The Utility of Conventional Dipsticks for Urinary Protein for Screening of Microalbuminuria in Diabetic Patients

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Abstract

The demonstration that microalbuminuria is predictive of overt diabetic nephropathy has created a demand for the routine measurement of urinary albumin in diabetic patients. We assessed the sensitivity, specificity, positive and negative predictive values of the conventional dipsticks for urinary protein (Ames Multistix, Bayer Diagnostic, Australia) as the screening test for microalbuminuria in diabetic patients compared with Micral-Test II (Boehringer Mannheim, Germany). Radioimmunoassay for albumin was taken as standard for comparison. With the urinary albumin concentration of 20 mg/L as a discriminating level of microalbuminuria, Micral-Test II had a sensitivity of 98.8 per cent and a specificity of 68.6 per cent whereas Ames Multistix had lower sensitivity but higher specificity. If urinary albumin concentration of 60 mg/L was used instead as a discriminating level of microalbuminuria, none of Ames Multistix by visual reading and only 5 of 32 (15.6%) of those by reflectance photometer had false negative results. By visual reading, the sensitivity of Ames Multistix was increased from 68.1 to 100 per cent with the drop in specificity from 85.7 to 50.2 per cent. On the other hand, the sensitivity was increased from 37.4 to 84.4 per cent but the specificity was maintained if reflectance photometer was used. In conclusion, Ames Multistix was less sensitive than Micral-Test II in detection of urinary albumin concentration above 20 mg/L. At higher urinary albumin concentration above 60 mg/L which indicates a clinically significant microalbuminuria, the sensitivity of Ames Multistix was increased to 100 per cent. Ames Multistix which is much less expensive than Micral-Test II, can be used as the screening test for significant microalbuminuria in clinical practice particularly in cases having financial problems.

Key word : Dipsticks, Microalbuminuria, Urinary Protein, Diabetes Mellitus

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Diabetic nephropathy is one of the major vascular complications of patients with longstanding diabetes. Microalbuminuria which is defined by the presence of urinary albumin excretion in the range of 20-200 μg/minute or 30-300 mg/24 hours or albumin concentration of 20-200 mg/L, is considered to be the earliest clinical manifestation of patients with diabetic nephropathy[1]. Microalbuminuria has been shown to be the predictor of the future development of clinical or overt diabetic nephropathy in both type 1 and type 2 diabetic patients[2-5]. Since rigid glycemic control and treatment with angiotensin converting enzyme inhibitors at stage of microalbuminuria can delay or may prevent the progression of diabetic nephropathy, [6-11] screening of diabetic patients for microalbuminuria is recommended and has been well regarded as part of routine standard diabetic care.

Microalbuminuria can be quantitatively measured by several methods, for example, radioimmunoassay (RIA), immunoturbidimetry and nephelometry methods. These methods are expensive and require special equipment, their routine use as a screening test of microalbuminuria is not cost-effective. Therefore, it is recommended that screening with reagent tablets or dipsticks for microalbuminuria may be carried out first and followed by more specific tests if the screening tests give positive results[12].

The conventional dipsticks for urinary protein which are routinely used in hospital laboratories is thought to be insensitive to detect protein in microalbuminuric range and not recommended as the screening test for microalbuminuria. The lowest concentration of urinary protein which can be detected by this method is ~200 mg/L. Since albumin comprises ~20 per cent of urinary protein, [13] urinary protein concentration of 200 mg/L should therefore be composed of 40 mg/L of albumin. Hence, any levels of albumin above 40 mg/L which include the microalbuminuric range, should be theoretically detectable by the conventional dipstick method.

In this study, we assessed the sensitivity, specificity, positive and negative predictive values of the conventional dipsticks for urinary protein (Ames Multistix, Bayer Diagnostic, Australia) as the screening test of microalbuminuria in diabetic patients compared with Micral-Test II (Boehringer Mannheim, Germany) which is widely used as the screening test of microalbuminuria in clinical practice. RIA for albumin was measured for all urine specimens as standard for comparison.

MATERIAL AND METHOD

First-voided morning urine specimens were collected from 196 diabetic patients. Each urine specimen was divided into 3 portions after being mixed well. The first portion was tested with Ames Multistix and Micral-Test II and interpreted by three of the investigators (S.S., A.T., N.T.) who were blinded to each other's results. The testing and handling procedure as well as visual reading technic were followed according to the manufacturer's operating manual. The corresponding results from at least two of the three investigators were regarded as the test results. Given possible disagreement between visual results and instrumental results, the second portion of the urine sample was tested with Ames Multistix using the Clinitek 200 urine chemistry analyzer, a reflectance photometer designed to read the strips with automated timing of color development. The first and second portion of the urine samples were tested at room temperature within 4 hours after voiding. The third portion of the urine sample was stored at 2-8°C for later quantitative measurement of albumin using RIA method (Diagnostic products Corporation, CA., USA). The detection limit of the assay was 0.3 mg/L. The intra-assay CV of the test at albumin concentrations of 9.5 and 53.7 mg/L were 4.8 and 4.1 per cent, respectively. All urine samples were tested within 2 weeks of collection.

