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Original Research Article

Microalbuminuria, Serum Creatinine and Other Biomarkers among T2 DM Patients in North India

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Abstract: ***Background:*** Diabetic nephropathy (DN) is a common finding in diabetic patients. Microalbuminuria is the earliest clinical evidence of DN. Early detection of microalbuminuria is very important; it allows timely interventions to prevent progression to macroalbuminuria and later end-stage renal disease (ESRD). ***Objectives:*** To determine the prevalence of microalbuminuria in diabetic patients and establish its association with traditional serum renal markers in assessment of incipient nephropathy. ***Methods:*** This cross-sectional study involved 213 participants with diabetes mellitus (DM) attending the diabetic clinic of MM Institute of MSR. ***Aim:*** We aimed to evaluate the levels of urine microalbumin, urine albumin creatinine ratio, plasma creatinine and glycosylated hemoglobin (HbA1c) among type 2 diabetic patients and assessed the correlation between microalbuminuria and plasma creatinine levels. Questionnaires were used to obtain participant data after obtaining written informed consent. ***Data collected included:*** age, sex, level of education, history of smoking and alcohol consumption, hypertension, body mass index, family history, and duration of DM. Morning spot urine samples were collected from each participant and blood drawn for analysis of other renal markers. Urine microalbumin was determined quantitatively using immunoturbidity assay (Microalbumin kit, Mindray). ***Results:*** Increase in mean level of plasma creatinine (138 µmol/L), urine microalbuminuria (310 mg/L), albumin creatinine ratio (52) and HbA1c (7.9%) was observed among type 2 DM patients. Moderate positive correlation was observed between microalbuminuria and urine albumin creatinine ratio (r = 0.643 P = 0.0008) and between urine albumin creatinine ratio and plasma creatinine (r = 0.645 P = 0.032). ***Conclusion:*** We concluded that type 2 DM patients who are at risk of developing renal impairment must be regularly monitored for microalbuminuria, urine albumin creatinine ratio, and HbA1c levels.

Keywords: Albumin creatinine ratio; diabetes mellitus type 2; microalbuminuria.

INTRODUCTION

The long-term deleterious effects of hyperglycemia on various end-organs necessitates regular monitoring of organ functions to initiate early intervention to prevent diabetes associated complications [1]. Diabetes mellitus (DM) is one of the primary risk factors for developing renal impairment globally [2, 3]. Both type 1 and type 2 DM may lead to chronic complication of diabetic nephropathy [4]. The presence of trace amount of albumin in urine (microalbuminuria) has a good prognostic value in predicting early renal damage (initial nephropathy) [5]. Approximately, one-third of diabetic patients develop microalbuminuria after 15 years of the onset of disease, whereas full nephropathy can develop in nearly half of the patients developing microalbuminuria [6] with collateral risks of developing cardiovascular disease [7, 8]. Abnormal albumin levels in urine can be detected in 30% of patients diagnosed with type 2 DM [9, 10]. Presence of protein in urine can speed up the development of the renal disorder and subsequently lead to end-stage renal failure [11]. However, several aspects of mechanisms leading to the development of albuminuria are actively researched.[1,3]

