Practical Aspects of Calculation, Expression and Interpretation Of Urine Albumin Measurement

Vilas U. Chavan,[*] Anjum K. Sayyed,[**] Pushpa P. Durgawale,[**] Ajit V. Sontakke,[**] Shreyasprasad D. Nilakhe[*]

*Department of Biochemistry, SMIMER, Surat, Gujarat, India, **Department of Biochemistry, KIMS, Karad, Maharashtra, India.

Abstract: There is a large variation for estimation of albumin in urine between different laboratories. Clinical practice guidelines for the urine albumin measurements have been issued by professional organizations in several countries. These guidelines are not uniform in recommendations regarding sample type, time of sample collection, units of reporting, reference intervals used for interpretation, nor methods used to measure albumin. The aim of this article is to provide practical information regarding laboratory measurement, calculations, reporting and interpretation of urine albumin excretion. For laboratory estimation of urine albumin one can follow clinical practice guidelines suitable for their region or country or recommended by professional organization. There is lot of confusion about reporting of results in different units. Ideally, International System of Units should be adopted. Also there should be agreement all over the world to use single system of units for expressing results for urine albumin measurement. At present in India there are no such clear guidelines about laboratory measurement of urine albumin.

Key words: Microalbuminuria, urine albumin measurement, albumin:creatinine ratio, calculation, interpretation.

Corresponding Author: Dr.Vilas U. Chavan, Assistant Professor, Department of Biochemistry, SMIMER, Umarwada, SURAT - 395010, Gujarat, India. E-mail: drvuchavan@yahoo.co.in

INTRODUCTION: Microalbuminuria described more than three decades ago as a predictor of nephropathy and associated with higher cardiovascular risk[1]. Once diabetic nephropathy develops, renal function deteriorates rapidly and renal insufficiency develops[2]. Microalbuminuria is recognized as a sign of abnormal vascular function and increased vascular permeability[3, 4]. However, it has also been considered the first indication of renal injury in patients with diabetes. Thus screening for microalbuminuria is currently recommended for all patients with diabetes or kidney disease[4]. In addition to the qualitative detection of overt microalbuminuria by dipstick methods, quantitative determination of albumin is essential for assessing the renal state, for optimizing diabetes care and for monitoring success of therapy[5]. Therefore precise assays for urinary albumin are now becoming inevitable in laboratory medicine[6].

Microalbuminuria is defined as urinary excretion of albumin that is persistently above normal, although below the sensitivity of conventional semi-quantitative test strips[7]. Microalbuminuria is currently defined as a urinary albumin excretion (UAE) of 30 to 300 mg/24 hours, if measured in a 24-hour urine collection, as urinary albumin excretion rate (AER) of 20 to 200 µg/min, if measured in a timed urine collection, or of 30 to 300 mg/g, if measured with the use of the urinary albumin:creatinine ratio (ACR) in a spot urine collection[8]. For quantitative estimation of urinary albumin and defining microalbuminuria, 24-hour urine sample is considered the 'gold standard'. However, 24-hour urine collections are cumbersome and subject to error [9, 10]. Currently, the National Kidney Foundation recommends the use of spot urine ACR obtained under standardized conditions to detect microalbuminuria. The ACR is a more convenient test for patients and may be less prone to errors due to improper collection

methods[11]. Measurement of a spot urine albumin concentration (UAC) only, without simultaneously measuring urine creatinine, is somewhat less expensive but susceptible to variation as a result of variation in urine concentration due to hydration and other factors[12]. For quantitative estimation of urine albumin in the laboratory, there are variations in sample used, methods, expression of data, units, normal range, cut off values and interpretation. Also there are various measures of albuminuria like ACR, UAC, UAE and AER. There is lot of confusion about how to measure urine albumin, calculate and express data among laboratory scientists. The aim of present article is to provide practical information about laboratory measurement of urine albumin measurement. For cutoff values used in the literature [8, 13, 14] (Table 1).

Calculation and expression of data:

In our laboratory urinary albumin and creatinine concentrations are measured as (mg/dl). ACR is reported in (mg/g). Albumin concentration in spot urine sample is reported as UAC (mg/L) and in 24-hour urine is reported as UAE (mg/24 hours). Urinary albumin excretion rate in timed urine is expressed as AER (µg/min).

