Original Research Article

Glomerular basement membrane damage and leaking of structural proteins in among different stages of type 2 diabetes mellitus patients

Raghavendra K[1], Shantharam V[2,*]

[1]Dept. of Anatomy, GR Medical College Hospital and Research Centre, Bangalore, Karnataka, India
[2]Dept. of Anatomy, Akash Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India

ABSTRACT

Background: Type 2 Diabetes mellitus (T2DM) is a syndrome of disorders characterized by hyperglycemia caused due to a relative or absolute deficiency of insulin, decreased glucose utilization. The metabolic derangements in diabetes lead to secondary complications that affect multiple organ systems particularly on kidney. For early detection of kidney damage in T2DM patients are important by using certain type of urinary proteins. We aimed to evaluate the glomerular basement membrane damage and leaking of structural proteins in among different stages of type 2 diabetes mellitus patients.

Materials and Methods: A total 150 type 2 diabetes mellitus patients were included in the present study and again sub classified into 2 types (Normoalbuminuria 50, Microalbuminuria 50) based on their urinary ACR and also included 50 age, gender matched healthy controls. The FBS, PPBS, Urea, Creatinine, HbA1C, Urinary Albumin was analysed by using laboratory standard methods and Urinary Adiponectin was measured by using were collected from the all subjects. A statistical was performed by using SPSS Version 21.0 and P value considers < 0.05 is statistically significant.

Results: The significantly elevated levels of plasma FBS, PPBS, Serum Urea, Creatinine, HbA1c, Urinary albuminuria and Urinary Adiponectin observed in two groups of type 2 diabetes mellitus when compared to healthy controls. The Urinary Adiponectin was positively correlated with blood sugar levels and urinary albumin in two groups of diabetes mellitus. The data showed a significantly damaged foot process of podocytes and glomerular basement membrane leads to leaking of certain urinary proteins.

Conclusion : The elevated levels of Urinary Proteins and HbA1c, Urinary Albuminuria directly indicate the damage of podocytes and glomerular basement membrane in the kidney. These studies suggest continuous monitoring of these parameters may helpful for progression of type 2 diabetes mellitus complications.

1. Introduction

Type 2 DM or Non-Insulin Dependent Diabetes mellitus (NIDDM) is a heterogeneous, multifactorial, polygenic disease characterized by a defect in insulin secretion and is associated with insulin resistance and qualitative and quantitative insulin deficiency caused by alterations in several gene products.[1,2] The prevalence of in 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) and the United States (17.7 million) in second and third place respectively. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease.[3,4] Diabetic nephropathy (DN) or diabetic kidney disease (DKD) is a common and severe microvascular complication of DM with adverse outcomes

* Corresponding author.
   E-mail address: shansdenovo@gmail.com (Shantharam V).

in the form of renal failure, cardiovascular disease, and premature mortality.[5] Diabetic nephropathy represents an important cause of chronic kidney disease (CKD) that frequently leads to end stage renal disease (ESRD). It is reported that up to 40% of patients with type 1 and type 2 Diabetes mellitus present first time with DN.[6–8] Diagnosis in incipient stages of DN will provide an opportunity for appropriate therapy that prevents or slows down the evolution of DN to ESRD.[9]

Microalbuminuria is currently considered as gold standard and is the earliest clinically available marker for detection of diabetic nephropathy. However, its low sensitivity and larger variability are the main drawbacks.[10] The factors that influence the excretion of albumin include plasma concentrations of atrial natriuretic peptide, vasopressin, aldosterone, arginine, angiotensin II, and fasting blood glucose, mean arterial blood pressure and glycated haemoglobin.[11] The inter-individual variation is as high as 47%. These factors make the use of albuminuria as the conventional biomarker for early detection of DN untenable.[12] Hence, there is a need for biomarkers with high sensitivity, specificity and high predictability for presence of diabetic nephropathy. We aimed to evaluate the glomerular basement membrane damage and leaking of structural proteins in among different stages of type 2 diabetes mellitus patients.

