Original Research

Study to determine urinary nephrin as an earlier marker in diabetic nephropathy than microalbuminuria.

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ABSTRACT

Background: Microalbuminuria was previously considered as a gold standard in early detection of diabetic nephropathy despite being a non-specific marker. Decrease in Nephrin expression has been suggested to precede podocyte loss and linked to the progression of kidney disease.Present study was aimed to determine urinary nephrin as an earlier marker in diabetic nephropathy than microalbuminuria.

Material and Methods: Present study was single-center, prospective, comparative study, conducted in patients with age > 18 years, Type 2 Diabetes, Diabetic Nephropathy, healthy individuals.Among the subjects, allocation was done into 3 groups based on history & investigations as Group DM MIC, Group DM NOR &Controls.

Results: In present study, 90 subjects were equally allotted to Group DM MIC (n=30), Group DM NOR (n=30)&Controls (n=30). Significant positive correlation of strong strength **of Urinary Nephrin levels was noted with**Duration of DM (yrs), SBP (mm Hg)DBP (mm Hg), FBS (mg/dL), PPBS (mg/dL), blood urea (mg/dl), BUN, Sr. creatinine (mg/dL), eGFR (ml/min)&UMCR (Microalbumin creatinine ratio).The cut off of U.NEPHRIN(ng/ml) for predicting Nephropathy in Diabetic Normal albuminuria is 3.33 which had a sensitivity of 96.7%, specificity of 100%, positive predictive value of 100%, negative predictive value of 96.81% and a diagnostic accuracy of 98.35%.The cut off of U.NEPHRIN (ng/ml) for predicting Group is 6.09 which had a sensitivity of 96.7%, specificity of 98.3%, positive predictive value of 96.6%, negative predictive value of 98.35% and a diagnostic accuracy of 97.77%.

Conclusion: Urinary nephrinhas greater diagnostic value in early detection of diabetic nephropathy compared to microalbuminas urinary nephrin levels were significantly increased in diabetic patients with nephropathy compared with diabetic patients without nephropathy and healthy non-diabetic individuals.

Keywords: Diabetes Mellitus, Urinary Nephrin, Diabetic Nephropathy, Microalbumin.

INTRODUCTION

Diabetes mellitus is one of the most common causes of End Stage Renal Disease (ESRD) and it accounts for 30 - 40 % of ESRD. Diabetic nephropathy is the most common cause for chronic renal failure, accounting for 45% of patients receiving renal replacement therapy. The complications of diabetes affect nearly every tissue of the body and is associated with both micro and macro vascular complications.[1] The most severe microvascular complication due to diabetes mellitus is Diabetic nephropathy (DN) which is characterized by the persistent albuminuria (>300 mg/d) that is confirmed on at least 2 occasions 3–6 months apart, progressive decline in GFR and elevated arterial blood pressure.[2,3]Microalbuminuria was previously considered as a gold standard in early detection of DN despite being a non-specific marker. Studies have shown a progressive decline in the number of podocytes and disappearance of foot processes which occur in the early stages of DN due to apoptosis or shedding of podocytes. Therefore, urinary podocytes and their specific proteins may be regarded as potential biomarkers of podocyte injury.[3,4]Nephrin (Nphs1) is a 180 KD trans-membrane adhesion protein that is expressed at the podocyte intercellular junction in the glomerulus. Nephrin is necessary for the proper functioning of the renal filtration barrier. Decrease in Nephrin expression has been suggested to precede podocyte loss and linked to the progression of kidney disease.[4,5]Present study was aimed to determine

urinary nephrin as an earlier marker in diabetic nephropathy than microalbuminuria.

MATERIAL AND METHODS

Present study was single-center, prospective, comparative study, conducted in department of General Medicine and Nephrology, at Thanjavur Medical College Hospital, Thanjavur, India. Study duration was of 2 years (January 2021 to December 2022). Study approval was obtained from institutional ethical committee.

Inclusion criteria

- Patients with Age > 18 years, Type 2 Diabetes, Diabetic Nephropathy, healthy individuals, willing to participate in present study

Exclusion criteria

- Pregnancy.
- Hypertension.
- Renal disease of Non Diabeticetiology

After getting an informed consent, the blood for both Fasting and Post prandial blood samples and Urine samples were collected from Type II Diabetes Mellitus and Healthy individuals. Among the subjects, allocation was done into 3 groups based on history & investigations.

1. Group DM MIC - DM patients with Microalbuminuria
2. Group DM NOR - DM patients with Normoalbuminuria
3. Controls - healthy subjects without history of DM & normal investigations.

