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ABSTRACT

Background: Microalbuminuria, though a relevant screening tool world wide, is scarcely reported with very sparse literature in our setting. Microalbuminuria is a marker of early renal involvement, compare to routine serum creatinine and electrolytes changes in hypertension and diabetes mellitus. This article attempts to review the significance, risk factors and methods of detection of Microalbuminuria.

Methods: Available publications from local and international journals in addition to Medline and Google search, particularly for local references were utilized. Other sources of our data included dissertations from the library of National post graduate medical college and text books of paediatric nephrology.

Results: Microalbuminuria is used extensively in diabetes mellitus as a sensitive test for the detection of preclinical kidney dysfunction prior to the development of overt proteinuria, and as a predictor of subsequent diabetic nephropathy. It has been found to be an important prognostic indicator in meningitis, malignancy and hypertension. It has been found to be useful in the monitoring of patients with renal scarring, unilateral nephrectomy and diabetes mellitus. It is also an important marker of glomerular injury, particularly in patients with sickle cell anaemia.

Conclusion: Microalbuminuria is an early maker of glomerular injury. It is important as a screening tool in a variety of disease conditions. Screening may be performed with a semiquantitative assay. If the screen is positive, UAE should be evaluated by a quantitative assay.

Key words: Microalbuminuria; Screening; Risk factors; Methods of detection.

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INTRODUCTION

Dipstick urinalysis is a valuable tool in the screening of urinary abnormalities,¹⁻⁵ as it detects proteinuria, haematuria, urinary specific gravity, leukocyturia among other things. It, however, cannot detect early renal changes that may be heralded by proteinuria in the range of 20-200mg/l (microalbuminuria). Long before one obtains a positive urine test for protein using the albutix, small

quantities of albumin can be detected in the urine using sensitive immunochemical techniques and this phenomenon is called Microalbuminuria.

MICROALBUMINURIA

Microalbuminuria (MA) which is a sub-clinical rise in the urinary albumin excretion has been defined variously by Marshall⁶, Mimran et al⁷, Winocour⁸ and Hasslacher et al⁹. Marshall⁶ defined MA in terms of timed overnight urine collections as an albumin excretion rate greater than 20µg/minute⁶. According to Mimran et al⁷, however the term MA refers to a situation where the urine is albutix negative but positive for albumin with an excretion rate in the range of 20-200µg/minute. (The lowest detection limit using conventional methods such as sulfosalicylic acid precipitation or bromophenol-blue reaction used in the albutix test is > 200µg/minute). Winocour⁸, on the other hand defined MA as a ratio of urinary albumin to urinary-creatinine greater than 3.0µg/mmol⁸ which can readily be done on spot urine samples^{10, 11}. While Hasslacher et al⁹ defined MA as albumin excretion in the urine within the range of 20mg/l to 200mg/l.

The concept of Microalbuminuria was introduced more than 30years ago when measures to detect urinary albumin below the level detectable by conventional urinary dipstick became available. MA was subsequently shown to be an early marker of diabetic nephropathy. It was also found, quite remarkably to be a stronger predictor of cardiovascular mortality than of renal events. In the Heart Outcomes prevention Evaluation (HOPE) study, Microalbuminuria was the strongest predictor of cardiovascular events in a high-risk population with underlying atherosclerosis. It was found to be stronger than other risk factors such as coronary artery disease and diabetes¹². It is important to appreciate Microalbuminuria not only predicts cardiovascular but also other atherosclerotic vascular events. Miettinen et al¹³ followed over 2,000 diabetic and non-diabetic patients for 7 years, looking at the association between the different degrees of proteinuria and atherosclerotic vascular events. In both groups, Microalbuminuria was associated with incidence of cardiovascular events stroke and aggregate vascular events. Compared to earlier documented traditional risk factors, Microalbuminuria has been shown to be a strong predictor of cardiovascular death even in healthy populations¹⁴. In a cohort of healthy individuals, Borch-Johnsen et al¹⁴ prospectively followed more than 2,000 patients for 10 years. The participants had no ischemic heart disease or diabetes. In this study, Microalbuminuria was associated with increased relative risk of cardiovascular death by 2.3-fold, independent of other risk factors.

