

## Original Article

# Urinary calcium levels in pre-eclampsia

Dasgupta Mandira, Adhikari Sudhir, Sanghamita Mamta.

*Department of Obstetrics and Gynecology Eden hospital, Medical College and Hospitals,  
88, College Street, Kolkata - 700 073.*

### Abstract

**Objectives :** To note the changes in calcium excretion in women with pre-eclampsia (PE), eclampsia and its role as its predictor, and to correlate the changes with renal function. **Methods :** A prospective study was conducted over a period of 1 year, on 60 women by 4 weekly clinical follow up and 24 hour urinary calcium and creatinine estimation. Thirtythree remained normotensive (Group I), while 27 developed PE in the later weeks (Group II). **Results:** . Twentyfour hour urinary calcium and calcium: creatinine ratio (Ca:Cr) decreased progressively in Group 2 from around the 32<sup>nd</sup> to 40<sup>th</sup> week and this was statistically significant. Urinary calcium excretion indices in Group II showed a steady decline in midtrimester. **Conclusions:** Hypocalciuria is a very good tool for prediction of PE and is independent of renal function. Ca:Cr and calcium excretion index are better than only calcium measurement.

**Key words:** pre-eclampsia, hypocalciuria, calcium:creatinine ratio, calcium excretion index

### Introduction

All obstetricians dread preeclampsia (PE) for its potential maternal (12.6% of maternal deaths)<sup>1</sup> and fetal complications. The predominant pathology i.e. endothelial dysfunction sets in as early as 8-18<sup>th</sup> week. However, the signs and symptoms appear in the late midtrimester, in the advanced stages of the disease. In order to arrest the disease process in the initial stages or to prevent complications especially in women predisposed to PE, various predictors have been proposed time and again. Hypocalciuria is one such predictor<sup>2</sup>.

However, we still do not know for sure the cause behind this change in calcium excretion. We studied the trends in urinary calcium levels in PE subjects and have envisaged finding a correlation between renal function and calcium excretion.

### Methods

This study was carried out from July 2000 to June 2001.

Sixty women were enlisted for this prospective study at 18-20<sup>th</sup> week of gestation, both from the indoor wards as well as the outpatient department and were followed through their pregnancy.

They were of similar age: mean age was 25.15 (SD 3.8) years in Group I and 24.74 (SD 3.68) years in Group II. At booking, body mass index (BMI) was 24.38 (SD 3.23) kg/m<sup>2</sup> in Group I. 24.55 (SD 3.93) kg/m<sup>2</sup> in Group II. Subjects with any past or present medical or surgical

*Paper received on 03/10/2006 ; accepted on 14/01/2008*

Correspondence :

Dr. Dasgupta Mandira  
559, Block N, New Alipore  
Kolkata - 700 053.

Tel. 033-24006133 Mobile : 9831106193

Email : mandiradasgupta@hotmail.com

problems - specially preexisting hypertension, diabetes mellitus, chronic renal disease, vasospastic or immunological disorders were excluded, so that no case of pregnancy aggravated hypertension was included in the study.

At the first visit, routine blood (hemoglobin, ABO-Rh typing, postprandial blood sugar, VDRL), urine (for protein, sugar, pus cells and epithelial cells) and stool (for ova, parasite, cyst) examinations were performed.

They were followed up every 4 weeks from 28<sup>th</sup> week (where possible) clinically and 24 hour urine samples were collected for biochemical evaluation for urinary calcium and creatinine.

Thirty three of them remained normotensive throughout their pregnancy (the control group, Group I), while 27 women developed PE in the later weeks (Group II). PE was defined as blood pressure (BP) of  $\geq 140$  mm Hg systolic (Korotkoff's I sound) and  $\geq 90$  mm Hg diastolic (Korotkoff's V sound) taken twice 6 hours apart, after 20 weeks of gestation with a total protein excretion of greater than 300 mg in 24 hours in a previously normotensive woman with normalization of BP following delivery, by 12 weeks.

Serum uric acid and liver function tests were performed in subjects with PE. Ultrasonography was performed as a routine procedure.

