ABI[a]	0.582 ± 0.36	0.62 ± 0.03	0.32 ± 0.08	**0.006**
				0.087–0.49
Applied wound care/ amputation/debridement procedure, n (%)	11 (8.33)	11 (9.9)	0 (0)	
Resting pain	33 (25)	30 (27)	3 (14.3)	
Wound cleansing/debridement	43 (32.57)	31 (27.9)	12 (57.1)	
Finger or small joint amputation	21 (15.90)	18 (16.2)	3 (14.3)	
Major joint amputation	9 (6.81)	6 (5.4)	3 (14.3)	
Trans metatarsal amputation	4 (3.03)	4 (3.6)	0 (0)	
Graft application	7 (5.30)	7 (6.3)	0 (0)	
Bypass	4 (3.03)	4 (3.69)	0 (0)	
Angioplasty				
hs-CRP[a], mg/L	1.52 ± 0.71	1.62 ± 0.76	1.29 ± 0.55	0.09 (−0.14)–0.78
Adiponectin[a], ng/mL	4.05 ± 0.35	4.07 ± 0.37	3.96 ± 0.29	0.17 (−0.047)–0.26
Resistin[a], pg/mL	4.08 ± 0.26	4.08 ± 0.26	4.08 ± 0.27	0.90 (−0.14)–0.12
Leptin[a], pg/mL	0.71 ± 0.65	0.82 ± 0.62	0.41 ± 0.64	**0.018**
				0.067–0.74

[a], mean ± SD; CI, confidence interval; SD, standard deviation; ABI, ankle brachial index; PVD, peripheral vascular disease; hs-CRP, high-sensitive C-reactive protein; [b], PVD and Buerger's disease groups were compared. Numbers in bold indicate statistically significant values.

Genotypings of *ADIPOQ-11377 C/G, LEP2548 G/A, RETN -420 G/C*

Primers and probes designed against gene regions for *ADIPOQ -11377 C/G rs266729, LEP2548 G/A rs7799039, and RETN -420 G/C rs1862513* in previously isolated DNA samples were genotyped in LightCycler 480 (Roche, Germany) [20–22].

Statistical analyses

In our study, parameters showing normal distribution from continuous data were evaluated with the student' *t*-test using the SPSS version 21 package program. In this study, hs-CRP, adiponectin, resistin, and leptin results, which did not show normal distribution in our groups, were converted to $\log_{10}$ transformation values. The chi-square test was used to evaluate categorical data. In the PVD and Buerger's disease, the distribution of adipokine genes within the group was analyzed to see if it was consistent with Hardy-Weinberg [23]. Pearson's correlation test was applied to evaluate statistical serum protein levels in each group. The Mann-Whitney U test was applied to evaluate the significance of parameters that did not show normal distribution. Using the G power 3.1.9.7 program, when the effect size was entered as 0.7 and the allocation ratio N2/N1: 0.1889, we obtained the power of our study as 0.917. $p<0.05$ was taken as the significance limit.

Results

Table 1 shows the demographic and clinical findings for all patients and patient groups with PVD and Buerger's disease. PVD patients (age range: 60.6–82.6) were found to be significantly older than the Buerger's disease (age range: 49.1–66.3) (p<0.001, 95 % confidence interval (CI): 8.9–18.6). When gender distribution between groups was examined, male gender was found to be higher in the Buerger's disease (p=0.014, 95 % CI: 0.66–0.86). It was found that smokers were higher in Buerger's disease, but it was not significant (p=0.053, 95 % CI: 0.34–0.83).

In PVD, 81 % had DM (p<0.001, 95 % CI: 1.90–11.39), and 58.5 % had hypertension (p=0.001, 95 % CI: 1.22–5.93), which was significantly higher than Buerger's disease. There were no significant differences in hyperlipidemia, coronary heart disease, or passed cerebrovascular disease between groups (p=0.256, 95 % CI: 0.37–19.75; p=0.108, 95 % CI: 1.03–8.66; and p=0.212, 95 % CI: 0.86–0.97, respectively) (Table 1).

When we examined the ABI values of two patient groups, they were found to be significantly higher in the PVD (p=0.006, 95 % CI: 0.087–0.49) (Table 1).

