Use of sulfosalicylic acid in the detection of proteinuria and its application to hypertensive problems in pregnancy

Jesús Arnulfo Velásquez Penagos[1], John Jairo Zuleta Tobón[2], José Duván López Jaramillo[3], Natalia Lucía Gómez Marulanda[3], Jáder Gómez Gallego[4]

SUMMARY

Objective: To determine the reliability and accuracy of the sulfosalicylic acid test in the fast detection and semi-quantitative analysis of proteinuria.

Methods: Prospective, descriptive study at *Hospital Universitario San Vicente de Paúl* in Medellín, Colombia. Ninety eight women with proteinuria and 129 without it were included. Turbidity test with sulfosalicylic acid was compared to 24-hour-proteinuria.

Results: The likelihood ratios (LR) for turbidity test results of 1+, 2+, 3+, 4+, and 5+ were 0.4, 0.7, 3.4, 6.7, and 39.1, respectively. Interclass correlation between the three evaluators of turbidity was 0.966. With the cut point set at 4+, sensitivity was 41.1% (CI 95%: 30.6-51.5), and specificity was 97.7% (CI 95%: 94.7-100). In hypertensive patients, positive predictive value was 95% (CI 95%: 87-100) and negative predictive value was 53.3% (CI 95%: 42.5-64).

Conclusion: Turbidity test is an easy, fast, cheap, and reproducible analysis. Because of its high specificity it is ideal to study pregnant women, particularly those with hypertension, for proteinuria.

KEY WORDS

Pregnancy-induced hypertension; Preeclampsia; Proteinuria; Sulfosalicylic acid test

[1] MD, Ginecólogo y Obstetra, cuidado intensivo obstétrico. Ginecobstetra del Hospital Universitario San Vicente de Paúl. Coordinador de vigilancia epidemiológica de la mortalidad materna y perinatal del Grupo NACER de la Universidad de Antioquia.

[2] MD. MSC, Ginecólogo y Obstetra. Epidemiólogo. Profesor del Grupo de Ginecología del Departamento de Ginecología y Obstetricia, Facultad de Medicina, Universidad de Antioquia. Integrante del Grupo NACER de la Universidad de Antioquia.

[3] MD, Ginecólogos y Obstetras, Universidad de Antioquia.

[4] MD, Ginecólogo y Obstetra, Profesor del Grupo de Perinatología, Departamento de Ginecología y Obstetricia, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. Ginecobstetra del Hospital Universitario San Vicente de Paúl, Medellín, Colombia.
Department of Obstetrics and Gynecology, Faculty of Medicine, University of Antioquia, Medellín, Colombia.
Contact: Jesús Arnulfo Velásquez; nacer@medicina.udea.edu.co
Universidad de Antioquia - Facultad de Medicina - Departamento de Ginecología y Obstetricia Carrera 51 D Nº 62 - 29 Oficina 283 CP 050010 Medellín - Colombia

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RESUMEN

Uso del ácido sulfosalicílico para la detección de proteinuria y su aplicación a problemas de hipertensión en el embarazo

Objetivo: determinar la confiabilidad de la prueba del ácido sulfosalicílico en la detección rápida y semicuantificación de la proteinuria.

Métodos: estudio descriptivo, prospectivo llevado a cabo en el Hospital Universitario San Vicente de Paúl, Medellín, Colombia. Se incluyeron 98 mujeres gestantes con proteinuria y 129 sin proteinuria. La prueba de turbidez con ácido sulfosalicílico se comparó con la medición de proteinuria en 24 horas.

Resultados: los coeficientes de probabilidad *(likelihood ratio)* para los resultados de la prueba fueron los siguientes: 1+ = 0,4; 2+: = 0,7; 3+ = 3,4; 4+ = 6,7 y 5+ = 39,1. La correlación entre los tres evaluadores de la turbidez fue 0,966. Con un punto de corte de 4+ la sensibilidad fue 41,1% (IC 95%: 30,6-51,5), la especificidad fue 97,7% (IC 95%: 94,7-100). Cuando la prueba se aplicó solamente a las pacientes hipertensas el valor predictivo positivo fue 95% (IC 95%: 87-100) y el valor predictivo negativo fue 53,3% (IC 95%: 42,5-64).

Conclusiones: la prueba de turbidez con ácido sulfosalicílico es fácil, rápida, económica y reproducible. Por su baja sensibilidad no es útil como método de tamización pero por su alta especificidad es ideal para la confirmación rápida de proteinuria en las gestantes hipertensas.