Albumin creatinine ratio in random urine samples is the most appropriate investigation to detect early renal impairment.[4] Annual screening for microalbuminuria (see Microalbumin) is recommended in all patients with diabetes. Measuring the albumin-to-creatinine ratio in a spot urine sample is probably the easiest method; the ratio, expressed in mg/g, is equivalent to albumin excretion in milligrams daily. A result greater than 30 mg/g indicates albuminuria, in which case a quantitation on a timed urine specimen (ie, overnight, 10 h, or 24 h) should be performed. Normal urine albumin excretion is defined as less than 30 mg daily. Microalbuminuria is defined as 30-300 mg daily (20-200 mcg/min). Diabetes is the leading cause Trusted Source of kidney disease. Some people may refer to diabetes-related kidney disease as diabetic nephropathy. The main functions of the kidneys include filtering waste products out of the blood and producing urine. The kidneys do these using filters known as nephrons. As a possible complication of diabetes, the nephrons may become thicker and develop scarring over time. The liver produces the protein albumin, which enters the bloodstream to carry substances, such as hormones, throughout the body. When a person is in good health, this protein typically remains in the blood, and there is little or no albumin present in the urine. Microalbuminuria is the term for when the quantity of albumin in the urine is more than the normal value but still lower than the amount a conventional dipstick may detect. Most laboratories define the rate of urine albumin excretion for microalbuminuria as 30–300 milligrams per day (7.9 mg/d) Trusted Source. Some labs may instead define it as 20–200 micrograms per minute. Lab technicians consider a result lower than 30 mg/d as normal and a value higher than 300 mg/d as macroalbuminuria. Microalbuminuria suggests a moderate increase in urinary albumin, which may indicate early-stage kidney disease. Macroalbuminuria may suggest kidney disease in the later stages. As such, it is advisable that people with diabetes undergo regular screening for kidney damage and take appropriate measures to lower their risk. Diabetic nephropathy (DN) on the other hand is a consequence of long-standing diabetes mellitus which is the leading cause of mortality among diabetic patients [4]. The disease is characterized by increased urinary albumin excretion in the absence of other renal diseases [5]. Microalbuminuria (MALB) is the appearance of albumin in mg/day or 20 μg/min and has been documented to be the earliest clinical evidence of diabetic nephropathy in DM patients [6]. Without specific intervention, patients with MALB have their urinary albumin excretions increased and eventually end up with end-stage renal disease [7]. Decreased glomerular filtration rate (GFR), elevated serum creatinine and uric acid levels, and electrolyte imbalances are key features in the laboratory diagnosis of DN here in Uganda hence termed traditional serum renal markers of nephropathy in this study. At MM Institute of Medical Sciences & Research (MALB) is not a routine test. Usually, the clinic requests for the traditional serum renal biomarkers (serum uric acid, urea, and creatinine levels), electrolyte levels. Although these tests are requested for by the attending physicians, they are not based on evidence to benefit the diabetic patients directly. In addition, glycated hemoglobin (HbA1c) is requested to confirm the newly diagnosed patients. When this occurs, the kidneys may not work as well, causing proteins, such as albumin, to leak into the urine. Therefore, doctors can measure the levels of this protein in the urine to help identify early signs of kidney damage.

MATERIALS & METHODS

Urine and Blood Sample Collection and Storage

A random spot urine sample [9, 10] as indicated in the testing procedure using the Mindray MALB reagent kit was collected in a clean, dry screw cap urine container. Participants were given clear verbal and written instructions on how to collect the required midstream urine sample into the container. Using a vacutainer needle and holder, 4 mL of venipuncture blood was drawn into a red top vacutainer tube. Blood samples were centrifuged at 12,000 revolutions per minute to obtain serum. Spot urine samples were aliquoted into cryotubes and frozen at -20°C. A single vial and cryotube for each participant was thawed once at 25°C and analyzed. The random urine samples were analyzed for microalbuminuria following the laboratory Standard Operating Procedures (SOPs). System compatible reagent of the Microalbumin (MALB) kit was used on a Chemistry Analyzer. The same analyzer was used to measure plasma creatinine, uric acid, and glucose level. Serum electrolytes (sodium, potassium, and chloride) were analyzed using a colorimeter (Environmental and Scientific Instruments Co., Digital Photo Colorimeter, India) at 630 nm, 500 nm, and 480 nm, respectively. All equipment was calibrated. Reference ranges used were those routinely used at KIUTH Laboratory. The types of albuminuria were defined as microalbuminuria (2-20 mg/dL), normoalbuminuric (<2 mg/dL), and macroalbuminuria (>20 mg/dL).

RESULTS

1. General Characteristics of the Study Participants

Of the 213 diabetic patients in this study, there were 145 (67.9%) females and 68 (32.1%) males. Most of the participants, 87 (29.3%), were aged between 45 and 54 years. Majority of the participants, 157 (54.3%), had received up to primary level education, 104 (74.3%) were married, 3 (2.1%) reported to be smoking, and 9 (6.4%) confirmed taking alcohol.

