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KEY WORDS: Type 2 Diabetes Mellitus (t2dm), Microalbuminuria, Micral Test, Retinopathy, Bmi. (body Mass Index)

MICROALBUMINURIA IN TYPE 2 DIABETES MELLITUS AND ITS ASSOCIATION WITH RETINOPATHY, BMI AND ITS RELATION TO HYPERTENSION: AN EXPERIENCE FROM A SINGLE CENTRE

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ABSTRACT

AIM: To study the presence of Microalbuminuria in patients with Type 2 DM and to find out its association with the duration of diabetes mellitus, Retinopathy, BMI and also its relation to Hypertension.

MATERIALS AND METHODS: One hundred patients of T2 DM admitted in our centre were included after careful exclusion. The selected patients were studied in detail with history and physical examination. Retinopathy examination was done by same qualified Ophthalmologist throughout the study. FBS, PPBS,HbA1C, blood urea, lipid profile, creatinine, complete urine examination, Microalbuminuria estimation with Micral test were done in all patients. Whereas Ultrasonography of the abdomen, an echocardiogram and chest x-ray were done in selected cases only.

RESULTS: 57 male and 43 female between 40-70 years were studied.38 and 44 patients are tested positive for Microalbuminuria and Retinopathy respectively. Incidence of Microalbuminuria is more likely for the age group above 50 years of age as compared to the age group <50 years of age with p=0.053. Incidence of Retinopathy is more likely for the age group above 50 years of age as compared to the age group <50 years of age with p=0.001. These two complications are significantly higher with higher HbA1C. (P values 0.016 and 0.001 respectively) and also with higher BMI. (P values 0.02 and 0.01 respectively).

The association of Microalbuminuria and Retinopathy with hypertension is significant with P values <0.005 and<0.001 respectively and the association between microalbuminuria is significant with P value of <0.001.

CONCLUSIONS: Microalbuminuria and Retinopathy show a direct relationship with increasing age of patients. An HbA1c value above 7 % is associated with increasing incidence of Microalbuminuria and Retinopathy. Patients with a BMI of more than 25 kg/m2 have a significant increase in the incidence of Microalbuminuria and Retinopathy. The incidence of Microalbuminuria and Retinopathy is significantly associated with presence of hypertension and there is a significant association between the presence of Microalbuminuria and Retinopathy.

INTRODUCTION:

Diabetes mellitus, the most common endocrine disorder is characterised by metabolic abnormalities and long-term microvascular and macrovascular complications. The epidemiology of diabetic nephropathy has been best studied in patients with type 1 disease, since the time of clinical onset is usually known. Approximately 20 to 30 percent will have Microalbuminuria after a mean duration of diabetes of 15 years[1,2], The reported prevalence of Microalbuminuria among patients with type 2 diabetes approximately 10 years after the diagnosis ranges from 25 to 40 percent and it varies with ethnicity being higher in Asians and Hispanics than in whites[3,4,5,6].

Diabetic nephropathy is a dreaded disease with progressive and continuous deterioration in glomerular function resulting in irreversible renal failure. Diabetic nephropathy is an important cause of morbidity and mortality and is now among the most common cause of end stage renal disease. However there is an early phase of diabetic renal disease called incipient diabetic nephropathy. In this stage there is a rise in urinary excretion of albumin i.e. Microalbuminuria. But the rise is detectable only by use of sensitive assays for urinary albumin. At this stage urine is negative for macro albumin and renal function is normal by standard clinical tests. 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.

Microalbuminuria can be measured qualitatively by several methods. Among them radioimmunoassay was the first and most widely used method [7], later various other methods were developed for detection of Microalbuminuria. In our study we have used Micral test for estimation of Microalbuminuria. Micral test (Boehringer Mannheim, Germany) is a dipstick method of estimation of Microalbuminuria. Test principle is immunochemical in nature. Sensitivity of Micral test was 93% ad its specificity was 93% when compared to radioimm unoassay in a study by Gilbert PE et al[8]. Micral test has also been compared with immunoturbidimetricassay and radioim munoassay methods. In all studies Micral test is comparable in sensitivity and specificity to the other methods of estim ation of Microalbuminuria. Patients with nephropathy and type 1 diabetes mellitus almost always have other signs of diabetic microvascular disease, such as Retinopathy and Neuropathy[9]. The relationship between diabetic Nephropathy and Retinopathy is less predictable in type 2 diabetes. In one study of 35 patients with diabetes and significant proteinuria (>300 mg/day), 27 (77 percent) were found to have diabetic Nephropathy by biopsy[10], diabetic retinopathy was present in 15 of the 27. (56 percent)

The aim of this study was to study the presence of Microalbuminuria in patients with Type 2 diabetes mellitus and to find out its association with the duration of diabetes mellitus, Retinopathy, BMI (body mass index) and its relation to Hypertension in these patients.