In patients aged more than 40 years, Yudkin et al¹⁵, observed that within a mean follow-up period 3.6 years, cardiovascular complications (coronary artery disease or peripheral arterial disease) were more frequent in patients with Microalbuminuria as compared with those without it; 30 percent versus 74 percent for coronary disease and 10 percent versus 44 percent for peripheral diseases. Moreover, death was more frequent in those with Microalbuminuria (2 percent versus 33)

MA has been found to be an important prognostic indicator in meningitis,¹⁶ malignancies¹⁷ and hypertension¹⁸ It has been found to be very useful in the monitoring of patients with renal scarring,¹⁹ unilateral nephrectomy^{20,21} and diabetes mellitus.^{22,23} MA has also been found to be an important marker of glomerular injury, particularly in patients with sickle cell anaemia prone to nephropathy

The term sickle cell nephropathy encompasses all the structural and functional abnormalities such as proteinuria with or without nephrotic syndrome,^{24,25} hyposthenuria,^{26,27} incomplete distal tubular acidosis,^{25,28} haematuria,^{24,29} immune complex glomerulonephritis,^{30,31} and progressive renal failure.^{28, 29} These manifestations are as a result of chronic renal micro-vascular occlusion by sickled erythrocytes.^{32, 33} the effect of which is accentuated during crises^{34,35}. The renal microvasculature is particularly vulnerable because of absence of collateral circulation³⁶ and the characteristic sickling promoting features of renal medulla, which include hypoxia, acidosis and hypertonicity^{18,25,37,38}. Sickle cell nephropathy may present as urinary abnormalities detectable by dipstick urinalysis³⁹ or in case of early glomerular involvement by MA detection.

Studies on urinary abnormalities among various populations of children in Nigeria have been carried out using the dipstick urinalysis⁴⁰⁻⁵⁰. Findings from these studies include, proteinuria in one to seventy percent of apparently normal neonates and children of varying ages⁴⁰⁻⁵⁰. Significant haematuria was reported in 0.6-4% of normal children studied⁴⁰⁻⁵⁰. Ugwu and Eke⁴⁰ in the course of their randomized study for suitable malaria prophylaxis amongst children with sickle cell anaemia (SCA) at the University of Port Harcourt Teaching Hospital did weekly interval urinalyses among 72 children aged 16months -16years and found significant haematuria and proteinuria of one and seven percent respectively; and all patients with abnormal urinary findings were above the age of 5yrs. In terms of MA, Ibadin et al⁵¹ in their study at the University of Benin Teaching Hospital (UBTH) to determine the prevalence of Microalbuminuria in adolescent/young adult offspring's of Nigerian hypertensive adults worked on 42 subjects aged between 13 and 24years, using micral-test strips. Nineteen percent had Microalbuminuria in their spot urine, as compared to 8 percent in controls. The incidence of Microalbuminuria was more in the age bracket 16-18years. It was also more in subjects with positive paternal history of hypertension (21.1% as against zero percent in those with positive maternal history of hypertension)

Microalbuminuria is believed to be an important marker of glomerular injury in patients with sickle cell anaemia. Few

studies on Microalbuminuria in sickle cell anaemia patients have been done. The prevalence of Microalbuminuria in sickle cell anaemia patients has been shown to vary in different settings. Studies^{22,23,52-54} have recorded prevalence rates of between 19 40%. The prevalence of Microalbuminuria was found to be 42.7%, 20.3% and 18.5% among paediatric patients with Sickle cell anaemia in Port Harcourt, Benin and Enugu respectively using the micral test strip⁵⁸⁻⁶⁰. Microalbuminuria occurs sufficiently enough in both children and adults to warrant routine screening. Such measures may assist in early detection and improve case management of patients who are prone to renal complications as interventional measures known to retard the rate of deterioration could be instituted.

RISK FACTORS FOR MICROALBUMINURIA;

Table 1: Risk Factors Associated with Elevated Albuminuria⁶¹.

Non modifiable	Modifiable	
	Well documented	Likely
Race/ethnicity	Diabetes	Hyperlipidemia
Male gender	Hypertension	High salt (and protein) diet
Older age	Obesity	Oral contraceptives
Low birth weight	Smoking	Hormone replacement therapy

Risk factors associated with elevated albuminuria are shown in Table I below.

