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Research Article

MICROALBUMINURIA: AN EARLY DETECTOR OF DIABETIC AND HYPERTENSIVE NEPHROPATHY

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ABSTRACT
Microalbuminuria is presence of albumin in urine above the normal level but below the detectable range of conventional urine dipstick methods. Its presence is an early marker of diabetic and hypertensive nephropathy. Our aim was to: 1. Detect prevalence of microalbuminuria in diabetic and non-diabetic, hypertensive (NDH) patients. 2. Correlate microalbuminuria with age, sex, duration of disease in diabetic and non-diabetic, hypertensive patients. 3. Correlate microalbuminuria with blood sugar (fasting and post-prandial) and blood pressure values in detecting renal damage. A cross-sectional study was carried out. Out of the total 85 cases, 39 cases were of diabetes mellitus (DM) and 46 NDH were taken into the study including newly diagnosed cases and those already diagnosed. None had overt albuminuria by dipstick method. Patients with acute illness and having both DM and hypertension were excluded from this study. Random urine sample was collected and microalbuminuria was detected by Micral test which includes test strips for the immunological, semi-quantitative in-vitro determination of urinary albumin up to a concentration of 100 mg/L. Blood samples were also collected for fasting and postprandial blood sugar (FBS and PPBS), urea and creatinine. The prevalence of microalbuminuriawas 71.8% and 54.3% in diabetic and NDH patients respectively. Prevalence of microalbuminuria increases with increase in duration of the disease in both DM and NDH. Positivity of microalbuminuria revealed higher range of urea and creatinine in DM and NDH patients. Our study concludes that microalbuminuria is essential for early detection of diabetic and hypertensive nephropathy. Therefore, it is recommended that microalbuminuria test should be done at regular intervals for DM and NDH patients.

Key Words: *Microalbuminuria, Diabetes Mellitus, Hypertension, Nephropathy*

INTRODUCTON
Microalbuminuria represents an abnormally elevated urine albumin level that cannot be detected with the use of a urinalysis dipstick, (Tobe, 2002) and is defined as urinary albumin excretion between 30 and 300 mg over a 24 hour period. (Jensen, 2000)

Microalbuminuria is present in very early stages of diabetesmellitus (DM) at a time when glomerular filtration rate (GFR) may be normal and when there is no evidence of abnormal glomerular filtration. Thus in early DM, microalbuminuria may be a marker of the subsequent development of proteinuria and diabetic nephropathy.

Diabetic nephropathy is characterized by proteinuria and is the leading cause of end-stage renal disease worldwide. It constitutes the major work load of dialysis centers. Diabetic subjects on dialysis and transplant recipients also have higher morbidity and mortality rates than their non diabetic counterparts. Progression to established diabetic nephropathy occurs through several stages. Microalbuminuria predicts future development of overt nephropathy. (Ahmedani, 2005)

Hypertensive nephropathy is a common cause of chronic kidney disease, in which chronic renal ischemia can remain unrecognized. Hypertension increases the glomerular ultrafiltration of albumin. (Badiger, 2012)

The purpose of this study was to evaluate the presence of microalbuminuria in patients admitted with DM and non-diabetic, hypertension (NDH) at a tertiary care hospital and a teaching institute in Pune, India, to ascertain its relationship to the duration, severity of the disease and extent of renal damage.

Research Article

MATERIALS AND METHODS

This cross-sectional study was carried out to detect the prevalence of microalbuminuria and to investigate correlation of microalbuminuria with age, sex, duration and markers of renaldamage. The study was approved by institutional ethical committee and oral informed consent was taken. Eighty-five patients were included in this study. There were 39 patients with DM and 46 with uncomplicated hypertension. All were without overt proteinuria (detectable by dipstick method). Patients from both sexes were included.

Patients with possible causes of proteinuria like evidence of urinary tract infection, urinary tract calculi, acute infections, pregnancy, severe physical exertion in recent past, malignancy, post-renal proteinuria were excluded. Patients with both DM and hypertension were also excluded from this study.