2. Materials and Methods

This is a prospective observational study conducted at akash institute of medical sciences and research centre, Bangalore, Karnataka. A total 150 patients diagnosed with type 2 diabetes mellitus according to American diabetic association (ADA) criteria and further sub grouped into two groups 50 T2DM with Normoalbuminuria (< 30 mg/dL) and 50 T2DM with Microalbuminuria (< 30 - 290 mg/dL) based on urinary albumin creatinine (ACR) ratio and also included 50 age and gender matched healthy controls. All the subjects were included after obtaining institutional ethical clearance and informed consent. Inclusion criteria all the subjects' age should be more than 30 and less than 70 and whoever the persons suffering with type1 diabetes mellitus, liver, kidney, thyroid cardiovascular, cerebrovascular and peripheral vascular diseases excluded from these study.

Four (4) mL of fasting venous blood sample was collected from all the subjects into two tubes: 1 mL into a tube containing (anti glycolytic and anticoagulant), and 3 mL into a plain tube. Plasma samples were separated immediately and plain samples were allowed to clot and separated by centrifugation at 3000 rpm for 15 min. The separated samples were transferred into appropriately labeled aliquots and stored at -800 C until biochemical analysis was done. Spot urine sample was collected along with the blood sample and was processed immediately for albumin and creatinine. The plasma fasting Blood Sugar,

post parandial blood sugar, HbA1c serum urea, creatinine, uric acid and urinary albuminuria (ACR) was measured by laboratory standard methods and Urinary Adiponectin was analysed by using Enzyme Linked Immunosorbent Assay (ELISA).

2.1. Statiscal analysis

The data was checked for normality of distribution using Kolmogorov Smirnov test. The data was expressed as mean ± standard deviation or median (inter quartile range), for normally and non-normally distributed data, respectively. Statistical analysis was performed using Microsoft excel spread sheets and SPSS for windows version 21.0. A p value <0.05 was considered statistically significant.

3. Results

Table 1 shows the assessment of data distribution in two groups of type 2 diabetes mellitus when compared to healthy controls by usingKolmogorov smrinov test. The parameters studied had at least one group with not normally distributed data. Hence, data was logarithmically transformed before applying parametric statistical tools.

Table 2 shows the biochemical parameters studied in controls and two groups oftype 2 diabetes mellitus patients. statistically significant elevated levels of FBS, PPBS, Urea, Creatinine, U. ACR, HbA1c and U. Adiponectin in patients with two groups of type 2 diabetes mellitus normoalbuminuria, microalbuminuria when compared to healthy controls, respectively p-value 0.0001**.

Table 3 shows thePearson's correlation analysis of the biomarkers in the study groups with characteristics of diabetes. The Urinary Adiponectin positively correlated with HbA1c, Urinary albuminuria, FBS, PPBS, Serum Urea, Creatinine, Uric acid among two groups of study subjects.

4. Discussion

Diabetes mellitus and the complications resulting from it are the third leading cause of death in the world. The epidemic of diabetes mellitus, especially T2DM, is already a major public health challenge with a prevalence of 6.6% (56) and according to recent estimations, it is likely to worsen to critical levels in the next few decades.[13] Therefore target organ complications secondary to diabetes will be one of the most important medical concerns in the near future. DN is the most frequent cause of ESRD functional and structural changes in the glomerulus, such as glomerular hyper filtration, thickening of glomerular basement membrane and an expansion of extra cellular matrix in masangial areas.[14,15] It has recently been recognised that proximal tubular cell atrophy and tubulointerstitial fibrosis are also important than glomerulosclerosis in terms of renal prognosis.

Table 1: Shows the assessment of data distribution in two groups of type 2 diabetes mellitus subjects by using Kolmogorov smrinov test.

Parameter	Group 1 (n=50)	Group 2 (n=50)	Group 3 (n= 50)
FBS	0.305†	0.186†	0.084†
PPBS	0.159†	0.058†	0.016†
S. Urea	0.242†	0.179†	0.165†
S. Creat	0.115†	0.842†	0.200†
HbA1c	0.274†	0.267†	0.200†
U.MA	0.246†	0.342†	0.064†
U. Adiponectin	0.179†	0.387†	0.200†

Table 2: Demographic characteristics and biochemical parameters studied in controls and type 2 diabetes mellitus patients

