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Original Paper

Lower Serum Irisin Levels Are Associated with Increased Vascular Calcification in Hemodialysis Patients

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Key Words
Irisin • Vascular calcification • Cardiovascular • Hemodialysis • Chronic kidney disease

Abstract
Background/Aims: Vascular calcification, which involves an active cellular transformation of vascular smooth muscle cells into bone forming cells, is prevalent and predicts mortality in dialysis patients. Its mechanisms are complex and unclear. We presume that irisin, a newly identified myokine also may play roles in vascular calcification in hemodialysis patients. This study aims to evaluate serum irisin levels and establish their relation to vascular calcification and other parameters in hemodialysis patients. ***Methods:*** A total of 150 patients on maintenance hemodialysis treatment and 38 age- and sex-matched healthy controls were enrolled in this cross-sectional study. Serum irisin concentrations were measured by ELISA. Vascular calcification was evaluated by abdominal aortic calcification scores. ***Results:*** **S**erum irisin concentrations were significantly lower in hemodialysis patients than in controls [52.8 (22.0, 100.0) vs. 460.8 (434.8, 483.4) ng/ml, $P<0.01$]. In addition, irisin was negatively correlated with the parathyroid hormone level (P=0.01). The HD patients with vascular calcification showed significantly lower serum irisin concentrations [39.0 (21.7, 86.2) vs.79.0 (39.5, 130.2) ng/mL, P<0.01]. Compared with the group without vascular calcification multivariate logistic regression analyses revealed that serum irisin, HD vintage and age were significant independent determinant factors for vascular calcification in HD patients. ***Conclusion:*** Our results are the first to provide a clinical evidence of the association between serum irisin and vascular calcification in HD patients. Lower irisin levels, long-term hemodialysis and old ages are independent risk factors in HD patients.

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Introduction

Cardiovascular disease is the leading cause of death in end-stage renal disease (ESRD) patients [1]. Vascular calcification is a well-accepted marker of increased cardiovascular risks, especially in chronic kidney disease (CKD) patients [2]. Vascular calcification presence and progression in incident dialysis patients predict cardiovascular complications [3] and all-cause mortality [4, 5]. The pathogenesis of vascular calcification is complex and unclear.

Irisin is a recently introduced myokine that drives brown-fat-like conversion of white adipose tissue involving in energy regulation [6]. Colaianni, et al. verified that irisin could induce the differentiation and increase activity of osteoblasts; moreover, irisin increased cortical bone mass [7-9]. That means irisin may be an inhibitor of vascular calcification for its capacity to promote bone formation. Our previous study showed that HD patients had lower irisin levels; especially HD patients with protein energy wasting had lower irisin level in comparison to patients without protein energy wasting [10]. According to these previous studies, we make the hypothesis that irisin might play a role in the pathogenesis of vascular calcification in hemodialysis patients. Thus, the purpose of this study is to identify the association between irisin and vascular calcification and to examine the principal risk factors related to vascular calcification in HD patients.

Materials and Methods

Subjects

This cross-sectional study enrolled 150 maintenance HD patients and 38 healthy controls in the Peking University Third Hospital between September and December 2016. Exclusion criteria were: (i) age<18 years old; (ii) less than 3 months after initiating HD; (iii) in the acute phase of infections, heart failure or some other complications; (iv) incomplete data for study.

The study complied with principles laid down by Declaration of Helsinki and was approved by the ethics committee at the Peking University Third Hospital. Written informed consent was provided by all participants. Authors did not have access to information that could identify individual participants during or after data collection.

Clinical and biochemical data collection

Data collected included patient demographics such as age, sex, underlying cause of end stage renal disease (ESRD) and hemodialysis vintage. Laboratory data collected at baseline included hemoglobin, albumin, corrected calcium, phosphorus, intact parathyroid hormone (iPTH) level, ultra-sensitive C reactive protein (usCRP), lipid profile, etc. Fractional urea clearance (Kt/V urea) was calculated by Daugirdas formula.

Measurement of serum irisin

Blood samples were collected in vacuum tubes without anticoagulant after fasting for at least 8 hours. Following centrifugation, serum was collected and stored with aprotinin at -80°C until analysis. Serum irisin was determined using enzyme linked immunosorbent assay (ELISA) kits (Phoenix Pharmaceuticals, USA). Interassay and intraassay coefficients of variation were less than 15% and less than 10% for irisin.

Assessment of Vascular Calcification

Vascular calcification was imaged within 1 week of enrollment: abdominal aortic calcification (AAC) by lateral lumbar radiography (Fig 1) with Kauppila scoring [11]. Patients were allocated to the group with vascular calcification if they had a AAC score≥4 [12, 13].

