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Original Research Article

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

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ABSTRACT

Background: Nephropathy is most common complication in type 2 diabeticsubjects. Urinary nephrin is a podocyte specific protein, can be serve as a predict early nephropathy and its progression of diabetic nephropathy.
Materials and Methods: A total 90 participants included were sub grouped as 60 cases and 30 healthy controls, a biochemical, clinical and experimental parameters were analyzed for all the study subjects. The data was interpreted by using statistics software SPSS and <0.05 was statistically significant.
Results: There were significantly increased levels of FBS, PPBS and glycated haemoglobin in type 2 diabetic cases when compared to controls. The urea, creatinine, uric acid and microalbumin were significantly elevated in type 2 diabetic with microalbuminuria when compared to type 2 diabetic with normoalbuminuria and controls. Additionally the urinary nephrin was significantly elevated in both groups of type 2 diabetics when compared to controls.
Conclusion: This study suggests determination of urinary nephrin can be used as an early predictable and prognostic marker for nephropathy in patients with type 2 diabetics.

1. Introduction

The Type 2 Diabetes Mellitus (T2DM) considered as a metabolic disorder caused by insulin resistance (IR).[1] The incidence of T2DM throughout the world, 469 million people million people were affected in 2019 and it will raise up to 2019 700 million by 2045.[2] In indian scenario 77 million people living with T2DM by 2019 and expected to reach 135 million by 2045.[3] Several metabolic changes occur in T2DM patient's results complications majorly on kidney.Hyperglycemia in patients with T2DM results production of free radicals results damage of kidney lead to diabetic kidney disease (DKD).[4]

The DKD is common most complication in patients with T2DM characterised by albuminuria.Microalbumin is considered as earliest clinical available for diabetic nephropathy. According to Kidney Disease Improvement Global Outcome (KDIGO) criteria the microalbuminuria is < 30 mg/g creatinine considered as normoalbuminuria, 30 – 299 mg/g creatinine considered as microalbuminuria and >300 mg/g creatinine considered as macroalbuminuria.[5,6] But a recent study was reported microalbumin is not a sensitive and specific biomarker for diagnosis and progression of diabetic nephropathy. Since it has a lot of flaws including blood sugar levels, glycated haemoglobin, hypertension, arginine, vasopressin, atrial natriuretic peptide and also the T2DM patients with normoalbuminuria only showed an advanced pathological events without microalbuminuria.[7–9] Hence there is a need best marker for diabetic nephropathy.

According to recent research studies are reported that podocyte proteins measurement can be used as early

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diagnosis and prognosis of diabetic nephropathy.[10] The nephrin one of the transmembrane podocyte protein forms the slit diaphragm and it act like a cytoskeleton of maintenance of glycocalyx of glomerular basement membrane prevent the excretion of protein in urine.[11–13] In T2DM patients due to the metabolic derangements glomerular damage will occur that leading to nephrinuria before appearance of microalbumin.[14–16] Recent experimental researchers observed significantly increased in T2DM patients with normoalbuminuria without microalbumin and also they suggested that to determination of nephrin in urine use for early clinical marker for diabetic nephropathy.[17–19] Based on this background, the current study evaluates the correlation of between urinary nephrin and microalbumin in type 2 diabetes mellitus patients.

2. Materials and Methods

The present study conducted in department of general medicine and collaborated with biochemistry in Sri BM Patil Medical College Hospital and Research Centre, Karnataka, India from 2018-2023. As per American Diabetes Association Criteria (ADA) 60T2DM patientswas recruited and these patients were separated to two subgroups with their microalbumin levels (Microalbumin: < 30 Mg/g Creatinine considered as Group 2: T2DM with normoalbuminuria and Microalbumin: 30-299 Mg/g Creatinine considered as Group 3: T2DM with microalbuminuria) according to KDIGO criteria. Along with that we also recruited 30 age, gender and BMI matched healthy controls and considered as Group 1. All the study subjects included were included after obtaining the institutional ethics committee approval and taken consent from the all individuals.

