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Screening for Microalbuminuria in Newly Detected Type 2 Diabetes Mellitus Patients

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ABSTRACT

Microalbuminuria is a major risk factor for various renal and cardiovascular events. The need for early detection and treatment of patients at risk for microalbuminuria is important to limit the excess renal and cardiovascular disease associated with type 2 diabetes mellitus (T2DM). Therefore, this study aimed to screen newly detected T2DM patients for the presence of microalbuminuria. A total of 120 patients diagnosed with T2DM were included in this prospective observational study. After collecting history, physical examination, and various biochemical investigations such as kidney function test, plasma blood sugars, lipid profile and glycosylated hemoglobin (HbA1c) were conducted. The detection of microalbuminuria was done by Micral test. Data were analyzed by the Chi-square test for significance of qualitative variables. A p-value of < 0.05 was considered significant. Prevalence of microalbuminuria among T2DM patients was observed to be 40%. HbA1c levels of females showed a significant association ($p=0.007$). Patients with BMI>25 kg/m^2 had a significant incidence of microalbuminuria ($p<0.001$). Urinary glucose was significantly higher ($p=0.024$) in 83.3% of patients with microalbuminuria. The mean fasting and postprandial blood glucose levels were considerably higher in patients with microalbuminuria ($p=0.004$, $p=0.002$). Blood urea and serum creatinine levels, too, were noted to be slightly higher in patients with microalbuminuria ($p=018$, $p=0.001$). Prevalence of microalbuminuria was slightly less in newly diagnosed T2DM patients. Early diagnosis and management of microalbuminuria in asymptomatic individuals could help in preventing worsening in renal function and progression of overt diabetic nephropathy.

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INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorders characterized by metabolic abnormalities and is one of the most challenging health problems worldwide. The prevalence of diabetes has increased rapidly over the past several decades. The global prevalence of diabetes among adults has increased from 4.7% to 8.5% in 2014.[1] One of the most severe complications of diabetes is the development of Diabetic Nephropathy and it is the leading cause of the end-stage renal disease (ESRD) worldwide (WHO, 2020). Almost 30% of the chronic renal failures in India are due to

diabetic nephropathy (Vishwanathan, 2003; Mani, 2003). Microalbuminuria appears to be a strong predictor of the subsequent development of overt diabetic nephropathy (Vijayakumar *et al.*, 2007; Association AD, 2004). The prevalence of microalbuminuria in patients with type II diabetes mellitus (T2DM) has been reported from 20% to 61% globally (Ghosh *et al.*, 2012; Ahmedani *et al.*, 2005).

Diabetic nephropathy in diabetic patients is characterized by increased rates of urinary albumin excretion with the initiation of normoalbuminuria followed by the progression of microalbuminuria, macroalbuminuria and eventually to ESRD (Varghese *et al.*, 2001; Debbarma *et al.*, 2015). Studies have demonstrated that without specific interventions, 20–40% of T2DM patients with microalbuminuria progress to overt nephropathy (Jong *et al.*, 2003). Hence, measurement of urine albumin is often used as a sensitive marker and predictor of overt nephropathy in patients with T2DM (Heerspink *et al.*, 2011).

The development of diabetic nephropathy is determined by a significant correlation between microalbuminuria and duration of diabetes. The longer the duration of diabetes, the higher the risk of microalbuminuria (Ahmedani *et al.*, 2005; Varghese *et al.*, 2001). The association between microalbuminuria and fasting blood sugar (FBS), age, and gender is debatable; the findings of various studies are contradictory (Varghese *et al.*, 2001). Several modifiable risk factors have been identified for the development of microalbuminuria and for progression to overt diabetic nephropathy, such as hyperglycemia, hypertension, dyslipidemia, smoking and genetic susceptibility (Ayodele *et al.*, 2004).