MATERIALS AND METHODS:

One hundred patients of Type 2 Diabetes mellitus admitted in our centre were studied. Some patients were diagnosed in the Outpatient department; Others were diagnosed elsewhere and presented with fever were admitted and evaluated, their

glycemic control was varied from good to poor at presentation (Microalbuminuria was estimated only after adequately controlling their blood sugars and hypertension with appropriate measures). Patients were included in the study based on WHO criteria for diagnosis of diabetes mellitus which is

1) Symptoms of diabetes mellitus plus a random glucose concentration >200 (11.1mmol/l). The classic symptoms of diabetes mellitus include polyuria, polydipsia and unexplained weight loss OR
2) Fasting blood glucose >126 mg/dl (7.0mmol/l). Fasting is defined as no caloric intake for at least 8 hours. OR 3) 2 hour post prandial glucose > 200mg/dl (11.1 mmol/l). Among diabetics, the above criteria were considered to include the patients for the study. The patients were excluded if they have macroalbuminuria, congestive cardiac failure, urinary tract infection, pregnancy, uncontrolled hypertension and chronic kidney disease (CKD).

The selected patients were studied in detail with history and physical examination. Hypertension was said to be present when there was a history of hypertension or the systolic blood pressure was recorded greater than 160mm of Hg and/or diastolic pressure greater than 90 mm of Hg on 3 consecutive occasions.These subjects received insulin treatment for the control of diabetes and hypertension was controlled by appropriate anti hypertensive medication, before the estimation of microalbuminuria. The following investigations were done in all the patients. Microalbuminuria was estimated by Micral test, fasting and postprandial blood sugar, Glycosylated hemoglobin (HbA1C), blood urea and serum creatinine, fasting lipid profile, urine routine and culture, Electrocardiogram. Whereas Ultrasonography of the abdomen, an echocardiogram and chest x-ray were done in selected cases only. Retinopathy examination was done by same qualified Ophthalmologist throughout the study and spectrum of retinopathy ranges from mild to severe NPDR or PDR.

ESTIMATION OF MICROALBUMINURIA BY MICRAL TEST:

All patients having overt macroalbuminuria detected by albustic were excluded from the study. Micral test, an immunological rapid dip stick semi qualitative technique for detection of microalbuminuria, was used for estimation of microalbuminuria.

MICRAL TEST COMPONENTS:

1 test strip contains monoclonal antibodies against human albumin (Immunoglobulin G) labeled with colloid gold
2. 2mg, fixed albumin 7.7 mg

TEST PRINCIPLE:

There is a serial arrangement of several reagent pads, which are in fluid communication by a reaction controlling chromatographic process. This step combines one step handling with a complex chemistry. The single reaction steps are as follows:

1) Urine of the sample is transported through the wick fleece to the buffer fleece, where acidic urine is adjusted to the proper pH.
2) Upon entering the conjugate fleece the antigen – antibody reaction takes place Albumin of the sample is specifically bound to a soluble conjugate of antibodies and marker enzyme resulting in an antigen – conjugate complex.
3) The excess antibodies are bound to immobilized albumin on the capture matrix and removed from the sample in this way.
4) Only the complex of conjugate with sample-albumin reaches the substrate pad. Here the colour reaction takes place, the marker enzyme B-Galactosidase cleaves off the purple dye chlorophenol red from the Yellow substrate (chlorophenol red galactoside) in a kinetic reaction. The intensity of the colour produced is proportional to the albumin concentration in the urine.

SPECIMEN COLLECTION:

All patients were afebrile during the course of collection of urine and were kept at rest during the collection of urine. Urine of the patient was first tested for albumin by albustix method. Patients who were negative for albumin by the albustix method were only included in this study.

