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Association of serum podocalyxin levels with peripheral arterial disease in patients with type 2 diabetes

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ABSTRACT

Objective: Renal podocalyxin is a marker for kidney diseases. Previous studies have shown the expression of serum podocalyxin (s-Podxl) in the endothelial cells of blood vessels. We aimed to investigate the association between s-podxl levels and peripheral arterial disease (PAD) in subjects with type 2 diabetes (T2DM).
Subjects and methods: Serum Podxl levels were analyzed in 69 subjects with normal glucose tolerance and PAD (NGT-PAD), 120 subjects with T2DM and PAD (D-PAD) and 36 subjects with T2DM without PAD (D-NPAD).
Results: In D-PAD Patients, s-Podxl was significantly higher (17.67 ± 20.7 ng/mL) than in D-NPAD subjects (9.97 ± 5.34 ng/mL; $P < 0.001$). Subjects with NGT-PAD had significantly higher s-Podxl levels (15.34 ± 18.21 ng/mL), than D-NPAD patients ($P < 0.001$). Subjects with D-PAD and medial calcific sclerosis (MCS) had significantly higher s-Podxl levels compared to the same group but without MCS ($P < 0.02$). In D-PAD patients, MCS ($P = 0.003$) and glycosylated hemoglobin ($P < 0.001$) were the two variables that had the strongest prediction for s-Podxl as revealed by regression analysis. Multivariate regression showed that an increase of one standard deviation in s-Podxl was associated with an odds ratio of 3.4 (95% confidence interval = 2.2–4.6, $P < 0.001$) for the prevalence of PAD.
Conclusions: This is the first study showing an association between s-Podxl and PAD in patients with T2DM. S-Podxl was higher in D-PAD patients than in D-NPAD subjects. In NGT-PAD patients, s-Podxl was also higher than in D-NPAD patients. In patients with D-PAD, s-Podxl was positively associated with MCS.

1. Introduction

It is reported that 200 million people suffer from peripheral arterial disease (PAD) worldwide.[1] The prevalence of PAD and associated complications are widespread due to the global increase in the prevalence of type 2 diabetes mellitus (T2DM) and the aging of the overall population.[2] Vessel wall stiffness in combination with T2DM is relevant for developing PAD.[3] Patients with PAD and T2DM have a cardiovascular mortality risk 5 times higher than those with single morbidity.[4–6] Monckeberg medial calcific sclerosis (MCS) is an arterial wall calcification at the level of the tunica media which is often observed in patients with diabetes and PAD.[7] In those high- risk patients, the risk of MCS may reach 30%.[5,8] Clinical symptoms are typically used for classification of PAD stages. One of the most well-known PAD staging systems is the Fontaine classification, under which increasing stages represent increased symptoms severity.[9]The diagnostic methods for PAD strongly influence the results of its prevalence studies. Low ankle-brachial index (ABI ≤0.9) is used as a marker for PAD in most of the relevant prevalence studies.[10] Podocalyxin (Podxl) is a sialomucin CD34 which is a major cell surface component that is expressed within the epithelial cells (podocyte) of the kidney glomerulus as a glycocalyx and was formerly known as sialylated protein.[11] Podxl maintains the slit diaphragm and podocytes' shape.[12,13] When podocytes are injured, Podxl is released from the vesicle-like structures or microvilli and excreted in urine.[14] Renal Podxl is used as an early marker for diabetic nephropathy and is considered a biomarker for glomerular disease.[15,16] Podxl also has a wide expression on endothelial cell surface all over the body.[17,18] Podxl is expressed within neurons,[19] mesothelial cells that line organs,[12] hematopoietic stem cells,[20] megakaryocytes,[21] and vascular endothelial cells[18]. A recent study showed that serum Podxl (s-Podxl) levels were correlated with carotid intimal medial thickness (cIMT).[22] As the expression of Podxl in vascular endothelial cells is evident, we assumed that s-Podxl level is related to endothelial injury. Nevertheless,

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there is no study investigating s-Podxl in patients with PAD or T2DM. Therefore, we sought to examine the association between s-Podxl levels as a marker of endothelial dysfunction and PAD in patients with T2DM.

