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The Use of Semi-Quantitative Urine Test-Strip (Micral Test) for Microalbuminuria Screening in Patients with Diabetes Mellitus

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ABSTRACT

Background: Microalbuminuria is an early marker of prognostic significance in diabetic renal disease. However, testing for microalbuminuria in a timed sample of urine using the double antibody radioimmunoassay (RIA) method is cumbersome and requires special laboratory facilities. Recently, a test strip for microalbuminuria, the Micral Test was available and we evaluated the performance of this test strip as a screening method for detection of microalbuminuria.

Methods: One hundred consecutive diabetic patients who were tested to be dipstick-negative (Albustix) for proteinuria were enrolled for the study. Micral Tests were performed on a paired first morning and random urine specimen from the same patient and the results compared with a timed 24-hour urine measurement of urine albumin excretion using the RIA method.

Results: Eighteen specimens were tested positive by the RIA method with a urinary albumin range of 32 – 177 mg/24 hours. With the Micral Test, the following sensitivity, specificity, positive and negative predictive values were obtained: 66.7%. 97.6%, 85.7% and 93.0% for the first morning urine specimens, and 77.8%, 91.5%, 66.7% and 94.9% for the random urine specimens.

Conclusion: These results suggest that Micral Test with either the first morning or random urine specimen offers a simple, reliable, rapid and convenient method for screening of microalbuminuria in the diabetic patient.

Keywords: microalbuminuria, Micral™ Test, diabetic nephropathy, screening

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INTRODUCTION

Microalbuminuria, defined as urinary albumin excretion rate of 20 – 200 μg/min or 30 – 300 mg/day predicts future development of overt or clinical diabetics nephropathy[1,2]. Overt nephropathy develops in one third of insulin-dependent diabetic patients[3] and approximately 20% in non-insulin-dependent diabetics (NI DDM)[2]. These patients have progressive loss of renal function and will eventually require renal replacement therapy[3]. Microalbuminuria has been found to be positively related to an increased mortality and morbidity in NIDDM mainly from cardiovascular disease[4].

Prevention, early detection and treatment of microalbuminuria is desirable and hence, screening of an elevated urinary albumin excretion rate has been recommended as a standard care for patients with diabetes mellitus[5]. In 1963, Keen and Chlouveraskis described the first specific radioimmunoassay (RIA) for albumin in the urine[6]. This technique was not widely used until the publication in 1980 of two longitudinal studies that an increased in urinary albumin excretion was strongly predictive of clinical diabetic nephropathy[1,2].

However, to permit frequent screening for microalbuminuria to be performed on a large scale and in the outpatient population, there is a need for methods which are easy to apply, inexpensive, and yield quick results. The defined levels of microalbuminuria are below the detection limit of conventional urine test strips such as "Albustix". The present study aims to evaluate a recent commercial semi-quantitative test strip, the "Micral Test". (Boehringer Mannheim, Germany) as a screening test for microalbuminuria. We also studied the suitability of using the spot first morning or random urine specimen in the detection of microalbuminuria.

PATIENTS AND METHODS

Patients and study design

One hundred consecutive diabetic patients seen at the outpatient Diabetes Clinic who were tested negative for clinical proteinuria (Albustix, Ames Co, USA) were studied. Patients were instructed to collect three urine specimens in the following sequence viz: (i) 24-hour collection on the day prior to clinic visit; (ii) first morning urine specimen, and (iii) spot random urine specimen on the day of the clinic visit.

Measurements of microalbuminuria

The Micral Test is an immunochemical strip specific for albumin. Albumin in the sample was bound by a soluble conjugate of antibodies and marker enzyme β-galactosidase. Conjugate-albumin complexes are separated and enzyme β-galactosidase reacts with a substrate to produce a red dye. The re-agent part of the test strip should be dipped into the urine for 5 seconds and then laid down horizontally and read after 5 minutes. The intensity of the colour produced is proportional to the albumin concentration in the

urine. The colour formed is compared with the reference chart on the vial. There are five colour blocks, reflecting categories of albumin concentrations of 0, 10, 20, 50, 100 mg/L. A measurement of ≥ 20 mg/L was considered positive. All visual assessments of the strips were performed by the same person trained to perform this test. All the first morning and random urine specimens were tested with the Micral Test.

