

Evaluation of Diabetic Nephropathy Using Selected Biochemical Markers in Settiyar (Vysya) Community of Nellore and Parkasam Districts of Andhra Pradesh, India

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Abstract: Diabetic nephropathy is a progressive kidney disease characterized by renal damage under high blood glucose. The early detection of DN may help in the prevention. Glomerular and tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine. Studies were conducted to evaluate the glomerular and tubular marker in urine as well as in serum of the control and selected community (Settiyar) people. Results have shown that all the markers except B2M were found to decrease in serum and a reverse was observed in urine in both KD and NDD subjects than the controls suggesting that the B2M can be effectively used in diagnosing the early kidney damage in the selected community.

Key words: Ace gene • Transferrin • Beta 2-microglobulin • Settiyar community

INTRODUCTION

Diabetic nephropathy is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to longstanding diabetes mellitus and is a prime indication for dialysis in many Western countries.

Racial differences in the prevalence of diabetic renal disease have been reported. Asian subjects have significantly ($p < 0.01$) higher prevalence (52.6%) of diabetic end stage renal disease (ESRD) when compared with the Caucasians (36.2%) migrant Asian [1]. Indians had 40 times greater risk of developing ESRD when compared with the Caucasians [2]. The prevalence of diabetic nephropathy in type 2 diabetic subjects is reported to be 5-9% from various Indian studies [3-5]. Patients with diabetic nephropathy, especially with type 2 diabetes, have a high cardiovascular risk. The risk for cardiovascular disease (CVD) was 3 fold higher in South Indian NIDDM subjects with nephropathy when compared with their non-nephropathic counterparts [6]. Thus, in type 2 diabetes, many patients may not reach end stage renal disease due to premature death from CVD.

Obesity is the main cause for several life threatening diseases like diabetes. Particularly in countries like India, where various forms of people live with a unity in diversity. Particularly some communities were more susceptible to diseases like diabetes due to their life style and habitates. In Andhra Pradesh, specifically the Vysya community (Settiyar) is more prone towards obesity due to their lifestyle. People belonging to this community are frequently suffering from diabetes and renal failures. In general most of the studies were conducted to know the ethnic differences between the countries. But studies related to the exact evidence of relation between the community and the diabetic nephropathy is not known. Hence the present study was aimed at the evaluation of Diabetic nephropathy in the selected community by using certain biochemical markers.

MATERIALS AND METHODS

A study was conducted in Nellore and Prakasam districts of Andhra Pradesh which is geographically southern part of India near to the Bay of Bengal and these districts having wide spread of selected community.

The lifestyle and from the reports of local medical laboratories made us to study the prevalence of DN among this particular community.

Selection of Patients: Eight hundred and twenty (n=820) type II diabetic subjects from 200 families of Shettiyar (Vysya) community in Nellore and Prakasam districts of Andhra Pradesh State were chosen randomly for the present study. A door to door survey with face-to-face interviews was carried out in the same community group to find out the known diabetic (KD) and newly diagnosed diabetic (NDD) subjects. The information collected was entered on a pre-coded questionnaire. Among the total number of samples 200 were KD subjects and remaining 620 were NDD. People suffering with regular renal failure with diabetes and newly diagnosed diabetic were separated.

Collection of Blood and Urine Samples: This study was conducted on around 200 families, who were suffering with renal problems associated with diabetes with KD and NDD. They were provided with explanations for all experimental procedures and informed consent was obtained before the beginning of the study. Blood and urine samples were collected from the subjects and preceded for further hematological and biochemical analysis.

Biochemical Analysis

Estimation of Random Blood Sugar: Random blood glucose was measured routinely by using "One Touch Ultra Blood Glucose Meter" (AccuChekGluco Meter, USA).

Estimation of Glomerular and Tubular Markers:

Estimation of Transferrin: The amount of transferrin was estimated using SRID kit in the first sample was 7.5 g. We used 10.5mg Fe and 11.44 anionic bicarbonate.

Estimation of IgG: The serum and urine IgGs were by Sandwich Elisa with one week storage using the reagents supplied by Genei, Bangalore, India.

