

Role of Urinary Neutrophil Gelatinase Associated Lipocalin as an Earlier Marker in Detecting Diabetic Nephropathy

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Abstract

Original Research Article

Diabetic Nephropathy also known as Kimmelsteil-wilson syndrome is the most common cause of End Stage Renal Disease (ESRD) and is the major cause of Diabetes related morbidity and mortality. Earlier, microalbuminuria is highly regarded to be the best indicator to assess the progression of diabetes to End Stage Renal Disease and cardiovascular complications. Now there are sensitive and specific markers available to help us in diagnosing renal damage and treating diabetic nephropathy even at an earlier stage to prevent further development into ESRD. This study is to evaluate the usefulness of urine Neutrophil Gelatinase Associated Lipocalin (NGAL) as a marker for the early detection of Diabetic Nephropathy. Study group comprised of 120 subjects; of whom Cases were 90 and classified into three groups Normoalbuminuric, Microalbuminuric and Macroalbuminuric based on Urine Albumin Creatinine Ratio. 30 healthy gender and age matched subjects were taken as control group. Urine NGAL was estimated by Enzyme Linked Immunosorbent Assay. Other parameters like Plasma Blood Glucose, Urine albumin and Urine Creatinine were estimated. Mean Fasting Blood Glucose, Urinary Albumin Creatinine Ratio and Urine Neutrophil Gelatinase Associated Lipocalin showed significant difference between groups ($p < 0.001$). Urine Neutrophil Gelatinase Associated Lipocalin had a positive correlation with Urine Albumin Creatinine Ratio. Urine Neutrophil Gelatinase Associated Lipocalin levels were higher in patients with Normoalbuminuria in whom the Urine Albumin Creatinine Ratio was normal. Thus, urine Neutrophil Gelatinase Associated Lipocalin can be used as a biomarker to detect Diabetic Nephropathy at an earlier stage ie Stage of Normoalbuminuria

Keywords: Type 2 Diabetes, Diabetic nephropathy, Normoalbuminuria, Microalbuminuria, Macroalbuminuria, Neutrophil Gelatinase Associated Lipocalin

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INTRODUCTION

Diabetes mellitus is one of the most common non communicable diseases affecting almost 7% of the world's population. Type 2 Diabetes Mellitus comprises 90–95% of all cases and has become an epidemic in developing countries[1]. The global incidence of type 2 Diabetes continues to rise due to the increase in obesity and aging population[2]. In 2000, the prevalence of diabetes was estimated to be 171 million (2.8%) worldwide. It is projected that 366 million (4.4%) people worldwide will have diabetes by 2030[3]. Diabetic nephropathy is a severe complication occurring in diabetic patients and it is associated with an increased risk of all-cause mortality, cardiovascular disease and End Stage Renal Disease (ESRD) requiring costly renal replacement therapy in the form of dialysis or transplantation [4, 5]. Diagnostic marker to detect Diabetic Nephropathy at an early stage is important as

early intervention can slow the loss of kidney function and reduces adverse outcomes. The appearance of small amount of albumin in urine called microalbuminuria has been accepted as the earliest marker for development of Diabetic Nephropathy. However, it has been reported that a large proportion of renal impairment occurs even before the appearance of microalbuminuria[6]. 29.1% - 61.6% of individuals with type 2 diabetes could have renal impairment even before the onset of microalbuminuria [7, 8]. Presence of albumin in urine of non diabetic subjects indicated the non specificity of albuminuria for accurate prediction of Diabetic Kidney disorder and Urinary biomarkers that predict DN at a very early stage even before the appearance of microalbuminuria are needed for optimal clinical management of diabetic patients[9]. Glomerular and tubulointerstitial injury plays a role in the pathogenesis of DN [10]. Within the diabetic kidney,