The 24-hour urine collection was tested with the gold standard RIA (Diagnostic Products Corp, USA) method which is a double antibody technique. Albumin in the sample competed with a fixed amount of ^{125}I-labelled albumin for the binding sites of the specific antibodies. Bound and free albumin were separated by addition of a second antibody immunoabsorbent, followed by centrifugation and decanting. The radioactivity in the pellet was measured with a γ-counter. Albumin concentration in the sample was inversely proportional to the radioactivity[7]. RIA were performed with no prior knowledge of sample's details. The sensitivity for the RIA method was 0.3 mg/L.

Analysis

Urine albumin concentration of ≥30 mg/24 hours measured by RIA method was considered positive for microalbuminuria. The Micral Test results obtained on the paired first morning and spot random urine samples were compared with the RIA results. Specificity, sensitivity, positive and negative predictive values of the Micral Test were calculated. The specificity is the proportion of true negative results among normoalbuminuric patients:

Specificity = true negative/(true negative + false positive)

The sensitivity is the proportion of true positive results among microalbuminuric patients:

Sensitivity = true positive/(true positive + false negative)

In calculating the positive predictive value, which is the probability of having microalbuminuria given a positive test result, the prevalence (= frequency of microalbuminuria in this group of diabetic patients) was used: positive predictive value = prevalence x sensitivity/[prevalence x sensitivity + (1-prevalence) x (1-specificity)]. The negative predictive value, which is the probability of having no microalbuminuria given a negative test result, was calculated as: negative predictive value = [(1-prevalence) x specificity]/{[(1-prevalence) x specificity] + [prevalence x (1-sensitivity)]}[8].

RESULTS

Fifty-one males and 49 females were studied. Thirty-eight of them were on insulin treatment. Mean age was 44 ± 13.5 years.

Eighteen specimens of the 24-hour urine collection tested positive for microalbuminuria. The mean albumin excretion was 80 ± 44 mg/24 hours with a range of 32 – 177 mg/24 hours.

Table I and II summarise the results of the Micral Test obtained with the first morning and spot random urine samples. With the first morning urine specimens, 14 specimens were tested positive with the Micral Test. Out of the 14 positive tests, two were falsely positive. This gives a sensitivity of 66.7%, and a specificity of 97.6%. Positive and negative predictive values were 85.7% and 93.0% respectively. Twenty-one of the random urine specimens were tested positive with the Micral Test of which 7 were falsely positive, giving a sensitivity of 77.8% and specificity of 91.5%. The positive and negative predictive values were 66.7% and 94.9% respectively.

DISCUSSION

We found that in our present study, the Micral Test has a good specificity and moderate sensitivity at the cut-off point of a urine albumin concentration of 20 mg/L. The frequency of false-positive of 4.5% was acceptable since the Micral Test is recommended to be a screening and not a diagnostic test for microalbuminuria. Urine samples screened positive with the Micral Test should be confirmed by a laboratory reference method for albumin using a timed urine sample. However, with a false-negative rate of 5%, Micral Test will miss out in a proportion of cases. In screening, a fairly high false-negative rate is only acceptable if, at the next screening, the missed cases are still detected at a pre-clinical stage. Cooper found that the mean duration of the microalbuminuric phase was 3 – 4 years, with considerable individual variation[9]. Hence, the use of the Micral Test is valid for clinical practice, if instructions for repeat measurement at regular intervals in patients tested negative are incorporated. The recommendation is for microalbuminuria screening to be performed at least once a year in those tested negative[10].

We have also shown comparable results in the two different urine specimens, as both showed satisfactory sensitivity and specificity. The random urine specimens attained better sensitivity over the first morning urine specimens but the latter had the advantage of a higher positive predictive value compared with the former. There appears to be no distinct advantage of either collection.

Table I – Performance of Micral Test with first morning urine specimens

	Positive	Negative
True	12	80
False	2	6

Table II – Performance of Micral Test with random spot urine samples

	Positive	Negative
True	14	75
False	7	4

In the busy outpatient setting, 24-hour urine collection may be cumbersome and inconvenient to both the patient and the clinic practice especially if repeated measurments are proposed. Hence, the Micral Test is a convenient, simple and useful screening test for detection of microalbuminuria, and either the first morning or random urine specimen may be used.

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