2. Clinical Characteristics of Diabetic Patients

Majority of the participants, 81 (57.9%), had normal blood pressure; their average systolic and diastolic pressure was 138.6mHg and 78.1 mmHg, respectively. One hundred and nine participants (77.9%) had a family history of DM, and the average duration of DM was 6.8 years. Seventy percent of study participants had normal BMI with the average being 24.4 kg/m2.

3. Estimated Laboratory Markers of Renal Function among Diabetic Patients

The mean values of the assessed biomarkers of renal function measured in serum and urine are shown in Table 2. The mean values of the traditional markers of nephropathy were within the laboratory reference intervals except for blood glucose which was elevated (9.3 mmol/L).

4. Estimated Categories of Albuminuria among Diabetic Patients

Majority of the 140 study participants, 32 (22.9%), had microalbuminuria, 107 (76.4%) normal abuminuria, and 1 (0.7%) macroalbuminuria (Table 3). There was no statistically significant association between microalbuminuria and gender (M) or with age (23). Microalbuminuria was found in 19 (20%, 95% CI (13.03, 29.44)) females and 13 (28.9%, 95% CI (17.21, 44.26)) males. In addition, microalbuminuria was mostly detected among participants aged 45-54 years, 10 (24.4%, 95% CI (13.3, 40.4), and the least affected age group was 18-34 years, 3 (23.1%, 95% CI (6.32, 57.2).

Table 2: Laboratory Renal Markers of the Study Population.

CHARACTERISTICS	MEAN (SD)	REFERENCE INTERVALS
CREATININE (MMOL/L)	109.8 (19.8)	74-127
URIC ACID (MMOL/L)	245.8 (74.6)	214-488
GLUCOSE (MMOL/L)	9.3 (5.4)	3.9-6.4
SODIUM (MEQ/L)	153.8 (8.5)	135-155
POTASSIUM (MEQ/L)	4.7 (0.8)	2-7
CHLORIDE (MMOL/L)	98.4 (12.5)	97-108
EGFR (ML/MIN/1.73M2)	66.0 (14.8)	60-90
NORMOALBUMINURIA (MG/DL)	0.68 (0.28)	<2
MICROALBUMINURIA (MG/DL)	6.52 (5.05)	2-20
MACROALBUMINURIA (MG/DL)	33.14 (0.00)	>20

5. Univariate and Multivariate Analysis for the Potential Association of Microalbuminuria

Linear regression analysis (Table 4) was performed with microalbuminuria levels as the dependent variable. A simple linear regression revealed serum creatinine (95% CI (0.005, 0.014), uric acid (95% CI (0.001, 0.003), and glucose (95% CI (0.011, 0.048) levels to have a positive correlation with microalbuminuria levels.

Table 3: Prevalence of Microalbuminuria and Its Distribution According To Age Group and Gender

PREVALENCE TYPE	N	% (95% CI)	P VALUE
MICROALBUMINURIA	32	22.9 (16.6, 30.6)	
NORMOALBUMINURIA	107	76.4 (68.6, 82.8)	
MACROALBUMINURIA	1	0.7 (0.1, 5.0)	
GENDER-SPECIFIC			0.242
MALE	13	28.9 (17.21, 44.26)	
FEMALE	19	20 (13.03, 29.44)	
AGE-SPECIFIC		23.1 (6.32, 57.2)	0.941
18-34	3		
35-44	5	20 (8.0, 41.7)	
45-54	10	24.4 (13.3, 40.4)	
55-64			
≥65	7		
PREVALENCE TYPE	7		
MICROALBUMINURIA			
NORMOALBUMINURIA	32		
MACROALBUMINURIA	107		
GENDER-SPECIFIC	1		
MALE			
FEMALE	13		
AGE-SPECIFIC	19		
55-64		19.4 (9.2, 36.5)	

