Original Research Article

Study of microalbuminuria as a nephropathic marker in type 2 diabetes mellitus and its correlation with the glycated hemoglobin

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ABSTRACT

Diabetic nephropathy is the main cause of morbidity and mortality in patients with diabetes mellitus. The results of many clinical studies shows variation in the correlation of HbA1c and microalbuminuria in type 2 diabetes mellitus. In the present study we tried to correlate the HbA1c levels and microalbuminuria with respect to duration in type 2 diabetes mellitus cases and also studied microalbuminuria as a marker of nephropathy in type 2 diabetes mellitus.

Materials and Methods: The study was conducted in the Department of Biochemistry at Raichur Institute of Medical Sciences. 100 subjects were recruited based on the inclusion and exclusion criteria, 50 were healthy controls and 50 were type 2 DM patients. FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sodium, serum potassium and urinary micro albumin were analyzed. Statistical analysis was done by using student ‘t’ test and Chi square test.

Results: A statistically significant difference was observed in values of FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sodium, serum potassium and urinary microalbumin levels in cases compared to controls. In our study the mean HbA1c values were 5.13±1.11% in controls and 7.51±1.19% in cases & mean HbA1c value in patients without microalbuminuria is 7.13±0.84 and in patients with microalbuminuria is 8.12±1.44 which is statistically significant (p=0.005).

Conclusion: The present study concluded that estimating glycosylated hemoglobin as an indicator of glycemic control and microalbuminuria in random urine sample for renal involvement in diabetic subjects provide a convenient 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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1. Introduction

Diabetic nephropathy is the main cause of morbidity and mortality in patients with diabetes mellitus (DM). Diabetic nephropathy is the leading cause of end stage renal disease worldwide. Nearly 30% of chronic renal failures in India are due to diabetic nephropathy. This complication is first manifested as an increase in urinary albumin excretion (microalbuminuria), which progresses to overt albuminuria and then to renal failure.¹ Improved glycemic control seems to delay or prevent the onset of microalbuminuria, but the development of cost-effective preventive strategies requires knowledge of the increase in the risk of nephropathy associated with each increase in the degree of hyperglycemia. Poor glycemic control has been identified as one of the risk factors of microalbuminuria, which hastens the progress of renal disease.²,³ Diabetic nephropathy is a common consequence of diabetes mellitus. Its pathogenesis appears to involve complex interactions between genetic and environmental factors.⁴,⁵ Thus study was designed to evaluate Microalbumin and HbA1C in patients with Type 2 DM and find its association with various other parameters.
2. Materials and Methods

This is a cross-sectional cases-control study, conducted at Department of Biochemistry in association with Department of General Medicine, Raichur Institute of Medical Sciences Teaching Hospital, Raichur. In this 100 subjects were included. Among them 50 were clinically diagnosed type 2 diabetes mellitus subjects as cases and 50 healthy age and sex matched subjects were taken as controls. Age of the subjects ranges from 30-80 years. The cases were selected on the basis of simple random sampling method. Patients with congestive cardiac failure, urinary tract infections, nephritic syndrome, chronic glomerulonephritis, ketoacidosis, pregnancy, alcoholics were excluded from the study. 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 2012 to November 2013. Under aseptic precautions 4 ml of fasting venous blood samples was taken from the study subjects, allowed to stand for 30 minutes and centrifuged for 10 minutes. The serum sample was used for the estimation of FBS, PPBS (GOD/PAP method), Creatinine (Jaffe’s method), Urea (Enzymatic Kinetic method), Sodium and Potassium (ISE method), whole blood sample was used for the estimation of HbA1c (Latex agglutination inhibition method). Early morning mid-stream urine sample (10 ml) in a sterile container without preservative was used for the estimation of urine microalbumin (Immunoturbidimetric method). The study parameters were estimated by using RANDOX – HA3830 autoanalyzer.

2.1. Statistical analysis
Data are expressed as Mean ± SD. Chi -square and Fisher Exact test have been used to find the significance of proportion of incidence of microalbuminuria with study parameters. 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. The Statistical software SPSS 11.0 and Systat 8.0 were used for the analysis of the data. P value <0.01 is considered statistically significant.

3. Results

In the present study, Microalbuminuria showed highly significant positive correlation with duration of diabetic mellitus, HbA1c, FBS, PPBS and serum creatinine in type 2 diabetes mellitus patients. It also revealed that Microalbuminuria showed no correlation with blood urea in cases. On the contrary, Microalbuminuria showed negative correlation with serum sodium and serum potassium levels. As illustrated in tables.

4. 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.6,7 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 get manifested in several forms. Microalbuminuria marks the onset of endothelial dysfunction related to the kidney.8–10 Since its original description by Mogensen, the estimation of microalbuminuria is made easy and practical. Microalbuminuria serves as a warning for imminent nephropathy. But its true value is that it heralds generalized endothelial dysfunction. Thus diabetic subjects with microalbuminuria not only have ongoing progressive nephropathy but are also likely to have retinopathy, neuropathy and cardiovascular problems including coronary artery disease and hypertension.11–13 An effort has been made in this study to highlight this issue. Even among randomly selected patients, an incidence of 38% for microalbuminuria is evident. Among various other studies the prevalence of microalbuminuria ranges from 25% to 35%.14–17 A slight increase in the percentage of microalbuminuria in our study can be attributed to several factors such as, large number of elderly patients, longer duration of diabetes and poor glyemic control.

