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Albuminuria and Proteinuria in Hospitalized Patients as Measured by Quantitative and Dipstick Methods

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We tested patients' urines for albumin, protein, and creatinine by quantitative and dipstick methods. The concentrations of these analytes were established by quantitative, cuvet-based chemistry methods that we assumed gave the "correct" values. There was good to excellent agreement of the dipstick results with the quantitative methods for the above three analytes. We found many patients who excreted pathological amounts of albumin and/or protein who did not have a diagnosis of kidney disease or other likely causes of proteinuria, suggesting that albuminuria and/or proteinuria were underdiagnosed in our group of patients. Those with cardiovascular disease, kidney disease, or diabetes showed the greatest predictive value of a positive test for albumin or protein by dipstick. Dipstick testing for albumin, protein, and creatinine had good or excellent agreement with quantitative methods. The dipstick tests were easy to use, simple, and low in cost, and can serve well for point-of-care testing. J. Clin. Lab. Anal. 15:295–300, 2001.

Key words: albumin/creatinine ratio; automated analysis; dye binding; hypertension; immunoassay; microalbuminuria; nephritis; protein/creatinine ratio; proteinuria; urinalysis

INTRODUCTION

Albuminuria and proteinuria are important findings, especially in patients with hypertension (HTN) and/or diabetes mellitus (DM) (1,2). Albuminuria in these individuals is a predictor of end-stage renal disease (3,4). Rachmani et al. (5) found that the higher the urinary albumin concentration, the faster the rate of decline of the glomerular filtration rate, and the greater the risk of having a cardiovascular event. In patients with DM or who undergo coronary artery bypass surgery, proteinuria is an important predictor of postoperative death (6). The Framingham study revealed that proteinuria is an independent risk factor for coronary artery disease (7).

Quantitative methods are available for urinary albumin, creatinine, and proteins; however, they are costly and time consuming. Immunonephelometry for albumin in urine is particularly costly. Dipsticks are easy to use and are excellent point-of-care (POC) tests (8,9). An algorithm used in screening 23,000 Japanese school children for kidney disease (such as nephritis) by dipsticks, and the necessary follow-up steps are described elsewhere (10). One of our goals in the testing of normal volunteers was to establish cutoff limits to guide caretakers in deciding whether further testing is appropriate. Another goal was to test patients with diagnoses other than kidney disease, HTN, or DM to identify some prevalent causes of undiagnosed albuminuria.

MATERIALS AND METHODS

Patients

Urine specimens from a total of 666 patients were collected at four sites (The Ohio State University, Columbus; Bowman Gray School of Medicine; South West Washington Medical Center; and University of Minnesota). Each site provided about 170 specimens. We tested these within 1 hr of collection or froze them if analysis was delayed. The primary diagnoses of our patients are given in Table 1.

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TABLE 1. Agreement of dipstick tests with quantitative methods

	Percent predictive value of negative or positive dipstick results in our population								
		Albumin		Albumin to creatinine ratio		Protein		Protein to creatinine ratio	
Population designation	No.	PVN[a]	PVP	PVN	PVP	PVN	PVP	PVN	PVP
Healthy volunteers	129	100	N/A[b]	100	N/A	100	N/A[b]	100	N/A
General hospital population[c]	310	99	82	89	84	95	67	87	84
Kidney disease	113	97	84	100	86	91	72	93	92
Diabetes mellitus	80	100	75	100	83	100	46	98	83
Cardiovascular disease	48	100	82	87	85	95	79	91	96
Cancer (all types)	31	100	43	100	43	89	57	94	71

[a]PVN is the predictive value of a negative result in percent, and PVP is the predictive value of a positive result in percent. The quantitative values were assumed to be correct in all cases, and the percents in the table give the agreement of the dipstick results with the quantitative assays. There were 84 patients with creatinines of ≤250 mg/l. They are not shown in the table, because we consider the PRO and quantitative laboratory results, or their ratios to creatinine, as unreliable.

[b]Not applicable because the controls showed only negative values.

