Original Research Article

A study on microalbuminuria as a nephropathic marker in type 2 diabetes mellitus and its correlation with the glycated hemoglobin

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Abstract

Introduction: Diabetic nephropathy is a leading cause of morbidity and premature mortality in diabetic subjects. Glycemic control is closely associated with renal involvement in diabetes and microalbuminuria can be used to detect the early stage also known as incipient nephropathy.

Objectives:

1. To estimate the microalbuminuria and HbA1c in patients with type 2 diabetesmellitus and in normal healthy controls.
2. To correlate HbA1c and microalbuminuria with duration of type 2 diabetesmellitus.
3. To study the correlation between HbA1c and microalbuminuria level in patients with Type 2 diabetes mellitus and normal healthy controls.

Materials and methods: It is cross sectional observational study, 50 patients with type 2 Diabetes Mellitus and 50, age and sex matched normal healthy controls were recruited. 5ml of fasting venous blood was collected and used for the analysis of plasma fasting and post prandial glucose and HbA1c was assayed in EDTA blood, serum was used to assay urea, creatinine, sodium, potassium, and early morning mid stream urine sample for estimation of the microalbuminuria. Data was statistically analyzed. **Results:** In the present study there was significant association (P<0.001) and positive correlation between microalbuminuria and HbA1c in cases as compared to controls. The occurance of microalbuminuria showed a direct relationship with increase duration of diabetes since diagnosis. We also found positive correlation between microalbuminuria with duration of diabetes. **Conclusion:** Current study shows elevated HbA1c and microalbuminuria with duration of diabetes. The risk of microalbuminuria increases with poor glycemic control are strongly associated with the presence of nephropathy, a microvascular complication of diabetes. Thus HbA1c and microalbuminuria may be considered as risk marker in diabetic nephropathy. Hence regular screening for microalbuminuria and HbA1c can help inclinical management for prevent of complication.

Keywords: Type 2 diabetes mellitus , HbA1c, Microalbuminuria, incipient diabeticnephropathy.

Introduction

Diabetes mellitus is one of the most challenging health problem in 21st century. Type 2 diabetes consists of 85-95% of all diabetes mellitus cases in developed countries. It is estimated approximately 285 million people worldwide in age group 20-79 years, have diabetes mellitus in 2010. If preventive measures not taken ,this number expected to increase to 438 million by 2030[1].

Type 2 diabetes mellitus is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which there is defect in insulin secretion, insulin action or most commonly both, resulting in hyperglycemia[2].

The national diabetes data group and world health organization have issued diagnostic criteria for diabetes mellitus, Symptoms of diabetes plus random blood glucose concentration ≥ 11.1mmol/L (200 mg/dl) or Fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dl) or Two hour post prandial glucose ≥ 11.1 mmol/L (200 mg/dl) .Patient with Type 2 diabetes mellitus often have long asymptomatic period of hyperglycemia and many have complications at the time of diagnosis.

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Diabetes mellitus is the leading cause of end- stage renal disease, non-traumatic lower extremity amputations and adult blindness[3].

Poor glycemic control has been identified as one of the risk factors of microalbuminuria which hastens the progress of renal disease[4].

Diabetic nephropathy is the leading cause of end stage renal disease worldwide. Nearly 30% of chronic renal failures in India are diabetic nephropathy[5].

Diabetic nephropathy is a common consequence of diabetes mellitus. Its pathogenesis appears to involve complex interactions between genetic andenvironmental factors[6].

The presence of microalbuminuria precedes the development of overt diabetic nephropathy by 10 to 15 years. It is at this stage that one can hope to reverse diabetic renal disease or prevent its progression by early therapeutic interventions.

The present study was designed to study the association of microalbuminuria with HbA1c and duration of disease in patients with type 2 Diabetes mellitus.

Objectives

1. To estimate the microalbuminuria and HbA1c in patients with type 2 DiabetesMellitus and in normal healthy controls.
2. To correlate HbA1c and microalbuminuria with duration of type 2 diabetesmellitus.
3. To study the correlation between HbA1c and microalbuminuria level in patients with Type 2 Diabetes Mellitus and normal healthy controls

Materials and methods

Source of data

The study was conducted at Maheswara Medical College. We collected data from 50 diagnosed cases of type 2 diabetes mellitus and 50 age and sex matched healthy individuals consenting outpatient Department of Medicine. The cases were selected on the basis of simple random sampling method. The study protocol was approved by the institutional ethical committee and informed consent was obtained from the subjects under study. The study was done from December 2020 to June 2021.

Study design

Observational cross-sectional study

Study subjects

Inclusion criteria: Following people were included in the study. Cases:

1. Patient with age group of 30 -80 years.
2. Diagnosed cases with fasting blood sugar > 126 mg/dl(7.0 mmol/l). Fasting is defined as no caloric intake for atleast 8 hours.
3. Patient with 2 hours post prandial glucose > 200 mg/dl (11.1 mmol/l).

