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URINARY PODOCALYXIN- A MARKER OF RENAL INSULT IN PREDIABETS AND DIABETES

Diabetology

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ABSTRACT

Introduction: Prediabtes and diabetes are associated with considerable risk of diabetic kidney disease. Microalbuminuria is used as an early marker for diagnosing diabetic nephropathy but lacks sensitivity and specificity. Podocalyxin is a podocyte related protein and is found to be present in urine in case of podocyte damage. **Aims and Objectives:** The present study was planned to explore utility of urinary podocalyxin as a marker of renal insult. **Material and Methods:** 60 patients each of prediabetes and diabetes diagnosed on the basis of American Diabetes Association criteria were enrolled along with 40 healthy controls. In addition to the routine investigations, estimated glomerular filtration rate(eGFR) was calculated by CKD-EPI equation and urinary podocalyxin levels were measured by ELISA. **Results and Conclusion:** A significant difference was found in mean levels of eGFR and urinary podocalyxin among the three groups with highest prevalence of hyperfiltration in prediabetes.

KEYWORDS

Prediabetes, eGFR, Urinary podocalyxin

Introduction

The prevalence of diabetes mellitus has risen dramatically and is expected to rise to 783 million by the year 2040.[1] Prediabetes is an intermediate condition in which glucose levels are above the levels found in healthy individuals but below the diagnostic cut off value of diabetes mellitus. In a cross sectional study carried out in 15 states of India, the prevalence of diabetes and prediabetes has been reported as 7.3% and 10.3% respectively.[2] Nearly 25% of prediabetes patients develop diabetes in 3-5 years and about 70% of patients with prediabetes develop diabetes in their life time. [3] The metabolic derangements in diabetes lead to secondary complications which affect multiple organ systems.[4] Prediabetes is also associated with significant risk of complications. In a prospective study, it was found that the 13.8% of prediabetes patients developed cardiovascular, chronic kidney disease or heart failure in follow up period of 11 years. However, amongst the above patients who had complications, only 12.4% progressed from prediabetes to diabetes. This provides an evidence that halting the progression of prediabetes to diabetes does not substantially decrease the complication rate.[5] The prevalence of diabetic kidney disease was found to be ranging from 4.5% to 26.0% in prediabetes.[6]

Microalbuminuria is stated to be an early marker for renal damage [7] and is reported to be present in prediabetes or at the time of diagnosis of diabetes mellitus. This reinforces the fact that renal pathological changes might set in much before the clinical diabetes. However, microalbuminuria lacks sensitivity and specificity for diagnosis of diabetic kidney disease as it has been documented that about 10% of diabetic patients with progressive renal disease have normal albumin levels and urine microalbumin levels can go back to normal without any treatment or intervention.[8]

A large metanalysis has reported modest association of prediabetes with chronic kidney disease.[9] However,, many cohort studies have reported that prediabetes was not associated with eGFR decline alone. [10] In a longitudinal study, it was found that prediabetes if adjusted for prognostic factors was not associated with eGFR decrease after a 2 years follow up period. [11] Some studies have also reported glomerular hyperfiltration in prediabetes[12] and it has been suggested that there is common pathway for hyperfiltration and albuminuria in prediabetes also as shown in the early stages of diabetes mellitus.[13] So, there is conflicting evidence regarding eGFR changes in prediabetes. Thus a need is also felt for a biomarker which can predict the changes in glomerular basement membrane at prediabetes stage. This would be of immense help in screening, timely intervention and prevention of progression of diabetic nephropathy.

The apical surface of podocytes faces the urinary space and is coated by a sialic acid rich glycocalyx that is designated epithelial polyanion and is composed mainly of podocalyxin.[14] Urinary podocalyxin has been found to be a marker of podocyte dysfunction as podocyte loss leads to denudation of glomerular basement membrane leading to abnormal filtration process.[15] Recently, few studies have investigated urinary podocytes and podocalyxin levels in diabetic patients, one such study reported the utility of urinary podocalyxin as early predictable marker of diabetic nephropathy [4] whereas other has documented that urinary podocalyxin could not predict the progression of diabetic kidney disease.[16]

However, to the best of our knowledge, urinary podocalyxin levels have not been studied in prediabetes so far. The onset and progression of nephropathy can be prevented if the focus is shifted from cure to prevention in terms of diagnosis at prediabetes stage and prompt treatment of early kidney abnormalities. So the present study was planned to estimate the levels of urinary podocalyxin in prediabetes and diabetes and compare with healthy controls.

