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SHORT COMMUNICATION

Evaluation of Microalb immunoturbidimetric test for albuminuria screening

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SUMMARY Microalb, an immunoturbidimetric test for screening urinary albumin levels was evaluated for its potential as a screening test for microalbuminuria in diabetic patients. It was compared with the current standard, the radioimmunoassay (RIA). The results showed that the test lacks sensitivity while its specificity was acceptable. Therefore, it can not replace the RIA as the screening method. This study also showed that the first early morning urine could be used if a 24- hour collection was not possible, as its albumin content fairly correlated (r= 0.78) with the 24-hour urine collection of the diabetic patients.

Microalbuminuria (MAU) has been recognized as an independent and reliable predictor for future development of overt proteinuria in diabetic patients.[1] It represents the condition in which minute quantities of albumin not detectable by simple urine dipstick are excreted into the urine. MAU is associated with a form of diabetic nephropathy that has a high morbidity and mortality[2-5] as well as with coronary artery diseases.[1] Furthermore, there have been many reports on the correlation of MAU with an increasing incidence of diabetic retinopathy.[1] Therefore, MAU is an important biomarker for predicting complications of diabetic patients. In cases where high blood sugar control was successful, MAU also decreased.[6] In this study, the new Microalb immunoturbidimetric test was evaluated as an initial screening test for MAU in urine samples collected from 3 different periods of 24-hours of diabetic patients in comparison with the standard method, radioimmunoassay (RIA).

MATERIALS AND METHODS

Subjects

Twenty-one patients (7 males and 14 females) aged 35-55 years who attended the Outpatient Department of the Diabetic Clinic of King Chulalongkorn Memorial Hospital were selected for this study. All had type 2 diabetes mellitus diagnosis based on World Health Organization criteria. All had been well followed-up for 5-10 years. None had any apparent proteinuria with the dipstick test (Albustix, Boeringher Manheim, Manheim, Germany). All subjects were asked to supply their informed consent before the study.

Sample collection

A 24-hour urine sample was collected from each subject. The urine collection was divided into three periods: 6.00 a.m.-2.00 p.m., 2.00 p.m.-10.00 p.m. and 10.00 p.m.-6.00 a.m. The volume of urine per period and the total 24-hour volume were recorded in each case. Collected urine samples were stored between 4 and 10°C without preservatives and sent to the laboratory within 2 hours after completion of the collection. Sample analysis was performed at the Central Laborato-

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Laboratory analysis

All urine samples were analyzed for microalbumin level by the immunoturbidimetric method using the Microalb kit and the RIA.[7] The sensitivity of RIA is 0.5 mg/l; the coefficient of variation (CV) is 7.0% at an albumin concentration of 21.4 mg/l and 5.5% at a concentration of 124 mg/l (normal value 0-15 μg/minute; 0-20 mg/l).

Microalb immunoturbidimetric test

Microalb kit (Miles, Canada) is an immunoturbidimetric test specific for albumin. The principle is that when human albumin reacts with its specific antibody, precipitating immunocomplexes are quickly formed in the presence of polyethylene glycol. The turbidity is then photometrically measured at 340 nm. All analytical procedures were performed according to the manufacturer's instructions. The normal value is 0-20 mg/l.

Sample analysis

In each case, 2-3 ml per period and the total 24-hour urine samples were used for the immunoturbidimetry test. Five milliliters of the total 24-hour urine sample was used for the RIA. The urine albumin concentrations were determined blindly by a technician. The reproducibility of the test was assessed by another observer on 10 occasions for precision analysis. The time dependence of the test was assessed by comparing the results obtained per period and the total 24-hour urine sample.

Statistical evaluation

Correlation coefficient (r) was used in determination of the relation between parameters.

RESULTS

Precision analysis of the Microalb test showed the during-run precision CV of 7.56% and the between-runs precision CV of 11.71%. The results of albumin determination by both RIA and Microalb test per period and the total urine sample in each case are presented in Table 1. According to the reference value of each method, MAU was detected in 3 cases by the Microalb test and in 7 cases by RIA.

Comparing the Microalb test with standard RIA method, only fair correlation was obtained ($r = 0.78$) (Table 2). Using the reference cutoff value of the Microalb test at 20 mg/l, the sensitivity, specificity, false positive and false negative values for determination of MAU in total 24-hour urine samples were 29.5%, 92.9%, 4.7% and 23.8%, respectively.

As to the time dependence of the Microalb test, it was demonstrated that urine collected during 10.00 p.m.-6.00 a.m. period correlates best with the total 24-hour urine ($r = 0.77$) (Table 2). All microalbumin determinations performed on periodic urine samples by the Microalb test showed less correlation with the standard RIA method compared with the total 24-hour urine (Table 2).

DISCUSSION

MAU, defined as an albumin concentration above normal but still negative by conventional dipstick testing, has recently been recognized as an early marker for diabetic complications especially for diabetic nephropathy.[2,3] In type 2, non-insulin-dependant diabetic patients, MAU can also serve as an indicator for mortality from cardiovascular diseases.[1] Therefore, identification of MAU in diabetic patients represents added value for diabetic care.

There are many methods for determining MAU. RIA is a widely used quantitative method for MAU detection. However, this method has limitations such as the long cycle time required and its radiohazard. Therefore, a number of alternative methods for detection of MAU have been developed. Although there are some semi-quantitative methods such as the Micro-Bumin test[8] and Micral-test strips[9] which have proven reliable, a major limitation of these tests is the inherent difficulty in an objective visual determination of the color reaction.