Therapeutic interventions that reverse microalbuminuria include use of angiotensin-converting enzyme (ACE) inhibitors and intensified glycemic control. These should be initiated in patients with T2DM with microalbuminuria to prevent progression to overt diabetic nephropathy (Perkins *et al.*, 2003). Screening for microalbuminuria is required in primary healthcare centers to prevent the progression of this serious complication of diabetic nephropathy. In India, there is a paucity of data on the prevalence and factors associated with microalbuminuria among newly diagnosed T2DM patients. Hence the present study aimed to study the occurrence of microalbuminuria in patients with newly diagnosed T2DMand also to find out its association with increased body mass index (BMI), lipid profile and glycated hemoglobin levels.

MATERIALS AND METHODS

This prospective observational study was conducted for 18 months in a tertiary care center. One hundred and twenty patients of NIDDM taken from wards of the hospital after obtaining a written informed consent were selected. The study was carried out after approval from the Institutional ethics committee. The sample size was calculated based on the chi-square test, with a 95% level of significance and 94% power to obtain a minimum sample size of 113 subjects. Patients with positive urinary sugar and fasting blood glucose $\geq$126 mg/dL and/or postprandial blood sugar $\geq$200 mg/dL were included in the study. Patients with known diabetics, macroalbuminuria, congestive cardiac failure, urinary tract infection or sepsis, ketonuria, pregnant women, patients with previously known renal dysfunction or renal pathology were excluded from the study.

All patients underwent a complete physical examination, and detailed history on smoking, alcohol consumption, bowel and bladder habits and drug intake, including details regarding presenting complaints were recorded in a standard proforma.BMI was calculated by weight in kg/height in m^2. Clinical examination with particular reference to the complications of diabetes like retinopathy, neuropathy, diabetic foot, and coronary artery disease was carried out.FBS and postprandial (PP) blood sugar, glycosylated hemoglobin (HbA1c), blood urea and serum creatinine, fasting lipid profile, urine routine and culture, and electrocardiogram was estimated in all the patients. For the evaluation of microalbuminuria, the Micral test was performed.

Estimation of microalbuminuria by Micral test

Micral test, an immunological rapid dipstick semi-qualitative technique for detection of microalbuminuria, was used for estimation of microalbuminuria. Each Urine Test Strip contains Monoclonal Antibodies: Anti-human albumin IgG labeled with colloidal gold (2.2 mg) and Fixed albumin (7.7 mg). All patients were afebrile during the course of collection of urine and were kept at rest during the collection of urine. Early morning mid-stream urine sample was collected in a sterile container and was used for screening microalbuminuria. The test strip was immersed in urine and the strip was withdrawn after 5 seconds. Strip was placed horizontally across the urine vessel and color change in the test zone was compared with color scale after one minute. Microalbuminuria was graded as Trace (30-50 mg/L): 1+; Mild (50-100mg/L): 2+; Moderate (100-200mg/L): 3+; Severe (200-300mg/L): 4+ depending on the color change in the strip.

Blood samples were taken from the patients after at least 10 hours of fasting to measure FBS, HbA1c, lipid profiles, blood urea, and serum creatinine levels. Severity of diabetes was graded based on HbA1c levels as mild (<7.0%), moderate (7.0% - 7.5%) and severe (>7.5%). Fasting lipid profiles were evaluated based on total cholesterol (<200 mg/dL as desirable). Blood urea (7 to 20 mg/dL as desirable) and serum creatinine levels (0.6 to 1.2 mg/dL in adult males and 0.5 to 1.1 mg/dL in adult females) were also estimated.

Data were analyzed using statistical software SPSS version 20 and Epi info version 7.2. Levene's test for equality of variances was used and equal variances were assumed within the groups. Independent sample test (Unpaired t-test) was used to compare categorical data. Chi-square test was used to find an association between qualitative variables. A p-value of < 0.05 was considered as significant.

RESULTS AND DISCUSSION

In a study population of 120 patients with T2DM, the majority of the patients were from 51-60 years of age and most of the patients included were male (57.5%). The mean BMI of the population was 24.06±4.01. The demographic characteristics is presented in Table 1.

