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REVIEW ARTICLE

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DIABETES MELLITUS AND MICROALBUMINURIA: FACTORS AFFECTING COLLECTION OF URINE SAMPLES FOR MICROALBUMINURIA

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ABSTRACT

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defective insulin secretion, action or both. Diabetic nephropathy is a multistage disorder and takes several years to progress from the stage of incipient nephropathy to end stage renal disease. Microalbuminuria is an early indicator of diabetic nephropathy. Urine examination for microalbumin is routinely done to monitor the progression of nephropathy. As many factors can interfere with the estimation of microalbumin, it is very important that high standards are maintained while estimating the MA levels.

KEY WORDS

Diabetes mellitus, microalbuminuria, nephropathy

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defective insulin secretion, action or both. The chronic complications of diabetes include damage, dysfunction and failure of various organs mainly eyes, kidneys, nerves, heart and blood vessels (1). Diabetes is the most common cause of end stage renal disease (ESRD) and is a major risk factor for development of cardiovascular disease and blindness (2).

The early sign of ongoing nephropathy is microalbuminuria (MA), which is defined as urinary excretion of albumin at the rate of 30-299 mg/24hrs or 20-199μg/min (3, 4, 5). The presence of microalbuminuria predicts worsening of renal disease to overt diabetic nephropathy (6) and the elevated risk of cardiovascular disease (7, 8, 9, 10). Up to 30% of people with newly diagnosed type 2 diabetes will already have abnormally high urine albumin levels; about 25% will have overt diabetic nephropathy (11 – 15).

Early detection of nephropathy through screening of diabetic patients allows early intervention and better control of progression of nephropathy.

Diabetic nephropathy

Diabetic nephropathy is a multistage disorder and takes several years to progress from the stage of incipient nephropathy to end stage renal disease. There are four main biochemical pathways linking hyperglycemia, which lead to the functional and structural changes in the kidney, these are: polyol pathway, non enzymatic glycation, glucose auto oxidation and de novo synthesis of di glycerol leading to protein kinase C and phospholipase A_2 activation.

Microalbuminuria

The term microalbuminuria has been used to describe an amount of albumin excreted in urine which is not detected by regular tests such as albustix. The gold standard for detection of microalbuminuria is collection of 24 hr urine sample which is cumbersome, and in older children is aesthetically unacceptable. Hence, a spot urine examination would be more acceptable and less time consuming or it can also be determined in random urine samples. Urine samples should be collected when patient is at rest and in diabetic patients when sugar levels are normal. No measurement should be done when the patient is having ketoacidosis and poor control of blood glucose. Microalbuminuria should be present in at least two of three samples collected over a period of several months (16, 17).

Normal and abnormal urinary albumin excretion values (18)

			First voided morning specimen	
Albumin Excretion	24 hr collection (mg/24h)	Timed collection (μg/min)	Urine Albumin concentration* (mg/l)	Urine Albumin:Creatinine ratio** (mg/mmol)
Normoalbuminuria	<30	<20	<20	<3.5 women <2.5 men
Microalbuminuria	30-300	20-200	20-200	3.5 to 35 women 2.5 to 25 men
Overt proteinuria	>300	>200	>200	>35 women >25 men

**urine albumin of 200mg/l is equivalent to 300mg/l of protein*
*** 3.5 as lower limit in females because of lower creatinine excretion*

Microalbuminuria and its association with physiological and pathological conditions:

Physiological conditions associated with microalbuminuria are (19)

- After exercise and high protein diet
- Pregnancy
- In short stature

Pathological conditions are (20, 21, 22)

- Diabetes mellitus
- Hypertension
- Micro and macro vascular complications of diabetes and hypertension including left ventricular failure.
- Inflammatory diseases like rheumatoid arthritis, pancreatitis or any systemic infection.
- Surgery
- Burns
- Dyslipidaemia (increased ApoB , lipoproteins & decreased HDL)
- Insulin resistance
- Increased prorenin (precedes microalbuminuria)

Methods of microalbuminuria estimation:

(A) Collection of sample

Albumin excretion varies with physiological factors like exercise posture, diuresis. Thus samples should not be collected after exercise, in the presence of urinary tract infection, during acute illness, immediately after surgery or after an acute fluid overload.

The following are considered acceptable (23):

- 24 hour collection is preferred by some centers
- Overnight (8 - 12 hour) urine sample collection
- Short term urine collection i.e. 1-2 hour collection (in laboratory or clinic)
- Early morning sample voided is usually rather concentrated and using this sample has good correlation between the excretion rate and concentration of albumin.

Many conditions can give a false positive value. Some of these common conditions are

- Hyperglycemia
- Hypertension
- Heavy exercise
- Urinary tract infection
- Cardiovascular
- Stress
- Febrile condition
- Contamination with seminal or menstrual fluid

B) Storage

Urine should be stored at 4 °C after collection. Alternatively, 2ml of 50 g /L sodium azide can be added per 500ml of urine. Bacterial contamination and glucose have no effect. Specimens are stable for at least 2 weeks at 4 °C and 5 months at -70 °C. Freezing samples may decrease albumin but mixing immediately before assay eliminates this effect (23).

