Journal of Clinical Laboratory Analysis 13:246–250 (1999)

Measurement of the Albumin Content of Urinary Protein Using Dipsticks

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An albumin selective urine strip based on bis (3′,3″-diiodo-4′,4″-dihydroxy-5′,5″-dinitrophenyl)-3,4,5,6-tetrabromo sulfonphthalein dye (DIDNTB) dye was examined in populations with clinical proteinuria. The relationship of albumin to the sum concentration of all protein in urine was found to vary widely even though the albumin concentration generally increased with the total protein concentration. The albumin reagent strips correlated well with immuno-nephrometric assays for albumin on specimens from hypertensives, diabetics, and renal disease which tended to have albumin contents of ≥ 50.0%. High proteinuria concentrations of > 250 mg/l, with low albumin contents of ≤ 30%, occurred more frequently in cases of cancer, infection, and myeloma. The albumin strip read higher than the immuno assay in samples with high proteinuria and low albuminuria. The albumin strip was also less affected by albumin fragmentation than by the immunological assay. Overall, the albumin strip gave a lower risk of false negatives than a protein strip based on tetrabromophenol blue (TBPB) dye and was more sensitive to disease condition. The protein strip was not sensitive to low levels of albumin and the agreement between TBPB dye strip and the quantitative analysis was not as affected by the albumin content. J. Clin. Lab. Anal. 13:246–250, 1999.

Key words: albuminuria; proteinuria, kidney disease; diabetes mellitus; hypertension; cancer; infection; myeloma; immunoassay; pyrogallol red

INTRODUCTION

Proteinuria is the result of collection of proteins from serum or renal origin and their degradation products in urine (1). In normal urine there are less than 150 mg of protein excreted per day, while clinical proteinuria is indicated at > 500 mg of protein per day (2). Tamm-Horsfall mucoprotein is the largest fraction of protein excreted under normal physiologic conditions, accounting for 70 mg per day, while all other serum proteins only account for 22 mg per day (1). Abnormal excretion of proteins occurs with impaired glomerular filtration or tubular reabsorption as the result of kidney disease (2). Albumin is excreted in the largest proportion and has been established as an appropriate marker of glomerular damage (3). Excretion of lower molecular weight proteins is typically associated with tubular and interstitial disease (4).

Protein excretion can also occur in the absence of renal abnormality. Intermittent proteinuria can result from strenuous exercise or orthostatic proteinuria (5). Proteins of renal origin like Tamm-Horsfall mucoprotein, urokinase, and secretory IgA can be shed into urine (6). Overflow proteinuria of Bence Jones protein, myoglobin, hemoglobin, and lysozyme can result from dyscrasias of prerenal origin such as multiple myeloma, muscle destruction, red cell lysis, and leukemia (1). Because of this lack of specificity, the evaluation of proteinuria requires consideration of a number of clinical conditions and relies on methodology to determine not only the amount of total protein, but also the identity of proteins excreted.

Urine protein reagent pads involve a very common methodology that measures 150 mg/l of protein as a "trace" and 300 mg/l protein as a positive (1,2,5,6). The evaluation of results must, however, consider the limitations of the methods. Albumin and most proteins are detected at levels of 200 to 400 mg/l, while mucoproteins and globulins are not detected at levels less than 600 mg/l or greater. Thus the test is an unreliable indicator of the absence of albumin; proteins other than albumin can cause positive results (7). This is evident at the trace level where as many as 48% of the specimens have less than 50 mg/l albumin present (8).

New strips that measure albumin concentrations down to 10 mg/l have become available and been shown to agree with

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Received 10 May 1999; Accepted 4 June 1999

laboratory method results when used in settings measuring microalbuminuria, i.e., patients without gross proteinuria (9). The technology of one of these strips is based on a new dye, DIDNTB with a tenfold higher affinity and selectivity for albumin (7,8).