First morning mid stream urine sample that was collected in a sterile container was used for determining Microalbu minuria. The Test strip was immersed in urine such that fluid level was between the two black bars provided on the strips. The strip was withdrawn after 5 seconds. The strip was placed horizontally across the urine vessel and colour change in the test zone was compared with colour scale after one minute. Sensitivity of the kit is 0.4ng/ml and measuring range is 0.8 to 10ng/ml.

RESULTS AND ANALYSIS

Table 1 shows the age and sex distribution of patients.

Table 1: Age and sex distribution

Age in yrs	Male		Female		Total
	No	%	No	%	No
40-50	23	40.26	16	37.21	39
51-60	15	26.32	16	37.21	31
61-70	14	24.56	6	13.95	20
>70	5	8.77	5	11.63	10
Total	57	100	43	100	100
Mean age±SD	54.82±12.38		54.82±11.08		54.87±11.59
P value	Mean age between male and female=0.965				

39 patients were in the age group between 40-50 years, amo ng whom 23 were male,16 were female patients. 31 patients were in the age group between 51 and 60 years, among whom 15 were male and 16 were female patients. 20 patients were in the age group between 61 and 70 years, among whom 14 were male and 6 were female patients. 10 patients were in the age group greater than 70 years, among whom 5 were male and 5 were female patients. The mean age of male patients in the study was 54.82 ± 12.38 years and that of the female patients was 54.82 ±11.08 years. The mean age of detection of diabetes mellitus among the male patients was 48.84± 10.11 years and in the patients was 48.75 ± 8.68 years. (Figure 1)

The mean age between male and female is not statistically significant with p=0.964.

Table 2: Number of patients with Microalbuminuria and Retinopathy

Microalbuminuria		Retinopathy	
-	+	-	+
62	38	56	44

There were 62 patients negative for Microalbuminuria.38 patients were positive for Microalbuminuria. There were 56 patients negative for Retinopathy, 44 patients were positive for Retinopathy. (Figure 2)

Table 3:Association of age with Microalbuminuria and Retinopathy

Age in years	Number of patients	Microalbuminuria		Retinopathy	
		-	+	-	+
41-50	39	31	8	29	10
51-60	31	20	11	19	12
61-70	20	10	10	8	12
>70	10	1	9	-	10

Among 39 patients in the age group between 40-50 years,8 patients had Microalbuminuria and 10 patients had Retinopathy. 31 patients were in age group between 51-60 years, among them 11 patients had Microalbuminuria and 12 patients had Retinopathy.20 patients were in the age group between 61 -70 years, among whom 10 patients had Microalbuminuria and 12 patients had Retinopathy.10 patients were in the in the age group of above 70 years , among whom 9 patients had Microalbuminuria and all 10 were positive for Retinopathy. (Figure 3)

Incidence of Microalbuminuria is more likely for the age group above 50 years of age as compared to the age group <50 years of age with p=0.053.Incidence of Retinopathy is more likely for the age group above 50 years of age as compared to the age group <50 years of age with p=0.001.

Table 4: Association of HbA1C with Microalbuminuria and Retinopathy

HbA1c	No of patients	Microalbuminuria		Retinopathy	
		-	+	-	+
<6.5	22	16	6	18	4
6.5-7	22	17	5	18	4
7-7.5	16	12	4	8	8
>7.5	40	17	23	12	28

22 patients had HbA_1C values less than 6.5% among them 6 were positive for Microalbuminuria and 4 were positive for Retinopathy. 22 patients had HbA_1C between 6.5% and 7%among them 5 were positive for Microalbuminuria and 4 were positive for retinopathy.16 patients had HbA1C levels between 7.0% and 7.5% among them 4 were positive for Microalbuminuria and 8 were positive for Retinopathy. 40 patients had HbA1c values more than 7.5%, among them 23 were positive for Microalbuminuria and 28 were positive for Retinopathy. (Figure 4)

The association of Microalbuminuria and Retinopathy with HbA1C is significant with P values 0.016 and 0.001 respe ctively.

