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Relationship between serum podocalyxin levels with the major risk factors of peripheral artery disease among patients with type 2 diabetes mellitus

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Abstract

Objective and aim: A positive association between serum podocalyxin (PCX) levels and peripheral artery disease has been reported. The aim of this study is to measure the level of serum PCX in patients with T2DM as well as looking for its relation to major PAD risk factors. Methods: Observational cross-sectional study was conducted on 200 patients with type 2 diabetes (T2DM) and a control group of 100 apparently healthy subjects (ages 30-65 years). All the participants were investigated for the major risk factors of peripheral artery disease (PAD) which included (cigarette smoking, diabetes mellitus, hypertension, dyslipidemia, obesity, and physical activity). Serum PCX, blood sugar, HbA1c, Lipid profile, C-reactive protein, body weight, and height, were measured in patients and healthy subjects. Results: Significantly higher PCX level (p=0.01) was found in patients compared to controls. Significant differences were found for PCX with body mass index and C − reactive protein (p=0.001 and <0.01 respectively). In the patient group, positive correlations of PCX were observed with BMI, HbA1c, and C-RP (p=0.00, p=0.01, and p=0.001 respectively). BMI was the best predictor of PCX level in both groups. Conclusion: Elevated S.PCX levels in conjunction with increased body weight may be strongly associated with peripheral artery disease in diabetic patients.

Keywords
Podocalyxin, dyslipidemia, HbA1c, and C-reactive protein

Podocalyxin (PCX) is a sialomucin CD34, it is a main component of the cell surface that is expressed in the renal glomeruli within the epithelial cells (podocyte) as a glycocalyx which was previously named a sialated protein[1]. PCX functions to maintain the slit diaphragm and the shape of the Podocyte and upon injury of the podocyte the PCX is released from the microvilli into the urine[2,3]. This excretion of PCX from the kidney can be used as an early marker for diabetic nephropathy and by itself it is a biomarker for glomerular disease[4]. PCX is widely expressed also on the endothelial cell surfaces all over the body like neurons, mesothelial cells lining organs, hematopoietic stem cells, megakaryocytes, and vascular endothelial cells[5].

Peripheral arterial disease (PAD) is a clinical condition that happened when blood flow to the limbs is reduced because of the narrowing of the arteries. [6] The major cause of such a condition is atherosclerosis in patients >40 years.[7] Low extremity PAD is common among the general population and is associated with a 2 to 5-fold increased risk of future cardiovascular morbidity and mortality.[8] It is recognized as a coronary heart disease risk equivalent and its prevalence is predicted to increase parallel to the aging of the population.[9] The prevalence of PAD is nearly

12% in adults with mostly equal in regards to men and women.[10] The prevalence of PAD is strongly associated with aging as about 20% of adults aged more than 70 years have PAD. [11] The common clinical presentation of PAD is claudication; though, it occurs less frequently than before. Patients may present with classic claudication, atypical leg pain, rest pain, ischemic ulcers, gangrene, or asymptomatic and up to 50% of PAD patients are asymptomatic.[12] Regarding the risk factors of PAD, the most common are increasing age, smoking, diabetes mellitus, obesity, hypertensive, and hyperlipidemia.[13] There is 2-4 times increase in the risk of PAD with smoking.[14] Those who have diabetes are at higher risk of developing atherosclerosis, the main cause of PAD and hypertension is a common and important risk factor for all vascular diseases including PAD.[15] The association with dyslipidemia appears to be multifaceted. High total cholesterol is associated with increased risk, whereas higher high-density lipoprotein (HDL) cholesterol is associated with decreased risk.[16]

Globally, the prevalence of PAD is high and reports showed that two hundred millions of people worldwide have PAD.[17] This increase in the prevalence of PAD globally is because of the widespread rise in the occurrence T2DM and aging of the population of the world.[18] Stiffness of the vessels' wall together with T2DM are considered for having PAD.[27] The mortality risk from cardiovascular diseases is five times more in patients with PAD and T2DM compared to those with single morbidity.[19]

Evidently, PCX is expressed in vascular endothelial cells and as for this it is assumed that serum PCX level is related to endothelial injury. Nevertheless, studies investigating serum PCX in patients with PAD are limited. Accordingly, the general aim of this study is to measure the level of serum PCX in patients with T2DM as well as look for its relation to major PAD risk factors.