PALABRAS CLAVE

Hipertensión inducida por el embarazo; Preeclampsia; Proteinuria; Prueba del ácido sulfosalicílico

INTRODUCTION

Worldwide, each year, hypertensive disorders during pregnancy are responsible for at least 200.000 maternal deaths, and preeclampsia-eclampsia affects approximately 10% of pregnant women. In Colombia, hypertensive disorders during pregnancy represent the second most common cause of maternal mortality (1). Most of the observed complications are secondary to inadequate of delayed treatments (2).

In order to establish the diagnosis of preeclampsia the finding of high blood pressure (above 140/90 mm Hg), and a level of 24-hour-proteinuria above 300 mg are required (3,4). The standard test to make the diagnosis of proteinuria is to measure it in urine during a 24 hour period. However, this is a lengthy process and the collection of the specimen is not easy thus leading to an important delay in establishing the diagnosis. Besides, measurement may not be available in institutions offering only basic clinical laboratory services. It would therefore be convenient to introduce a low cost test with high sensitivity and specificity, easy to use and to read that can be implemented in the basic health care services and the obstetrics emergency units. These goals could be achieved with the use of the sulfosalicylic acid test; this chemical reagent is able to produce protein precipitation through urine acidification ant it provides prompt results.

The major objective of this study was to determine the reliability and accuracy of the sulfosalicylic acid test in the fast measurement of proteinuria.

MATERIALS AND METHODS

Type of study: prospective-descriptive.

Population: The criterion for inclusion was to have more than 20 weeks of gestation.

Setting: The study was done at *Hospital Universitario San Vicente de Paúl,* a high level institution serving as referral center in Medellín, Colombia. It has 32 beds in the obstetrics area and an average of 1.500 deliveries per year.

To increase the chances of including women with proteinuria, 135 hypertensive pregnant patients were selected to enter the study regardless of the stage of their condition. Ninety two pregnant patients with a low chance of having proteinuria were also included; the latter group was made up of women with medical complications different from hypertensive disorders. Patients with vaginal bleeding were excluded, because of the potential contamination of the urine with blood. Proteinuria was defined as the presence

of 300 mg or more of protein in urine in a 24 hour period.

At admission to the emergency service, a 5 mL urine sample was collected in an assay tube and mixed with 5 ml of 3% sulfosalicylic acid. In the following two minutes the mixture was gently agitated for 60 seconds. The degree of turbidity was read against a visual scale chart available in the emergency room. Scores were assigned depending on the ability to read a text (letter size 12 points) through the tube at a distance of 30 cm. Values were as follows:

0 = negative (turbidity 1).

+ = low turbidity but the whole text can be read (turbidity 2).

++ = moderate turbidity; only some parts of the text can be read (turbidity 3).

+++ = the text is illegible but some dots or lines can be seen (turbidity 4).

++++ = it is not possible to read through the tube because of strong turbidity, either with a spotted or coagulated pattern (turbidity 5).

Three different observers independently evaluated the turbidity degree: the intern, the obstetrics and gynecology resident and the obstetrician. Each one deposited his/her diagnosis in a closed urn, one for each group of observers. Only the investigators had access to the results.

Twenty four hour proteinuria, urine pH and urine density were measured in all patients at the central laboratory of the hospital. Technologists did not know the results of the turbidity measurements. When patients delivered during the time of urine collection, this was continued during the postpartum period. Measurement of proteinuria was done by the Vitros method.

Statistical analysis

Size of the sample: based on an expected 90% sensitivity, 95% specificity, 5% maximum error, 95% confidence, and a ratio of 0.7 between women with and without proteinuria, the Epidat 3.1 program calculated a sample size of 135 patients with proteinuria and 92 without it. All information was downloaded into a data base designed in the Excel 2000 program and the analysis was done with the SPSS 14.0 and Epidat 3.1 programs.

The summary of the quantitative variables was done with mean and standard deviation or median and percentiles based on the distribution found after the application of the Kolmogorov Smirnov test. Qualitative variables are given as absolute frequencies and percentages. The operative characteristics of the test were calculated on the basis of universally accepted definitions and are presented with their respective confidence interval (CI) of 95%. Likelihood ratios (LR) can deal with tests with more than two possible results (not just normal/abnormal). LR higher than one increase the likelihood, those lower than one decrease the likelihood, values near one indicate a result that does not substantially change disease likelihood. The Mann-Whitney U test and the Rho Spearman coefficient were used to compare the quantitative variables because their distribution was not normal.

