



## Original Article

# High serum leptin level is associated with peripheral artery disease in adult peritoneal dialysis patients

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## INTRODUCTION

Peripheral artery disease (PAD) is a disease in which atherosclerotic plaques cause arterial obstruction and reduce blood flow [1]. PAD represents systemic atherosclerosis which can affect the functional activity of the limbs and heightens the risk of cardiovascular disease [2]. Escalated morbidity and 2–3-fold higher overall mortality were found in patients with PAD compared to patients without PAD [3].

PAD is more common in patients with end-stage renal disease (ESRD) than in the general population [4]. In the general population, the prevalence of PAD increases with age, smoking, and diabetes [5,6]. The duration of dialysis is another risk factor in ESRD patients [7]. A recent study revealed that the prevalence of PAD was 27.4% among all patients on maintenance ambulatory peritoneal dialysis (PD) and was higher (45%) in patients older than 70 years [8]. The ankle–brachial pressure index (ABI) is a reliable, initial test to diagnose PAD if a patient has abnormal physical findings, such as claudication, impaired walking function, or ischemic

## ABSTRACT

**Objective:** Peripheral artery disease (PAD) is associated with systemic atherosclerosis and indicates an increased risk of mortality in peritoneal dialysis (PD) patients. A high leptin level accelerates atherosclerosis in apoE-deficient mice. The purpose of this study was to examine the association of serum leptin level and PAD in adult PD patients. **Materials and Methods:** The clinical characteristics of sixty PD patients recruited from June 2015 to October 2016 were obtained. Serum leptin concentrations were determined. Ankle–brachial index (ABI) values were measured and those with a left or right ABI <0.9 were defined as the low ABI group. **Results:** Twenty of these 60 PD patients (33.3%) had diabetes mellitus and 32 patients (53.3%) had hypertension. Thirteen PD patients (21.7%) were in the low ABI group. Higher serum leptin ( $P = 0.002$ ) and C-reactive protein (CRP,  $P < 0.001$ ) levels were found in the low ABI group compared with those in the normal ABI group. More number of patients with diabetes ( $P = 0.015$ ) and current smokers ( $P = 0.037$ ) were noted in the low ABI group than in the normal ABI group. After adjustment for factors that were significantly associated with PAD in multivariate logistic regression analysis, each increase of 1 ng/mL in the serum leptin level (odds ratio [OR], 1.062; 95% confidence interval [CI], 1.014–1.114;  $P = 0.012$ ) and each increase of 0.1 mg/dL in the serum CRP level (OR, 1.107; 95% CI, 1.011–1.211;  $P = 0.028$ ) were found to be independent predictors of PAD in PD patients. **Conclusion:** Higher serum leptin and CRP levels correlated positively with the diagnosis of PAD in PD patients.

**KEYWORDS:** C-reactive protein, Leptin, Peripheral artery disease, Peritoneal dialysis

rest pain. An ABI <0.9 is an indicator of PAD when assessing asymptomatic patients [9].

Leptin is a kind of adipokine which is produced and secreted by white adipocytes [10,11]. Several studies reported that leptin is related to the pathogenesis of atherosclerosis and coronary artery disease from its actions to stimulate oxidative stress, vascular inflammation, and vascular smooth muscle hypertrophy [12–14]. Hyperleptinemia has been associated with atherosclerosis, endothelial dysfunction, and insulin resistance [15].

The relationship of serum leptin and PAD in PD patients is not clear. Therefore, our study aimed to examine the correlation between serum leptin and ABI in PD patients.

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## MATERIALS AND METHODS

### Patients

Sixty PD patients were recruited from Hualien and Dalin Tzu Chi Hospital from June 2015 to October 2016. All patients had undergone regular PD for more than 3 months. Twenty-six patients were men and 34 were women with ages ranging from 23 to 89 years. Exclusion criteria included acute infection, malignancy, acute myocardial infarction, pulmonary edema, or heart failure at the time of blood sampling or refusal to sign informed consent. The mean duration of PD treatment was  $53.92 \pm 41.65$  months. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Protection of Human Subjects Institutional Review Board of Tzu-Chi University and Hospital (IRB103-136-A). Informed written consent was obtained from all patients prior to their enrolment in this study. Among the patients, 42 received continuous ambulatory PD (Dianeal, Baxter Health Care, Taiwan), with 3–5 dialysate exchanges per day, while the other 18 patients underwent 4–5 dialysate exchanges each night with an automated device (automated PD). The weekly fractional clearance index for urea, total clearance of creatinine, and peritoneal clearance of creatinine were obtained from the medical records.

