

Hypocalciuria in pre-eclampsia and gestational hypertension due to decreased fractional excretion of calcium

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Abstract

Introduction: This study investigated whether reduction in urinary calcium excretion is due to decreased fractional excretion of calcium, and evaluated the potential of urinary calcium to creatinine ratio as a screening test for pre-eclampsia.

Material and methods: This prospective study involves 83 pregnant subjects: 29 with pre-eclampsia, 27 with chronic hypertension and 27 normotensive pregnant controls. Serial blood and urine specimens were obtained and analyzed in the third trimester (32 weeks to term).

Results: The serum levels of calcium, phosphate, urea and uric acid were not different among the groups. In contrast, the serum albumin was significantly reduced in pre-eclamptic women and chronic hypertensive women, compared with normotensive pregnant controls. Pre-eclamptic women had the lowest urinary calcium excretion rate (1.70 ± 0.26 mmol/day) compared with chronic hypertensive women (4.23 ± 0.59 mmol/day, $p < 0.05$) and normotensive pregnant women (4.43 ± 0.60 mmol/day, $p < 0.05$). Hypocalciuria in pre-eclamptic women was associated with decreased fractional excretion of calcium of 1.45 ± 0.05 mmol/day compared with 4.02 ± 0.09 mmol/day in normotensive women ($p < 0.05$). There was decreased urinary calcium to creatinine ratio (0.17 ± 0.03 mmol/mmol) in pre-eclamptic women compared with normotensive women (0.45 ± 0.08 mmol/mmol, $p < 0.05$).

Conclusions: These findings suggest that hypocalciuria in pre-eclampsia is associated with decreased fractional excretion of calcium, suggesting a mechanism which may involve increased tubular reabsorption of calcium. Urinary calcium to creatinine ratio may be a useful potential screening test for pre-eclampsia.

Key words: pre-eclampsia, hypocalciuria, chronic hypertension, fractional excretion, calcium to creatinine ratio.

Introduction

Hypertension occurs in 5 to 10% of pregnant patients and is an important cause of both perinatal and maternal morbidity and mortality [1]. One of the ways to reduce the impact of arterial hypertension on material mortality is to establish early and correct diagnosis of pre-eclampsia, followed by early intervention [2].

Calcium and phosphate metabolism during normal pregnancy are characterized by minor changes in the serum levels of calcium and phosphate. Urinary calcium and phosphate however, increases [3]. Urinary calcium excretion in normal pregnancy is 350-620 mg/day, compared with 100-250 mg/day in non-pregnant women [4]. Excretion usually increases during each trimester, with maximum levels reached during the third trimester [5]. Aberrations in calcium homeostasis have been recognized in hypertension in general and specifically in pre-eclampsia [6]. Abnormalities of calcium metabolism in pre-eclampsia include hypocalciuria, decreased 1,25-dihydroxyvitamin D, elevated parathyroid hormone (PTH), decreased urinary cyclic adenosine monophosphate, increased levels of intracellular calcium, and reduced levels of ionized calcium [7-9].

Studies using measurements of 24-h urinary calcium excretion have documented decreased excretion in women with pre-eclampsia [5, 10], while there are others who have found no such correlations [11]. Hypocalciuria can be due to low dietary calcium intake [6], increased foetal extraction, or primary renal dysfunction. Rodriguez *et al.* [12] found that the calcium to creatinine ratio may be a useful screening tool in predicting the development of pre-eclampsia. Based on the observation that urinary calcium excretion is reduced in pregnant patients with pre-eclampsia, this study investigated whether alterations in urinary calcium excretion, calcium to creatinine ratio and fractional excretion of calcium would distinguish women with pre-eclampsia from normal pregnant women or those with chronic hypertension in a black population at the University Hospital of the West Indies.

Material and methods

Hypertension in pregnancy is defined as the presence of a blood pressure of 140/90 mm Hg taken twice 6 h apart, or a rise of 30 mm Hg in systolic pressure or 15 mm Hg in diastolic pressure [13]. The first and fifth Korotkoff auscultatory sounds were used to determine the systolic and diastolic components. Pre-eclampsia is defined as hypertension associated with excretion of more than 300 mg of urinary protein per day or a rise in serum uric acid level of more than 1 mg per decilitre, decrease in the platelet count of more than 50,000 per cubic millimetre, or both [14, 15]. The classification followed the guidelines of the American College of Obstetricians and Gynecologists [16]. Chronic hypertension was defined as the presence of a documented blood pressure before pregnancy of more than 140/90 mm Hg.

