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Biochemistry

Effect of Age and Diabetic Duration on Levels of Microalbuminuria among Type 2 Diabetic Patients

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Abstract: Out of 10, one adult will encompass diabetes by 2030; facts indicate that the figure of persons inhabiting with this disorder is anticipated to go up from 366 million in 2011 to 552 million by 2030, if no critical act is undertaken. During the first 5 years of diabetes, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial volume expansion occur as the GFR returns to normal. After 5–10 years of type 1diabetes, nearly 40% of individuals begin to excrete small amounts of albumin in the urine.[1] Microalbuminuria is present in 20-30% of all patients with type 2 diabetes mellitus, and is especially common in those with hypertension, endothelial dysfunction and other features of insulin resistance. Thus, the present study was conduct to estimate microalbuminuria among type 2 diabetes mellitus and to correlate age and diabetic duration with the levels of microalbuminuria among such patients. The case-control study was conducted in the Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana (Ambala), India by taking fifty diagnosed cases of type 2 diabetes mellitus and fifty age and sex matched apparently healthy controls. It was found that the prevalence of microalbuminuria was 52 %. In type 2 diabetic patients, mean FPG and microalbuminuria were higher as compared with healthy controls. Thus, there was significant difference ($p<0.001$) in levels of FPG and microalbuminuria. Further, there was strong correlation between microalbuminuria and glycemic control. Also, there was significant correlation of microalbuminuria with age and the duration of diabetes in type 2 diabetic patients. With the increase in duration of diabetes, there was significant progression of microalbuminuria to macroalbuminuria (>300 mg/day). Hence, it was concluded that the early determination of microalbuminuria should be implemented in clinical practice for overall risk evaluation, at least in diabetic patients.
Keywords: Type 2 diabetes mellitus, Microalbuminuria, Age, Duration of diabetes, Albumin.

INTRODUCTION

Microalbuminuria is defined as urinary-albumin excretion (UAE) between 30-300 mg/day, if measured in a 24 hour urine collection; 20-200 µg/min, if measured in a timed urine collection or 30-300 mg/gm, if measured with use of urinary albumin to creatinine ratio (UACR) in spot urine collection. Any urinary albumin value below these limits is considered as normal UAE, whereas any value above them reflects the presence of macroalbuminuria or clinical proteinuria [1,2]. Diabetes mellitus (DM) is a state of metabolic disturbance caused by a complex interaction of genetics and environmental factors resulting in hyperglycemia due to reduced insulin secretion, decreased glucose utilization, and increased glucose production. Insulin resistance and abnormal insulin secretion are central to the development of type 2 DM. During the first 5 years of DM, thickening of the glomerular basement membrane, glomerular hypertrophy, and mesangial volume expansion occur as the GFR returns to normal. After 5–10 years of type 1 DM, nearly 40% of individuals begin to excrete small amounts of albumin in the urine [1]. Microalbuminuria is present in 20-30% of all patients with type 2 diabetes mellitus, and is especially common in those with hypertension, endothelial dysfunction and other features of insulin resistance. Although, microalbuminuria is a predictive of worsening micro vascular disease in kidney (5-10% per year progress to overt diabetic nephropathy), an increased albumin excretion rate reflects a