Table5: Association of BMI with Microalbuminuria and Retinopathy

Body mass index(kg/m^2)	Number of patients	Microalbuminuria		Retinopathy	
		-	+	-	+
<25	78	53	25	49	29
>25	22	9	13	7	15

78 patients had a BMI less than $25kg/m^2$, out of them 25 patients were positive for Microalbuminuria and 29 patients were positive for Retinopathy.

22 patients had a BMI above 25 kg/m^2, out of them 13were pos itive for Microalbuminuria and 15 were positive for Retino pathy. The association of Microalbuminuria and Retinopathy with BMI is significant with P values 0.02 and 0.01 respectively. (Figure 5)

Table 6: Association of hypertension with incidence Microalbuminuria and Retinopathy

	Number of patients	Microalbuminuria		Retinopathy	
		-	+	-	+
Non Hypertensives	77	57	20	54	23
Hypertensives	23	5	18	2	21

23 patients had Hypertension, among them 18 were positive for Microalbuminuria and 21 patients were positive for Retinopathy. The association of Microalbuminuria and Retinopathy with hypertension is significant with P values <0.005 and<0.001 respectively. (Figure 6)

Table 7: Association between microalbuminuria and retinopathy

	Microalbuminuria +	Microalbuminuria -
Retinopathy +	31	13
Retinopathy -	7	49

31 subjects had evidence of both Microalbuminuria and Retinopathy,13 patients had retinopathy without microalb uminuria,7 patients had only Microalbuminuria without Retinopathy. The association between Microalb uminuria is significant with P value of <0.001. (Figure 7)

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 the duration of the diabetes. The clinical markers of the generalized endothelial dysfunction become manifest in several forms.

Microalbuminuria marks the onset of endothelial dysfunction related to the kidney. Since its original description by Mogensen, the estimation of Microalbuminuria has been 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 and cardiovascular problems including coronary artery disease and hypertension. 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%.[11,12,13,14] A slight increase in the percentage of Microal buminuria in our study can be attributed to several factors such as, large number of elderly patients, longer duration of diabetes and poor glycemic 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 and Retinopathy tended to be 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 beta cell function, which occurs with increasing duration of diabetes, is likely to contribute to worsening of glycemic control. Poor values of HbA1c are known to be associated with increasing incidence of Microalbuminuria and Retinopathy. In our study only 11 out of 44 patients who had a normal HbA1c (< 7.0%) manifested Microalbuminuria, whereas with HbA1c values more than 7, 27 out of 56 (nearly 50%) had Microalbuminuria. Also nearly 64% (36/56) patients with HbA1c >7 had evidence of Retinopathy. It is seen from the above result that even small increments of HbA1cmore than 7.0% result in almost doubling of the incidence of Microalbuminuria and Retinopathy.

This study has also brought out a significant association of Microalbuminuria with body mass index of more than 25kg/m2. Of the 22 patients with BMI of more than 25, 13had Microalbuminuria (52%). 15 out of 22 (68%) patients with BMI > 25 had Retinopathy. Similar findings have been brought forth by other studies[12,13,14].

The possible explanation for this could be 1) Increasing body mass index is a reflection of insulin resistance which in turn leads to endothelial dysfunction and Microalbuminuria. 2) Associated hypertension may also be responsible for Microalbuminuria and retinopathy. 3) Poor glycemic control which in turn is an outcome of insulin resistance is also held responsible. There was a significant correlation between the

prevalence of Microalbuminuria, Retinopathy and the presence of hypertension. In our study 23 patients had hypertension, among them 18 patients had Microalbuminuria and 21 patients had Retinopathy. In our study of 100 patients 31 patients had evidence of both Microalbuminuria and Retinopathy, 13 patients had retinopathy without Microalbuminuria,7 patients had only Microalbuminuria without evidence of Retinopathy. Thus Retinopathy and Microalbuminuria have a high concordance rate.

LIMITATIONS OF THE STUDY:

The Study was done in a small group, so we require large scale studies to prove this association. Microalbuminuria estimation was done by Micral test, not by the gold standard radioimmunoassay method and it was measured only once in our patients.

CONCLUSIONS:

Microalbuminuria and Retinopathy show a direct relationship with increasing age of patients. An HbA1c value above 7 % is associated with increasing incidence of Microalbuminuria and Retinopathy. Patients with a BMI of more than 25 kg/m^2 have a significant increase in the incidence of Microalb uminuria and Retinopathy. The incidence of Microal bu minuria and Retinopathy is significantly associated with presence of hypertension and there is a significant association between the presence of Microalbuminuria and Retinopathy.

