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Correlation of urinary nephrin with albuminuria to predict early onset of nephropathy in patients with type 2 diabetes mellitus

Veluri Ganesh

PhD Scholar, Department of Biochemistry, Arupadai Veedu Medical College, Puducherry, Vinayaka Mission's Research foundation (Deemed to be university), Salem – 636308, Tamilnadu, India

Murugan M.

Professor, Department of Biochemistry, Arupadai Veedu Medical College, Puducherry, Vinayaka Mission's Research foundation (Deemed to be university), Salem – 636308, Tamilnadu, India
Email: ganeshbabu370@gmail.com

Veerabhadra Goud GK.

Associate Professor, Department of Biochemistry, Andaman & Nicobar Islands Institute of Medical Sciences, Port Blair, Andaman and Nicobar Islands.

Vijay Kumar Gangannagari

Tutor, Department of Microbiology, Akash Institute of Medical Sciences and Research Centre, Karnataka, India

Abstract---Nephropathy is a major microvascular complication in patients with type 2 diabetes mellitus (T2DM), where urinary albumin a gold standard and clinically using marker for detection of nephropathy. Some of the studies reported urinary albumin is not a marker for detection of nephropathy. Moreover, recent studies reported urinary nephrin was considered to be a predictable marker for nephropathy. The present study has been designed to evaluate the status of urinary albumin and nephrin to predict early onset of nephropathy in T2DM. The present study included 120 subjects, among these 80 subjects with T2DM again sub grouped into 2 based on ACR ratio (40 T2DM with Normoalbuminuria, 40 T2DM with Microalbuminuria) and 40 healthy controls. Routine biochemical parameters, urinary albumin were analysed by using standard laboratory methods and Urinary nephrin were analysed by using ELISA and statistical analysis was done by using Microsoft Excel Spread Sheets and SPSS Version 20.0. In T2DM Patients with

Normoalbuminuria shows a significant elevation of urinary nephrin but no variation in urinary albumin levels. The urinary albumin levels were significantly elevated in Group 3 subjects when compared to Group 1 and Group 2 ($P < .0001^{**}$). The U. Nephrin was positively correlated with glycosylated hemoglobin and urinary albumin ($r = 0.688, 0.722, P < .0001^{**}$). We found that urinary nephrin elevated in patients with T2DM with Normoalbuminuria than urinary albuminuria. We concluded that urinary nephrin might be a better marker than urinary albumin to predict early onset of nephropathy in T2DM Patients.

Keywords---type 2 diabetes mellitus, urinary nephrin, urinary albumin, HbA1c.

Introduction

T2DM caused by Hyperglycemia due to both defect in insulin secretion from the beta cells of pancreas and inactivation of insulin leads to insulin resistance (1). T2DM is one of the most leading disorder in the worldwide population, 415 millions of people were affected by the year 2015 and estimated to reach 650 million by 2030, in Indian scenario 65 million peoples were affected by the year 2013 and estimated to reach 103 million by the year 2030 (2-3). In patients with T2DM leads to many complications like Micro and Macro vascular, this will affect many organs like Kidney, Retina of the eye, Brain, Lungs and Heart etc and most of the diabetic people are affected with nephropathy. Diabetic nephropathy is the major microvascular complication in patients with T2DM due to hyperglycemia in the blood and hypoglycemia in the skeletal muscles leads to excess mobilization of lipids and proteins results production of reactive oxygen species is more and reduced antioxidants (4-6). Currently urinary albumin used for diagnosis of nephropathy in patients with T2DM. However, some of the recent researchers reported that microalbuminuria is not a gold standard, sensitive and specific biomarker for diagnosis of nephropathy in patients with T2DM, because it is also elevating in other disorders like obesity, hypertension, different types of kidney problems etc. In addition to that some of the patients diagnosed with microalbuminuric stage directly converting into normoalbuminuric stage due to treatment modalities (7-9).

Along with that some of recent studies found urinary nephrin levels used for early detection of nephropathy in patients with T2DM. Nephrin is the transmembrane protein of the immunoglobulin super family and also it is very essential component of slit diaphragm between the foot processes of the podocytes in the kidney (10). The physiological action of nephrin is to maintain the size selectivity of the slit diaphragm and form a network of interdigitating foot processes of the podocytes in the kidney results prevent the leaking of proteins (11). Dysregulation of nephrin due to hyperglycemia in podocytes lead to nephrinuria in T2DM patients with normoalbuminuric patients, preceding microalbuminuria (12). According to my knowledge very few studies has been done on urinary nephrin in south indian population and a study is required to evaluate sensitive and specific biomarker (urinary albumin and nephrin) for early detection of nephropathy in

T2DM Patients. Hence we aimed to evaluate the “the status of urinary albumin and nephrin to predict early onset of nephropathy in T2DM”.