1.1. Subjects and methods

We recruited 225 adults who were examined at the outpatient clinic of Zagazig University Hospital, Egypt. The study was approved by the ethics committee (Institutional review board Zagazig university - Faculty of medicine). Signed informed written consent was sought from all patients prior to their participation in the study. The study was performed in agreement with the standards of the Helsinki Declaration. Subjects were divided into three groups as follows: 189 patients with PAD, who were divided into 2 groups: T2DM with PAD (D-PAD, $n = 120$) and normal glucose tolerance with PAD (NGT-PAD, $n = 69$) in addition to 36 patients with T2DM without PAD (D-NPAD). To exclude vascular complications in D-NPAD, inclusion criteria were as follows: Toe brachial index (TBI) >0.70 and/or ankle-brachial index (ABI) >0.90, albumin excretion rate < 30 mg/day/24-h urine and creatinine <1.4, glycated hemoglobin (HbA1c) <8%, no insulin intake, maximum one oral antidiabetic drug and duration of T2DM <5 years. The use of current medications and detailed history for current and previous diseases were obtained. The smoking behavior questionnaire was used to assess the smoking status. Present smoking was defined as occasional or current daily smoking. Exclusion criteria: hormone replacement therapy, critical illness within the last 6 months, connective tissue disease, malignancy, significant respiratory or hepatic disease, creatinine clearance <45 mL/min or serum creatinine >2 mg/dL, type 1 diabetes, acute limb ischemia, percutaneous angioplasty to the lower limb arteries or prior bypass surgery, and PAD Fontaine stage (PAD-FS) III or IV. As a manifestation of atherosclerosis, we identified patients with atherosclerotic morbidity such as stroke or myocardial infarction (MI), cerebrovascular disease (CVD), and coronary heart disease (CHD) in our study. Patients answered a questionnaire about symptoms of PAD including resting pain, ischemic claudication, numbness, and chillness. Previous histories of amputation for PAD or intervention (bypass surgery or percutaneous peripheral intervention) were also registered. Full clinical examination was done for all patients including skin lesions, such as gangrene and ulcers, pulse exam of posterior tibial artery, dorsal artery, popliteal artery and femoral artery, warmth and skin color. Patients' symptoms, physiological examinations including TBI and ABI and previous histories of amputation or intervention, were obtained to determine limb ischemia according to the criteria for classification of PAD severity developed by Fontaine et al.[9] Patients who suffered from any symptoms of limb ischemia and had apparent previous history of amputation of lower limbs or intervention or at least one abnormality among TBI ≤0.6, ABI ≤0.9, they were defined as having PAD. Blood pressure (BP) was assessed in the seated position using a standard blood pressure measuring device. Body weight and height were assessed for all subjects and body mass index (BMI) was defined as the patient's weight in kilograms divided by their height (in meters) squared. Fundus examination was carried out for all subjects. Blood samples were collected after a 12-h fast. All subjects were subjected to an oral glucose tolerance test and were classified according to the American Diabetes Association diagnostic criteria[16] as follows: Normal glucose tolerance: fasting blood glucose (FPG) < 100 mg/dL with a 2-h postload glucose (2 h-PLG) of <140 mg/dL) or T2DM: (FPG ≥ 126 mg/dL or 2 h-PLG ≥ 200 mg/dL). Patients with impaired glucose tolerance or impaired fasting glucose are excluded from our study. The measurement of serum podocalyxin was done by ELISA kit (MyBioSource, CA, USA) according to the manufacturer's instructions. Triglycerides (TG), Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL—C) were estimated on a HITACHI 7450 analyzer (HITACHI, Tokyo, Japan). Glycated hemoglobin was measured by a turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics). Plasma glucose was assessed by hexokinase method on a Hitachi 7170 analyzer (Boehringer Mannheim, Mannheim, Germany). The level of high-sensitivity C-reactive protein (hs-CRP) was analyzed using a nephelometric method. Estimated glomerular filtration rate (eGFR) was calculated using the simplified Modification of Diet in Renal Disease (MDRD) formula.