An observational controlled and prospective study in pregnant patients admitted to maternity

wards of the University Hospital of the West Indies was conducted between April and December 2006. The test population consisted of 83 pregnant women during the third trimester of pregnancy: 29 with pre-eclampsia, 27 with chronic hypertension and 27 normotensive controls matched by gestational age and maternal weight. Patients who had a history or evidence of renal disease, diabetes, proteinuria or other chronic medical illnesses were excluded. None of the subjects received diuretics during the study. The patients were instructed to continue their usual diets and physical activities.

Serial venous blood samples were collected and assayed for serum levels of albumin, calcium, magnesium, phosphorous, uric acid, urea and creatinine utilizing a multi-channel autoanalyzer (c8000, Abbott Diagnostics, USA). Urinary creatinine, sodium, calcium, and protein were also measured using this analyzer. The fractional excretion of calcium was computed by dividing calcium clearance by creatinine clearance and multiplying by 0.60 [17].

Data were analyzed by the Student's *t*-test for independent samples and the Fisher test for independent test with the level of significance set at 5%. Statistical significance was assessed by the Mann-Whitney U test [18]. $P < 0.05$ was considered significant. Statistical significance of the differences between the various groups was determined by one-way analysis of variance. The results are reported as mean \pm S.E. Data were stored and later analyzed using the Statistical Package for Social Sciences (SPSS version 12). This study was approved by the University of the West Indies Ethics Committee.

Results

Table I presents the clinical characteristics of the groups. The study consisted of 83 pregnant women – 27 normal, 27 with chronic hypertension and 29 with pre-eclampsia. The maternal age and gestational ages did not differ significantly among the groups. Patients with pre-eclampsia and chronic hypertension were delivered at an earlier gestational age. Both the chronic and pre-eclampsia groups had higher mean, systolic and diastolic pressures than the normotensive women ($p < 0.05$). The infants born to pre-eclamptic women were smaller than those born to normotensive women ($p < 0.05$) or to women with chronic hypertension ($p < 0.05$).

Table II shows the laboratory data obtained before delivery. Pre-eclamptic women and those with chronic hypertension had higher serum uric acid concentrations than those in the normotensive group; however, the differences were not statistically significant. The concentrations of serum total calcium and phosphorous did not differ among

the groups. Pre-eclamptic women, however, had lower serum albumin and total protein concentrations than the normotensive pregnant controls ($p < 0.05$).

Pre-eclamptic women excreted significantly more protein (2.56 ± 0.42 g/day) than women with chronic hypertension (0.35 ± 0.14 g/day) and normotensive women (0.14 ± 0.03 g/day). Women with pre-eclampsia and those with chronic hypertension had reduced creatinine clearances compared with normotensive women (Table III). Pre-eclamptic women had significantly lower urinary excretion of calcium (1.70 ± 0.26 mmol/day) compared with women with chronic hypertension

(4.23 ± 0.59 mmol/day) and normotensive women (4.43 ± 0.60 mmol/day, $p < 0.05$). The urinary excretion of sodium in pre-eclamptic women was significantly lower than in normotensive women. In contrast the potassium excretion rates did not differ significantly among the three groups.

To determine whether the decreased urinary calcium excretion in women with pre-eclampsia could be attributed to decrease glomerular filtration or whether there was evidence of increased tubular reabsorption, we calculated the fractional excretion. It was significantly lower in the women with pre-eclampsia ($1.45 \pm 0.05\%$, $p < 0.05$) compared with women with chronic hypertension

Table I. Description of the study subjects and the outcome of their pregnancies

	Normotensive (N = 27)	Chronic hypertensive (N = 27)	Pre-eclampsia (N = 29)
Maternal age [years]	34.33 \pm 1.25	34.20 \pm 1.49	31.60 \pm 1.58
Gestational age [wk]	35.56 \pm 1.25	34.40 \pm 1.22	34.56 \pm 1.02
Systolic blood pressure [mm Hg]	121.38 \pm 3.60	138.33 \pm 3.84*	143.63 \pm 5.41 ⁺
Diastolic blood pressure [mm Hg]	75.83 \pm 2.64	91.53 \pm 2.68*	91.06 \pm 3.70 ⁺
Gravida	3.69 \pm 0.47	3.54 \pm 1.02	2.42 \pm 0.31
Para	2.27 \pm 0.36	1.33 \pm 0.17	1.78 \pm 0.36
Birth weight [g]	3.14 \pm 0.24	2.27 \pm 0.28*	1.88 \pm 0.24 ⁺

