



## Evaluation of Urinary Calcium – Creatinine Ratio in Pre-Eclampsia-A Study from Kerala, Southern India

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### ABSTRACT

**Background:** Hypertensive disorders still continues to be a major killer of mothers around the globe. Its complex pathophysiology starting with abnormal placentation with endothelial dysfunction, still remains unclear. This study aims to know alteration in serum and urinary calcium levels in cases of Gestational Hypertension and Pre-eclampsia. Primary objective was the evaluation of urinary calcium and creatinine excretion levels and find out calcium-creatinine ratio in patients with Gestational Hypertension and pre-eclampsia and compare it with normal pregnant women.

**Method:** 54 Gestational hypertensive cases and 54 normal pregnant women were included in the study based on inclusion and exclusion criteria. Urine calcium and creatinine and serum uric acid, total protein, albumin and serum sodium were estimated in corresponding samples collected from patients. Urinary calcium was estimated by Arsenazo method and creatinine by modified Jaffe's method.

**Result:** It was found that urinary calcium was decreased in both pre-eclampsia and gestational hypertensive patients compared with the controls, and a significant decrease is seen in urine CCR. The CCR had a sensitivity and specificity of 90.9% and 76.7% respectively.

**Conclusion:** Decreased urinary calcium excretion in pre-eclampsia and pregnancy induced hypertensives compared to normal pregnant women found to be significant and urine CCR is significantly decreased in pre-eclampsia patients compared to pregnancy induced hypertensives.

**Keywords:** GHTN, PET, BMI, Hypocalciurea, CCR.

### Introduction

In developing nations, the incidence of the disease is reported to 4% - 18% <sup>1</sup> and is the second most common obstetric cause of stillbirths and early neonatal deaths. Besides having increased maternal morbidity and mortality accounting for 40000 maternal deaths annually<sup>2</sup>. Maternal

mortality ratio averages at 16 per lakh live births in developed regions. While in developing regions it is 440 for the same number of live births <sup>3</sup>.

In the Indian scenario, the incidence of pre-eclampsia is 4.47% in primigravida and 2.8% in multigravida. It accounts for 44.44% of all cases of hypertensive disorders of pregnancy <sup>4</sup>.

### Calcium excretion in pre-eclampsia

Several researchers have reported that hypocalciurea is associated with pre-eclampsia due to derangement in calcium metabolism associated with this condition<sup>5</sup>. Also hypocalciurea could be preceded by the development of pre-eclampsia. Low serum calcium may cause high blood pressure by stimulating para thyroid hormone and renin release and also by increasing magnesium levels<sup>6</sup>. Hypocalciurea has been found in women with de novo pre-eclampsia (PE), and PE superimposed on chronic hypertension and a calcium deficiency has been suggested as having a role in the development of pre-eclampsia<sup>7</sup>. Hence the decrease in a calcium – creatinine ratio (CCR) could be considered as a risk factor for the development of pre-eclampsia in pregnancy. Rodriguez et al evaluated the role of decreasing calcium creatinine ratio and microalbuminuria in prediction of preeclampsia<sup>8</sup>. In Majority of pre eclamptic 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 hypocalciurea of PE<sup>9</sup>.

As a good predictor of pre-eclampsia urinary calcium a creatinine ratio was proposed in some studies. These studies were conducted to know alterations in serum and urinary calcium level in all pregnancy induced hypertension cases including pre-eclampsia.

This study assess whether urinary calcium creatinine ratio can be considered as a useful tool for the early diagnosis of pre-eclampsia also whether there is any considerable changes in some other biochemical parameters like serum uric acid, total protein albumin and serum sodium.

### Materials and Methods

A descriptive study was conducted to evaluate the difference in urinary calcium- creatinine ratio in gestational hypertensive patients and normal pregnant women attending Obstetrics O.P,

Department of Obstetrics & Gynaecology, SAT, Govt. Medical College, Thiruvananthapuram. Cases and controls were selected based on inclusion-exclusion criteria. This study was completed in Clinical Biochemistry laboratory, SAT Govt. Medical College, Thiruvananthapuram

### Study Population

- All patients between 24-34 weeks of gestation, attending obstetrics O.P, SAT, Trivandrum, during the study period.