It is very well recognized that microalbuminuria occurs more commonly in diabetic subjects who are more than 50 years of age. In our study microalbuminuria tended to be 2.54 times more common in the age group of above 50 years as compared to the age group of less than 50 years. There are many reasons for this phenomenon. Firstly deterioration in the β-cell function, which is the likely factor to contribute to worsening glycemic control. Poor values of HbA1c are known to be associated with increasing incidence of microalbuminuria.

Our study also correlated FBS value in diabetic patients with or without microalbuminuria and the study concluded that mean FBS value in patients without microalbuminuria is 121.19 ± 78.29 and in 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 patient with or without microalbuminuria and the study concluded that mean PPBS value in a patient without microalbuminuria is 251.87 ± 28.98 and in 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
Table 1: Comparison of Biochemical parameters between the two study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD Cases (n=50)</th>
<th>Mean±SD Controls (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dL)</td>
<td>186.4±29.24</td>
<td>83.24±13.88</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>PPBS (mg/dL)</td>
<td>260.72±34.62</td>
<td>122.26±8.43</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.512±1.198</td>
<td>5.132±1.11</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Blood Urea (mg/dL)</td>
<td>29.56±6.63</td>
<td>13.28±2.61</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>1.028±0.184</td>
<td>0.86±0.307</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Serum Sodium (mEq/L)</td>
<td>128.16±4.44</td>
<td>138.34±2.97</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Serum Potassium (mEq/L)</td>
<td>2.744±0.37</td>
<td>3.836±3.308</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Microalbuminuria (mg/L)</td>
<td>44.08±62.33</td>
<td>10.58±4.75</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

(*** highly significant)

Table 2: Frequency and percentage distribution of HbA1c and Microalbuminuria at different levels in the two study groups

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Cases (n=50)</th>
<th>Controls (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 6.5 %</td>
<td>40 (80%)</td>
<td>6 (12%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>&lt; 6.5 %</td>
<td>10 (20%)</td>
<td>44 (88%)</td>
<td></td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 mg/l</td>
<td>31 (62%)</td>
<td>50 (100%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>&gt; 20 mg/l</td>
<td>19 (38%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

(*** highly significant)

Table 3: Mean pattern of parameters and their correlation in presence and absence of microalbuminuria in cases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD Microalbuminuria</th>
<th>Significance by student t test</th>
<th>r-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dL)</td>
<td>121.19 ±78.29</td>
<td>P&lt;0.001**</td>
<td>0.17</td>
</tr>
<tr>
<td>PPBS (mg/dL)</td>
<td>251.87 ±28.98</td>
<td>P=0.014*</td>
<td>0.228</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.13 ±0.84</td>
<td>P=0.005**</td>
<td>0.225</td>
</tr>
<tr>
<td>Blood Urea (mg/dL)</td>
<td>19.64 ±6.64</td>
<td>P=0.45</td>
<td>0.11</td>
</tr>
<tr>
<td>S. Creatinine (mg/dL)</td>
<td>1.01 ±0.19</td>
<td>P=0.022*</td>
<td>0.26</td>
</tr>
<tr>
<td>S. Sodium (mEq/L)</td>
<td>130.58 ±3.25</td>
<td>P&lt;0.001**</td>
<td>0.027</td>
</tr>
<tr>
<td>S. Potassium (mEq/L)</td>
<td>2.97 ±0.21</td>
<td>P=0.078*</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 4: Pearsons correlation co-efficient (r)

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbuminuria and duration of DM</td>
<td>0.810</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and FBS</td>
<td>0.356</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and PPBS</td>
<td>0.487</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and HbA1c</td>
<td>0.574</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and blood urea</td>
<td>0.024</td>
<td>0.053</td>
</tr>
<tr>
<td>Microalbuminuria and Sr. creatinine</td>
<td>0.917</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and Sr. sodium</td>
<td>-0.732</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Microalbuminuria and Sr. potassium</td>
<td>-0.808</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

results in kidney damage by directly altering intercellular signaling pathways and via many biochemical pathways. Microalbuminuria is an earliest indicator of diabetic nephropathy. It is associated with poor glycemic control and the present study also concluding the same. Varghese et al study also showed the poor glycemic control is associated with microalbuminuria.