Blood pressure was measured in subjects in the sitting position on the left upper arm after a 5-minute rest. Body mass index was calculated as weight divided by height in meter squared. Collected serum & urine samples were analysed for Urine Nephrin (by Enzyme-linked Immuno Assay), Urine Microalbumin (by Turbidimetry method), Urine creatinine (by Modified Jaffe's method), Blood glucose (FBS, PPBS) (by Glucose-oxidase / peroxidase method), Blood urea (by Urease Glutamate Dehydrogenase Method), Serum Creatinine (mg/dl) (by Modified Jaffe's method), eGFR (mL/min/1.73 m2) was calculated using Cock Croft – Gault Equation, BUN, UMCR were calculated using formula.Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant. Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

RESULTS

In present study, 90 subjects were equally allotted to Group DM MIC (n=30), Group DM NOR (n=30)&Controls (n=30). The mean Age among DM MIC was 46.23 years which is higher than mean among Controls which was 45.33 years followed by DM NOR with a mean of 44.23 years but the difference was not statistically significant ($p > 0.05$). Gender distribution was not statistically significant ($p > 0.05$). The mean BMI among DM NOR was 32.25 which is higher than mean among DM MIC which was 30.75 followed by Controls with a mean of 26.9 and the difference was statistically significant ($p < 0.05$). The mean Duration of Diabetes among DM MIC was 12.13 (± 3.68) years which is higher by 4.5 years and statistically significant compared to 7.63 (± 1.54) years in DM NOR ($p < 0.05$).The mean Systolic Blood Pressure among DM MIC was 122.33 mm/hg which is higher than mean among DM NOR which was 120.67 mm/hg followed by Controls with a mean of 113 mm/hg and the difference was statistically significant ($p < 0.05$). The mean Diastolic Blood Pressure among DM MIC was 84.67 mm/hg which is higher than mean among DM NOR which was 77.33 mm/hg followed by Controls with a mean of 75.67 mm/hg and the difference was statistically significant ($p < 0.05$).

Table 1: General characteristics

	DM MIC	DM NOR	Controls	p value
Mean age (in years)	46.23 ± 7.92	44.23 ± 8.54	45.33 ± 11.27	0.710
Gender				
Male	11 (36.66%)	13 (43.33%)	19 (63.33%)	0.099
Female	19 (63.33%)	17 (56.66%)	11 (36.66%)	
BMI	30.75 ± 3.80	32.25 ± 3.52	26.90 ± 4.01	0.001
Duration of Diabetes (Years)	12.13 ± 3.68	7.63 ± 1.54	---	0.001
Blood pressure				
Systolic Blood Pressure (mm/hg)	122.33 ± 13.57	120.67 ± 16.80	113.00 ± 14.18	0.040
Diastolic Blood Pressure (mm/hg)	84.67 ± 10.08	77.33 ± 12.02	75.67 ± 8.98	0.003

The mean Fasting Blood Sugar among DM MIC was 171.6 mg/dl which is higher than mean among DM NOR which was 159.5 mg/dl followed by Controls with a mean of 84.6 mg/dl and the difference was statistically significant ($p < 0.05$). The mean Post Prandial Blood Sugar among DM MIC was 253.57

mg/dl which is higher than mean among DM NOR which was 202.63 mg/dl followed by Controls with a mean of 126.1 mg/dl and the difference was statistically significant ($p < 0.05$).The mean Serum Urea among DM MIC was 86.17 mg/dl which is higher than mean among DM NOR which was 55.83 mg/dl followed by Controls with a mean of 30.53 mg/dl and the difference was statistically significant ($p < 0.05$). The mean Blood Urea Nitrogen among DM MIC was 40.26 which is higher than mean among DM NOR which was 26.09 followed by Controls with a mean of 14.27 and the difference was statistically significant ($p < 0.05$). The mean Serum Creatinine among DM MIC was 3.11 mg/dl which is higher than mean among DM NOR which was 1.45 mg/dl followed by Controls with a mean of 0.75 mg/dl and the difference was statistically significant ($p < 0.05$).The mean eGFR among Controls was 107.55 ml/min which is higher than mean among DM NOR which was 67.04 ml/min followed by DM MIC with a mean of 29.37 ml/min and the difference was statistically significant ($p < 0.05$). The mean UMCR among DM MIC was 101.99 mg/g which is higher than mean among DM NOR which was 15.49 mg/g followed by Controls with a mean of 2.78 mg/g and the difference was statistically significant ($p < 0.05$). The mean U.NEPHRIN among DM MIC was 8.69 ng/ml which is higher than mean among DM NOR which was 4.66 ng/ml followed by Controls with a mean of 0.22 ng/ml and the difference was statistically significant ($p < 0.05$).

