

**Original article:**

## **A study of urine microalbumin in non insulin dependent diabetes mellitus**

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### **Abstract:**

**Background and objectives:** Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India. Urinary albumin excretion are found to be increased in various conditions like diabetes mellitus, cardiovascular diseases, cancer etc. In diabetes mellitus, acute phase reactants are considered as the indicators of microvascular angiopathy. Microalbuminuria is a predictor of incipient nephropathy and coronary vascular disease in the diabetic patients. Therefore our study was undertaken to understand the association levels in incipient diabetic nephropathy patients and to assess the correlation microalbuminuria with glycemc control.

**Methods:** Present study involved 90participants of which 60 were non insulin dependent diabetes mellitus (NIDDM) patients studied for their urinary microalbumin, fasting blood glucose and serum creatinine levels. Analysis was performed by categorized them based on their albumin excretion (normoalbuminuric and microalbuminuric). 30 non diabetic age and sex matched healthy subjects were taken as a control group. Blood samples were drawn and urine samples were collected under aseptic precautions from study subjects. The values were tabulated for cases and controls.

**Results:** Serum creatinine shows significant increase only in NIDDM with microalbuminuric patients . **CONCLUSIONS:** The study concludes that elevated microalbumin levels are strongly associated with the progression microvascular complications such as of diabetic nephropathy.

**Key words:** FBS, Creatinine, Microalbuminuria; Diabetes mellitus.

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### **Introduction:**

Diabetes mellitus is a metabolic disorder of multiple etiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.<sup>(1)</sup>

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90%of cases globally<sup>(2)</sup> It is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia.<sup>(3)</sup> All forms of diabetes, both inherited and acquired, are characterised by hyperglycemia, a relative or absolute lack of insulin, and development of diabetic specific microvascular pathology in retina, renal glomerulus, and peripheral nerve.<sup>(4)</sup>

International diabetic federation (IDF) estimates the total number of diabetic subjects in India to be around 40.9million and this is further set to raise to 69.9million by the year 2025.<sup>(5)</sup> Large prospective clinical studies show a strong relationship between glycemiaand diabetic microvascular complications in both type1 diabetes mellitus and type2 diabetes mellitus. There is a continuous, though not linear, relationship between level of glycemia and risk of development and progression of these complications.<sup>(4)</sup>

It has been proposed that inflammatory process play an important role in the developmentof diabetes and its late complications. Various acute phase reactants are being studied in diabetic process as indicators or predictors of diabetic microvascular complications.<sup>(6)</sup>

Diabetic nephropathy remains a major cause of morbidity and mortality for the persons either T1DM, or T2DM.<sup>(4)</sup> Diabetic nephropathy occurs in about 25-30% of diabetic patients. However there is an early phase of diabetic renal disease called incipient diabetic nephropathy characterised by increased albumin excretion in the range of 30-300mg/day microalbuminuria.<sup>(9)</sup> Incipient diabetic nephropathy has been proved to be reversible with tight glycaemic control and ACE inhibitors.<sup>(10)</sup>

The study was undertaken to correlate microalbuminuria which are the markers of early renal damage to establish the role of estimation of microalbumin in NIDDM. Incipient diabetic nephropathy has been proved to be reversible with tight glycaemic control and ACE inhibitors.

#### **Materials and methods:**

##### **Setting:**

A Case control study was conducted during march 2013-September 2014, in the Department of Clinical Biochemistry, Osmania General Hospital, Hyderabad; Telangana.

##### **Sources of Samples and Data;**

- The established NIDDM patients attending the OPD of Osmania General Hospital, and Department of Clinical Biochemistry Osmania General Hospital, Hyderabad.

##### **Control Group:**

Consists of Age and gender matched healthy controls. None of the patients had a history of Diabetes mellitus, Hypertension, Hepatic, Renal, any other systemic illnesses. Also they were judged to be free of any illness by clinical examination.

