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Original Article

Detecting proteinuria: A comparison of diagnostic tests

Jeanida Mauliddina, Rosmayanti Siregar, Oke Rina Ramayani, Rafita Ramayati, Rusdidjas

Abstract

Background Proteinuria is a condition when protein is found in urine, a common symptom in children with renal disorders. Proteinuria can also be found in normal children and in those with non-renal disorders. A high sensitivity test is needed to detect proteinuria. Spectrophotometry has been used as a standard to detect proteinuria, however, it is expensive and not readily available in health clinics. We tested the use of 20% sulfosalicylic acid to detect proteinuria, and compared it to spectrophotometry. The sulfosalicylic acid test is inexpensive, rapid, and easily performed in primary community health centers.

Objective To compare 20% sulfosalicylic acid test to spectrophotometry as a diagnostic test for proteinuria.

Methods We conducted a cross-sectional study in Adam Malik Hospital from September 2009 until December 2009. Inclusion criteria were children aged 3 to 18 years who experienced kidney disease. We collected twenty-four hour urine specimens from 55 children by consecutive sampling. Urine specimens were tested for proteinuria by 20% sulfosalicylic acid test and spectrophotometry.

Results Sensitivity and specificity of 20% sulfosalicylic acid test compared to spectrophotometry were 88.1% and 69.2%, respectively, with a positive predictive value and a negative predictive value of 90.2% and 64.3%, respectively.

Conclusion The sulfosalicylic acid test had low sensitivity and specificity for detecting proteinuria, but it was more practical and less expensive compared to spectrophotometry. **[Paediatr Indones. 2011;51:17-21]**.

Keywords: *Sulfosalicylic acid, spectrophotometry, proteinuria.*

For over 150 years, proteinuria has been known to be related to kidney disease, as well as non-renal diseases, such as febrile seizures, congestive heart failure, changes in posture, and emotional stress.[1] Proteinuria incidence among children is 1-10% of diseases.[2-7] Normal urine in children may contain protein, nearly 60% of which is derived from plasma proteins, while the remaining 40% comes from secretions of the urinary tract.[8] Renal abnormalities are a common cause of proteinuria.[9] Proteinuria can be caused by excessive concentration of high molecular weight protein in plasma and through the borderline of tubular reabsorption during protein filtration.[10-12]

Sulfosalicylic acid and spectrophotometry examinations are two ways to examine proteinuria.[13-15] Spectrophotometry is a quantitative method,[9,16.17] and an often used standard for detecting proteinuria. This tool is highly sensitive and makes use of the light spectrum, but is rarely available in primary community health centers because spectrophotometers are

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From the Department of Child Health, Medical School, University of North Sumatra, Adam Malik Hospital, Medan.

Reprint request to: Jeanida Mauliddina, MD, Department of Child Health, Medical School, University of North Sumatra, Adam Malik Hospital, Jl. Bunga Lau No.17 Medan 20136. Ph. +62 (061) 8361721/8365663, Fax. +62 (061) 8361721. E-mail : dinashadiq2530@yahoo.co.id

expensive.[6,18,19] The 20% sulfosalicylic acid test can be an inexpensive and practical way to detect proteinuria. This test has been shown to be more accurate than the urine dipstick test.[12,17]

Our study was conducted to compare the usefulness of the 20% sulfosalicylic acid test for proteinuria to spectrophotometry in children with suspected kidney disease, who were referred to our hospital from primary community health centers due to abnormal urinalyses.

Methods

We conducted a cross-sectional study in the Pediatrics outpatient and Pediatrics ward of Adam Malik Hospital, Medan, North Sumatera from September until December 2009. We included children aged 3 to 18 years with suspected kidney disorders at the time of enrollment. Informed consent was obtained by parents. This study was approved by the Research Ethics Committee.

We recorded the name, age/date of birth, gender, address, and telephone number of subjects, as well as the name of parent/guardian. A plastic urine container containing thymol was given to parents / guardians / caregivers, with an explanation of how to collect their children`s urine. Urine was collected for 24 hours, started at 08.00 AM. The first urine void sample was discarded and subsequent urine voids were collected. Urine volume was measured and recorded after 24 hours. From each specimen, 4 ml of urine was used for a semi-quantitative examination using a 20% sulfosalicylic acid, while the remainder was sent to a laboratory to be examined by spectrophotometry.