We wanted to determine whether the albumin strip could be used in a population with proteinuria. Total protein and albumin were measured using laboratory methods and dry reagent strips to analyze 301 hospital specimens. The bias between laboratory methods and new albumin reagent was compared to the albumin percentage of total urinary proteins. Glycoslation and fragmentation of albumin was also examined for the occurrence of any potential bias between strip and laboratory method results. Cases of clinical proteinuria (n = 151) were categorized by disease and albumin content. The effectiveness of the albumin and protein strips in predicting the laboratory results was compared.

MATERIALS AND METHODS

Equipment

Quantitative measurements of urine total protein were made on the RX-40 analyzer (Nihon Denshi, Tokyo, Japan) and quantitative measurements of urine albumin were made on the Olympus AU 800 (Olympus Optical Co., Tokyo, Japan) by following the respective reagent instructions for implementation on the instrument. Urine strips were read with a CLINITEK® 200+ urine analyzer. SDS-PAGE electrophoresis was carried out exactly according to the instructions of Pharmacia (Piscataway, NJ), with the PhastSystem using their gels, SDS buffer strips, and fast Coomassie staining procedure.

Reagents

CLINITEK Microalbumin and MULTISTIX 10SG reagent strips (Bayer Corp Elkhart, IN). were used according to manufacturer's instructions (9). The former used bis(3′,3″-diiodo-4′,4″-dihydroxy-5′,5″-dinitrophenyl)-3,4,5,6-tetrabromo sulfonephthalein dye (DIDNTB) for the detection of albumin concentrations from 10 to 150 mg/l (8–10). The latter used tetrabromo phenol blue dye (TBPB) for the detection of protein concentrations from 150 mg/l to 10 g/l (9). The Micro TP-AR (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used to measure total protein by the pyrogallol red method on the lab analyzer. The quantitative measurement of albumin was performed using an immunonephelometry method (BML, Inc., Saitama, Japan). Quality control results for quantitative methods and urine strips have been described elsewhere (9).

Hospital Specimens

Random or first-morning specimens and adult screening samples were collected at Minoh City Hospital from inpatients and outpatients (n = 301) with various clinical conditions. The specimens were frozen on the day of collection, thawed overnight at 4°C, and tested the following day. Specimens were normal in terms of color, turbidity, or mucus. The urines were assayed in duplicate with urine strips and quantitative methods. Specimens were grouped by patient disease for diabetes mellitus, hypertension, renal diseases, kidney stones, cancer, urinary tract infection, myeloma, or other as the primary diagnosed disease condition. The other category included gout, asthma, pregnancy, toxemia of pregnancy, inguinal hernia, prostatic hypertrophy, subarachnoidal hemorrhage, cataract, neurosis, anemia, and cerebral infarction.

Preparation of Modified Albumin

Fresh urine from normal adults was pooled and any protein present removed by ultrafiltration with 10,000 dalton cutoff filters from Amicon, Inc. (Beverly, MA). Contrived urines were prepared by adding 65 mg/l of PENTEX human serum albumin (Bayer, cat. no. 82-301) or glycated albumin (SCIPAC, Sittingbourne, Kent, UK) to the urine pool. The concentration of albumin was traceable to available standard materials (8).

Fragmentation of albumin was initiated at 25°C by mixing 2.0 mL of contrived urine with 200 µL of 20 mg/l proteinase in water. Fungal Aspergillus acid Type XIII proteinase was used for complete digestion and Newlase Type XVIII proteinase was used for partial digestion. Digestion was moni-