Materials and Methods

Study population

A total of 317 subjects aged 30-65 years were examined. Of these, 217 patients with type 2 DM and 100 apparently healthy subjects (controls) of comparable age and sex were enrolled in this study. Then 17 patients were excluded because of chronic renal and liver disease.

Study design

An observational cross-sectional study performed from September 2021 to March 2022 at the department of Medical Laboratory Technology, College of Health and Medical Techniques – Shekhan, Duhok Polytechnic University, Duhok, Kurdistan Region, Iraq.

Sample selection

A consecutive sampling procedure was applied to select patients with type 2 DM from the Diabetic center and Gulan Hospital. The healthy subjects were selected and recruited by personal request from the staff and sub-staff of the Azadi teaching hospital and Gulan Hospital. Recruitment of patients was done among those who visited the diabetic center at Azadi teaching hospital from (9:00 am to 11:00 am). Consent to participate in the research was assigned by each subject.

Inclusion and Exclusion criteria

Inclusion criteria were patients with type 2 DM without a history of chronic diseases (renal disease, liver diseases, thyroid dysfunction, cardiovascular and malignant diseases) pregnant women were also excluded from the study. Apparently healthy subjects with a history of a family history of diabetes were excluded from the study.

Anthropometric measurements

A questionnaire included age, gender, exercise, family history of diabetes, diabetes duration, and anthropometric parameters. For assessing the smoking status, we used the questionnaire of smoking behavior (smoking twenty or more cigarettes per day was defined as heavy smokers). The blood pressure was measured while the patient is sitting using a standard sphygmomanometer and the hypertension was defined as SBP ≥140 mmHg and DBP ≥90 mmHg[28]. We measured the height and weight of all subjects and calculated the body mass index (BMI). The BMI was defined as less than 18.5 kg/m^2 being underweight, between 18.5-25.0 Kg/m^2 being normal weight, between 25.0-30 $Kg/m^2$is overweight and more than 30 Kg/m^2 is obese.

[29] We used waist circumference to determine central obesity. Waist circumference was measured by measuring tape positioned at the high point of the iliac crest, central obesity was defined as female with a waist circumference of more than 88 cm and male with more than 102 cm. [30] Physical activity defining as doing at least 150−300 minutes of moderate-intensity aerobic physical activity per week. [31] A family history of diabetes defines as a positive and negative family history, while the duration of diabetes is classified as a long duration of more than years and a short duration of less than years. Hyperlipidemia was defined as serum cholesterol of more than 200 mg/dl and triglyceride of more than 150 mg/dl. [32]

Biochemical Measurements

A blood sample was taken from participants at the Clinical Biochemistry section of the laboratory of Azadi Hospital at the morning after about 12-14 hours of fasting overnight and with no heavy physical activity 2 hours before the examinations. Six ml of blood was taken from each patient of which 2 ml of them put in the EDTA tube to measure the HbA1c. The remaining 4 ml were transferred into a gel tube that was kept for 15 minutes, then the tube was placed in a HITACHI centrifuge at 3000 rpm for 10 minutes to separate serum. Half of the serum was kept in the freezer (-20 C) for measuring podocalyxin. Biochemical measurement was done for HbA1c, FBS, CRP, TG., and cholesterol by Roche/Hitachi Cobas series 6000 (auto analyzer biochemical machine). Elisa methods were used for measuring human podocalyxin in serum by the Human PCX (Podocalyxin) ELISA Kit (Catalog No: E-EL-H2360, Sensitivity: 0.10.

Ethical considerations

The study got ethical approval from the research ethics committee of the Directorate General of Health in Duhok on 18 August 2021 (18082021-8-24).

Statistical analysis

Analysis of the study data was done by the Statistical Package for Social Silence SPSS version 18.0. Frequency tables and calculation of mean and standard deviation were obtained for description of data. The differences of means of continuous data between groups were evaluated using Student's-t-test. The relationship between PCX levels and different variables was determined using a correlation analysis by Pearson's correlation coefficient (r) value. The level of statistical significance (p-value) was set at $\leq$0.05.