the glomeruli and tubules are subjected to damage from hyperglycemia, advanced glycosylation products, activation of inflammatory cytokines and microalbuminuria which ultimately develop into renal fibrosis with renal failure [11, 12]. Several glomerular and tubular biomarkers predicting onset or progression of nephropathy in patients with diabetes have been identified and are become increasingly important in clinical diagnostics [10, 13]. Recent studies have demonstrated that urinary biomarkers are significantly elevated in normoalbuminuric type 2 diabetic patients compared with non diabetic control subjects and could be used as markers for earlier, specific and accurate prediction of Diabetic Nephropathy [14]. Among them is Neutrophil Gelatinase Associated Lipocalin (NGAL), which is produced in epithelial cells and neutrophils [15]. NGAL, a glycoprotein belonging to the Lipocalin superfamily is one of the most commonly studied novel biomarkers for renal impairment. NGAL can be expressed in various cells. Matrix metalloproteinases (MMPs) are a class of zinc-dependent proteinases that act in the degradation and turnover of extracellular matrix proteins. Of the many mammalian MMPs currently discovered, MMP-9 was noted to be in higher concentrations in persons with Obesity, Metabolic Syndrome and Type 2 Diabetes mellitus [16-18]. Binding of NGAL with MMP-9 has been demonstrated to decrease the degradation of these complexes and enhance their proteolytic activity [19]. The stimuli that induce epithelial damage lead NGAL in high expression and then increase the baseline serum level. NGAL filtered by the glomerulus will be captured by the proximal tubules and only a minimal amount is excreted in the urine. Tubular injury results from ischaemia, inflammation and hyperglycemia leads to a decrease in NGAL reabsorption and increase in NGAL secretion by tubular cells [20]. NGAL detection in urine has been shown to be sensitive for renal damage in Type 2 Diabetes mellitus. Both urinary and serum NGAL predicted Chronic Kidney Disease progression independently of age and Estimated GFR [21]. NGAL has been evaluated in several studies of Diabetic subjects. NGAL was 5-10 folds higher in normo or Microalbuminuric patients as compared to controls. This indicates that tubular injury (proximal and distal) occurs early and perhaps albumin excretion in patients with emerging Diabetic nephropathy [22]. These results were confirmed in another study of Type 2 Diabetes mellitus patients with normo, micro and Macroalbuminuria where urine NGAL was positively correlated with CystatinC, BUN, Serum Creatinine as well as albuminuria and negatively with GFR [23].

MATERIALS AND METHODS

This is a Cross sectional Case Control Study and was conducted after getting Institutional Ethical Committee approval. The Study composed of a total number of 120 subjects, of which the apparently normal subjects who formed the control group were 30 comprised of volunteers from Master Health Checkup and the staffs

of Madras Medical College, Chennai. The remaining subjects were those with Type 2 Diabetes mellitus, enrolled into the study as Cases. These individuals were from among the Type 2 DM individuals attending the Diabetology Outpatient clinic in Rajiv Gandhi Government General Hospital, Chennai.

Inclusion Criteria were Patients with Type 2 Diabetes mellitus diagnosed based on American Diabetes Association Classification on treatment with Oral Hypoglycemics. Controls were Age and Gender matched individuals without Diabetes mellitus. Patients with Acute illness, Urinary tract infection, Inflammation, Neoplastic disorder and Cardiac disorders were excluded from the study.

Cases were classified into three groups based on Urine Albumin Creatinine Ratio (UACR) and each group comprising of 30 subjects

Group I – Type 2 Diabetic patients with Normoalbuminuria (UACR <30mg/g of Creatinine)

Group II – Type 2 Diabetic patients with Microalbuminuria (UACR 30-300mg/g of Creatinine)

Group III – Type 2 Diabetic patients with Macroalbuminuria (UACR >300mg/g of Creatinine)

Sample collection

Urine

Early morning Mid-stream urine specimen was collected and the following investigations were performed. Urine albumin concentration was estimated using Semiautoanalyser by Latex agglutination/Immunoturbidimetry and the kit from Biosystems Reagents and Instruments. Urine Creatinine was estimated by Modified Jaffe's method.

Urine Albumin Creatinine Ratio is calculated by the following equation:

$$\text{UACR} = \frac{\text{Urine Albumin (mg/L)}}{\text{Urine Creatinine (g/L)}}$$

$$\text{UACR} = \text{mg/g of Creatinine}$$

After classifying the individuals enrolled in the study based on UACR into three groups, Urine Neutrophil Gelatinase Associated Lipocalin (NGAL) was estimated by Quantitative Sandwich Enzyme Immunoassay. Kit from R&D systems. Urine for estimating Neutrophil Gelatinase Associated Lipocalin (NGAL) was stored at -40°C to -80°C and analysed within 15 days.