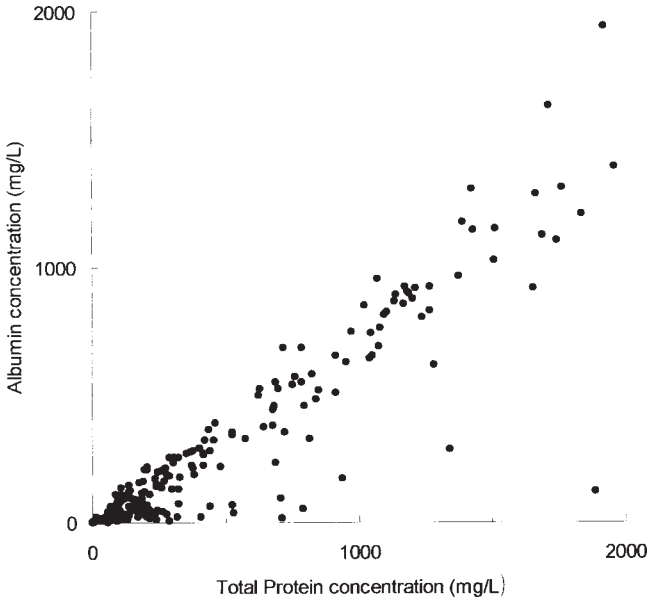

Fig. 1. Relation between total protein and albumin. Total protein concentration (x) as compared to albumin concentration (y) in 301 specimens. The correlation coefficient, r, was 0.94 and y was 0.88x – 143. The total protein concentration levels were 0 to 35,240mg/l. When total protein concentration was 0–999mg/l (n = 216), the correlation coefficient, r, was 0.86 and y was 0.59x – 16.

TABLE 1. Classification by Total Protein and Albumin Content

Albumin content (%)[a]	Total protein concentration (mg/l)					
	0–99	100–299	300–999	1,000–2,999	≥ 3,000	Total[b]
< 20	29	20	9	2		60
20–40	26	19	3	1		49
40–60	8	14	14	5	2	43
60–80	6	16	26	35	15	98
≥ 80	7	12	7	11	14	51
Average[c]	35.0	46.3	57.1	70.5	79.9	53.3
SD[c]	28.7	30.4	24.5	17.7	18.4	29.8
Total	76	81	59	54	31	301

[a]Albumin content (%) = mg/l albumin by immunoassay divided by mg/l total protein by pyrogallol red assay.
[b]Specimens were divided into five groups by 20 percentage-point increments in accordance with the albumin content percentage and the total protein concentration.
[c]Average albumin content percentage and standard deviation shown for each total protein concentration range.

tored by sodium dodecyl sulfate polyacrylamide gel electrophoresis at 0-, 3-, 6-, 9-, 12-, and 24-hour intervals. Samples were assayed by strip and immuonephrometric method for albumin in duplicate.

RESULTS AND DISCUSSION

Proportion of Albumin in Total Protein

The relationship between total protein and albumin, as measured by the quantitative methods, is shown in Figure 1. This figure indicates that levels of albumin and total protein are both predictive of each other. Several have suggested the replacement of total protein measurements with albumin for the assessment of glomerular permeability (10,11). However, the albumin content of the total urinary protein was highly variable and decreased dramatically as total protein concentration decreased (see Table 1). The nonuniform relationship between albumin and total protein would be expected to impact the results obtained with urine strips based on dye binding.

Effects of Albumin Contents on Urine Test Strips

Traditional urine reagent strips detect proteinuria at ≥ 250 mg/l using TBPB dye while the albumin reagent strips detect microalbuminuria at 20 mg/l with DIDNTB dye (9). The albumin selectivity of the DIDNTB dye is tenfold better when compared in urine standards for each individual protein (7). Comparisons using single protein standards do not account for the mixtures of proteins that are commonly encountered. To address this concern, the agreement of results from the reagent strip with the new dye to an immunological method has been tested using clinical urines (9). In urine without frank proteinuria, the agreement was > 89.2% at a threshold of 20 mg/l albumin with a sensitivity of 89.1% and a specificity of 89.4% (9). Proteinuria of ≥ 250 mg/l was observed in over 50% of the 301 specimens shown in Figure 1. In these proteinuria specimens, the overall agreement of the new dye remained ~90%; however, the sensitivity was increased to 97.3% and the specificity was reduced to 75.6%.