Mechanisms underlying progressive loss of renal function.

It has been documented that the haemodynamic adaptations of glomerular hypertension and hyperfiltration in remnant nephrons (i.e. those nephrons not damaged by the initiating renal disease) ultimately prove detrimental⁶². They suffer progressive glomerulosclerosis, a process that sets into motion a vicious cycle of nephron loss. The more initial nephrons lost, the more the haemodynamic burden to the remaining ones⁶². The ensuing protein leakage through these affected glomeruli results in enhanced tubule protein reabsorption, which initiates progressive tubule atrophy and interstitial fibrosis⁶³. Clinically, the most important factors promoting this final common pathway of progressive nephron loss are hypertension⁶⁴, proteinuria⁶⁵, hyperlipidemia⁶⁶, and genetic factors such as race⁶⁷. Other factors such as obesity⁶⁸, smoking⁶⁹, low birthweight⁷⁰, male gender⁷¹ and high salt intake are also to be associated with a worse outcome in subjects with preexisting renal disease. These are shown in Table I above.

Nonmodifiable risk factors associated with a higher urinary albumin excretion

a) Race/ethnicity: Various reports⁷²⁻⁷³ have documented a higher prevalence of an elevated albumin excretion in specific ethnic groups. Recent studies suggest that the racial and geographic disparities in ESRD, and the increasing incidence rates, may have a fetal origin as indicated by an association with birth weight (BW)⁷²⁻⁷⁴. Brenner et al⁷⁵, proposed a mechanism involving impaired kidney development in utero as an explanation for reduced renal function later in life. b) Male Gender/Old age : It has been documented that

elevated albumin excretion is found more frequently in men than women⁷⁴⁻⁷⁶, this difference is age dependent.

Urinary albumin excretion is significantly higher in men than women, especially at older age⁷⁴⁻⁷⁷. In the normal population, GFR decreases from the age of 30 by about 0.8ml/min/year⁷⁸. Assuming that a 30-year-old subject has a normal GFR of about 120ml/min, his/her GFR will be about 70ml/min at age of 80. A renal biopsy from that 80-year-old person will typically reveal some atrophic glomeruli with tubule atrophy, other glomeruli showing signs of glomerulosclerosis and still others showing glomerular enlargement and hypertrophy.

c) Low Birth Weight: With respect to low birth weight, an inverse association between Microalbuminuria and height was found, arguing that factors operating in utero or early childhood influence urinary albumin excretion in later life^{71,74,79}. Brenner et al⁷⁵, proposed a mechanism involving impaired kidney development in utero as an explanation for reduced renal function later in life. Hoy et al⁷¹, proposed that the association between low birth weight and renal disease might be mediated through impaired nephrogenesis caused by intrauterine malnutrition.

Modifiable Factors that have been well documented in Relation to Albuminuria.

- a) Diabetes: Both glomerular hyperfiltration and a slightly elevated albumin excretion rate have been found to predict progressive renal failure in both type I and type 2 diabetes^{27, 28, 80-82}. Albuminuria in both type I and type 2 diabetes is associated with widespread endothelial dysfunction, manifest not only in the glomerular vasculature, but in other vascular beds as well⁸³⁻⁸⁴.
- b) Hypertension: Increased urinary albumin loss has also been linked to essential hypertension⁸⁵⁻⁸⁶. Just as in diabetes, Microalbuminuria in essential hypertension has been taken to reflect widespread endothelial dysfunction⁸⁷. Albuminuria in essential hypertension is associated not only with left ventricular hypertrophy, but also with glomerular hyperfiltration⁸⁸.
- c) Obesity: Elevated albumin excretion is frequently found in nondiabetic obese subjects^{70, 89-91}. In obese subjects, the risk for glomerular hyperfiltration and hyperperfusion is enhanced⁸⁹⁻⁹¹. The risk for glomerular hyperfiltration seems to be especially evident in cases of abdominal obesity^{82, 89}.
- d) Smoking: Smoking is also associated with an increased risk for albuminuria. It has been shown that smoking is associated with an increased risk for both hyperfiltration and impaired filtration⁹². Life time tobacco exposure, but not current level of smoking, is associated with renal function impairment and proteinuria⁹³.

