The Association Between Serum Levels of Leptin and Lipid Profiles in Cardiovascular Patients With Valve Calcification

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Abstract- Adipose tissue-derived hormones known as adipokines, like leptin, have multiple bioactions. Notwithstanding the key roles of leptin in regulating energy homeostasis and metabolism, its cardiovascular functions are complex and not fully understood. This study aimed to investigate the association between serum concentrations of leptin and lipid profiles in patients with valve calcification. Seventy-two patients with valve calcification and 72 healthy individuals participated in this case-control study. The serum levels of biochemical markers and leptin were measured by the standard enzymatic methods and enzyme-linked immunosorbent assay (ELISA) technique, respectively. Significantly increased serum concentrations of FBS (P=0.001), urea (P<0.0001), creatinine (P=0.018), P (P<0.0001), LDL-C (P=0.011) and lower Ca (P=0.006), and HDL-C (P<0.0001) levels were observed in patients compared to controls. There was no significant difference in the serum level of TG and TC of patients than controls. Systolic and diastolic blood pressures were significantly increased in patients relative to controls (P<0.0001). However, a significantly diminished serum level of leptin was observed in patients than controls (P<0.0001). The correlation analysis demonstrated that the serum leptin concentration is negatively correlated with creatinine, but it is positively correlated with systolic blood pressure (P=0.0302, P=0.0362, respectively). There was no statistically significant association between serum levels of leptin and lipid profiles. Our findings indicated dyslipidemia and reduced serum leptin concentrations in patients with valve calcification, suggesting the role of lipid abnormalities and reduced leptin levels in the development and pathogenesis of valve calcification diseases.

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Keywords: Leptin; Cardiovascular; Adipokines; Valve calcification; Lipid profiles

Introduction

The increasing prevalence of cardiovascular complications, particularly aortic and mitral valves calcification (AMVC) diseases, has considered as one of the most predominant health problems in different populations around the world (1,2). Aortic valve calcification (AVC) disease is the most common valve disease in developed countries, which is characterized by active inflammatory and cellular processes similar to atherosclerosis, along with the accumulation of lipoproteins, chronic inflammation, and calcium deposition in the valve leaflets (3). Mitral annular calcification (MAC) disease is a degenerative process that tends to be chronic. MAC progresses with an increase in age and predominantly affects the elderly subjects and women compared to men individual. It mainly involves the posterior part of the annulus than anterior part and is considered as the result of rheumatic heart disease (4).

Dyslipidemia that is denoted predominantly by enhanced levels of low-density lipoprotein-cholesterol (LDL-C) and/or reduced levels of high-density lipoprotein-cholesterol (HDL-C) is recognized as a potent risk factor for the development of cardiovascular diseases (CVDs) (5-7). Leptin- a 16 kDa hormone belonging to the adipokines family- is produced from adipose tissue. Adipose tissue-derived adipokines have

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pro-inflammatory and/or anti-inflammatory roles (8,9). Interestingly, leptin has been found to exert pro-inflammatory roles that include increasing monocyte-mediated production of pro-inflammatory cytokines, namely, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, besides stimulating the macrophage-mediated generation of CC-chemokine ligands, namely, CCL3, CCL4, and CCL5, through activating the Janus kinase 2-signal transducer and activator of transcription 3 (JAK2-STAT3) pathway (9-11).

In humans, leptin levels increase with obesity and are higher in females relative to male individuals (1,12). It has been illustrated that leptin, a central satiety signal, may be a better risk factor for cardiovascular complications than body mass index (BMI), reflecting the combined mass of adipose and non-adipose tissues, including bones and muscles (13). Several studies reported that leptin has pleiotropic effects, and hyperleptinemia has been recognized as a risk factor for CVDs (14-20). It has been demonstrated that treatment with exogenous leptin augments the calcification of atherosclerotic lesions without raising lesion size and enhances valvular calcification in apolipoprotein E (Apo-E)-deficient mice (21). According to the literature, the association between hyperleptinemia and the risk of CVDs is controversial, and the lack of association between them has been shown in several studies (1,22-24). Additionally, leptin has been reported to regulate and even enhance the calcification of vascular cells (21,25,26).