Based on the distribution of patients on various clinical manifestations, most of the patients (60%) tested negative for urine microalbumin. Whereas 40% of patients tested positive for microalbuminuria. Majority of the patients (29.2%) tested negative for the presence of urinary glucose, and 88.3% of patients were noted to have a normal fundus with no signs of diabetic retinopathy. In terms of the presence of other chronic diseases, 62.5% of patients had no comorbidities. Coronary artery disease (CAD) was the most common comorbidity (25.8%) followed by chronic obstructive pulmonary disease in 10.8% of patients. Majority of the patients (84.2%) had normal mean serum cholesterol levels. High risk of high-density lipoprotein (HDL) levels was observed in 65.5% of patients. Most of the patients (61.7%) had normal triglycerides (TGL). All the patients (100%) had normal low-density lipoprotein (LDL) levels. With respect to HbA1c levels, the majority of the subjects (47.5%) had 7.5-10 of HbA1c levels. High serum creatinine and high blood urea were noted in 36.7% and 17.5% of patients, respectively.

In terms of the association between cholesterol levels with gender, it was observed that abnormal cholesterol was more in females as compared to males (55.6% vs 44.4%). Whereas, normal and abnormal TGL levels were found to be higher in males compared to females (51.9% vs 48.1%). With regards to HDL, the relation of males with both borderline and high HDL levels were higher as compared to females (57.5% vs 42.5%). Similar findings were observed with respect to serum creatinine levels (56.8% vs 42.2%) and blood urea levels (57.1% vs 42.9%) which showed higher levels in males compared to females. In terms of the association between BMI levels with gender, the relation of males with obesity was much higher as compared to females (77.8% vs 22.2%); However, the difference was not statistically significant. With respect to HbA1c levels, the relation of males with normal HbA1c levels was much higher than that compared to females which showed a statistically significant difference (p=0.016). Females were noted to have higher HbA1c levels as compared to males.

Gender wise comparison of different parameters is depicted in Table 2. Among all the parameters, the HbA1c levels were found to be significantly higher in females (9.35 gm%) as compared to males (8.49 gm%). The difference in HbA1c levels of the two genders was observed to be statistically significant (p=0.007).

With respect to the association between urinary microalbumin level and gender, the proportion of abnormal urinary microalbumin was higher (41.2%) in females as compared to males (39.1%). However, there was no significant association observed between gender and urinary microalbumin levels. In terms of the association of grading of urinary microalbumin and gender, the proportion of both normal urinary microalbumin was higher in males while 1+ and 2+ microalbumin levels were higher in females and 3+ and 4+ were absent in females. The difference was observed to be insignificant.

Comparison of various biochemical parameters in two groups based on normal and abnormal urinary microalbumin levels was presented in Table 3. The mean BMI of abnormal microalbumin was significantly higher than the normal group (p<0.001). The average FBS levels and PP blood glucose levels were considerably higher in the abnormal microalbumin group as compared to the normal group (p=0.004, p=0.002). Blood urea levels and serum creatinine levels were very high in the abnormal microalbumin group as compared to the normal group and were statistically significant (p=018, p=0.001). Other parameters like age, HbA1c, cholesterol, TGL, HDL and LDL levels were also compared in normal and abnormal microalbumin levels; however no statistical association between urinary microalbumin and

Table 1: Demographic characteristics

Variables	Number of subjects (%)
Age (years)	
30-40	9 (7.5)
41-50	22 (18.3)
51-60	53 (44.2)
61-70	28 (23.3)
71-80	5 (4.2)
>80	3 (2.5)
Gender	
Females	51 (42.5)
Males	69 (57.5)
BMI (kg/m2)	
Underweight (<18.50)	4 (3.33)
Normal (18.50-24.99)	75 (62.5)
Overweight (25-29.99)	31 (25.83)
Obese class-I (30-34.99)	10 (8.33)