Modifiable Factors which are likely to be Related to Albuminuria

- a) Hypercholesterolemia: The Gubbio study, showed that the risk for elevated albumin excretion increased 2-fold for each 40mg/dl increase in plasma cholesterol⁹¹. Moreover, it has been shown that hypertensive subjects with high cholesterol levels have a more rapid decline in

GFR over time⁹⁴.

- b) Dietary salt intake: A higher salt intake is independently associated with a higher urinary albumin excretion⁹⁵. It also predicts mortality and the risk for coronary disease⁹⁶.
- c) Oral Contraceptives and Hormone Replacement Therapy: The use of oral contraceptives and hormone replacement therapy⁹⁷⁻⁹⁸, is also associated with an enhanced urinary albumin excretion. Users of oral contraceptives had an increased renal vascular resistance and filtration fraction⁹⁹.

Factors Affecting Urine Albumin Excretion in Children

Albuminuria is affected by exercise and posture¹⁰⁰. They cause increase in the excretion of albumin in urine. Albumin demonstrates a day to day variability¹⁰¹. Urine albumin excretion (UAE) tends to be approximately 25 percent greater during the day than the night and shows a daily variation of 40-100 percent in children¹⁰². Other factors which increase UAE are obesity¹⁰³, puberty^{74, 104}, excessive alcohol intake and smoking¹⁰⁵. Physiological factors such as exercise, posture and diuresis increase UAE other factors like urinary tract infection and acute illness also increase UAE¹⁰⁶. In children age 5-18 years UAE is higher in females than males¹⁰⁷ and does not differ with age in either sex below the age of 5 years¹⁰⁷. In adults the converse is true; i.e. women have lower albumin excretion than men⁷⁴. A negative correlation has been shown between UAE and height⁷⁴.

Review of Methods Available for the Detection and Measurement of Albumin:

Variations in urine flow rate in a person may be corrected by the expression of albumin as a ratio to creatinine (that is albumin/creatinine)

All the following urine samples are currently acceptable:

1. 24-hour collection
2. Overnight (8-12-hour) collection
3. 1-2-hour collection (in laboratory or clinic)
4. First morning sample for simultaneous albumin and creatinine measurements.

The timed specimen (24-hour or overnight) are the most sensitive but the albumin-to-creatinine ratio is more practical and convenient for the patient. At least three separate samples should be assayed because of the high intraindividual variation (30% -50%) and diurnal variation (50% to 100%) higher during the day. Urine should be stored at 4°C after collection. Alternatively, 2ml of 50g/l sodium azide can be added per 500ml of urine, but preservatives are not recommended for some assays. Bacterial contamination and glucose have no effect.

Semiquantitative assays.

A number of semiquantitative assays for screening for increased UAE are available. These test strips, most of which are optimized to read "positive" at a predetermined albumin concentration, are suitable for screening programs. Because of the wide variability in UAE, a "normal" value does not exclude renal disease. Because these assays measure albumin concentration, dilute urine may yield a false negative result. Refrigerated urine samples should be allowed to reach at least 10°C before analysis. Albu Screen and Albu Sure detect urinary albumin concentration above 20 and 30 mg/l,

respectively. The assay is a latex agglutination inhibition test. Briefly, one drop of urine is mixed with one drop of goat antihuman albumin, the titer of which is adjusted so that all anti-body binding sites are occupied at urinary albumin concentration of 30mg/l or greater. Excess albumin binding sites are detected by the addition of one drop of albumin-coated latex microspheres and subsequently rocking for 2 minutes. Albumin concentrations less than 20mg/l produce agglutination. Sensitivity and specificity are reported to be approximately 90% to 95%. Micro-bumintest uses bromophenol blue in an alkaline matrix to detect albumin concentration exceeding 40mg/l. The assay sensitivity is approximately 95%, but specificity is approximately 80%.

