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Original Article

Urinary podocalyxin as a marker of preeclampsia in a Hispanic population

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Abstract: Background: Preeclampsia is associated with significant materno-fetal morbidity and mortality. Podocyturia due to podocyte damage seems to be associated with the disease. The objective of this study was to evaluate the usefulness of urinary podocalyxin as a marker of preeclampsia in a Hispanic population. Methods: 63 patients were studied. 25 patients had preeclampsia/eclampsia (PE-E). 38 patients had normal pregnancies and served as control group. 24 hour proteinuria, urine protein/creatinine (UPC), urinary podocalyxin and perinatal outcomes were measured. A Podocalyxin ELISA test was used to detect podocyturia. Results: Mean age (years), mean±SD was 30.5±5.4 in normal patients vs 30.6±5.8 in PE-E, p=0.98. Median gestational age (weeks) was, 38 (range 21-42) for normal pregnancies and 36 (range 24-40) for patients with PE-E, <0.001. Urine podocalyxin/creatinine on admission (ng/mg), median [IQR] in normal patients was 55.9 [29.4, 74.9] vs 109.7 [63.8, 234.1] in PE-E, p=0.001. After adjusting for admission proteinuria, urinary podocalyxin remained independently associated with preeclampsia: OR=1.0040 (95% CI 1.0003-1.0078), p=0.03. There was low to moderate correlation between UPC and urinary podocalyxin, Spearman's =0.31, p=0.01. In PE-E, post-partum urine podocalyxin was lower, median [IQR]: 69.7 [32.7, 184.8] p=0.19 vs admission. There was a trend towards more podocyturia and proteinuria in patients with eclampsia, comparing to those with preeclampsia. There was no association observed between podocyturia and neonatal mortality, IUGR or Apgar scores. Conclusions: Significantly higher levels of urinary podocalyxin are seen in preeclampsia/eclampsia. They tend to normalize after delivery.

Keywords: Podocalyxin, preeclampsia, proteinuria, hypertension, biomarkers

Introduction

Preeclampsia affects 3-10% of pregnancies. Its prevalence has been increasing and is associated with significant maternal-fetal morbidity and mortality [1].

This condition affects women during their pregnancies and it may be associated with cardiovascular and chronic kidney disease later in life.

Although preexisting hypertension, kidney disease, and diabetes mellitus are risk factors for preeclampsia, the large majority of women with preeclampsia are healthy, primiparous, without significant medical problems [2].

Even though appropriate prenatal care has reduced the number and extent of poor outcomes, serious maternal-fetal morbidity and mortality still occurs.

As a result, there has been a search for diagnostic markers to improve our understanding of the disease.

Podocytes are highly specialized glomerular visceral epithelial cells. Their function is the stabilization of the glomerular capillaries and partici-

pation in the barrier function of the glomerular filter. A growing body of evidence suggests that podocyte damage occurs in preeclampsia, and is reflected by the abnormal shedding of podocytes in the urine [3].

Several markers of podocyte injury are available (i.e. nephrin, sinaptopodin, podocalyxin, podocin). Some of them are more specific to the podocytes, and some are also expressed in other tissues [4, 5].

Different methods are used to detect podocyturia. They include immunohistochemistry after cell culture, ELISA and polymerase chain reaction types. A prior study has demonstrated that urinary podocalyxin, detected by ELISA is characteristic of preeclampsia [6, 7].

To date, most studies on podocyturia and preeclampsia have been performed in populations in Asia, Europe and North America. There is paucity of data in other populations. This is the first study enrolling a predominantly Hispanic population (Paraguayan women).

The objective of the study was to validate, in a Hispanic population, prior findings that suggest that urine podocalyxin (detected by ELISA) is more elevated in preeclampsia.

Material and methods

After obtaining institutional review board approval, this prospective pilot study was performed between March 2013 and January 2014.