1.2. Assessment of TBI and ABI

For assessment of blood flow in the lower limbs, we performed measurements for TBI and ABI. Blood pressure was recorded simultaneously in the four limbs. In a room with constant temperature of 25 °C and after bed rest for 5 min, all subjects underwent measurements of TBI and ABI by a well-trained radiologist. The toe was encircled by the cuff in accordance with the manufacturer's instructions. TBI and ABI were estimated as the ratio of toe and ankle to brachial systolic blood pressure (SBP), respectively. Normal values of TBI and ABI were reported to be >0.6 and 0.91 to 1.2, respectively. Therefore, the TBI and ABI cut-off values **for** PAD diagnosis were determined at ≤0.6 and ≤ 0.9, respectively. An ABI value of ≥1.3 suggests incompressible vessels and MCS.[23] Treadmill stress test was done for all subjects. The maximal walking distance was defined when the patient was unable to walk further because of pain. A post exercise ABI decrease >20% or A post exercise ankle SBP decrease >30 mmHg are diagnostic for PAD.[24]

1.3. Statistical methods

Statistical evaluation was done by SPSS version 22 (IBM, Inc) and included a univariate and multivariate, multinominal, linear and logistic regression analysis, ANOVA, Mann- Whitney U, Kruskal-Wallis test, chi-square test and independent-sample Student *t*-test as appropriate. The data were shown as the mean ± standard deviation or as a number with a percentage for categorical variables. A change in β of >10% was used to identify confounding in multivariate analysis.[25] **For adjusted age and sex groups,** an age cut-off value of 67 years was determined. $P < 0.05$ was considered statistically significant.

2. Results

Table 1 shows the general characteristics of the participants. There were statistically significant differences among patient groups with respect to HbA1c, lipids, eGFR, BMI, and age. Table 2 shows that differences in HbA1c, BMI and lipids remained even after sex and age adjustment was applied to the groups.

2.1. s-Podxl levels

The highest s-Podxl level was found in D-PAD patients (17.67 ± 20.7 ng/mL) followed by subjects with NGT-PAD (15.34 ± 18.21 ng/mL). Moreover, s-Podxl concentrations in D-PAD patients were higher than in D-NPAD subjects (9.97 ± 5.34 ng/mL; $P < 0.001$). In subjects with PAD but without diabetes (NGT-PAD), s-Podxl was higher compared with those with diabetes without PAD (D-NPAD) ($P < 0.001$). To study the relationship between s-Podxl and atherosclerosis, we determined subgroups of D-PAD: patients who had other atherosclerotic diseases in addition to PAD such as MI, stroke, CVD or CHD ($n = 57$) and patients with only PAD ($n = 63$). There were no significant differences in s-Podxl levels between both groups (16.46 ± 22.25 vs 15.8 ± 17.8 ng/mL; $P = 0.53$). Moreover, in all subjects ($n = 225$), s-Podxl levels were significantly associated with the number of sites affected by atherosclerotic disease in univariate regression analysis ($\beta = 0.341$, $P < 0.001$). Such association became more powerful ($\beta = 0.432$, $P < 0.001$) in the sex and age adjusted subjects (Table 2). To evaluate whether s-Podxl level was significantly associated with vascular calcification, we tested the association between s-Podxl level and MCS in D-PAD patients. Subjects with D-PAD and MCS ($n = 56$) had higher s-Podxl levels than those without MCS ($n = 64$), 17.52 ± 20.65 vs

Table 1
Biochemical and anthropometric characteristics of three studied groups.