[c]For example, if the patient is a member of the "general hospital population," then the patient has a 99% chance of having a negative albumin dipstick test in the absence of disease and a 82% chance of having a positive dipstick test in the presence of disease. All urines had been tested by quantitative methods that were used to classify patients as either negative or positive. Here, a patient was deemed "positive" if any of the following were above our cutoff limits as assayed by quantitative methods: albumin, or albumin/creatinine ratio; protein or protein/creatinine ratio. The above classification was assumed to be correct, and the dipstick results were compared to these quantitative values.

Control Subjects

We obtained urine specimens from 129 individuals presumed to be in good health. None had HTN, DM, or kidney disease. The control subjects were nearly all clinical-laboratory workers.

Assays for Albumin, Protein, and Creatinine by Dipsticks

Multistix PRO™ ("PRO") dipsticks (Bayer Corp. Elkhart, IN) were used according to the manufacturer's instructions. The strips include a reagent pad using bis-(3′,3″-diiodo-4′,4″-dihydroxy-5′,5″-dinitrophenyl)-3,4,5,6-tetrabromo sulfonephthalein dye (DIDNTB) for the detection of albumin at ≥80 mg/l (11). Also present on the dipsticks is a pad for protein that is based on a color change of tetrabromophenol blue (TBPB) for detection of protein at ≥300 mg/l, and a pad for creatinine that uses the peroxidase activity of copper-creatinine complexes (12). With these dipsticks, it is possible to determine if the albumin is ≥80 mg/g of creatinine or if the urinary protein is ≥300 mg/g creatinine. A negative protein result with a ≤250 mg/l creatinine value indicates a specimen that may be too dilute to analyze, in which case accurate values for the albumin/creatinine ratio or the protein/creatinine ratio cannot be obtained. Such specimens arise owing to contamination with water and/or acute diuresis of any cause.

Specimens were analyzed in duplicate by the dipsticks and also by the three quantitative methods. We assumed that the latter gave accurate results in all cases. The dipsticks were read on a Clinitek® 50 reflectance photometer. Quality control data for the dipsticks and for the quantitative methods are given elsewhere (13). Earlier, we found good agreement between a visual and a reflectometer reading. A reflectometer speeds up testing, standardizes the reading, and provides automation in that it can be readily linked to a laboratory information system (14).

Quantitative Assays for Albumin, Protein, and Creatinine

Immunonephelometric methods were used for all determinations of albumin according to the manufacturers' instructions. The Beckman Array® (Fullerton, CA) was used at two hospitals. The Roche COBAS Integra® (Nutley, NJ) and Dade Behring Paramax® (Miami, FL) were used at the other hospitals. Total protein was measured by a pyrogallol red method ("Microprotein-PR" reagent No 611-A; Sigma Chemical Co., St. Louis, MO) in the Beckman CX3® analyzer, in the Bayer Opera® analyzer, the Roche Cobas Integra, and the Roche Cobas Mira® analyzer. All four sites used a rate-Jaffe procedure for creatinine. By "quantitative methods" we always mean cuvet-based, quantitative methods.

Statistical Methods

We used the Mann-Whitney test to evaluate the data for significant differences or agreement between two groups. There was no statistical difference ($P > 0.05$) between the quantitative interlaboratory results for the albumin, protein, or creatinine controls, allowing merging of the quality control data and the data from the healthy volunteers and patients at the four sites.

RESULTS AND DISCUSSION

Healthy Control Subjects

Albuminuria or proteinuria, i.e., abnormally increased values, were absent in all of the 129 healthy controls. Eleven

subjects showed trace proteinuria by dipstick, the upper threshold of normal, and we chose to retain these individuals in the control population. For the entire group of 129, the average quantitative values (SD) were albumin: 11.6 mg/l (9.5); protein: 67.7 mg/l (38.4); albumin/creatinine ratio: 9.5 mg/g (10.2); and protein/creatinine ratio: 49.5 mg/g (32.9). The urine creatinines ranged from 0.295 g/l to 4.220 g/l; the mean (SD) for creatinine was 1.699 g/l (1.005). A more detailed discussion of reference values for the above tests in healthy controls and athletes is provided in Ref. 15.

