High Risk Factors for Elevated Excretion of Albumin In Diabetic Subjects

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Abstract: Background: Microalbuminuria refers to the excretion of albumin in the urine at a rate that exceeds normal limits but is less than the detection level for traditional dipstick methods and is considered as a marker of diabetic nephropathy. Aims: To establish the prevalence of elevated urinary albumin levels (microalbuminuria) in a sequential sample of diabetic patients and to determine its relationship with known and putative risk factors, to ascertain relationship of serum angiotensin converting enzyme (ACE) activity with diabetic incipient nephropathy. Study design: This cross-sectional analytical study included 100 control and 325 diabetic patients (180 type 2 and 145 type 1 diabetic patients) subjects attending outpatient department of the hospital. Patients having clinical albuminuria and with other causes of proteinuria were excluded. Result: Microalbuminuria was observed in 34.48% in patients with type 1 and 28.33% in patients with type 2 diabetes mellitus respectively. Having the condition was significantly associated with advanced age, poor glycaemic control, dyslipidemia (with respect to total cholesterol, triglycerides and LDL-C), smoking, body mass index and coexisting hypertension. The duration of diabetes was a significant correlate in type 1 DM subjects only. No significant association with gender, HDL-C levels, age at onset of DM, mode of treatment, socio-economic status and other lifestyle variations was found. All clinical and biochemical parameters in patient with microalbuminuria was more adversely affected than patients with normoalbuminuria. Serum angiotensin converting enzyme (ACE) levels were significantly elevated ($P<0.001$) in both of the diabetic groups, moreover, its levels were higher in subjects with microalbuminuria than in those without this complication ($P<0.05$). Conclusions: Microalbuminuria in diabetes, which represents an earlier phase in the development of clinical nephropathy, is associated with many potentially modifiable risk factors. In estimating diabetic nephropathy risk, AER is most important and should be done frequently but there are gains to be made in predictive precision by considering family history, smoking habits, glycemia, B.P.,BMI lipid levels and ACE activity. Early screening for incipient diabetic nephropathy and aggressive management of these risk factors is important in optimising the renal outcome of patients with diabetes mellitus. [Parchwani D et al NJIRM 2012; 3(1) : 1-7]

Key Words: Diabetes mellitus, Microalbuminuria, High risk factors, Angiotensin converting enzyme

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Introduction: Diabetic nephropathy is the major cause of morbidity and mortality in patients with diabetes mellitus. This complication is first manifested as an increase in urinary albumin excretion (microalbuminuria) which progresses to overt albuminuria and then to renal failure[1]. Detection of microalbuminuria is important from a clinical standpoint because, once detected, it is an indication for initiation of appropriate therapy for the purpose of preventing or delaying the advance of progressive diabetic nephropathy. Despite the degree of interest shown in the detection of microalbuminuria and their prognostic implication, only recently has there been greater focus on the determinants of elevated urinary excretion of albumin but many questions in this field remained unanswered. A better understanding of such factors may influence the nature of therapeutic intervention in patients with or even before development of renal impairment. Thus in present study an attempt has been made to establish the prevalence of elevated urinary albumin levels (microalbuminuria) and to determine the relationship between microalbuminuria and known and putative risk factors. In addition, serum angiotensin converting enzyme (ACE) level was investigated to ascertain its relationship with diabetic incipient nephropathy.

Material and Methods: This study was designed to determine the prevalence of microalbuminuria and the associated risk factors in patients with diabetes mellitus. 425 subjects with either sex (283 males and 142 females) of varying age were included and were categorized into 3 main groups which include:
GROUP I : healthy controls.

n = 100 (61 males and 39 females)
GROUP II : type 2 diabetes mellitus.
n = 180 (120 males and 60 females)
GROUP III : type 1 diabetes mellitus.
n = 145 (102 males and 43 females)

Exclusion criteria were:

- Patients with overt diabetic nephropathy.
- Patients who visited the pregnancy clinic or who had given both within the preceding six weeks.
- Patients who were taking drugs which are known to influence angiotensin converting enzyme.
- Lack of approval by physician.
- Pre-existing macro vascular condition (such as CVD).
- Urinary tract infection or recent intercurrent illness.
- Subjects showing disinterest.