Therefore, the current study aimed to investigate the serum concentrations of leptin and biochemical markers in patients with valve calcification and to examine the association of leptin serum levels with the biochemical parameters and BMI in a population from western Iran.

Materials and Methods

Study population and ethical considerations

In this case-control study, the patient group was comprised of 72 patients with valve calcification (divided into three groups: 41 AVC, 22 MAC, and 9 AMVC) admitted to the Imam Ali hospital in Kermanshah province located in the west of Iran. All patients were diagnosed by the heart and vessel specialist according to history, clinical experiments, angiography, and echocardiography. The control group consisted of 72 age-and sex-matched healthy volunteers without any chronic inflammatory disease. This study was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Kermanshah University of Medical Sciences (KUMS). Written informed consent was also signed by all participants who contributed to this study.

Blood sample collection

After overnight fasting, about 10 ml of fresh venous blood was collected from each participant in a vacuum tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant agent. The serum samples were separated by centrifugation at 4000 rpm for 6 min. Subsequently, the isolated serum samples were aliquoted and stored at -70° C, pending the performance of experiments.

Biochemical measurements

The serum samples were thawed, and then serum level of fasting blood sugar (FBS) and leptin was measured by the glucose oxidase kit (Pars Azmoon Co., Iran) and by the enzyme-linked immunosorbent assay (ELISA: Human Leptin ELISA kit purchased from EASTBIOPHARM company, China) according to the instructor of the manufacture, respectively. Also, the serum levels of total plasma cholesterol (TC), triglyceride (TG), urea, creatinine, calcium (Ca), phosphor (P), LDL-C, and HDL-C were measured by the standard enzymatic methods (Pars Azmoon Co., Iran).

Statistical methods

The statistical software package version SPSS 24 (SPSS Inc, Chicago, IL, USA) and GraphPad Prism software version 6 (GraphPad Software, La Jolla, CA, USA) were used to all of the statistical analyses. One sample Kolmogorov-Smirnov (1-sample K-S) normality test was used to assess the normality of quantitative variables, which are expressed as mean±standard error of the mean (SEM). One-way ANOVA and independent-samples T-test were used to analyze the data with normal distribution, and Kruskal-Wallis and Mann-Whitney U tests were used to analyze the data with non-normal distribution. The correlations analysis was performed by Spearman rank correlation analysis. The results were considered statistically significant at the P of less than 0.05 (P<0.05).

Results

Participant's characteristics and laboratory measurements

The demographic characteristics and laboratory measurements of patients and healthy subjects are

represented in Table 1. Significantly increased serum concentrations of FBS (103.86 versus (vs) 91.74, *P*=0.001), urea (34.92 vs 28.46, *P*<0.0001), creatinine (0.96 vs 0.88, *P*=0.018), P (4.8 vs 4.35, *P*<0.0001), LDL-C (93.49 vs 71.51, *P*=0.011) and lower Ca (8.0457 vs 8.7540, *P*=0.006), and HDL-C (44.08 vs 55.28, *P*<0.0001) levels were observed in patients compared to controls. There was no significant difference in the serum level of TG (127.32 vs 117.79, *P*=0.913) and TC (161.53 vs 147.21, *P*=0.297) between patient and control groups. Systolic (125.75 vs 108.33, *P*<0.0001) and diastolic (79.07 vs 71.00, *P*<0.0001) blood pressures were significantly increased in patients relative to healthy subjects.

Serum concentrations of leptin protein

A significantly decreased serum level of leptin was observed in patients compared to controls (*P*<0.0001), as shown in Figure 1a. Among patients, the serum level of leptin was significantly reduced in AVC and MAC patients relative to controls, but not in AMVC patients and in the comparison between other groups (Figure 1b). Also, leptin levels were significantly higher in females than in males (214.02 vs 152.14, *P*=0.003).