BMI:Body mass index

Table 2: Gender wise comparison of different parameters

Parameters	Male (n=69) Mean±SD	Females (n=51) Mean±SD	p-value
Age (years)	58±11.012	56.27±9.39	0.35
BMI (Kg/m2)	24.13±4.27	23.98±3.68	0.83
FBS (mg%)	162.13±48.32	160.14±49.38	0.82
PP (mg%)	217.60±70.22	236.54±82.57	0.19
HbA1c (%)	8.49±1.58	9.35±1.78	0.007*
Blood urea (gm%)	34.33±11.87	33.35±12.48	0.66
Serum creatinine (mg%)	1.28±0.33	1.28±0.40	0.98
Cholesterol (mg%)	156.91±40.22	168.02±45.35	0.17
Triglycerides (mg%)	142.87±57.96	164.21±70.02	0.08
HDL (mg%)	35.01±9.80	36.03±7.68	0.52
LDL (mg%)	75.01±28.17	78.88±27.58	0.45

BMI: Body massindex, FBS: Fasting blood sugar, HbA1c: Glycosylated hemoglobin, HDL:High-density lipoprotein, LDL: Low-density lipoprotein, PP: Postprandial bloodsugar
p<0.05considered statistically significant

these parameters were observed.

The proportion of both abnormal urinary microalbumin was maximum in patients with abnormal urinary glucose (83.3%). Among patients with normal microalbumin levels, urinary glucose was abnormal in 62.5% as compared to 37.5% of patients with normal urinary glucose. The difference was observed to be statistically significant (p=0.024) (Table 4).

The Pearson's correlation coefficient was calculated for various possible pairs of the age of patients, BMI and biochemical parameters. There was a positive correlation observed between FBS and PP sugar levels (p=0.0002), PP and HbA1c levels (p=0.00036), blood urea levels and serum creatinine (2.22E-15), cholesterol and HDL (p=0.00276), cholesterol and LDL (1.3E-05), TGL and HDL (p=0.00082), TGL and LDL (0.01947) and HDL and LDL (p=0.00236) (Table 5).These variables were found to be statistically significant.

Diabetic nephropathy is a severe complication of T2DM, and early detection is of paramount importance. Microalbuminuria screening test is cost-effective and easy to perform and thus it will help to relieve some of the burden in our health care sys-

Table 3: Different parameters according to normal and abnormal urinary microalbumin

Parameters	Normal (n=72) Mean±SD	Abnormal (n=48) Mean±SD	p-value
Age (years)	57.81±11.05	56.46±9.24	0.487
BMI (Kg/m2)	22.47±2.98	26.10±4.55	<0.001*
FBS (mg%)	150.92±38.93	176.83±57.21	0.004*
PP (mg%)	208.01±61.99	252.12±87.25	0.002*
HbA1c (%)	8.67±1.58	9.14±1.87	0.148
Blood urea (gm%)	31.79±10.38	37.10±13.79	0.018*
Serum creatinine (mg%)	1.19±0.30	1.41±0.40	0.001*
Cholesterol (mg%)	162.00±42.02	161.08±44.02	0.909
Triglycerides (mg%)	149.01±64.39	156.33±63.77	0.541
HDL (mg%)	36.31±9.46	34.17±8.02	0.200
LDL (mg%)	75.93±28.89	77.75±26.53	0.728

BMI:Body mass index, FBS: Fasting blood sugar, HbA1c: Glycosylated hemoglobin, HDL:High-density lipoprotein, LDL: Low-density lipoprotein, PP: Postprandial bloodsugar
p<0.05considered statistically significant