2.1. Inclusion criteria

The study participants age should be 30-70 years without any illness consider as controls. For T2DM diagnosed according to ADA criteria and KDIGO criteria.

2.2. Exclusion criteria

The subjects with pregnancy, h/o smoking and alcoholism, other types of diabetes mellitus, hypertension, thyroid diseases, liver diseases, kidney diseases, cardiovascular diseases, vascular diseases, infectious diseases, cerebro vascular diseases were excluded from the study.

2.3. Sample collection

Five (5) millilitres (ml) 8 to 12 hours overnight fasting venous blood sample was collected from all the study participants, later 2 ml were transferred to anti-coagulant and anti-glycolytic (Sodium Fluoride) tube and remaining 3 ml transferred to clot activator tube. Second time another 2 ml of blood sample was collected after 2 hours of breakfast. All the samples was separated by the process of centrifugation at 5000 rotation per minute and plasma, serum transferred to bullet vailes and stored until analysis was done. Urine also obtained from all the participants, processed centrifugation and immediately microalbumin was analysed and separated 1 ml of urine into properly labelled aliquots for nephrin.

2.4. Statistical analysis

The distribution of data was tested by using kolmogorov smrinov test and expressed normally distributed data into mean ± standard deviation (SD). Analysis of Variance followed by posthoc analysis was done for comparison of variables between the groups. The pearsons correlation analysis was done to correlate between the parameters. Statistical analysis was done by SPSS and P value is < 0.05 was considered significant.

3. Results

The height shows moderate significance (P=0.04*) and the weight, body mass index, fasting blood sugars, post parandial blood sugars, urea, creatinine. Uric acid, microalbumin and urinary nephrin was shown significantly very high (P=0.001**) and the age not shown any significance (P=0.63) between healthy controls and T2DM patients.

We observed that in anthropometric parameters the weight, BMI showed high significant (P = 0.001**) and height shown moderate significant(P = 0.03*) between the study groups. Along with the fasting blood sugars, post parandial blood sugars, urea, creatinine, uric acid and microalbumin shown very high significant (P=0.001**). The nephrin also shown a highly significance between the both the study groups (P= 0.001**) (Table 2).

In Table 3 represents the comparison of biochemical variables studied between the groups by using post hoc analysis. The BMI, FBS, PPBS, urinary nephrin showed a high significant(P =0.001**) and the urea, creatinine, uric acid and microalbumin not shown any significant (P = 0.92,0.86, 0.81 and 0.94) observed between group 1 and group 2. We observed there was highly significant of BMI, fasting blood sugars, post parandial blood sugars, urea, creatinine, uric acid and urinary nephrin between group 1 vs group 3 and group 2 vs group 3 (P=0.001**).

Table 4 shows the correlation of anthropometric, demographic, biochemical parameters with urinary nephrin among the study subjects. The urinary nephrin was highly significant positively correlatedand with weight, BMI, fasting blood sugars, post parandial blood sugars, urea, creatinine, uric acid, microalbumin (P = 0.001**) and moderate significant positive correlation with height

Table 1: Distribution of demographic, anthropometric, biochemical andexperimental parameters between cases and controls

Parameters	Healthy Controls			Patients with T2DM			P- Values
Age (Years)	52.40	±	7.00	53.62	±	6.61	0.63 †
Height (CM)	163.27	±	4.76	165.43	±	5.37	0.04*
Weight (Kg)	57.50	±	3.67	88.02	±	9.60	0.001**
BMI (Kg/m^2)	21.58	±	1.18	32.09	±	2.36	0.001**
FBS (Mg/dl)	92.27	±	1.60	160.65	±	10.60	0.001**
PPBS (Mg/dl)	124.60	±	8.08	316.02	±	51.97	0.001**
S. Urea (Mg/dl)	29.97	±	3.65	60.50	±	32.07	0.001**
S. Creatinine (Mg/dl)	0.61	±	0.19	3.65	±	1.59	0.001**
S. Uric Acid (Mg/dl)	4.61	±	0.44	8.02	±	3.50	0.001**
Microalbumin (Mg/g creatinine)	7.26	±	1.27	40.94	±	35.62	0.001**
Urinary Nephrin (ng/ml)	5.11	±	1.34	40.12	±	11.81	0.001**

Table 2: Comparison of anthropometric, biochemical andexperimental parameters between cases and controls by ANOVA.