Estimation of Antitrypsin: Oxidative antitrypsin (AT) was estimated using Elisa with a monoclonal antibody against oxidized alpha 1 antitrypsin in which chloramine T oxidized alpha 1 antitrypsin was the antigen [7]. The sensitivity of oxAT measurement was 1.0ng/ml with an inter CV of <6.7%.

Beta 2 Microglobulin Assay: The samples were analyzed by using Enzyme linked immunosorbent assay (B2-microglobulin EIA kit, Immunotech, France). 2.4mg/L was used as upper limit, when 97% of normal values are below this cutoff value.

Estimation of Serum and Urinary ACE Levels: Serum or urine Ace levels were measured by a colorimetric method (Colorimetric assay Kit, Fujizoki assay, Tokyo, Japan) using p-hydroxyhippuryl-L-histidyl-L-leucine as the substrate [8].

Statistical Analysis: Statistical work was carried out by using SPSS software for Windows 10.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel. Values were reported as mean \pm standard deviation. SD was not more than 10%.

RESULTS AND DISCUSSION

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide and it is estimated that ~20% of type 2 diabetic patients reach ESRD during their lifetime [9]. Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard, or reverse the progress of the disease. As hyperglycemia is the preliminary biomarker for the identification of diabetes and developing disease progression, studies were explored in the selected populations under fasting and postprandial visits in terms of blood tests. Results showed that the selected community is having higher glucose levels of 138.59 \pm 18.06 mg/dL fasting and 228.32 \pm 7.54 mg/dL of postprandial glucose levels, stating that the selected subjects were belongs to diabetic (Table 1). These people considered as KD (n=408), where already they were identified and under treatment category. The other 412 patients were newly discovered that they are diabetic. Hence they were considered as NDD. Results showed that 142.21 \pm 10.87 mg/dL of fasting glucose and 239.47 \pm 6.12 mg/dL postprandial glucose levels are observed in the NDD category. These results were matched with their family history and life style (Table 1).

The routine classical evaluation of nephropathy (any type of renal problems) includes the identification of glomerular and tubular markers in the patient's serum and urine. The normal individual doesn't contain this content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferrin, IgG,

Table 1: Analysis of the blood sugar and serum creatinine of the normal and selected community (Shettiyar) peoples

		Random Blood Sugar (mg/dL)		Serum Creatinine(mg/dL)
		Fasting	PP	
Normal value		70-100 mg/L	<150 mg/L	0.5 to 1.0 mg/dL
Control (n=200)		83.58±15.83	133.58±5.21	1.03±0.35
Shettiyar community	KD (n=408)	138.59±18.06	228.32±7.54	2.78±0.24
	NDD (n=412)	142.21±10.87	239.47±6.12	2.91±0.76
Significance		P<0.001	P<0.001	P<0.001

antitrypsin, β -2-microglobulin and angiotensin converting enzyme (ACE). Recent studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine [10].

Thus studies were conducted to evaluate the glomerular and tubular marker in urine as well as in serum of the control and selected community (Shettiyar) people. Figure 1 shows the analysis of serum glomerular and tubular markers in the control and test samples. Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. The blood transferrin level is tested for diverse reasons like to determine the cause of anemia, to examine iron metabolism (for example, in iron deficiency anemia) and to determine the iron-carrying capacity of the blood. Low transferrin can impair hemoglobin production (since to make hemoglobin, you have to have iron) and so lead to anemia. Low transferrin can be due to poor production of transferrin by the liver (where it's made) or excessive loss of transferrin through the kidneys into the urine. Here in this present study the level of transferrin seems to be low when compared to that of control (Figure1). That indicates the chance of anemia due to diabetes. Low levels of IgG occur in macroglobulinemia. In this disease, the high levels of IgM antibodies suppress the growth of cells that produce IgG. Other conditions that can result in low levels of IgG include some types of leukemia and a type of kidney damage (nephrotic syndrome). Here we can find the low levels of serum IgG, but within the normal range indicating the altered renal function. Alpha 1-antitrypsin (A1AT) is produced in the liver. Accumulation of this in liver causes lower levels of A1AT in blood results in the development of liver cirrhosis. Excessive excretion of A1AT through urine indicates the loss of renal function. In present case