The reduced specificity was examined by classifying albumin strip results into high- and low-bias groups relative to the albumin content (see Table 2). The loss of specificity between the albumin test strip and laboratory method was attributable to a response to high concentration levels of proteins other than albumin. The dipsticks prepared with DIDNTB are not specific, but are selective for albumin and other proteins can give positive results (7). To be detected, the urine

TABLE 2. Effect of Albumin Contents on Urine-Test-Strips Agreement

Albumin contents (%)[a]	n[d]	Albumin agreement[b]					Protein agreement[c]				
		–2[e]	–1	Agreed	+1	+2	–2	–1	Agreed	+1	+2
< 20	60	0	0	32	19	9	0	9	48	3	0
20–40	49	0	2	39	8	0	0	2	43	4	0
40–60	43	0	5	36	2	0	0	2	35	6	0
60–80	98	0	8	89	1	0	0	1	89	7	1
≥ 80	51	0	8	42	1	0	0	0	42	9	0

[a]Specimens were divided by increments of 20% in albumin content using laboratory assays. Albumin content (%) = mg/l albumin by immunoassay divided by mg/l total protein by pyrogallol red assay.
[b]Agreement between albumin reagent strip and immunological lab assay level by level.
[c]Agreement between protein reagent strip and pyrogallol red lab assay level by level.
[d]n = number of urine specimens.
[e]–2 = An albumin strip result that was two strip results lower than expected based on the laboratory method.

TABLE 3. The Effect of Albumin Modification on Strip and Laboratory Results

	Bias[a]	
	Immuno-assay	Albumin strip
Albumin partial digestion to 30,000 daltons[b]	–93%	–15%
Albumin complete digestion to 10,000 daltons[b]	–100%	–89%
Glycated albumin[c]	–64%	–40%

[a]The difference between assay result and expected value.
[b]Human serum albumin (65 mg/l) added to filtered urine and digested with protease for 24 hours with fragmentation followed by SDS-PAGE electrophoresis and albumin assays.
[c]Glycated human serum albumin (65 mg/l) added to filtered urine.

protein levels must be present at a much greater level than the normal excretion rate (12). Glycosyl albumin, beta-2-microglobulin, transferrin, haptoglobin, hemoglobin, and myoglobin were detected at lower concentrations than other proteins.

Specimens containing less than 40% albumin gave a positive bias to the laboratory albumin method results, while specimens containing more than 60% albumin gave a negative bias. The bias was within one level 97% ([301–9] / 301) of the time. As the albumin strip is used as a yes-or-no screening test, the effect of the bias at the 20 mg/l albuminuria threshold is only 2.7% false negatives (6 FN/ 223 TP) and 24% false positives (19 FP/78 TN). As the threshold increases towards 150 mg/l, the false results are reduced to 5.1% (8 FP/ 155 TP) and the false negatives remained low at 5.5% (8 FN/146 TP).

The traditional protein test strip also showed bias when compared to the protein lab method, relative to albumin content, but to a much lesser extent (Table 2). The protein-strip agreement with the pyrogallol red method was > 94.4% at a threshold of 250 mg/l protein, with a sensitivity of 96.8% and a specificity of 91.4%. When affected, the protein strip gave lower results on specimens containing less than 20% albumin and higher results on those containing ≥ 60% albumin (Table 2). Of the 146 negative samples for protein, 49.3% contained > 20 mg/l of albumin; i.e., the strip failed to detect clinically significant albuminuria. The albumin strip was more sensitive to the albumin contents and less likely to fail to detect albuminuria.

Effect of Albumin Modification

In vivo modification of albumin leading to loss of immulogical response is a possible reason for positive bias observed with the albumin strip. Gylcated albumin is common in diabetic serum and albumin fragments are expected in urine due to proteinase activity. The response of albumin reagent strips and the immuno-tubrimetric assay to glycated albumin and albumin after proteolytic digestions was measured (see Table 3). The largest albumin fragment needed to maintain dye response was determined. Cleavage of tyrosine groups was used to divide albumin into eighteen fragments for complete digestion to 10,000 daltons (13). Partial digestion to 30,000 daltons was accomplished by following proteolysis by SDS-PAGE electrophoresis. The immunoassay was unable to detect albumin after fragmentation to 30,000 daltons. The albumin strip was only slightly affected by fragmentation down to 30,000 daltons. A response by the DIDNTB dye was not observed after complete digestion to fragments of less than 10,000 daltons. Detection of albumin fragments in urine offers another potential explanation for a positive albumin result, unconfirmed at the 20 mg/l threshold, by immunoassay. Glycation of albumin reduced both albumin immunological assay and reagent strip results, the latter to a slighty less degree.