Inclusion criteria

Pregnant patients, admitted to the Obstetrics service at the IPS (Instituto de Prevision Social) Hospital in Asuncion, Paraguay, who had a thorough physical examination, urine and blood tests at the time of admission and dismissal (within one week post-partum). These patients signed an informed consent before being admitted to the service and undergoing medical tests and treatment.

Mild preeclampsia was defined as the new development of hypertension (BP=140/90 mmHg) on 2 occasions at least 6 hours apart, without evidence of end-organ damage (no evidence of chronic hypertension), in a woman who was normotensive before 20 weeks' gestation, along with proteinuria ≥300 mg.

Preeclampsia superimposed to chronic hypertension was defined as an increase from baseline systolic blood pressure (SBP) of 30 mmHg or an increase in diastolic blood pressure (DBP) of at least 15 mmHg along with proteinuria ≥300 mg/24 hs in a patient with a prior medical history of hypertension without prior evidence of proteinuria or edema.

Severe preeclampsia was defined as preeclampsia complicated by either a SBP ≥160 mm or a DBP ≥110 mmHg on 2 occasions at least 6 hours apart and/or pulmonary edema and/or oliguria (<400 mL of urine output in 24 hours) and/or persistent headaches, neurological symptoms and/or epigastric pain and/or impaired liver function and/or thrombocytopenia and/or oligohydramnios, decreased fetal growth, or placental abruption and/or HELLP syndrome (hemolysis, elevated liver enzyme, low platelets).

Eclampsia was defined as seizures that cannot be attributable to other causes in a woman with preeclampsia [8].

Normal pregnancy control

Patients without a diagnosis of preeclampsia or eclampsia. Patients without the conditions listed in the exclusion criteria.

Exclusion criteria

Patients in whom urine or blood test were not available at the time of the study, patients with prior history of chronic kidney disease, glomerulonephritis, hematuria, autoimmune disorders, cancer or diabetes mellitus.

Variables collected

Age, blood pressure, serum creatinine, serum uric acid, proteinuria at the time of the admission and dismissal (after delivery), urine podocalyxin at the time of admission and dismissal (post-partum), combined Apgar score (sum of the 1 and 5 minutes Apgar scores), intrauterine growth restriction (IUGR) and neonatal death.

Estimation of podocyturia

Random urine (10 ml) was collected in plastic tubes, without preservative. If necessary, they were clarified by centrifugation (at 3,000 rpm for 5 min). The urine was kept at 4°C for up to 1 week. Prior to the assay, the samples were

Table 1. Baseline characteristics of the study population upon admission

	Normal Pregnancy. N=38	Preeclampsia/Eclampsia N=25	*p* value
Age (years), mean±SD	30.5±5.4	30.6±5.8	0.98
Creatinine (mg/dl) on admission, median [IQR]	0.6 [0.58, 0.70]	0.73 [0.59, 0.89]	0.05
Uric acid (mg/dl) on admission, median [IQR]	3.6 [3, 4.27]	3.8 [2.7, 6.6]	0.27
Urine protein/creatinine on admission (mg/g), median [IQR]	144.6 [49.5, 226.5]	703.5 [246.5, 2762.8]	<0.0001
Urine podocalyxin/creatinine on admission (ng/mg), median [IQR]	55.9 [29.4, 74.9]	109.7 [63.8, 234.1]	0.001
24 hour urine protein (mg/24 hr), median [IQR]	NA	1019 [511, 3390]	NA
Gestational age (wks), median (range)	38 (21-42)	36 (24-40)	<0.001

SD: standard deviation; IQR: interquartile range.

allowed to thaw at room temperature (24°C). All the assays were completed using duplicate wells for each dilution of the standard and each dilution of the sample.

A commercially available Podocalyxin ELISA test (Exocell Inc.) was used. This assay is designed to measure podocalyxin in urine or tissue extract samples of rodent or human origin. The assay range is 0.156 ng/ml-10.0 ng/ml. The intra- and inter-assay precision for samples within the assay range has a C.V. of <7%. Each sample was measured in duplicate. The values are expressed as ng/ml.