Controls

Age and sex matched healthy individuals are taken as controls.

Exclusion criteria

Patients with congestive cardiac failure, urinary tract infections, nephritic syndrome, chronic glomerulonephritis, ketoacidosis, pregnancy, alcoholics

Sample collection

1. After due consent, under aseptic precautions 4 ml of fasting venous blood samples will be taken for the estimation of,
   - Glycated hemoglobin.
   - Fasting blood sugar
   - Serum creatinine
   - Blood urea
   - Serum sodium, potassium
2. 1 ml of venous blood in fluoride bulb taken after 2 hours of meals for theestimation of post prandial blood sugar.
3. Urine microalbuminuria: early morning mid-stream urine sample(10 ml) in a sterile container without preservative for the estimation ofmicroalbuminuria.

Method of analysis

Estimation of blood glucose[7]

Blood glucose was estimated by GOD/PAP method using RANDOX KIT-GL3815 in the Randox Daytona auto analyze

Estimation of glycated hemoglobin (HbA1c)[8]

HbA1c was estimated by Latex agglutination inhibition method using RANDOX KIT – HA 3830 in the Randox Daytona auto analyser.

Estimation of blood urea[9]

Blood urea was estimated by Enzymatic Kinetic method using RANDOX KIT-UR 3825 in the Randox Daytona auto analyzer.

Estimation of serum creatinine[10]

Serum creatinine was estimated by Jaffe´s method using RANDOX KIT –CR3814 in the Randox Daytona autoanalyzer.

Electrolytes (sodium, potassium,)[11,12]

An ion selective electrode makes use of unique properties of certain membrane materials to develop an electric potential (electromotive force) for the measurement of ions in the solution.

Estimation of microalbumin in urine[13]

Microalbumin in urine was estimated by immunoturbidimetric method usingRANDOX KIT – MA 2426 in Randox autoanalyzer.

Statistical methods

Chi-square and Fisher Exact test have been used to find the significance of proportion of incidence of microalbuminuria with study parameters namely Age, Duration of DM, GHB %. The Odds ratio has been used to find the strength of relationship between the incidence of microalbuminuria and other study parameters. Student t test has been used to find the significance of mean levels of lab parameters between the presence and absence of microalbuminuria

Statistical software

The Statistical software SPSS 11.0 and Systat 8.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Results

Table 1: Age distribution of study group

Age in years	Cases		Controls	
	No.	%	No.	%
30-40	7	14	9	18
41-50	12	24	10	20
51-60	15	30	15	30
61-70	10	20	10	20
>70	6	12	6	12
Total	50	100.0	50	100.0
Mean age ±SD	54.9±11.19		54.2±10.78	
P value	P=0.375			

Table 1 shows the distribution of subjects in different agegroups with the mean age of 54.2±10.78 in controls and 54.9±11.19 in cases. The cases and controls were age matched (p=0.375).

Table 2 : Gender distribution of study group

Gender	Cases		Controls	
	No	%	No	%
Male	28	56	28	56
Female	22	44.0	22	44.0
Total	50	100.0	50	100.0
P value	P= 0.50			

The following table 2 shows the gender distribution pattern of diabetes mellitus cases and controls under study. The study included with 56% males and 44% females in both cases and controls. The sample are gender matched with P= 0.50.

Table 3 : Frequency and percentage distribution of HbA1c at different levels in two groups

Study variables	Cases (n=50)	Controls (n=50)	P value
HbA1c (%)			
>6.5 %	40 (80%)	6 (12%)	<0.001**
<6.5 %	10 (20%)	44 (88%)	

As represented in table 3 shows 12% of controls and 80% of cases of diabetes mellitus showed HbA1c value >6.5% whereas 88% of controls and 20% of cases of diabetes mellitus showed values <6.5% . From this it can be inferred that there is a statistically significant increase in the percentage distribution of HbA1c value in cases as compared to controls (p<0.001).

Table 4: Frequency and distribution of Microalbuminuria at different levels in two groups

Study variable	Cases (n=50)	Controls (n=50)	P value
Microalbuminuria			
<20 mg/l	31 (62%)	50 (100%)	<0.001**
>20 mg/l	19 (38%)	0	

As represented in table 4 , 62 % of controls and 100 % of cases of diabetes mellitus showed Microalbuminuria values <20 mg/l whereas 38 % of cases of diabetes mellitus showed values >20 mg/l and no controls showed values >20 mg/l . From this it can be inferred that there is a statistically significant increase in the percentage distribution of Microalbuminuria value in cases as compared to controls (p<0.001).