Statistical analysis

Results are expressed as proportions (percentages) for categorical variables, mean ± standard deviation for continuous normally distributed variables, and median with interquartile ranges for continuous non-normally distributed variables. Kendall's tau-b correlations were performed to assess correlation of irisin

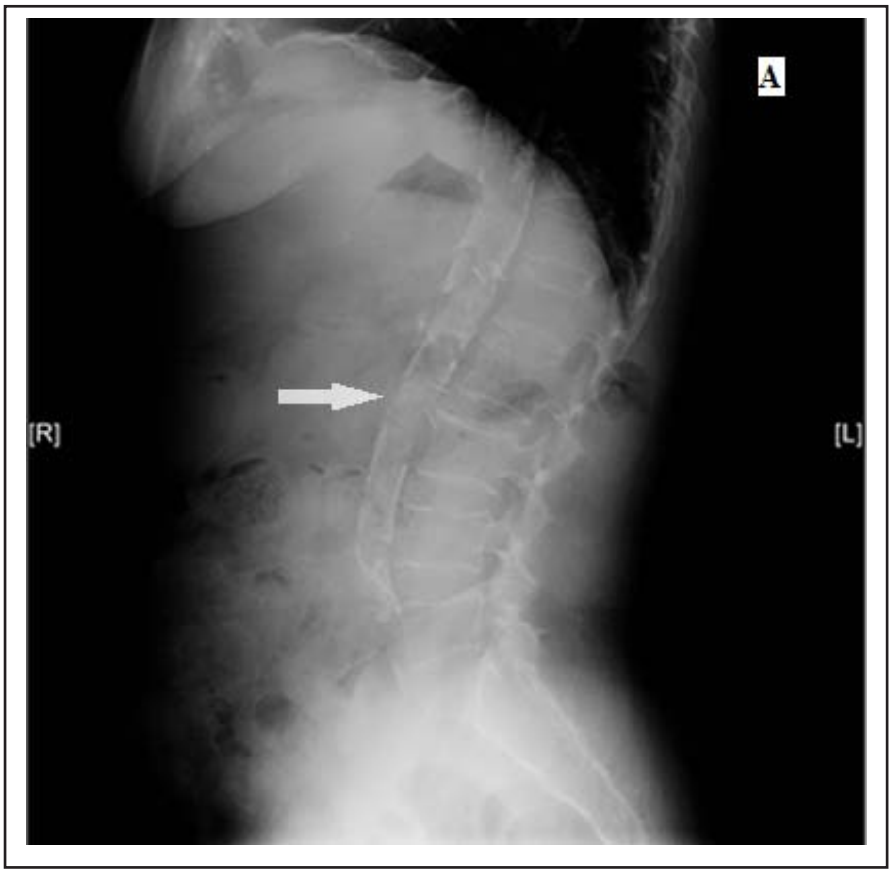

Fig. 1. Vascular calcification image in hemodialysis patients (A) abdominal aortic calcification (arrow) by lateral lumbar radiography.

with different laboratory data. The Student's t-test was used to compare differences between the two groups for normally distributed data, while the Mann-Whitney U test was used for non-normal data. Categorical data were compared using the Chi-square test. Multivariate logistic regression analyses were employed to select variables independently related to vascular calcification. All analyses were two-tailed, and a $p<0.05$ was considered statistically significant. SPSS Software, version 16.0 was used for all statistical analysis.

Results

Baseline characteristics of subjects

150 HD patients were recruited according to the inclusion and exclusion criteria. The demographic and clinical characteristics of those patients and 38 healthy controls are shown in Table 1. The mean age of HD patients was 64.5 ± 14.7 years. The median HD vintage was 61.9 (31.1, 108.5) months. The primary renal diseases were diabetes (44 patients), chronic glomerulonephritis (57 patients), hypertensive glomerulosclerosis (21 patients), other causes (20 patients), and unknown causes (8 patients). As shown in Table 1, there were significant differences in hemoglobin, serum creatinine, serum glucose, serum ultra-sensitive C reactive protein, serum lipids between HD patients and normal controls. There were no significant differences in age, gender between the two groups. Notably, serum irisin concentrations were significantly lower in HD patients than that of control subjects [52.8 (22.0, 100.0) ng/ml vs. 460.8 (434.8, 483.4) ng/ml, $P<0.01$].