Study questionnaire and Performa were written for each patient including registration number, name, age, sex, present complaints, past history, family history, drug history and duration of illness. Blood pressure (BP), urine microalbuminuria (spot test), fasting blood sugar (FBS), post prandial blood sugar (PPBS), serum blood urea nitrogen (Sr. BUN) and serum creatinine were estimated.

This study was based on Micral test using random urine sample which contains test strips for the immunological, semi-quantitative in vitro determination of urinary albumin up to a concentration of 100 mg/L (ACCU-CHEK product, Roche Diagnostics Australia Pty. Ltd.). Test principle is based on the immunological detection of human albumin by means of soluble antibody-gold-conjugate. Excess conjugate is retained in separation zone containing immobilized human albumin. Cross-reactions with other human proteins, such as hemoglobin, transferrin, Bence-Jones protein, α1-antitrypsin, acidic α1-glycoprotein, α-amylase, Tamm-Horsfall protein and retinol-binding protein, as well as with lgG, IgA, human leukocytes and erythrocytes have been found to be < 0.5% with this method.

Procedure:

1. The test strip was dipped in the urine so that the fluid level was just between the two black bars making sure that it does not touch the side of the vessel in the process. The test strip was withdrawn after 5 seconds and placed across the top of the urine vessel.
2. After 1 minute the colour of the test pad was compared with the colour scale on the test strip container label. If colour development is slightly uneven, the average colour was taken into consideration. In case it was unclear which colour matches the test pad, a range was chosen, e. g. 20-50 mg/L or 50-100 mg/L.

Figure 1 shows a negative dipstick test (small arrow) and a positive Micral test (big arrow).

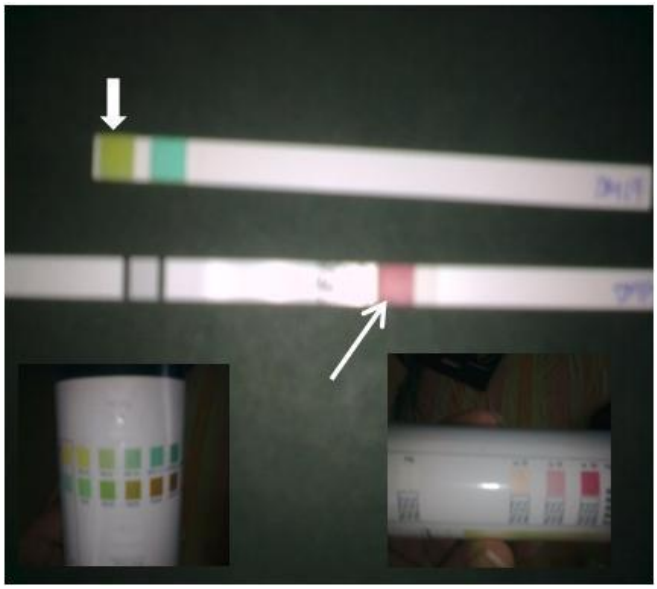

Figure 1: Sources of Error

For reliable results on a concentration basis a normal fluid intake prior to testing is important (1.5 to 2 L fluids per day). A very low or very high fluid intake can lead to false-positive or false-negative results. False-negative results can be produced by residual quantities of strongly oxidizing cleaning agents in the urine collection vessel or if the test strip is immersed too far into the urine.

Statistical Analysis

Apart from Bar Chart Distribution Analysis, Statistical Correlation Test and Regression Equation Analysis have been used to analyze microalbuminuria with independent variables like age, sex, duration of DM or hypertension, urea and creatinine values.

RESULTS AND DISCUSSION

Out of the total of 85 patients investigated, the prevalence of microalbuminuria was more in diabetic patients (71.8%) as compared to NDH patients (54.3%). The overall percentage of the microalbuminuria positive patients was 60.4%.