### Anthropometric analysis

All anthropometric factors were measured three times, during the morning after overnight fasting, without dialysate in the abdominal cavity. Body weight was measured in light clothing and without shoes to the nearest 0.5 kg; height was measured to the nearest 0.5 cm. Body mass index was calculated as weight (kg) divided by height in meters squared [16-18].

### Biochemical investigations

Biochemical parameters were determined in the morning before dialysis exchange. Blood samples (~5 mL) were immediately centrifuged at  $3000 \times g$  for 10 min. Serum samples were stored at 4°C and used for biochemical analyses within 1 h of collection. Serum levels of blood urea nitrogen, creatinine, fasting glucose, albumin, total cholesterol, triglycerides, total calcium, phosphorus, and C-reactive protein (CRP) were measured using an autoanalyzer (Siemens Advia 1800, Siemens Healthcare GmbH, Henkestr, Germany). Serum leptin (SPI-BIO, Montigny le Bretonneux, France) concentrations were checked using a commercially available enzyme immunoassay [16,19-21]. Serum intact parathyroid hormone levels (iPTHs) were measured using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Webster, Texas, USA) [16,19-21].

### Ankle-brachial index measurements

ABI values were obtained using an oscillometric method. The ABI-form device (VaSera VS-1000; Fukuda Denshi Co., Ltd., Tokyo, Japan) measures blood pressure (BP) in both arms and ankles automatically and simultaneously [22]. During examination, participants were in the supine position and cuffs were placed tightly around the four extremities. Meanwhile, an electrocardiogram was obtained and heart sounds were recorded for at least 10 min. The ABI was derived from the

calculation of the ratio of the ankle systolic BP (SBP) divided by the arm SBP. Diagnostic criterion for PAD was an ABI  $<0.9$  and our study adapted left or right ABI values  $<0.9$  to define the low ABI group.

### Statistical analysis

The Kolmogorov–Smirnov test was used to examine the normal distribution of data. Normally distributed data were expressed as means  $\pm$  standard deviation and comparisons between patients were performed using the Student's independent *t*-test. Nonnormally distributed data were expressed as medians and interquartile ranges and comparisons between patients were performed using the Mann–Whitney U-test. Data expressed as the number and percentage of patients were analyzed by the Chi-square test. The fasting glucose, iPTH, and leptin datasets showed skewed nonnormal distributions and therefore were recalculated by transformation to the logarithm to the base 10; after this transformation, the log-glucose, log-iPTH, and log-leptin became normally distributed. Variables that were significantly associated with PAD were tested for independence using multivariate logistic regression analysis (adapted factors: gender, smoking, age, diabetes, hypertension, CRP, and leptin). The receiver operating curve (ROC) was used to calculate the area under the curve (AUC) to identify the log-leptin levels and serum log-CRP levels which predicted PAD in PD patients. SPSS for Windows (version 19.0; SPSS Inc., Chicago, IL, USA) was used for all statistical analyses and  $P < 0.05$  was considered statistically significant.

## RESULTS

Table 1 shows the laboratory and clinical characteristics of the sixty enrolled PD patients. Examination of their medical histories indicated that twenty patients (33.3%) had diabetes mellitus (DM) and 32 (53.3%) had hypertension. Thirteen PD patients (21.7%) were included in the low ABI group. PD patients in the low ABI group had higher serum CRP ( $P < 0.001$ ) levels and higher serum leptin ( $P = 0.002$ ) levels than those in the normal ABI group. Compared with the normal ABI group, there were statistically significant differences in diabetes ( $P = 0.015$ ) and current smoking ( $P = 0.037$ ) in the low ABI group among PD patients.

Adjustment of the factors significantly associated with PAD (gender, smoking, age, diabetes, hypertension, CRP, and leptin) in multivariate logistic regression analysis revealed that each increase of 1 ng/mL in the serum leptin level (odds ratio [OR], 1.062; 95% confidence interval [CI], 1.014–1.114;  $P = 0.012$ ) and each increase of 0.1 mg/dL in the serum level (OR, 1.107; 95% CI, 1.011–1.211;  $P = 0.028$ ) were independent predictors of PAD in PD patients [Table 2]. ROC curve analysis was applied to estimate the optimal log-leptin levels [Figure 1a] and log-CRP levels [Figure 1b] predicting PAD in PD patients. The AUC for log-leptin was 0.777 (95% CI: 0.651–0.875,  $P < 0.001$ ) and for log-CRP was 0.930 (95% CI: 0.833–0.980,  $P < 0.001$ ).