##### **Cases:**

###### **The diabetic patients:**

- Age between 40 to 60 yrs of both Genders.
- The Classification of Subjects in to Diabetic and Non-diabetic Groups was based on American Diabetes Association Criteria 2011.

##### **Exclusion Criteria:**

- Type 1 diabetic cases.
- Urinary albustic positive cases of NIDDM
- Cases with inflammatory disorders like eczema, secondary hyperglycemic states like hypothyroidism,
- Proteinuric conditions like congestive cardiac failure, renal failure, and pregnancy.
- Female patients with menstrual disorder
- Cases with severe combined immune deficiencies.
- **Study Grouping**
- All the study subjects (60 cases + 30 controls) / participants were explained the nature of the study. Informed consent was obtained from all the 90 subjects.
- **The study sample**
- In the present study 90 subjects were selected and divided into two age and sex matched type 2 diabetic cases and non diabetic control groups.
- **Group I:** Includes 30 type 2 DIABETIC CASES with urine microalbumin negative.
- **Group 2:** Includes 30 type 2 DIABETIC CASES with urine microalbumin positive

- **Group 3:** Includes 30 NON DIABETIC CONTROLS.
- **Sample collection:**
- Participants were in overnight fasting status. They were in supine position for 5 to 10 minutes before venipuncture and 3ml to 4ml venous blood was drawn and collected into three tubes. One containing sodium fluoride and potassium oxalate (grey top), and the other was a plain tube (red top). The blood in plain tube was allowed to clot to separate serum. Serum, plasma was separated within one hour after sample collection. Care was taken to avoid Hemolysis. All icteric, hemolysed samples were ignored. Serum for other parameters was stored at -20°C.
- Blood samples were analysed for fasting blood glucose, serum creatinine, and serum sialic acid.
- Early morning Urine samples were collected under aseptic precautions for estimation of urinary microalbumin.
- **Samples from all 90 subjects were analyzed for the following parameters:**
- 1. Plasma fasting Blood Sugar
- 2. serum creatinine
- 3. urine microalbumin

#### **ESTIMATION PROCEDURES FOR ANALYTES:**

**GLUCOSE:** (GOD-POD METHOD) <sup>(13,14,15)</sup>

**Method:** GLUCOSE OXIDASE PEROXIDASE METHOD.

#### **ESTIMATION OF SERUM CREATININE:**

**Method:** modified Jaffe's reaction <sup>(16,17,18,19)</sup>

#### **ESTIMATION OF URINE MICROALBUMIN:**

**Method:** TURBILATEX METHOD (immune turbidometric method) <sup>(16,17,18,19)</sup>

Type 2 diabetes usually develops in obese patients who are over 40 years old. Its pathogenesis involves a combination of insulin resistance and impairment of insulin secretion. Insulin resistance in several tissues like skeletal muscle, adipose tissue and liver leads to increased insulin secretion from pancreas. This compensatory hyperinsulinemia maintains glucose levels within normal range but individual is at high risk of developing diabetes. Beta cell function eventually declines and leads to development of impaired glucose tolerance and eventually overt diabetes mellitus. <sup>(11)</sup>

Environmental influences, such as dietary habits and sedentary life styles, clearly have a role which becomes evident when obesity is considered. Genetic factors are even more important in type 2 than in type 1 diabetes. <sup>(12)</sup>

#### **Obesity and insulin resistance:**

Insulin resistance is the link between obesity and diabetes. The risk for diabetes increases as the body mass index (a measure of body fat content) increases, suggesting a dose response relationship between insulin resistance and body fat. <sup>(12)</sup>

**Diabetes mellitus is a metabolic disorder of multiple etiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.** <sup>(1)</sup>

#### **Results:**

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad.

A total of 90 subjects were recruited for the study which included 30 healthy individuals as controls, 30 T2DM patients with normoalbuminuria, 30 patients T2DM with normoalbuminuria.

The following parameters were analysed.