All of them received routine iron and calcium (500 mg/day) supplementation from the 20<sup>th</sup> week of gestation. Those who developed PE received antihypertensive therapy (as necessary) and aspirin. None of them were given the high dose calcium therapy.

#### *Urine sample analysis*

Samples were analyzed by the colorimetric method by the semi-autoanalyzer. Calcium levels were estimated by the OCPC method at 575 nm.

The formula used was calcium concentration (mg/dL) = (Reading of test material /reading of standard) x concentration of standard (10mg/dL)

Creatinine level was measured by the alkaline picrate method by the Jaffe reaction at 520nm. The formula used was creatinine concentration = (AS2-AS1)/(ASt2-ASt1) x concentration of standard (100 mg/dL) (AS= absorbance of the sample, ASt = absorbance of the

standard). Blood was also analyzed for albumin, creatinine, uric acid and liver function tests.

#### **Statistical analysis**

The data have been assumed to be normally distributed and the distribution parameters are expressed in terms of their mean value and standard deviation (SD).

Analysis of serial measurements of urinary calcium and creatinine levels was by summary measures. A linear regression was fitted for each subject's data over time. The slope of the line, representing the rate of change of the measurements per week, was taken as the summary measures for the subjects in each group. The summary measures of the two groups were compared for statistical significance using unpaired student's *t* test. The levels in each group were compared by the paired *t* test. P values of less than 0.05 were considered statistically significant.

#### **Results**

In the control group majority (69.97%) of the subjects were second gravidas, while in the study group most of them (59.26%) were primigravidas.

There were no statistically significant differences between the maternal ages, booking BMI and booking blood pressures, hematocrit, serum creatinine and liver function tests between the two groups.

The 24 hour urinary calcium and calcium:creatinine ratio (Ca:Cr) did not change appreciably till term in the control group (urinary calcium levels: 185.26 $\pm$ 107.48 vs 197.22 $\pm$ 152.02; Ca:Cr 0.22 $\pm$ 0.12 vs 0.21  $\pm$ 0.10), while it decreased progressively in PE subjects (urinary calcium levels; 271.26 $\pm$ 222.77 vs 104.41 $\pm$ 63.9 and Ca:Cr 0.26  $\pm$ 0.10 vs 0.12  $\pm$ 0.11 respectively) (Figure 1 (a) and (Figure 1 (b)).

This change was significant when we compared Group II with Group I (P=0.001 for both urinary calcium and Ca:Cr) which was more prominent midtrimester onwards (Figure 3 and 4).

Analysis at each time point for Group II revealed significant decrease from around the 32<sup>nd</sup> to 40<sup>th</sup> week (P=0.03, 0.0004, 0.0002 and P=0.03, 0.000007, 0.00002 for calcium and Ca:Cr respectively at 32, 36 and 40 weeks respectively). The initial i.e. 28 weeks levels were almost similar in the two groups.