It was found that hs-CRP between groups was insignificantly higher in PVD (p=0.09, 95 % CI: (−0.14)–0.78) (Table 1). The levels of adiponectin were higher in PVD, but did not reach significance (p=0.17, 95 % CI: (−0.047)–0.26); there was also no significant difference between groups in terms of resistin values (p=0.90, 95 % CI: (−0.14)–0.12). Leptin levels were significantly lower in Buerger's disease than in PVD (p=0.018, 95 % CI: 0.067–0.74) (Table 1).

When we compared hs-CRP and serum adipokine between groups with ABI values below 0.6, we found that hs-CRP, leptin, and adiponectin were higher in PVD (p=0.105, 95 % CI: (−0.18)–1.45; p=0.138, 95 % CI: (−0.13)–0.81; p=0.278, 95 % CI: (−0.069)–0.31, respectively). Resistin was found to be similar between groups with ABI values below 0.6 (p=0.71, 95 % CI: (−0.23)–0.12) (Table 2).

There was a significant difference in adiponectin between the groups in terms of the presence of diabetes (p=0.048, 95 % CI: 0.006–0.75), and hs-CRP was found higher in PVD (p=0.052, 95 % CI: (−0.015)–1.49). There were no differences in resistin and leptin between groups (p=0.73, 95 % CI: (−0.34)–0.24; p=0.069, 95 % CI: (−1.04)–26.65, respectively) (Figure 1).

When we selected hypertensive patients in PVD and Buerger's disease, leptin was found to be significantly higher in PVD (p=0.017, 95 % CI: 0.13–1.31). Resistin and adiponectin were similar in the hypertensive PVD and Buerger's disease (p=0.86, 95 % CI: (−0.29)–0.24; p=0.81, 95 % CI: (−0.12)–0.16, respectively) (Figure 2).

Table 2: Demographic, clinical, and biochemical evaluations of PVD and Buerger's disease groups according to ABI classification.

	PVD			Buerger's disease		
	ABI<0.6	ABI=0.6–0.9	ABI>0.9	ABI<0.6	ABI=0.6–0.9	ABI>0.9
n (%)	56 (57.7)	24 (24.7)	17 (17.5)	11 (84.6)	1 (7.7)	1 (7.7)
Gender (male/Female)	(47/9)	(12/12)	(12/5)	(10/1)	(1/0)	(1/0)
(% male)	(83.9)	(50)	(70.6)	(90.9)	(100)	(100)
Diabetes mellitus n (%)	44 (80.0)	20 (83.3)	16 (94.1)	2 (18.2)[b]	0 (0)	0 (0)
Hypertension n (%)	32 (61.5)	14 (58.3)	8 (47.1)	3 (27.3)[c]	0 (0)	1 (100)
Hyperlipidemia n (%)	6 (13.3)	2 (9.2)	1 (7.7)	1 (100)	0 (0)	0 (0)
Passed cerebrovascular disease n (%)	5 (11.6)	3 (13.6)	–	0 (0)	0 (0)	–
Coronary heart disease n (%)	21 (39.6)	9 (37.5)	5 (31.2)	1 (10)	0 (0)	1 (100)
Applied wound care/amputation/debridement procedure n (%)	4 (66.7)	2 (33.3)	–	–	–	–
Resting pain	13 (56.5)	3 (13)	7 (30.4)	1 (50)	–	1 (50)
Wound cleansing/debridement	11 (42.3)	9 (34.6)	6 (23.1)	3 (75)	1 (25)	–
Finger or small joint amputation	12 (85.7)	1 (7.1)	1 (7.1)	–	–	3 (100)
Major joint amputation	3 (50)	2 (33.3)	1 (16.7)	–	–	2 (100)
Trans metatarsal amputation	2 (50)	1 (25)	1 (25)	2 (50)	1 (25)	1 (25)
Graft application	5 (83.3)	1 (16.7)	–	–	–	–
Bypass application						
hs-CRP[a], mg/L	1.69 ± 0.72	1.76 ± 0.50	2.17 ± 0.23	1.05 ± 0.68	1.09 (1)	–
Adiponectin[a], ng/mL	4.04 ± 0.35	4.02 ± 0.51	4.09 ± 0.26	3.91 ± 0.25	4.38 (1)	3.95 (1)
Resistin[a], pg/mL	4.07 ± 0.31	4.11 ± 0.19	4.04 ± 0.36	4.13 ± 0.21	0.19 (1)	3.77 (1)
Leptin[a], pg/mL	0.67 ± 0.65	1.04 ± 0.59	0.74 ± 0.58	0.33 ± 0.63	0.20 (1)	0.49 (1)