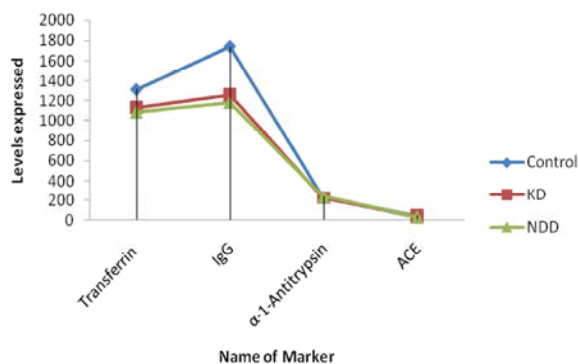


Fig 1: Levels of glomerular and tubular markers in serum

there is no difference with the control value. It seems to be almost equal, that indicates the normal functioning of liver (Figure 1). Beta 2-microglobulin is a protein found on the surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma or kidney disease. B2M can be an early marker to diagnose renal failure under fluoride toxicity [11] and other heavy metal toxicity. In our study interestingly rapid enhancement of B2M was noticed. The control subjects showed 2.60 ± 0.99 g/ml, where the KD people showed a maximum increase of B2M to 10.89 ± 2.08 g/ml and NDD people showed 11.56 ± 1.88 g/ml. That shows a drastic increase which indicates the altered renal activity in the selected group of people (Figure3). There was a significant ($P < 0.001$) change was noticed compared to the normal. This altered range is more supportive for further analysis for the diabetic nephropathy in Shettiyar community. The angiotensin-converting enzyme test is used to measure the blood level of angiotensin-converting enzyme, which converts angiotensin I to angiotensin II and controls blood pressure. Angiotensin-converting enzyme and ACE2 are highly expressed in the kidney. The role of ACE in the development of renal damage is generally accepted. Here in the present study the ACE level seems to be decreased when compared to that of control individuals (Table 12). Control individuals having a concentration of 44.97 ± 8.72

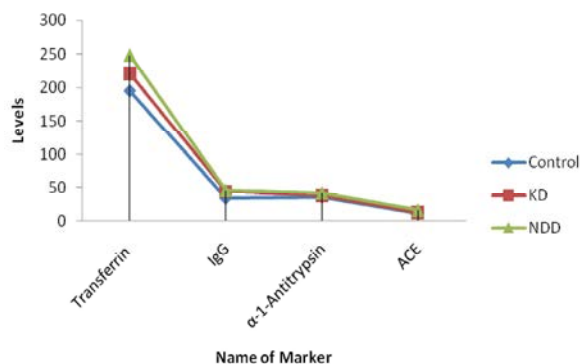


Fig 2: Urinary levels of selected glomerular and tubular markers

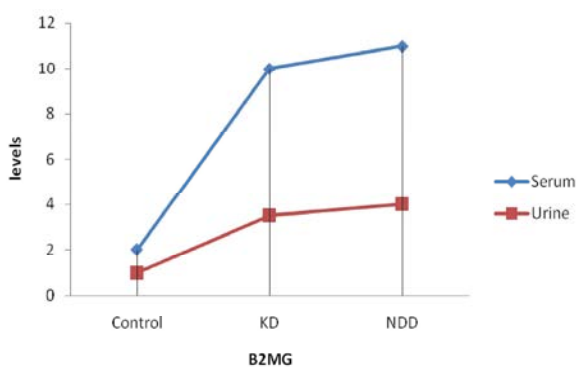


Fig 3: β2- microglobulin levels in urine and serum

and selected group of people belongs to KD are showing 37.07 ± 12.68 and NDD people are showing a concentration of 32.51 ± 10.23 indicating a significant ($P < 0.001$) decrease. This indicates the accumulation of angiotensin I.