1. Fasting plasma glucose
2. Serum creatinine
3. Urine microalbumin

The results were expressed in milligrams /deci liter for Fasting plasma glucose, Serum creatinine, and milligrams/litre for urine microalbumin.

The data was analysed using GraphPad Prism software version 6.0.

Descriptive results are expressed as mean and SD of various parameters in different groups.

Student's t-test was used for testing the significance difference in mean scores of various bio-chemical parameters between case and control groups. Results indicated that the mean scores on various biochemical parameters differ significantly between cases and controls.

Pearson correlations were computed to see the association between different biochemical parameters for case and control groups. Significance of the correlations was indicated with (\*) for  $p < 0.05$  and (\*\*) for  $p < 0.01$ .

The statistical significance was set at minimum 5 percent ( $p < 0.05$ ). Results were represented in the form of tables and bar diagrams.

**Table 1: mean and sd values of parameters in three groups**

parameters	FBS		Microalbumin		Sr.creatinine	
	mean	Sd	Mean	s.d.	mean	s.d
<b>Microalbuminurics</b>	145.4	40.24	49.02	23.46	1.206	0.2834
<b>Normoalbuminurics</b>	134.1	43.14	12.49	4.468	1.061	0.2320
<b>Controls</b>	88.90	10.53	9.130	3.724	0.9445	0.1402

Table 1 showing mean  $\pm$  sd of FBS ,serum creatinine,urine micro albumin levels in controls and NIDDM patients with micro albuminurics and normo albuminurics.All parameters found to be increased in NIDDM patients.

**Table 2: Comparison of p values between three groups**

Parameter	Controls vs normoalbuminurics	Controls vs microalbuminurics	Normoalbuminurics vs microalbuminurics
<b>FBS</b>	< 0.0001	< 0.0001	0.3013
<b>Serum creatinine</b>	0.2917	0.0759	0.0572
<b>Micro albumin</b>	0.062	< 0.0001	< 0.0001

Table 2 shows urine microalbumin levels showed significant difference between controls and microalbuminurics ( $p < 0.0001$ ) and in between normoalbuminurics and microalbuminurics( $p < 0.0001$ ) whereas not significant between controls and normoalbuminurics.

Fasting glucose levels showed no significant difference in their levels when compared between normoalbuminurics (134.1 ±43.14) and microalbuminurics (145.4 ± 40.24) and p value (0.301) also not significant.

**Table 3:Pearsons Correlation between different parameters in control group**

parameter	FBS	MICROALBUMIN	SR.CREATININE
FBS		0.148	0.142
		0.428	0.446
MICROALBUMIN	0.148		0.092
	0.428		0.624
SR.CREATININE	0.142	0.092	
	0.446	0.624	

**Table 4 : Pearsons correlation between different parameters in normoalbuminurics group**

parameter	FBS	MICROALBUMIN	SR.CREATININE
FBS		0.395	0.192
		0.031	0.311
MICROALBUMIN	0.395		0.283
	0.031		0.130
SR.CREATININE	0.192	0.283	
	0.311	0.130	

In table 4 there is significant positive correlation between microalbumin excretion and and FBS in normoalbuminurics(p 0.006 and r 0.492).

**Table 5 .Pearsons Correlation between different parameters in microalbuminurics group**

parameter	FBS	MICROALBUMIN	SR.CREATININE
FBS		0.032	0.111
		0.866	0.559
MICROALBUMIN	0.032		0.176
	0.866		0.353
SR.CREATININE	0.111	0.176	
	0.559	0.353	

In table 5 shows there is significant positive correlation microalbumin excretion ( $p < 0.0001$  and  $r = 0.71$ ); serum creatinine levels ( $p = 0.05$  and  $r = 0.36$ ).

