COMPARATIVE STUDY OF URINARY CALCIUM/CREATININE RATIO BETWEEN PREGNANT WOMEN WITH AND WITHOUT PIH

Sharwan Kumar[1], Shyam Sunder Mittal[2], Vipul Garg[3], Balveer Singh[4*], Shakuntala Saini[5]

1. PG Resident, *2., 3*. Senior Demonstrator, *4*. Assistant Professor, *5*. Senior Professor, Department of Biochemistry, SMS Medical College, Jaipur.

*Corresponding author – **Dr. Balveer Singh**

Email id – drbsdangas@gmail.com

ABSTRACT

Background: Pregnancy Induced Hypertension (PIH) is elevated BP resulting from gravid state and It includes gestational hypertension and preeclampsia. Estimation of urinary Calcium Creatinine Ratio (CCR) is an easy, non-invasive and inexpensive method and spot urinary CCR result reflects 24 hour urinary calcium level. **Objective:** To compare urinary calcium/creatinine ratio (CCR) between PIH women with proteinuria and PIH women without proteinuria and in healthy pregnant women in spot urine sample. **Material and Method:** A cross sectional study was undertaken at SMS Medical College, Jaipur in the year 2018. Written consent was taken from all subjects and ethical clearance was obtained from the ethics committee. Hypertensive pregnant women of 21-35 years of age and 24-32 weeks gestational period and having BP more than 140/90 mm of were taken as cases and these cases by absence or presence of protein in their urine by dipstick method were divided into PIH cases without proteinuria (gestational hypertension GH group) and PIH cases with proteinuria (preeclampsia group). Healthy normotensive pregnant women of same age and same gestational period were taken as control. All subjects divided into 3 groups each group having 40 women. Spot urine sample was taken and urinary calcium and urinary creatinine was measured in mg/dl and urinary CCR was calculated. **Results:** It was noted that urinary CCR was low in GH group (.09±.04) compared to control group (0.12±0.1) and this difference was statistically significant. Mean urinary CCR was also low in preeclampsia group (0.04±0.02) compared to GH group and control group and difference was statistically significant. **Conclusion:** Analysis of urinary CCR is an easy, non-invasive and inexpensive method. Spot urinary CCR result are equivalent to 24 hour urinary calcium level. So this test can be used as a predictive test to identify pregnant women having high risk of developing PIH and timely prophylactic interventions.

Key words: PIH, CCR, urinary calcium, proteinuria.

INTRODUCTION

Pregnancy Induced Hypertension (PIH) or Hypertensive Disorder of Pregnancy (HDP) includes four main categories:- 1. Gestational hypertension 2. Pre-eclampsia 3. Chronic hypertension and 4. Pre-eclampsia superimposed on chronic hypertension **(1)**. Gestational hypertension is defined as arterial hypertension (systolic BP ≥ 140 mmHg and / or diastolic BP ≥ 90 mmHg) appearing after gestational week 20. Pre-eclampsia is defined as gestational hypertension plus proteinuria. (≥300 mg/24 hrs, dipstick analysis of ≥ 1+) **(2)**.

Preeclampsia is a pregnancy-related condition affecting up to 8 % of pregnancies worldwide. Incidence of HDP in India is found to be 10.08 % **(3)**. Etiology of hypertensive disorder in pregnancy have been studied by various workers over past twenty years, the etiology remains unclear even today. Prediction of pregnancy induced hypertension earlier is useful in early intervention and prevention of PIH. So many researchers are more towards the way of predicting PIH early and preventing it timely. A number of predictive tests for PIH has been studied like roll over test, angiotensin sensitivity test, uterine artery doppler, platelet volume, antithrombin III level, serum fibronectin, urinary kallikerin excretion but none has been proved to be very sensitive and specific to predict PIH. Different other laboratory parameters that have also been studied by various workers like blood uric acid, serum creatinine, blood urea, serum and urinary creatinine, serum calcium and urinary calcium, Results of present study were comparable to other study **(4, 5, 6, 7)**. Taufield et al **(4)** noted that five women with GH had urinary calcium excretion similar to that of normal pregnancies. Hutchesson et al **(5)** noted a reduction in urinary calcium in pregnant women with hypertension having renal dysfunction but in that study there were no reduced urinary calcium level in pregnant women with gestational hypertension. Results of this study were comparable to study by Roelofsen et al **(6)** and Graves et al **(7)**. Our results were similar to Sanchez-Ramos et al **(8)** who also noted that the women with preeclampsia had significantly less excretion of total calcium than normotensive pregnant women or pregnant women having gestational hypertension.

Several studies have been done in the past to find out the role of decreased calcium excretion in early detection of preeclampsia. In the early 1980 they have shown that these investigations may be used as a screening method in predicting PIH. Several other studies have failed to show the effectiveness of the low calcium excretion as a screening method.