	D-NPAD	NGT-PAD	D-PAD	P(all groups)	P(1 vs. 2)	P(1 vs. 3)	P(2 vs. 3)
n	36	69	120				
Male sex (%)	19 (54)	38 (56)	87 (73)	0.014*	0.684	0.019*	0.017*
Age (years)	61.9 ± 9.8	67.2 ± 9.7	69.6 ± 8.4	<0.001*	<0.001*	<0.001*	0.072
BMI (kg/m^2)	29.8 ± 4.7	26.4 ± 3.3	32.6 ± 6.7	<0.001*	<0.001*	<0.001*	<0.001*
LDL-C (mg/dL)	108.3 ± 30.9	119.8 ± 38.6	100.5 ± 38.6	0.001*	0.183	0.191	<0.001*
TCH (mg/dL)	197.2 ± 34.8	204.9 ± 46.4	181.7 ± 46.4	0.003*	0.472	0.211	0.001*
HDL-C (mg/dL)	50.3 ± 15.5	58 ± 11.6	50.2 ± 11.7	0.002*	0.162	0.251	<0.001*
TG (mg/dL)	194.8 ± 124	159.4 ± 79.7	177.2 ± 106.2	0.231	0.078	0.352	0.072
eGFR (mL/min/1.73 m^2)	72.9 ± 17.8	84.2 ± 39.6	68.3 ± 20.4	<0.001*	0.029*	0.005*	0.038*
SBP (mmHg)	143 ± 20	137 ± 18	144 ± 21	0.118	0.148	0.887	0.041*
DBP (mmHg)	88 ± 9	76 ± 12	77 ± 13	<0.001*	<0.001*	0.003*	0.955
HbA1C (%)	6.8 ± 1.2	5.6 ± 0.3	7.3 ± 1.4	<0.001*	<0.001*	<0.001*	<0.001*
hsCRP (mg/L)	–	2.5 (1.4, 4.2)	3.1 (1.3, 5.9)	–	–	–	0.084
Serum podocalyxin (ng/mL)	9.97 ± 5.34	15.34 ± 18.21	17.67 ± 20.7	<0.001*	<0.001*	<0.001*	<0.001*

Data represent mean ± standard deviation unless otherwise stated; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TCH, Total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein.
* $P < 0.05$ is considered statistically significant.

14.9 ± 17.7 ng/mL; $P < 0.02$, but patients without MCS were still having higher levels of s-Podxl than patients with D-NPAD ($P < 0.001$). All subjects were classified into three groups according to the tertiles of s-Podxl level (Table 3). There were statistically significant differences in the number of patients with CHD ($P = 0.007$) and MI ($P = 0.019$) between the highest and lowest tertile of s-Podxl level. We observed that subjects with no vascular complications (D-NPAD) constitutes 41% of the patients in the first tertile of s-Podxl. Until now, there is no data on the relationship between s-Podxl concentrations and the clinical stages of PAD. In Table 3, patients with advanced PAD-FS were significantly higher in the 3rd tertile compared with the 1st or 2nd tertile of S-Podxl concentrations ($P < 0.001$).

2.2. Predictor variables of s-Podxl in regression analysis

In all study subjects, univariate regression analysis showed that no statistically significant associations between s-Podxl levels and all other available quantitative parameters. Also, multivariate regression analysis demonstrated the same unpredictability for s-Podxl after adjusting for confounding.[25] Multivariate regression analysis for sex and age adjusted subjects, didn't obtain any significant prediction for s-Podxl. In subjects with PAD (NGT-PAD/D-PAD), the univariate model of analysis showed that s-Podxl levels were associated with smoking (pack-years) ($\beta = 0.172$, $P = 0.042$) and HbA1c ($\beta = 0.189$, $P = 0.006$). In multivariate model, smoking (pack-years) and HbA1c remained significant predictors for s-Podxl, independently of each other. In D-PAD group, the univariate regression analysis showed that s-Podxl levels were associated with MCS ($\beta = 0.279$, $P = 0.003$) and HbA1c ($\beta = 0.352$, $P < 0.001$). In multivariate regression, both variables remained significant and the strongest predictors for s-Podxl.