Diabetic nephropathy is the most frequent cause of end stage renal disease. Microalbuminuria is the first clinical detectable sign of involvement of the kidney. It affects between 20-40% of subjects, 10-15 years after the onset of DM. Once microalbuminuria is present, it progresses over 5-10 years to proteinuria in 20-50% of subjects. With microalbuminuria, the decline in renal functions varies but average reduction in glomerular filtration is around 10-12 ml/min/year. Progression to end stage renal disease is accelerated by hypertension. The process of renal involvement is step wise and microalbuminuria (also referred to as incipient nephropathy) is potentially reversible. Microalbuminuria is also strongly associated with traditional cardiovascular risk factors and cardiovascular complications. (Ahmedani, 2005)

Logistic regression modelingidentified HBA1c, duration of DM, systolic blood pressure, serum creatinine, smoking and waist circumference as independent risk factors associated with albuminuria. (Meisinger 2008)

The prevalence of microalbuminuriawas 71.8% for diabetic patients. In a study on Type 2 diabetic subjects having poor metabolic control, the prevalence of microalbuminuriawas about 20% and was associated with components of the metabolic syndrome. (Sheikh, 2009) In other studies, prevalence of 34% and 27.2% were reported. (Ahmedani, 2005 and Meisinger, 2008) In our study, higher prevalence of microalbuminuria in DMmay be due to the fact that most diabetics were on poor glycemic control and due to the small sample size. This range of prevalence probably relates to differences in patient selection criteria and/or techniques used to measure Albumin Excretion Rate (AER). Morningurine sample was collected for analysis of creatinine and microalbumin in Sheikh Study (2009). In some studies, albumin-creatinine ratio was estimated as a measure of microalbuminuria. (Meisinger 2008 and Escobedo 2010) In another study, microalbuminuria was defined as a urinary albumin >50mg/L. (Ahmedani, 2005) The present study was also not population-representative, as the patients were selected from the admissions and out-patient care of the hospital. Different ethnicities can affect the results too.

With the increasing age, the percentage of microalbuminuria positive cases of diabetic patients increases. This trend is clearly visible from 40 years and above age groups. Also, there seems to be some correlation between age and severity of microalbuminuria in case of diabetic patients as the microalbumin level increases with age in case of diabetic patients as shown in Figure 2.

This is in agreement with previous studies (Ahmedani, 2005) where microalbuminuria positive group was older as compared to microalbuminuria negative group.

In Diabetic patients, male patients (88.2%) have been found to be more susceptible to microalbuminuria as compared to female patients (59.1%) which is in accordance with Ahmedani study (2005) as well.

Our study confirms the positive relationship of microalbuminuria with the duration of DM. Other studies have also highlighted this observation. (Sheikh, 2009 and Meisinger, 2008) Duration of DM, microvascular complications and microalbuminuria are positively correlated as indicated by other workers (Escobedo, 2010).