Disease classification: The history of diabetes mellitus was based on patient self report of a prior physician diagnosis and was treatment under oral anti-diabetic agents and/or insulin. Hypertension was defined as self-report of a physician diagnosis. All subjects were studied as outpatient. Participant's examination included interviews for medical and nutritional history. Present and past history of each case was recorded in detail on a pre-tested proforma regarding their general information i.e. name, age, sex, address, religion, occupation, economic status, nutritional and personal habits, education and history suggestive of any systemic illness. Each subject was then examined for various anthropometric parameters: Weight (Kg) and height (metres) were measured in normal light clothing without shoes. Body Mass Index (BMI) was calculated by Weight (Kg) / height squared (m^2).

Urine collection: All 425 subjects were asked to collect a 24 hour urine sample for analysis of albumin excretion. Urine collection was carried out during unrestricted daily life activity. The urinary albumin concentration was determined by Micral test[2] using commercially available assay kits from Roche Diagnostics (Mannheim, Germany). Normoalbuminuria was defined as Albumin Excretion Rate (AER) < 30 mg/24 hr, and Microalbuminuria as AER 30- 300 mg/24 hr. Results were confirmed after 2 measurements done in a space of 6 months. If the results of a 2nd measurement placed the patient in a different category from that based on the first measurement, a 3rd urine sample was obtained to confirm either the first or second measurement.

Collection of blood samples of patients and controls: 10 ml of blood from the control as well as from the patients was withdrawn with an aseptic technique and was collected in plain, sodium fluoride – citrate, and heparin vial. Serum and plasma was separated by centrifugation of blood sample and were subjected for analytical procedures. Glucose[3], cholesterol[4], triglycerides[5], HDL-C[6], $HbA1_C$[7], angiotensin converting enzyme[8], creatinine[9] were estimated. LDL and VLDL cholesterol[10] were calculated. This study was carried out from November 2009 to May 2011 and was approved by Institutional Ethical Committee.

Statistical analysis: Data analyses were performed with the SPSS statistical software. The results for continuous variables are mean ± SD. The two tailed (unpaired) student's test for independent samples, analysis of variance (ANOVA) was used, in assessment of the significance of difference between group means. The Chi square test was used for evaluating differences in proportions between groups. For all analyses, the nominal level of statistical significance was<0.05.

Result: 325 patients (145 type 1 DM and 180 type 2 DM) were evaluated for Albumin Excretion Rate (AER). Microalbuminuria was observed in 34.48% in patients with type 1 DM and 28.33% in patients with type 2 DM respectively. The trend was similar when the sexes were examined separately. In both types of diabetes, HbA_{1C} levels, hypertension, dyslipidemia (with respect to total cholesterol, triglycerides and LDL-C), age, obesity and smoking habits were significantly related to microalbuminuria. The duration of diabetes was a significant correlate in type 1 DM subjects only. However, no relation of microalbuminuria with gender, HDL-C levels, age at onset of DM, mode of treatment, socio-economic status and other lifestyle variations was found.

Mean values of selected variables according to urinary albumin concentration were compiled (Table 1).

Table1: Selected characteristics of all subjects.

Parameters	Control (n=?)	NAU* (n=?	MAU† n=?
Total cholesterol (mg/dl)	171.6±14.7	204.3±46.6	242.1±37.8‡
Triglycerides (mg/dl)	102.2±12.9	195.7±91.0	245.6±82.4‡
HDL-C (mg/dl)	49.6±6.2	46.2±5.7	42.6±4.5
LDL-C (mg/dl)	96.9±16.4	119.2±46.1	150.3±37.6‡
VLDL-C (mg/dl)	20.4±2.5	39.1±18.3	49.1±22.5‡
TC/HDL-C	3.2±0.6	4.7±1.8	5.9±1.6‡
LDL-C/HDL-C	1.8±0.5	2.8±1.5	3.8±1.5‡
Glucose-F(mg/dl)	76.9±10.3	128.5±16.1	170.2±21.6§
Glucose-2hrPP(mg/dl)	95.2±18.0	173.7±17.3	240.6±16.8§
HbA1C (%)	5.1±1.2	7.6±1.7	8.6±1.2§
BMI(kg/m^2)	22.4±1.8	24.1±2.3	26.2±3.6‡
ACE(U/L)	18.4±2.3	40.7±3.0	44.4±4.7‡
BP-systole (mm/Hg)	122.2±10.2	136.4±16.4	144.8±16.2‡
BP-diastole (mm/Hg)	78.4±6.2	84.4±10.6	88.6±8.2‡
Age(years)	44.2±10.0	42.8±8.7	49.6±7.5‡
Duration of DM	-	6.6±4.0	9.6±5.2‡