**Discussion:**

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both.<sup>(1)</sup>

Type2 Diabetes mellitus is the predominant form of diabetes worldwide, accounting for 90% of cases globally.<sup>(2)</sup> All forms of diabetes, both inherited and acquired, are characterised by hyperglycemia, a relative or absolute lack of insulin, and development of diabetic specific microvascular pathology in retina, renal glomerulus, and peripheral nerves.<sup>(2)</sup>

Diabetic nephropathy is the most common cause of end stage renal disease requiring dialysis. The incidence of diabetic nephropathy has increased substantially in this country over the past few years. Advanced diabetic nephropathy is also the leading cause of glomerulosclerosis and end stage renal disease worldwide. Between 20% and 40% of patients with diabetes ultimately develop nephropathy.

Diabetic nephropathy has several distinct phases of development and multiple mechanisms contribute to the development of the disease and its outcomes. The natural history of nephropathy in T2DM has more similarities than differences from that seen in T1DM. Approximately 7% of patients with T2DM already have microalbuminuria at the time of diagnosis. This may be partly related to the fact that most of these patients have had untreated diabetes for 10 years (on average) before diagnosis. Within 5 years after diagnosis of T2DM, up to 18% of patients have microalbuminuria, especially those with poor metabolic control and high blood pressure levels.<sup>(4)</sup> Functional changes in the nephron occur at the level of glomerulus, including glomerular hyperfiltration and hyperperfusion, before the onset of any measurable clinical changes. The next stage is silent in which there is no overt evidence of any form of renal dysfunction. Patients usually have normal GFR with no evidence of albuminuria. However this phase is associated with significant structural changes, including basement membrane thickening and mesangial expansion.<sup>(4)</sup>

The third phase is known as microalbuminuria or stage of incipient nephropathy. In this stage urinary albumin excretion rate has increased into the microalbuminuric range of 20-200 µg/min or 30-300mg/24 hours. Persistent microalbuminuria, if left untreated, it is incumbent on clinicians to perform serial measurements and to repeat the measurement if there is an isolated elevation in urinary albumin excretion.<sup>(4)</sup> The next stage is the macroalbuminuria phase or overt nephropathy. This stage represents the phase that has been

previously described as diabetic nephropathy and is highly predictive of subsequent renal failure if left untreated. It is characterised by a urinary albumin excretion rate greater than 300 mg/24 hrs.<sup>(4)</sup>The final stage is uremic phase, which can occur in up to 40% of T1DM subjects requires the institution of renal replacement therapy.<sup>(4)</sup> After development of nephropathy decline in the rate of kidney function is influenced by additional factors, including blood pressure and glycemic control. Incipient diabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors.<sup>(10)</sup> Olmesartan increases the time to the onset of microalbuminuria in patients with microalbuminuria in patients with type 2 diabetes, even when blood pressure control is excellent according to current recommendations.<sup>(71)</sup> In the present study we took type 2 diabetes mellitus patients one group of urinary microalbumin positive 30 cases, urinary microalbumin negative 30 cases on urine dipstick method who attended Osmania General Hospital and the study was compared with 30 healthy controls. In both cases and controls FBS, serum creatinine, urinary microalbumin levels were measured.

#### **Blood glucose:**

Diabetes mellitus is a group of metabolic disorder of carbohydrate metabolism in which glucose is underutilized producing hyperglycemia. The diagnosis of DM solely depends on the demonstration of hyperglycemia. The criteria for the diagnosis of diabetes mellitus is when the fasting blood glucose level is  $\geq 126$ mg/dl or 2hr postload plasma glucose concentration of  $\geq 200$ mg/dl during the oral glucose tolerance test or the classic symptoms of diabetes mellitus with casual plasma glucose concentration  $\geq 200$ mg/dl.

Hyperglycemia is a causative factor in the pathogenesis of diabetic nephropathy. Glucose reacts non enzymatically with primary amines of proteins forming glycated compounds. Hyperglycemia exerts toxic effects and results in kidney damage by directly altering intracellular signaling pathways and via many biochemical pathways.