In our study the mean blood urea values were 13.28±2.61mg/ dl in controls and 29.56±6.63 mg/ dl in cases which is statistically highly significant (p<0.001). Our study also correlated blood urea value in diabetic patients with or without microalbuminuria and the study concluded that mean blood urea value in patients without microalbuminuria is 1 9.64±6.64 and in 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, serum creatinine and microalbuminuria.\textsuperscript{20} 

In our study the mean serum creatinine values were 0.86±0.307 mg/dL in controls and 1.028±0.184 mg/dL in cases which is statistically significant (p<0.001). Our study also correlated blood urea value in a diabetic patient with or without microalbuminuria and the study concluded that mean blood creatinine value in patients without microalbuminuria is 1.01±0.19 and in patients with microalbuminuria is 1.05±0.17 which is statistically significant (p=0.022).

Shehnaz AS et al. showed statistically significant increase in serum creatinine in diabetes mellitus when compared to controls.\textsuperscript{21} 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, serum creatinine and microalbuminuria.\textsuperscript{20}

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.\textsuperscript{22}

Formation of HbA1c is essentially irreversible and the concentration in blood depends on both the life span of the red blood cells (average 120 days) and glucose concentration. As the rate of formation of HbA1c is directly proportional to the concentration of glucose in blood, the HbA1c concentration represents the integrated values for glucose over the preceding 6-8 weeks.\textsuperscript{22} In patients with poorly controlled DM, values may extend to twice the upper limit of the reference interval or more but rarely exceed 15%. There is no specific value of HbA1c below which the risk of diabetic complications is eliminated completely. The ADA states that the goal of treatment should be to maintain HbA1c <7% ADA recommends that HbA1c should be routinely monitored at least every 6 months in both type 1 and type 2 DM.\textsuperscript{22} The mean HbA1c values were directly proportional to the risk of developing retinopathy and nephropathy.

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 50 mg/L and 4 had microalbuminuria touching 300 mg/L.

Our study correlated HbA1c value in diabetic patients with or without microalbuminuria and the study concluded that mean HbA1c value in a patients without microalbuminuria is 7.13±0.84 and in a patients with microalbuminuria is 8.12±1.44 which is statistically significant (p=0.005). Although this is a cross sectional study, these findings raise concern regarding the strong association between poor glycemic control and microalbuminuria in a rural setting.

Our study is in accordance with the study done by Naveen P et al. who showed significant correlation between microalbuminuria and HbA1c.\textsuperscript{23} Ido Gone ES et al. in their study showed the mean HbA1c was the highest in diabetic with microalbuminuria when compared with diabetic without microalbuminuria.\textsuperscript{24} 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.\textsuperscript{25}

In our study the observed reduction in serum sodium and potassium in diabetic subjects might be a result of electrolyte loss which arises due to dehydration or a result of kidney dysfunction caused by diabetes.\textsuperscript{26} As the body tries excrete out excess glucose due to hype glycemia, water is also excreted out continuously through the kidney tubules. Sodium and potassium depletion is a common feature of type 2 diabetes.\textsuperscript{27}

In our study the mean serum sodium values were 138.34±2.97 mEq/L in controls and 128.16±4.44 mEq/L in cases which is statistically significant (p<0.001). The mean serum potassium values were 3.836±0.308 mEq/L in controls and 2.744±0.37 mEq/L in cases which is statistically significant (p<0.001).

Our study also correlate d serum electrolyte level in diabetic patients with or without microalbuminuria and the study concluded that mean serum sodium level in patients without microalbuminuria is 130.58±3.25 and mean serum potassium level in patients without microalbuminuria is 2.97±0.21 and in patients with microalbuminuria is mean serum sodium level is 124±3.10 and mean serum potassium level in patients with microalbuminuria is 2.68±0.81 and which are statistically significant.

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.\textsuperscript{28}

In our study the mean values of urinary microalbumin were 10.58±4.75 mg/L in controls and 44.08±62.33 mg/L in cases which is statistically highly significant (p<0.001).
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 control group.\textsuperscript{20} The protein excretion increases in diabetic subjects compared to controls due to renal involvement caused by prolonged glycermia. This further increases with duration of diabetes.

In the present study, statistically highly significant positive correlation was found between the microalbuminuria and the duration of diabetes ($r=0.810$) that was consistent with findings of past studies. Huraib et al.\textsuperscript{30} Varghese et al.,\textsuperscript{19} Mather et al.,\textsuperscript{31} reported a significant correlation between microalbuminuria and the duration of diabetes.

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.\textsuperscript{18} However, Huraib et al.,\textsuperscript{31} 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.,\textsuperscript{18} Shehnaz AS et al.\textsuperscript{20} and the study conducted in Yazd. However, Huraib et al.\textsuperscript{20} and Afkhami AM et al.\textsuperscript{31} reported there was no statistically significant correlation between the microalbuminuria and HbA1c in diabetes mellitus patients.

In the present study, statistically highly significant positive correlation was found between the microalbuminuria and serum creatinine ($r=0.917$). In the present study, statistically negative correlation was found between microalbuminuria with serum sodium ($r=-0.732$) and serum potassium ($r=-0.808$).

5. Conclusion
The present study concluded that estimating glycosylated hemoglobin as an indicator of glycemic control and microalbuminuria in random urine sample for renal involvement in diabetic subjects provide a convenient 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.

6. Source of funding
None.

7. Conflict of interest
None.

References


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