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Evaluation of Dirui® H13-Cr and H12-800MA strips for albuminuria detection

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Abstract

Increased urine albumin excretion is suggested to be an early marker of glomerular damage. Evaluation of a test's performance characteristics such as diagnostic sensitivity and specifictiy is a necessity before application into routine practice. To our knowledge up to now there is no study evaluating Dirui test strips for albuminuria determination. Therefore the objective of this study was to test these strips for agreement with the established method used in our routine laboratory. Freshly collected 177 urine specimens were included in the study. Strips were read on instruments Dirui H-500 and Dirui H-800 using reflectance photometry. For DiruiH13-Cr strip albumin to creatinine ratio (ACR) and for DiruiH12-800MA strip albumin determinations were compared with the analyses performed using immunoturbidimetry and kinetic Jaffe method. Sensitivity values for estimated ACR using DiruiH13-Cr strip and albumin concentration using DiruiH12-800MA strip were calculated as 80%, 72%, respectively. Specificity values were as; 71% for Dirui H13-Cr strip and 78% for Dirui H12-800MA strip. Screening tests using urine test strips should be validated before acceptance in the routine use. Dirui strips evaluated in this study seem to have a lower sensitivity than suggested and excess number of false negatives seems to limit utility of these strips.

Keywords: Urine albumin, albumin to creatinine ratio, strip, sensitivity, screening

Introduction

Increased urine albumin excretion is known as an early marker of kidney damage. Albuminuria assessment is used for the definition, classification, and predicting the prognosis of chronic kidney disease (CKD) [1]. Since diabetes is the leading cause of CKD in developed countries and rapidly becoming the major cause in developing countries [2] annual screening for albuminuria is advocated by American Diabetes Association for early determination of renal impairment in all type 2 diabetic patients starting at the diagnosis [3]. There is also improving data about that albuminuria is related with increased risk for adverse cardiovascular events [4,5].

Today urine albumin is measured using a variety of methods such as immunoassays including turbidimetric and nephelometric procedures and size exclusion chromatography [6]. At this time, the Joint Committee for Traceability in Laboratory Medicine web site does not list a reference measurement procedure for urine albumin [7].

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Persistent albuminuria in the range of 30-300 mg/24 h which corresponds to urine albumin to creatinine ratio (ACR) between 30-300 mg/g and was formerly called ''microalbuminuria'', is termed as ''moderately increased'' albuminuria. Since conventional strips used in routine urinalysis are known to be relatively insensitive for detecting moderately increased albuminuria they do not have recommended usage for this purpose. [1,3]. Today there is an ongoing demand for practical and convenient method alternatives for urine albumin analysis.

To our knowledge no study evaluating the performance of Dirui H13-Cr and H12-800MA strips has been published up to date. Therefore the aim of this study was to test these strips for the agreement with the method used in our laboratory considering the diagnostic performance measures such as sensitivity and specificity for detecting albuminuria.

Material and Method

Samples

Freshly collected 177 spot urine specimens which belong to all of the subjects undergoing evaluation for urine albumin determination in our hospital within a period of 5 days were included in the study. The participants aged between 19-85 years old and were being evaluated for diabetes (n=80), chronic renal disease (n=77), hypertension (n=10) and cardiovascular diseaseas (n=10).

All of the analyses were completed within two hours of urine collection. The study was approved by the Ethical Committee of Ankara Numune Research and Training Hospital.

Laboratory analysis

Analyses using Dirui H13-Cr strip were performed on Dirui H-500 Urine Analyzer (Dirui Industrial, Co. Ltd. China). The reaction principle depends on high sensitivity of Sulfone phthalein dye to microalbumin by the method of protein error. Method for measuring creatinine is based on its reaction with 3,5-dinitro-benzoic acid in an alkaline medium to yield a colored compound. Albumin and creatinine results are reported as 10, 30, 80, 150 mg/L and 10, 50, 100, 200, 300 mg/dL respectively. Albumin to creatinine ratios <30 mg/g and ≥30 mg/g were accepted as negative and positive, respectively.

Analyses using reagent strip Dirui H12-800MA were performed on Dirui H-800 Urine Analyzer (Dirui Industrial, Co. Ltd. China). The reaction principle depends on high sensitivity of Sulfone phtalein dye to microalbumin by the method of protein error. Results are reported as negative, 150 mg/L and >150 mg/L. A strip result of 150 mg/L was accepted as an indicator of clinical albuminuria according to the manufacturer's recommendations.

Both of the strips also contain reagent areas impregnated for other urinalysis parameters including urobilinogen, bilirubin, ketone, blood, protein, nitrite, leukocytes, glucose, specific gravity, ph and ascorbic acid. Implementation is practical, provides rapid detection and needs no centrifugation step. Presence of urinary tract infection or hematuria with microalbuminuria invalidates the microalbumin test result [8]. Since other parameters of urinalysis are also impregnated on the strip, evaluation of urine for urinary tract disorders is also possible.