Results

Table 1, shows the baseline characteristics of healthy subjects and patients with type 2 diabetes mellitus. The differences found were not significant in regards to age, smoking, and cholesterol between the control and patient groups. Significantly higher PCX, BMI, HbA1c, C-RP, triglycerides, and less physical activity levels were observed in patients compared to controls (p=0.01 for all parameters).

Table 1. Baseline characteristics of patients and controls

Characteristics	Controls (100)	Patients (200)	p-value
Age (years)	48.38 ±11.4	52.28 ±7.4	0. 07
BMI (kg/m²)	23.1 ±3.8	28.36 ±4.7	0. 01
Hypertension	7.0(7.0)	46.0(23.0)	0.01
Smoking n(%)	14(14.0)	32 (16.0)	0.12
Physical activity n(%)	29(29.0)	27(13.5)	0.01
FBS (mg/dl)	89.13 ±9.17	191.26 ±80.38	0.01
HbA1c %	5.16 ±0.41	8.98 ±1.99	0.01
C-reactive protein (mg/L)	1.77±2.27	5.89±9.86	0.01
Cholesterol(mg/dl)	182.18±29.8	187.07±38.6	0.22
Triglycerides(mg/dl)	114.47±48.7	219.07±162	0.01
S.PCX (ng/ml)	92.68 ±30.3	139.46 ±43.83	0.01
Results presented as mean + SD, significant p<0.05			

Table 2. Shows serum PCX level stratified by major risk factors of peripheral artery disease. As shown, the major risk factors of peripheral artery disease were significantly associated with serum PCX level in diabetic patients, PCX level was significantly associated with BMI and C-RP (P<0.01). No significant association was observed between PCX level and age, hypertension, smoking habits, blood sugar, HbA1c, cholesterol and triglyceride.

Table 2 Serum PCX level in diabetic patients stratified by all parameters.

S.PCX(ng/ml)			
Variables	N	Mean ± SD	P-value
Age (years)			
<50	69	139.65±46.9	0.94
=>50	131	139.12 ±23.5	
BMI (kg/m^2)			
Normal weight	37	123.35±338	
Over weight	84	131.38±37.6	0.01
Obese	79	155.59±49.3	
Hypertension			
Hypertensive	46	150.26±45.5	0.05
Normotensive	154	136.24±37.5	
Smoking habits			
Smokers	169	137.50±43.3	0.14
Non-smokers	31	150.12±46.9	
Physical activity			
Active	27	138.93±43.5	0.93
Sedentary life	173	139.85±45.2	
Fasting blood sugar (mg/dl)			
<125	42	136.9±31.3	0.67
≥125	158	140.1±46.8	
HbA1c (%)			
<6.5	9.0	129.7±13.7	0.09
≥ 6.5	191	139.9±44.84	
C-reactive protein(mg/L)			
<6.0 mg/L	150	135.08±43.6	0.01
≥6.0 mg/L	50	152.61±58	
Cholesterol (mg/dl)			
<200 mg/dl	131	135.50 ±37.16	0.08
≥200 mg/dl	72	146.94 ±54.0	
Triglyceride (mg/dl)			
<150	70	143.7±46.8	0.31
≥150	130	137.1±42.3	

The relationship between PCX and studied parameters in controls and diabetic patients is presented in Table 3. As shown, in the patient group positive correlations of PCX were observed with BMI, HbA1c, and C-RP (p=0.01, p=0.01, and p=0.01 respectively). No significant correlation was found between serum PCX and other parameters.