In the study the mean FBS values were in controls ( $88.90 \pm 10.53$ ), in cases with normoalbuminurics ( $134.1 \pm 43.14$ ) and microalbuminurics ( $145.4 \pm 40.24$ ) which are statistically highly significant ( $P < 0.001$ ). FBS values were higher than the cutoff value of 110mg/dl in cases which correlated well with the clinical diagnosis. The potential biochemical pathways leading to diabetic nephropathy are polyol pathway, nonenzymatic glycation, glucose auto-oxidation and de novo synthesis of diglycerol leading to protein kinase C and phospholipase A2 activation. Hyperglycemia and insulin resistance could also promote inflammation, and may be a factor linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress.

#### **Serum creatinine:**

Serum creatinine is the most important indicator of renal function. Creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). The measurement of GFR is clinically important as it is a measure of renal function.

In the present study the mean serum creatinine values were  $0.944 \pm 0.140$  in controls, in normoalbuminurics is  $1.061 \pm 0.232$  and in microalbuminurics is  $1.206 \pm 0.283$  which are showing the progressive increase but are statistically not significant, indicating that gradual progression of kidney damage with increased albumin excretion.

In the study of Krishnamurthy et al showing that serum creatinine increased, in microalbuminurics and normoalbuminurics but significant increase only in microalbuminuric patients when compared with normoalbuminurics and controls, but not in between normoalbuminurics and controls suggesting its importance only after the onset of nephropathy.

In the study of Ashok kumar et al showing serum creatinine levels were found to be increased in type 2 DM without any complications and type 2 DM with nephropathy when compared to controls.

In the study of Shahid et al showing that serum creatinine levels were found to be increased in type 2 DM without any complications and with nephropathy and showing p value showing significant only with diabetic nephropathy patients.

In the study of Divija showing that serum creatinine levels were increased and statistically significant in diabetic nephropathy patients when compared with healthy individuals.

In another study, Shahid SM and Mahaboob T, showed significant increase in serum creatinine levels in diabetic nephropathy patients as compared to diabetes without nephropathy and controls.

Our study is in accordance with several studies, which have shown increase in serum creatinine levels in type 2 DM with normoalbuminurics and microalbuminuric type 2 diabetic patients compared to healthy controls.

#### **Urine microalbumin:**

Microalbuminuria is an important risk factor for cardiovascular disease and progressive renal impairment. Microalbuminuria arises from the increased passage of albumin through the glomerular filtration barrier which results from ultrastructural changes rather than alterations in glomerular pressure or filtration rate alone.<sup>(88)</sup> Microalbuminuria predicts the development of overt diabetic nephropathy in type 1 and type 2 DM but the relationship is less clear in type 2 because of heterogeneity and presence of other risk factor for microalbuminuria in these elderly patients. Glomerular structural changes typical of diabetic nephropathy are established by the time microalbuminuria becomes apparent.

In the study of Prajna K. et al showed that the urine microalbumin levels were found to be increased, in type 2 diabetics without any complications and type 2 diabetics with nephropathy when compared to controls, which was statistically significant. The increase in urine albumin in the diabetics can be interpreted as an early sign of nephropathic changes in those individuals. Increase in urine albumin seen with diabetic nephropathy can be attributed to degradation of the glomerular basement membranes and hypertension, both characteristic of diabetic nephropathy. The presence of microalbuminuria is a marker of endothelial dysfunction, and indicates the rate of progression of increased risk of generalized atherosclerosis and increased mortality from cardiovascular disease.

With the increase in protein excretion there is a tendency of GFR to fall to lower level but not below the normal range during the 2<sup>nd</sup> and 3<sup>rd</sup> stages of diabetic nephropathy. With the onset of persistent proteinuria, GFR progressively falls and culminates in the end stage renal disease (ESRD) in months to a year if left untreated. Raised albumin excretion is also associated with HbA1c in patients with incipient nephropathy. The rate of progression of nephropathy is correlated with metabolic control.