Correlations analysis

The correlations between serum concentrations of leptin with BMI, FBS, urea, creatinine, Ca, P, LDL-C, HDL-C, TG, TC, disease severity, systolic, and diastolic blood pressures are indicated in Table 2. In patients, the serum concentration of leptin was negatively associated with creatinine (r= -0.2557, *P*=0.0302), but it was positively associated with systolic blood pressure (r=0.2473, *P*=0.0362). There was no statistically significant correlation between serum levels of leptin and lipid profiles. In AVC patients, serum level of leptin was positively correlated with systolic and diastolic blood pressures (r=0.3692, *P*=0.0175, and r=0.3485, *P*=0.0256, respectively). Likewise, the serum concentration of leptin was positively correlated with LDL-C (r=0.4229, *P*=0.0499) and TG (r=0.4484, *P*=0.0363) in MAC patients. In AMVC patients, the serum level of leptin was negatively correlated with BMI (r= -0.7167, *P*=0.0369).

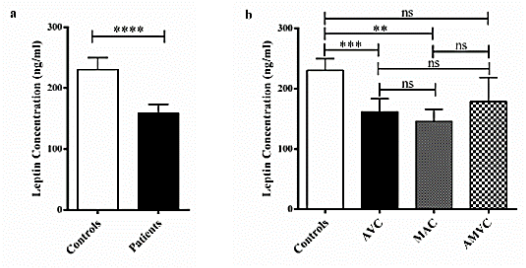

Figure 1. Comparison of serum leptin levels in control, patient, and between patient groups. a) A significantly lower serum level of leptin was observed in patients compared to healthy individuals. b) Leptin serum level was significantly diminished in AVC and MAC patients relative to controls, but not in AMVC patients and in the comparison between other groups, as shown in the graph. Data are expressed as mean±SEM; **** *P*<0.0001; *** *P*<0.001; ** *P*<0.01; ns, not statistically significant. AVC, Aortic valve calcification; MAC, Mitral annular calcification; AMVC, Aortic and mitral valve calcification

Table 1. Comparison of serum level of lipid profiles, biochemical variables, and other demographic characteristics between controls and AVC, MAC, and AMVC patients, respectively.

Variable	Controls (n=72)	Patients (n=72)			*P*
		AVC (n=41)	MAC (n=22)	AMVC (n=9)	
Sex (female/male) (%)	45 (62.5%)/27 (37.5%)	18 (43.9%)/23 (56.1%)	11 (50%)/11 (50%)	5 (55.6%)/4 (44.4%)	0.215
Age, years	66.02 ± 1.23	65.21 ± 1.57	63.36 ± 2.44	66.22 ± 2.69	0.311
BMI, kg/m^2	25.49 ± 0.35	24.00 ± 0.46	23.80 ± 0.65	25.81 ± 1.38	0.075
FBS, mg/dL	91.74 ± 1.03	101.24 ± 3.52	103.59 ± 4.54	116.44 ± 13.84	0.001
Urea, mg/dL	28.46 ± 0.75	34.88 ± 1.13	33.82 ± 1.51	37.78 ± 2.88	<0.0001
Creatinine, mg/dL	0.88 ± 0.02	0.94 ± 0.03	0.94 ± 0.04	1.06 ± 0.06	0.018
Ca, mg/dL	8.75 ± 0.20	8.00 ± 0.10	8.20 ± 0.14	7.86 ± 0.25	0.006
P, mg/dL	4.35 ± 0.03	4.84 ± 0.15	4.80 ± 0.12	4.60 ± 0.21	<0.0001
LDL-C, mg/dL	71.51 ± 3.88	87.58 ± 7.21	94.76 ± 8.56	117.24 ± 17.88	0.011
HDL-C, mg/dL	55.28 ± 1.80	43.00 ± 1.12	45.77 ± 1.69	44.89 ± 1.89	<0.0001
TG, mg/dL	117.79 ± 3.66	128.68 ± 8.66	125.41 ± 9.89	125.78 ± 18.37	0.913
TC, mg/dL	147.21 ± 4.36	157.90 ± 7.44	156.00 ± 7.97	191.56 ± 19.45	0.297
Smoking %	16.28 %	24.39 %	9.09 %	11.11 %	0.809
Severity %	--	Mild: 51.22 % Moderate: 34.15 % Severe: 14.63 %	40.91 % 31.82 % 27.27	22.22 % 33.33 % 44.45 %	--
Systolic blood pressure (mmHg)	108.33 ± 1.25	127.56 ± 3.11	120.32 ± 3.50	130.78 ± 4.87	<0.0001
Diastolic blood pressure (mmHg)	71.00 ± 0.84	79.83 ± 1.81	77.36 ± 2.12	79.78 ± 3.12	<0.0001