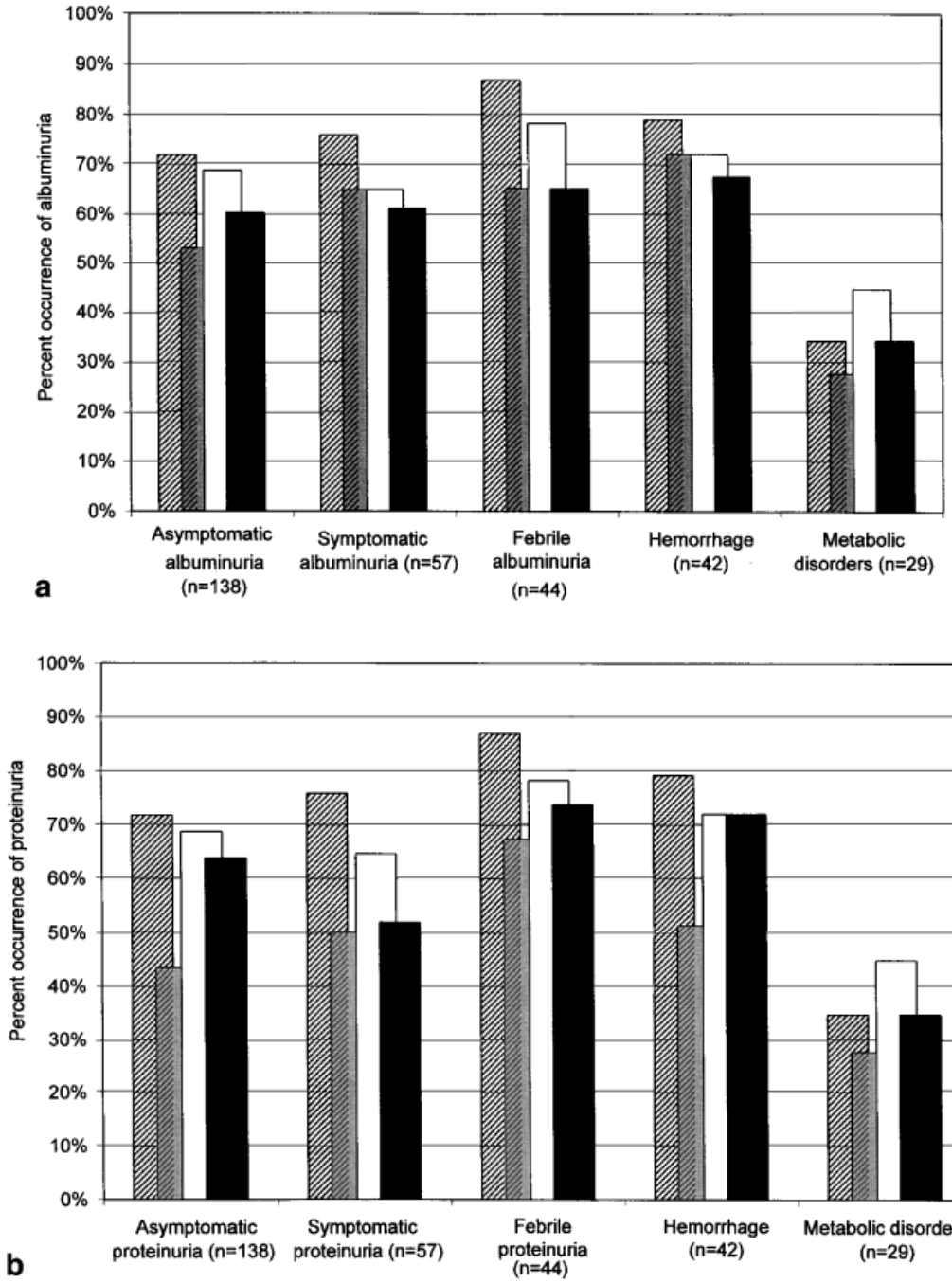

Fig. 1. **a:** Occurrence of albuminuria in various diagnostic groups. Bars with diagonal lines: positive albumin by dipstick; gray bars: positive albumin by quantitative method; white bars: positive albumin/creatinine ratios by dipstick; and black bars: positive albumin/creatinine ratios by quantitative methods. We found no statistically significant differences between any pairs of sets in the five sets of bars. **b:** Occurrence of proteinuria in various diagnostic groups. Bars with diagonal lines: positive protein by dipstick; gray bars: positive protein by quantitative methods; white bars: positive protein/creatinine ratios by dipstick; and black bars: positive protein/creatinine ratios determined by quantitative methods. We found no statistically significant differences between any pairs of sets in the five sets of bars.