Table 5 : Comparison of study variables in two groups

Study variables	Cases(n=50)	Controls(n=50)	P value
FBS (mg/dl)	186.4±29.24	83.24±13.88	<0.001**
PPBS (mg/dl)	260.72±34.62	122.26±8.43	<0.001**
HbA1c%	7.512±1.198	5.132±1.11	<0.001**
Blood urea (mg/dl)	29.56±6.63	13.28±2.61	<0.001**
Serum creatinine (mg/dl)	1.028±0.184	0.86±0.307	<0.001**
Serum sodium (mEq/L)	128.16±4.44	138.34±2.97	<0.001**
Serum potassium (mEq/L)	2.744±0.37	3.836±3.308	<0.001**
Microalbuminuria (mg/l)	44.08±62.33	10.58±4.75	<0.001**

The above table representing the mean values of FBS was 83.24±13.88 in controls and 186.4±29.24 in diabetes mellitus cases. From this it concludes that there is a statistically significant increase in mean value of FBS in cases compared to controls (p<0.001). The mean value of PPBS was 122.26±8.43 in controls and 260.72±34.62 in cases. This also concludes that there is a statistically significant increase in mean values of PPBS in cases as compared to controls (p<0.001). The mean values of HbA1c% was 5.132±1.11 in controls and 7.512±1.198 in cases. This also concludes that there is a statistically significant increase in mean value of HbA1c in cases as compared to controls (p<0.001). The mean values of Blood urea was 13.28±2.61 in controls and 29.56±6.63 in cases. This also concludes that there is a statistically significant increase in mean values of blood urea in cases as compared to controls (p<0.001.) The mean value of serum creatinine was 0.86±0.307 in controls and 1.028±0.184 in cases. This also concludes that there is a statistically significant increase in mean value of serum creatinine in cases as compared to controls (p<0.001). The mean value of serum sodium was 138.34±2.97 in controls and 128.1±4.44 in cases. This concludes that there is a statistically significant decrease in mean values of serum sodium in cases as compared to controls (p<0.001). The mean values of serum potassium was 3.836±0.308 in controls and 2.744±0.37 in cases. This concludes that there is a statistically significant decrease in mean value of serum potassium in cases as compared to controls (p<0.001). The mean value of Microalbuminuria was 10.58±4.75 in controls and 44.08±62.33 in cases. This concludes that there is a statistically significant increase in mean values of Microalbuminuria in cases as compared to controls (p<0.001).

Table 6: Duration of diabetes mellitus since diagnosis

Duration of DM(in years)	Male (n=28)		Female (n=22)		Total (n=50)
	No	%	No	%	No
≤5.0	17	60.71	12	54.54	29
5.1-10.0	5	17.86	5	22.72	10
10.1-15.0	3	10.71	4	18.18	7
>15.0	3	10.71	1	4.54	4

A total of fifty patients diagnosed with diabetes and their duration of diabetes is taken since diagnosis. Among 50 diabetic patients, twenty eight were male and twenty two female patients. Twenty nine patients had less than five years of duration since diagnosis .Ten patients had duration of diabetes between five years and ten years since diagnosis among which five were male and five were female patients. Seven patients had duration of diabetes between ten years and fifteen years since diagnosis among which three were male and four were female patients. Four patients had duration of diabetes >15 years since diagnosis among which three were male and one were female patients. The mean duration of diabetes since diagnosis was 6.32±4.75 years among the male patients and 6.41±4.51 years among the female patients. The mean duration of DM between male and female patients not showed any statistical significance with p=0.947.

Discussion

Type 2 diabetes mellitus is being increasingly recognized as a

disease, which is characterized by dysfunction of the endothelium. Endothelial dysfunction occurs in a generalized and widespread manner in diabetic subjects. The severity of the dysfunction is directly proportional to the age of the patient and duration of the diabetes. The clinical markers of the generalized endothelial dysfunction becomes manifest in several forms. Microalbuminuria marks the onset of endothelial dysfunction related to the kidney.

In our study 50 cases of diabetes mellitus and 50 healthy controls. In both cases and controls FBS, PPBS, HbA1c, blood urea, serum creatinine, serum sodium, serum potassium, urinary microalbumin levels were measured.

Blood glucose

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized producing hyperglycemia. The diagnosis of DM solely depends on the demonstration of hyperglycemia. In our study the mean FBS values were 83.24±13.88 mg/dl and 186.4±29.24mg/dl in controls andcases which is statistically highly significant (p<0.001).

Our study also correlated FBS value in a diabetic patients with or without microalbuminuria and the study concluded that mean FBS value in a patients without microalbuminuria is 121.19 ± 78.29 and in a patients with microalbuminuria is 198±29.30 which is statistically significant (p<0.001).

Similarly the mean PPBS values were 122.26±8.43 mg/dl and 260.72±34.62 mg/dl in controls and cases which is statistically highly significant (p<0.001).