Accuracy of the test is measured by the area under the ROC curve, and depends on how well it separates the group being tested into those with and those without the disease in question. A rough guide for classifying the accuracy of a diagnostic test is the traditional academic point system: 0.90-1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair, 0.60-0.70 = poor, 0.50-0.60 = fail.

Ethical considerations

The protocol for this study was approved by the ethics and research review boards of the *Universidad de Antioquia* and *Hospital Universitario San Vicente de Paúl.*

RESULTS

Twenty four hour proteinuria (more than 300 mg) was present in 84 of the 135 patients with hypertension (62.2%) and in 14 (15.2%) of the 92 that were non-hypertensive. Regardless of the diagnosis at admission, proteinuria was present in 98 patients and absent in 129. Table n.º 1 presents the general characteristics of the group.

The most frequent diagnoses at admission were preterm labor (42 cases; 18.5%), gestational hypertension

in study (41 cases; 18.1%), severe preeclampsia (57 cases; 25.1%), moderate preeclampsia (20 cases; 8.8%), premature rupture of membranes (PROM) (10 cases; 4.4%). In 57 (25.1%) patients other diagnoses were done.

Interclass correlation coefficient among the three observers for the turbidity test was 0.966 (CI 95%: 0.958-0.973). Due to this high level of agreement, only the turbidity reported by one of them (the resident) was taken into account in the analysis.

Table n.º 1. General characteristics of the group

	Without proteinuria Median (P_{25}-P_{75})	With proteinuria Median (P_{25}-P_{75})
Proteinuria mg/dL	137 (91.5-185.5)	901.5 (429.3-2153.5)
Age	23 (19-29.8)	24 (19-31)
Gestational age (weeks)	33 (29-35)	33.5 (30-36)
Systolic blood pressure at admission (mm Hg)	120 (110-140)	150 (140-160)
Diastolic blood pressure at admission (mm Hg)	80 (70-95)	100 (90-100)
Number of previous pregnancies	2 (1-4)	2 (1-3)
Urinary pH	6 (5.5-6.5)	6 (5.5-6.5)
Urinary density	1.015 (1.010-1.023)	1.015 (1.010-1.020)

Tabla n.º 2. Percentiles for 24-hour-proteinuria for each degree of turbidity detected with sulfosalicylic acid test

Turbidity	Percentile 25	Percentile 50	Percentile 75
1	90	137	275
2	139	189	357
3	229	693	1.060
4	308	1.484	2.910
5	1.207	2.365	6.893

Table n.º 2 and figure n.º 1 present the values of 24-hour-proteinuria for a given turbidity value. Table n.º 3 shows the probability coefficient (LR) for each turbidity value. Figure n.º 2 shows the respective ROC curve, in which the area under the curve was of 0.77 (CI 95%: 0.71-0.84).

Table n.º 3. Probability coefficient for each degree of turbidity

Turbidity	Proteinuria + (n = 95)*	%	Proteinuria - (n = 129)	%	LR†
1	18	18.9	65	50.4	0.4
2	28	29.5	56	43.4	0.7
3	10	10.5	4	3.1	3.4
4	10	10.5	2	1.6	6.7
5	29	30.5	2	1.6	39.1