[a], mean ± SD; SD, standard deviation; ABI, ankle brachial index; CI, confidence interval; hs-CRP, high-sensitive C-reactive protein; PVD, peripheral vascular disease. [c]p<0.05, versus PVD patients with ABI less than 0.6, 95 % CI: 0.84–6.064; [b]p<0.001, versus PVD patients with ABI less than 0.6, 95 % CI: 1.24–15.52. ABI classification was made reference to the Ostergren et al. (Reference [16] of this study).

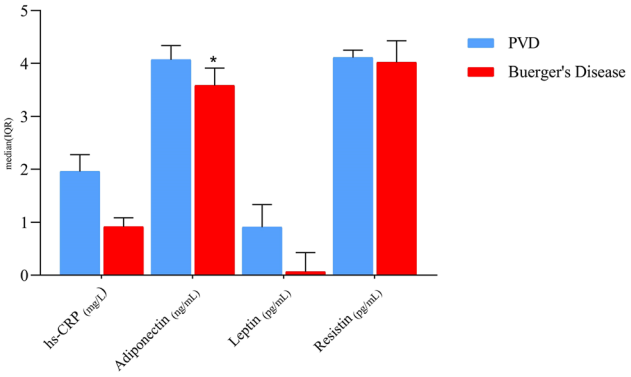

Figure 1: The levels of hs-CRP, adiponectin, leptin, and resistin in PVD and Buerger's disease groups with diabetes mellitus. *: p=0.048.

Among those with an ABI value less than 0.6, it was observed that those with diabetes were 80 % in the PVD and 18.2 % in the Buerger's disease (p<0.001, 95 % CI: 1.24–15.52). A significant difference was found in the proportion of hypertensive patients with an ABI value below 0.6, 61.5 % in PVD, and 27.3 % in Buerger's disease (p=0.038, 95 % CI: 0.84–6.064) (Table 2).

Leptin G2548A rs77999039

LEP G2548A genotype and allele distribution frequencies were found to be similar in groups. The distributions of LEP G2548A within the groups were found to be in accordance with Hardy-Weinberg (for PVD: X^2=0.579, p=0.446 and for Buerger's: X^2=0.211, p=0.64, respectively) (Table 3).

RETN -420 G/C rs1862513

When the *RETN -420 G/C* genotype and allele distributions were examined, the *GG, GC, CC* genotypes, and G, C alleles were observed to be different in PVD and the Buerger's disease, but they were not significant. It was observed that the Hardy-Weinberg distribution was not appropriate within the groups, with X^2=3.948, p=0.0469 in PVD; while it conformed to the Hardy-Weinberg with X^2=0.00143, p=0.969 in Buerger's disease (Table 3).

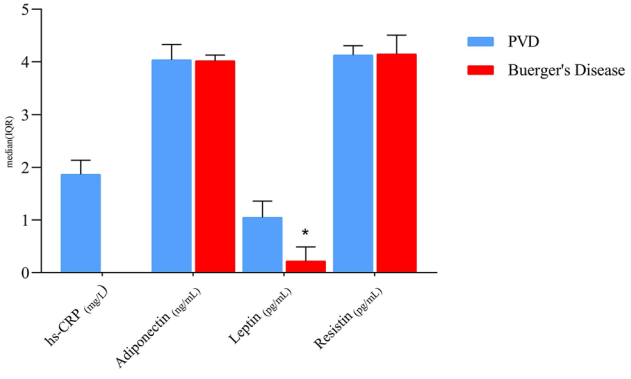

Figure 2: The levels of hs-CRP, adiponectin, leptin, and resistin in PVD and Buerger's disease groups with hypertension. *: p=0.017.

Table 3: Genotype and allele distributions of ADIPOQ, RETN, and LEP in PVD and Buerger's disease groups.