For a predictor of diabetic nephropathy to be optimally useful, it should identify individuals at an increased risk of the development of serious diabetic renal disease early enough in the natural history of the disorder that the evolution of the process can be influenced by interventional strategies. Microalbuminuria in terms of predictive power is still the strongest broadly available marker or predictor of diabetic nephropathy. In diabetic pregnancy, an increase of microalbuminuria predicts complications.

In the present study the mean values of urinary microalbumin were  $9.13 \pm 3.72$  in controls, in patients of diabetes with normoalbuminurics  $12.49 \pm 4.46$  and in patients of diabetes with microalbuminurics  $49.02 \pm 23.46$  in cases which is statistically highly significant ( $P < 0.001$ ). Urinary microalbumin values were higher than the cut off value of 20mg/l in cases with microalbuminurics which is statistically correlated well.

In a study done by Melidonis A, Tournis S, it was shown that urinary albumin levels were higher in type 2 diabetic patients with signs of nephropathy compared to those without signs of nephropathy and control group.

Chen JW, Gall MA in their study, demonstrated increase in urinary albumin levels in NIDDM patients with microalbuminuria and diabetic nephropathy patients compared to controls. But the increase was far more significant in diabetic nephropathy patients compared to diabetic with microalbuminuria.



In the study of shivananda nayak, Heidi Duncan, sunita increased urinary albumin excretion levels diabetes without nephropathy and diabetes with nephropathy when compared with healthy control group.

Our study is in accordance with Krishnamurthy U, Halyal SS, Jayaprakash murthy, who demonstrated increase in urinary microalbumin levels in NIDDM patients with microalbuminuric when compared to normoalbuminuric and healthy controls and found significant positive correlation between microalbumin excretion and sialic acid.

#### **Summary and conclusion:**

Type 2 Diabetes mellitus is the predominant form of diabetes worldwide, and the most common endocrine disorder characterized by metabolic abnormalities and long term complications such as retinopathy, nephropathy and neuropathy. Diabetic nephropathy remains a major cause of morbidity and mortality for the persons either T1DM, or T2DM. Diabetic nephropathy occurs in about 25-30% of diabetic patients.

As the progression of diabetic nephropathy is slow, it is possible to be detected at an early stage. Microalbuminuria is an early indicator of diabetic nephropathy. 'Prevention is better than cure' holds good for diabetic nephropathy as the best way of treatment for this disease is to control the risk factors such as increase in blood glucose and blood pressure level. Incipient diabetic nephropathy has been proved to be reversible with tight glycemic control and ACE inhibitors. The pathophysiology of the disease has to be well known, in order to prevent a disease. Various hypotheses have been proposed till date on how diabetic nephropathy progresses in human subjects. These include the involvement of renin-angiotensin system, advanced glycation end product formation (AGE), endothelial dysfunction and oxidative stress.

The present study was undertaken to study the levels of microalbuminuria to assess whether there is a relationship between these parameters with FBS and serum creatinine in diabetic patients towards the development of diabetic nephropathy, before landing into the stage of macroalbuminuria and end stage renal disease. 30 clinically diagnosed cases of diabetes with microalbuminuria and 30 clinically diagnosed cases of diabetes with normoalbuminuria who attended outpatient department at Osmania general hospital were taken for case study. 30 age and sex matched healthy persons were taken as controls.

A statistically significant difference was observed in values of FBS, serum creatinine, and urinary microalbumin levels in cases with microalbuminuria when compared to cases with normoalbuminuria and controls. In our study positive correlation was observed in urinary microalbumin in cases. It was also observed that creatinine and urine albumin excretion for the development of micro and macrovascular complications. It was also observed that urine microalbumin excretion was positively associated with glycemic status and serum creatinine.

It is concluded that increase in circulating serum sialic acid is an early manifestation of diabetic renal disease (microvascular complications) and hence estimation of both microalbuminuria and serum sialic acid levels in NIDDM is helpful in assessing the progress of disease and identifying the risk category for complications, such as diabetic nephropathy which are main causes for mortality and morbidity among diabetes mellitus patients.

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