Urine creatinine was measured by the Jaffe reaction on the same aliquot of urine. To further adjust for urine creatinine, the ratio of urinary podocalyxin to creatinine (ng/mg) was used.

Estimation of renal function

Estimation of glomerular filtration rate (GFR) in pregnant patients is difficult. Studies have shown that formulas calculating GFR based on plasma will overestimate or underestimate greatly GFR in pregnant women. That is why renal function is only expressed as serum creatinine (mg/dL). No creatinine, inulin or iothalamate clearance was performed in this study [9, 10].

Estimation of proteinuria

Random urine total protein-to-creatinine ratio (UPC, expressed as mg/g) was obtained on admission and dismissal on patients with preeclampsia/eclampsia and on admission in normal pregnant patients. Total protein was measured by the Pyrogallol red dye method. Urinary creatinine was measured by the Jaffe reaction on the same aliquot of urine.

A 24 hour urine protein (mg/24 hours) collection was performed using the benzethonium chloride assay on admission and whenever possible on dismissal on patients with preeclampsia and eclampsia.

Statistical analyses

Data are presented as mean and standard deviation if normally distributed and median [25% and 75% percentiles], (range) if not.

Differences in means were compared by the Student's t test with equal variance not assumed and if more than two groups, by one-way ANOVA. For highly skewed data, the Mann-Whitney U test and Kruskall-Wallis tests were used. For paired data, the Wilcoxon signed rank test was used.

Spearman's p was used to test correlations. Differences in proportions were assessed by the Fisher's exact test.

For multivariate analyses, logistic and linear regression models were used when applicable.

P values lower ≤0.05 were considered statistically significant. All the analyses were performed using SOFA Statistics version 1.4.0 (Paton-Simpson & Associates Ltd, Auckland, New Zealand) and OpenStat software program.

Results

Seventy-one patients were enrolled. Of these, 8 patients were excluded from the final analysis (one patient was diagnosed with gestational diabetes and seven patients had hematuria on admission). Sixty three patients were part of the final study.