Abbreviations: AVC, Aortic valve calcification; MAC, Mitral annular calcification; AMVC, Aortic and mitral valve calcification; BMI, Body mass index; FBS, fasting blood sugar; Ca, Calcium; P, Phosphor; LDL-C, Low-density lipoprotein-cholesterol; HDL-C, High-density lipoprotein-cholesterol; TG, Triglyceride; TC, Total plasma cholesterol.

Table 2. The correlation between serum concentrations of leptin with BMI and other biochemical parameters in patients

Variable	Leptin level in patients	Leptin level in AVC patients	Leptin level in MAC patients	Leptin level in AMVC patients
Age, years	r=0.1041, *P*=0.3842	r=0.01631, *P*=0.9194	r=0.2577, *P*=0.2469	r= -0.2594, *P*=0.4978
BMI, kg/m^2	r= -0.04868, *P*=0.6847	r= -0.002483, *P*=0.9877	r=0.1158, *P*=0.6079	r= -0.7167, *P*=0.0369
FBS, mg/dL	r=0.01434, *P*=0.9048	r= -0.03905, *P*=0.8085	r=0.1397, *P*=0.5352	r=0.0500, *P*=0.9116
Urea, mg/dL	r=0.009960, *P*=0.9338	r= -0.07092, *P*=0.6595	r=0.2560, *P*=0.2501	r= -0.05858, *P*=0.8868
Creatinine, mg/dL	r= -0.2557, *P*=0.0302	r= -0.2504, *P*=0.1143	r= -0.2981, *P*=0.1778	r= -0.06932, *P*=0.8651
Ca, mg/dL	r= -0.2013, *P*=0.0899	r= -0.2231, *P*=0.1608	r= -0.2112, *P*=0.3453	r=0.1500, *P*=0.7081
P, mg/dL	r=0.07247, *P*=0.5452	r= -0.04060, *P*=0.8010	r=0.2384, *P*=0.2853	r=0.0500, *P*=0.9116
LDL-C, mg/dL	r=0.07076, *P*=0.5548	r=0.01363, *P*=0.9326	r=0.4229, *P*=0.0499	r= -0.2167, *P*=0.5809
HDL-C, mg/dL	r= -0.1707, *P*=0.1516	r= -0.06537, *P*=0.6847	r= -0.3733, *P*=0.0870	r= -0.1526, *P*=0.7094
TG, mg/dL	r=0.04491, *P*=0.7080	r= -0.02074, *P*=0.8976	r=0.4484, *P*=0.0363	r= -0.4167, *P*=0.2696
TC, mg/dL	r= -0.03083, *P*=0.7971	r= -0.1682, *P*=0.2931	r=0.4131, *P*=0.0560	r= -0.4500, *P*=0.2298
Smoking	r= -0.06429, *P*=0.5916	r= -0.1104, *P*=0.4920	r= -0.09969, *P*=0.6589	r=0.5477, *P*=0.2222
Severity	r= -0.07437, *P*=0.5347	r= -0.1985, *P*=0.2134	r= -0.03429, *P*=0.8796	r=0.4009, *P*=0.2825
Systolic blood pressure	r=0.2473, *P*=0.0362	r=0.3692, *P*=0.0175	r=0.06797, *P*=0.7638	r= -0.2929, *P*=0.4422
Diastolic blood pressure	r=0.1409, *P*=0.2377	r=0.3485, *P*=0.0256	r= -0.09235, *P*=0.6827	r= -0.3950, *P*=0.2909