Calculations (formulae):

Albumin:creatinine ratio (ACR): It is ratio of urinary albumin to urinary creatinine; usually it is expressed as milligram of albumin excreted per gram of urinary creatinine.

$$\text{ACR (mg/g)} = \frac{\text{Albumin (mg/dl)}}{\text{Creatinine (mg/dl)}} \times 1000.$$

ACR (mg/g) can be calculated by albumin (mg/dl) divided by creatinine (g/dl).

Urinary albumin concentration (UAC): It is concentration of albumin present in one litre of urine or albumin excreted per litre of urine. It is expressed as (mg/L).

$$\text{UAC (mg/L)} = \text{Albumin (mg/dl)} \times 10.$$

Urinary albumin excretion (UAE): It is excretion of albumin in urine per day (24 hours), expressed as (mg/24 hours).

$$\text{UAE (mg/24 hours)} = \text{Albumin (mg/dl)} \times \text{Volume of 24-hour urine (dl).}$$

Urinary albumin excretion rate (AER): It is rate of albumin excretion per minute time. This is calculated in timed urine collection, expressed as (microgram/min). This is also termed as urinary albumin excretion rate (UAER).

$$\text{AER (µg/min)} = \frac{\text{Albumin (mg/dl) x volume of urine in timed collection (dl) x1000}}{\text{Time period of urine collection (min)}}$$

This is also calculated and expressed in 24-hour urine collection in some studies. Actually in 24-hour urine sample UAE (mg/ 24 hours) and AER (µg/min) are same only difference is earlier is total albumin concentration in 24 hours of urine while later is

Table 1. Cut off values indicating normoalbuminuria, microalbuminuria and macroalbuminuria.

Terms	24-hour urine sample UAE (mg/24 hours)	Timed Overnight urine sample AER (µg/min)	Spot (random) urine sample	
			UAC (mg/L)	ACR (mg/g)*
Normoalbuminuria	< 30	< 20	< 20	< 30
Microalbuminuria	30 to 300	20 to 200	20 to 200	30 to 300
Macroalbuminuria	>300	> 200	>200	> 300

*ACR (mg/g) values are for both males and females (gender independent).[8, 13, 14]

albumin excretion rate per minute (24 hours = 1440 minutes).
In case of 24-hour of urine collection:

$$\text{AER } (\mu g/min) = \frac{\text{UAE (mg/24 hours)} \times 1000}{24 \times 60}$$

Albumin (protein): creatinine ratio: This is simple ratio of albumin or protein to creatinine in urine unlike ACR (mg/g). For this calculation both albumin and creatinine are in the same unit. Mostly used for assessment of proteinuria rather than albuminuria.

Sometimes protein: creatinine ratio is also used.

$$\text{ACR (simple ratio)} = \frac{\text{Albumin (mg/dl)}}{\text{Creatinine (mg/dl)}}$$

DISCUSSION: Normal individuals usually excrete very small amounts of protein in the urine. Persistently increased protein excretion is usually a marker of kidney damage. The excretion of specific types of protein, such as albumin, depends on the type of kidney disease that is present. Increased excretion of albumin is a sensitive marker for chronic kidney disease due to diabetes, glomerular disease, and hypertension. Albuminuria refers specifically to increased urinary excretion of albumin. Microalbuminuria refers to albumin excretion above the normal range but below the level of detection by routine dipstick tests for total protein. Patients with a positive dipstick test (1+ or greater) should undergo confirmation of albuminuria by a quantitative measurement of albumin-to-creatinine ratio within 3 months[13].

Macroalbuminuria (UAE > 300 mg/24 hours, corresponding to a total protein excretion > 500 mg/24 hours) will eventually lead to end-stage renal insufficiency within 10 to 20 years[15]. Prevalence of diabetes, hypertension, obesity, and chronic kidney disease is increasing markedly in many developing countries, and all of them contribute to cardiovascular diseases. By 2020, it is predicted that 80% of the global burden of cardio vascular disease will be borne by developing countries [16].

Measurement of albumin in urine has important role in secondary prevention, to decide treatment and monitor response to treatment. The measurement of albumin in urine is not standardized. There is a large variation for estimation of albumin in urine between different laboratories and between different methods. Furthermore, there is no consistency among laboratories regarding sample type, units of reporting, and reference intervals or cutoff values[17]. Clinical practice guidelines for the use of urine albumin measurements have been issued by professional organizations in several countries. These guidelines are not uniform in recommendations regarding sample type, time of sample collection, units of reporting, reference intervals or cut points used for interpretation, nor methods used to measure albumin and creatinine[18].