Blood

Blood was collected after an overnight fast for

8-12 hours and Fasting Plasma was analysed on the same day within 4 hours of collection. Fasting Plasma glucose level was estimated using Semiautoanalyser by Glucose oxidase and Peroxidase method.

Statistical analysis

Statistical analysis was performed using SPSS software. Gender and risk factors like alcohol, Smoking and hypertension were compared using chi square test. One – way ANOVA was used to compare the mean

values of Age, BMI and biochemical parameters like Urine Albumin Creatinine Ratio, urine Neutrophil Gelatinase Associated Lipocalin and Fasting blood sugar between controls, Normoalbuminuria, Microalbuminuria and Macroalbuminuria. Pearson coefficient correlation was done to measure the linear relationship between UACR and urine NGAL.

RESULTS

Table-1: Characteristics of different groups of patients with Diabetic nephropathy and controls

Variables	Controls	Normo albuminuria	Micro albuminuria	Macro albuminuria	P-value
Age	56.07±10.34	55.57±11.047	55.97±10.217	55.27±10.079	0.990-NS
Sex – male	16(53.33%)	18(60%)	14(46.67%)	13(43.33%)	
Female	14(46.67%)	12(40%)	16(53.33%)	17(56.67%)	0.579-NS
Smoking	7(23.33%)	7(23.33%)	8(26.67%)	8(26.67%)	0.981-NS
Alcohol	7(23.33%)	7(23.33%)	8(26.67%)	9(30%)	0.923-NS
Hypertension	10(33.33%)	10(33.33%)	12(40%)	18(60%)	0.117-NS
BMI	28.64±2.51	29.51±2.44	29.08±2.69	30.01±2.57	0.198-NS
Fasting Blood sugar	88.77±12.41	138.77±53.19	194.37±72.79	204.43±87.84	<0.001-S
Urine albumin creatinine ratio	2.62±0.74	16.24±8.29	150.14±75.55	551.02±161.26	<0.001-S
Urine neutrophil gelatinase associated lipocalin	15.83±10.83	66.83±17.08	263.57±117.68	672.48±138.18	<0.001-S

NS – not significant; S-significant

Table-2: Comparison of NGAL between groups

Groups	N	Mean	Std. Dev	Min	Max	P-Value
Control	30	15.83	10.83	4.00	39.38	
Normoalbuminuria	30	66.83	17.08	37.98	94.08	
Microalbuminuria	30	263.57	117.68	126.05	500.05	<0.001
Macroalbuminuria	30	672.48	138.18	494.60	990.00	
Total	120	254.68	274.65	4.00	990.00	

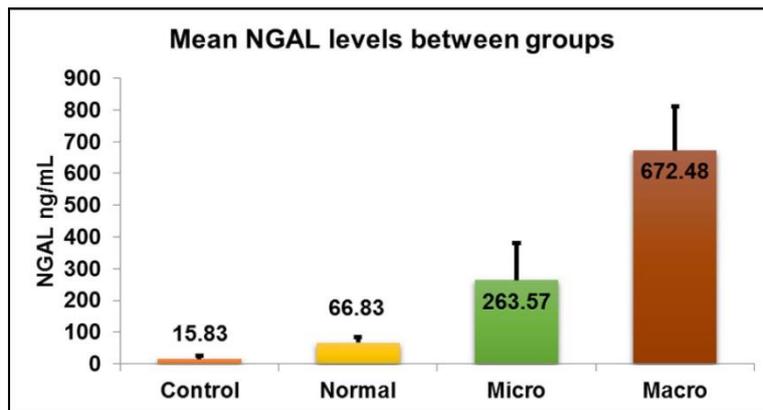


Fig-1

Table-3: Pearson correlation co-efficient to measure the relationship between NGAL and uacr

	Control	Groups	

		Normo	Micro	Macro
		Albuminuria	Albuminuria	Albuminuria
Correlation	0.180	0.637	0.938	0.849
P-value	0.342	<0.001	<0.001	<0.001
N	30	30	30	30