generalized abnormality of vascular function and is associated with 2-4 fold increase in cardiovascular and all-cause mortality. The extent to which microalbuminuria is a risk factor independent of other variables in type 2 diabetes, e.g. blood pressure and smoking, has been highlighted by recent cohort studies, e.g. Heart Outcome Prevention Evaluation Study and The Wisconsin Epidemiological Study [3]. Microalbuminuria in patients with type 2 diabetes is an important predictor of progression for diabetic nephropathy, and a worsening of albuminuria has been reported as associated with a rapid decline in renal function [4]. In patient with DM 2, it has become evident that microalbuminuria is a predictor of cardiovascular death. However, the presence of microalbuminuria in DM 2 may be more reflective of generalized vascular disease than diabetic glomerulopathy. The development of microalbuminuria is associated with established extracellular matrix expansion in the glomerular and tubulointerstitial compartments of the kidney. The natural history results in progressive glomerular sclerosis, increasing proteinuria, chronic renal failure. The long period between the onset of microalbuminuria and chronic renal failure (in years) offers a substantial window for therapeutic intervention [5]. Albumin is a relatively large, negatively charged protein (molecular weight 69 kilo Dalton; size36 Å). The filter through which albumin must pass before entering the urine, the glomerular capillary wall is size and charge selective. Microalbuminuria is thought to be a consequence of an increased albumin leakage through the glomerular capillary wall as a result of increased permeability of the wall, an increased intraglomerular pressure, or both. For example, hyperglycemia and high BP are generally accepted risk factors for development of microalbuminuria. Both can increase intraglomerular pressure. In addition, hyperglycemia can alter the charge selectivity of the glomerular capillary wall, thereby increasing its permeability. In a healthy kidney, >99% of filtered albumin is reabsorbed in the proximal tubules. Some data suggest that microalbuminuria, at least in patients with type 2 diabetes, is associated not only with increased glomerular protein passage but also with an absence of a compensatory increase in tubular reabsorption of albumin. A pronounced increase in albumin filtered by the glomerulus will lead to excessive supply of albumin to the renal tubule, eventually exceeding tubular reabsorptive capacity, and thus to increased albumin excretion in the urine [6]. Thus, present study was designed to assess microalbuminuria among patients of type 2 diabetes mellitus, and to correlate the degree of microalbuminuria with age and diabetic duration among such patients.

MATERIALS AND METHODS

The present case-control study was conducted in the Department of Biochemistry, MMIMSR (Maharishi Markandeshwar Institute of Medical Sciences and Research), Mullana (Ambala), India. Simple random sampling method was adopted for the selection of patients. Type 2 diabetic patients were taken from Medicine outpatient and indoor patient departments. Subjects included for the study were categorized into 2 groups: Group 1 included 50 (fifty) type 2 diabetic patients and 50 (fifty) age and sex matched apparently healthy controls. The cases were diagnosed as type 2 diabetes mellitus with the help of following criteria.

Criteria for diabetes mellitus [1]

Fasting plasma glucose level ≥ 126 mg/dl
Post prandial plasma glucose level ≥ 200 mg/dl.

All the type 2 diabetic patients included in the study were in the age range of 40-60 years. Any patients with urinary infections, any endocrinal disorder like thyroid hormone disorder etc. and gestational diabetes were excluded from the study.

Biochemical Investigations

Fasting blood glucose was measured by glucose oxidase/peroxidase (GOD/POD) method [7]. Glucose oxidase (GOD) converts glucose into gluconic acid. Hydrogen peroxide formed in this reaction, in presence of peroxidase (POD), oxidatively couples with 4-aminoantipyrine / phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm (500-550nm). The intensity of colour is directly proportional to the glucose in specimen.

$$\beta\text{-D Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{POD} \text{Red dye} + H_2O$$

Fasting blood glucose was calculated by the following formula.

$$\text{Glucose in mg \%} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Expected Values

Fasting Blood Glucose	:	60 to 100 mg%
Postprandial Blood Glucose	:	< 140 mg%

24-hours urinary albumin for microalbuminuria was estimated by Pyrogallol red method [8]. Proteins in the test sample form a blue - purple complex when reacted with a combination of Pyrogallol red dye and molybdic acid at p^H 2.2. The concentration of the protein in the sample is obtained by measuring the absorbance at 600 nm and calculated by the following formula.

$$\text{Protein concentration (mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

Normal Range
Urine: 30- 300 mg/day

STATISTICAL ANALYSIS

Data obtained were analyzed as per standard statistical methods. Mean and standard deviation for all parameters was calculated. Correlation between various parameters was calculated by using Pearson correlation coefficient.