* Three patients were not read by the resident

† LR: likelihood ratio, probability coefficient

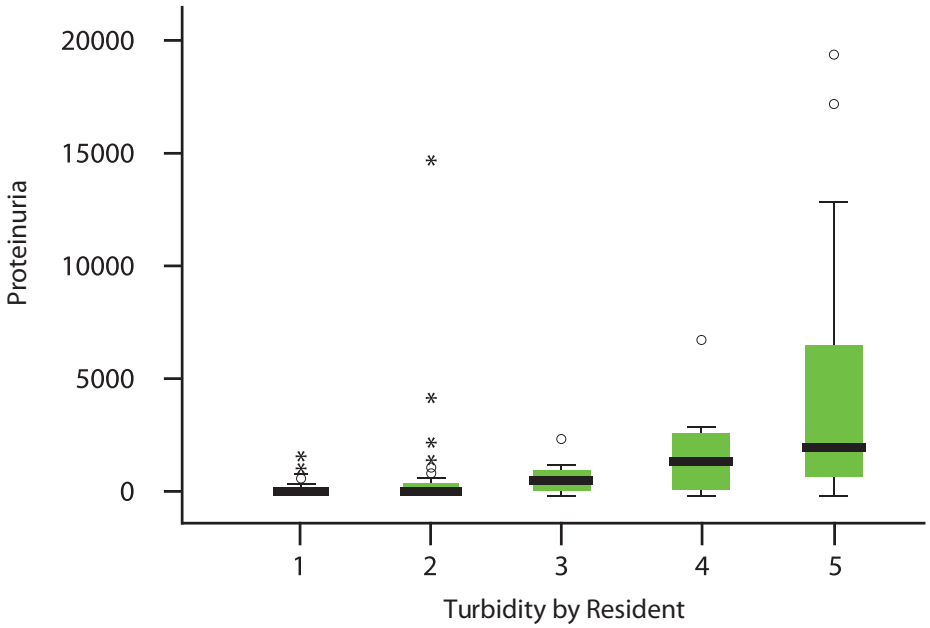

Figure n.° 1. Turbidity of the urine as evaluated by the resident physician vs the 24 hour proteinuria determination

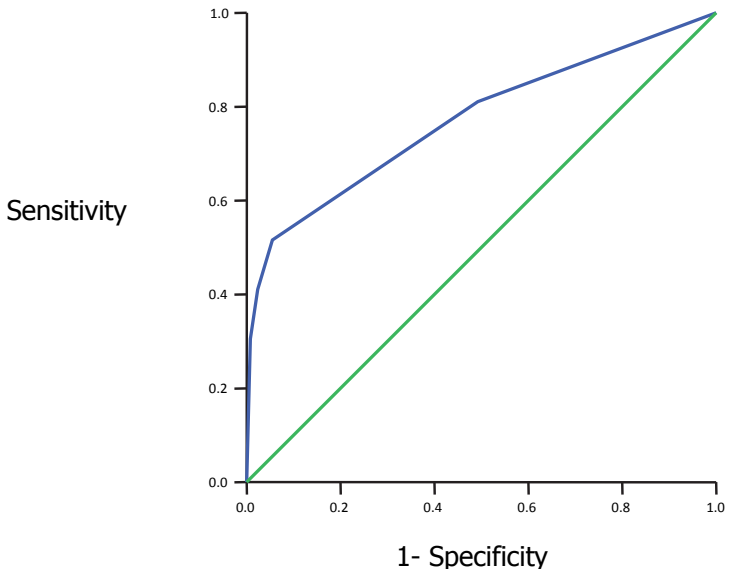

Figure n.° 2. ROC curve of turbidity

Test results were dichotomized: a turbidity value of 4 + or more was assumed as positive for proteinuria, while lesser values were considered negative. After this, sensitivity, specificity and predictive values were calculated. Results are presented in table n.º 4.

Table n.º 4. Sensitivity, specificity, positive predictive value, negative predictive value for a turbidity of 4+or 5+

Variable	Value (CI 95%)
Sensitivity	41.1% (30.6-51.5)
Specificity	97.7% (94.7-100.0)
Positive predictive value	95.0% (87.0-100.0)
Negative predictive value	53.3% (42.5-64.0)

Analysis of the test performance in those women in which it would have been really employed in the clinical practice, namely, hypertensive patients with suspected preeclampsia, revealed the following data: sensitivity 46.9% (CI 95%: 35.4-58.4), negative predictive value 53.3% (CI 95%: 42.5-64.0), specificity 96.1% (CI 95% 89.8-100), positive predictive value 95% (CI 95% 87-100), and a positive LR of 12 for threshold turbidity level of 4+. The respective LR obtained in this subgroup are shown in table 5.

Table n.º 5. LR in hypertensive patients*

Turbidity	Proteinuria + (n = 81)	%	Proteinuria - (n = 51)	%	LR
1	11	13.6	22	43.1	0.3
2	23	28.4	26	51.0	0.6
3	9	11.1	1	2.0	5.6
4	9	11.1	1	2.0	5.6
5	29	35.8	1	2.0	17.9

*Three patients were not read by the resident

Urine pH did not affect the turbidity or proteinuria results and did not act as a confusion variable: there were neither statistically significant differences of pH between the groups with or without proteinuria (p 0.23 Mann-Whitney test) nor correlation with the turbidity levels (Spearman rho -0.027). The same situation was found with the urinary density: there were neither differences of density between the groups according to the presence of proteinuria (p 0.25 Mann-Whitney test) nor correlation with the levels of turbidity (Spearman rho 0.026).

DISCUSSION

The sulfosalicylic acid test is useful for a quick diagnosis of proteinuria. In a hypertensive pregnant patient the finding of a turbidity level of 4+ or 5+ would confirm the diagnosis of preeclampsia in 98% of the cases. Our results show a specificity rate close to 100% with a cut point of 4+ or more; however, it has to be emphasized that even with a turbidity of 3+ more than 50% of the patients will have a significant proteinuria.