### Inclusion criteria

**Cases:** Include normotensive pregnant women between 24-34 weeks of gestation who developed pregnancy induced hyper tension later. The cases were having one or more high risk factors like-primi gravida, history of pregnancy induced hypertension in past pregnancy. Twin gestation in present pregnancy.

**Controls:** Include age matched pregnant women between 24-34 weeks of gestation, without any major illness/risk factors stated above. Exclusion criteria-Patients with hypertension prior to pregnancy / use of any anti-hypertensivedrugs, Patients with diabetes. Patients with renal diseases / or on any diuretics. Patients with other chronic medical illness., Patients with history suggestive of thrombophilia / coagulopathies. Patient refusal. Sampling-Consecutive cases coming to O.P.D, during study period were considered as cases.

### Sample analysis

Urine is diluted 1 in 5 in which calcium and creatinine were estimated. Serum is separated from aseptically collected sample in which uric acid, totalprotein, albumin and serum sodium were measured. Both urine and serum were analyzed in fully automated analyzer -Olympus AU 400 (open system).

**Assay Method**-fully automated system

- a) Estimation of urinary calcium by Ortho-Cresolphthalein complex one method
- b) Estimation of urinary creatinine by Jaffe's reaction (End point method)

- c) Estimation of serum uric acid by Uricase enzymatic method
- d) Estimation of serum total protein by Biurete method
- e) Estimation of serum albumin by BCG method
- f) Estimation of serum sodium by ISE method

**Data Analysis**

Data were entered in Microsoft Excel and data analysis was performed using SSPE software. Qualitative variables were described by percentage distribution and quantitative variables were described by Mean, SD, Minimum and Maximum

**Observations and Results**

The data for biochemical analysis was subjected to standard statistical analysis using Statistical Package for Social Science (SPSS) software for windows version 17.0. Chi-square test and anova tests were used to analyze the results. If anova test (for multiple group comparison) is seen as significant, post Hochberg's test is used to compare between groups.

The study population is grouped into three, i.e, Group: 1 (GHTN-PE) include gestational hypertensive patients who developed pre-eclampsia, Group:2 (GHTN) include patients who remained as hypertensive till term and Group:3 include normal pregnant control. Both GHTN-PE and GHTN together forms the case group.

Out of 54 cases with PIH (pregnancy induced hypertension) 11(20.3%) developed Pre-eclampsia, 43(79.6%) cases remained as hypertensives.

**Table 1:** Distribution of study population according to different age groups

Age group (years)	GHTN-PE		GHTN		Control	
	N	%	N	%	N	%
18-24	3	27.3	13	32.6	16	29.6
25-34	6	54.5	25	58.1	35	64.8
35-44	2	18.2	4	9.3	3	5.6
Total	11	100.0	43	100.0	54	100.0

Table 1 shows the percentage distribution of study population according to different age groups in the case and control subjects. The difference between groups is not significant (p= 0.699) indicating that

the groups are of comparable age. Maximum study subjects are in age group of 25-34 followed by age group of 18-24...

**Table 2:** Distribution of study subjects according to gestational age.

Gestational Age group	GHTN-PE		GHTN		Control	
	N	%	N	%	N	%
>24-28 weeks	4	36.4	17	39.5	29	53.7
>28 weeks	7	63.6	26	60.5	25	46.3
Total	11	100.0	43	100.00	54	100.0

P=0.298

Table 2: shows the percentage distribution of study population according to gestational age groups in the case and control subjects. The difference between groups is not significant (p= 0.298) indicating that the groups are comparable

with respect to gestational age, even though there is a difference in distribution i.e., in case group majority of study subjects fall in the group above 28 week of gestation while most of the control subjects are in group 24-28 week of gestation.