In the Micral test strip a monoclonal antialbumin IgG is complexed to β -galactosidase. The albumin in the urine binds to the antibody-enzyme conjugate in the test strip. Excess conjugate is retained in the separation zone containing immobilized albumin, and only albumin bound to antibody-enzyme immunocomplex diffuses to the separation zone. There it reacts with a buffered substrate (chlorophenol red galactoside) to produce a red colour when the β -galactosidase hydrolyzes galactose. The test strip is dipped into the urine for 5 seconds, and the intensity of the color after 5 minutes is proportional to the urinary albumin concentration. Direct visual comparison is made with printed color blocks-yellow, light brown, medium brown, brick red, and burgundy, representing 0,10,20,50 and 100mg/l respectively. Comparison with a reference method demonstrates a sensitivity and specificity of approximately 100% and 91% respectively. Both the time the stick is in contact with the urine and the time of reading are critical.

A modification (Micral 11) uses gold-labeled instead of enzyme-labeled antibody. This method enhances the stability, allowing the strip to be read at any time from 1 to at least 60 minutes. Urine specimens with albumin concentrations greater than 100-300mg/l may be diluted and reassayed. The assigned concentration of the color block is multiplied by the dilution factor to obtain the concentration in the sample. These semiquantitative assays are suitable for screening only and are not sufficiently accurate for regular monitoring of patients.

Quantitative Assays:

All the sensitive, specific assays for urine albumin use immunochemistry with antibodies to human albumin. Four methodologies are available RIA, ELISA, radioimmunodiffusion and immunoturbidometry. Each method has advantages and disadvantages, and the choice depends on local experience and technical support. All methods have similar precisions, sensitivity, and range. Although dye-binding and protein precipitation assays have been described, these are insensitive and nonspecific and should not be used.

Radial immunodiffusion:

This is a reliable and inexpensive method but is unlikely to gain wide acceptance because it requires a long incubation period and high level of technical skill and cannot be automated. The antibody is incorporated into an agar gel. Aliquots of samples and calibrators are added to wells and

allowed to diffuse into the agar. Antigen-antibody complexes precipitate at equilibrium, and the distance of migration is measured after staining.

Radioimmunoassay (RIA):

Standard RIA is performed in the liquid phase in the presence of excess antigens. ¹²⁵I-labeled albumin and antialbumin antiserum are used, with separation of bound from free by the double-antibody technique. The sample values are determined by comparison with a calibration curve. Assays are sensitive, precise and inexpensive but reagents are radioactive and have short shelf lives. Commercial kits are available.

Enzyme-linked immunosorbent assay:

Both competitive and “sandwich” ELISAs are available. Although the competitive ELISA is faster because it uses only one incubation with antibody, it is reportedly less sensitive and exhibits large variance. ELISA can be performed on a microplate reader, allowing semiautomation. In the sandwich assay the primary antibody (antialbumin antiserum) is fixed on the plastic plate, which is then washed. Samples, controls and calibrators are added, and the complexes detected and quantified by a second antibody are conjugated to an enzyme label.

Immunoturbidometry:

Albumin in the urine sample forms an insoluble complex with antibodies to human albumin. The turbidity caused by the complexes is spectrophotometrically measured at 340nm and provides a measure of albumin concentration. The background absorbance of the initial urine sample is automatically subtracted. This method is simple and less expensive than RIA, and rapid analysis of large numbers of samples is possible. The assays may be performed as either kinetic or equilibrium reactions. Kits are commercially available to be used on automated analyzers.

High antigen concentrations may cause a “hook” effect, resulting in falsely low concentrations. This effect can be avoided by screening of urine samples with a dipstick but may not be necessary if the analyzer automatically test for antigen excess.

A urinary tract infection or contamination with seminal or menstrual fluid may produce false-positive results. High

Condition	g/min	mg/24h	Corrected(mg/g urine creatinine)
Normal	<20	<30	<30
Increased UAE	20-200	30-300	30-300
Macroalbuminuria	>200	>300	>300

physiological Ca²⁺ concentrations in the urine falsely increase albumin values. The interference is abolished by the addition of EDTA, which has been incorporated into commercial assay.

Reference: intervals interval for Microalbuminuria is as shown below:

CONCLUSION

Microalbuminuria is an early maker of glomerular injury. Screening may be performed with a semiquantitative assay. If the screen is positive, UAE should be evaluated by a quantitative assay.

If the confirmatory test is positive, treatment with an angiotensin-converting enzyme (ACE) inhibitor would retard the Microalbuminuria and prevent progression to overt renal failure.

Recommendation

Paediatric patients should undergo periodic screening for Microalbuminuria.

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