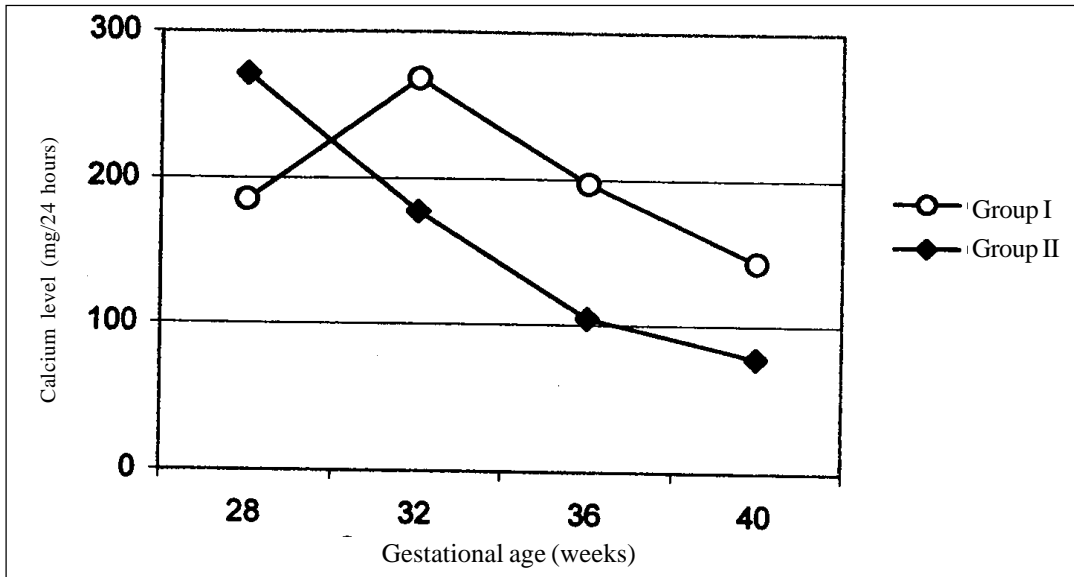


Figure 1. Urinary Calcium levels in 24 hours

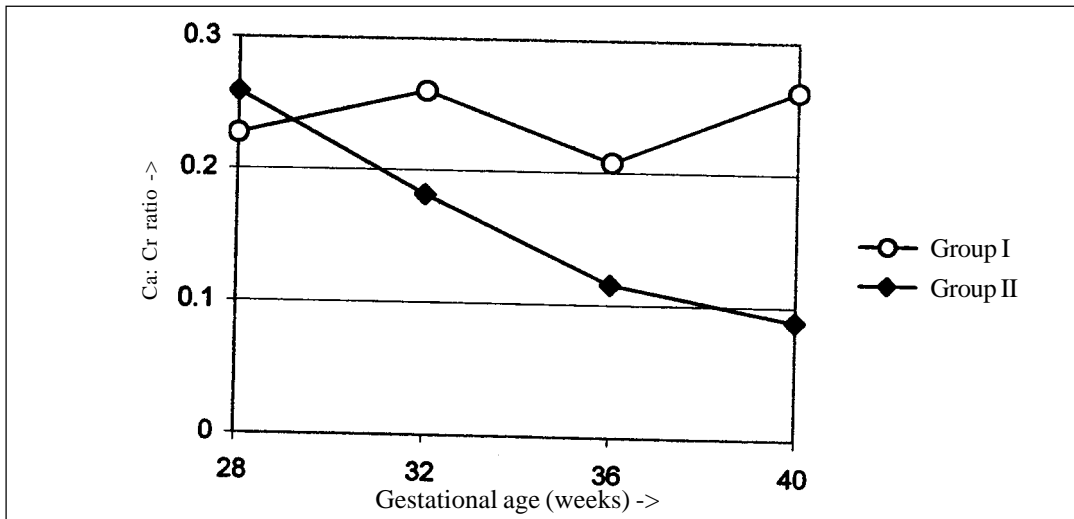


Figure 2. Urinary Calcium : Creatinine (Ca:Cr) ratio

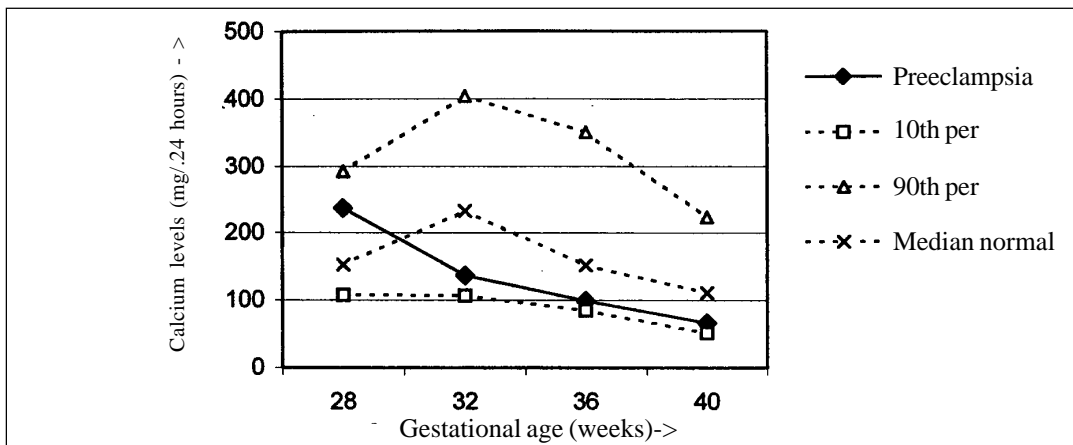


Figure 3. Median Urinary Calcium levels in Group II.

