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Comparison study of semiquantitative test strips for detecting human serum albumin (HSA) in urine specimens

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Abstract

Background: Microalbuminuria (MAU) is a powerful predictive marker of early diabetic nephropathy, progressive cardiovascular disease in diabetic and hypertensive patients as well as in the general population.
Methods: This study was designed to compare the diagnostic performance of the two semi-quantitative immunostrip tests for rapid detection of urinary albumin based on monoclonal antibody (mAb) conjugated with colloidal gold particles (AuNPs) (test A) and MCM-41 mesoporous nanoparticles conjugated with HSA (test B). The measurements of the analyte were compared with the values obtained by immunoturbidimetry (IT) assay (test C).
Results: 30 randomly chose urine samples were analyzed by tests A, B and C. Resemble to the gold standard, 10 patients were normoalbuminuric, 18 were microalbuminuric and 2 were macroal-buminuric by test B. By comparison with IT, the immunostrip tests had a sensitivity of 90% and 100% for test A and B, respectively. False-negative results were yielded in 6.6% of specimens in the normoalbuminuric group, by using test A. No false-negative results occurred in the group of macroalbuminuric and microalbuminuric patients by this test. The agreement degree of the test A and B was 86% which were classified as substantial with regard to Kappa index (κ=0.857).
Conclusion: Our findings suggest that the immunostrip test with MCM-41 is a very reliable method for detecting urine albumin in the range of 0 to >200mg/L. This test with sensitivity of 100% is easy to perform, low-cost and rapid as compared to other sophisticated tests which are time-consuming and expensive to perform.

Keywords: Semiquantitative test strips, Albuminuria, Comparison study

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Introduction

Since detection and quantification levels of certain proteins can be very complex, expensive and time consuming, there is a high demand for convenient methods to detect and measure such proteins in the biological specimens. Human serum albumin (HSA) is the most abundant plasma protein synthesized exclusively in the liver. Under physiological conditions, urinary protein excretion is less than 150 mg/day, of which about 10 mg is albumin. Persistent albuminuria in the range of 30-300 mg/day known as microalbuminuria (MAU), which is recognized as one of the powerful predictive markers of early diabetic nephropathy and progressive cardiovascular disease in diabetic and hypertensive patients (1-4) as well as in the general population (5,6). Since medical intervention, including control of blood glucose, cholesterol and blood pressure at the onset of MAU has shown to be critical in reducing adverse outcomes, current consensus guidelines recommend the annually screening for MAU (7). However, studies of primary care physicians in the United States reveal astonishingly low levels of albuminuria screening even in high risk diabetic patients. Explanations for the poor performance have focused on the lack of immediate results for medical decision making in addition to the associated cost, patient inconvenience and patient non-compliance with the modern and sophisticated methods (8, 9). Therefore, these kinds of problems have spurred the development of point-of-care testing systems with the advantages of reliability and rapidity. In addition, they can conveniently be used in a clinical laboratory setting. Immunostrip test is one of those tests which recently there has been a growing trend toward it. They allow a physician too quickly and accurately detection of analytes on small sample volumes from a patient at the time of the visit as well as by the patient at home. In the present study we focused our investigation on comparing the diagnostic performance of the two in-house semi-quantitative immunochromatographic (ICG) tests, based on conjugated monoclonal antibody gold nanoparticles (mAb–AuNPs) and mobile crystalline material (MCM)-41–HSA bio-conjugate, for the rapid detection of urinary albumin, which have developed in our previous work (10,11).

Methods

Recently, we successfully constructed two competitive in-house immunostrip tests in our laboratory by mAb conjugated with 20-nm colloidal gold particles (test A) and MCM-41 mesoporous nanoparticles conjugated with HSA (test B). After optimization and validation, they applied to analyze 30 different urine samples from 30 patients who had referred to the Endocrinology and Metabolism Research Center (EMRC) of Tehran University of Medical Sciences, in order to measure urine albumin. Details for preparation process of anti –HSA mAb, Ab-AuNPs, MCM 41- HSA conjugates, and immunostrips development, detection and signal intensity acquisition are provided in our previous reports (10-12). Briefly, urinary albumin was measured by three tests including immunoturbidimetry (IT) (test C), immunostrip tests with and without MCM-41 (test B and A respectively). Method C was considered as the reference standard (comparison method). This study dealt with the performance characteristics of test A and B regarding diagnostic accuracy and their agreement percentage for detecting urinary albumin concentration, then the yielded results were compared with the values obtained by the widely regarded IT assay as a gold standard.

Statistical analysis

Sensitivity of the test was determined using the Med-Calc-statistical software. The kappa coefficient was used to measure the degree of agreement between the immunostrip tests.