DISCUSSION

This cross-sectional study conducted at MM Institute of Medical Sciences & Research, Mullana-Ambala provides the prevalence of microalbuminuria among diabetic patients, and its performance against traditional markers of renal function in assessment of incipient nephropathy. Microalbuminuria was detected in 32.7% of participants in this study. The authors observed a prevalence of 43% microalbuminuria in 202 newly diagnosed diabetic patients, which was more than double than what we observed in the current study. This study was conducted at Superspecialist Hospital was done to assess microalbuminuria as a contributor to echocardiographic abnormalities among newly diagnosed diabetic patients and not a marker of nephropathy Several epidemiological studies have reported the prevalence rates of MALB as ranging between 29% and 71% in patients with diabetes [4, 9, 10]. A study conducted in Senegal by Djiby *et al*., [5] to assess the prevalence of microalbuminuria and associated risk factors in a population of diabetics followed at the Marc Sankale Center of Dakar reported a prevalence rate of 25.45% among 213 participants. Variable Univariate analysis value Multivariate analysis value.

Table 4: Univariate and Multivariate Analysis for Potential Association Showing the Relationship between Microalbuminuria and the Traditional Serum Biomarkers

VARIABLE UNADJUSTED COEFF. (95% CI)	**UNIVARIATE ANALYSIS ADJUSTED COEFF. (95% CI)**	**P VALUE**	**MULTIVARIATE ANALYSIS**	**P VALUE**
CREATININE	0.010 (0.005, 0.014)	0.0001	0.006 (0.001, 0.011)	0.012
URIC ACID	0.002 (0.001, 0.003)	0.0071	0.001 (0.0002, 0.002)	0.117
GLUCOSE	0.030 (0.011, 0.048)	0.0017	0.027 (0.008, 0.0452)	0.005
SODIUM	0.0002 (-0.011, 0.011)	0.9685		
POTASSIUM	0.096 (-0.021, 0.213)	0.1067		
CHLORIDE	0.004 (-0.003, 0.012)	0.2417		
VARIABLE	UNIVARIATE ANALYSIS	VALUE	MULTIVARIATE ANALYSIS	VALUE
UNADJUSTED COEFF. (95% CI)	ADJUSTED COEFF. (95% CI)			

Variations in the prevalence of microalbuminuria has been attributed to several factors like difference in populations, the definition of microalbuminuria by different laboratories, and the method of urine collection and of measurement of microalbuminuria [6]. Nevertheless, microalbuminuria in adults who formed the majority of our study participants is believed to be an early risk marker for nephropathy [7]. In this study, the definition of microalbuminuria of 2-20 mg/dL in a spot urine sample has been used elsewhere [6] and recommended by others [8, 9]. A study in India by Chowta *et al*., [2] reported a prevalence of microalbuminuria of 37% in 100 diabetic patients. In this same study, there were 20 (51.87%) males and 17 (35.67%) females with microalbuminuria. In comparison to our study, females, 19 (23%), dominated with microalbuminuria while males were only 13 (21.87%). Several epidemiological studies have reported MALB prevalence rates ranging from 21.76% to 54% in diabetic patients [3-5]. Several epidemiological studies have reported MALB prevalence rates ranging from 25% to 54% in diabetic patients [7, 8, 4]. Djiby *et al*., conducted a study in Senegal to assess the prevalence of microalbuminuria and associated risk factors in a diabetic population followed at the Marc Sankale Center in Dakar and found a prevalence rate of 27.14% among 221 participants. Variations in the prevalence of microalbuminuria have been attributed to a variety of factors, including population differences, different laboratories' definitions of microalbuminuria, and the method of urine collection and measurement of microalbuminuria [11]. Nonetheless, microalbuminuria is thought to be an early risk marker for nephropathy in adults, who made up the majority of our study participants [7]. In this case, this cross-sectional study conducted at MM Institute of Medical Sciences & Research, Mullana-Ambala provides the prevalence of microalbuminuria among diabetic patients, and its performance against traditional markers of renal function in assessment of incipient nephropathy. Microalbuminuria was detected in 22.9% of participants in this study. we observed a prevalence of 54% microalbuminuria in 202 newly diagnosed diabetic patients, which was more than double than what we observed in the current study. This study was conducted at Superspecialist Hospital was done to assess microalbuminuria as a contributor to echocardiographic abnormalities among newly diagnosed diabetic patients and not a marker of nephropathy. Several epidemiological studies have reported the prevalence rates of MALB as ranging between 20% and 61% in patients with diabetes [7, 8, 4]. A study conducted in Senegal by Djiby *et al*., [5] to assess the prevalence of microalbuminuria and associated risk factors in a population of diabetics followed at the Marc Sankale Center of Dakar reported a prevalence rate of 27.14% among 221 participants. Variations in the prevalence of microalbuminuria has been attributed to several factors like difference in populations, the definition of microalbuminuria by different laboratories, and the method of urine collection and of measurement of microalbuminuria [6]. Nevertheless, microalbuminuria in adults who formed the majority of our study participants is believed to be an early risk marker for nephropathy [5]. In this study, the definition of microalbuminuria of 2-20 mg/dL in a spot urine sample has been used elsewhere [6] and recommended by others [8,