Data are presented as mean \pm SE

⁺ $p < 0.05$ – pre-eclampsia group compared with normotensive group

* $p < 0.05$ – chronic hypertensive group compared with normotensive group

Table II. Serum analyses of the study subjects

	Normotensive (N = 27)	Chronic hypertensive (N = 27)	Pre-eclampsia (N = 29)
Sodium [mmol/l]	135.33 \pm 3.57	137.00 \pm 0.86	136.74 \pm 3.57
Potassium [mmol/l]	3.88 \pm 0.17	3.94 \pm 0.09	3.87 \pm 0.09
Phosphorous [mmol/l]	1.14 \pm 0.08	1.17 \pm 0.13	1.15 \pm 0.17
Magnesium [mmol/l]	0.88 \pm 0.09	0.84 \pm 0.04	0.81 \pm 0.05
Uric acid [mmol/l]	0.24 \pm 0.03	0.28 \pm 0.02	0.27 \pm 0.02
Calcium [mmol/l]	2.15 \pm 0.05	2.03 \pm 0.07	1.95 \pm 0.09
Albumin [g/l]	36.27 \pm 0.90	36.20 \pm 0.97	32.36 \pm 1.34 ^a
Total protein [g/l]	65.00 \pm 2.03	60.67 \pm 1.93*	54.06 \pm 1.86 ⁺
Urea [mmol/l]	2.60 \pm 0.27	3.34 \pm 0.48	3.26 \pm 0.37
Creatinine [mmol/l]	64.60 \pm 2.72	70.19 \pm 3.30	68.11 \pm 4.78
AST [U/l]	36.42 \pm 3.57	38.80 \pm 6.72	36.42 \pm 3.57

Data are presented as mean \pm SE

⁺ $p < 0.05$ – pre-eclamptic group compared with normotensive group

* $p < 0.05$ – chronic hypertensive group compared with normotensive group

^a $p < 0.05$ – pre-eclamptic group compared with chronic hypertensive group

Table III. 24-h urine collections of the study subjects

	Normotensive (N = 27)	Chronic hypertensive (N = 27)	Pre-eclampsia (N = 29)
Sodium [mmol/day]	144.42 ±16.37	106.46 ±14.98	100.33 ±16.61 ⁺
Potassium [mmol/day]	42.39 ±3.81	42.39 ±3.81	35.27 ±2.93
Calcium [mmol/day]	4.43 ±0.60	4.23 ±0.59*	1.70 ±0.26 ⁺
Phosphorous [mmol/day]	11.52 ±1.89	10.89 ±1.43	10.95 ±1.66
Creatinine [mol/day]	12.20 ±1.30	13.70 ±5.40	12.50 ±6.50
Protein [g/day]	0.14 ±0.03	0.35 ±0.14*	2.56 ±0.42 ⁺
Creatinine clearance [ml/min]	118.56 ±20.76	115.46 ±7.06	94.19 ±7.78
Calcium – creatinine ratio [mmol/mmol]	0.45 ±0.08	0.36 ±0.05*	0.17 ±0.03 ⁺
Fractional excretion of calcium [%]	4.02 ±0.09	2.14 ±0.08	1.45 ±0.05 ⁺

Data are presented as mean ± SE

⁺p < 0.05 – pre-eclampsia group compared with normotensive group

*p < 0.05 – chronic hypertensive group compared with normotensive group

(2.14 ±0.08%) and normotensive women (4.02 ±0.09%). The mean calcium to creatinine ratio was found to be significantly lower in the pre-eclamptic women (0.17 ±0.03, $p < 0.05$) compared with chronic hypertensive women (0.36 ±0.05) and the normotensive controls (0.45 ±0.08).

Discussion

In this study pre-eclamptic women had reduced urinary excretion of calcium compared with those with chronic hypertension and normal pregnancies. Our observation that pre-eclamptic women have hypocalciuria is in accord with reports from other investigators [7, 12, 19]. These authors suggested that the urinary calcium excretion levels may serve as a diagnostic tool for differentiating between the various forms of hypertension in pregnancy. Sanchez-Ramos *et al.* [20] found a relative risk of 9.4 of predicting pre-eclampsia when the patients show urinary calcium excretion lower than 195 mg/day.

Serum calcium concentrations in pre-eclamptic women appear no different from values in the chronic hypertensive and normotensive women; however, the serum albumin was significantly lower in pre-eclamptic women. The concentration of total calcium in maternal serum declines during pregnancy, reaching its lowest value during the third trimester and rising slightly thereafter [21]. The pattern of decline of serum calcium is parallel to that of serum albumin, suggesting that the fall largely involves the protein bound fraction [20].

