Original Article

PREVALENCE OF MICROALBUMINURIA AS A MARKER OF INCIPIENT NEPHROPATHY IN TYPE 2 DIABETES PATIENTS AT SMIMER HOSPITAL, SURAT

Vilas U. Chavan¹, DVSS Ramavataram², Kinjal L. Miyani³, Hasit D. Lad⁴, S.D. Nilakhe⁴

ABSTRACT

Background: Diabetic nephropathy (DN) is one of the most significant long-term complications in diabetes mellitus (DM). The study was primarily aimed to screen type 2 DM patients for renal damage and to find out the prevalence of MAU as marker of nephropathy in the SMIMER hospital, Surat.

Methods: We studied 82 known cases of type 2 DM and 50 healthy subjects as control. Plasma glucose, serum lipid profile, urea, uric acid, creatinine and urine albumin and creatinine were measured in subjects. Based on urine albumin and albumin:creatinine ratio (ACR) stages of nephropathy were diagnosed. Data was expressed as mean ± SD and data was compared by using unpaired student ‘t’ tests for independent sample.

Results: We found significantly higher levels of fasting and post prandial glucose (P<0.05), urea (P<0.01), uric acid (P<0.03), urine albumin and ACR (P<0.05) in DM patients compared to controls. We observed increased level of total cholesterol (P<0.05), triacylglycerol (P<0.05) and non significant decrease in HDL level (P=0.11) in DM patients compared to controls. In DM patients the prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria were 21.95%, 62.19% and 15.85% respectively.

Conclusion: Our study concludes that MAU is a reliable marker of DN in type 2 DM patients. MAU and dyslipidemia together may be more potent risk factor for complications in type 2 DM. Therefore regular screening for MAU is recommended for all asymptomatic DM patients for reducing cardiovascular risks and slowing the progression to end-stage renal disease to reduce the socioeconomic burden of DM.

Key words: Type 2 Diabetes mellitus, Microalbuminuria, Nephropathy, Prevalence, Lipid profile.

INTRODUCTION

Diabetes mellitus (DM) is now one of the most common non-communicable diseases globally. It is epidemic in many low and middle-income countries and major cause of blindness, renal failure, amputation, coronary artery, peripheral vascular disease and stroke (1, 2). It is estimated that there are approximately 285 million people (6.4%) with diabetes worldwide in 2010 and this number is expected to increase to 438 million (7.7%) of the adult population by 2030. The largest increases will take place in the developing countries (1, 3). World Health Organisation (WHO) projects that diabetes will be the
7th leading cause of death in 2030 (2). India has highest number of people with diabetes in the world, according to the International Diabetes Federation (IDF); the disease affects more than 50 million Indians comprising 7.1 % of the nation’s adult (20-79 age groups) population and kills about 1 million Indians per year (1). The high incidence in India is attributed to increased life expectancy in India, economic growth, lifestyle changes, intake of high calorie food and combination of genetic susceptibility (4, 5).

Diabetic nephropathy (DN) is one of the most significant long-term complications in terms of morbidity and mortality for individual with diabetes and is leading cause of chronic kidney disease in the United States. Diabetes is responsible for 20-40% of all end-stage renal disease (ESRD) cases in the United States (6-8). The incidence of diabetes is increasing worldwide with subsequent increase in the incidence of DN.

Microalbuminuria (MAU) is an increased excretion of albumin in urine above physiological levels. MAU has been considered the first indication of renal injury in patients with diabetes (9). MAU is now widely recognized as a sign of abnormal vascular function and increased vascular permeability (10).

MAU is defined as a urinary albumin excretion (UAE) of 30-300 mg/day, when measured in a 24 hour urine collection. It is also defined as values between 20-200 mg/L or 30-300 mg/g, if measured with the use of the urinary albumin:creatinine ratio (ACR) in a spot or random urine samples (9,11). Level of urine albumin below these limit are considered normal, whereas any albumin excretion above this limits represents macroalbuminuria or clinical proteinuria (Table 1).

Currently, the National Kidney Foundation (NKF) recommends the use of spot urine ACR obtained under standardized conditions to detect MAU (13, 14). MAU is an independent risk factor for cardiovascular events, therefore, a strategy to detect early diabetic kidney disease by screening for albuminuria is important step in diabetic kidney disease (10). Additionally there is remarkable lack of awareness among diabetic patients regarding DN. Based on present knowledge our study was primarily aimed to screen type 2 DM patients for renal damage and to find out the prevalence of MAU as marker of nephropathy in the SMIMER hospital, Surat. Secondary objectives were to measure the lipid profile and other biochemical parameter in study subjects.

METHODS
Presence study was carried out in the department of Biochemistry at Surat Municipal Institute of Medical Education and Research (SMIMER), Surat, Gujarat, India, over a period of 6 month from January to June 2013. Study was approved by institutional ethical committee and informed consent was taken from all participants.