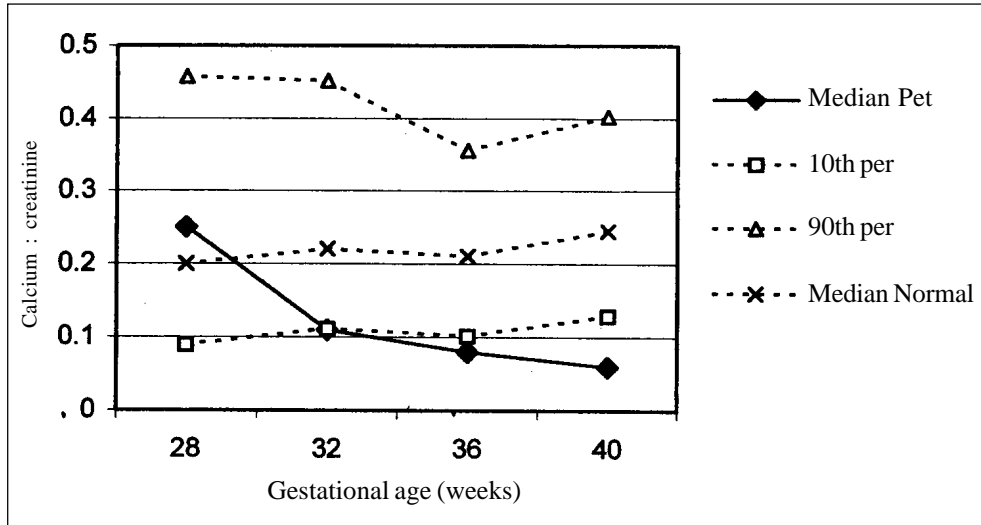


Figure 4. Median urinary Calcium : Creatinine (Ca:Cr) ratio in Group II

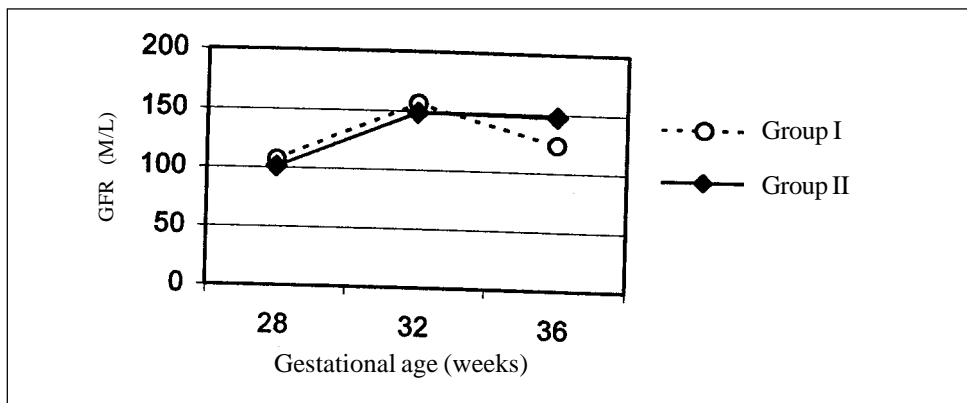


Figure 5. Glomerular filtration rate

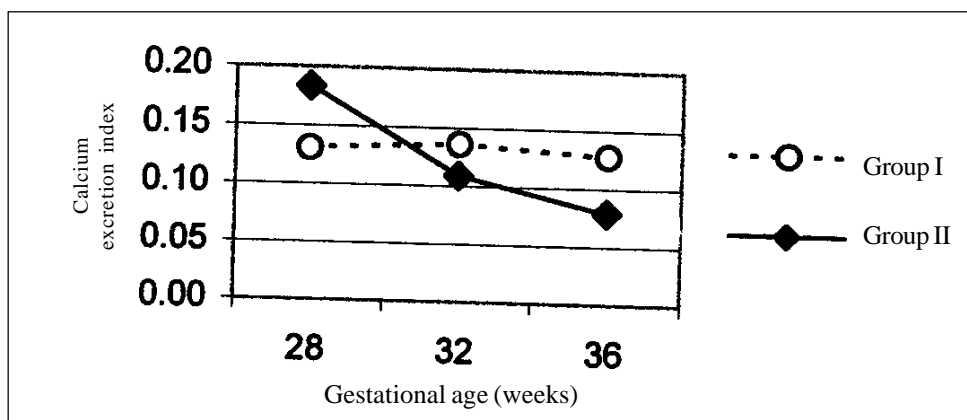


Figure 6. Calcium excretion index

However, no significant correlation was observed between urinary calcium, Ca:Cr and creatinine levels.

Glomerular filtration rates increased moderately in the midtrimester in both the groups with no significant difference between Group I and II (Figures 5 and 6).

Calcium excretion indices i.e. excretion of calcium/urinary creatinine\* serum creatinine, which measures calcium excretion independent of the GFR were calculated. This showed almost no alteration in the calcium excretion in Group I. However, urinary calcium excretion indices in Group II showed a steady decline in midtrimester ( $P=0.008$  and  $0.0002$  at 32 and 36 weeks respectively, on comparison with initial levels). The rate of fall was approx.  $0.1 \text{ mg/mL GFR}$  per week of gestation (i.e.  $0.04/4$  weeks).

## Discussion

These data show that urinary calcium and Ca:Cr levels in women destined to develop PE progressively diminish till term. This finding is in unison with findings of several researchers<sup>3-6</sup>.

As regards the Ca:Cr values, we have demonstrated a markedly declining trend in PE subjects. This ratio is easy to measure and has been used as a test predicting PE by some<sup>7</sup>. Few others have however contradicted this finding<sup>8</sup>.

In majority of preeclamptic women, mild to moderately diminished glomerular filtration appears to result from a reduced plasma volume. Intrinsic renal changes caused by severe vasospasm may cause severe fall in GFR in some cases. Thus, this might be responsible for hypocalciuria of PE.