As there are differences in clinical practice guidelines by professional organizations in different countries for urine albumin measurement. There is also poor agreement as to whether proteinuria should be defined in terms of albumin or total protein loss, with a different approach being used to stratify diabetic and non-diabetic nephropathy [19]. Based on present knowledge and situation we planned to measure albumin using different urine samples and some practical aspects are discussed here. For laboratory estimation of urine albumin one can follow clinical practice guidelines suitable for their region or country or recommended by professional organization. Few points are discussed below.

Samples: Various methods for urine collection are used in clinical practice to measure albumin in urine[2]. The amount of albumin excreted in urine during a 24-hour period has been considered the "gold standard" [10]. 24-hour urine collections may be associated with significant collection errors, largely due to improper timing and missed samples, leading to over-collections and under-collections. Timed overnight collections or shorter timed daytime collections may reduce the inconvenience of a 24-hour collection, but are still associated with

collection errors. In addition, errors due to incomplete bladder emptying are relatively more important in shorter collection intervals[13]. More practical and easier alternatives are collection of a first morning void or a spot (random) urine sample. It has been suggested that a first morning void is to be preferred over a spot urine sample, because the former is less influenced by factors such as hydration status and physical activity, reducing the variability that is caused by these factors. From a practical point of view, however, spot urine samples are preferred because they can be collected during consultation at the doctor's office and therefore pose the least inconvenience for patients[20].

American Diabetes Association (ADA) guidelines for detection of microalbuminuria permit the use of 24-hour collections, timed specimens taken over a period of less than 24 hours, and untimed random spot specimens[4]. According to the National Kidney Foundation (NKF), clinical practice guidelines, under most circumstances, untimed spot urine samples should be used to detect and monitor proteinuria in children and adults. It is usually not necessary to obtain a timed urine collection (overnight or 24-hour) for these evaluations in either children or adults. First morning specimens are preferred, but random specimens are acceptable if first morning specimens are not available [13].

Container and storage: For routine clinical laboratory testing, fresh urine collected from midstream is preferable. Albumin is generally stable in urine stored at 2–8 °C for 7 days[21]. Precipitates often form in refrigerated or frozen urine, and their effect on albumin measurement has not been thoroughly investigated. Precipitates frequently redissolve when the urine is warmed for analysis. Centrifugation of cloudy urine is needed to remove insoluble material before measurement. Long term storage of urine samples at temperatures above -80 °C, particularly at -20 °C, has been reported to produce falsely low values of albumin concentration[22]. Albumin is reported to adsorb to plastic surfaces. The allowable sample storage time is unknown. Freezing at -20 °C is known to be unsatisfactory. Storage below −70 °C is recommended when the measurement cannot be performed promptly, but this is impractical in clinical practice [23].

Factors affecting: Because of variability in urinary albumin excretion, two of the three specimens collected within a 3 to 6 month period should be abnormal before diagnosis of microalbuminuria. Exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, pyuria, and hematuria may elevate urinary albumin excretion over baseline values [4].

Reporting and Expression of data: There are variations in expression and reporting of results. The absence of recognized standard methods for reporting results reduce the use of this test in clinical and research settings [18]. Confusing reporting methods make the test difficult for the users of laboratory services [17]. Reported results may be milligrams albumin per gram (or µg/mg) or milligrams of albumin per millimole of creatinine, and the meaning of neither are obvious to nonspecialists. Clinically, healthcare providers may have difficulty in interpreting results. Different ways of reporting urine albumin results were: concentration (mg/L), excretion per 24 hours (mg/24 hours), excretion per minute (µg/min), and ACR (mg/ mmol or mg/g) [18].

Units of measurements: The units of measure for ACR used as milligrams per gram[12,24, 25] or milligrams per millimole or both[14].

Interconversion of units: ACR (1 mg/g = 1 µg/mg = 0.113 mg/mmol)[18]. Dividing the ACR by 8.84 converts the units (from µg/mg or mg/g to mg/mmol)[26]. There is conversion factor for creatinine in various units [27, 28]. Another easy way of conversion of creatinine is to convert mg/dl to g/L.

CONCLUSIONS: The term microalbuminuria is a confusing. The term urine albumin is recommended, instead of microalbumin. There is lot of confusion about reporting of results in different units. Ideally, International System of Units should be adopted. Also there should be agreement all over the world to use single system of

units for expressing and reporting results for urine albumin measurement. Uniform guidelines should be followed in a country or universally. At present in India there are no such clear guidelines about laboratory measurement of urine albumin. Here we feel need to set clinical practice guidelines for laboratory measurement of urine albumin for diagnosis of microalbuminuria in India.

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