	PVD, n (%)	Buerger's disease, n (%)	p-Value
ADIPOQ -11377 C/G	58 (49.6)	9 (45)	NS
rs266729	49 (41.9)	9 (45)	NS
CC	10 (8.5)	2 (10)	
CG	165 (70.5)	27 (67.5)	
GG	69 (29.5)	13 (32.5)	
C	X^2=0.0059	X^2=0.0131 p=0.908	
G	p=0.938		
RETN -420 G/C	60 (51.3)	7 (36.8)	NS
rs1862513	41 (35.0)	9 (47.4)	NS
GG	16 (13.7)	3 (15.8)	
GC	161 (68.8)	23 (60.5)	
CC	73 (31.2)	15 (39.5)	
G	X^2=3.948	X^2=0.00143 p=0.969	
C	p=0.0469		
LEP G2548A	24 (20.7)	5 (25)	NS
rs77999039	53 (45.7)	11 (55)	NS
GG	39 (33.6)	4 (20)	
GA	101 (43.5)	21 (52.5)	
AA	131 (56.5)	19 (47.5)	
G	X^2=0.579	X^2=0.211 p=0.64	
A	p=0.446		

NS, non-significant; PVD, peripheral vascular disease. p<0.05 was accepted as a significance value. Courtlab -HW calculator was used for compliance with X2 Hardy-Weinberg Equilibrium. (Reference [23] of this study).

ADIPOQ -11,377 C/G rs266729

Genotype distributions were detected as *CC*, *CG*, and *GG* (for PVD: 49.6 %, 41.9 %, 8.5 %, respectively) (for Buerger's disease: 45 %, 45 %, 10 %, respectively) in both groups; there were no significant differences. It was found to be similar distributions of C, G allele frequencies in PVD (70.5 %, 29.5 %, respectively) and Buerger's disease (67.5 %, 32.5 %, respectively). It was determined that both groups conformed to the Hardy-Weinberg, with X^2=0.0059, p=0.938 in the PVD, and X^2=0.0131 and p=0.908 in Buerger's disease (Table 3).

A significant relationship was detected between age and continuous variables in PVD, a negative relationship with leptin (r= –0.264, p=0.047), and ABI (r= –0.206, p=0.045), and a positive relationship with adiponectin (r=0.210, p=0.026).

There was a significant inverse relationship between resistin and leptin (r= −0.508, p=0.019) in Buerger's.

According to *ADIPOQ -11,377 C/G* genotype distributions, leptin was found to be significantly lower in PVD carrying *GG* (p=0.019, 95 % CI: 2.40–18.55) (Figure 3). Serum leptin was also found to be significantly higher in PVD compared to the Buerger's disease in those with the *RETN -420 GG* genotype (p=0.030, 95 % CI: 0.078–1.307) (Figure 4), and there was a limit of significance in those with the *LEP2548 GA* genotype (p=0.054, 95 % CI: (−0.010)–1.056) (Figure 5). Serum adiponectin was higher in those carrying the *LEP2548 AA* genotype in the PVD, but were not found to be significant (p=0.063, 95 % CI: (−0.026)–0.93) (Figure 5).

Discussion

According to the main findings, leptin in PVD were found to be significantly higher than the values in Buerger's disease. Adiponectin and leptin were found to be higher in diabetic and hypertensive PVD patients. Among those with ABI values below 0.6, 80 % of diabetic patients were in PVD, and 61.5 % of hypertensive patients were in PVD. It was observed that leptin was significantly lower in PVD carrying the *ADIPOQ -11,377 GG*, while leptin in those carrying the *RETN -420 GG* wild-type genotype formed a significant difference.

When we evaluated the age ranges and gender distributions of PVD and Buerger's disease, we found that the average age was younger in Buerger's disease, and the male gender was lower in PVD. Buerger's disease is more common in men than in women, and young and middle-aged men are more affected in the population; the disease rate is three times higher in men than in women [24].