Table 1. Comparison of parameters between hemodialysis patients and normal controls. HD: hemodialysis, HDL-C: High density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, Ln (irisin): the natural logarithm of irisin, usCRP: ultra-sensitive C reactive protein

Characteristics	Hemodialysis patients (n=150)	Normal controls (n=38)	P value
Age (years)	64.5±14.7	62.2±7.4	0.191
Male/female (n)	87/63	21/17	0.761
HD Vintage(month)	61.9(31.1,108.5)		
Hemoglobin (g/l)	114.0±9.4	147.7±12.4	<0.001
Serum creatinine (μmol/l)	902.6±215.3	75.6±11.2	<0.001
Serum calcium (mmol/l)	2.4±0.2	2.4±0.1	0.135
Serum phosphate (mmol/l)	1.9±0.5	1.3±0.1	<0.001
Serum albumin (g/l)	43.1±3.0	44.1±5.1	0.011
Serum glucose (mmol/l)	6.8±2.7	5.3±0.5	<0.001
Serum usCRP (mg/l)	3.0(1.5,5.1)	0.6(0.3,2.1)	<0.001
Serum LDL-C (mmol/l)	2.3±0.7	3.0±0.7	<0.001
Serum HDL-C (mmol/l)	0.8±0.3	1.2±0.3	<0.001
Serum triglycerides (mmol/l)	2.0(1.3,2.7)	1.4 (1.0,2.2)	0.072
Serum irisin(ng/ml)	52.8(22.0,100.0)	460.8 (434.8,483.4)	<0.001
Ln(irisin)	3.7±1.4	6.1±0.1	<0.001

Correlation analysis of circulating irisin with other parameters in HD patients (Kendall's tau-b analysis)

Bivariate correlation analysis revealed that circulating irisin was negatively correlated with serum creatinine, iPTH and vascular calcification. We did not observe a significant correlation between irisin and age, HD vintage, hemoglobin, serum calcium, phosphate, alkaline phosphatase, Kt/V urea, lipids, etc. See Table 2.

Comparison of irisin levels and other established parameters between the group with vascular calcification and the group without vascular calcification

Finally, 114 HD patients performed vascular calcification assessment (had AAC scores). Prevalence of vascular calcification was 63.1 %(72/114) in our current study. Compared with the group without vascular calcification, the HD patients with vascular calcification showed significantly lower serum irisin concentrations [39.0 (21.7, 86.2) vs.79.0 (39.5, 130.2) ng/mL, P <0.01]. Hemodialysis patients with vascular calcification had lower serum creatinine, lower serum albumin levels in comparison to those without vascular calcification. However, there were significantly older age, longer HD vintage and higher serum ultra-sensitive C reactive protein level in HD patients with vascular calcification than those patients without vascular calcification. Additionally, there were no differences in serum calcium, phosphate, lipids levels, Kt/V urea and plasma iPTH levels between HD patients with vascular calcification and the group without vascular calcification. See Table 3.

Independent determinant factors for vascular calcification by multiple regression analysis in HD patients

A logistic regression model was performed to find the independent determinant factors associated with vascular calcification in HD patients. The variables which significantly related to cardiovascular calcification in Table 3 (Age, HD Vintage, serum creatinine, serum albumin, serum usCRP) and some previous reported variable such as serum calcium, phosphate, KT/V and iPTH entered the analysis as candidate variables. In the analysis, serum albumin, usCRP, calcium, phosphate, iPTH and Kt/V urea levels were excluded from the model. Serum irisin, HD vintage and age were independently associated with vascular calcification in HD patients (see Table 4).

Table 2. Correlation analysis of serum irisin with other parameters in HD patients (n=150). HD: hemodialysis; usCRP: ultra-sensitive C reactive protein; HDL-C: high-density lipoprotein cholesterol; iPTH: intact parathyroid hormone; Kt/V urea: fractional urea clearance; LDL-C: low-density lipoprotein cholesterol

Variables	(Irisin)r	P value
Age (years)	0.020	0.727
Gender	0.106	0.114
HD Vintage(month)	-0.026	0.647
Hemoglobin (g/l)	0.042	0.472
Serum creatinine (μmol/l)	-0.118	0.040
Serum calcium(mmol/l)	0.049	0.391
Serum phosphate(mmol/l)	-0.066	0.248
Serum albumin (g/l)	-0.111	0.058
Serum glucose (mmol/l)	-0.121	0.052
Serum usCRP (mg/l)	-0.019	0.760
Serum LDL-C (mmol/l)	0.013	0.890
Serum HDL-C (mmol/l)	0.173	0.070
Serum triglycerides (mmol/l)	-0.107	0.262
Serum alkaline phosphatase(mmol/l)	-0.034	0.567
Serum iPTH (pg/ml)	-0.153	0.010
Kt/V urea	0.040	0.546
Vascular calcification(0=no,1=yes)	-0.193	0.029