For the semi-quantitative 20% sulfosalicylic acid method, 4 ml of urine was divided into 2 small tubes so that each contained 2 ml of urine. We added 8 drops of 20% sulfosalicylic acid to one of the tubes before gently mixing. We compared the two tubes by visual observation in front of a black background and graded them according to the scale and conversion values reported by Schumann *et al*. Interpretation of the results, shown in Table 1, was as follows: (1) negative, no turbidity or unchanged turbidity (protein level <0.050 g/dl); (2) trace positive, perceptible turbidity (protein level 0.020 g/dl); (3) +1, distinct turbidity but no discrete granulation (protein level 0.050 g/dl); (4) +2, turbidity with granulation but no flocculation (protein level 0.20 g/dl); (5) +3, turbidity with granulation and flocculation (protein level 0.5 g/dl); (6) +4, clumps of precipitated protein or solid precipitate noted (protein level 1.0 g/dl).[3]

For the quantitative method of assessing proteinuria, we used 20% sulfosalicylic acid test and the clot was examined by spectrophotometry. A test tube with 2 - 4 ml of urine was heated in a 100^0C water bath for 5 - 10 minutes. If the sample was positive for proteinuria, we added 2 to 3 drops of 6%-acetic acid and reheated to determine the degree of proteinuria. If the sample was negative, no further urine dilution was performed. If the sample was positive (+1) for proteinuria, the urine was diluted 1:5 (1 ml of urine and 4 ml aquadest/demineralized water) and retested. If this dilution was positive (+2), urine was diluted 1:10 (1 ml of urine and 9 ml aquadest). If the 1:10 dilution was positive (+3 or +4), urine was diluted 1:40 (1 ml of urine added to 39 ml aquadest) , 4 ml of which were added to 1 ml 5.12 M TCA (trichloraceticacid). The sample was mixed and incubated 5 - 10 minutes at room temperature. As a standard, 20 ul of normal serum was added to 5 ml aquadest, 4 ml of which was then added to 1 ml 12.5 M TCA. The standard was mixed and incubated for 5 - 10 minutes at room temperature. Spectrophotometry readings were made with a wavelength of G 420 (F1000).

To determine the sensitivity, specificity, positive predictive value, and negative predictive value of 20% sulfosalicylic acid test compared to spectrophotometry, we used the Chi-Square test. Differences were considered significant at $P < 0.05$.

Results

There were 55 children enrolled this study, 32 males (58.2%) and 23 females (41.8%). There were 29 children aged 3 to 7 years (52.7%), 22 children aged 8 to 12 years (40.0%), and 4 children > 12 year-old (7.3%). There were 11 children (20%) in preschool, 32 children (58.2%) in primary school, and 12 children (21.8%) in junior high school.

From 55 children, we found 37 children (67.3%) had nephrotic syndrome. (**Table 2**) By using spectrophotometry to test for proteinuria, we found that 42 children (76.4%) tested positive and

Table 1. Conversion values.[3]

Spectrophotometry	20% sulfosalicylic acid
< 0.050 g/dl	-
0.020 g/dl	Trace
0.050 g/dl	+1
0.20 g/dl	+2
0.5 g/dl	+3
1.0 g/dl	+4

negative predictive value was 64.3%. (**Table 4**)

Discussion

An accurate and rapid assay for proteinuria is needed in order to diagnose renal and other diseases and to determine the prognosis of various kidney disorders. In

Table 2. Comparison of spectrophotometry and 20% sulfosalicylic acid tests for proteinuria

Diagnosis	Total		Spectrophotometry				20% sulfosalicylic acid			
			Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%	N	%
Nephrotic Syndrome	37	67.3	27	49.1	10	18.2	27	49.1	10	18.2
Hydronephrosis	2	3.6	1	1.8	1	1.8	1	1.8	1	1.8
CHF	9	16.4	8	14.5	1	1.8	7	12.7	2	3.6
SLE	2	3.6	2	3.6	0	0	2	3.6	0	0
Complicated meningitis with UTI	3	5.5	3	5.5	0	0	3	5.5	0	0
Glomerulonephritis	2	3.6	1	1.8	1	1.8	1	1.8	1	1.8
Total	55	100	42	76.4	13	23.6	41	74.5	14	25.5