Measurement of urinary Calcium Creatinine Ratio (CCR) is a simple, non-invasive, easy and inexpensive method. Spot urinary CCR result are equivalent to 24 hour urinary calcium level. So the present study was designed with objective to compare urinary calcium/creatinine ratio (CCR) between PIH women with proteinuria and PIH women without proteinuria and in healthy pregnant women in spot urine sample.

MATERIAL AND METHODS

A cross sectional study was undertaken in the Department of Biochemistry and Mahila Chikitsalaya attached to SMS Medical College, Jaipur in the year 2018. Informed consent was taken from all subjects and ethical clearance was obtained from the ethics committee. Newly diagnosed hypertensive pregnant women of 21-35 years of age and gestational period of 24-32 weeks having BP more than 140/90 mm of Hg attending ANC clinic of Mahila Chikitsalaya were taken as cases and these cases by absence or presence of protein in their urine by dipstick method (at least 1+) were divided into PIH cases without proteinuria (gestational hypertension group) and PIH cases with proteinuria (preeclampsia group). Healthy normotensive pregnant women of same age and same gestational period were taken as control. A total of 120 pregnant women were considered for doing this study and divided them into 3 groups. Each group having 40 women. Group 1 or control - involved healthy pregnant women. Group 2 or GH group- involved pregnant women having PIH without proteinuria (Gestational Hypertension) and Group 3 or preeclampsia (PE) group- involved PIH women with proteinuria (Pre-Eclampsia).

Patients with history of chronic hypertension or use of any hypertensive drugs, patients with endocrine disorder /renal disease /hepatic disease were excluded from the study. The spot urine sample from all subjects of case and control group was collected in calcium free vials. Urine protein was analysed by dipstick method. Then 0.2 ml nitric acid was added to 10 ml of urine to prevent calcium salt precipitation. Blood sample was taken from case and control group. Collected blood and urine samples were centrifuged and separated and analyzed on fully automated analyzer (Beckman Coulter AU680). Urinary protein was analysed by using urine strip of Siemens Uristix 2857 by reagent strip method. Estimation of urine calcium was done by Calcium Arsenazo III

Colorimetric method (Reagent kit of Reactive GPL) and urinary creatinine by Modified Jaffe's reaction (reagent kit of Erba). Urinary CCR was analysed by simply dividing the urine calcium (mg/dl) by urine creatinine (mg/dl). Continuous variables were expressed as mean and standard deviation and were analyzed by using one way ANOVA test and Post Hoc Tukey'sHSD test. Nominal/categorical variables were expressed as proportions and were analyzed by using chi square test/fisher exact test. Medcalc 16.4 version software was used for all statistical calculation. P value <0.05 was taken as significant and inference was drawn.

RESULTS

It was noted in present study that mean age was 24.08±2.71 years in control group, 25.58±3.16 years in gestational hypertension (GH) group and 25.60±4.08 in preeclampsia (PE) group and all 3 groups were found alike with respect to age. (Table 1)

Table 1 : Comparison of mean age of control group, GH group and preeclampsia group

	Group	N	Mean	SD	'p' value[*]
Age in years	Group 1 Control	40	24.08	2.71	p value is .07, difference in mean age of all 3 groups are non significant
	Group 2 GH	40	25.58	3.16	
	Group 3 Preeclampsia	40	25.6	4.08	

* One way ANOVA

It was observed that mean urine calcium in spot urine was low in GH group (10.89±6.30) compared to control group (12.04±8.55) but this was not statistically significant. Urine calcium in preeclampsia group (6.25±4.98) was low compared to GH group and control group and this was statistically significant. Urine creatinine in spot urine was almost similar among 3 groups and this was not significant as p value by ANOVA was 0.96. (Table 2)

Table 2- Mean urine calcium and mean urine creatinine level comparison between 3 groups

	GROUP	N	MEAN	SD	'p' value[*]
Mean urine calcium in mg/dl	Group 1 control	40	12.04	8.55	
	Group 2 GH	40	10.89	6.3	NS Vs 1 and S Vs 3
	Group 3 preeclampsia	40	6.25	4.98	S Vs 1 and 2
Mean urine creatinine in mg/dl	Group 1 control	40	109.9	54.47	All 3 groups are alike as p value by ANOVA is 0.96
	Group 2 GH	40	112.6	41.53	
	Group 3 preeclampsia	40	112.4	46.23	

* One way ANOVA and Post Hoc Tukey test, NS- Non Significant, S- Significant

It was noted that urinary CCR was low in GH group (.09±.04) compared to control group (0.12±0.1) and this difference was statistically significant as p value by tukey test was <0.05. Mean urinary CCR was also low in preeclampsia group (0.04±0.02) compared to GH group and control group and difference was statistically significant. (Table 3)

Table 3- Urinary CCR comparison between 3 groups

	Group	N	Mean	SD	'p' value[*]
Urinary CCR	Group 1 Control	40	0.12	0.1	
	Group 2 GH	40	.09	0.04	S Vs 1 AND 3
	Group 3 Preeclampsia	40	.04	0.02	S Vs 1 and 2

* One way ANOVA and Post Hoc Tukey test, NS- Non Significant, S- Significant. p value by one way ANOVA is <0.001.