Subjects: Total 132 subjects of which 82 known cases of type 2 DM attending the Medicine department were taken for this study and 50 age and gender matched healthy subjects were taken as control. Control group comprised of relatives of patients and from general population. Patients were on oral hypoglycemic agents and attending for follow up treatment in diabetes. Selection of patients was random and no specific sampling criteria were applied. Patients having past history of hospitalisation for kidney diseases, other metabolic, cardiovascular diseases, tuberculosis, and thyroid disorders were excluded from study.

Laboratory analysis: Blood sample was collected after overnight fasting and spot urine sample was collected in morning (between 8.00 am – 9.00 am). Laboratory analysis was done in the Clinical Biochemistry laboratory on the same day by using commercially available kits on fully automated clinical chemistry analyzer Erba-XL 300 (Transasia Bio-Medicals Ltd. Mumbai, India). Plasma glucose was estimated by the Glucose oxidase/Peroxidase (GOD-POD) method (15). Estimation of total cholesterol, triglycerides and HDL cholesterol were measured by enzymatic method (Aspen Laboratories Pvt. Ltd; India) [16]. Other biochemical parameters were estimated by using commercial reagent kits (Pathozone Diagnostics, India), Urea by enzymatic (urease/glutamate dehydrogenase, kinetic) method [17], uric acid by the uricase/PAP (peroxidase coupled with 4-aminophenazone) enzymatic method [18], creatinine by modified Jaffe’s fixed time kinetic method [19], sodium (Na⁺), potassium (K⁺) and chlorides (Cl⁻) collectively called as electrolytes were measured by ion selective electrode method [20] using Combisys-II, Eischweiler BGA plus E instrument (Eischweiler automatic analyzing systems, Eschweiler GmbH & Co. Germany). Urinary albumin was determined by immunoturbidimetry method (2) (Tu-
lup Diagnostics (P) Ltd. Goa, India). Urine was diluted as 1:50 with distilled water before urine creatinine analysis.

**Statistical analysis:** Data was expressed as mean ± SD. Comparisons of diabetic subjects with control was performed using unpaired student ‘t’ tests for independent sample; a level of P < 0.05 was considered as statistical significant. Statistics were computed using GLM unisporate.

**RESULTS**

The present study comprised of 82 type 2 DM patients and 50 healthy controls (Table 2). The study subjects were divided in to two groups according to age, group 1(age 20-40 years) and group 2 (age 41-60 years) In case of DM 26 (15 males and 11 females) were of 20-40 years age group and 56 (32 males and 24 females) were 40-60 years age group.

We found significantly higher values of fasting and post prandial glucose (P<0.05), urea (P<0.01), uric acid (P<0.03), urine albumin and ACR (P<0.05) in DM patients compared to controls (Table 3). We observed increased level of total cholesterol (P<0.05), triacylglycerol (P<0.05) and non significant decrease in HDL level (P=0.11) in DM patients compared to controls.

Table 1. Cut off values used in literature for indicating normal, microalbuminuria (MAU) and macroalbuminuria (9, 11-13).

<table>
<thead>
<tr>
<th>Terms</th>
<th>24-hour urine Sample UAC (mg/24 hours)</th>
<th>Spot morning/random urine sample UAC (mg/L)</th>
<th>ACR* (mg/g)</th>
<th>ACR* (mg/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;30</td>
<td>&lt;20</td>
<td>&lt;30</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30 to 300</td>
<td>20 to 200</td>
<td>30 to 300</td>
<td>3 to 30</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>&gt;300</td>
<td>&gt;200</td>
<td>&gt;300</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

*ACR (mg/g) values are for both males and females (gender independent) (9, 12).

Table 2. Age and gender distribution of study subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>20-40 year</th>
<th>40-60 year</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>15</td>
<td>11</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>17</td>
<td>5</td>
<td>21</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3: Baseline laboratory data of study subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diabetic subjects (Mean ± SD) (n=82)</th>
<th>Control subjects (Mean ± SD) (n=50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma FBS (mg/dl)</td>
<td>169.21±64.89</td>
<td>93.38±16.55</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Plasma PPBS (mg/dl)</td>
<td>261.81±232.94</td>
<td>112±19.20</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.71±0.31</td>
<td>0.71±0.24</td>
<td>0.99</td>
</tr>
<tr>
<td>Serum Urea (mg/dl)</td>
<td>27.65±11.09</td>
<td>22.20±3.75</td>
<td>0.01*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.37±1.64</td>
<td>4.56±1.23</td>
<td>0.03*</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>137.65±4.72</td>
<td>141.58±20.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.19±0.8</td>
<td>9.51±26.41</td>
<td>0.06</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>96.31±6.17</td>
<td>101.46±3.29</td>
<td>0.21</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>195.5±50.15</td>
<td>160.94±21.35</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>190.73±73.47</td>
<td>132.96±42.90</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>46.72±10.46</td>
<td>60.40±12.71</td>
<td>0.11</td>
</tr>
<tr>
<td>Urine albumin (mg/L)</td>
<td>66.39±84.70</td>
<td>9.02±4.39</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Urine Creatinine (gm/L)</td>
<td>0.95±0.37</td>
<td>1.05±0.38</td>
<td>0.81</td>
</tr>
<tr>
<td>A/C ratio (mg/g)</td>
<td>79.34±109.74</td>
<td>9.75±5.84</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

Significant *
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Diabetes is major cause of chronic kidney disease
DISCUSSION

There was no significant change in electrolytes (sodium, potassium and chlorides) between cases and controls. We compared significant increase in level of total cholesterol (P<0.05), triacylglycerol (P<0.05) and non significant decrease in HDL level (P=0.23), urine albumin and ACR (P<0.001) in each age group of cases compared to controls (Table 4).