Reference Ranges

In an earlier screening study by our group of 23,000 Japanese school children, ages 6–18 years, we estimated the upper reference limits for albumin to be 23 mg albumin/g creatinine; for protein they were 115 mg protein/g creatinine. The values are means + 2 SD (9). We report here similar reference values for adults: our cutoff values, as determined earlier, are ≤80 mg albumin/l, ≤300 mg protein/l, ≤80 mg albumin/g creatinine, and ≤300 mg protein/g creatinine. We suggest that there is a "gray" zone for albumin that is between 30 mg/g and 80 mg/g creatinine; for protein it is between 115 and 300 mg/g creatinine.

Predictive Values of Dipstick Results

The predictive values of a positive dipstick test (PVP) and the predictive values of a negative test (PVN) are shown in Table 1 for each of the major disease groups. We found that most patients with kidney disease of any cause and/or DM showed the best agreement with the quantitative methods. We also found generally better agreement for any abnormally increased values.

General Hospital Population

In 310 hospitalized patients, we found 218 to be positive by the quantitative method for albumin; 224 were positive by the PRO dipsticks. We found 138 patients for whom no comment was made in the medical record of the dipstick protein value when it was abnormally increased. None of these individuals had a diagnosis of kidney disease. Using our cutoffs, we found that 100 of these 138 patients had asymptomatic proteinuria, i.e., no painful urination, hematuria, pyuria, "burning" sensation during voiding, or other symptoms. The patients were typically first identified in a POC setting by the dipstick test.

We concluded that the 100 patients had a transient or persistent albuminuria or proteinuria and should have had further evaluation or at least repeat dipstick tests. The remaining hospitalized patients had disorders that were expected to exhibit the usual finding of symptomatic albuminuria or proteinuria (Fig. 1a and b). In this group, albuminuria or proteinuria was present in 35 of 57 patients with urinary tract infections who were symptomatic. Febrile albuminuria or proteinuria was present in 38 of 44 patients with fevers owing to influenza, pneumonia, septicemia, and other causes. Albuminuria or proteinuria was associated with hematuria in 32 of 42 patients with kidney stones, internal bleeding, or trauma. Positive results were less likely in patients with functional disorders of the liver, adrenals, digestive organs, or endocrine glands (13 of 29 patients).

Patients With Kidney Disease, DM, Cardiovascular Disease, or Cancer

In Table 2, we summarize our findings of the fraction of selected patients with positive quantitative or PRO dipstick

TABLE 2. Occurrence of positive quantitative or PRO dipsticks creatinine ratios[a]

Patient group[b]	Number of patients	Number of patients with abnormal albumin/creatinine quantitative assay	Number of patients with abnormal protein/creatinine quantitative assay	Number of patients abnormal by PRO dipstick
Kidney diseases				
All cases	113	73	81	86
With CRF or CRI	65	39	43	45
Without CRF or CRI	48	34	38	41
With HTN	13	7	9	8
Without DM & HTN	33	21	26	27
With DM & HTN	67	45	46	51
Diabetes mellitus				
All cases	80	19	20	23
With HTN	24	8	8	8
Without complications	43	5	6	9
Poorly controlled diabetes	13	6	6	6
Cardiovascular disease				
All cases	48	28	28	26
Peripheral vascular disease	10	7	7	7
CAD and HTN	13	8	8	6
CHF	15	10	10	11
≥1 week post CABG	10	3	3	2
Cancer				
All cases	31	6	11	14
Multiple myeloma	8	1	2	2
Other malignancies	23	5	9	12

[a]Albumin/creatinine >80 mg/g or protein/creatinine >300 mg/g.
[b]Patients may be present in more than one group.
PRO, Multistix PRO; CRF, chronic renal failure; CRI, chronic renal insufficiency; HTN, hypertension; DM, diabetes mellitus; CAD, coronoary artery disease; CHF, congestive heart failure; CABG, coronary artery bypass graft.