Table 2: Investigations

	DM MIC	DM NOR	Controls	p value
Fasting Blood Sugar (mg/dl)	171.60 ± 11.45	159.50 ± 18.25	84.60 ± 7.77	0.001
Post Prandial Blood Sugar (mg/dl)	253.57 ± 37.25	202.63 ± 18.51	126.10 ± 9.72	0.001
Serum Urea (mg/dl)	86.17 ± 17.48	55.83 ± 8.89	30.53 ± 2.89	0.001
Blood Urea Nitrogen	40.26 ± 8.17	26.09 ± 4.16	14.27 ± 1.35	0.001
Serum Creatinine (mg/dl)	3.11 ± 0.68	1.45 ± 0.11	0.75 ± 0.11	0.001
eGFR (ml/min)	29.37 ± 6.56	67.04 ± 9.02	107.55 ± 10.85	0.001
UMCR (mg/g)	101.99 ± 33.83	15.49 ± 5.06	2.78 ± 2.02	0.001
U.NEPHRIN (ng/ml)	8.69 ± 1.63	4.66 ± 0.90	0.22 ± 0.06	0.001

The area under the curve for U.NEPHRIN(ng/ml) in predicting Nephropathy in Diabetic Normal albuminuria is 1 (1 - 1). The cut off of U.NEPHRIN(ng/ml) for predicting Nephropathy in Diabetic Normal albuminuria is 3.33 which had a sensitivity of 96.7%, specificity of 100%, positive predictive value of 100%, negative predictive value of 96.81% and a diagnostic accuracy of 98.35%.

Table 3: ROC for predicting Nephropathy in Diabetic Normal albuminuria using U.NEPHRIN

Test Result Variable(s)	Cut off	Sensitivity	Specificity	PPV	NPV
U.NEPHRIN (ng/ml)	3.33	96.70%	100.00%	100.00%	96.81%

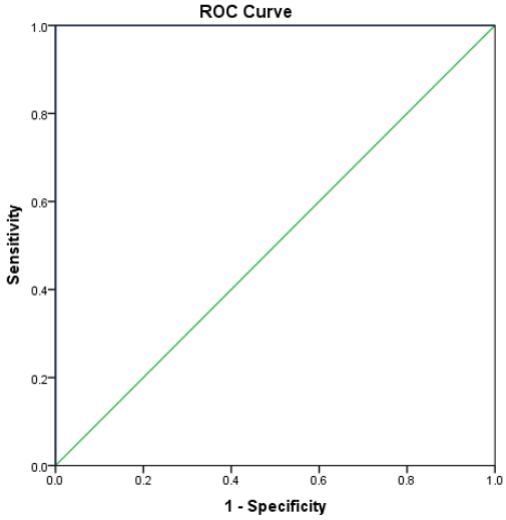

Figure 1: ROC for predicting Nephropathy in Diabetic Normal albuminuria using U.NEPHRIN

The area under the curve for U.NEPHRIN (ng/ml) in predicting Group is 0.997 (0.99 - 1). The cut off of U.NEPHRIN (ng/ml) for predicting Group is 6.09 which had a sensitivity of 96.7%, specificity of 98.3%, positive predictive value of 96.6%, negative predictive value of 98.35% and a diagnostic accuracy of 97.77%.

Table 4: ROC for predicting Diabetic Nephropathy using U.NEPHRIN

Test Result Variable(s)	Cut off	Sensitivity	Specificity	PPV	NPV
U.NEPHRIN (ng/ml)	6.09	96.70%	98.30%	96.60%	98.35%