Table 3. Proteinuria level in the five main disease conditions in this study

	Light Proteinuria *Trace* - +1	Heavy Proteinuria +2 - +4
Nephrotic syndrome	-	37
CHF	9	-
SLE	-	2
Complicated meningitis	3	-
Glomerulonephritis	-	2

Table 4. Diagnostic test and correlation between 20% sulfosalicylic acid and spectrophotometry

Proteinuria using 20% sulfosalicylic acid	Proteinuria using spectrophotometry					
	Positive		Negative		Total	
	N	%	N	%	N	%
Positive	37	67.3	4	7.3	41	74.5
Negative	5	9.1	9	16.4	14	25.5
Total	42	76.4	13	23.6	55	100

13 children (23.6%) tested negative. Using the 20% sulfosalicylic acid assay for proteinuria, we found 41 children (74.5%) tested positive and 14 children (25.5%) tested negative. (**Table 4**)

In comparing the 20% salicylic acid test to spectrophotometry, we found the sensitivity and specificity were 88.1% and 69.2%, respectively. Similarly, positive predictive value was 90.2% and

children, protein excretion greater than 4 mg/m^2 per hour is considered abnormal. Protein excretion of more than 40 mg/m^2 per hour is considered nephrotic proteinuria.[16] Persistent proteinuria can cause progressive kidney injury and some renal structure abnormalities are associated with proteinuria.[5,14,16] Proteinuria also may indicate an underlying kidney disease and is an important factor to look at for renal

trauma and its prognosis.[16] Protein analysis of urine collected for 24 hours is the best method to evaluate proteinuria.16 The prevalence of mild proteinuria (30 – 100 mg/dL) is as much as 4.9%. Furthermore, 60.7% of these cases proved to have significant glomerulopathy[20]

Of the 55 children in our study, we found 42 children with heavy proteinuria and 13 children with light proteinuria. Thirty-seven of 41 children with heavy proteinuria were diagnosed with nephrotic syndrome. We used spectrophotometry as a standard for evaluating proteinuria, because it is believed to be the most accurate method of monitoring proteinuria during treatment and is used worldwide. However, spectrophotometry is less practical because it requires a 24-hour urine collection to determine proteinuria.[21-23] A study to detect microalbuminuria in spot urine samples using a spectrophotometer found a sensitivity of 87.8%, specificity 89.3%, positive predictive value 29.3% and negative predictive value 96.2%. For protein creatinine ratio values, the sensitivity was 87.8%, specificity 89.3%, positive predictive value 29.3%, and negative predictive value 96.2%.[11]

Sulfosalicylic acid may provide a less expensive and more practical tool for evaluating proteinuria than spectrophotometry. The sulfosalicylic acid test is sensitive to protein concentrations from 0.02 g/dL to 0.1 g/dL and has a 95% predictive value. Therefore, a negative result can rule out microalbuminuria. In addition, the sulfosalicylic acid method is accurate and specific for several types of proteins compared to urine dipsticks. However, a limitation of this method is that turbidity of the sample can be inhibited by the higher detergent concentration.[24] Therefore, we did not use any detergent to prevent bias.

A study in Japan reported that sulfosalicylic acid can be used for screening proteinuria in primary school children.[25] Other research in comparing the urine dipstick to sulfosalicylic acid as diagnostic tests, found that sulfosalicylic acid was better than the urine dipstick in detecting proteinuria in concentrated urine, but the sulfosalicylic acid was less accurate in estimating protein concentration.[26-28] An Australian study compared six methods for proteinuria examination and concluded that the sulfosalicylic acid method was easier but required a larger volume of urine. Although the method was more practical and less biased, it was imprecise in estimating the concentration of albuminuria in the absence of a control.[31]

A study of 221 children for microalbuminuria screening using 20% sulfosalicylic acid, reported a sensitivity value of 76.7%, specificity 75.4%, positive predictive value 32.9% and negative predictive value 95.4%.[29] In the same country, Lyon *et al*. compared four methods to detect albumin in dog and cat urine and found a sensitivity of 28.7%, specificity 94.2%, positive predictive value 65.2% by using the sulfosalicylic acid method.[30] We found that the 20% sulfosalicylic acid had a sensitivity value of 88.1%, specificity 69.2%, positive predictive value 90.2%, and negative predictive value 64.3%.

In conclusion, the 20% sulfosalicylic acid test has low sensitivity and specificity for detecting proteinuria, but is more practical and less expensive than spectrophotometry.

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