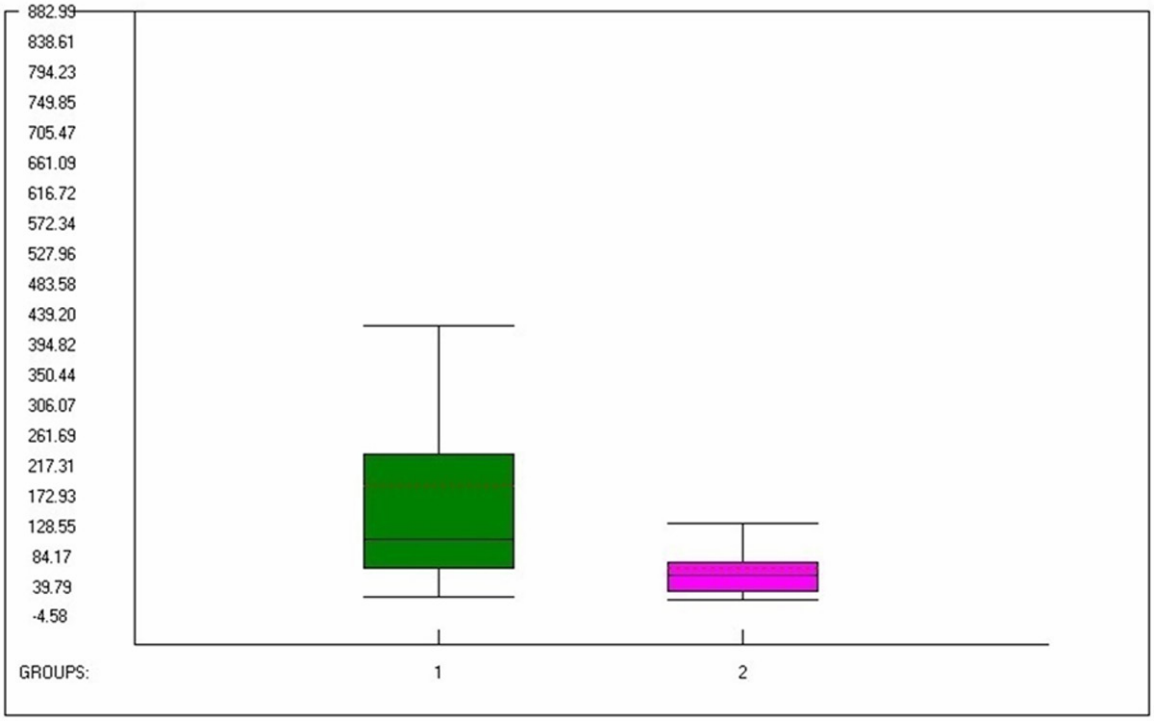

Figure 1. Podocyturia on admission. Preeclampsia/Eclampsia =1; Normal pregnancies =2. Urine podocalyxin is expressed as ng/mg on the y axis.

Patients with preeclampsia and eclampsia had more podocyturia and proteinuria compared to pregnant patients without preeclampsia. Of note, most of the patients with PE-E were already receiving treatment with blood pressure medications (**Table 1**, **Figure 1**).

Fifteen (60%) of the patients with preeclampsia/eclampsia were primiparous.

There was no statistical significant difference in podocyturia between patients with preeclampsia/eclampsia and those with hematuria.

There was a low to moderate correlation between admission UPC and urinary podocalyxin. No correlation was noted between urinary podocalyxin and 24 hour proteinuria. There was good correlation between 24 hour proteinuria and UPC (**Table 2**).

Serum creatinine had a low to moderate correlation with both UPC and 24 hour proteinuria. There was a trend towards correlation between urinary podocalyxin and serum creatinine, and between UPC and uric acid (**Table 2**).

When compared to values obtained on admission, blood pressure, proteinuria and urinary podocalyxin improved after delivery, although only the changes in blood pressure readings became statistically significant (**Table 3**). Fifty-five percent of patients with PE/E had podocyturia less than 75 ng/mg on dismissal.

Upon dismissal, no correlation was found between UPC and urine podocalyxin: Spearman's =-0.23, p=0.34.

On multivariate analysis, after adjusting for admission proteinuria (UPC), urine podocalyxin on admission remained independently associated with preeclampsia/eclampsia: OR=1.0040 (95% CI 1.0003-1.0078), p=0.03.

UPC also remained independently associated with preeclampsia: OR=1.0005 (95% CI 1.0002-1.0007), p=0.001.

In subgroup analyses, compared to those with preeclampsia, there was a trend towards more podocyturia and proteinuria in patients with eclampsia, although the sample is very small to draw a definite conclusion (**Table 4**, **Figure 2**).

Patients with intrauterine growth restriction (IUGR) had a tendency towards increased proteinuria (median UPC=2542 mg/g, range 201-

Table 2. Correlation between proteinuria, podocyturia and other clinical and laboratory variables in the entire study population. N=63

	Urine Podocalyxin (ng/mg)	Urine protein/creatinine (mg/g)	24 hour proteinuria (mg/24 hours)
24 hour proteinuria (mg/24 hours)	0.09 (0.68)	0.65 (<0.001)	NA
Urine protein/creatinine (mg/g)	0.31 (0.01)	NA	0.65 (<0.001)
Serum creatinine (mg/dl)	0.23 (0.10)	0.34 (0.01)	0.41 (0.04)
Serum uric acid (mg/dl)	-0.084 (0.56)	0.27 (0.06)	0.17 (0.42)
SBP (mmHg)	-0.23 (0.26)	0.34 (0.09)	0.17 (0.42)
DBP (mmHg)	-0.34 (0.09)	0.28 (0.17)	0.13 (0.53)

Spearman's correlation. *p* values in parenthesis. Values are upon admission. SBP=systolic blood pressure. DBP=diastolic blood pressure.