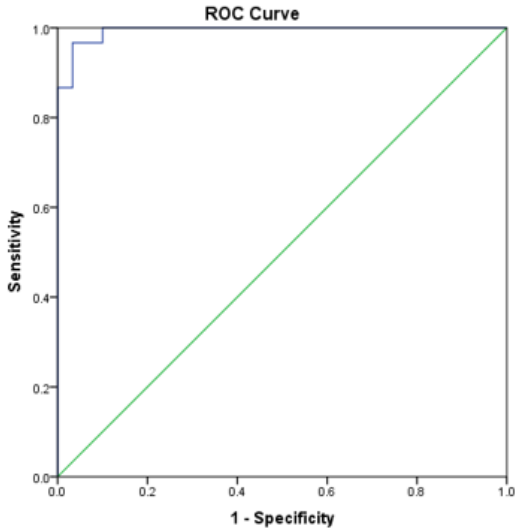

Figure 2: ROC for predicting Diabetic Nephropathy using U.NEPHRIN

DISCUSSION

Type 2 Diabetes Mellitus (DM) is the most common cause of End-Stage Renal Disease (ESRD). ESRD in almost half of patients is due to Diabetic Nephropathy (DN), and these cases have the worst outcome compared to patients with other causes of ESRD. DN may occur as a result of the interplay between genetic and nongenetic factors, such as the metabolic and hemodynamic ones. Reason for poor outcome in patients includes inadequate markers for early diagnosis and the complicated mechanisms of DN.Currently, the severity of DN is staged according to Urine albumin excretion. In persons without diabetes, urinary albumin excretion is generally lower in levels than seen in Diabetes.[6,7] Microalbuminuria, the most commonly used marker to predict onset and progression of DN is present in 30% of middle-aged patients with either T1 DM or T2 DM.[8]However, this traditional marker lacks both sensitivity and specificity to detect early stage of DN. Furthermore, some DN patients with ESRD do not present with significant albuminuria. Some studies have noted the existence of pathological change before the appearance of microalbuminuria. Nephrin, a transmembrane protein with a large extracellular portion, self-associates in a zipper-like arrangement through homophilic dimerization, forming the molecular substrate of the slit diaphragm.[9,10] The presence of Nephrin in urine may indicate damaged podocytes, as Nephrin is expressed on Podocytes and is intimately involved in podocyte dysfunction.[11,12]Moreover, in previous studies, a marked reduction and redistribution of Nephrin was also observed in glomeruli of patients with Diabetes and microalbuminuria without significant histological glomerular lesions. Thus, it is thought that podocyte damage is present before the appearance of microalbuminuria and proteinuria and hence, podocyte proteins such as Nephrin are considered as earlier and more specific markers for diagnosis of DN compared to microalbuminuria.Nephrin measurement was carried out using commercially available ELISA KIT. In this study, Urinary Nephrin level was significantly higher in Diabetic patients with both normoalbuminuria and microalbuminuria. A statistically significant elevation in SBP, DBP, FBS, PPBS, Blood urea, Serum creatinine, BUN and UMCR and significant decline in eGFR were noted in Diabetic patients with normoalbuminuria and microalbuminuria when compared with the healthy controls.In the present study, Urinary Nephrin levels had a strong positive correlation with Age, Duration of Diabetes, SBP, DBP, FBS, PPBS, Blood urea, Serum creatinine, BUN and UMCR. These results were consistent with the study results of Wang *et al.*,[12] and Chang *et al.*,[13] which suggested that Nephrinuria correlated with proteinuria. Similarly, a negative correlation is observed between Urinary Nephrin and eGFR in this study indicating that Nephrinuria is a marker of disordered renal function, which is in accordance with the study results of Jim *et al.*,[14]ROC curve analysis was done to find out the predictive value of urinary Nephrin in Diabetic Nephropathy patients. The cut off of U.NEPHRIN (ng/ml) for predicting Diabetic Nephropathy (Normoalbuminuric and Microalbuminuric) is 6.09.The area under the curve for U.NEPHRIN (ng/ml) in predicting Diabetic Nephropathy in Normal albuminuric patients is 1 (1 - 1). The optimal cut off of U.NEPHRIN (ng/ml) is 3.33 which had a sensitivity of 96.7%, specificity of 100%, positive predictive value of 100%, negative

predictive value of 96.81% and a diagnostic accuracy of 98.35%.

An elevated level of U.NEPHRIN was found in 96.6% of Diabetic Nephropathy patients with Normoalbuminuria. This is similar to the results obtained in the study conducted by Irena Kostovska *et al.*,[15] which highlighted elevated concentration of urinary Nephrin in 82% of normoalbuminuric subjects (97).This study showed high sensitivity and specificity of Nephrin as a urinary biomarker in early detection of Diabetic Nephropathy. Confirmation of Nephrinuria as a marker of preclinical Diabetic Nephropathy would replace the present gold standard marker of Diabetic Nephropathy, Microalbumin with Urinary Nephrin in routine laboratory practice.Limitations of the study were cross sectional design with small sample size. The results of this study could not determine whether the early Nephrinuria will consistently predict progression of Diabetic Nephropathy. Hence, a large prospective study is needed to further assess the value of Urinary Nephrinin early detection of Diabetic Nephropathy.

CONCLUSION

Urinary Nephrinhas greater diagnostic value in early detection of Diabetic Nephropathy compared to Microalbumin as Urinary Nephrin levels were significantly increased in Diabetic patients with Nephropathy compared with Diabetic patients without Nephropathy and Healthy Non-Diabetic individuals. Urinary Nephrincould be very important in early detection of DN, due to High percent of normoalbuminuric subjects of Diabetes Mellitus with elevated levels of Urinary Nephrin& negative correlation between Urinary Nephrinconcentration and GFR and High diagnostic sensitivity and specificity of Urinary Nephrin in patients with Diabetic Nephropathy.

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