2.3. Predictor variables of PAD

We found that the prevalence of PAD increases with the increase tertiles of s-Podxl, therefore, we investigated whether s-Podxl can predict PAD in multivariate or univariate regression models. Univariate regression analysis showed that, each 0.5 ng/mL higher in the s-Podxl level leads to a 12% higher in odds ratio (95% confidence interval = 7.0–17, $P < 0.001$) for progressive PAD (progression from without PAD up to PAD-FS II). Multivariate regression analysis controlled for sex, age, BMI, HbA1c, LDL, HDL, and eGFR, showed that, there was an improvement in predictive power of s-Podxl by 27%: each 0.5 ng/mL higher in s-Podxl level now leads to a 15% higher in odds ratio (95% confidence interval = 8–24, $P < 0.001$) for progressive PAD. In multivariate fashion, we noticed that age was significantly positively predictive of PAD: Each unit increase in age, leads to a 9% increase in PAD progression (95% confidence interval = 1–16, $P = 0.029$). Therefore, we compared the predictive power of s-Podxl and age for PAD by calculating the odds ratio per one standard deviation change: one standard deviation increase of s-Podxl results in an odds ratio of 1.4 (1.2–1.9, $P < 0.041$) for the prevalence of MI and odds ratio of 3.4 (95% confidence interval = 2.2–4.6, $P < 0.001$) for progression of PAD; one standard

Table 2
Baseline variables of sex and age adjusted subjects in the three studied groups.

	D-NPAD	NGT-PAD	D-PAD	P(all groups)	P(1 vs. 2)	P(1 vs. 3)	P(2 vs. 3)
n	36	40	51				
Male sex (%)	18 (52)	26 (65)	35 (70)	0.277	0.411	0.20	0.617
Age (years)	61.9 ± 9.8	60.2 ± 5.8	61.1 ± 4.2	0.713	0.865	0.495	0.427
BMI (kg/m^2)	29.8 ± 4.7	27.5 ± 3.1	33.6 ± 6.9	<0.001*	<0.001*	0.042*	<0.001*
LDL (mg/dL)	108.3 ± 30.9	119.8 ± 46.4	96.6 ± 42.5	0.034*	0.362	0.224	0.018*
TCH (mg/dL)	197.2 ± 34.8	204.9 ± 54.1	181.7 ± 50.2	0.047*	0.455	0.232	0.014*
HDL (mg/dL)	50.3 ± 15.5	54.1 ± 11.6	46.4 ± 11.7	0.003*	0.412	0.054	<0.001*
TG (mg/dL)	194.8 ± 124	159.4 ± 88.5	203.7 ± 106.2	0.176	0.189	0.844	0.060
eGFR (mL/min/1.73 m^2)	72.9 ± 17.8	89.7 ± 38.4	76.6 ± 20.6	0.061	0.231	0.074	0.534
SBP (mmHg)	143 ± 20	137 ± 19	140 ± 15	0.475	0.342	0.281	0.756
DBP (mmHg)	88 ± 9	80 ± 12	77 ± 13	0.001*	0.038*	0.001*	0.214
HbA1C (%)	5.9 ± 1.2	5.5 ± 0.2	7.7 ± 1.3	<0.001*	<0.001*	0.047*	<0.001*
hsCRP (mg/L)	–	2.6 (1.4, 4.3)	3.4 (1.5, 6.8)	–	–	–	0.085
serum podocalyxin (ng/mL)	9.97 ± 5.34	14.49 ± 15.8	18.4 ± 21.8	<0.001*	<0.001*	<0.001*	0.062

Data represent mean ± standard deviation unless otherwise stated; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TCH, Total cholesterol; HDL—C, high-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein.
* $P < 0.05$ is considered statistically significant.

Table 3
Characteristics of study subjects according to Serum Podxl tertile levels.