Parameter	Healthy Controls			T2DM with Normoalbuminuria			T2DM with Microalbuminuria			P – Value
Age (Y)	51.04	±	7.00	53.53	±	6.65	53.70	±	6.68	0.72 †
Height (CM)	163.27	±	4.76	164.20	±	4.69	166.67	±	5.79	0.03*
Weight (Kg)	57.50	±	3.67	84.43	±	8.63	91.60	±	9.31	0.001**
BMI (Kg/m^2)	21.58	±	1.18	31.26	±	2.22	32.92	±	2.23	0.001**
FBS (Mg/dl)	92.27	±	1.60	151.37	±	1.99	169.93	±	6.80	0.001**
PPBS (Mg/dl)	124.60	±	8.08	276.27	±	19.73	355.77	±	42.84	0.001**
S. Urea (Mg/dl)	29.97	±	3.65	29.40	±	2.13	91.60	±	9.31	0.001**
S. Creatinine (Mg/dl)	1.24	±	0.19	1.16	±	0.22	8.14	±	0.99	0.001**
S. Uric Acid (Mg/dl)	4.61	±	0.44	4.78	±	1.22	11.26	±	1.28	0.001**
Microalbumin (Mg/g creatinine)	7.26	±	1.27	8.14	±	1.10	73.73	±	18.82	0.001**
Urinary Nephrin (ng/ml)	5.11	±	1.34	29.07	±	2.88	51.18	±	4.74	0.001**

Table 3: Comparision of biochemical variables studied in between sub groups of the study.

Parameter	Group 1 Vs Group 2	Group 1 Vs Group 3	Group 2 Vs Group 3
BMI (Kg/m^2)	0.001**	0.001**	0.004**
FBS (Mg/dl)	0.001**	0.001**	0.001**
PPBS (Mg/dl)	0.001**	0.001**	0.001**
S. Urea (Mg/dl)	0.92	0.001**	0.001**
S. Creatinine(Mg/dl)	0.86	0.001**	0.001**
S. Uric Acid(Mg/dl)	0.81	0.001**	0.001**
Microalbumin (Mg/g creatinine)	0.94	0.001**	0.001**
U. Nephrin (ng/mL)	0.001**	0.001**	0.001**

Table 4: Correlation of anthropometric, demographic, biochemical parameters with urinary nephrin among the study subjects.

Parameter	Urinary Nephrin (ng/ml)	
	r- Value	P – Values
Age (Y)	0.096	0.36
Height (CM)	0.298	0.04
Weight (Kg)	0.857	0.001**
BMI (Kg/m^2)	0.867	0.001**
FBS (Mg/dl)	0.943	0.001**
PPBS (Mg/dl)	0.944	0.001**
S. Urea (Mg/dl)	0.829	0.001**
S. Creatinine (Mg/dl)	0.826	0.001**
S. Uric Acid (Mg/dl)	0.804	0.001**
Microalbumin (Mg/g creatinine)	0.804	0.001**

(P=0.04*). The nephrin is not correlated with age (P=0.366).

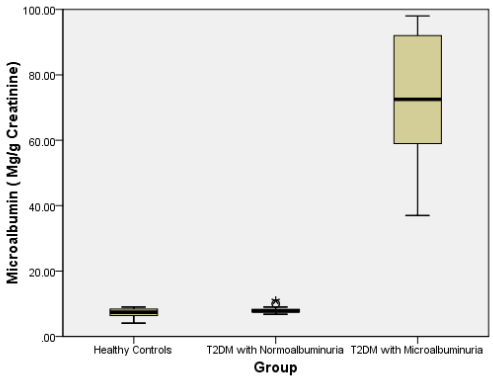

Figure 1: Shows the microalbumin was significantly increased in type 2 diabetes with microalbuminuria than type 2 diabetes with normoalbuminuria and healthy controls.