Dirui H-500 is a semi-automated benchtop urine chemistry analyzer. For each sample, test strip was dipped into the specimen briefly. After all the test pads were completely immersed, excess urine was removed and the strip was placed on the conveyor of the instrument by the same laboratory operator. Dirui H-800 Urine Analyzer is a fully automated urine chemistry analyzer. Both of the instruments take reflectance readings at 525, 572, 610 and 660 nm.

Quantitative urinary albumin determinations were performed on Roche Modular® P800 Autoanalyzer using Tina-quant Albumin reagent belonging to the same manufacturer (Roche Diagnostics, Mannheim, Germany). Anti-albumin antibodies in the reagent bind to albumin in the sample. After the agglutination these immuncomplexes are detected with turbidimetric principle. Urinary creatinine measurements were performed using kinetic Jaffe method on the same system. An ACR greater than 30 mg/g was considered positive for albuminuria.

Precision study

Precision studies were performed with the Dirui® Urinalysis Control containing low and high level control materials. Ten and twenty replicates of each level were run on both instruments for within-day and between day studies respectively. Results were registered as reflectance reading values.

Statistical analysis

2x2 tables were used to compare the performance of the tests [9].

Figure 1 Results of a new test presented as a 2x2 table [9].

Sensitivity and specificity were assessed according to the following equations [10]:

Sensitivity = [TP / (TP+FN)] x 100
Specificity = [TN / (TN+FP)] x 100

Results

Precision study

Precision study revealed quite different coefficient of variation (CV) values for the instruments indicating a better performance for Dirui H-500 Urine Analyzer. Results of the precision study are presented in Table 1.

Table 1. Precision study results for Dirui H-500 and Dirui H-800 Urine Analyzer

	Dirui H-500 Urine Analyzer.		Dirui H-800 Urine Analyzer.	
	Within-day	Between Day	Within-day	Between Day
Sample	CV %	CV %	CV %	CV %
Low control	1.05	0.97	6.38	4.18
High control	5.44	9.09	18.42	43.02

Parameters of the efficacy of Dirui H13-Cr and Dirui H12-800MA Strips

Measured albumin concentrations ranged from 3 mg/L to 2886 mg/L.

Table 2 illustrates the results of Dirui H13-Cr strip on Dirui H-500 Urine Analyzer comparing with the reference method. Sensitivity and specificity were calculated as 80%, 71% respectively.

Table 2. Results of Dirui H13-Cr strip on Dirui H-500 comparing with the albumin:creatinine ratio by immunoturbidimetry and kinetic Jaffe

Dirui H13-Cra	Albumin:creatinine ratio[a]		
	Positive	Negative	Total
Positive	75	24	99
Negative	19	59	78
Total	94	83	177

[a] Values <30 mg/g considered negative and ≥30 mg/g considered positive

Table 3 illustrates the results of Dirui H12-800MA strip on Dirui H-800 Urine Analyzer comparing with the reference method. Sensitivity and specificity were calculated as 72%, 78% respectively.

Overall agreement concerning ACR between the reference test and Dirui H13-Cr strip was 76%. Dirui H12-800MA strip had a similar agreement such as 75% with the reference test.

Table 3. Results of Dirui H12-800MA strip on Dirui H-800 comparing with the albumin:creatinine ratio by immunoturbidimetry and kinetic Jaffe

Dirui H12-800MA [b]	Albumin:creatinine ratio[a]		
	Positive	Negative	Total
Positive	68	18	86
Negative	26	65	91
Total	94	83	177

[a] Values <30 mg/g considered negative and ≥30 mg/g considered positive
[b] Values <150 mg/L considered negative and ≥150 mg/L considered positive

Discussion

Chronic kidney disease is an insidious, burdensome health problem. Some studies reported the prevalence of this disease as high as 10-16% overall the world [11-13]. Identification of subjects in the early stages of CKD provides opportunity for treatment, delay in the disease progression and prevention for some of the complications. Therefore screening for albuminuria is recommended in high risk groups such as diabetes mellitus and hypertension [1,3,14-19].

In the literature there is an ongoing concern about the ability of point-of-care (POC) tests for albuminuria detection. POC tests designed for this purpose use mainly immunometric methods or specific dye binding property. Strip based methods may be evaluated by visual inspection manually or read on small POC devices besides fully automated urinalysis instruments. In a recent review it was reported that there has been a considerable variation in the sensitivity and specificity estimates of the studies assessing the semiquantitative strip tests using a dye-binding albumin method. Sensitivity was reported to range from 18% to 92.9% and specificity from 60% to 100% [20]. But to our knowledge there is no study evaluating the performance of Dirui microalbumin strips for urine albumin determination up to now.