In this study, a new quantitative method, the Microalb immunoturbidimetric test has been evaluated. This test is based on the precipitation of the albumin by immunologic reaction and subsequent quantitative spectrophotometrical determination. The process requires about 45 minutes less than the RIA.

The evaluation of this study has shown a fair correlation between the Microalb and the RIA for microalbuminuria detection. Using the manufacturer's reference at 20 mg/l, the test lacks the sensitivity but the specificity is acceptable. Therefore, Microalb can be used only as a selective method for MAU detection but it cannot replace the RIA or be used as a screening method.

It has also been shown that urine collected from 10.00 p.m.-6.00 a.m. contained a representative amount of albumin compared with the total 24-hour sample. This result

Table 1 Urine microalbumin detection per period (period 1: 6.00 a.m.-2.00 p.m., period 2: 2.00 p.m.-10.00 p.m. and period 3: 10.00 p.m.-6.00 a.m.) and the 24-hour samples examined by Microalb immunoturbidimetric test and RIA

Subject	Sex	Creatinine Concentration (mg/dl)	Total urine volume (ml)	Microalbumin (mg/l)				
				Microalb test				RIA*
				Period 1	Period 2	Period 3	24-hours	
1	Male	69.0	2,280	9.0	4.5	4.0	3.0	4.5
2	Male	102.3	1,496	9.0	16.0	2.0	5.5	5.2
3**	Male	66.0	1,767	29.5	44.3	17.5	32.0	37.7
4	Male	64.9	1,826	50.0	10.5	20.5	24.0	6.6
5	Male	67.1	1,647	11.0	8.5	0	3.5	4.2
6**	Male	49.5	2,800	11.5	5.0	4.0	5.5	22.5
7	Male	4.4	1,906	30.0	5.0	4.0	5.5	7.1
8	Female	19.8	2,896	2.0	17.0	1.5	1.7	1.3
9	Female	35.2	2,606	7.2	7.5	9.0	12.0	12.0
10	Female	22.0	8,16	12.5	18.0	5.0	10.0	11.0
11**	Female	90.2	2,051	83.0	19.5	15.5	24.0	37.2
12	Female	67.1	2,451	68.0	22.0	22.0	16.0	2.3
13**	Female	22.0	1,220	40.0	40.0	12.0	15.5	14.4
14**	Female	96.8	1,159	9.0	15.5	22.0	14.5	20.5
15	Female	53.9	1,589	16.5	6.5	1.5	7.0	8.4
16	Female	79.2	1,211	8.5	7.0	8.0	12.0	11.0
17	Female	49.5	1,780	27.0	10.5	2.0	5.0	6.3
18**	Female	53.9	2,092	13.0	15.0	4.5	10.5	12.8
19**	Female	92.4	1,377	10.0	13.0	19.0	13.5	17.9
20	Female	100.1	8,50	4.0	7.0	13.0	5.0	7.2
21	Female	47.3	1,232	2.5	1.0	2.0	1.5	1.2

* already transformed from μg/minute to mg/l
** cases withMAU (urine albumin ≥ 20 mg/l by RIA)

Table 2 Correlation coefficient of each period and total 24-hour urine microalbumin detection by Microalb immunoturbidimetric test and RIA

Microalb test (period)	Correlation coefficient (*r*)	
	Microalb test (24-hours)	RIA
6.00 a.m.-2.00 p.m.	0.64	0.39
2.00 p.m.- 10.00 p.m.	0.73	0.63
10.00 p.m.- 6.00 a.m.	0.77	0.50
24-hours	-	0.78

is similar to a previous study by Eshoj *et al.*[10] Therefore, the first early morning urine can be used as a proper spot urine specimen for determination of MAU in diabetic patients.

Since the Microalb test does not require any sophisticated equipment, therefore, it can be used in rural hospital laboratories. Although some automatic-analyzer-based methods for detection of MAU have been developed very recently, the equipment are still expensive and not cost effective for small laboratories. The results of this pilot study, nevertheless, may provide information to help physicians for planning of urine specimen collection and selection of the proper method to monitor MAU and hence, provide better care to the diabetic patients.

REFERENCES

1. Mattock MB, Morrish NJ, Viberti GC, Keen H, Fitzgerald AP, Jackson G. Prospective study of microalbuminuria as predictor of mortality in NIDDM. Diabetes 1992; 41: 736-41.
2. Viberti GC, Hill RD, Jarett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982; 1: 1430-2.
3. Matheisen ER, Oxenboll B, Johansen K, Svendsen PA, DEckert T. Incipient nephropathy in type 1 (insulin-dependent) diabetes. Diabetelogia 1984; 26: 406-10.
4. Mogensen CE, Christensen CK. Predicting diabetic nephropathy in insulin-dependent patients. N Eng J Med 1984; 311: 89-93.
5. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity of onset diabetes. N Eng J Med 1984; 310: 356-60.
6. Feldt-Rasmussen B, Mathiesen ER, Deckert T. Effect of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. Lancet 1986; 2: 1300-4.
7. Christensen C, Orskov C. Rapid screening PEG radioimmunoassay for quantification of pathological microalbuminuria. Diabetic Nephrop 1984; 3: 92-7.
8. Tai J, Tze WJ. Evaluation of Micro-Bumin test reagent tablets for screening of microalbuminuria. Diabetes Res Clin Pract 1990; 9: 137-42.
9. Marshall SM, Shearing PA, Alberti KGMM. Micral-test strips evaluated for screening for albuminuria. Clin Chem 1992; 38: 588-91.
10. Eshoj O, Feldt-Rasmussen B, Larsen ML, Mogensen EF. Comparison of overnight, morning and 24-hour urine collection in the assessment of diabetic microalbuminuria. Diabetic Med 1987; 4: 531-3.