Materials and Methods

This is a case control study conducted in “Basaveshwara Institute of Medical Sciences and Research Centre” from 2018 – 2021. A total 120 subjects with age group of 30 to 70 years were included in the study among these, 80 T2DM subjects, 40 age and gender matched healthy controls. T2DM subjects are further divided into two groups based on urinary albumin values [Group 1: T2DM Patients with Normoalbuminuria (< 30 mg/dL); Group 2: T2DM Patients with Microalbuminuria (30 – 299 mg/dL)]. All the T2DM subjects were diagnosed with different stages of nephropathy according to American Diabetic Association Criteria (ADA) (13) are included in the study and whoever having smoking, alcoholism, liver diseases, hypertension, cardiovascular, cerebrovascular, thyroid, peripheral vascular diseases and those are in treatment on thiazolidine and anti inflammatory were excluded from the study.

Seven milliliters (mL) of fasting (overnight 8- 12 hours) venous blood sample and Three mL of post prandial blood sample was collected from all the subjects after obtaining informed consent from. Two mL of blood sample transferred into tube containing anticoagulant sodium fluoride, Two mL transferred into tube containing anticoagulant EDTA and remaining Three mL transferred into plain tube. Anticoagulant tubes separated immediately and Plain tube allowed 10 mins at room temperature for clotting. All the samples centrifuged at 3000 RPM for 10 mins, after centrifugation separated samples (Plasma & Serum) transferred into properly labeled aliquots and stored at deepfreeze -80°C until biochemical analysis was done. Along with the blood sample urine sample also collected from all the subjects, centrifuged at 3000 RPM for 10 mins, after centrifugation of urine sample, 1 mL separated into labeled aliquots, stored at deepfreeze -50°C and remaining urine sample immediately processed Urinary albumin. Plasma Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Glycosylated Hemoglobin (HbA1c), Serum Urea, Creatinine and Urinary Albumin analysed by using laboratory standard methods. Urinary Nephrin was analysed by using Enzyme Linked Immuno Sorbent Assay (ELISA).

Statistical analysis

The data distribution was tested using Kolmogorov Smirnov test. Continuous variables are expressed as Mean $\pm$ standard deviation (SD). Comparison between among the three groups were analysed by using One Way Analysis of Variance (ANOVA). Pearson correlation analysis was done to test the correlation of urinary Nephrin with other biochemical parameters. The data was analysed by using Microsoft Excel Spread Sheets and IBM Statistical Package for the Social Sciences (SPSS) Version 20.0. “p” Value < 0.05 was consider as Statistically Significant.

Results

Table 1: Shows the normal distribution of data in different groups of the study by using Kolmogorov – Smirnov Test, some of the parameters were not normally

distributed. Hence, data was logarithmically transformed before applying parametric statistical tools.

Table 1
Distribution of biochemical and Urinary Nephtrin data among T2DM subjects and healthy controls

Parameter	Group 1 (n=40)		Group 2 (n=80)		P – Value
Age (years)	48.10	± 9.56	56.60	± 6.59	--
FBS (mg/dL)	90.63	± 7.31	138.35	± 26.03	0.0001**
PPBS (mg/dL)	114.70	± 13.39	224.84	± 84.66	0.0001**
Serum Urea (mg/dL)	24.60	± 9.73	46.70	± 24.48	0.0001**
Serum Creatinine (mg/dL)	1.21	± 0.23	3.60	± 2.84	0.0001**
HbA1C (%)	4.35	± 0.73	6.33	± 1.88	0.0001**
U. Albumin (mg/dL)	3.42	± 2.64	79.80	± 87.54	0.0001**
U Nephtrin (ng/mL)	48.80	± 4.03	119.47	± 49.44	0.0001**

* Significant at the 0.05 probability level, † NS- Not Significant at the 0.05 probability level. Group 1 Healthy Control, Group-2 Type 2 Diabetes. FBS: Fasting Blood Sugar, PPBS: Post Parandial Blood Sugar, HbA1C: Glycosylated Haemoglobin, U.ACR: Urinary Albumin: Creatinine Ratio.

Table 2: Shows the comparison of the routine biochemical parameters and urinary biomarkers across the study groups. There is statistically significant elevation of plasma FBS, PPBS, HbA1c, Serum urea, creatinine, Urinary albumin and nephtrin were observed in patients with T2DM subjects compared to healthy controls, respectively P .0001**.