Table 4: Association between urinary microalbumin level and urinary glucose

Microalbumin	Urinary glucose Normal (%)	Abnormal (%)	Total (%)	p-value
Normal (%)	27 (37.5)	45 (62.5)	72 (100)	p=0.024*
Abnormal (%)	8 (16.7)	40 (83.3)	48 (100)	
Total (%)	35 (29.2)	85 (70.8)	120 (100)	

p<0.05 considered statistically significant

tem by early detection. This further helps the physicians to prevent progressive renal disease in many patients with diabetes. Hence, our study was conducted to screen newly diagnosed T2DM patients for the presence of microalbuminuria. Prevalence of microalbuminuria was observed to be 40% in T2DM patients (Harris and Eastman, 2000). Various other studies reported 25%-35% prevalence of microalbuminuria in T2DM patients which was almost similar to the current study (Voulgari *et al.*, 2011; Copeland *et al.*, 2013). A slight increase in the percentage of microalbuminuria in the present study could be attributed to several factors such as a larger number of elderly patients, longer duration of undiagnosed diabetes and poor glycemic control. In the present study, gender comparison with various parameters demonstrated that HbA1c levels were found to be significantly higher in females as compared to males. In contrast, a study carried out by Habib et al. reported more than 8% higher levels of HbA1c in males compared to female T2DM patients (Habib and Akbar, 2018). Similarly, Geetha et al. also reported significantly higher levels of HbA1c levels in males compared to females in T2DM patients in the study (Geetha and Shanmugasundaram, 2017). The differences in results could be probably due to the selection of samples or certain differences in population. It could also be an indication that higher levels of HbA1c could be due to poor diabetic control in patients.

HbA1c levels showed a statistically significant difference in gender-wise distribution though it was not significant when compared with normoalbuminuria and microalbuminuric patients. In contrast, a study conducted by Geetha et al. reported no statistically significant difference observed with HbA1c levels when compared gender-wise (Geetha and Shanmugasundaram, 2017). The reason for higher levels of HbA1c levels in microalbuminuric patients could be due to endocrine or behavioral factors involved in women which could have affected the outcome of reporting high HbA1c levels in females compared to males in the present study.

Mean BMI of patients with microalbuminuria was significantly higher than that of normoalbuminuric patients in the present study. Agarwal et al. reported similar observation in a study, wherein the incidence of nephropathy increased with increase

Table 5: Correlation between different parameters using Pearson's Correlation

	PP	HBA1C	UREA	Sr. Cr.	HDL	LDL
Age	0.01	-0.11	0.06	0.06	0.14	-0.07
p-value	0.93	0.23	0.48	0.83	0.11	0.41
BMI	-0.01	0.07	0.06	0.08	-0.04	0.01
p-value	0.86	0.42	0.63	0.38	0.66	0.94
FBS	0.33	0.04	0.18	0.14	-0.062	0.1
p-value	0.01*	0.63	0.04	0.11	0.50	0.27
PP		0.32	0.16	0.17	0.01	0.08
p-value		0.01*	0.07	0.06	0.91	0.33
HBA1C			-0.02	-0.02	0.11	0.16
p-value			0.75	0.75	0.22	0.06
UREA				0.64	0.03	-0.02
p-value				<0.01*	0.70	0.79
Sr.Cr					-0.05	-0.11
p-value					0.56	0.22
CHOL					0.27	0.38
p-value					<0.01*	<0.01*
TGL					-0.30	-0.21
p-value					<0.01*	<0.01*
HDL						0.27
p-value						<0.01*

BMI:Body mass index, Chol: Cholesterol, FBS: Fasting blood sugar, HbA1c:Glycosylated hemoglobin, HDL: High-density lipoprotein, LDL: Low-densitylipoprotein, PP: Postprandial blood sugar, Sr Cr: Serum creatinine, TGL:Triglycerides
*p<0.05considered statistically significant

in BMI (Agarwal *et al.*, 2011). Another study conducted by Debbarma et al. also reported similar results concurrent to the present study (Debbarma *et al.*, 2015). The possible explanation could be attributed to the fact that is increasing BMI is a reflection of insulin resistance which further leads to endothelial dysfunction of kidneys and glomerular lesions by multiple complex pathogeneses resulting in microalbuminuria (Unger and Orci, 2002; Tchernof and Despres, 2013).