Table 3. Comparison of irisin levels and other parameters between hemodialysis patients with vascular calcification and without vascular calcification. AAC: abdominal aortic calcification; HDL-C: high-density lipoprotein cholesterol; iPTH: intact parathyroid hormone; Kt/V urea: fractional urea clearance; LDL-C: low-density lipoprotein cholesterol; usCRP: ultra-sensitive C reactive protein; VC: vascular calcification

Characteristics	With VC	Without VC	P value
Cases(n)	72	42	
Age (years)	63.8±11.0	46.6±14.7	＜0.001
Male/female (n)	44/28	32/10	0.099
Diabetic nephropathy (%)	37.5	24.2	0.188
HD Vintage(month)	72.5(29.2,117.1)	32.1(22.9,47.8)	＜0.001
Hemoglobin (g/l)	115.6±7.9	116.7±9.9	0.609
Serum creatinine (μmol/l)	933.9±179.9	1070.4±246.9	0.019
Kt/V urea(per week)	1.3±0.2	1.4±0.2	0.340
Serum glucose (mmol/l)	7.8±3.5	6.3±1.8	0.528
Serum albumin (g/l)	43.6±2.5	45.0±3.4	0.050
Serum corrected calcium (mmol/l)	2.4±0.2	2.3±0.2	0.438
Serum phosphate (mmol/l)	2.0±0.6	2.0±0.5	0.889
Serum usCRP (mg/l)	3.4(2.3,6.1)	2.3(1.1,3.5)	0.019
Plasma iPTH (pg/ml)	202.4(92.8,397.9)	188.8(103.9,363.6)	0.986
Serum LDL-C (mmol/l)	2.4±0.7	2.3±0.6	0.349
Serum HDL-C (mmol/l)	0.8±0.2	0.8±0.2	0.963
Serum triglycerides (mmol/l)	2.0(1.3,2.8)	1.9(1.2,3.3)	0.789
Serum irisin(ng/ml)	39.0(21.7,86.2)	79.0(39.5,130.2)	0.008
AAC (score)	7(4,10)	0(0,1.5)	＜0.001

Table 4. Independent determinant factors for vascular calcification by multiple regression analysis in HD patients. CI: confidence interval

Variables	B	P value	Exp(B)/OR	95%CI for Exp(B)
Serum irisin (ng/ml)	-0.012	0.042	0.988	0.977-0.999
HD vintage (months)	0.048	0.004	1.050	1.016-1.084
Age (years)	0.113	0.002	1.120	1.044-1.201
Constant	-7.233	0.001	0.001	

Discussion

In the current study, we verified that 63.1% hemodialysis patients suffered from vascular calcification, and irisin, a novel myokine, was significantly lower in HD patients compared with controls. Moreover, we demonstrated for the first time that irisin was significantly lower in patients with vascular calcification in comparison to those patients without vascular calcification. Lower irisin is independent risk factors of vascular calcification along with old age and long term hemodialysis in HD patients.

Irisin is a recently verified myokine that drives brown-fat-like conversion of white adipose tissue involving in energy regulation [6]. In our current study, we found that circulating irisin concentrations were significantly lower in 150 HD patients than 38 ages- and sex- matched healthy controls. The results were in accordance with previous studies, which also showed that serum irisin levels were decreased in nondialysis chronic kidney disease (CKD), hemodialysis patients, and peritoneal dialysis patients [14-18]. However, so far, the underlying mechanism for decreased irisin in CKD patients is not fully elucidated, which may be multifactorial. Firstly, previous studies proposed that uremia can be a possible candidate. Wen et al. [14] showed that indoxylsulfate, a well-known uremic toxin, decreased fibronectin type III domain containing protein 5 (FNDC5) expression in skeletal muscle cells and irisin concentrations in cell culture medium. Secondly, protein energy wasting (PEW) and muscle atrophy are prevalent among HD patients, whereas irisin is secreted by myocytes and related to muscle volume. In our previous study [10], lower irisin levels were correlated with protein energy wasting. .

The most important finding of our study was that reduced irisin concentrations were independently associated with increased vascular calcification in HD patients, which is demonstrated for the first time in HD patients. Generally, vascular calcification includes atherosclerotic intimal calcification and arterial medial calcification. Vascular calcification, especially media calcification, is common among patients with CKD [2]. The severity of vascular calcification is associated with increased risk of cardiovascular events and mortality in dialysis patients [3, 4]. But it is still unclear about the underlying mechanism between irisin and cardiovascular disease. So based on the above data including our results, we speculated that irisin may affect HD patients' future cardiovascular outcomes through the vascular calcification pathway.