Table 3. Admission vs post-partum characteristics in patients with preeclampsia and eclampsia. N=18

	Admission	Post-partum	*p* value
Creatinine (mg/dl), median [IQR]	0.73 [0.59, 0.89]	0.70 [0.60, 0.80]	0.14
Uric acid (mg/dl), median [IQR]	3.8 [2.7, 6.6]	3.6 [3, 4.3]	0.28
Urine protein/creatinine (mg/g), median [IQR]	703.5 [246.5, 2762.8]	495.3 [307.4, 2446.3]	0.13
Urine podocalyxin/creatinine (ng/mg), median [IQR]	109.7 [63.8, 234.1]	69.7 [32.7, 184.8]	0.19
Systolic BP mmHg, median [IQR]	156 [132, 174]	122 [114, 131]	<0.001
Diastolic BP mmHg, median [IQR]	100.6 [89, 110]	79 [71, 84]	<0.001

IQR: interquartile range.

5096) when compared to those without this problem (UPC=395 mg/g, range 135-10790), p=0.11. No difference was noted in urine podocalyxin: 141 ng/mg (2.14-863) vs 97 ng/mg (23-417) p=0.93.

No difference in levels of urinary podocalyxin was noted in patients with a neonatal demise: 125.8 ng/mg (range 37-214) vs 114 ng/mg (range 2.14-863) in those with an alive newborn p=0.84.

There was a trend towards higher proteinuria in those with neonatal mortality: UPC=2831 mg/g (range 565-5096) vs 757 mg/g (range 135-10790) p=0.42.

Neither urine podocalyxin nor proteinuria was associated with the combined Apgar score (Apgar score and podocyturia: R=0.09 p=0.28, Apgar score and UPC: R=0.07 p=0.28).

In patients with preeclampsia/eclampsia, 23 underwent a C-section (96%) and 1 (4%) had a normal vaginal delivery. One patient left the service before delivery. No proteinuria or podocyturia on dismissal was obtained on this patient.

Discussion

In Paraguay, the prevalence of preeclampsia is 10%. Early detection of preeclampsia will allow health professionals to deliver better medical care and will improve the morbidity-mortality associated with this disease [11, 12].

Endothelial dysfunction is thought to play a role in the pathogenesis of preeclampsia along with podocyturia.

Although proteinuria has always been used as a marker of preeclampsia, this is a later event in a process that started earlier. Other proposed biomarkers of preeclampsia include serum angiogenic markers (placental growth factor, fms-like tyrosine kinase receptor-1 for vascular endothelial growth factor, endoglin) and urinary podocytes [13, 14].

Podocytes are normally absent or seen in small numbers in the urine of healthy individuals or those with inactive kidney disease. It is believed that podocyturia only appears when there is ongoing renal disease, and it tends to disappear in response to successful therapy. On the other hand, proteinuria can be seen as an

Table 4. Patient characteristics in preeclampsia, preeclampsia superimposed to chronic hypertension and eclampsia. N=25

	Mild preeclampsia N=3	Severe preeclampsia N=8	Preeclampsia superimposed to chronic hypertension N=12	Eclampsia N=2	*p* value
Admission urine podocalyxin/creatinine (ng/mg), median (range)	382.4 (73- 457)	134.1 (28.7-318.4)	65.2 (2.1-416.6)	488.4 (114-863)	0.23
24 hour urine protein (mg/24 hr), median (range)	497 (288-705)	1758 (358-3942)	932 (271-3524)	6587 (6517-6656)	0.14
Admission urine protein/creatinine (mg/g), median (range)	268.4 (242-541.5)	1850 (135-10790)	667 (194.5-6374)	2070 (395-3745)	0.67
Admission creatinine (mg/dl), median (range)	0.79 (0.77-0.8)	0.66 (0.4-0.9)	0.72 (0.5-1.2)	0.75 (0.4-1.1)	0.68
Dismissal urine podocalyxin/creatinine (ng/mg), median (range)	186.2 (37-335)	96.6 (7.6-203)	53.4 (19-183)	423 (94-751)	0.46
Dismissal urine protein/creatinine (mg/g), median (range)	928 (320-1537)	1690 (242-5641)	378 (192-2842)	1623 (256-2991)	0.76
Dismissal creatinine (mg/dl), median (range)	0.55 (0.5-0.6)	0.64 (0.6-0.8)	0.75 (0.5-0.9)	0.64 (0.6-0.7)	0.25