DISCUSSION

It was observed that mean urine calcium in spot urine was low in GH group (10.89±6.30) compared to control group (12.04±8.55) but this was not statistically significant. Urine calcium in preeclampsia group (6.25±4.98) was low compared to GH group and control group and this was statistically significant. Urine creatinine in spot urine was almost similar among 3 groups and this was not significant as p value by ANOVA was 0.96.

Results of present study were comparable to other study (4, 5, 6, 7). Taufield et al (4) noted that five women with GH had urinary calcium excretion similar to that of normal pregnancies. Hutchesson et al (5) noted a reduction in urinary calcium in pregnant women with hypertension having renal dysfunction but in that study there were no reduced urinary calcium level in pregnant women with gestational hypertension. Results of this study were comparable to study by Roelofsen et al (6) and Graves et al (7). Our results were similar to Sanchez-Ramos et al (8) who also noted that the women with preeclampsia had significantly less excretion of total calcium than normotensive women or those with GH.

Different predictive value of urinary calcium for pre-eclampsia by different workers have been used (9-12). Xie et al (13) found that the urinary calcium excretion could be used as an indicator for HDP from a spot urine sample or 24 hour urinary calcium excretion. Dasgupta et al (14) used predictive value of urinary calcium and concluded that hypocalciuria can be a good tool for prediction of PIH. Sirohiwal et al (15) found that a decrease in 24 hour urinary calcium between 20-28 weeks gestation was predictive of preeclampsia later.

Many studies have noted reduced excretion of calcium in urine to be associated with development of preeclampsia. The pathology behind this is said to be the decreased intestinal absorption, increased calcium uptake by growing fetus or increased tubular reabsorption of calcium (16). It was noted by Ingec et al that reduced urinary calcium excretion were due to increased reabsorption of calcium from renal tubules (17). During pregnancy higher intracellular calcium level in vascular smooth muscle cell (VSMC) were noted by Myatt et al and this resulted in vasoconstriction and hypertension (18). Changes in the calcium balance in the microenvironment may be cause of this lower urinary calcium level. Normally intracellular calcium level in pregnancy increases and this increase is noted more in PIH due to a increase in the membranous calcium (19). Distinct changes on cellular calcium metabolism has been noted by sera from pregnant women without PIH and pregnant women with PIH in normal VSMC (18). Sera from normotensive pregnant women amplify the voltage dependent calcium channels (VDCC) whereas sera from PIH women reduce it.

In present study urine creatinine in spot urine was almost similar among 3 groups and this was not statistically significant as p value by ANOVA was 0.96. Contrast to this study many authors noted significant decrease of urinary creatinine in pre eclampsia (14,15,20,21,22,23) and some others like Mittal Shilpa et al (24) KazemiAFN et al,(25) and MoniSY et al (26) observed significant increase in urinary creatinine in preeclamptic patients.

It was noted that urinary CCR was low in GH group (.09±.04) compared to control group (0.12±0.1) and this difference was statistically significant as p value by tukey test was <0.05. Mean urinary CCR was also low in preeclampsia group (0.04±0.02) compared to GH group and control group and difference was statistically significant.

In preeclamptic women urinary calcium/creatinine ratio was significantly decreased. This finding is well correlated with different worker's findings (**11, 12, 27, 28, 29**). But one study by Huikeshoven and Zuijderhout (**30**) is in contrast, they did not found any difference in the urinary calcium creatinine ratio between gestational hypertensives and normal pregnant women. Another study by Duttvivvan et al (**31**) also noted decreased urinary CCR in preeclampsia cases but not significant (p value = .072) compared to control.

CONCLUSION

Measurement of urinary calcium creatinine ratio is a non-invasive, inexpensive and is an easy to carry out method. Spot urinary CCR result are equivalent to 24 hour urinary calcium level. So this test can be used as early predictors to identify pregnant women having high risk of developing PIH and thereby initiating timely prophylactic interventions, thus minimizing the severity of preeclampsia. Although further studies are needed on a large sample and followup type before firm conclusion can be made for the diagnostic assessment of PIH.

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