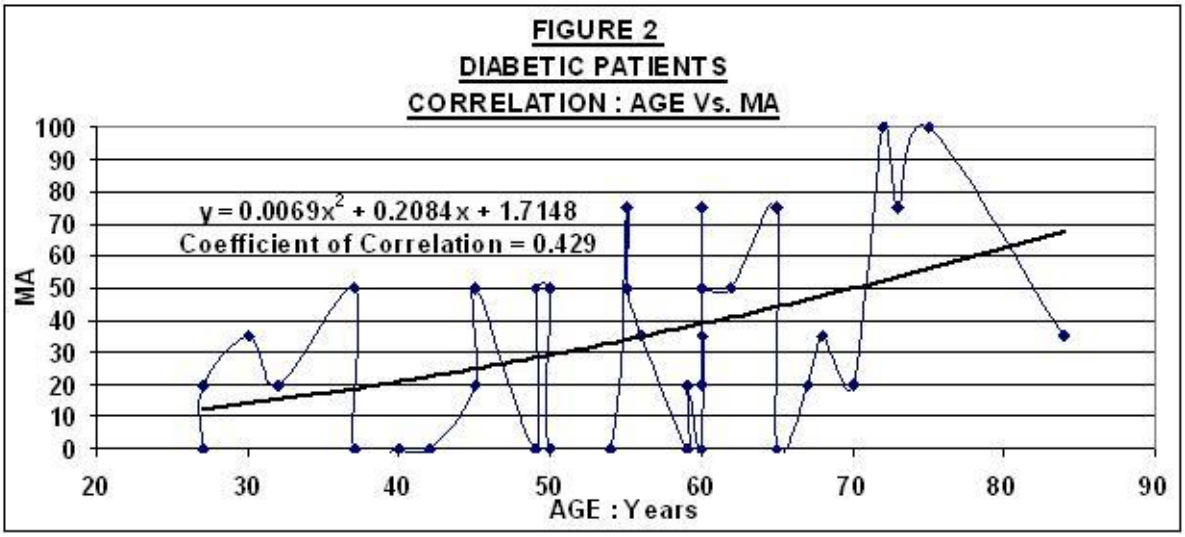

Figure 2: Diabetic Patients Correlation

Blood glucose is a continuous variable, rising and falling about two-fold throughout the day in people without DM and up to some 10-folds in people with DM. (Sheikh, 2009) Although the emergence of proteinuria requires years of poor glycemic control, short-term changes in blood glucose values can also alter the rate of urinary protein excretion. (Escobedo, 2010)

Prevalence of microalbuminuria with FBS and PPBS in diabetic patients is shown in Table 1 and 2. There is no significant correlation between increased FBS and degree of microalbuminuria.

Table 1: Correlation between FBS and Microalbuminuria

Severity	FBS	No. of patients	Positive for Microalbuminuria	Prevalence of Microalbuminuria (%)
Mild	**< 100**	**4**	**2**	**50.0**
Moderate	**100-120**	**3**	**2**	**66.7**
Severe	**> 120**	**32**	**24**	**75**
TOTAL		**39**	**28**	**71.8**

Table 2: Correlation between PPBS and Microalbuminuria

PPBS SEVERITY		No. of patients	Positive for Microalbuminuria	Prevalence of Microalbuminuria (%)
Mild	**< 160**	**4**	**2**	**50.0**
Moderate	**160 - 210**	**10**	**7**	**70.0**
Severe	**> 210**	**25**	**19**	**76**
TOTAL		**39**	**28**	**71.8**

This is in contrast to other studies in which a highly significant correlation was found. (Sheikh, 2009 and Kassab (2008) However in a study conducted by Meisinger (2008), a strong association between HbA1c and albuminuria was found. This lack of association could be due to the method of estimating blood glucose. Earlier studies have used glycosylated hemoglobin as a diagnostic test for Type-2 DM, instead of relying only on FBS. (Sheikh, 2009 and Meisinger, 2008) Treatment of the patients in the study could affect the results, as confirmed by a study conducted by Meisinger (2008).

The effect of BUN with microalbuminuria in diabetic and NDH patients is shown in Figure 3. There was also positive correlation between high BUN and incidence of microalbuminuria in both DM and NDH patients.

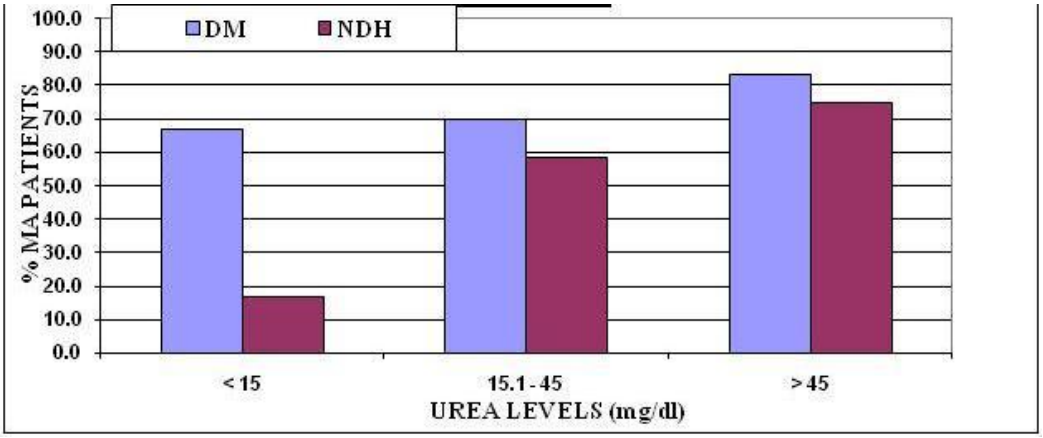

Figure 3: Urea Levels in Microalbuminuria (MA) Patients

The effect of Sr. creatinine with microalbuminuria in DM and NDH patients is shown in Figure 4.