Ames Multistix is a chemically impregnated dipstick. The degree of proteinuria is determined from a colorimetric reaction of an indicator dye (0.3%w/w tetrabromphenol blue). The color is compared to a color chart on the bottle label. The test assay levels are negative, trace (10-20 mg/dl), 1+ (30 mg/dl), 2+ (100 mg/dl), 3+ (300 mg/dl), and 4+ (1,000 mg/dl). The results are considered positive when the tests produce a reaction color corresponding to trace or more.

Micral-Test II is gold-labelled optically read immunoassay. The color produced permits the visual determination of urinary albumin concentration by comparison with color blocks on the bottle label after a 1-minute incubation time. The test assay levels are 0, 20, 50 and 100 mg/L albumin. Reaction color lighter than the color scale of 20 mg/L indicates negative result. The results are positive when the tests produce a reaction color

corresponding to 20 mg/L or more of albumin. The sensitivity of Micral-Test II in detection of microalbuminuria is claimed to be higher than the first-generation Micral-Test,[14-16] the reported sensitivity of which ranged from 63 per cent to as high as 100 per cent[17-23].

To determine the diagnostic accuracy of each test, results obtained by Ames Multistix and Micral-Test II were interpreted with regard to their ability to detect urinary albumin concentration of ≥ 20 mg/L as determined by RIA. Urine samples that contained albumin out of microalbuminuric range or > 200 mg/L were excluded from the analysis.

RESULTS

The albumin concentrations of 196 urine samples measured by RIA ranged from 1.2 to 189.7 mg/L. Of these, 105 (53.6%) had urinary albumin concentration < 20 mg/L and 91 (46.4%) had urinary albumin concentration ≥ 20 mg/L or within microalbuminuric range. As seen in Tables 1 and 2, Micral-Test II had lower rate of false negative results, whereas, Ames Multistix had a lower rate of false positive results in detection of urinary albumin concentration of ≥ 20 mg/L. The higher false negative rate of Ames Multistix was greater among those performed by reflectance photometer. With the urinary albumin concentration of 20 mg/L as an upper limit of normal or a discriminating level of microalbuminuria, Ames Multistix could not detect the presence of microalbuminuria in 29 (46.8%) samples if read by investigators' eyes (visual reading) and 57 (62.6%) samples if read by reflectance photometer, whereas, only one (1.1%) sample was missed by Micral-Test II. Therefore,

Table 1. Comparison of the ability of Ames Multistix and Micral-Test II in detection of microalbuminuria of various ranges.

Test	urinary albumin concentration by RIA (mg/L)		
	< 20 (n=105)	20-60 (n=59)	> 60 (n=32)
Ames Multistix			
Visual reading			
Negative	90	29	0
Positive	15	30	32
Reflectance photometer			
Negative	104	52	5
Positive	1	7	27
Micral-Test II			
Negative	72	1	0
Positive	33	58	32

RIA : radioimmunoassay method

Table 2. Comparison of diagnostic accuracy of Ames Multistix and Micral-Test II according to cutoff levels of microalbuminuria.

Test	MAU level (mg/L)	Sensitivity	Specificity	PPV	NPV
		(%)			
Ames Multistix					
Visual reading	≥ 20	68.1	85.7	80.5	75.6
	> 60	100.0	50.2	41.6	100.0
Reflectance photometer	≥ 20	37.4	99.0	97.1	64.6
	> 60	84.4	95.1	77.1	96.9
Micral-Test II	≥ 20	98.9	68.6	73.2	98.6

MAU : microalbuminuria, PPV : positive predictive value, NPV : negative predictive value

Micral-Test II had higher negative and lower positive predictive values than Ames Multistix in this regard.

From Table 1, regarding the Ames Multistix results, it was notable that all 119 samples tested negative by visual reading and 156 of 161 (96.9%) samples tested negative by reflectance photometer had urinary albumin concentration < 60 mg/L. If urinary albumin concentration of ≥ 60 mg/L was used instead as a discriminating level of microalbuminuria, none of Ames Multistix by visual reading and only 5 of 32 (15.6%) of those by reflectance photometer had false negative results. By visual reading, the sensitivity of Ames Multistix was increased from 68.1 to 100 per cent with the drop in specificity from 85.7 to 50.2 per cent (Table 2). On the other hand, the sensitivity was increased from 37.4 to 84.4 per cent with a slightly decrease in specificity from 99.0 to 95.1 per cent if reflectance photometer was used. With the use of urinary albumin concentration of 60 mg/L as a cutoff level of microalbuminuria, Ames Multistix, either by visual reading or reflectance photometer, had high negative predictive power (100 *vs* 96.9%) although their positive predictive power was not as high (41.6 *vs* 77.1%).