Figure1 : Age and sex distribution.

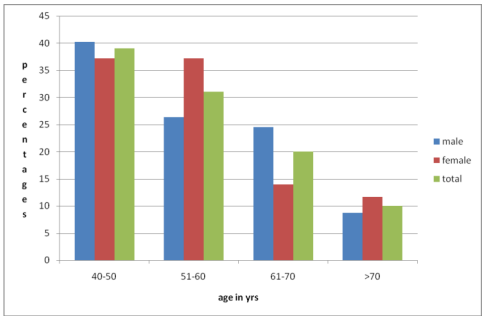


Figure 2: Number of patients with microalbuminuria and retinopathy.

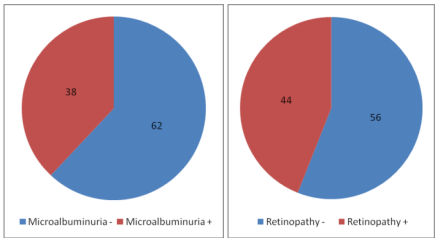


Figure 3: Association of age with microalbuminuria and retinopathy.

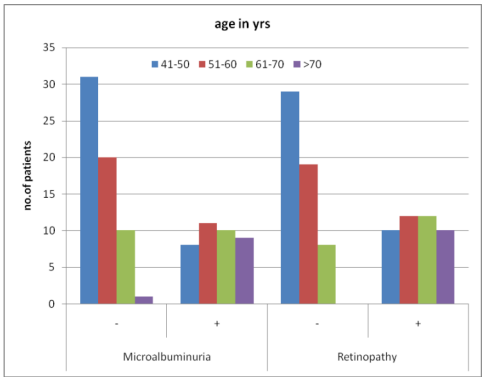


Figure 4: Association of HbA1c with microalbuminuria and retinopathy.

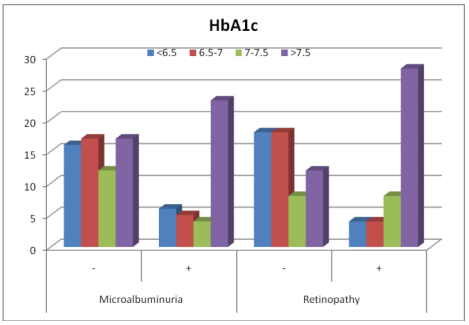


Figure 5: Association of BMI with microalbuminuria and retinopathy.

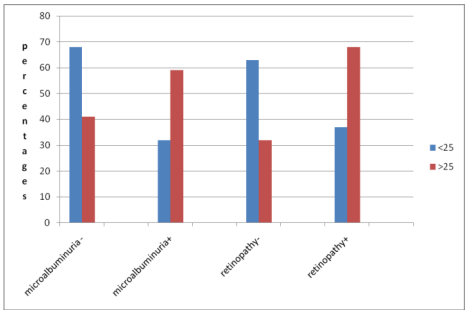


Figure6: Association of hypertension with incidence microalbuminuria and retinopathy

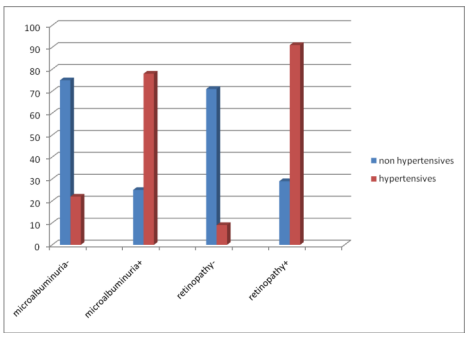
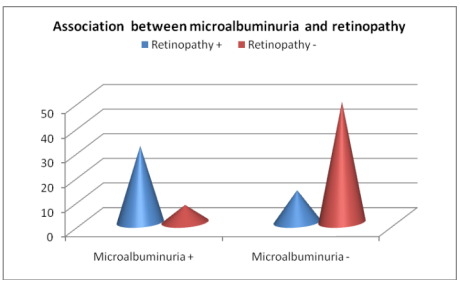


Figure 7: Association between microalbuminuria and retinopathy.



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