C) Estimating microalbuminuria

1) Semiquantitative methods:

a) Micral microalbumin urine test strip (Roche Diagnostic):

This test is an immunochemical strip specific for albumin. Albumin in the sample is bound by soluble conjugate of antibodies and the β-galactosidase enzyme marker. Conjugate-albumin complexes are separated and the β -galactosidase enzyme reacts with a substrate to produce a red dye. The intensity of the color produced is proportional to the albumin concentration in the urine.

b) Clinitec Microalbumin (Bayer Diagnostic):

This test strip is based on dye binding by albumin method. It uses the high affinity dye bis (3,3'-diiodo- 4, 4'-dih ydroxy-5, 5'-di nitrophenyl)-3,4,5,6-tetrabromosulfonephthalein. At a constant pH, the strip turns blue in the presence of albumin, and color is directly related to albumin concentration in the urine sample.

2) Quantitative

There are many methodologies available but most frequently used are: radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), radioimmunodiffusion (RID), and immunoturbidimetry. All these four methods have similar sensitivity, precision and range.

a) Immunoturbidimetry

In this process turbidity is produced by an immune complex reaction. This causes a reduction in the intensity of light as it passes through the solution. Turbidimetry is the measurement of this loss in intensity because of scattering, absorption or reflection of the incident light in the angle/direction of the incident light.

Most colorimeters and spectrophotometers can measure turbidity with good precision and accuracy. This is the most widely used test as it can be done on most semi auto chemistry analyzers. It can even be done on automated chemistry analyzers (23,24).

b) Nephelometry

This assay is also based on scatter detection but unlike turbidimetry it measures scattered light at 90°C to the incident light. The instrument is called a nephelometer. It is more sensitive than turbidimetry (23,24).

c) Radio immunoassay (RIA)

This assay procedure involves competitive binding between radio labelled and unlabelled molecules of antigen to high affinity, specific antibody. The amount of unlabelled antigen present in the specimen is measured by its competitive effect on the labelled antigen for limited antibody sites. It involves the use of radio isotopes like tritium (^{3}H), ^{131}I or ^{125}I as labels. It has high sensitivity and specificity (23,24). The sample values are determined by comparison with a calibration curve. The advantages are sensitivity, precision and inexpensiveness, whereas the disadvantage is short shelf life and radioactivity of the reagents.

d) Chemiluminescent immunoassay (CLIA)

Chemiluminescence is a chemical reaction that emits energy in the form of light. When used with immunoassay technology, the light produced by the reaction indicates the amount of analyte in a sample. This again is of two types:

- ***Luminescent Immunoassay (LIA):*** Here the labelled and unlabelled antigen competes for the limited binding sites on the labelled antibody. An inverse relationship exists between concentration of labelled antibody bound to the antigen and the unlabelled antigen.

- ***Immuno Chemiluminometric assay (ICMA) :*** This is a sandwich assay in which unlabelled antigen is sandwiched between antibody bound to paramagnetic particles and antibody labelled Acridinium ester (AE). A direct relationship exits between the concentration of antigen in the patient sample and the amount of light emitted during oxidation of the AE (23,24).

Both RIA and CLIA are preferred globally for their sensitivity, specificity and reproducibility but unavailability, cost factor, big infrastructure, government permission for use of radioactive materials are the limiting factors

SUMMARY AND CONCLUSION

Nephropathy is a common complication among diabetics which increases their mortality. As the progression of diabetic nephropathy is slow, it is possible to be detected at an early stage. Microalbuminuria is an early indicator of diabetic nephropathy. Urine examination for microalbumin is routinely done to detect and monitor the progression of nephropathy. As many factors can interfere with the estimation of microalbumin, it is very important that high standards are maintained while estimating the MA levels. Various studies have proved the advantages of using a spot

urine sample over the conventional 24 hrs collected urine. Pre-analytical care has to be taken regarding the time of collection, ensuring that the patient is at rest and not suffering from any other disease or condition which could give a false positive result. Ideally fresh urine samples should be used. In case if the urine has to be stored, proper temperature and preservatives should be added to prevent oxidation and contamination as it again may alter the albumin levels.

Semiquantitive dipstick methods are useful for screening. It can be done in the clinics and bedside of the patient. Although it is a rapid method, it seldom indicates the severity of the disease. Quantitative methods like immunoturbidimetry, chemiluminescense, nephlometry and RIA are more reliable and sensitive methods. Immunoturbidimetric method is one of the most commonly used as it is highly sensitive and is more cost effective. RIA and chemiluminescence have high sensitivity but also have disadvantages of radiation hazards and high cost respectively.

Since early detection of microalbuminuria can help in early diagnosis of diabetic nephopathy, adequate care and precautions has to be taken while estimating it.

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