DISCUSSION

Our study agreed with others that Micral-Test II, a semi-quantitative immunoassay for urinary albumin, is appropriate as a screening test of microalbuminuria in diabetic patients considering its high sensitivity and high negative predictive power. The drawback of this test in terms of its poor positive predictive power or having a high false positive rate was also confirmed in our study which means that it requires other specific or repeated testing to confirm the diagnosis. Furthermore, our study also agreed that the conventional dipstick for urinary protein was less sensitive than Micral-Test II in detection of urinary albumin concentration above 20 mg/L. However, the results of our study were in contrast with Pegoraro et al's study[24] which showed the high sensitivity of Chemstrips, a dipstick for urinary protein, comparable to Micral-Test (90.0 *vs* 96.7%). The fewer number of samples containing urinary albumin concentration > 20 mg/L (~14 *vs* ~46% of our study) may have possibly contributed to the unexpectedly high sensitivity of Chemstrips in the latter study. Our results were also contradictory to results from Bloomgarden's study[25] in which the fairly high sensitivity but low specificity of Ames reagent strips, a similar test-strips used in our study, either by visual reading or by reflectance photometer were demonstrated. The higher range of urinary albumin concentration approaching 300 mg/L in a significant number of samples may be responsible for the high sensitivity of this test in Bloomgarden's study since the chance of having positive results could be increased. However, with the same instrument as ours, the false positive rate particularly by visual reading was much higher in his study. This discrepant result would propably be due to the subjective variability in color perception.

It is well known that the intraindividual variability of urinary albumin excretion particularly at the lower levels is very high[26,27]. Patients who test positive for microalbuminuria on one occasion may turn out to be negative on other occasions and vice versa. In addition to biologic variation of urinary albumin excretion, it should be recognized that other factors, for instance, heavy exercise, poor glycemic control, acute febrile illness, cardiac failure, urinary tract infection may transiently increase urinary albumin excretion. Therefore, the finding of urinary albumin excretion slightly above the normal range may not represent pathological microalbuminuria. Furthermore, the association between the presence of lower levels of microalbuminuria and the progression of nephropathy is not strong. In Steno studies where the development and progression of diabetic nephropathy in patients with type 1 diabetes was prospectively followed,[28] Feldt-Rasmussen et al reported the progression to clinical nephropathy in only one of 32 patients who had an initial urinary albumin excretion rate of 30-99 mg/24 hours (or urinary albumin concentration of ~20-60 mg/L), whereas, such progression was demonstrated in 12 of 19 patients who had an initial urinary albumin excretion rate of 100-300 mg/24 hours after 5-8 years of follow-up. In another long-term study of normotensive type 2 diabetic patients who had persistent microalbuminuria reported by Ravid et al,[9] it was found that all patients who progressed to clinical macroalbuminuria had initial urinary albumin excretion greater than 89 mg/24 hours (or urinary albumin concentration of ~60 mg/L). Mathiesen et al[10] found that of 7 patients with type 1 diabetes who developed clinical nephropathy (urinary albumin > 300 mg/24 hours) during 4 years of

follow-up, only one had a baseline urinary albumin excretion of 54 mg/24 hours and all of the rest had baseline urinary albumin excretion of > 100 mg/24 hours.

Therefore, it could be stated that urinary albumin excretion above 90-100 mg/24 hours or urinary albumin concentration above ~60-70 mg/L signifies a clinically meaningful albuminuria which can predict the progression of diabetic nephropathy[29]. Our study showed that Ames Multistix particularly by visual reading had high sensitivity and high negative predictive power to detect urinary albumin concentration above this level. Although the sensitivity of Micral-Test II at this level of urinary albumin concentration was not tested in our study and cannot be compared with Ames Multistix, it should not be superior to Ames Multistix given the high sensitivity of the latter. When the cost per test of Ames Multistix (0.25 US$ per test; 40 Baht = 1 US$) and Micral-Test II (1.13 US$ per test) was taken into account, it would be more cost effective to use Ames Multistix as a screening test for significant microalbuminuria. Diabetic patients who test negative with Ames Multistix should be rescreened annually and those who test positive with the results of "trace" or more should be confirmed by measuring albumin excretion rate with a more specific method.