Abbreviations: AVC, Aortic valve calcification; MAC, Mitral annular calcification; AMVC, Aortic and mitral valve calcification; BMI, Body mass index; FBS, fasting blood sugar; Ca, Calcium; P, Phosphor; LDL-C, Low-density lipoprotein-cholesterol; HDL-C, High-density lipoprotein-cholesterol; TG, Triglyceride; TC, Total plasma cholesterol.

Discussion

The present study investigated the association between serum concentrations of lipid profiles and leptin in patients with valve calcification compared to healthy individuals. Valve calcification diseases have a considerably increased incidence and are considered as one of the most important public health problems (2,4). In our study, a significant increase in serum concentrations of FBS, urea, creatinine, P, LDL-C and lower Ca, and HDL-C levels were observed in patients (mean age=65) compared to control group (mean age=66). Moreover, systolic and diastolic blood pressures were significantly increased in patients compared to healthy subjects. It has been reported that a potent risk factor for the development of CVDs is dyslipidemia, which is predominantly denoted by increased levels of LDL-C and/or diminished levels of HDL-C (5–7), similar to our findings. Based on research reported by Lozano *et al.*, (2004), lipid abnormalities (low HDL-C and/or abnormal TG levels, as well as abnormal LDL-C levels) are plentiful in the elderly individuals (age ≥60) and are related to a higher incidence of CVDs (7).

According to our correlation analysis, the serum concentration of leptin in patients was negatively correlated with creatinine, but it was positively correlated with systolic blood pressure. In our study, the serum leptin concentrations were strikingly decreased in patients relative to controls. Leptin, produced from adipose tissue, emerges as an adipokine with pleiotropic effects (9). Besides, hyperleptinemia has been considered as a risk factor for CVDs (14-20). However, the association between leptin concentration and risk of CVDs is controversial (1,22-24). Lieb *et al.*, (2009) showed that higher plasma levels of leptin are related to a greater risk of congestive heart failure (CHF) and CVDs. Likewise, this study illustrated that the relationship between log-leptin and mortality is U-shaped so that increased risk of death is evidenced at both low and high leptin concentrations (13). Furthermore, treatment with recombinant murine leptin has been shown to enhance the calcification of vascular cells (21,26). In contrast, *LDL receptor (LDLR)*$^{-/-}$ (knockout) mice lacking leptin develop more atherosclerotic lesions relative to *LDLR*$^{-/-}$ control mice, indicating the protective role of leptin against atherosclerosis (27). Also, it has been reported that a low plasma level of leptin predicts cardiovascular mortality in 207 women with normal glucose tolerance, disturbed glucose tolerance, or type 2 diabetes mellitus (T2DM) during a seven-year follow-up period (28). Therefore, the cardiovascular roles of leptin are complex and not fully clear (29). Taken together, it can be suggested that both low and high leptin concentrations may contribute to the development of CVDs, as well as valve calcification diseases. Indeed, every factor has a specified balance for exerting its correct and normal

functions, and disturbance in this balance can lead to the development of pathologic conditions and, subsequently, disease.

To conclude, our findings indicated dyslipidemia and reduced serum concentrations of leptin in patients with valve calcification that suggest the role of lipid abnormalities and reduced leptin levels in the development and pathogenesis of valve calcification diseases. Further studies with larger sample sizes are needed to confirm our results and to decipher the precise physiological and pathological roles of leptin and lipid profiles in the pathogenesis of valve calcification diseases.

Some main limitations of this study as follow: First, we did not compare the effects of lipid profile variation due to medication, food consumption, physical activities, or other confounding factors. Second, our studied-sample size was small and may not be representative of all patients with valve calcification in Kermanshah province. Also, the study of the serum levels of other adipokines and main polymorphisms of leptin, as well as its receptor genes, might be useful to a better interpretation of our findings.

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