## DISCUSSION

The results of our study revealed higher serum leptin and CRP levels in the low ABI group than the normal ABI

**Table 1: Comparison of clinical variables of sixty peritoneal dialysis patients in the normal and low ankle-brachial index groups**

Characteristics	All participants (n=60)	Normal ABI group (n=47)	Low ABI group (n=13)	P
Age (years)	56.88±14.75	56.36±15.47	58.77±12.15	0.607
Peritoneal dialysis duration (months)	53.92±41.65	54.09±35.64	53.31±60.49	0.953
Height (cm)	160.27±8.46	160.26±8.31	160.31±9.32	0.984
Body weight (kg)	63.77±14.07	62.56±13.74	68.17±14.93	0.207
Body mass index (kg/m <sup>2</sup> )	24.85±3.91	24.79±3.87	25.06±4.22	0.826
Left ankle-brachial index	1.09±0.17	1.15±0.13	0.87±0.14	<0.001*
Right ankle-brachial index	1.10±0.16	1.16±0.11	0.88±0.07	<0.001*
SBP (mmHg)	145.90±23.46	147.87±21.62	138.77±29.08	0.219
Diastolic blood pressure (mmHg)	85.35±12.65	87.00±11.32	79.38±16.46	0.058
Total cholesterol (mg/dL)	169.32±35.84	171.53±38.16	161.31±25.39	0.367
Triglycerides (mg/dL)	190.77±127.94	188.55±122.38	198.77±151.60	0.801
Fasting glucose (mg/dL)	106.00 (97.00-141.25)	104.00 (96.00-143.00)	122.00 (105.00-142.50)	0.154
Albumin (mg/dL)	3.71±0.34	3.72±0.35	3.69±0.33	0.788
Blood urea nitrogen (mg/dL)	58.73±19.87	59.94±21.19	54.38±13.92	0.377
Creatinine (mg/dL)	11.24±3.02	11.54±2.97	10.14±3.03	0.140
Total calcium (mg/dL)	9.05±0.71	9.13±0.72	8.78±0.64	0.118
Phosphorus (mg/dL)	5.18±1.28	5.23±1.23	5.00±1.51	0.567
CRP (mg/dL)	0.22 (0.07-0.43)	0.12 (0.06-0.30)	1.58 (0.47-2.06)	<0.001*
Intact parathyroid hormone (pg/mL)	259.70 (138.60-505.42)	253.43 (116.93-466.89)	313.60 (184.40-606.43)	0.370
Leptin (ng/mL)	22.55 (11.02-56.43)	19.76 (7.88-43.72)	61.12 (35.24-79.73)	0.002*
Weekly Kt/V	2.15±0.36	2.17±0.38	2.07±0.29	0.416
Peritoneal Kt/V	1.83±0.43	1.85±0.46	1.76±0.29	0.551
Total clearance of creatinine (L/week)	57.88±24.79	58.31±27.40	56.35±11.87	0.804
Peritoneal clearance of creatinine (L/week)	41.61±13.58	41.32±14.15	42.66±11.71	0.757
Female, n (%)	34 (56.7)	26 (55.3)	8 (61.5)	0.689
Diabetes, n (%)	20 (33.3)	12 (25.5)	8 (61.5)	0.015*
Hypertension, n (%)	32 (53.3)	27 (57.4)	5 (38.5)	0.225
Smoking, n (%)	8 (13.3)	4 (8.5)	4 (30.8)	0.037*
CAPD model, n (%)	42 (70.0)	35 (74.5)	7 (53.8)	0.151

\*Values of  $P < 0.05$  were considered statistically significant. Values for continuous variables are shown as mean±SD after analysis by Student's *t*-test; variables not normally distributed are shown as median and interquartile range after analysis by the Mann-Whitney U-test; values are presented as *n* (%) after analysis by the Chi-square test. ABI: Ankle-brachial index, CAPD: Continuous ambulatory peritoneal dialysis, CRP: C-reactive protein, Kt/V: Fractional clearance index for urea, SBP: Systolic blood pressure, SD: Standard deviation

**Table 2: Multivariate logistic regression analysis of the factors correlated to peripheral artery disease among sixty peritoneal dialysis patients**

Variable	OR	95% CI	P
Leptin (ng/mL) (each increase of 1 ng/mL)	1.062	1.014-1.112	0.012*
CRP (mg/dL) (each increase of 0.1 mg/dL)	1.107	1.011-1.211	0.028*