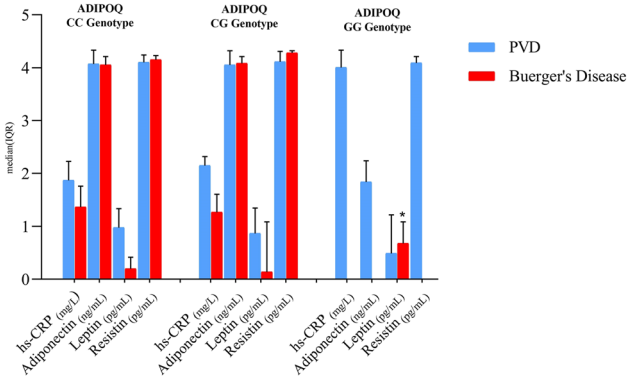

Figure 3: The levels of hs-CRP and adipokines in patients with PVD and Buerger's disease according to *ADIPOQ -11377 C/G rs266729* genotype distributions. *: p=0.019.

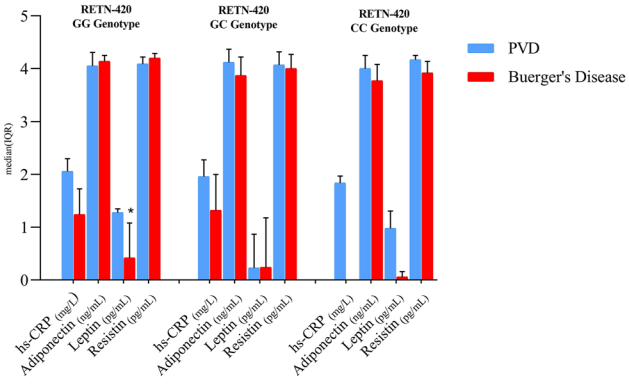

Figure 4: The levels of hs-CRP and adipokines in patients with PVD and Buerger's disease according to *RETN -420 G/C rs1862513* genotype distributions. *: p=0.030.

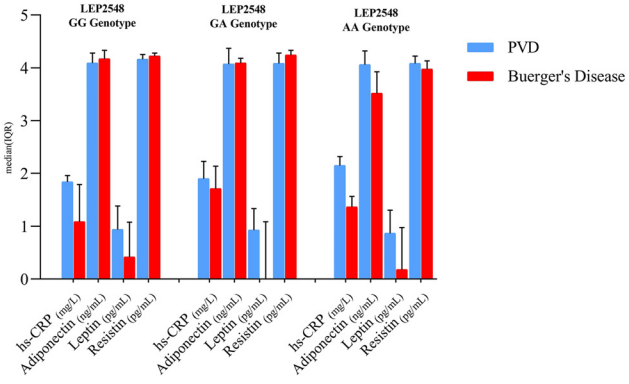

Figure 5: The levels of hs-CRP and adipokines in patients with PVD and Buerger's disease according to *LEP2548 G/A rs7799039* genotype distributions.

In our study, we found that smoking was higher in the Burger's disease and that the difference was close to the significance limit in the comparison between the groups. The presence of a smoking history in the pathophysiology of Buerger's disease has been associated with the incidence and progression in male patients [25].

In this study, we found that hs-CRP was higher in PVD, but it was found to be insignificant. The role of hs-CRP in linking fibrosis and inflammation in the pathophysiology of PVD was demonstrated in patients with PAD studied in the ARIC study [26].

Leptin levels were significantly lower in Buerger's disease than in PVD, while the decrease in adiponectin levels did not reach a significant level. It was found that serum resistin levels were similar in both PVD and Buerger's disease.

In this study, depending on the presence of DM, adiponectin and leptin levels were found to be lower in the Buerger's group than in PVD. Low adiponectin is linked to vascular

endothelial dysfunction [5–7]. Low adiponectin in our Buerger's disease group may be related to the development of inflammatory reactions [27]. Our study also found that serum leptin was positively associated with PVD in hypertensive patients. There are studies indicating that the leptin system is related to the development of atherosclerosis, which are shown as risk factors in vascular diseases, including PVD and Buerger's.

Resistin, in contrast, has emerged as a significant player in vascular inflammation and dysfunction. It is secreted by adipocytes and inflammatory cells, particularly in the context of obesity [28]. Resistin has been shown to promote endothelial cell activation, leading to increased expression of adhesion molecules and pro-inflammatory cytokines, which can contribute to atherosclerosis and vascular remodeling [29]. Elevated levels of resistin have been linked to increased risk of cardiovascular events, including ischemic stroke and CAD [30, 31]. Furthermore, resistin's role in promoting vascular smooth muscle cell migration and proliferation underscores its contribution to the pathogenesis of PVD [32, 33]. In our study, it was found similar resistin values between the PVD and Buerger's disease groups.