results vs. what was present in the patients' medical records. With all the testing methods, patients in Group 1 (with kidney disease) had significantly higher dipstick values ($P < 0.05$) than those in Groups 2, 3, or 4. Group 2 patients with poorly controlled DM or who also had HTN were more likely to have an abnormal albumin or protein excretion. Group 3 patients with cardiovascular diseases had a significant proportion of cases with abnormal results, especially those with congestive heart failure (CHF). Albuminuria and proteinuria were found in some Group 4 patients and those with cancers of all types. These values may or may not reflect some renal pathology. Tissue breakdown following chemotherapy often leads to proteinuria. In a limited number of patients with multiple myeloma, no albuminuria (<35 mg/g creatinine) or no proteinuria (<161 mg/g creatinine) were found unless the patient had renal failure (two patients). Both the dipsticks and cuvet-based dye-binding methods have limited chemical sensitivity for Bence-Jones light chains, and immunochemical assays such as immunonephelometry should be used (11,16).

Patients Producing a Dilute Urine

We concluded that patients producing a urine with a creatinine of ≤250 mg/l were undergoing diuresis owing to endogenous or exogenous causes. Contamination with water will produce the same result. We found 84 patients producing a highly dilute urine (see Fig. 2). Based on our past experience, the assay of albumin or protein is unreliable in highly dilute urines, even when ratios to creatinine are used (16).

CONCLUSIONS

Albuminuria and proteinuria were common in our selected group of patients (Table 1). The highest prevalence of positive protein excretion occurred in patients with kidney disease of any type. In the presence of HTN, the albumin and/or protein excretion were even greater. Dipstick testing should identify patients with protein-wasting disorders. A finding of albuminuria and/or proteinuria warrants further investigation. The dipsticks are easy to use and are suitable for POC testing (17). The agreement with the quantitative assays was good to excellent, especially for positive values, and dipstick results can be obtained at the bedside.

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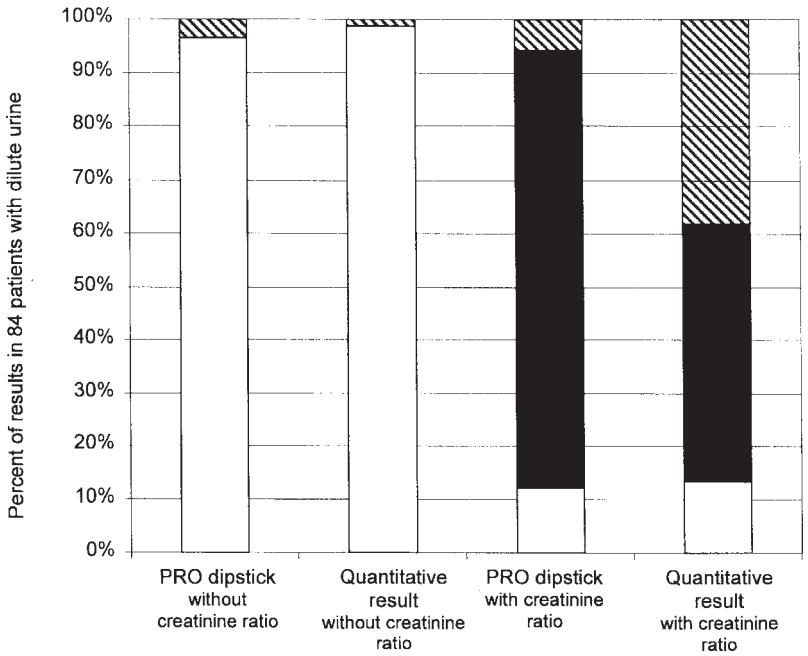

Fig. 2. Albumin and protein findings in urines with creatinine concentrations ≤250 mg/l (left to right): **Bar 1,** white portion: negative PRO dipstick (i.e., negative albumin); striped portion: positive PRO dipstick. **Bar 2:** Same as Bar 1, but quantitative assays for albumin and creatinine were carried out. **Bar 3,** white portion: negative PRO dipstick/creatinine ratio; black portion: dilute specimen by PRO dipstick/creatinine ratio; striped portion: positive PRO dipstick/creatinine ratio. **Bar 4:** Same as Bar 3, but quantitative assays for albumin and creatinine were carried out. More dilute specimens were found with the PRO dipsticks than by the quantitative methods.

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