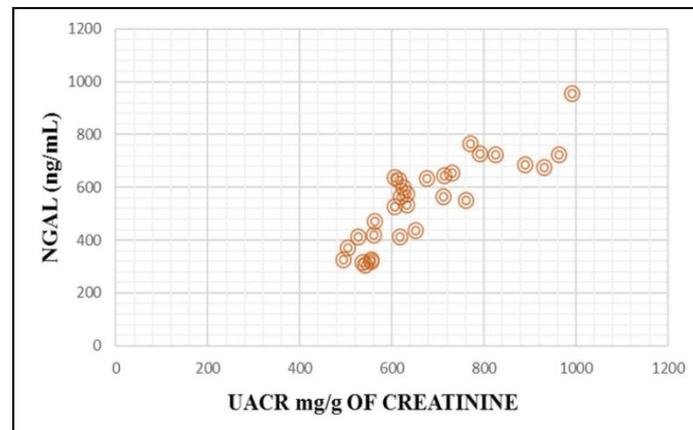


Fig-2

DISCUSSION

Diabetic Nephropathy is a major cause of illness and death in diabetes. Mortality due to DN occurs mainly in patients with Proteinuria because of End Stage Renal Disease (ESRD) and Cardiovascular complication [24-26]. Blood urea nitrogen, serum creatinine and analysis of urine are the gold standard markers routinely used to diagnose kidney damage. But, they don't detect kidney dysfunction at the earliest so that therapeutic interventions can be done. In order to avoid mortality due to the above mentioned complications, it is better to identify the diseased state at an earliest. Recently, Biomarkers have gained importance to the dysfunction earlier enough for therapeutic intervention.

In the present study, urine Neutrophil Gelatinase Associated Lipocalin (NGAL) levels were estimated in patients with Diabetic Nephropathy and Controls to assess the utility of NGAL as a biomarker in patients with DN.

In this study, we recruited 120 subjects and divided them into four groups. Cases were categorized into 3 groups namely Patients with i) Normo albuminuria, ii) Microalbuminuria and iii) Macro albuminuria and Age and Gender matched Controls in one group.

In the present study, the mean of urine NGAL was observed to be increased gradually from the Normoalbuminuric patients (66.83 ± 17.08) to Macroalbuminuric patients (672.48 ± 138.18) with significant increase in patients with macro albuminuria.

Similar result were observed by the study done by Mohamad Fathimah *et al.* [27]. This finding

implies that urine NGAL can be used to assess degree of renal impairment.

In this study, Urine NGAL was found to be elevated in patients with Normoalbuminuria (66.83 ± 17.08) when compared with UACR. It is within the normal range (16.24 ± 8.29). Bolognani *et al.* in his study has found that urine NGAL levels are elevated even in patients with Normoalbuminuria in spite of normal UACR in the same group [28]. So, NGAL can be also used as a marker for the early detection of diabetic nephropathy.

In the present study, correlation was done to measure the linear relationship between NGAL and UACR. A moderate positive linear correlation was observed in Normoalbuminuria. A strong positive linear correlation was observed in Microalbuminuria and Macro albuminuria. This indicates that as the concentration of UACR increases, the levels of NGAL also increases.

CONCLUSION

Diabetes has been recognised as a worldwide health problem affecting people at variable ages with increasing incidence and prevalence leading to various complications. One of the major complications is Diabetic Nephropathy. The interaction of metabolic, hemodynamic pathways and inflammatory pathways in Diabetic Nephropathy lead to the development of basement membrane damage, tubular injury, tubule interstitial fibrosis and finally resulting in irreversible Renal Impairment. Biomarkers provide important information regarding the pathogenesis of Diabetic Nephropathy, identification of subjects at the earliest in order to intervene therapeutic measures. One such marker is Neutrophil Gelatinase Associated Lipocalin.

From this study, it is concluded that

Early diagnosis of diabetic nephropathy

Urine NGAL levels can be measured routinely in Patients with Diabetes mellitus so that early identification of risk groups who are prone to develop Diabetic Nephropathy could be identified. It is the appropriate time to carry out therapeutic interventions and the mortality due to complications can be prevented

Assessing severity of renal impairment

Urine NGAL levels are increased gradually from Normoalbuminuria to Macroalbuminuria. The values are higher in case of Macroalbuminuria. By estimating the concentration of NGAL, we can assess the degree of renal impairment.

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