SCREENING FOR MICROALBUMINURIA IN THE EARLY DETECTION OF DIABETIC NEPHROPATHY: A CHEAP AND SIMPLE METHOD

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Abstract- ***Prompt detection of renal involvement through evaluation of microalbuminuria can both reduce mortality in diabetic patients and significantly reduce the cost of managing these patients. To this end, 'Micral' test strips have been used as a screening tool in this group of patients though not yet in our country. The present study aimed to evaluate this test and to obtain a simpler and less expensive method of screening for microalbuminuria.***

In this study, 200 type 1 and type 2 diabetic patients referred to the Endocrine & Metabolism Research Centre of the Tehran University of Medical Sciences were evaluated for microalbuminuria. Entry criteria consisted of a history of recognised diabetes longer than or equal to 5 years. Exclusion criteria included development of urinary tract infection, pyrexia, and a history of uncontrolled hypertension. Every patient first completed a data questionnaire and then provided a first-void urine sample, which was tested for microalbuminuria with a urinary protein test strip (an Iranian-made Uri-Yab and a German-made BM-test-GP strip) and the sulphasalicylic acid chemical method. Negative samples were tested further using Micral test strips (based on gold-label immunochromatography) and the microalbumin measurement kit manufactured by Dako, Germany (based on turbidometry using a Hitachi autoanalyser).

126 women and 74 men were recruited into the study. The average age of the sample was 40.7 years (range = 16 to 63 years); 17 patients had IDDM and 173, NIDDM. Average duration of recognised diabetes was 8.75 years. Based on results obtained using chemical analysis and foreign-made tests strips, 46 patients (23%) had macroalbuminuria, though the detection rate using Iranian-made test strips was 18 percent (36 patients).
A further 16.2 percent of patients had microalbuminuria, with an average urinary albumin excretion of 28.7 mg per litre. Compared with colorimetric methods, the Micral test yielded a sensitivity of 93% and a specificity of 87 percent. Furthermore, the negative predictive value of the Micral test was 0.92, compared with a figure of 0.94 for a combination of the sulphasalicylic acid method and the BM-Test-GP strips.

The results we obtained for the Micral test as a screening tool concurs with the results of numerous other studies. Diurnal variations in albumin excretion dictate the performance of screening tests on three different occasions at specified regular intervals. Given the relatively high cost of Micral strips, it seems that a simpler and less expensive method should be devised for our country. We observed a higher negative predictive value for the combined sulphasalicylic + BM-Test-GP method than for the Micral strips. The cost of the latter combination comes to 2,000 Rials (≅0.25 US$), which is easily affordable for most patients; the methodology falls comfortably within the expertise of medical diagnostic laboratories in Iran. More detailed studies, using 24-hour urine collection, are needed to confirm this approach.
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Key Words: ***Diabetic nephropathy, microalbuminuria, Micral test***

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INTRODUCTION

Diabetic nephropathy is the commonest cause of renal failure in End-Stage Renal Disease [ESRD] (1). In developed countries alone, more than 5 billion US dollars are spent each year on the management of ESRD. Mortality rates in these patients during dialysis are 50% higher than they are for non-diabetic cases (2). At present, once total proteinuria is discovered, renal failure is the inevitable outcome, whereas early detection of renal involvement through testing for microalbuminuria can prevent disease progression even before onset of clinical symptoms, thereby leading to increased survival and lower treatment costs (1).

Microalbuminuria is defined as when urinary albumin excretion increases but remains undetectable by conventional laboratory methods, such as routine urine testing strips. Screening for microalbuminuria is performed every 6 months and only then in patients who have had diabetes for longer than 5 years and who show no evidence of macroalbuminuria (2). The latter is in general screened for using a semi-quantitative method (e.g. urine strips) and, in patients with at least two positive

out of three screening tests, a standard quantitative method, usually immunoassay, is then used as confirmation (3). Among the various urine test strips available such as Albustix™, Microalbutest™, Albuscreen™, and Micral Test II™- the last is generally more accepted and widely used worldwide. The sensitivity and specificity of the above test strips have been confirmed by numerous investigators, namely Gilbert et al (sensitivity=93%, specificity=93%), Morgensen et al (sensitivity=92%, specificity=97%), Perograro et al, etc (4- 6). On the other hand, several studies, including Webb et al in 1997, have questioned the diagnostic value of the Micral Test because of false positive results (7). The uniformly high cost of these strips, however, is a major drawback. In general, therefore, this method has two main disadvantages:

1.High cost (US$2.00 per strip), and
2.False positive results (2).

This has meant that they have not so far been widely adopted in Iran. The present study was performed by screening patients using the Micral test strip and comparing the outcome with standard immunoturbidometry, with the goal of evaluating the Micral test strip and obtaining a simple, rapid and inexpensive screening tool.