Table 5. Distribution and prevalence of DN in diabetes patients.

<table>
<thead>
<tr>
<th>Stage of Nephropathy</th>
<th>Diabetic subjects</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>18</td>
<td>21.95%</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td></td>
<td>51</td>
<td>62.19%</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td></td>
<td>13</td>
<td>15.85%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>82</td>
<td>100%</td>
</tr>
</tbody>
</table>

In DM patients the prevalence of normoalbuminuria, microalbuminuria and macroalbuminuria were 21.95%, 62.19% and 15.85% respectively (Table 5). Out of 82 DM patients 60 patients had diseases duration < 5 year, out of which 14, (23.33%) had normoalbuminuria, 42 (70 % ) had MAU and 4 (6.66%)had macroalbuminuria. 22 DM patients had diseases duration > 5 year, out of which 4 (18.18%) had normoalbuminuria, 9 (40.99 % ) had MAU and 9 (40.99%)had macroalbuminuria (Table 6).

D I S C U S S I O N

Diabetes is major cause of chronic kidney disease and is recognized as the most common causes of end stage renal disease in the world both in developed and developing countries. (6, 22). It has been reported that 40% of adults with diagnosed or undiagnosed diabetes have some degree of chronic kidney disease (23, 24). Increased excretion of albumin is sensitive marker for chronic kidney disease due to diabetes. This stage of renal involvement was termed MAU or incipient nephropathy (13). DN is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases and categorized into three stages: microalbuminuria (MAU), macroalbuminuria and overt nephropathy or clinical nephropathy (table 1) (25).The rates of progression of newly diagnosed type 2 diabetes between the stages of normoalbuminuria, MAU,
macroalbuminuria and renal failure were 2-3% per year (26). DN is more prevalent among African Americans, Asians, and Native Americans than Caucasians (27).

Out of 82 diabetic subjects 64 subjects had increased excretion of albumin in urine which includes 51 patients (62.19%) who had microalbuminuria 13 patients (15.85%) who had macroalbuminuria and remaining 18 had normal albuminuria. Our observation is supported by other studies (28-31). Kedak et al. (28) observed 50% prevalence of MAU in cross sectional hospital study (28), and Thakkar et al. (29) observed 54.09% of MAU prevalence in newly diagnosed type 2 DM in India. Similar observations were found in general population in India (30), UAE (31) and hospital based study in Pakistan (32) supports our observations.

We observed similar prevalence of macroalbuminuria (15.85%) in our study like Kanakamani et al. study (33) from hospital based study from north India, but they have screened patients by deep stick method and not quantitatively measured urine albumin unlike us.

In our study the prevalence of MAU in diabetic subjects is found higher compared to other studies in India (34-36). Higher prevalence in the present study may be due to the fact that most of the patients were with poor glycaemic control. A significant correlation was found between the prevalence of MAU and the duration of diabetes in our study supported by other studies (36-37)

We observed significant increase in total cholesterol, triacylglycerol and non significant decrease in HDL in DM patients. Dyslipidemia is common in diabetic patients (38) and further predisposes the diabetic patients to cardiovascular disease. Significant alterations in the lipid levels were found to be influenced by a glycaemic control. The MAU is independent risk factor for cardiovascular diseases in type 2 diabetic patients. (28).

Al-Shaikh et al. study (32) observed cardiovascular diseases and retinopathy were significantly more in patients with MAU than those with normal microalbuminuria. Hence micro- or macroalbuminuria patients should undergo evaluation for assessment of renal function and the presence of other co-morbid conditions (25). Persistent hyperglycemia known to cause the impairment of renal functions and this is assessed by measuring using the serum urea, creatinine and urine albumin and ACR in our study. Our study concludes that MAU is a reliable marker of DN in type 2 DM patients. We recommend that all patients with type 2 DM should be screened at the time of diagnosis and yearly thereafter. MAU and dyslipidemia together may be more potent risk factor for complications in type 2 DM. Patients with micro- and macroalbuminuria should undergo an evaluation regarding the presence of co-morbid conditions. Therefore regular screening for MAU is recommended for all asymptomatic DM patients for reducing cardiovascular risks and slowing the progression to end-stage renal disease to reduce the socioeconomic burden of DM.

REFERENCES