*NAU=Normoalbuminuria,†MAU=Microalbu-inuria, ‡p<0.05(MAU Vs NAU), §p<0.01(MAU Vs NAU)

The data definitely indicate a microalbuminuria related differences in lipid parameters and endothelial dysfunction (reflected by increased ACE activity). These observations partly explain why patients with microalbuminuria are at higher risk of developing cardiovascular disease. Despite being under treatment these patients did not had adequate control of sugar and might be responsible for exaggerate lipid profile.

Discussion: Microalbuminuria was observed in 34.48% in patients with type 1 DM and 28.33% in patients with type 2 DM respectively. Various epidemiological and cross sectional studies have reported marked variation in the prevalence of microalbuminuria (from 7% to 42%)[11,12,13,14]. So, first part of the study resulted in two subgroups namely normoalbuminuric and microalbuminuric patients.

The patients were further segregated on the basis of gender, age, glycaemia status, blood pressure status, residence, socio-economic status, work, personal habits, dietary habits, weight, levels of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, duration of diabetes to ascertain any possible relationship with microalbuminuria (Table 2 & 3)

Relationship between prevalence of microalbuminuria and various parameters:

Glycemic status (measured as HbA_{1C}): HbA_{1C} an integrated measure of the level of glycemia, was positively associated with the prevalence of abnormal albumin excretion among diabetic patients, it was observed that patients who had HbA_{1C} levels <8%, the risk of microalbuminuria, varied little, although it was higher than in control subjects. In contrast, in patients with HbA_{1C} values above 10% the risk of microalbuminuria rose steeply (Table 2).

Table 2: **Prevalence of microalbuminuria in diabetic subjects according to blood pressure, glycemic status and duration of disease.**

	Type 2 DM*			Type 1 DM*		
	n	MAU† n(%)	Odds Ratio	n	MAU† n(%)	Odds Ratio
B.P.						
Normal	80	12(15)	1.00	59	10(16.9)	1.00
Borderline	68	20(29.4)	2.40	58	23(39.6)	2.40
Hypertensive	32	19(59.3)	4.75	28	17(60.7)	4.75
HbA_1C						
<8%	107	19(17.7)	1.00	81	15(18.5)	1.00
8-10%	37	11(29.7)	2.69	37	18(48.6)	2.87
>10%	36	21(58.3)	9.74	27	17(62.9)	11.7
Duration of DM						
<5 years	71	15(21.1)	1.00	51	7(13.7)	1.00
5-10 years	67	25(37.3)	2.98	59	25(42.3)	3.03
>10 years	42	11(26.1)	1.56	35	18(51.4)	3.99

***DM=Diabetes Mellitus, † =Microalbuminuria**

These results are consistent with those of DCCT (Diabetes Control and Complications Trial)[15]. The distinctly different risks of microalbuminuria in patients with low HbA_{1C} values and those with high values suggest that diabetes damages the kidney through several mechanisms. The mechanism operating below the HbA_{1C} value 8% seem to be independent of the level of hyperglycemia and may be influenced by other components of the diabetic

milieu — such as, abnormalities in plasma insulin concentrations[16]. At high HbA_{1C} values, which are indicative of high blood glucose concentrations, microalbuminuria is most likely caused by the deleterious effects of hyperglycemia on cell functions and extracellular structures such as hypertrophy and basement membrane thickening[17], increased endothelial cell permeability to albumin[18], increased matrix protein synthesis[19]and increased production of vasodilatory prostaglandins, which contribute to renal hyper perfusion, intraglomerular hypertension, and increased hyperfiltration[20].