Material and Methods

The study design was observational and cross sectional. 60 patients each of diabetes and prediabetes diagnosed on the basis of criteria given by American Diabetes Association (ADA)2016 [17] were recruited after taking informed consent. 40 apparently healthy individuals formed the control group. Detailed general physical examination along with anthropometric measurements were done. A fasting blood sample was taken for estimation of plasma glucose, serum urea, serum creatinine and glycated hemoglobin . Plasma glucose, serum urea and serum creatinine were estimated by spectrophotometric method on fully automated chemistry analyzer (Beckman Coulter AU5800).Glycated hemoglobin was measured by high performance liquid chromatography using automated analyzer Biorad D10. The calculation of eGFR was done by using CKD-EPI equation.[18]

A spot urine sample was also taken for urinary albumin and podocalyxin estimation. Urinary microalbumin concentration was measured by immunoturbidimetric method on automated chemistry analyzer. Urinary microalbumin concentration if found to be less than

30ug/mg of creatinine was considered as normoalbuminuria, 30-300ug/mg of creatinine- microalbuminuria present and if >300ug/mg of creatinine means macroalbuminuria is present. Urinary podocalyxin levels in the spot urine sample were measured by ELISA BT BioLaboratory, China).

Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed as number and proportion. Chi-square test was used for the categorical variables and Student's t-test (ANOVA for 3 groups) was used to compare variables between controls, prediabetic and diabetic group. Post hoc analysis was done to find out specific differences between the groups. A p-value <0.05 was considered as significant.

Results

The control, prediabetes and diabetes group comprised of 32.5% males and 67.5% females, 41.6% males and 58.4% females and 53.3% males and 46.6% females respectively. The mean age (years) was found to be 47.15±10.82, 49.3± 12.32 and 54.73± 8.13 in control, prediabetes and diabetes group respectively. Table 1 shows the various biochemical investigations in the three groups. Table 2 shows the post hoc analysis of urinary podocalyxin levels among the three groups.

In diabetes group, 36.6% and 35% patients were found to have microalbuminuria and macroalbuminuria respectively as compared to 23.3% and 3.3% in prediabetes. No consensus is available for the definition of hyperfiltration and GFR varies with age, sex and race. So the values above 95 percentile for age and gender specific values were considered as hyperfiltration (Shilpasree et al)and by applying this criteria, 8.3% diabetic, 13.3% prediabetic patients and 5.2% healthy controls were found to have hyperfiltration. A significant positive correlation was found between serum creatinine levels and urinary podocalyxin (r=0.264, p=0.001) and negative correlation between eGFR and urinary podocalyxin levels (r = -0.242, p=0.002) was observed (Figure 1 and 2 respectively).

Table 1-Biochemical investigations in control, prediabtes and diabetic group patients

	Control Group n=40	Prediabetic Group n=60	Diabetic Group n=60	p value
FBS (mg/dL)	92.07± 6.70	111.65± 11.21	163.9± 43.27	0.000
HbA1c (%)	5.27± 0.20	5.87± 0.45	9.07± 1.89	0.000
Serum Urea (mg/dL)	26.4± 9.13	27.4± 16.28	35.88± 22.94	0.011
Serum Creatinine (mg/dL)	0.86± 0.23	0.93± 0.48	1.19± 0.56	0.001
Urinary Podocalyxin levels (ng/mL)	1.41±0.39	1.52±0.55	1.80±0.61	0.001
eGFR(ml/min/1.73m^2)	96.20 ± 17.70	94.16 ± 15.94	74.26 ±29.30	0.000