The aetiology of hypocalciuria in pre-eclamptic patients is unknown. It has been speculated that hypocalciuria may result from decreased dietary

intake, decreased intestinal absorption, increased calcium uptake by the fetus and placenta, or intrinsic renal tubular dysfunction [22]. Taufield *et al.* [7] observed marked hypocalciuria in patients with hypertensive disorders of pregnancy and suggested increased distal tubular reabsorption of calcium as a possible mechanism. Pedersen *et al.* [5] in a longitudinal study reported that urinary calcium excretion was considerably lower in the third trimester of pre-eclamptic pregnant women than in both pregnant subjects and non-pregnant controls. They suggested that these changes could partly be related to a decrease in the glomerular filtration in pre-eclampsia [5]. The authors also found reduced tubular reabsorption in pre-eclamptics because the fractional excretion was decreased, a finding which is similar to ours in this study.

Urinary excretion of calcium represents a balance between glomerular filtration and tubular reabsorption. Increased calcium reabsorption in the distal tubule [23] is regulated by parathyroid hormone (PTH) [23]. Pederson *et al.* [5] found that PTH and calcitonin levels were not altered in patients with pre-eclampsia and concluded that the differences in calcium metabolism were not related to alterations in the secretion of these hormones [5]. In a later study by Frenkel *et al.* [22], PTH levels were lower in pre-eclamptic pregnant women, possibly because of increased renal calcium reabsorption, thereby reaching a steady state of normocalcaemia associated with lower PTH levels [22]. Considering the normal serum phosphorous and decreased creatinine clearance observed in this study, it seems unlikely that altered sensitivity to PTH accounts for the lower urinary calcium excretion.

Other investigations have shown that a renal tubular dysfunction is not the likely cause of hypocalciuria. August *et al.* [8] and Seely *et al.* [9] reported decreased levels of 1,25-dihydroxyvitamin D and increased serum PTH in pre-eclamptic patients compared to normotensive controls. These findings led to the speculation that hypocalciuria in patients with pre-eclampsia may result from decreased intestinal absorption of calcium as a secondary result of decreased 1,25-dihydroxyvitamin D, rather than decreased dietary intake or increased transfer to the fetus [8, 9]. However, a later study by Tolaymat *et al.* [24] found similar intestinal absorption of calcium in pre-eclamptic and normotensive control groups, but significantly lower levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the pre-eclamptic group. They suggested that the lower levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in pre-eclamptic patients could be the result of a reduction in vitamin D binding protein in the maternal serum [24]. Additional studies of these hormones need to be performed in normal pregnant women and those with chronic hypertension and pre-eclampsia.

The urinary calcium to creatinine ratio was significantly lower in pre-eclamptic patients compared with those patients with chronic hypertension, and the pregnant controls. This suggests that the calcium to creatinine ratio may be useful in predicting pre-eclampsia. Rodriguez *et al.* [12] evaluated the calcium to creatinine ratio and the presence of microalbuminuria in 88 normotensive pregnant women. They found that the urinary calcium to creatinine ratio proved to be a better predictor of pre-eclampsia than microalbuminuria and compared favourably to the combination of tests [12]. This finding was supported by Huikeshoven and Zuijderhoudt [10], who concluded that the measurement of urinary calcium excretion is of value for the study of pregnant patients with arterial hypertension both in terms of 24-h excretion and the calciuria/creatinuria ratio of a single urine sample [10]. However, Baker and Hackett [25] did not find significantly predictive values in the measurement of the albumin to creatinine and/or calcium to creatinine ratio in relation to the diagnosis of pre-eclampsia [25].

Reduced calcium excretion may result from dietary variation. We did not monitor the calcium intake in our patients. However, because we did not advise any of the patients in the study to alter their diet, it is unlikely that dietary calcium intake played a major role in our findings.

In conclusion, the study showed lower urinary excretion of calcium in pre-eclamptic women compared with chronic hypertensive women or normotensive controls. The reduced urinary

excretion of calcium is attributed to decreased creatinine clearance and fractional excretion of calcium. Reduced urinary calcium to creatinine ratio was a key finding and may be a useful predictor of pre-eclampsia. As a potential diagnostic test for pre-eclampsia urinary calcium excretion is easy to carry out, non-invasive and inexpensive. However, further prospective studies are required to elucidate its mechanism and pathophysiology.

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