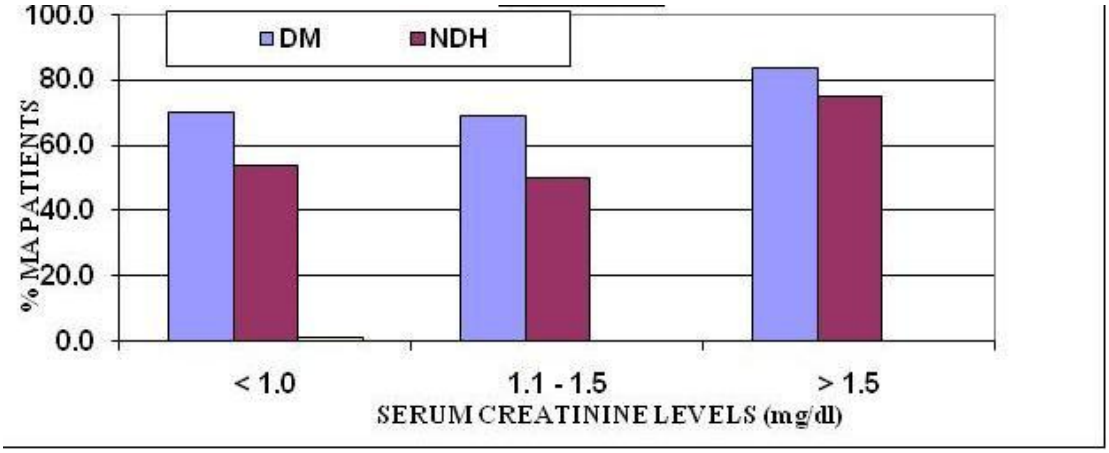

Figure 4: Creatinine Levels in Microalbuminuria (MA) Patients

The measurement of serum creatinine concentration is widely used clinically as an index of renal function. It is widely affected by age, sex and body weight. Microalbuminuria and serum creatinine increase significantly in Type 2 DM as reported in an earlier study. (Sheikh, 2009) We found that there is only slight correlation of creatinine with microalbuminuria values. This is in contrast with Sheikh Study (2009) where a highly significant correlation between microalbuminuria and serum creatinine level was seen.

Serum creatinine measurement is convenient and inexpensive methods of assessing renal function and consistently elevated levels indicate chronic kidney disease. However some patients have a substantial decrease in glomerular filtration rate, while their serum creatinine concentration remains within the normal range. Hence it is a poor screening test for mild kidney function. Minimizing microalbuminuria and having a tight glycemic control is an important treatment goal for patients with DM. (Sheikh 2009) the present study was cross-sectional in design, thus, longitudinal trends in the management of DM and modifiable risk factors could not be taken into account.

Recently, a higher prevalence of cardiovascular disease has been described in patients with less severe renal impairment. In the Framingham study, patients with mild renal dysfunction showed an almost 2-times higher prevalence of coronary heart disease, congestive cardiac failure, ischemic stroke and left ventricular hypertrophy as compared to control subjects. Renal dysfunction entails the presence of several metabolic and hemodynamic abnormalities, which may have a negative impact on long-term cardiovascular complications. Overt proteinuria is a strong independent risk factor for cardiovascular disease both in diabetic and non-diabetic populations. Over the last several years, the association between urinary protein excretion and cardiovascular events has been extended to low-grade albuminuria (i.e. microalbuminuria). A strong relation between microalbuminuria, mild reduction in creatinine clearance and the presence of cardiac and vascular hypertrophy could explain the excess morbidity and mortality rates observed in patients with renal dysfunction. This could further support the role of the kidneys as an integrated sensor of cardiovascular risk. (Leoncini, 2003)