Table 2- Post hoc comparison of urinary podocalyxin among the three groups

Control Group	Prediabetes	Diabetes	0.328 0.001
Prediabetic Group	Control	Diabetes	0.328 0.006
Diabetic Group	Control	Prediabetes	0.001 0.006

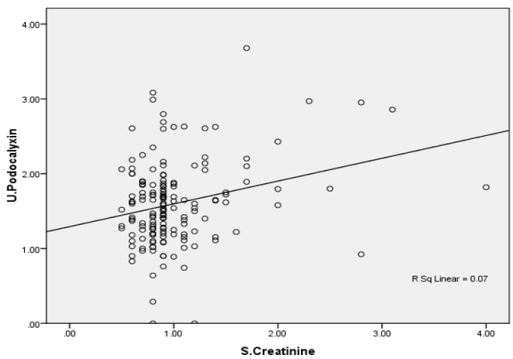

Figure 1: Scatter plot showing correlation between urinary podocalyxin (ng/mL) and serum creatinine (mg/dL) levels

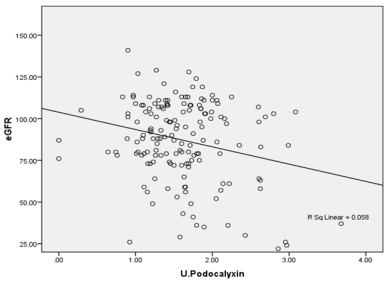

Figure 2: Scatter plot showing correlation between urinary podocalyxin (ng/mL) and eGFR(mL/min/1.73m^2)

DISCUSSION

Renal hyperglycemic injury leads to changes in several cellular events and activation of signalling pathways resulting in renal fibrosis, mesangial expansion and glomerular hypertrophy. [19] In the current study, a significant difference was found in the prevalence of hyperfiltration among the three groups. Shilpasree et al also reported significantly high prevalence of hyperfiltration in prediabetes and diabetes as compared to controls.[3] However, mean levels of eGFR in prediabetes and diabetes were found to be decreased as compared to the healthy controls and similar findings were observed in the present study.

The predictive value of urinary protein is limited in the diagnosis of diabetic nephropathy. It has been elucidated that pathological changes may have existed much before the presence of clinical symptoms.[20] So there is a need for new methods of diagnosis and new targets for prevention and treatment. During the occurrence and progression of diabetic nephropathy, podocytes suffer both functional and structural damage.[8] Therefore, detection of urinary podocytes and its related proteins is seen as potential marker of early diagnosis. A review article has also cited podocyturia to be present in active disease and emphasised that it appears before proteinuria.[21] Podocalyxin is a negatively charged sialoglycoprotein, expressed in the membrane of podocytes and plays an essential role in maintaining the function of glomerular podocytes foot processes stability and permeability of glomerular capillary wall.[22] In the present study, urinary podocalyxin levels were measured in the patients of diabetes, prediabtes and healthy controls and the difference in mean levels among the three groups was found to be statistically significant. Although the mean levels were high in prediabetes as compared to the control group, on post hoc analysis, the difference did not achieve the level of significance whereas significant difference was found between healthy controls and diabetes patients. The studies with larger sample size may be carried out to further explore the utility of urinary podocalyxin as marker of renal damage in prediabetes.

A significant negative correlation was found between urinary podocalyxin levels and eGFR and positive correlation between urinary podocalyxin and serum creatinine levels. Another study has also reported positive correlation of urinary podocalyxin with the progressively increasing serum levels of creatinine and negative correlation was seen with eGFR during the deterioration of diabetic kidney disease.[23]

CONCLUSION

The results of our study showed increased mean levels of urinary podocalyxin in prediabetic and diabetic subjects as compared to healthy controls but reached statistical significance only in diabetics. Urinary podocalyxin levels were also found to be negatively associated with eGFR in suggesting its role as a marker of diabetic nephropathy.

The main limitation of this study is that the number of patients was relatively small and homogenous. Therefore, the statistical analysis of the results could be underpowered. Further more extensive prospective studies with larger sample size should be planned to further explore the utility of urinary podocalyxin as a marker of renal insult in prediabetes.

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