	1st Tertile $n = 75$	2nd Tertile $n = 74$	3rd Tertile $n = 76$	P (all 3 Tertiles)	P (1 vs. 3)
Serum podocalyxin (ng/mL)	≤11.56	11.57–15.86	≥15.87		
Male sex (%)	48 (64)	46 (63)	49 (65)	0.892	0.887
Age (years)	67.5 ± 11	68.6 ± 9.1	68.1 ± 9.2	0.159	0.142
BMI (kg/m^2)	28.8 ± 4.3	29.6 ± 4.8	30.6 ± 5.2	0.184	0.087
LDL (mg/dL)	108.2 ± 46.4	100.5 ± 34.8	108.2 ± 38.6	0.415	0.896
TCH (mg/dL)	197.2 ± 58	185.6 ± 38.6	193.3 ± 42.5	0.458	0.731
HDL(mg/dL)	54.1 ± 15.4	50.3 ± 11.6	51.4 ± 12.7	0.561	0.335
TG (mg/dL)	168.2 ± 106.2	177.1 ± 106.2	186.02 ± 88.5	0.482	0.223
eGFR (mL/min/1.73 m^2)	78.2 ± 33.7	66.5 ± 19.3	68.7 ± 16.4	0.034*	0.059
SBP (mmHg)	138 ± 19	145 ± 21	141 ± 20	0.152	0.632
DBP (mmHg)	79 ± 11	77 ± 9	79 ± 16	0.498	0.583
HbA1C (%)	6.9 ± 1.3	6.5 ± 1.2	6.7 ± 1.2	0.079	0.436
hsCRP (mg/L)	2.9 (1.0, 5.7)	2.3 (1.0, 4.5)	3.0 (1.6, 5.6)	0.264	0.843
D-NPAD	30(41)	4 (6)	2 (3)		
D-PAD	24 (33)	42 (58)	54(72)	<0.001*	<0.001*
NGT-PAD	16 (22)	33 (45)	20 (27)		
PAD					
Non-PAD	30(41)	4 (6)	2 (3)		
Fontaine Stage I	18 (24)	34 (47)	34 (46)	<0.001*	<0.001*
Fontaine Stage II	24 (33)	35 (48)	44 (58)		
Stroke	7 (10)	9 (13)	6 (9)	0.672	0.768
CHD	11(15)	22 (30)	25 (33)	0.021*	0.007*
MI	7 (10)	17(24)	15(21)	0.039*	0.019*
CAD	11 (15)	13 (18)	15 (20)	0.646	0.372

Data represent mean ± standard deviation unless otherwise stated; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TCH, Total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, Glycated hemoglobin; hs-CRP, high-sensitive C-reactive protein; D-NPAD, type 2 DM with no PAD; D-PAD, PAD and type 2 DM; NGT-PAD, PAD with normal glucose tolerance; PAD, Peripheral Arterial Disease; CHD, coronary heart disease; MI, myocardial infarction; CAD, carotid artery disease.

* $P < 0.05$ is considered statistically significant.

deviation increase of age results in an odds ratio of 1.8 (95% confidence interval = 1.2–2.7, P < 0.001) for progression of PAD. In the unadjusted model, each one-unit increase in the standard deviation of s-Podxl leads to an odds ratio of 2.8 (95% confidence interval = 2.1–3.6, P < 0.001) for the PAD prevalence. Control of sex, age, SBP, HbA1C, TC, TG, LDL, HDL, eGFR, alkaline phosphatase, γ-glutamyl transferase, alanine transaminase, and aspartate transaminase, has made the association more powerful: odds ratio per one standard deviation increase of s-Podxl became: 3.9 (95% confidence interval = 2.6–5.1).