This test for proteinuria is easy to use and has minimal variability among observers. For these reasons, it can be performed by inexperienced personnel making it useful in emergency services and obstetric wards, regardless of the complexity level of the health care institution. Besides, it is inexpensive: the cost of the reagent per test is only around US$ 0,14.

Some authors have suggested the use of quick tests such as the urine strips (urinary dipsticks) which can suggest the presence of proteinuria; however, a meta-analysis about the use of this method in pregnant patients affected by hypertensive disorders concluded that it has a limited role when the result is 1+ (5). Because this result does not rule out the possibility of having a clinically significant proteinuria (more than 300 mg in 24 hours), there are limits on its clinical use and for the decision making process. Besides, the use of urinary dipsticks to make the diagnosis of proteinuria is affected by both false positive and false negative results due to several factors such as the presence of an active urinary tract infection

and changes in urinary density and pH (6-9). Finally, assessment of proteinuria by this method is based solely on the detection of albumin while proteinuria in patients with preeclampsia is not selective; therefore, there may be false negative results.

We acknowledge the existence of different types of tests also able to offer the accuracy needed to make the correct diagnosis of significant proteinuria, including the albumin/creatinine ratio; however, this test requires automated methods, it is semi-quantitative and the specimen has to be obtained at the time of the first morning micturition (10) because results are not equally good and may be confusing in specimens obtained during the day (11). Contrariwise, sulfosalicylic acid test can be performed in random urine specimens obtained at admission.

The low negative predictive value found with the sulfosalicylic acid test, even in patients with hypertension, makes it necessary to confirm negative results with a 24 hour proteinuria determination. Despite this limitation, use of the sulfosalicylic acid test reduces the total number of quantitative 24-hour-proteinuria measurements required.

The sensitivity of this test is not high enough; however, assessment for proteinuria in a pregnant patient without hypertension or as a screening test for preeclampsia does not have any value.

The possibility of improving early diagnosis of proteinuria in pregnant women controlled as outpatients has recently been suggested in order to reduce the mortality rate due to preeclampsia. This is achieved by providing patients, especially those facing difficulties of access to health care, a urinalysis dipstick system or a similar test that they can use frequently to check for the onset of proteinuria. This is a sound proposal but it has not been clinically evaluated. The sulfosalicylic acid test is innocuous and easy to use and could become the ideal test under these circumstances (12). Good sensitivity was demonstrated in a study carried out in Africa with the sulfosalicylic acid test: it was able to detect 5 to 10 mg/dL of protein in urine. In comparison, dipsticks are able to detect values starting at 20 to 30 mg/dL. Moreover, the new test also corroborated its ability to detect all kinds of urinary proteins and was regarded as simple to use for health care providers having minimal training (13). However, in this study the test was not used as a diagnostic tool but only for screening purposes, and results were not compared with those of a 24-hour-proteinuria determination.

Certain conditions have been described as potential causes for alteration of the turbidity results, among them: use of contrast agents, antibiotics (tetracycline), penicillamine, and changes in urinary pH and density (14). In our study no patient was exposed to such agents and the analyses performed ruled out changes in urinary pH or density that could have modified the results.

In our series 15% of patients without hypertension presented significant proteinuria but no clinical follow-up was done because it was not part of the study; however, it is an important finding when defining the normal limits of proteinuria.

This study has the strength of its methodological rigor: independent and blinded evaluation of the test and the inclusion of women with and without proteinuria. The range of proteinuria values was representative of what really happens in the daily medical practice. Our patients had different degrees of severity of their hypertensive syndrome. The sample size was calculated to obtain accurate confidence intervals and the test reproducibility was confirmed among people with different training levels. Being a new test, it was necessary to rule out the possibility that the acid was chemically reacting with substances different from proteins; therefore, it made sense to include women in whom the test would not have been routinely used; that was the case of patients without suspicion of hypertensive syndrome associated to pregnancy.

Before recommending the routine use of the sulfosalicylic acid test to detect proteinuria in pregnant patients affected by hypertensive disorders, it is mandatory to confirm that the promising results found in this study can be reproduced.

CONFLICTS OF INTEREST

The authors of this manuscript do not have any conflict of interest which may arise from their participation in the study. The *Pedro Nel Cardona Foundation* does not have any particular interest in the chemical reagent that was utilized.

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