When we calculated the calcium excretion index i.e. excretion of calcium/urinary creatinine, serum creatinine, which measures calcium excretion independent of the GFR there was a statistically significant fall in calcium excretion 32nd week onward in PE subjects. The calcium excretion in the Group I remained almost unaltered upto 36<sup>th</sup> week.

Thus, although the physiological calciuria of the control group can be explained by an increased GFR, the fall in calcium excretion in PE subjects is independent of the glomerulopathy and altered filtration rate.

The changes in urinary calcium levels are thus basically

a reflection of the alterations in the calcium homeostasis in the microenvironment.

Normally there is an increase in the level of intracellular (i) calcium ( $\text{Ca}^{2+}$ )<sub>i</sub> in pregnancy. This effect is exaggerated in preeclampsia due to a significant increase in the membranous calcium content<sup>9</sup>. Conversely the membranous and intracellular magnesium concentrations were decreased in these patients<sup>10</sup>.

It has been demonstrated that sera from normotensive pregnant women and preeclamptic women (as opposed to sera from normal nonpregnant women and nonpregnant hypertensive women) exert distinct changes on cellular  $\text{Ca}^{2+}$  metabolism in normal vascular smooth muscle cells (VSMC)<sup>11</sup>.

Normal pregnant sera amplify, whereas preeclamptic sera blunt the voltage dependent calcium channels (VDCC).

Normally calcium ions are released from intracellular source in response to agonist-stimulated production of  $\text{IP}_3$ . Thus, it is possible that PE sera (compared to normotensive pregnant sera) exert an inhibitory effect on  $\text{IP}_3$  generation or cause a resistance to the effect of  $\text{IP}_3$ . Moreover, the agonist-induced release of calcium from internal stores triggers a capacitative influx of extracellular  $\text{Ca}^{2+}$  across the plasma membrane<sup>12</sup>.