In addition, in our current study, we found that irisin was negatively correlated with the intact parathyroid hormone (iPTH) level in HD patients. High level of iPTH activates bone resorption through enhancing osteoclast formation by increased osteoblastic production of the receptor activator of nuclear factor kappa-B ligand (RANKL), a master regulator of osteoclastogenesis [19]. It results in increased release of serum phosphate and calcium from bone, which also contributed to vascular calcification [20]. This finding was in accordance with our results of the relation between irisin and vascular calcification. However, in another study, no association was observed between irisin and iPTH levels by Tan, et al. [21]. The differences may be explained by the different study populations evaluated. Besides, cardiovascular disease association with low- and high-bone-turnover disease is a biphasic relationship [22], which also is another explanation for the difference. Otherwise, in our study irisin may not affect vascular calcification in HD patients through iPTH pathways, for we found not PTH but irisin was associated with vascular calcification independently.

There is controversy concerning the association between circulating irisin and fasting glucose or lipids levels. While Timmons et al. revealed no association between serum irisin and fasting glucose in a diabetic population [23], Huh et al. found a positive association between the two parameters among healthy women [24]. Our study showed circulating irisin levels were not inversely associated with fasting glucose levels significantly in HD patients (P=0.052), but nearly reached the significant differences. Additionally, irisin was positively associated with high density lipoprotein cholesterol (HDL-C) in CKD patients in a study by Wen et al. [14]. However, in our study, serum irisin did not show a significant

association with HDL-C (P=0.07). The disparity among studies can be partly explained by different study populations and sample sizes. Also differences in renal replacement therapy modality (nondialysis, hemodialysis, or peritoneal dialysis) and different proportions of patients with diabetes mellitus and patients taking lipid-lowering drugs might contribute to these discrepancies. Theoretically, irisin is proposed to affect insulin resistance and glucose intolerance [6].

Risk factors for vascular calcification include traditional cardiovascular risk factors such as aging, smoking, dyslipidemia, as well as CKD-related risk factors such as phosphate retention, excess calcium load, chronic inflammation and prolonged dialysis time [25]. However, we did not find vascular calcification was associated with calcium, phosphate and iPTH in our current study, the reason might be that we used only single calcium, phosphate and PTH levels whereas vascular calcification is a pathological process in long term excessive calcium, phosphate loading. Alternatively, the well-controlled serum calcium and phosphorus levels in our patients and the relatively small sample size may have limited our ability to find the association.

The pathogenesis of vascular calcification in CKD and dialysis patients is complicated. Now, it is believed that vascular calcification involves a tightly regulated transformation of vascular smooth muscle cells to an osteo/chondrocytic cell that expresses Runt-related transcription factor 2 (Runx2) and produces matrix vesicles under hyperphosphate circumstance [26-29]. Recently, Chen [30], et al. reviewed that high mobility group box 1 have a series of pro-calcification effects, such as promoting vascular smooth muscle osteo/chondrogenic differentiation, apoptosis and release of calcific extracellular vesicles, inflammation, oxidative stress and autophagy signaling, these indicated that inhibition of high mobility group box 1 may be a potential therapy for attenuating vascular calcification. Thus a new concept of bone-vascular axis appears.

On the other hand, the imbalance of promoters (such as phosphate load and excess calcium) [31, 32] and inhibitors (e.g. fetuin-A, matrix-gla protein, osteoprotegerin and osteocalcin) [33-36] is critical in the development of vascular calcification. It is reported that irisin was shown to increase cortical bone mass and strength in mice by stimulating bone formation [8]. Moreover, irisin plays a role in all stages of osteoblast differentiation [8, 9]. We demonstrated lower irisin are associated with calcification in HD patients. The mechanisms were unclear. According to previous studies, we speculated the mechanisms for irisin protecting blood vessels from calcification is through decreasing circulating calcium load by enhancing osteoblast differentiation, or inhibiting transformation of vascular smooth muscle cells to an osteo/chondrocytic cell, or improving endothelial dysfunction, for Lu, et al. reported that irisin could decrease endothelial apoptosis [37]. It is worthy of the further study in next step.

This study has several limitations. This was a cross-sectional, observational study and thus could not provide causal relationships for the findings. The sample size of this study was relatively small, and there were many confounding factors affecting our results in hemodialysis patients.

Conclusion

Our results are the first to provide a clinical evidence of the association between serum irisin and vascular calcification in HD patients. Lower irisin levels, long- term hemodialysis and old ages are independent risk factors in HD patient patients.

Disclosure Statement

None.

Acknowledgements

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