Our study also correlated PPBS value in a diabetic patients with or without microalbuminuria and the study concluded that mean PPBS value in a patients without microalbuminuria is 251.87 ± 28.98 and in a patients with microalbuminuria is 275.15 ± 38.83 which is statistically significant (p=0.014).

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 intercellular signaling pathways and via many biochemical pathways[14].

Blood urea

In our study the mean blood urea values were 13.28±2.61mg/dl in controls and 29.56±6.63mg/dl in cases which is statistically highly significant (p<0.001).

Our study also correlated blood urea value in a diabetic patients with or without microalbuminuria and the study concluded that mean blood urea value in a patients without microalbuminuria is 19.64±6.64 and in a patients with microalbuminuria is 29.42±6.8 but the value is not statistically significant (p=0.45).

Shahid SM and Shaik R, in their study showed a significant increase in blood urea levels in incipient diabetic nephropathy patients when compared to controls and the study also demonstrated direct proportional linearity between blood urea, serumcreatinine and microalbuminuria[15].

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 refelects the glomerular filtration rate (GFR). The measurement of GFR is clinically important as it is a measure of renal function.

In our study the mean serum creatinine values were 0.86±0.307mg/dl in controls and 1.028±0.184 mg/dl in cases which is statistically significant (p<o.oo1).

Our study also correlated blood urea value in a diabetic patients with or without microalbuminuria and the study concluded that mean blood urea value in a patients without microalbuminuria is 1.01±0.19 and in a patients with microalbuminuria is 1.05±0.17 which is statistically significant (p=0.022).

Shehnaz AS et al.(2009) showed statistically significant increase in serum creatinine in diabetes mellitus when compared to controls[16].

Glycated hemoglobin

Glycated hemoglobin is effective in monitoring long term glucose control in patients with diabetes mellitus. The complication of diabetes depends not only by the duration of diabetes mellitus but also by the mean average level of chronic glycemia as measured by glycated hemoglobin level[17].

In our study the mean HbA1c values were 5.132±1.11% in controls and 7.512±1.19% in cases which is statistically highly significant (p<0.001). In cases HbA1c values >6.5% were higher which correlated well with the clinical diagnosis.

In our study among the cases only 5 out of 21 patients who had a normal HbA1c (< 7.0%) manifested microalbuminuria, whereas with HbA1c values more than 7, 14 out of 29 (nearly 50%) had microalbuminuria. It is seen from the above result that even small increments of HbA1c more than 7.0% result in almost doubling of the incidence of microalbuminuria.

It is also interesting to note that when HbA1c rises above 7.0%, 10 out of 14 patients tended to have more than 50mg/l and 4 out of 14 had microalbuminuria touching 300mg/l.

Shivananda nayak B and Geetha Bhaktha, also showed increased HbA1c levels in diabetic nephropathy patients and diabetic patients without any complications compared to healthy controls[18].

Seum electrolytes

In our study the mean serum sodium values were 138.34±2.97mEq/L in controls and 128.16±4.44mEq/L in cases which is statistically significant (p<o.oo1). The mean serum potassium values were 3.836±0.308mEq/L in controls and 2.744±0.37mEq/L in cases which is statistically significant (p<o.oo1).

Rao GM. In their study showed statistically significant difference in the mean serum sodium and potassium in diabetes mellitus when compared to controls[19]

Microalbuminuria

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 heterogenesity 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[115].

In the present study, statistically highly significant positive correlation was found between the microalbuminuria and the FBS (r=0.356), PPBS (r=0.487) which was consistent with the findings reported in Varghese et al[21].However, Huraib et a1[22]. reported no statistically significant correlation between the microalbuminuria and FBS.

In the present study, statistically highly significant positive correlation was found between microalbuminuria and HbA1c (r=0.574) which was similar to findings reported by Varghese et al[21], Shehnaz AS et al[16] and the study conducted in Yazd. However, Huraib et al[22] and Afkhami AM et al[23] reported there was no statistically significant correlation between the microalbuminuria and HbA1c in diabetes mellituspatients.

In the present study, statistically highly significant positive correlation was found between the microalbuminuria and serum creatinine (r=0.917). Which was similar to the finding reported by Shehnaz AS et al[24].

In the present study, statistically significant negative correlation was found between microalbuminuria with serum sodium(r= -0.732) and serum potassium (r= -0.808) which was similar to the findings reported by Rao GM[19]

Conclusion

The present study concluded that estimating glycosylated haemoglobin as an indicator of glycemic control and microalbuminuria in random urine sample for renal involvement in

diabetic subjects provide a convienient method for early diagnosis and intervention. Thus the study suggests microalbuminuria as a nephropathic marker in type 2 diabetes mellitus. The possibility, delay or reverse the progression of diabetic nephropathy can be achieved only by perfect long term metabolic control.

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