The prevalence of microalbuminuria in NDH patients in the present study is 54.3% which is higher than that in the study by Jalal *et al.*, (2001), wherein reported prevelance was 37.5%. Our result is in concordance with a study by Badiger *et al.*, (2012) which showed prevalence of 63%.This observation of high prevalence of microalbuminuria in patients with essential hypertension, must alert the clinicians to the high prevalence of subclinical chronic kidney disease. (Badiger 2012) Higher prevalence in the present study may be due to the irregular treatment and small sample size. This range of prevalence probably relates to differences in patient selection criteria and/or techniques used to measure AER. (Jensen, 2000) Furthermore, in another study (Pontremoli 1997 and Viazzi 2010), microalbuminuria was measured in a first morning urine sample, which can yield different results as compared to random urine sample which can give higher values. Ethnic differences, age of subjects, selection bias and different assay methods may have contributed to the differences. (Gould, 1993)

There was no correlation between microalbuminuria and age in NDH patients in this study. This was the observation of Jalai *et al.*, (2001) too. We observed that the sex of the patient had little correlation with presence of microalbuminuria unlike the observation of Gould (1993) who found microalbuminuria prevalence more in males. (Gould, 1993 and Romundstad, 2003) However the correlation between the presence of microalbuminuria and duration of hypertension in the study was as observed by others. (Badiger, 2012) There was no correlation with the severity of hypertension though Jalal (2001) noted a positive correlation. Our observations are depicted in Table 3 and 4.

Table 3: Correlation between systolic BP and microalbuminuria

Severity	Systolic BP	No. of patients	Positive for Microalbuminuria	Percentage with Microalbuminuria
Mild	**< 160**	**37**	**21**	**56.8**
Moderate	**160 - 200**	**6**	**3**	**50**
Severe	**> 200**	**3**	**1**	**33.3**
TOTAL		**46**	**25**	**54.3**

Table 4: Correlation between diastolic BP and microalbuminuria

Severity	Diastolic BP	No. of patients	Positive for Microalbuminuria	Percentage with Microalbuminuria
Mild	**< 100**	**35**	**20**	**57.1**
Moderate	**100 - 120**	**11**	**5**	**45.5**
Severe	**> 120**	**0**	**0**	**-**
TOTAL		**46**	**25**	**54.3**

One reason for lack of correlation between severity of hypertension and microalbuminuria is that we had fewer cases of newly detected severe hypertension in our study.

A linear relation between BUN levels and prevalence of microalbuminuria was observed in hypertensive patients. It is interesting to note that increase in urea may increase prevalence of microalbuminuria in both diabetic and hypertensive patients. Similar observations were noted with S. creatinine. However there was no correlation of creatinine with microalbuminuria values in NDH patients. Serum creatinine is a less accurate indicator of renal function as compared to creatinine clearance, since the latter takes into account age, gender and body weight, variables that are known to influence glomerular filtration rate. (Mattix, 2002) Furthermore, calculated creatinine clearance is easily obtained and obviates the inaccuracy and practical obstacles related to 24-hour urine collection (Leoncini, 2003). Jalal *et al.*, (2001) failed to document any significant difference in creatinine clearance between microalbuminuric and normo-albuminuric hypertensive patients. There is a possibility that Asian population including Japanese might be more susceptible to microalbuminuria as compared with the population in North America and Europe. Moreover, Osicka and Comper reported the existence of immunochemically non-reactive urinary albumin. Thus, some patients who have increased creatinine clearance but no microalbuminuria might have predominantly excreted an immunochemically non-reactive urinary albumin (Konta, 2006). Possibly, such factors can influence the overall prevalence observed in this study.

Microalbuminuria reflects a renal and systemic transvascular albumin leakage that is perhaps due to the low vessel wall content of heparan sulfate. In animal models, it has been shown that the transvascular leakage of albumin and lipoproteins are tightly correlated and that both are elevated in atherosclerosis and in atherosclerosis-prone sites of arteries (Jensen, 2000). Hypertension is associated with functional and morphological alterations of the endothelium, which disturbs delicate balance of endothelium derived factors resulting in endothelial dysfunction. The endothelial dysfunction results in elevated peripheral resistance, leading to atherosclerosis (Badiger, 2012). Hence, it is very important to screen the patients with essential hypertension for microalbuminuria in early stages, which if treated in time, can prevent atherosclerotic processes in the entire vascular system (Badiger 2012).

We conclude that microalbuminuria is essential for early detection of diabetic and hypertensive nephropathy. Hence, it is recommended that microalbuminuria test should be a part of routine checkup for DM and NDH patients.

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