9]. A study in India by Chowta *et al*., [2] reported a prevalence of microalbuminuria of 37% in 100 diabetic patients. In this same study, there were (54.05%) males and 17 (45.95%) females with microalbuminuria. In comparison to our study, females, (20%), dominated with microalbuminuria while males were only (28.9%). Adjusting for age and sex did not show any statistically significant association with microalbuminuria in this study population. Lack of association between age and microalbuminuria may be because majority of our patients were above 30 years of age. It has been suggested that sex hormones possibly play a role in incidence of microalbuminuria in young diabetic patients; however, this is significant for pubertal onset diabetes; prepubertal [11] and post pubertal onset of diabetes do not increase the risk of microalbuminuria [2]. We did not exclude female patients of reproductive age who may have been in their menstruation cycle from participation. Overall, our study population was comprised of mostly adult patients: 102 (72.8%) above 45 years of age. Of these, 61 (59.8%) were above 55 years of age. In view of this, it is unlikely that the possibility of some women being in menstrual cycle could have significantly affected the observed prevalence of microalbuminuria. A statistically significant correlation between microalbumin levels in urine and serum creatinine levels, serum uric acid levels, and serum glucose levels at univariate analysis. Microalbuminuria was not associated with sodium, potassium, and chloride levels. If serum creatinine is to be used as a renal function marker among diabetic patients, it should be combined with microalbuminuria. This is primarily important in children and elderly diabetic patients who are likely to have lean muscle mass. Although microalbuminuria may be asymptomatic in diabetic patients, monitoring glucose levels and measurement of microalbuminuria should be carried out with the purpose of preventing end-stage renal disease (ESRD) among these patients. Our study did not estimate urinary albumin/creatinine ratio (ACR) which is regarded as a reflection of albumin excretion rate (AER) [3] that can be measured in untimed spot urine samples. This is because urinary creatinine reflects muscular mass and so the latter may affect ACR. This means that low muscular mass can be a confounding in the use of urinary ACR to estimate AER. We found no association between electrolytes and microalbuminuria. This is in contrast to what was reported by Kumari *et al*., [3] who investigated the association of serum electrolytes with renal function in DM. We found sex and family history of DM to have a correlation with microalbuminuria at univariate analysis but only family history of DM remained statistically significant at multivariate analysis. The rest of the factors, which is, age, hypertension, systolic and diastolic blood pressure, duration of DM, alcohol consumption, smoking, and body mass index showed no correlation with microalbuminuria. However, the weak association we observed in our study population should not be ignored because it can progress through the three developmental stages of diabetic nephropathy [11], and the patient finally ends up with ESRD, macroalbuminuria, and diminished eGFR [10]. End-stage renal disease is characterized with irreversible renal damage.

CONCLUSION

We concluded that type 2 DM patients who have a considerable risk factor for developing renal impairment should be regularly monitored for more sensitive biomarkers of nephropathy such as microalbuminuria, urine albumin creatinine ratio, and HbA1c levels to facilitate early detection of diabetes-induced nephropathy. The prevalence of microalbuminuria among diabetic patients at MM Institute 0f MSR was 22.9%. This prevalence is substantial considering that it can develop into macroalbuminuria and its associated complications. Monitoring blood glucose, creatinine, and uric acid levels even in the absence of reduced eGFR should be considered for patients with microalbuminuria.

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