Hyperglycemia also causes the release of cytokines such as transforming growth factor - β, by glomerular endothelial, epithelial, mesangial, and tubular cells, which result in mesangial hypertrophy[21]. Elevated glucose levels also cause production of advanced glycosylation end products, which alter structural proteins and cause vascular dysfunction, glomerular lesions, proteinuria and renal failure[21].

Blood pressure: A relationship between excessive albumin excretion and blood pressure has been described previously in non-diabetic subjects[22]. In the present study, higher mean blood pressure was associated with higher prevalence rates of microalbuminuria (Table 2). The biological meaning of the relationship between blood pressure and diabetic renal disease, however, is difficult to assess in cross-sectional studies because elevated blood pressure may cause abnormal albumin excretion, but may also be a consequence of progressive diabetic renal disease. Schmitz et al[22] found that type 1 diabetic subjects with normal urinary albumin excretion rates, who progressed to incipient nephropathy developed significantly higher blood pressure than subjects who remained normal. In the Pima Indians, however, elevated blood pressure before the onset of diabetes has been shown to predict elevated albumin excretion after the onset of diabetes[23]. Thus, higher blood pressure seen in diabetic nephropathy is not entirely the result of the renal disease, but may precede and contribute to it. The mechanism by which increased B.P. damages kidney includes hyperfiltration and hemodynamic abnormalities, which contribute to the development of glomerular damage and microalbuminuria[19].

Duration of diabetes mellitus: The duration of diabetes mellitus was a significant correlate of microalbuminuria in type 1 subjects but not in type 2 subjects (Table 2):

(1) Prevalence of microalbuminuria increased with increase in duration of type 1 diabetes mellitus.

(2) The prevalence of microalbuminuria in type 2 DM was highest for diabetic subjects with a known duration of diabetes of 5-15 years, and then it decreased for subjects with longer duration.

These findings for type 1 and 2 DM are conflicting, perhaps because it is impossible to accurately date the onset of type 2 DM – known duration is at best a crude underestimate. Similar results were reported by Suzuki et al[24] who showed a relationship between urinary albumin excretion and diseased duration in patients with type 1 DM but not with type 2 DM.

Association with serum lipid levels: Lipid abnormalities are strongly associated with diabetic nephropathy. Prevalence of microalbuminuria increased with increase in dyslipidemia (with respect to Total cholesterol, Triglyceride and LDL-C); however it was independent of HDL-C levels.(Table3). The plasma lipid levels have emerged as potentially important predictors of diabetic nephropathy risk. Although not differently expressed at baseline in the steno type 1 diabetes cohort study[25], serum cholesterol, later on, was statistically significantly higher before microalbuminuria onset than in patients who still had normoalbuminuria. This was also true in the Joslin clinic study, in which serum cholesterol and triglyceride levels were independent predictors of return from microalbuminuria to normoalbuminuria after 6 year of follow up[26].

Smoking: Current smoking was significantly associated with the prevalence of microalbuminuria in present study (Table 3). Although this finding is consistent with the results of previous studies[27,28]. In this study it was also found that smokers had poorer glycemic control than non-smokers. These results indicate that hyperglycemia (as reflected in a higher HbA_{1c}) and current smoking may impact the risk of microalbuminuria through different pathophysiological mechanisms. An evidence to support this view that the effect of smoking on the risk of microalbuminuria is magnified in patients

with poor glycemic control, is that of DCCT investigators [15] who found higher risk for current smokers in conventional treated group (HbA_{1c} 9.0%) than patients (smokers) treated with intensive regime (HbA_{1c} 7%). Baggio et al[27], have reported in a large group of patients with type 2 diabetes, most of who had microalbuminuria that cigarette smoking was an independent determinant of increased GBM width, providing a possible explanation for the link between smoking and abnormal AER.

Other factors : A progressive increase in the prevalence of microalbuminuria with advancing age and BMI was observed, however no significant difference in prevalence of microalbuminuria with gender and any life style variation (i.e. levels of income, dietary habits, physical activity or different residing areas) was observed.