Results

The urine samples were divided into three groups: normo-, micro-, or macroalbuminuric on the basis of immunoturbidimetric albumin measurement. Of 30 randomly chose urine samples analyzed by test C, 10 were classified as normoalbuminuric (<18 mg/l), 18 as microalbuminuric (20–200 mg/l), and 2 as macroalbuminuric (>200 mg/l). Urine albumin determination by IT, immunostrip tests with and without MCM-41 is provided in Table 1. Both micro- and macro-albuminuria were considered as positive result with regard to having albuminuria and normoalbuminuria was considered as negative result. By comparison with the IT regarding its ability to detect a urinary albumin concentration

>20mg/l, the immunostrip test without MCM-41 (test A) had a sensitivity of 90% with 6.6% of false negative results in normoalbuminuric. No false negative results were found among the group of micro and macroalbuminuric patients by this test. The sensitivity of the immunostrip test with MCM-41 (test B) was 100% with 0% of false negative results. The agreement degree of the test A and B was 86%, which were classified as substantial with regard to Kappa index (κ=0.857). The diagnostic accuracy of the two strip tests by comparison with the IT assay is illustrated in Table 2.

Table 1. Determination of urine albumin by immunoturbidimetry, immunostrip tests with and without MCM-41

Type of assay	Total number	Normal (<18 mg/L)	Microalbuminuria (20–200 mg/L)	Macroalbuminuria (>200 mg/L)
Immunoturbidimetry	30	10	18	2
Immunostrip test without MCM-41	30	12	16	2
Immunostrip test with MCM-41	30	10	18	2

Table2. The diagnostic accuracy of the two immunostrip tests. Immunoturbidimetry assay was considered the gold standard for detecting microalbuminuria

Immunoturbidimetry	S (%)	FN (%)	κ
Immunostrip test without MCM-41	90	6.6	0.86
Immunostrip test with MCM-41	100	0	

S = Sensitivity, FN= False-negative result, κ = Kappa index

Discussion

The diagnostic performance of two in-house strip tests developed in our previous study was compared and IT test was chosen as a gold standard. The results of this study suggest that, compared to the gold test, our newly developed ICG strip test with MCM-41 (test B), with equal sensitivity of 100% to the gold standard seems to be valid for the detection of albuminuria (>20mg/l). This is pivotal for a screening test since costs for confirmatory testing have to be considered, especially when the indication for albuminuria screening is extended to a population less likely to have elevated urinary albumin (13, 14). On the other hand, this study demonstrates that test A with a sensitivity of 90% and diagnostic agreement of 80% with test B can also be a reliable test for screening HSA. Sensitivity of 90% assures that only a few patients with elevated urinary albumin are overlooked.

As it mentioned in our previous report, the analytical sensitivity of test B was greatly higher than test A. This test with the detection limit of 100 ng/ml was superior to the test A (18 µg/ml) and also most other commercial urinary albumin immunostrip tests (10). Considering to the fact that we did not evaluate the tests in healthy individuals, specificity, false positive results, positive predictive value and negative predictive value could not be calculated for each test, and it can be considered as a limitation of our study. However, we will deal with this issue in subsequent studies.

At present, various antibody-based methods are used to measure lower levels of urinary albumin. These include radioimmunoassay (RIA), nephelometry, IT, and enzyme-linked immunosorbent assay (ELISA). Although some investigators argue for the widespread adoption of techniques based on high-performance liquid chromatography (HPLC) to improve accuracy (15, 16), HPLC may need experienced staff to interpret and it is not practical in most clinical laboratory settings (17, 18). In spite of the fact that immunoassay methods provide the advantages of sensitivity and specificity with a small sample volume for analysis, they often need long reaction time and involved multiple steps. It is noteworthy that recent studies have claimed the accuracy of some of these assays such as immunochemical methods and HPLC have come in to debate (19).

In recent years, immunostrip tests have practical advantages in clinical diagnostic and have extensively been used as a popular detection method in clinical chemistry for various biomarkers (10, 11, 20, 21). Therefore,

rapidly available, cost-effective and sensitive tests are clinically needed to detect MAU and initial early prevention strategies. There are various rapid and easy performing strip tests commercially available for albuminuria, among those, Micral® II (Roche Diagnostics) based on gold-label ICG, is generally more accepted and widely used worldwide. Several studies of different size have evaluated this strip test. The sensitivity of the Micral® II test as a rapid and accurate method for detecting MAU is reported to be within the range of 64-97% and reaches 100% in non-diabetic patients with kidney diseases. Specificity is reported to be in the range of 71-93% (22-26). While the Micral test strips provide a rapid approach to detecting MAU, unfortunately, high costs and the reported range of sensitivity, specificity and predictive values for this test strip have raised some concerns of reliability (8, 22, 23).

In conclusion, our findings suggest that the immunostrip test with MCM-41 is a very reliable method for detecting albuminuria in the range of 0 to >200mg/L. This test with sensitivity of 100% is easy to perform, low-cost and rapid compared to other sophisticated tests which are time-consuming and expensive to perform. Therefore, it may be a useful and valid method for the screening of elevated urinary albumin excretion and also to limit costs for the identification of false positive results. Nevertheless, further studies with larger sample size are needed to confirm the results we have obtained and definitive judgments.

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