In summary, our study showed that Ames Multistix, a conventional dipstick for urine protein was less sensitive than Micral-Test II, a dipstick for microalbuminuria in detection of urinary albumin concentration above 20 mg/L. At higher urinary albumin concentration above 60 mg/L which indicates a clinically significant microalbuminuria, the sensitivity of Ames Multistix was increased to approach 100 . Since urinary albumin excretion above this level predicts the progression of diabetic nephropathy, we suggest that Ames Multistix or other brands of dipstick for urinary protein, if confirmed, can be used as the screening test for significant microalbuminuria in clinical practice particularly in some rural areas or some developing countries where Micral-Test or other tests for microalbuminuria are not available or too expensive.

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การตรวจไมโครอัลบูมินในปัสสาวะด้วยแถบตรวจโปรตีนในปัสสาวะในผู้ป่วยเบาหวาน

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ในปัจจุบันการตรวจหาอัลบูมินในปัสสาวะ ถือเป็นส่วนหนึ่งในการดูแลผู้ป่วยเบาหวาน เนื่องจากการตรวจพบไมโครอัลบูมินในปัสสาวะ (microalbuminuria) ในผู้ป่วยเบาหวานสามารถพยากรณ์การเกิดไตเสื่อม (diabetic nephropathy) ในทางปฏิบัตินิยมใช้แถบตรวจไมโครอัลบูมินในปัสสาวะเป็นการตรวจกรอง (screening test) ไม่แนะนำให้ใช้แถบตรวจโปรตีนในปัสสาวะซึ่งมีใช้กันอย่างแพร่หลายและราคาถูกกว่า เนื่องจากมีความไวในการตรวจไมโครอัลบูมินในปัสสาวะไม่เพียงพอ ข้อเสียของการใช้แถบตรวจไมโครอัลบูมินคือราคาค่อนข้างแพงและมีไม่แพร่หลายโดยเฉพาะในโรงพยาบาลขนาดเล็ก คณะผู้วิจัยจึงได้ศึกษาผลการตรวจไมโครอัลบูมินในปัสสาวะในผู้ป่วยเบาหวานด้วยแถบตรวจไมโครอัลบูมิน (Micral-Test II, Boehringer Mannheim, Germany) และแถบตรวจโปรตีน (Ames Multistix, Bayer Diagnostic, Australia) โดยใช้การตรวจด้วยวิธี radioimmunoassay เป็นมาตรฐาน ผลการศึกษาพบว่า ถ้าใช้ระดับอัลบูมินในปัสสาวะที่ 20 มก/ล เป็นเกณฑ์ขั้นต่ำของไมโครอัลบูมินในปัสสาวะ การตรวจด้วย Micral-Test II มีความไวร้อยละ 98.8 และความจำเพาะร้อยละ 68.6 ในขณะที่การตรวจด้วย Ames Multistix มีความไวน้อยกว่าแต่ความจำเพาะมากกว่า ถ้าใช้ระดับอัลบูมินที่ 60 มก/ล เป็นเกณฑ์ขั้นต่ำของไมโครอัลบูมินในปัสสาวะ พบว่าการตรวจด้วย Ames Multistix โดยการอ่านผลด้วยตาจะมีความไวเพิ่มขึ้นจากร้อยละ 68.1 เป็นร้อยละ 100 ความจำเพาะลดลงจากร้อยละ 85.7 เป็นร้อยละ 50.2 และไม่มีผลลบปลอมเลย แต่ถ้าอ่านผลด้วยเครื่อง reflectance photometer จะมีความไวเพิ่มขึ้นจากร้อยละ 37.4 เป็นร้อยละ 84.4 ความจำเพาะไม่เปลี่ยนแปลงและมีผลลบปลอมร้อยละ 15.6 โดยสรุป การตรวจไมโครอัลบูมินในปัสสาวะด้วย Amex Multistix มีความไวน้อยกว่า Micral-Test II ถ้าใช้ระดับอัลบูมินในปัสสาวะที่ 20 มก/ล เป็นเกณฑ์ขั้นต่ำของไมโครอัลบูมินในปัสสาวะ แต่ถ้าใช้ระดับอัลบูมินในปัสสาวะที่ 60 มก/ล ซึ่งเป็นระดับที่มีความสำคัญทางคลินิกเป็นเกณฑ์ขั้นต่ำของไมโครอัลบูมินในปัสสาวะ การตรวจด้วย Ames Multistix มีความไวเพิ่มขึ้นเป็นร้อยละ 100 ดังนั้นในทางปฏิบัติอาจใช้ Ames Multistix ซึ่งมีราคาถูกกว่า Micral-Test II มาก เป็นการตรวจกรองสำหรับไมโครอัลบูมินในปัสสาวะ โดยเฉพาะในรายที่มีปัญหาด้านเศรษฐกิจ

คำสำคัญ : แถบตรวจ, ไมโครอัลบูมินในปัสสาวะ, โปรตีนในปัสสาวะ, เบาหวาน

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