MATERIALS AND METHODS

The present study was performed on 200 type 1 and type 2 diabetic outpatients at the Endocrinology and Metabolism Research Centre of Tehran University of Medical Sciences. Entry criteria consisted of absence of confounding factors such as hematuria, fever, uncontrolled hypertension and urinary tract infection, together with a history of diabetes longer than or equal to 5 years (1). Having completed a questionnaire revealing data such as age, duration and type of diabetes, treatment regimen, etc, patients were asked to provide a 'first-void' urine sample. Samples were first tested for macroalbuminuria with urinary protein testing strips (homemade Uri-Yab and German-made Böhringer-Mannheim-Test-Glucose-Protein [BM-Test-GP] strips) and the sulphasalicylic acid chemical method, Which is based on protein precipitation in acid media. In routine urine testing strips, colorimetry is based on the principle of 'protein error indicator', a phenomenon that involves colour change of certain pH indicators in the presence of protein. Generally, at a pH of between 3 to 4, the indicator changes colour from yellow to blue or green. In the presence of protein, however, the colour change takes place at between pH 2 and 3. Thus, the presence of protein induces an error in the behaviour of the indicator, the change in colour correlating with the amount of protein present. The test strip is extremely sensitive to albumin, but responds less well to the other urinary proteins, such as γ-globulin, glycoprotein, ribonuclease, lysozyme, hemoglobin, Tomm-Horsfall mucoprotein, and Bence-Jones Protein (2). The above strips can only detect albumin in quantities above 250 mg/L, i.e. macroalbuminuria. A negative result does not therefore preclude the presence of other proteins, or of small amounts of albumin. Since acid-based tests react to all types of protein, all positive results with sulphasalicylic acid – an anionic precipitant – underwent further confirmatory testing. With the latter method, the degree of turbidity caused is proportional to the concentration of protein present in solution. A negative result means that protein is present in quantities inferior to 50 mg/L.

All samples negative for macroalbuminuria were tested for microalbuminuria using Micral II strips and compared with standard immunoturbidometry using a microalbumin kit manufactured by Dako Co.

The Micral test is based on a change in colour of the indicator present on the test strip when urinary albumin binds to a specific gold-bound antibody within a chromatographic system, such that urinary albumin (acting as antigen) initially binds to an 'absorbent layer'. It then enters a 'buffer zone' in order to attain optimal conditions. Subsequently, within a 'conjugate zone', it is attached by an enzyme specifically to a conjugated antibody, thereby generating an antigen (albumin)-conjugated antibody complex. In the next stage ('capture matrix'), excess antigen-conjugated antibody complexes are removed by an immobilising antibody, when the remaining antigen-conjugated antibody complexes enter the reacting colour layer, which contains the substrate needed to generate a colour change in direct proportion to the concentration of albumin in urine. The cross-reaction rate with other proteins, such as hemoglobin, transferrin, Bence-Jones protein, α_1-antitrypsin, α_1-glycoprotein, α-amylase, Tomm-Horsfall protein, retinol-binding protein, IgG, IgA, but also leukocytes and erythrocytes – has been given as less than 0.5 percent. Each strip contains 6 μg/cm² of monoclonal IgG anti-human-albumin antibody covered by a colloidal collapse and 9.5 μg of fixed albumin. The above strips can detect albumin in concentrations varying from 20 to 200 mg/L. To perform the test, strips are dipped at the correct point in the sample of urine for 5 seconds, avoiding contact with the walls of the sampling vessel. If albumin is present, a colour change should be observed after 1 minute, and the results will be recorded as 0, 20, 50 or 100 mg/L. If albumin is present in amounts in excess of 100 mg/L, the initial sample will need to be diluted with distilled water (8).

To confirm the results obtained, quantitative methods were used to test for microalbuminuria in all samples. In general, immunoassay methods are the

standard for measurement of microalbuminuria, and comprise four different techniques:

1. RIA (radioimmunoassay)
2. ELISA (enzyme-linked immuno-sorbent assay)
3. RID (radioimmunodiffusion)
4. Immunoturbidometry

These four methods have a similar level of sensitivity and specificity, and are used in different areas according to the resources available (9). Immunoturbidometry is the one more frequently used because of its greater simplicity. Also in our study, all negative patients for macroalbuminuria were evaluated by immunoturbidometry using a kit manufactured by Dako Co. and a Hitachi autoanalyser. The basis of this method is the reaction between urinary albumin and anti-albumin antibody, the resulting turbidity being measured by spectrometry at a wavelength of 340nm. The information obtained was processed using the Chi-square and Fisher tests of the 'SPSS for Windows' software. Quantitative variables were compared using the 'student t-test', and intra-group comparisons were performed using the 'paired t-test' and the repeated measure with "ANOVA test".