Distribution of Albumin Contents by Disease, and the Effects on Test Strips

Patients with albuminuria at > 20 mg/l, or frank proteinuria at > 250 mg/l, were examined for 8 disease cat-

TABLE 4. Distribution of Specimens by Albumin Contents and Disease

		Albumin contents[a]						
	n[b]	<20%	20–40%	40–60%	60–80%	> 80%	Mean[c]	SD[c]
Diabetes mellitus	22	0	6	6	7	3	58.9	21.6
Hypertension	6	0	1	2	1	2	63.3	26.2
Renal disease	34	3	4	5	11	11	67.4	30.5
Kidney stones	7	1	0	1	4	1	62.8	22.4
Cancer	12	5	2	4	1	0	30.4	20.1
Infection	25	12	9	2	2	0	25.3	18.3
Myeloma	5	4	0	1	0	0	14.7	14.8
Other	40	9	8	8	5	10	50.4	33.3

[a]Specimens were divided by increments of 20% in albumin content using laboratory assays. Albumin content (%) = mg/l albumin by immunoassay divided by mg/l total protein by pyrogallol red assay.
[b]n = number of urine specimens.
[c]Average albumin content percentage and standard deviation shown for each disease group.

TABLE 5. Laboratory and Strip Results Compared by Disease[a]

	Number of urines by classification							
	mg/l Albumin in urine				mg/l Protein in urine			
By laboratory	< 20		≥ 20		< 250		≥ 250	
By strip	< 20	≥ 20	< 20	≥ 20	< 250	≥ 250	< 250	≥ 250
Diabetes mellitus	2	0	2	18	14	1	1	6
Hypertension	0	0	0	6	5	0	0	1
Renal disease	2	3	1	28	15	4	0	15
Kidney stones	0	0	1	6	4	1	0	2
Cancer	0	2	2	8	4	0	3	5
Infection	3	8	0	14	16	1	1	7
Myeloma	0	1	0	4	0	0	0	5
Other	1	5	0	34	25	5	0	10

[a]Shows the comparison between the strip test results and the quantitative analyses of protein and albumin by disease category for 151 patient urines.

egories using the albumin strip results. The albumin content of the total protein was calculated for each group and comparisons made. Albumin contents were much lower in urine from patients with myeloma, infection, and cancer (Table 4). The average albumin contents in these cases ranged from 14.7 to 30.4%. Higher albumin contents were observed in hypertension, diabetes, renal, and kidney cases, with all averages exceeding > 58.9%.

Albumin and protein strip results are compared, by disease group, to reference assays, in Table 5. Albuminuria constitutes the critical factor in the screening test for glomerular kidney damage, especially in the case of hypertension and diabetes. The albumin reagent was positive in 86% of these cases and agreed with reference methods 93% of the time. The protein reagent was only positive in 29% of hypertension and diabetic cases and only increased to 56% as kidney damage progressed to renal disease. The albumin content of the total protein became less pronounced in cases of infection and cancer-protein overflow. However, the albumin reference assay still was more frequently positive than the protein reference assay in these disease cases (65 vs. 43%) (Table 5).

CONCLUSIONS

The albumin strip is suitable for microalbumin testing in diabetics and hypertensives and has less risk of false negatives than does the traditional protein test when used in samples with or without proteinuria. This strip has the lower threshold of 20 mg/l albumin and more clinical sensitivity than the protein reagent, which has a threshold of 250 mg/l protein. Use of the albumin strip in samples with proteinuria could lead to more positive results, which would be unconfirmed by immunoassay methods at the 20 mg/l threshold, than could samples without proteinuria. Unconfirmed results could possibly be due to the dye responding to proteins other than albumin at pathological levels or to fragments of albumin.

ACKNOWLEDGMENT

We thank Yoshihide Ohta of Bayer-Sankyo, Tokyo, Japan, for his many contributions that made this study possible.

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