With respect to the FBS and PP blood glucose levels, the present study reported considerably higher levels of FBS and PP in microalbuminuria patients compared to normoalbuminuric patients. Studies conducted by Kundu et al. and Mir et al. reported similar results with higher levels of FBS and PP in patients with microalbuminuria compared to normoalbuminuric patients (Kundu *et al.*, 2013; Mir *et al.*, 2019). Debbarma et al. reported significantly higher FBS and PP in microalbuminuric patients compared to normoalbuminuric patients (Debbarma *et al.*, 2015). Hyperglycemia is a crucial factor in the development of diabetic nephropathy. It is associated with an increase in mesangial cell proliferation and hypertrophy. Also, it results in increased matrix production and membrane thickening (Kovacs, 2009). Hence, tight glycemic control and monitoring on a regular basis should be the primary goal for any patients with diabetes.

Significantly higher levels of blood urea and serum creatinine was reported in the abnormal microalbumin group as compared to the normal group in the present study. Zakerkish et al. reported similar results with increased blood urea and serum creatinine levels in patients with diabetes mellitus (Zakerkish *et al.*, 2013). These findings could be attributed to the fact that higher levels of serum creatinine and blood urea levels could manifest diabetic nephropathy at a later stage, and hence screening for microalbuminuria should be given importance in future. Statistically, a significant association was observed between higher abnormal microalbumin levels with abnormal urinary glucose in the current study. Hence, it was concluded that the appearance of glycosuria in diabetic patients could be due to impairment of renal tubular function. With prolonged glycosuria, development of glomerulus and a decrease in pore size occurs, which ultimately results in albuminuria as a late complication.

In the present study, a significant correlation between FBS with blood urea was observed, which

proved that FBS and PP contributed to the overall hyperglycemic status in the progression of severe diabetes mellitus. With regards to the significant correlation between FBS and blood urea in the present study, a requirement in the assessment of renal dysfunction in diabetic patients was noted. A significant correlation between PP and HbA1c was observed in the current study. This could possibly indicate the development of micro and macroangiopathic complications in T2DM patients (Landgraf, 2004). Similarly, a statistically significant correlation between high blood urea levels and serum creatinine levels was observed in the present study. This proved that these could be simple biomarkers as a predictor and prognostic tests of renal failure in diabetic patients which helps in early detection of renal failure. Also, in the present study, a significant correlation was observed between cholesterol with HDL and LDL. These results conclude that lipoprotein levels and triglyceride levels in type 2 diabetes patients with microalbuminuria are known risk factors for CAD (Singla *et al.*, 2009).

T2DM patients with microalbuminuria have a significant relationship with HDL, poor glycemic control, and body mass index. However, the current study has a few limitations. Firstly, the study failed to follow up with the patients who were put on ACE inhibitors. It could be possibly due to the shorter duration of the current study to comment on the benefits of ACE inhibitors related to cardiovascular and renal effects. Hence, further studies are warranted to prove the response level of patients to drug therapy on reducing the urinary microalbuminuria. Secondly, confounding factors like data on diet, smoking habits, and occurrence of anemia in patients were not collected as the study was more concentrated on factors associated to T2DM with microalbuminuria which can cause false-positive correlations rather than false-negative results.

The findings of this study provide support for comprehensive screening for diabetes-related complications, especially microalbuminuria at the time of diagnosis of T2DM. Despite its role as an independent predictor of renal and cardiovascular outcomes, the importance of monitoring microalbuminuria and to act as a modifiable risk factor is still underestimated. Therefore, large-scale clinical trials to establish a relation between elevated microalbumin levels and T2DMare worth undertaking.

CONCLUSIONS

Prevalence of microalbuminuria in newly diagnosed type 2 diabetes mellitus patients were found to be slightly less in the current study. Early screening for microalbuminuria is strongly recommended for all newly diagnosed T2DM patients to prevent the development of diabetic nephropathy. The use of easy, cheap, and reliable semi-quantitative methods such as micral test seems to be a promising strategy for screening microalbuminuria in primary healthcare centers.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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