RESULTS AND DISCUSSION

Increased excretion of albumin into the urine is thought to occur as a result of increased systemic capillary leakiness in the kidney resulting in increased passage of albumin through the glomerulus. This is believed to occur as a result of endothelial cell injury, but there is also the possibility that increased urinary excretion of albumin occurs owing to decreased reabsorption by the renal proximal tubular epithelial cells. Whichever is the primary mechanism it is now accepted beyond doubt that increased excretion of albumin into the urine carries a significant increased risk of progressive renal disease, whether associated with diabetes or not. As there are potentially different mechanisms associated with the development of increased albumin excretion it is feasible that the development of microalbuminuria in patients with diabetes may be an aggregate of other risk factors or markers and show little independent association with significant clinical outcomes when these other risk factors are adjusted for [9].

The present study of effect of age and diabetic duration on levels of microalbuminuria among type 2 diabetic patients was conducted in 50 diabetic patients and 50 healthy controls and significant differences were found in the two groups in the parameters of sex, age, fasting plasma glucose, duration of diabetes and microalbuminuria and its progression to macroproteinuria. In the present study, all the subjects were between 40-60 years of age. The distribution of all subjects was shown in table 1.

Table-1: Grouping of total subjects according to sex and their number

Group	Sex		Total
	Males	Females	
Healthy Control	30	20	50
Type 2diabetic Patients	21	29	50
Total	51	49	100

In present study, males and females were equally affected with microalbuminuria in the range of 30-300 mg/day, male: female ratio being 1:1. But, males were commonly affected in progression of microalbuminuria to macroalbuminuria i.e. >300 mg/day, male: female ratio being 1.75: 1. (Table 2)

Microalbuminuria in type 2 diabetes mellitus was significant as it was found that out of 50 diabetic cases, 24 cases were microalbuminuric. The prevalence rate of microalbuminuria in the present study was 48 %.(Table 3)

Table-2: Degree of Microalbuminuria

Range of Microalbuminuria (mg/day)	Healthy Control		Type 2 Diabetic Patients	
	Male	Female	Male	Female
Below 30	30	20	9	18
30-300	Nil	Nil	5	7
>300	Nil	Nil	7	4
Total	30	20	21	29

Table-3: Microalbuminuria in Type 2 diabetes mellitus

S.No.	Microalbuminuria	Frequency
1	Positive (≥30 mg/day)	26
2	Negative (<30 mg/day)	24
3	Total	50

These findings are consistent with the other studies. Data from large population-based studies in the United States, Europe and Australia showed that the prevalence of microalbuminuria is 5%–15% in the general population, 20%–30% in diabetics and 11%–17% in patients with hypertension [10]. Microalbuminuria is highly prevalent in several disease states. Widely known is the high prevalence in individuals with diabetes. A recent worldwide survey showed that in 40% of the patients with diabetes and without known kidney disease, the levels of urinary albumin were in the microalbuminuric range [11]. Similar data (20%) were found in a large population study (Australian Diabetes, Obesity, and Lifestyle Study [Aus-Diab]). This Australian population-based study shows that albuminuria is common among patients with established diabetes, is present before the onset of diabetes, and becomes more prevalent with worsening glucose tolerance [12]. The transition from normo- to microalbuminuria is frequent despite adequate treatment: 2 to 2.5% per year [13, 14]. The National Urban Diabetes Study (2000) showed the prevalence of diabetes in a population older than 40 years to be 23.8% in 6 cities in India including Chennai, and more recently, the Chennai Urban Rural Epidemiology Study (2003-2004) estimated the prevalence in those older than 40 years to be 30.1% [15].

The prevalence of microalbuminuria in the general population is in the range of 5 to 7% according to several large cohort studies: Prevention of Renal and Vascular End stage Disease (PREVEND), Nord-Trøndelag Health Study (HUNT), AusDiab [16, 17]. Recent data from PREVEND show that the incidence of an individual's moving from a normoalbuminuric to a microalbuminuric classification occurs at a rate of approximately 8% in 4 years, which is surprisingly close to that of treated diabetes. Most frequently, the individuals moved from high normal albumin levels to microalbuminuria [18].