Table 2
Comparison of the routine biochemical parameters and urinary biomarkers across the study groups

Parameter	Group 1 (n=40)		Group 2 (n=40)		Group 3 (n=40)		P- Value
Age (years)	49.45	± 10.73	57	± 6.63	56.6	± 6.63	-
FBS (mg/dL)	97.23	± 8.87	126.40	± 14.45	150.30	± 29.51	0.0001**
PPBS (mg/dL)	119.73	± 21.21	160.50	± 26.93	289.18	± 72.82	0.0001**
Serum Urea (mg/dL)	24.60	± 9.73	26.70	± 9.10	66.70	± 17.61	0.0001**
Serum Creatinine (mg/dL)	1.21	± 0.23	0.98	± 0.31	6.23	± 1.46	0.0001**
HbA1C (%)	4.35	± 0.73	4.71	± 0.81	7.90	± 1.13	0.0001**
U. ACR	3.42	± 2.64	10.27	± 8.95	147.60	± 75.49	0.0001**

(mg/dL)				
U Nephtrin (ng/mL)	48.80 ± 4.03	81.47 ± 6.15	157.48 ± 44.16	0.0001**

* Significant at the 0.05 probability level, Group 1 Healthy Controls, Group-2 Type 2 Diabetes with Normoalbuminuria and Group 3- Type 2 Diabetes with Microalbuminuria. Fasting Blood Sugar, PPBS: Post Parandial Blood Sugar, HbA1C: Glycosylated Haemoglobin, U.ACR: Urinary Albumin: Creatinine Ratio, mg/dL: Milligrams per decilitre, %: Percent, ng/dL: Nanograms per decilitre.

Table 3: Shows the comparison of the routine biochemical parameters and urinary biomarkers across the study groups by one-way ANOVA. There is statistically significant elevation of plasma FBS, PPBS, HbA1c, Serum urea, creatinine, Urinary albumin and nephrin were observed in two groups of T2DM subjects compared to healthy controls, respectively P .0001**. It was also observed that T2DM subjects with microalbuminuria have high level of urinary nephrin then T2DM subjects with normoalbuminuria, respectively P .0001** shows statistically highly significant.

Table 3

Shows the Pearson Correlation between the Urinary Nephrin with other routine biochemical analysis

Parameter		FBS	PPBS	UREA	CREATININE	HbA1C	U. ACR
	R	0.629	0.742	0.688	0.756	0.688	0.722
U. NEPHRIN	P = Value	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**	0.0001**

** Significant at the level of 0.05, r: Rho Factor, HbA1C- Glycosylated Haemoglobin, and U ACR: Urinary Albumin Creatinine Ratio (ACR).

Table 4: Shows the correlation of urinary nephrin with other routine biochemical parameters of the subjects by using Pearson Correlation analysis. We observed that urinary nephrin was positively significant correlation with plasma FBS, PPBS, HbA1c, Serum urea, creatinine and Urinary albumin (r = 0.403, 0.494, 0.461, 0.478, 0.554 and 0.398) respectively P .0001**.

Figure 1: Shows the graphical representation of urinary nephrin distribution in all the three groups of study subjects. Significantly an elevated level of urinary nephrin in T2DM subjects with normoalbuminuria and microalbuminuria was observed then healthy controls.

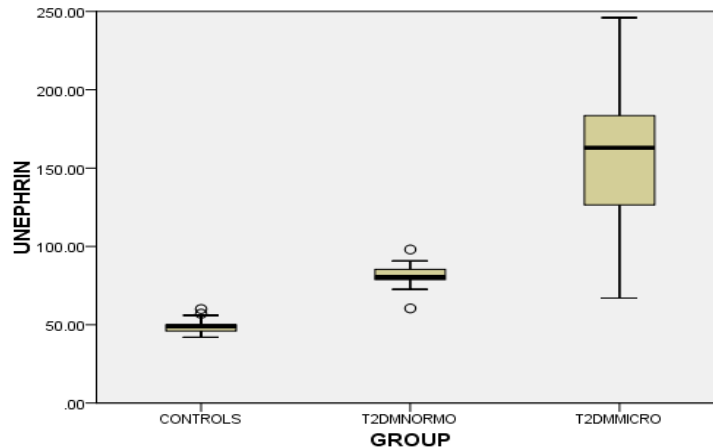


Figure 1. Shows the distribution of Urinary Nephryn levels in three groups of study subjects

Figure 2: Shows the distribution of urinary albumin levels in different groups of study subjects. Where T2DM subjects with microalbuminuria have significantly increased levels of urinary albumin when compared to T2DM subjects with normoalbuminuria and healthy controls.