**Table. 3:** Distribution of study population according to parity.

Parity	Category						Total	
	GHTN-PE		GHTN		Control		N	%
	N	%	N	%	N	%		
Primigravida	9	81.8	24	55.8	29	53.7	62	57.4
2 <sup>nd</sup> gravida	2	18.2	15	34.9	16	29.6	33	30.6
3 <sup>rd</sup> gravida	0	0.0	4	9.3	9	16.7	13	12.0
Total	11	100.0	43	100.0	54	100.0	108	100.0

$X^2 = 4.673$

df=4

p=0.323

Table. 3: Shows the distribution of control and case groups according to parity. It is seen that maximum number of cases in GHTN-PE (81.8%) occur in primiparas, (18.2%) belongs to G2 (second gravida) and no cases were present in G3 (third gravida) and above.  $X^2 = 4.673$ ;  $p = 0.323$  suggest that the case and control groups are homogenous when grouped according to parity. The mean age  $\pm$  SD in years in the case and control group GHTN –PE group shows a mean

age of  $29 \pm 5.23$  followed by control group ( $26.76 \pm 4.28$ ) Mean age of GHTN group is found to be 26.23. The difference between groups is not significant ( $p = 0.226$ ) indicating that the groups are of comparable age. The mean of gestational age  $\pm$ SD in weeks in the case and control group. The difference between groups is not significant ( $p = 0.702$ ) indicating that the groups were comparable according to gestational age

**Table 4 :** Comparison of mean and standard deviation of BMI in case and control groups

Category	N	BMI		ANOVA		Post Hoc analysis	
		Mean	SD	F	P	Multiple comparison	P
GHTN-PE	11	29.13	4.79	3.941	0.022	Group I & Group II	.064
GHTN	43	26.86	3.80			Group I & Group III	.007
Control	54	25.88	3.11			Group II & Group III	.183
Total	108	26.60	3.68				

Table 4. shows mean $\pm$ SD of body mass index among case and control groups. The mean value of BMI in Group 1 (GHTN-PE) was  $29.13 \pm 4.79$  and in Group 2 (GHTN),  $26.86 \pm 3.8$  while in

control group (normal pregnant women) - it was  $25.88 \pm 3.1$ . The difference of mean between the GHTN-PE group and control group was statistically significant with a p value of 0.007.

**Table 5:** Urinary calcium excretion among study population.

Category	N	U.Ca		ANOVA		Post Hoc analysis	
		Mean	SD	F	P	Multiple comparison	P
GHTN-PE	11	4.32	2.66	5.694	0.004	Group I & Group II	.140
GHTN	43	8.56	7.07			Group I & Group III	.004
Control	54	12.62	10.01			Group II & Group III	.021
Total	108	10.16	8.80				

Table 5 shows mean $\pm$ SD of urinary calcium excretion among the study subjects. The mean value of urinary calcium in Group 1 was found to be  $4.32 \pm 2.66$ , in Group 2,  $8.56 \pm 7.07$  and in control group it was  $12.62 \pm 3.11$ . The difference of mean between the groups were statistically significant with a p value of 0.004.

Comparing the mean values between Group 1 & 2 shows no significant difference ( $p$  value= 0.14), But comparing with control both Group 1 & 2 shows a significant decrease in the urinary calcium excretion ( $p < 0.05$ ).

**Table 6:** Urinary creatinine excretion among study population

Category	N	U.Cr		ANOVA		Post Hoc analysis	
		Mean	SD	F	P	Multiple comparison	P
GHTN-PE	11	102.64	47.56	8.687	<0.001	Group I & Group II	.018
GHTN	43	70.94	47.20			Group I & Group III	<0.001
Control	54	51.94	28.38			Group II & Group III	.019
Total	108	64.67	41.60				

Table 6 shows mean±SD of urinary creatinine excretion among study subjects. The mean value of urinary creatinine in Group 1 was found to be 102.64±47.56, in Group 2 , 70.94±47.20 and in control group (Group 3) it was 51.94±28.38. The

difference of mean between the study groups were statistically significant with a p value<0.001 .All the three groups show significant difference with a tendency to show an increased level of urinary creatinine in Group 1

**Table 7:** Urinary calcium-creatinine ratio among study population

Category	N	U.Cr		ANOVA		Post Hoc analysis	
		Mean	SD	F	P	Multiple comparison	P
GHTN-PE	11	0.04	0.02	36.792	<0.001	Group I & Group II	.018
GHTN	43	0.13	0.09			Group I & Group III	<0.001
Control	54	0.23	0.07			Group II & Group III	.019
Total	108	0.17	0.10				