In type 2 diabetic patients, mean FPG and microalbuminuria were higher as compared with healthy controls (Table 4). Thus, there was significant difference ($p<0.001$) in levels of FPG and microalbuminuria. Further, there was strong correlation between microalbuminuria and glycemic control ($p<0.001$ highly significant), as shown in table 5.

Also, there was significant correlation of microalbuminuria with age and the duration of diabetes in type 2 diabetic patients, as shown in table 6. With the increase in duration of diabetes, there was significant progression of microalbuminuria to macroalbuminuria (>300 mg/day).

Table-4: Comparison of mean value, standard deviation and p value of age, duration, FPG and microalbuminuria between the healthy control and type 2diabetic patients.

Parameters	Group	N	Mean ± SD Deviation	P value
Age	Healthy Control	50	48.48±6.876	>0.001
	Type 2 Diabetic patients	50	51.38±7.920	
Duration	Healthy Control	0[a]	.	.
	Type 2 Diabetic patients	50	58.28±70.181	
FPG	Healthy Control	50	80.30±7.733	<0.001
	Type 2 Diabetic patients	50	189.76±78.494	
Microalbuminuria	Healthy Control	50	13.73±5.372	<0.001
	Type 2 Diabetic patients	50	379.18±691.147	

a. t cannot be computed because at least one of the groups is empty.

Table-5: Correlation between microalbuminuria and glycemic control

Parameter	FPG< 126	FPG> 126	Total
Microalbuminuria <30 mg/day	4	20	24
Microalbuminuria ≥30 mg/day	2	24	26
Total	6	44	50

Chi-square: 49.887, df: 1, $p<0.001$ highly significant.

Table-6: Correlation among type 2 diabetic patients

Parameter		Age	Diabetic Duration	FPG
Microalbuminuria	Pearson Correlation	0.336*	0.940**	0.090
	Sig. (2-tailed)	0.017	0.000	0.533
	N	50	50	50
*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).				

These findings are in consistent with previous studies. Similar findings were also found in other studies [19]. The frequency of microalbuminuria increases with the increase in duration of diabetes**.** Shehnaz *et al.* found significant correlation of microalbuminuria with duration of diabetes [20]. Unnikrishnan *et al* have found significantly higher age of the patient, duration of disease, body mass index (BMI), waist circumference, blood pressure, FBS, HbA1c in case of microalbuminuria and macroalbuminuria when compared with normoalbuminuria [21]. Similarly, Bessie *et al.* [22] have found significantly high level of age, duration of disease, total cholesterol, and triglyceride, LDL-C, HbA1c and FBS. Chronic renal insufficiency is reported twice as frequently in persons with diabetes. A study done in Nepal found that about 18% of the patients admitted for haemodialysis were diabetic. It has been shown that the risk of developing diabetic nephropathy in type 2diabetes is 30–40%. Albuminuria in type 2 diabetic patients is the predominant renal risk marker for nephropathy and cardiovascular complications [23].

CONCLUSION AND FUTURE PERSPECTIVES

The early determination of microalbuminuria should be implemented in clinical practice for overall risk evaluation, at least in all diabetic patients. The test is inexpensive, easy to obtain in the clinical setting and the results are rapidly available. Further research should define in detail the populations that would benefit from this measurement and future guidelines should provide clear recommendations for their clinical use. The renal biomarker albuminuria predicts renal and cardiovascular complications in patients with diabetes beyond the set of traditional cardiovascular biomarkers. The short-term (treatment-induced) change in albuminuria indicates the long-term changes in renal and cardiovascular risk. This feature provides further clinical usefulness to this biomarker.

Limitations to this study are the information such as the glycosylated hemoglobin ($HbA1_c$), hyperhomocysteinemia, body mass index (BMI), serum lipid profiles, and history of smoking, degree of insulin resistance, C - reactive protein, obesity, endothelial dysfunction, and genetic factors. Also the patients included for the present study were taken from those visiting the medicine OPD, and it, probably may not be suitable for the general population.

CONFLICT OF INTEREST

There is no conflict of interest in completing of the research study.

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