Serum ACE levels were elevated in diabetic subjects compared with control populations and an additional significant increase was noted in patients with microalbuminuria (Table 1). This diabetes-related ACE elevation confirms previous findings[29,30]. Angiotensin converting enzyme is secreted by the vascular endothelium and increased serum activity may reflect endothelial damage. Although a pathogenetic role for these enzymic alterations is not proven, they provide support for a role of ACE in modulating renal hemodynamic alterations in diabetic subjects The presence of endothelial dysfunction (reflected by increased serum ACE activity) in normoalbuminuric diabetic patients suggests it could precede microalbuminuria and thus may serve as an early risk marker for later development of overt diabetic nephropathy in diabetic patients.

Thus high risk patients are those with the following characteristics(Table 3): B.P. > 160/95 mmHg, total cholesterol > 240 mg/dL, triglycerides > 200 mg/dL, LDL-C > 150 mg/dL, HbA_{1c} > 10%, older age, longer duration of diabetes(In type 1 DM only), obese, smoking habits and increased ace activity.

Conclusion: Findings of this study have implications for the care of patients with diabetes. Patients and care providers should give the highest priority to improve glycaemia control sufficiently to maintain

Table3:Association of various parameters with risk of microalbuminuria in diabetic subjects.

Parameter	Type 2 DM		Type 1 DM	
	No. of patient	No(%)with MAU*	No. of patient	No(%)with MAU*
Male	120	35(29.16)	102	37(36.27)
Female	60	16(26.66)	43	13(30.23)
<50 years	162	43(26.54)	121	38(31.4)
≥50 years	18	08(44.44)†	24	12(50.0)‡
Non smoker	105	20(19.04)	83	20(24.09)
Smoker	75	31(41.33)‡	62	30(48.38)‡
B.P. <160/95	148	32(21.62)	117	33(28.20)
≥160/95	32	19(59.37)‡	28	17(60.71)‡
HbA_1C<10%	144	30(20.83)	118	33(27.96)
HbA_1C>10%	36	21(58.33)‡	27	17(62.96)‡
TC<240mg/dl	128	21(16.4)	112	29(25.89)
TC≥240mg/dl	52	30(57.69)‡	33	21(63.63)‡
TG<200mg/dl	137	30(21.89)	104	27(25.96)
TG≥200mg/dl	43	21(48.83)‡	41	23(56.09)‡
LDL-C				
<150mg/dl	148	32(21.62)	102	22(21.56)
≥150mg/dl	32	19(59.37)‡	43	28(65.11)‡
HDL-C				
<35mg/dl	29	10(34.48)	11	04(36.36)
≥35mg/dl	151	41(27.15)	134	46(34.32)
BMI<25kg/m^2	133	35(26.31)	114	37(32.45)
BMI≥25kg/m^2	47	16(34.04)†	31	13(41.93)†
Duration of				
DM. <5 years	71	15(21.12)	51	07(13.72)
≥ 5 years	109	36(33.02)	94	43(45.74)‡
Urban society	136	39(28.67)	114	37(32.45)
Rural society	44	12(27.77)	31	13(41.93)
Vegetarian	102	29(28.43)	89	32(35.95)
Non veg.	78	22(28.20)	46	18(39.13)
Middle class	54	16(29.62)	50	18(36.0)
Upper middle + upper class	126	35(27.77)	95	32(33.68)
Physical activity				
Sedentary	83	24(28.91)	73	27(36.98)
Moderate to severe	97	27(27.83)	72	23(31.94)†

*MAU: microalbuminuria, †p<0.05, ‡p<0.01

HbA_{1c} values below 8% along with other modifiable risk factors such as hypertension, smoking, and lipid levels. If this can be achieved, the number of patients, in whom microalbuminuria develops, should decline. Substantially, this should, in turn, lower the number in whom overt macroalbuminuria and end-stage renal disease develop. Although the concept of microalbuminuria was a major advance in this field, it is also suggested in studies that the

predictive precision of this test alone is not adequate for a disease of such dire consequences. What is argued here is that we can do better in estimating diabetic nephropathy risk by looking at the whole patient, not just their AER measurements. Although AER is important and should be measured frequently, there are gains to be made in predictive precision in considering family history, smoking habits, glycemia, ACE activity, BMI,B.P. and lipid levels.

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