Table shows mean±SD of urinary calcium-creatinine ratio in spot urine sample among study subjects. The mean value of urinary CCR in Group 1 was found to be 0.04±0.02, in Group 2, 0.13±0.09 and in control group it was 0.23±0.07. The difference of mean between different groups

was statistically significant with a p value <0.001.Urine CCR is significantly decreased in pre-eclamptic group (Group 1) when compared with Group 2 as well as Group 3.GHTN category (Group 2) also showed a decreased urine CCR comparing with the control group (Group 3).

**Table :8 :** Distribution of patients on the basis of urine CCR

Category	Ca Creatinine ration				Ratio	
	≤0.055		≥0.055		Total	
	N	%	N	%	N	%
GHTN-PE	10	50.0	1	1.1	11	10.2
GHTN	10	50.0	33	37.1	43	39.8
Control	0	0.0	54	61.4	54	50.0
Total	20	100.0	88	100.0	108	100.0

Table 8. shows distribution of patients on the basis of CCR which reveal 90.9% ofGHT-PE category fall in the group with CCR ratio ≤0.055(True positive) and 76.7% of GHTN fall in the group with CCR>0.055(True negatives)

**Discussion**

A descriptive study titled "Evaluation of urinary calcium creatinine ratio as an indicator of pre-eclampsia" was conducted in Department of Obstetrics and Gynaecology, SAT, Thiruvananthapuram. The study population included 108 patients attending the Obstetrics O.P, i.e,54 cases

and 54 controls during the study period. Urine and blood samples were collected after getting patient consent, for assessing the biochemical parameters. Estimation of calcium and creatinine were done in urine while uric acid, total protein, albumin and serum sodium are estimated in serum sample of the patients.

All data were entered in Microsoft Excel and data analysis was performed using SSPE software. Qualitative variables were described by percentage distribution and quantitative variables were described by Mean and SD.

Most of the patients who developed pre-eclampsia in this study population belongs to the age group of 25-34 (54.5%) followed by the age group 18-24 (27.3%). Similar pattern is seen in GHTN group and also in control group so that the distribution is similar among groups with a p value of 0.699.

When this study population was grouped according to gestational age, most of the patients who developed pre-eclampsia and patients in GHTN category were fall in with gestational age group >28 weeks. In control group even though slightly higher percentage of subjects were present in gestational age group of 24-28weeks, the difference between the groups are not significant (p value>0.05).

In the present study out of 54 cases 33 (61 %) were primigravida, 17 (31%) were second gravida and 4 (8%) were third gravida. It is found that out of total GHTN-PE cases 81.8% (9 no's) were primigravida, 18.2% (2 no's) were second gravida and no cases found in third gravida. In control group also a higher percentage (53.7%) of study subjects were primigravida.

It is evident from this study that BMI shows a significant difference between Group 1 (GHTN-PE) and Group 3(control) with a p value of 0.007. In GHTN-PE group the mean value of BMI was  $29.13 \pm 4.79$  while in control group it was  $25.88 \pm 3.11$ . The difference between mean BMI in Group 2 and Group 3 was not significant (p>0.05).

The present study showed 91% of the GHTN-PE category had CCR < 0.05. The cut off value (CCR < 0.05) was similar with that of various studies<sup>10, 11</sup>. It is observed that the urinary excretion of calcium and creatinine is increased during pregnancy but when pregnancy is associated with high risk factors like hypertension, their excretion is reduced resulting in development of pre-eclampsia. So, this study shows a definite relationship between low calcium creatinine ratio and development of pre-eclampsia. Out of 43 GHTN cases analyzed 33 (76.7%) have urine CCR > 0.05.