The relationship between these adipokines and PVD is complex and multifaceted. While adiponectin generally exerts protective effects, leptin and resistin are often associated with adverse vascular outcomes. For example, resistin has been implicated in the development of endothelial dysfunction and hypertension, which are critical components of PVD [34, 35]. Studies have demonstrated that high serum resistin levels correlate with increased severity of CAD and PAD, suggesting that resistin may serve as a potential biomarker for vascular damage [36, 37].

It has been shown that an amino acid change occurring in the *ADIPOQ* gene at the promoter position 11,377 (-11,377 C/G), where cytosine is replaced with guanine, is associated with T2DM. In addition, the G allele of the *ADIPOQ* rs266729 may appear important in associations with T2DM risk in various populations [38]. Resistin gene is located on chromosome 19p13.2 and spans 1,369 bp with four exons and three introns [39]. *RETN -420 C/G* in the promoter region of the resistin gene has been identified as the main determinant of plasma resistin concentration and expression in humans [40, 41].

In the PVD group, while leptin was significantly negatively related to age, adiponectin was positively related to age. In studies conducted, higher serum adiponectin in older men and women may be associated with an age-related increase in visceral adipose tissue [42]. The age-related alteration in leptin levels is attributable to changes in fat mass in women and probably also in men [43]. In *in vitro* studies, Wabitsch et al. [44] demonstrated a direct long-term inhibitory effect of testosterone on leptin production from human adipocytes in culture. It can be explained that the age-related decrease in leptin in the PVD group may be due to the decrease in subcutaneous fat tissue with aging [45].

There was a significant and inverse relationship between age and ABI values in PVD. In a cross-sectional study conducted in Scotland involving 28,980 men and women over the age of 50 without cardiovascular disease, they found that ABI values decreased proportionally with increasing age [46]. In our study, we found that the frequency of diabetics with ABI values lower than 0.6 was significantly higher in PVD. The relationship we found between them can be explained by the fact that the ABI value was reported to be important in the evaluation of diabetic PAD patients, especially in the lower extremities, in cohort studies [47]. We found that patients with hypertension in PVD with an ABI value of less than 0.6 were significantly more likely to have hypertension compared to the Buerger group. In studies, patients with an ABI value of<0.9 were associated with hypertension and a history of acute coronary syndrome were associated with a higher risk of developing heart failure [48].

It was found a difference in serum leptin between PVD and Buerger's groups carrying *RETN wild GG*, suggesting that leptin resistance may be more prominent in PVD than in Buerger's disease, as resistin affects the development of obesity and hyperinsulinemia through glucose and lipid efflux from adipocytes with its physiological effect on leptin. It was observed that the resistin and leptin were significantly inversely correlated in Buerger's disease. It has been reported that acute resistin treatment inhibited leptin-mediated STAT3 phosphorylation [49]. The inverse relationship between resistin and leptin levels obtained in Buerger's disease may be due to the central metabolic effect of resistin on leptin, known as leptin resistance, from the finding by Asterholm et al. [50].

The limitation of our study can be shown as the lack of a healthy control group with age and gender-matched with the study groups. The study has an objective cross-sectional study feature for both patient groups. The fact that the study is not a patient-control study eliminates the examination of comparisons with the healthy group. Since there are significant differences between these groups in peripheral vascular diseases, their cross-sectional examination in this way also makes an important contribution to the study.

Conclusions

In conclusion, the roles of adiponectin, leptin, and resistin in both PVD and Buerger's disease illustrate the intricate balance between protective and harmful effects of adipokines on vascular health. The higher leptin levels we detected in the Buerger group carrying the *ADIPOQ GG* genotype, and in

the PVD group carrying the *RETN GG* suggest that they may be related to the etiopathogenesis of both diseases. Our study is the first to examine the serum levels and genetic variations of adipokines in PVD and Buerger's disease in the Turkish population. In addition, these main findings may contribute to the investigations of potential biomarker proteins or genetic variants related to atherosclerosis and inflammation that may be effective in the etiopathogenesis of both PVD and Buerger's disease.

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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