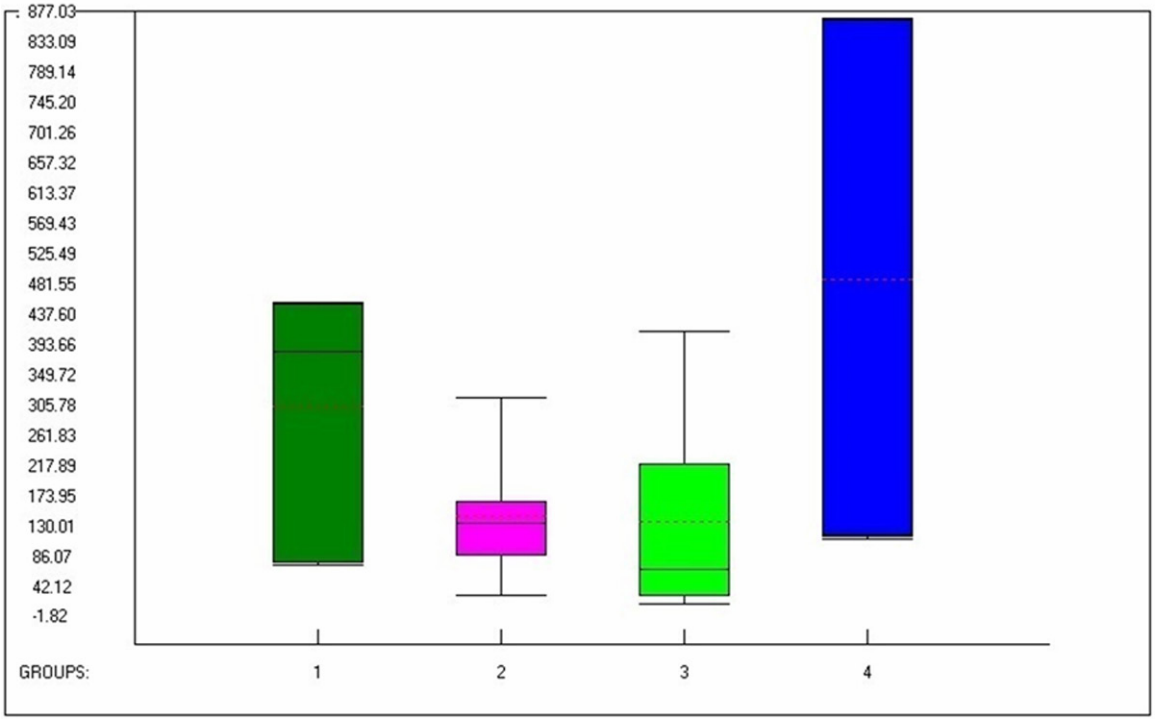

Figure 2. Podocyturia in patients with preeclampsia, severe preeclampsia, preeclampsia superimposed to chronic hypertension and eclampsia. Mild preeclampsia =1; Severe preeclampsia =2; Preeclampsia superimposed to chronic hypertension =3; Eclampsia =4. Urine podocalyxin is expressed as ng/mg on the y axis.

acute finding suggesting ongoing glomerular disease or as a chronic marker of a scarred, sclerotic glomerulus [15, 16].

It has been proposed that podocyturia appears before preeclampsia, and eventually could prove useful as a screening method [17].

Podocalyxin is the major sialoglycoprotein present on the surface of podocytes. The amount of its urinary excretion is associated with the degree of podocyte damage in glomerular diseases. Quantification of urinary sediment podocalyxin by ELISA is a reliable marker of active glomerular disease. Urinary podocalyxin ELISA assays are readily available, cheaper and easier to use in the standard laboratory setting in contrast to other methods of podocyturia detection or angiogenic markers. Although it is not podocyte specific, it is the marker used most frequently to diagnose podocyturia [18-22].