When the level of urinary calcium excretion is analyzed it is found that in GHTN-PE category urinary calcium excretion was significantly decreased when compared to normal pregnant women, with a p value of 0.004. The mean value of urinary calcium excretion in Group 1 was found to be  $4.32 \pm 2.66$ , in Group 2,  $8.56 \pm 7.07$  and in control group it was  $25.88 \pm 3.11$ . The difference between Group 1 & 2 was shown to be not significant (p value > 0.05). A significant decrease in urinary excretion of calcium was found in patients with PIH (Group 2) when compared with control group.

Regardless of its mechanism and pathophysiologic importance, hypocalcemia may be a reliable sign distinguishing more benign forms of PIH. In normal pregnant women during late gestation, urinary calcium excretion has been reported in different studies both to increase and to decrease (Pitkin et.al)... A recent large cross sectional study (Hogarth ATI Morgan DB, Payne RB) showed urinary excretion of calcium in late pregnancy and its relation to creatinine clearance.

Urinary creatinine excretion in category 1 and 2 was found to be high when compared to control group as in the study of Sheela. C.N et.al<sup>12</sup> and the difference is seen to be significant. Also the difference in the decrease was significant in Group 1 compared to Group 2.

This study reveals a significant decrease in urinary CCR in GHTN-PE category when compared to GHTN category and normal control group. Even though the difference in urinary excretion of calcium in GHTN-PE category and GHTN category was found to be not significant, the difference in CCR was found to be significant.

Serum uric acid among case and control groups showed a mean value of serum uric acid in Group 1  $4.87 \pm 1.49$  and in Group 2,  $3.75 \pm 1.03$  while in control group it was  $3.53 \pm 0.63$ . Group 1 showed statistically significant increase in serum uric acid when comparing with Group 2 & 3. Though the difference between the groups are small it is statistically significant with a p value of 0.001.

The mean value of serum total protein in Group 1 was  $6.49 \pm 0.35$  and in Group 2,  $6.65 \pm 0.63$  while in control group it was  $6.49 \pm 0.43$ ... The mean value of serum albumin in Group 1 was  $3.34 \pm 0.31$  and in Group 2,  $3.42 \pm 0.39$  while in control group it was  $3.39 \pm 0.36$ . There is a difference of mean value between groups, a decreased value is observed in pre-eclampsia.

Serum sodium value also showed a slight decrease in pre-eclampsia group with a value  $133.64 \pm 1.91$  and in Group 2,  $133.98 \pm 1.64$  while in control group  $134.63 \pm 1.55$ . Data of study population shows other serum parameters such as SGOT, SGPT, Serum ALP, Serum urea, Serum creatinine and Serum potassium were in normal range for case and control group.

### Conclusion

Current study shows hypocalcaemia precedes emerging pre-eclampsia in pregnancy induced hypertensive women, though cause of this pathophysiology is not clear. A cut off value of 0.055 for urinary CCR obtained from receiver operator in case group. Use of urinary CCR in predicting pre-eclampsia shows a specificity of 76.7% and sensitivity of 90.9%. Hypocalcaemia is independent of renal function and reflects a complex altered calcium homeostasis at cellular level.

An increased BMI of  $29.13 \pm 4.79$  is found to be significant in pre-eclampsia group.

Urinary creatinine excretion may be high or low in pre-eclampsia. Incidence of pre-eclampsia was high in primigravida. Maximum number of pre-eclampsia patient in an age group of 25-43. Increased serum uric acid level is seen in pre-eclampsia group compared to the other two groups. The study revealed:

Urinary CCR is decreased in pre-eclampsia cases due a decreased urinary calcium excretion. A urinary CCR  $\leq 0.005$  is suspicious of pre-eclampsia in gestational hypertensive patients. This study confirms the finding that an increase in BMI as predisposing factor for the development of pre-eclampsia. This study reveals that

hyperuricemia is an indirect risk factor for pre-eclampsia. It is found that primigravida and increased age are predisposing factors for development of pre-eclampsia.

Early therapeutic use of calcium shows its beneficial role in reducing morbidity and mortality due to pre-eclampsia. Estimating urinary CCR is simple as well as easy to be done. Thus this test may be used as a screening tool for early diagnosis of pre-eclampsia along with the routine tests. Further studies are required to investigate the altered cellular mechanisms leading to altered calcium excretion in PE.

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