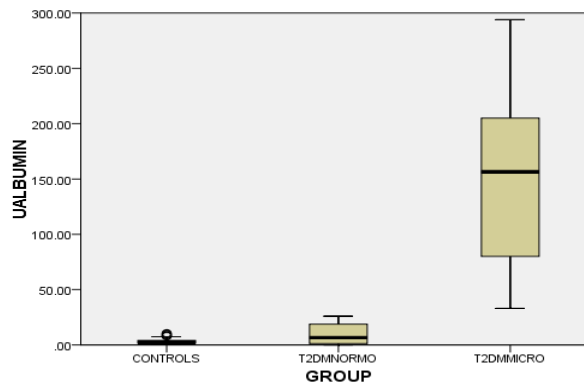


Figure 2. Shows the distribution of Urinary Albumin levels among the study subjects

Discussion

Diabetic kidney disease (DKD) is one of the major microvascular complications in patients with T2DM and now a day's more than 40 percent of the DKD patient's leads to End Stage Renal Disease (ESRD) (14). In this condition damage of glomerular filtration barrier and tripartite system have fenestrated endothelial cells of Glomerular Basement Membrane (GBM) and podocytes of kidney. The physiological action of these barrier is selective filtration of water and solutes, and impermeable to leaking of macromolecules like proteins (15-16). In T2DM

Patients, hyperglycemia in the blood and hypoglycemia in tissues triggers lipolysis, proteolysis results production of reactive oxygen species and advanced glycation end products. These will bind to the advanced glycation end product receptors and triggering downstream signaling facilitates generation of free radicals, activates inflammatory cells, increase synthesis of angiotension II and production of growth factors like Vascular Endothelial Growth factors (VEGF) and Transforming Growth Factor Beta (TGF- β) finally lead to proteinuria (17-18).

The urinary albumin estimation used for functional capacity of kidney, when the patients showed less than 30 mg/dL of albumin is normoalbuminuria, 30 to 299 mg/dL of albumin is microalbuminuria and more than 300 mg/dL is macroalbuminuria, that depends on long standing of diabetes and later for further progressed by Estimating Glomerular Filtration Rate (e GFR) (19). Some of the recent studies reported that urinary microalbuminuria is not a sensitive and specific biomarker for detection of nephropathy in T2DM patients because; it has low sensitivity and larger variability. The present study also shown a significantly elevated levels of urinary albumin in Group 3 when compared to Group 1 and 2 (Table 2). The following factors are influenced to excrete albumin in urine include plasma concentrations of fasting blood sugar, mean arterial blood pressure, glycosylated hemoglobin, aldosterone, arginine, atrial natriuretic peptide, vasopressin and angiotension II. However earlier studies was observed that some of the T2DM Patients with microalbuminuria revert back to normoalbuminuria (20-22).

If DN can be detected before the appearance of microalbuminuria enabling early intervention to halt or reverse the process. Glomerular biomarker of renal injury is being studied as alternative markers for prediction of DN risk. Some of the recent studies found sensitive, specific biomarker for early detection of nephropathy in T2DM Patients. The nephrin is the protein located in the podocytes of glomerular basement membrane of the kidney. Any pathological changes occur in the podocytes these podocytes protein will excrete through the urine. The studies are very limited and some of the studies found significantly elevated levels of urinary nephrin in T2DM and other studies reported the urinary nephrin levels were decreased in patient with T2DM (23-26). The present study also analysed urinary nephrin, the significantly elevated levels observed in patients with two groups of T2DM when compared to healthy controls and also we found significantly increased levels of urinary nephrin in T2DM Patients with normoalbuminuria when compared to healthy controls. Similarly other studies also reported podocytes are present GBM of kidney and play a good role in maintaining glycocalyx of glomerulus and maintained the slit diaphragm size by specific proteins like nephrin and also they suggested elevated levels of urinary nephrin were useful for early detection of nephropathy in T2DM Patients.

We also found that urinary nephrin was positively correlated with blood sugars, glycosylated hemoglobin, urea, creatinine and urinary albumin (Table 3). Similarly another recent study also reported that association of proximal tubule dysfunction with podocytes damage and dysfunction of proximal convoluted tubule proteins could be validate as an early diagnosis and progression of nephropathy in patients with T2DM (27-28). The elevated levels of urinary nephrin in T2DM patients with normoalbuminuria stage and these levels were

increased more than two times in T2DM with microalbuminuria showed a disease progression towards end stage kidney disease. This study suggests that estimation of urinary nephrin may help to predict early onset of nephropathy in T2DM subjects.

Conclusion

This study concluded that urinary nephrin might be a better marker than urinary albumin to predict early onset of nephropathy in T2DM Patients because urinary nephrin elevated in T2DM patients with normoalbuminuria but the urinary albumin levels were elevated in T2DM patients with Microalbuminuria and also some of the patients its revert to normoalbuminuria.

Conflict of Interest

No Conflict of Interest

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