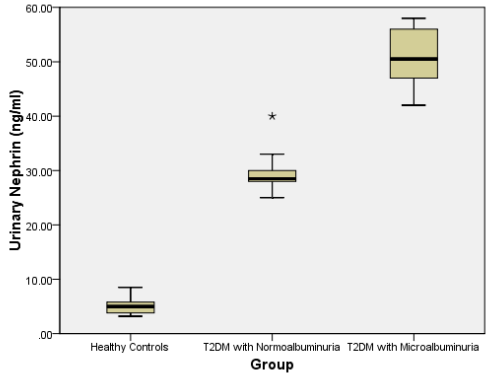

Figure 2: Shows the significantly and drastically elevated concentrations of refrain in type 2 diabetes with normo, micro than healthy controls.

4. Discussion

Recently diabetic nephropathy is most common complication associated with type 2 diabetes mellitus, the metabolic derangements in T2DM patients leads to damage of kidney particularly podocytes.[20] Podocytes are very essential components of glomerulus of kidney and it will maintain the slit diaphragm allow the leakage of water and small molecules by podocyte proteins.[21] Nephrin is one of the podocyte protein very important for maintenance of foot process of podocytes and forms the calyx of glomerulus prevent the excretion of protein in urine.[22]

In T2DM patients with hyperglycemia and dyslipidaemia leads to production of excess free radicals lead to damage

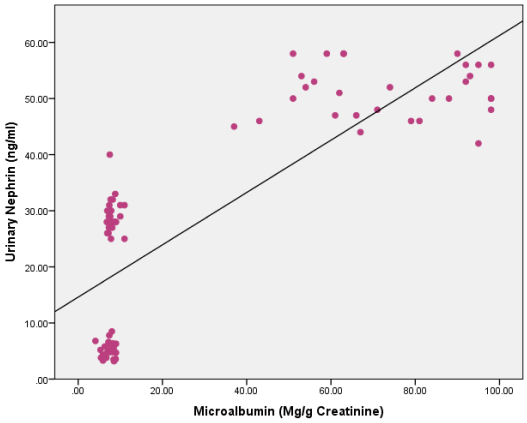

Figure 3: Shows the scatter plots revealed that there was a positive association between nephrin and microalbumin between the groups.

of podocytes. In starting stage of kidney damage plasma protein cannot be excreted due to its size and simultaneously podocyte protein nephrin will be excreted since its having less size lead to nephrinuria.[23,24] The nephrinuria in patients with T2DM patients with normoalbuminuria without microalbumin can be used as early predectible marker for kidney damage in type 2 diabetic patients.

Present study found the highly significant increased levels of nephrin in the type 2 diabetic subjects with normo and microalbuminuria than in healthy controls (P = 0.001**).

T2DM patients with normoalbuminuria shown significant high concentrations of nephrin without change ofmicroalbumin levels. Similarly other recent studies also reported thetype 2 diabetes with normoalbuminuria shown nephrinuria and there were no change in microalbumin levels.[25] Along with that significantly increased levels of urinary nephrin elevated levels of urinary nephrin has capable to predict early nephropathy in T2DM than microalbumin. Another case control study reported that nephrinuria can be used detection and progression of podocyte damage, kidney injury and diabetic nephropathy.[26]

Additionally we observed there was a significant positive correlation between nephrin and fasting blood sugars, post prandial bold sugars, blood urea, creatinine, uric acid, microalbumin and glycated hemoglobin. Previous study also reported that expression of specific protein particularly nephrin positively correlated with albuminuria and can be used as a pre clinical biomarker for diabetic nephropathy.[27] Another case control study also reported nephrin positively correlated with microalbumin have consider early predict and prognosticmarker for diabetic nephropathy.[28] Additionally the present study also observed scatter plots revealed the significant nephrinuria and positively correlated with microalbumin might be used as

early predectible marker for diabetic nephropathy.

5. Conclusion

Based on our findings we suggest significantly and drastically elevated concentrations of nephrin in type 2 diabetes with normo and microalbuminuria used as early predectible and prognostic marker for nephropathy.

6. Source of Funding

None.

7. Conflict of Interest

None.

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