Based on this recently gathered data, one could speculate that the reduced calcium release by the preeclamptic sera is directly related to an inhibition of calcium influx as well.

The distinct effects of sera from preeclamptic patients and normotensive pregnant women on ( $\text{Ca}^{2+}$ )<sub>i</sub> responses in VSMC, when taken together with the data showing that the effect of the PE sera disappears shortly after delivery, suggest the presence of a putative serum factor. It has been speculated that in women with preeclampsia, the putative serum factor has a suppressive effect on ( $\text{Ca}^{2+}$ )<sub>i</sub> in VSMC, and operates as defense mechanism against the intense vasoconstriction so characteristic of preeclampsia. This factor is deficient in normal pregnancy, which then allows for the augmentation of the ( $\text{Ca}^{2+}$ )<sub>i</sub> signals, thereby counterbalancing the reduced peripheral vascular resistance often seen in normal pregnancy. This explains the role of dietary calcium in prevention and treatment of PE.

Our study suggests that hypocalciuria in PE is independent of renal function, and as such is a reflection of a complex alteration in calcium homeostasis at the cellular level. This is a very good tool for prediction of PE, as various authors have previously suggested. The use of Ca:Cr and calcium excretion index are better than only calcium measurements. Since these values can be measured in random samples, without the need for measuring 24 hour urine output, they are much more convenient to measure and interpret. We however could not propose a cut-off value for the above parameters. Further studies are required to investigate these altered cellular mechanisms leading to altered calcium excretion in PE.

### Conclusion

Hypocalciuria in preeclampsia is independent of renal function and reflects a complex alteration in calcium homeostasis at the cellular level. It can be used for predicting preeclampsia.

### References

1. Gelband H, Liljestrand J, Nemer L et al. The evidence base for interventions to reduce maternal and neonatal mortality in low and middle-income countries. CMH Working Paper Series, WG5 Paper No.5.
2. Taufield PA, Ales KL, Resnick LM et al. Hypocalciuria in pre-eclampsia. *N Engl J Med* 1987;316:715-8.
3. Sanchez-Ramos L, Sandroni S, Andres FJ et al. Calcium excretion in pre-eclampsia. *Obstet Gynecol* 1991;77:510-3.
4. Ramos JG, Martins-Costa SH, Kessler JB et al. Calciuria and pre-eclampsia. *Braz J Med Biol Res* 1998;31:519-22.
5. Ingec M, Nazik H, Kadanali S. Urinary calcium excretion in severe preeclampsia and eclampsia. *Clin Chem Lab Med* 2006;44:51-3.
6. Suarez VR, Trelles JG, Miyahira JM. Urinary calcium in asymptomatic primigravidas who later developed preeclampsia. *Obstet Gynecol* 1996;87:79-82.
7. Ozcan T, Kaleli B, Ozeren M et al. Urinary calcium to creatinine ratio for predicting preeclampsia. *Am J Perinatol* 1995; 12:349-51.
8. Phuapradit W, Manusook S, Lolekha P. Urinary calcium/creatinine ratio in the prediction of preeclampsia. *Aust NZJ Obstet Gynaecol* 1993;33:280-1.
9. Kosch M, Hausberg M, Louwen F et al. Alterations of plasma calcium and intracellular and membrane calcium in erythrocytes of patients with preeclampsia. *J Hum Hypertens* 2000;14:333-6.
10. Kisters K, Barenbrock M, Luwen F et al. Membrane, intracellular, and plasma magnesium and calcium concentrations in pre-eclampsia. *Am J Hypertens* 2000;13:765-9.
11. Green J, Assady S, Nakhoul F et al. Differential effects of sera from normotensive and hypertensive pregnant women on Ca<sup>2+</sup> metabolism in normal vascular smooth muscle cells. *J Am Soc Nephrol* 2000;11:1188-98.
12. Berridge MJ. Capacitative calcium entry. *Biochem J* 1995;312:1-11.