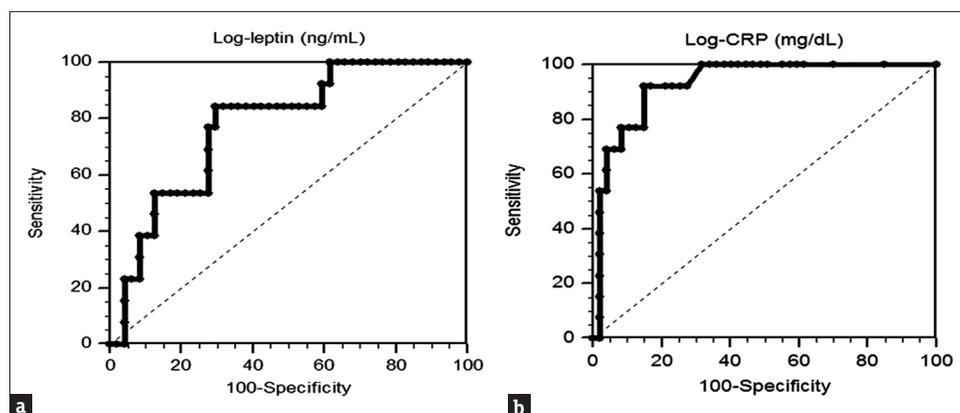
\*Values of  $P < 0.05$  were considered statistically significant. Analysis of the data was done using multivariate logistic regression analysis (adopted factors: gender, smoking, age, diabetes, hypertension, CRP, and leptin). OR: Odds ratio, CI: Confidence interval, CRP: C-reactive protein

group in these adult PD patients. In addition, significantly higher percentages of patients with DM and smoking were noted in the low ABI group than that of the normal ABI group.

No prior studies have investigated the association between serum leptin and PAD in PD patients. Some studies have revealed a positive correlation between serum leptin and cardiovascular disease [23-25]. The serum leptin level was also considered an independent predictor of arterial stiffness in patients with a history of cardiovascular disease [16], possibly due to its atherogenic and thrombotic physiological actions [13,26,27].

Leptin, a 16-kDa peptide product of the *ob* gene, is produced from white adipocytes [28]. Several studies have explained the possible pathophysiology of the atherogenic and thrombotic properties of leptin. First of all, leptin may stimulate the production of nitric oxide (NO) by upregulating inducible NO synthase, which may induce atherogenesis and injure endothelial function due to reactive oxidative stress [29]. Leptin may also increase oxidative stress by several different mechanisms [15]. Second, leptin potentiates the production of cytokines and growth factors to promote the pro-inflammatory signaling pathway (or pro-inflammatory signals) which may relate to atherosclerosis and endothelial dysfunction [30-32]. In addition, leptin may calcify vascular smooth muscle cells by stimulating osteoblastic differentiation and hydroxyapatite production [33]. Moreover, leptin correlates to thrombosis which may be due to platelet hyperactivity and loss of the balance between coagulation and fibrinolysis [15]. Finally, angiotensin II (Ang II) escalates leptin synthesis and both Ang II and leptin potentiate sympathetic activity [34]. Leptin has insulin-resistant properties which lower the antioxidative and lipogenic effects of insulin [35].

Older age, hypertension, dyslipidemia, cigarette smoking, and diabetes are established risk factors for PAD [36]. High



**Figure 1:** Receiver operating characteristic curve analysis to predict peripheral artery disease in sixty peritoneal dialysis patients. (a). The area under the receiver operating characteristic curve indicates the diagnostic power of log-leptin to predict peripheral artery disease in peritoneal dialysis patients. The area under the curve for log-leptin was 0.777 (95% confidence interval: 0.651–0.875,  $P < 0.001$ ). (b). The area under the curve indicates the diagnostic power of log-CRP to predict peripheral artery disease in peritoneal dialysis patients. The area under the curve for log-CRP was 0.930 (95% confidence interval: 0.833–0.980,  $P < 0.001$ ). CRP: C-reactive protein

levels of inflammatory markers such as CRP have been associated with accelerated functional decline in PAD patients [1,36]. Higher CRP levels were associated with PAD in a cross-sectional study among 1611 US adults free of cardiovascular disease, diabetes, and hypertension [37]. In our study, more patients with DM and current smoking and higher serum CRP levels were noted in the low ABI group compared with the normal ABI group among these PD patients. Our results are similar to those in a previous study which showed that PAD is a common disease in PD patients and DM is an independent risk factor for PAD in PD patients [8]. This could be explained by the pathophysiological correlation of arterial stiffness and DM reported in previous studies [16,38]. DM was found to be an independent risk factor for PAD among all ESRD patients in another single-center study in Taiwan; however, DM was not a significant risk factor in the subgroup of PD patients. Possible explanations for this finding are the lower age of the PD patients and lower percentage of PD patients with DM in this study [39].

The limitations of this study include the small number of patients and the cross-sectional design. Therefore, further cohort studies should be conducted to confirm the cause-effect relationship between serum leptin and PAD in PD patients.

## CONCLUSION

The present study revealed that serum leptin and CRP levels correlated positively with a diagnosis of PAD in PD patients.

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## Conflicts of interest

There are no conflicts of interest.

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