To assess an acceptable analytical CV, a goal of an 18% CV has been offered considering the biological variation of urine albumin excretion [21]. Furthermore the analytical CV for albumin when it is used to estimate the ACR is recommended to be less than 15% for the methods aim to measure low levels of albuminuria by The National Academy of Clinical Biochemistry (NACB) [19]. Evaluation of the reflectance reading values revealed that both of the strips had less than 15% CV for the low level control. Nevertheless using the high level control the CV results of Dirui H12-800MA strip were unsatisfactory. In a study of Decavele et al the protein test of Combur 10-test M strips (Roche) on the automated urine strip reader Cobas U411(Roche) was evaluated using the quantitative reflectance readings. This protein test was based on the principle of the protein error of a pH indicator and was supposed to be particularly sensitive to albumin and providing reliable data on albumin. Reported within- and between-day CV values for low concentration control were both <2% and for high concentration control material <5% [22].

High sensitivity (low false negatives) is a desirable characteristic of screening tests. Identification of the patients correctly provides treatment possibility for them. On the contrary high false negatives due to misclassification of real patients are related with poor outcomes such as deprivation of treatment and consequently the progression of disease. Sensitivity was higher for Dirui H13-Cr with a rate of 80%. Accordingly 19 out of 94 positive samples were misclassified as negative. NACB states that to be useful, semiquantitative or qualitative screening test must be shown to be positive more than 95% of patients with albuminuria [19].

Increased albumin excretion should be persistent for the definition of albuminuria. Therefore three sampling within a 6 month period is recommended and at least two of these samples should be positive [8]. In this study only one sample belonging to each subject was analysed. This is a limitation since multi sample evaluation may improve the sensitivity.

A positive result of a screening test should be confirmed with a diagnostic test to establish a correct diagnosis. Therefore to obtain an optimal benefit cost ratio specificity should be high especially for prevalent diseases. However for less frequent disorders lower specificity may provide sufficient performance. Albumin to creatinine ratios of false positives ranged between 2.54 mg/g and 29.5 mg/g for Dirui H13-Cr strip and 3.28-29.5 mg/g for Dirui H12-800MA.

NACB states that a strip to detect low levels of albuminuria must have a sensitivity greater than 95% at 20 mg/L concentration and suggests chemical-strip methods to be tested in the interval of 20-50 mg/L urine albumin concentration [19]. Except one study in the literature no strip is reported to meet the sensitivity requirement of NACB up to now. Davidson et al showed that ImmunoDip®, a test utilizing an immune method, met this condition as evidenced by one false negative result in 35 samples with albumin concentrations of 20–50 mg/L [23]. In our study 48 of 177 samples had an albumin concentration between 20-50 mg/L. Seven and 36 of them were classified as false negative by Dirui H13-Cr and H12-800MA strip, respectively. Accordingly within this concentration range, sensitivity rate for Dirui H13-Cr was calculated as 85.4%, yielding a better result. However for H12-800MA within this range sensitivity was lower; 25%.

Wilde HM et al reported that when investigating a community cohort of 83 referrals using both Bayer® microalbumin/creatinine urine reagent strips (albumin by dye binding and creatinine by color production of the reaction between diisopropyl-benzene dihyperoxide and 3,3,5,5-tetramethylbenzidine catalysed with a copper creatinine complex) on the Clinitek® 50 urine analyzer and the Roche Integra® 700 (microalbumin by immunoturbidimetry and creatinine by Jaffe-kinetic) the sensitivity (52.4%) of the dipstick has remained less than optimal and the specificity (97.6%) has achieved a clinically acceptable result [24]. In a study of Graziani et al urine samples collected from 201 consecutive subjects enrolled in an epidemiological study and 259 type 2 diabetic patients were evaluated using the Siemens Clinitek Microalbumin strips (albumin by dye binding and creatinine by the peroxidase like activity of the copper creatinine complex) on a bench-top reflectance meter [25], Clinitek Status against the albumin measurement results of an immunochemical method on the Immage 800 nephelometer and creatinine results on the RxL Dimension using an alkali picrate method. In the general population the sensitivity and specificity of the strip method were 90% and 91%, respectively. Results obtained from the diabetic group were similar, with a 91% sensitivity and a 92% specificity.

In the literature there is an ongoing debate about the evaluation of ACR results considering the between person variation of urine creatinine excretion [6,22,26-28]. The ACR is excepted to vary with age, gender, and ethnicity. Therefore different decision thresholds appropriate for different subgroups are supposed to need further investigation [6,26,27] as well as an adjustment for muscle mass into the reported ACR is suggested to achieve an improvement in accuracy of ACR interpretation [28]. In this study we used only single threshold value for ACR. This preliminary study may be followed with further studies investigating the most appropriate ACR considering the between person variation of creatinine excretion and evaluating the effect of this on the

performance measures of Dirui strips.

Conclusion

In conclusion, screening tests using urine test strips should be verified before acceptance in the routine use. Dirui strips evaluated in this study seem to have a lower sensitivity than suggested and excess number of false negatives seems to limit the utility of these strips.

Competing interests
The authors declare that they have no competing interest

Financial Disclosure
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