In this study, podocyturia (expressed as urinary podocalyxin) was shown to be elevated in cases of preeclampsia and eclampsia. This association remained independent of proteinuria and correlated with UPC. Also, its values appeared to normalize after delivery (to levels comparable to those seen in normal pregnancies), in the same manner as the proteinuria and blood pressure readings. This finding is somewhat similar to the only other study of urine podocalyxin (detected by ELISA) in preeclampsia published so far, by Wang et al. Nonetheless, it is noted that in that study, patients with chronic hypertension also had a tendency to higher levels of urine podocalyxin [7].

In our study, there was a trend to higher levels of urinary podocalyxin in eclampsia compared to other cases, suggesting these higher levels may indicate increasing severity of disease. This finding needs to be explored further, since the number of patients in this study is small.

Some of the studied patients had persistent podocyturia even after delivery. The significance of this finding is unknown, but some hypotheses can be entertained. One of them is that persistent podocyturia might identify a subgroup of patients who are prone to chronic kidney disease in the future. Currently, there is controversy about the association of pre-

eclampsia/eclampsia with chronic kidney disease later in life. If podocyturia indicates ongoing podocyte injury, then one would assume that these patients will go on to develop chronic kidney disease. It is important to remember that it can take up to 2 years for hypertension and proteinuria to resolve, therefore longer follow up is needed to answer this question [23].

The fact that podocyturia remained independently associated with preeclampsia/eclampsia further supports its diagnostic value. This might enable us to use it as a screening marker of women who develop the disease later on. In order to do this, a larger study will need to be performed in which patients are screened earlier in their pregnancies.

No specific diagnostic cutoff of urinary podocalyxin can be recommended from this study, although values greater than 75 ng/mg have a sensitivity and specificity of 64% and 77% for preeclampsia/eclampsia.

It is important to note that podocyturia is not unique to preeclampsia. It is seen in lupus nephritis, diabetic nephropathy, IgA nephropathy and FSGS. In this study 7 patients with hematuria had high degree of podocyturia and were not included in the final analysis, suggesting this as a confounding factor as well. One patient with gestational diabetes had podocyturia levels compared to those found in normal pregnancies.

Podocalyxin can also be detected in the endothelium, platelets and in some malignancies. In preeclampsia, where there appears to be endothelial damage, along with podocyte affectation, one would assume this marker will be elevated [4, 5, 24].

In this study, urine podocalyxin did not associate with perinatal outcomes (death/IUGR/Apgar score).

There was a trend towards association of proteinuria (UPC) with perinatal outcomes, but the sample is too small to study this association.

In the non-pregnant population, the use of UPC is standard of care. It is easy to use and reproducible. On the other hand, 24 hour urine collection is cumbersome and time consuming [25].

In this study, both methods of measuring proteinuria were used, mostly upon admission. They correlated fairly well in this setting. Prior studies report conflicting evidence about how useful UPC is, especially when results are equivocal. Most misclassifications tend to occur in women with borderline proteinuria (250 to 400 mg/d). More studies will be needed in this area before a center fully commits to one method, but our results support some role for UPC, especially when a proper 24 hour urine collection is not feasible [26, 27].

The strengths of the study include the ability to measure proteinuria and podocyturia on admission and post-partum, the use of a technically easy to perform ELISA assay to detect urine podocalyxin, the ability to obtain perinatal data on these patients and the use of both UPC and 24 hour urine proteinuria. This is the first study of its kind enrolling a predominant Hispanic population.

Weaknesses include the small sample size, although most similar studies suffer from the same bias, along with lack of statistical power to perform reliable subgroup or multivariate analyses. Taken together, these findings need to be tested in a larger population.

In conclusion, in this Hispanic population, urinary podocalyxin appears to be more elevated in preeclampsia/eclampsia independent of proteinuria. Its values tend to normalize after delivery, in the same fashion as the proteinuria and blood pressure readings.

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Disclosure of conflict of interest

None.

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