RESULTS

Our sample consisted of 126 women and 74 men. Patients age ranged from 16 to 63 years, with a mean of 40.7 years. 17 patients had IDDM and the rest, NIDDM. Mean duration of disease since diagnosis was 8.75 years.

Chemical testing and foreign-made strips showed that 46 patients (23%) had macroalbuminuria. The detection rate for macroalbuminuria using homemade strips was only 18 percent (36 patients) (Table 1). Using standard methodology, 16.2% of patients had microalbuminuria, with an average urinary albumin excretion of 28.7 mg/L.

To determine the sensitivity and specificity of our Micral test strips, the results obtained with them were compared with those obtained by standard methodology (Table 2). 121 patients had negative results with both methods, with 8 patients having false positive results (i.e. Micral-positive and standard-negative). Similarly, 62 patients had positive results with both methods and 9 had false negative outcomes (Micral-negative, standard-positive). Thus, our Micral strips had a sensitivity of 93% and a specificity of 87%. Similarly, the negative predictive value of the Micral test was calculated as 0.926, and its positive predictive value as 0.88 (Table 2).

In comparison, the results obtained using BM-Test-GP strips and the sulphasalicylic acid methods were 135 double negatives and 26 double positives. Seventeen samples yielded positive results with BM-Test-GP strips but not with the acid method, with 2 samples vice versa.

To determine the negative predictive value of the combination of the last two methods in comparison with standard methodology, the aforementioned 17 samples were excluded from calculations because of mismatch and the results of the remaining samples were compared with those obtained by standard methodology. Based on the information given in table 3, out of the 135 double negatives reported above, 7 samples turned out positive using standard methodology: the remaining 128 samples being in effect triple negatives. The negative predictive value of the combined BM-Test-GP/sulphasalicylic acid method was therefore calculated as 0.948.

Table 1. Detection Rate for Various Tests

Method	Macroalbuminuria >300 mg/L	Microalbuminuria 20-300 mg/L
Uri-Yab	18%	---
BM-Test-GP	27%	---
Sulphasalicylic Acid	23%	---
Standard Immunoassay	---	16.2%

Table 2. The comparison of Micral test to standard immunoturbidometry

Standard Immunoturbidometry	Micral Test		Total
	≤20	>20	
≤20	121	8	129
>20	9	62	71
Total	130	70	200
	Sensitivity = 93% Specificity = 88% Negative Predictive Value = 0.92		

Table 3. The comparison of combination of BM-Test-GP and sulphasalicylic acid standard immunoturbidometry

Standard Immunoturbidometry	Combination of BM-Test-GP and Sulphasalicylic Acid	
	Both Negative	Both Positive
Negative	128	0
Positive	7	46

DISCUSSION

Given the worldwide high prevalence of both diabetes and hypertension, and of renal involvement in both disorders, it is important to detect renal disease promptly–through screening for microalbuminuria–when it is still at the reversible stage, in order to reduce both mortality and treatment cost in those affected (10-12). Sample collection, determination of albumin-to-creatinine ratios, and measurement of albumin concentration in first void urine samples are all both time-consuming and expensive, especially when large numbers of patients are involved. There is also often a need to repeat tests because of day-to-day variations in albumin excretion, which further accentuates these problems. Consequently, much research in developed countries has been and still is focused on increasing the sensitivity of screening tests whilst reducing their cost and time. Micral II strips are used routinely for screening purposes in many parts of the world (US, Germany, and the UK…), but high costs and the false negative results reported by certain investigators have brought their use under question. Our study has confirmed the sensitivity and specificity of Micral II strips in comparison with standard immunoturbidometric methodology. The advantage of the above method lies in the fact that it does not require 24-hour urine collection and that it is quick (3 minutes in total). Against this, however, one must count its high cost. In a study by Alfredo Pegoraro and colleagues in 1997, a combination of the sulphasalicylic acid and 'Chemstripe' strips had a higher negative predictive value than Micral strips (6). In our study too, the combined sulphasalicylic acid/BM-Test-GP method had a higher negative predictive value (0.94) than Micral strips (0.92). Use of this combined method will therefore lead to a reduction in the number of false negative results from 5 to 2 cases per 10,000. Both methods take approximately three minutes to perform, and easily fall within the technical expertise of medical diagnostic laboratories in Iran. The cost of the combined method is significantly less than that of Micral strips. Further studies are needed to confirm the results we have obtained. Accordingly, in view of the absence of a consensus or protocol for microalbuminuria screening in Iran, we propose that the combined method outlined above be used until less expensive and more sensitive, or at least Micral test strips, become available in the country.

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