After identifying the glomerular and tubular markers in the serum studies has been made to know the status of the same in the urine to confirm the diabetic nephropathy in Shettiyar community people. From the table 1 it is clear that transferrin levels are hiked in the Shettiyar community people. Control people are showing a value of 195.50 ± 29.30 and the KD people are showing 221.43 ± 49.48 and NDD are showing 248.29 ± 38.21 . From the earlier table it is clear that transferrin concentration seems to be low in the serum and now it increases in the urine indicates the loss of renal function (Figure 1 and 2). Similar results were found in the case of IgG in the urine as well as in serum. Here also we can find the decreased concentration of serum IgG and increased levels of urinary IgG indicates the renal alterations. The control urinary IgG seems to be 34.54 ± 2.37 and in the selected KD people it reaches to 45.41 ± 7.71 and in NDD it reaches to 46.98 ± 5.55 . This shows a significant ($P < 0.001$) increase (Table 1). Similarly AIAT was also changed with slight modifications, where the serum AIAT is not having

any significant change. But here we can clearly find altered values of AIAT. The control individuals are showing 36.61 ± 8.38 AIAT, where the KD people are showing an increased value of 39.96 ± 6.54 and NDD up to 42.15 ± 5.88 indicates the increase in the excretion of AIAT due to renal failure (Table 1). Here B2M also showing similar pattern of over excretion. Here we can find 3.64 ± 0.97 in the KD people and 4.21 ± 0.66 in NDD people, where the control value is 1.24 ± 0.98 . Hence, it can be concluded that, these values are drastically increased in the serum as well as in urine of the selected community (Shettiyar) people. The same was also found with ACE levels here the control value is 11.46 ± 0.84 and the KD people are showing 13.77 ± 1.46 and the NDD are showing 16.74 ± 0.89 , which means over excretion indicates the renal problems (Figure 2).

B2M is normally cleared by the kidneys at a rate comparable to GFR Karlsson *et al.* [12], then reabsorbed and catabolized in the tubules and serum levels are inversely related to GFR [12]. Clearance by conventional dialyzers is negligible as these membranes are impermeable to β2m. Production of β2m in normals is 9 mg/ hr/70 kg [13]. Production may be increased in proliferative disorders [14] and rheumatoid arthritis [15] as indicated by high serum levels in the presence of normal renal function.

Angiotensin-converting enzyme (ACE) is a risk factor for DN. Its plasma levels have been reported to be associated with DN but not with diabetic retinopathy in type 1 diabetes patients [16]. ACE modulates the generation of angiotensin II, which increases intraglomerular hydraulic pressure [17], leading to glomerulopathy. ACE inhibition strongly modifies renal hemodynamics in animals [18] and the course of DN can be considerably improved by treatment with ACE inhibitors, in patients with type 1 diabetes [19]. Plasma ACE concentrations are stable in individuals [20] and are partly under genetic control [21]. Similar results were found in the case of IgG in the urine as well as in serum. Here also we can find the decreased concentration of serum IgG and increased levels of urinary IgG indicates the renal alterations. Similarly AIAT was also changed with slight modifications, where the serum AIAT is not having any significant change. But here we can clearly find altered values of AIAT. Here B2M also showing similar pattern of over excretion. Hence, it can be concluded that, these values are drastically increased in the serum as well as in urine of the selected community (Shettiyar) people. The same was also found with ACE levels here the control value is 11.46 ± 0.84 and the KD

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Individual differences in renal ACE activity predict the susceptibility for proteinuria-associated renal damage in experimental conditions [22]. Furthermore, Ang II is increased in damaged tubules and is suggested to be a possible mediator of renal damage in experimental and human renal disorders [23, 24]. Blockade of the actions of Ang II by ACE inhibitors or AT₁ receptor blockers has been proven to effectively reduce blood pressure and proteinuria [25], thereby providing renoprotection. A disrupted balance between intrarenal ACE and ACE2 with consequent low levels of Ang II and high levels of Ang (1-7) might contribute to the renoprotective mechanisms of ACE inhibitors [26-29].

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