Table 3. Pereson's correlation coefficient (r) between serum PCX and other parameters in patients and controls

		Controls (100)		Patients (200)
Variables	r	P-value	r	P-value
Age	0.18	0.07	-0.12	0.11
BMI (kg/m2)	0.10	0.32	0.282	<0.01
Hypertension	0.39	0.00	-0.135	0.05
Smoking	-0.01	0.94	0.104	0.14
Physical activity	0.36	0.00	-0.01	0.94
Fasting blood sugar	-0.32	0.00	0.17	<0.01
HbA1c	0.01	0.98	0.169	<0.01
C-reactive protein	0.19	0.055	0.202	<0.01
Cholesterol mg/dl	0.035	0.73	0.039	0.58
Triglyceride mg/dl	-0.113	0.26	-0.10	0.16

Table 4 shows the linear regression analysis with PCX as the dependent variable; BMI, fasting blood sugar, HbA1c, CRP (p<0.01) as well smoking (p<0.01) were significant predictors of the PCX level. While age and lipid parameters (serum cholesterol and triglyceride levels) have no predictor in all participants.

Table 4. A Linear Regression Analysis with PCX as Dependent Variable

Independent variable	B	B	p-value
Age	0.54	0.08	0.17
BMI	4.51	0.53	<0.01
Hypertension	-10.33	-0.10	0.13
Smoking	3.061	0.253	<0.01
Physical activity	14.88	0.116	0.09
Fasting blood sugar	0.22	0.38	<0.01
HbA1c	8.33	0.45	<0.01
C-reactive protein	1.50	0.27	<0.01
Cholesterol	0.087	0.073	0.25
Triglycerides	0.026	0.083	0.15
B: regression coefficient			

Discussion

To our knowledge, this is the first study to examine the level of serum PCX in patients with type 2 diabetes mellitus and to analyze its

relation to major risk factors for peripheral artery disease. Hence the study focused on serum PCX levels because PCX is expressed in vascular endothelial cells.

The important finding of the research shows significantly higher levels of serum PCX in diabetic patients compared with levels in healthy subjects. The results confirm a correlation between obesity and peripheral artery disease as the mean PCX level observed in the obese diabetic patient was significantly higher than that observed in normal weight. These results are in accordance with the previous study.[20] Furthermore, in diabetic patients a positive significant correlation was found between serum PCX and fasting blood sugar and HbA1c[21]. Nevertheless, linear regression analysis of our study confirms a significant association between PCX and BMI as well as smoking habits. Age and lipid parameters (serum cholesterol and triglyceride level) had no predictor in all participants. These findings are in agreement with recent studies.[20]

A significant association between PCX and the anthropometric measurement of BMI and CRP was observed also. In this study, BMI correlated with PCX in diabetic patients, the association was less significant with CRP, suggesting that the more metabolic risk factors, the more level of podocalyxin.[34] A new study found that urinary PCX levels were significantly higher in obese patients compared to non-obese. Interestingly, PCX cells showed a good correlation with BMI in obese adults.[23] One of the results reached by researchers recently found that patients with existing weight mainly adults with obesity, had increased amounts of PCX, and these finding supports the hypothesis that early podocyte injury and podocyturia can occur in individuals with obesity. [24], and suggested an important mechanism throw which obesity can influence a higher risk of PAD in diabetic patients.[25] It is essential to see stronger associations between endothelial dysfunction initiating atherosclerosis and obesity, as well as chronic persistent inflammation have been reported for type 2 diabetic patients.[26] A study by Horrillo et al. mentioned that conditional knocks out of the PCX gene in the endothelial cells in murine elevate the C-reactive protein levels and non-specific inflammatory infiltrate within the vessels or vasculitis.[27] Although, it is still not clear what kind of stimulation lead to increased serum PCX concentration. More investigations are needed to see how serum PCX concentration increases in conjunction with intima-media thickness. However, no firm conclusion can be drawn from this study, but the findings may explain some of the thoughts regarding this issue. Further study on large scale may be necessary to confirm our observation.

Conclusions

High serum levels of PCX are associated with the risk factor of PAD. Elevated serum PCX levels in relation with increased body weight may be strongly associated with PAD in diabetic patients.

Limitations of the study

Because of the design of this research which is a cross-sectional one, it is not easy to determine the causal association between serum PCX and having PAD in diabetic patients. On the other hand, the relatively small sample size of the current study could limit the outcomes. So, it is recommended to do experimental studies and clinical trials to examine more the relation between serum PCX and complications of T2DM specifically the macrovascular ones.

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Conflict of interest

I declare that I have no potential conflicts of interest to disclose.

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