3. Discussion

As far as we know, this is the first study that has investigated the associations between s-Podxl levels and PAD in patients with T2DM. We showed that s-Podxl levels were significantly higher in patients with T2DM and PAD compared with subjects with T2DM without any vascular complications. In the present study, we found that the PAD prevalence increases with each one increase of the standard deviation of s-Podxl and this association became more powerful after adjustment for multiple variables. A recent study reported an association between s-Podxl concentrations and markers of CVD. Shoji et.al claimed that s-Podxl levels were significantly associated with cIMT and this association remained significant even after controlling the common CVD risk factors such as diabetes, dyslipidemia, hypertension, sex, age and current smoker.[22] In addition, the number of cases with CHD increased with increasing tertiles of s-Podxl from 11(15%) in the 1st tertile, over 22 (30%) in the 2nd tertile, to 25 (33%) in the 3rd tertile, ($P = 0.021$) (Table 3). In our cross-sectional study, the prevalence of MI was only 17%, but after multivariate control for sex, HbA1C, eGFR, LDL and age, we found that one-unit increase of s-Podxl tertiles category results in an odds ratio of 1.76 (95% confidence interval = 1.1–2.8, $P = 0.043$) for MI. Previous study showed that the release of urinary Podxl from injured podocytes occurs as vesicle-like structures and/or as a result of shedding microvilli.[26] Various types of cells and tissues express Podxl including neurons, lungs, platelets and vascular endothelial cells.[27] Our results of high s-Podxl levels in patients with PAD and MI might be due to its release from injured endothelium through a mechanism which may be similar to that of urinary podxl release from injured podoctyes. Previous studies have shown the role of Podxl in endothelial function and vascular inflammation. It has been shown that endothelial cells lacking in Podxl show a weak ability to spread on a laminated dish.[28] Another study showed that, there was an increase in non-specific inflammatory infiltrates within the vessels and CRP level in murine endothelial cells after conditional knock out of the Podxl gene. Also, the study reported that, after thrombin stimulation, endothelial cells lacking in Podxl show persistent F-actin stress fibers and delayed recovery of vascular endothelial cadherin cell contacts.[29] In our study, we found higher levels of s-Podxl in patients with PAD (D-PAD and NGT-PAD) compared with subjects without PAD suggesting that s-Podxl might be associated with CVD independently of diabetes. However, we observed that, subjects with MCS which is a common feature in diabetic patients with PAD,[30] demonstrated significantly higher levels of s-Podxl compared with subjects without MCS and these levels were also significantly higher compared with patients with diabetes alone (D-NPAD), presenting the possibility that high s-Podxl might have a pathological role in the process of vascular calcification. The present study showed that, the total smoking dosage in patients with PAD (NGT-PAD and D-PAD) was significantly and positively associated ($\beta = 0.172$, $P = 0.042$) with s-Podxl. Smoking is known to be a risk factor for calcification of the coronary artery along with age, hypertension and diabetes,[31] as it reduces the level of nitric oxide, leading to vasomotor and endothelial dysfunction.[32] Previous long term study has shown that active cigarette smoking is an independent risk factor for the annual increase in total calcification volumes in carotid arteries.[33] We can't confirm whether smoking increases s-Podxl releases by injuring the endothelial layer during vascular calcification process because our study is cross-sectional. However, our results suggest that s-Podxl may play a role in the pathogenesis of PAD in patients with T2DM by increasing the vascular calcification process.

4. Conclusion

This is the first study report that high s-Podxl levels were associated with PAD in patients with T2DM. We showed that PAD and MI are

associated with higher s-Podxl values. Also, we demonstrated, the association between s-Podxl levels and MCS in patients with T2DM and PAD. We think that s-Podxl can be used either alone or with other markers for CVD to determine diabetic patients with high risk of PAD and other macrovascular complications of diabetes in the hope of avoiding or reducing such complications and its related mortality.

4.1. Study limitations

First: The causal relationship between s-Podxl levels and the development of PAD in patients with T2DM could not be established due to the cross-sectional design of our study. Second: The sample size of the study was relatively small. Therefore, interventional studies and Large prospective trials are recommended to further examine the relationship between s-Podxl levels and macrovascular complications in patients with T2DM.

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