Parameter	Group 1 (n=50)	Group 2 (n=50)	Group 3 (n = 50)	P – Value
Age	49.33 ± 6.22	46.33 ± 9.17	51.00 ± 10.45	0.48
FBS	86.36 ± 32.79	136.36 ± 23.14	173.46 ± 81.46	0.0001**
PPBS	134.46 ± 69.79	179.46 ± 57.64	252.93 ± 98.27	0.0001**
S. Urea	24.63 ± 6.60	39.63 ± 5.55	109. 53 ± 48.37	0.0001**
S. Creat	1.01 ± 0.23	0.89 ± 0.62	14.85 ± 1.68	0.0001**
U.MA	18.70 ± 4.93	22.70 ± 6.12	108.26 ± 31.08	0.0001**
HbA1c	3.92 ± 0.48	6.92 ± 0.59	10.77 ± 3.06	0.004**
U. Adiponectin	2.46 ± 0.69	9.46± 1.45	22.36 ± 6.47	0.0001**

Table 3: Pearson's correlation analysis of the biomarkers in the study groups with characteristics of diabetes

Parameter		FBS	PPBS	Urea	Creatinine
HbA1c	R	0.475	0.532	0.324	0.399
	Significance	0.0001**	0.0001**	0.0001**	0.0001**
Urinary ACR	R	0.547*	0.274	0.763	0.914
	Significance	0.0001**	0.0001**	0.0001**	0.0001**
Urinary Adiponectin	R	0.789	0.897	0.966	0.987
	Significance	0.0001**	0.0001**	0.0001**	0.0001**

The genesis and progression of diabetic nephropathy is due to interactions between metabolic and hemodynamic pathways, which are often disturbed in the setting of diabetes. The metabolic and hemodynamic abnormalities seen in diabetes interact with each other and pathways linked to ROS generation.[16,17] The consequences of these lead to functional and structural changes that clinically become manifest as diabetic nephropathy, characterized by increasing albuminuria and declining renal function.

Micro albuminuria, the current gold standard for early detection of diabetic Nephropathy, has its limitations due to low sensitivity and larger variability. Moreover, it has a number of drawbacks. Its excretion can be affected by several factors including plasma concentrations of atrial natriuretic peptide, arginine vasopressin, angiotensin II, aldosterone and fasting blood glucose, glycated haemoglobin, and mean arterial blood pressure.[18] The inter-individual variation is as high as 47%. These factors make the use of albuminuria as the conventional biomarker for early detection of DN untenable. Hence, there is a need for biomarkers with high sensitivity, specificity and high predictability for presence of diabetic Nephropathy. Also, it would be better prognostically, if diabetic nephropathy

can be detected before the appearance of micro albuminuria enabling early intervention to halt or reverse the process. As a result, glomerular or tubular biomarkers of renal injury are being studied as alternative markers for prediction of DN risk. Adiponectin was shown to play a protective role on kidneys through several mechanisms and protects against the development of albuminuria. The development of albuminuria, which is a marker of diabetic kidney disease was found to be influenced by adiponectin.

In the present study, Urinary adiponectin levels were measured in patients with type 2 diabetes mellitus who were classified further based on the amount of protein excretion into three groups and the levels were compared with age, BMI and gender matched healthy controls. Analysis of variance (ANOVA) showed significant difference in Urinary adiponectin levels across the Three study groups (p<0.0001). Several studies have evaluated Urinary adiponectin levels in patients with diabetes mellitus in relation to the complications associated with diabetes. Accordingly, increased levels of adiponectin were reported in diabetes patients with nephropathy, mainly in patients in advanced stages of diabetic nephropathy.[19,20] Similarly other recent studies also reported the urinary

Adiponectin was positively correlated with glycemic control and Urinary albumin in patients with type 2 diabetes mellitus and also they reported elevated levels of urinary adiponectin was useful for early detection and progression of nephropathy.[21,22] The present study also supports the previous studies, the significantly elevated levels of urinary adiponectin may be useful for clinical and earliest biomarker for nephropathy in different stages of type 2 diabetes mellitus.

5. Conclusion

A case control study was taken up to investigate the utility of urinary markers for the early diagnosis of DN in T2DM diabetes patients in comparison with healthy controls. The elevated levels of urinary adiponectin results damage of glomerular basement membrane in the kidney and also suggest to estimate these urinary adiponectin may be useful for early detection of nephropathy in T2DM because it is elevated in Normoalbuminuria.

6. Conflict of Interest

The authors declare that there are no conflicts of interest in this paper.

7. Source of Funding

None.

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Author biography

Raghavendra K, Associate Professor

Shantharam V, Professor