

STUDY OF URINARY CALCIUM AND URINARY CREATININE LEVELS AND URINARY CALCIUM/CREATININE RATIO IN GESTATIONAL HYPERTENSIVE PATIENTSSwapna V. S¹, Triveni Jambale², Jayaprakash Murthy D³**HOW TO CITE THIS ARTICLE:**

Swapna V. S, Triveni Jambale, Jayaprakash Murthy D. "Study of Urinary Calcium and Urinary Creatinine Levels and Urinary Calcium/Creatinine Ratio in Gestational Hypertensive Patients". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 53, July 02; Page: 9145-9150, DOI: 10.14260/jemds/2015/1329

ABSTRACT: BACKGROUND: Gestational hypertension (BP>140/90mmHg) or pregnancy induced hypertension usually precedes pre-eclampsia (BP>140/90mmHg associated with proteinuria). Many tests to predict pre-eclampsia are coming up on the horizon. Hypocalciuria is one such test to predict pre-eclampsia which may be present before other clinical signs and symptoms. **AIM:** To determine urinary calcium, urinary creatinine and urinary calcium/creatinine ratio in pregnancy induced hypertension and in normal pregnant women. **MATERIAL AND METHODS:** The study included 100 subjects with gestational age between 24-38 weeks and divided into two groups viz. control group and study group. The control group included 50 subjects who were normal pregnant women and study group included 50 subjects who were gestational hypertensive patients. Urinary calcium was estimated by Ortho-Cresolphthalein Complexone (CPC) method, urinary creatinine was estimated by Jaffe's method in all the subjects. **RESULTS:** The estimated mean levels (Mean±SD) of urinary calcium, creatinine in control group were 263.0±80.3, 0.95±0.16 respectively and in patients with gestational hypertension they were 86.2±19.5, 1.21±0.37 respectively. The statistical analysis by unpaired t-test shows that the levels of urinary calcium were significantly decreased (p<0.001) and the urinary creatinine were significantly increased (p <0.001) in gestational hypertensive patients when compared to healthy controls. The mean urinary calcium/creatinine ratio in control group were 0.28±0.08 and in gestational hypertensive patients were 0.07±0.03. The statistical analysis by unpaired t-test shows that the levels of urinary calcium/creatinine ratio were significantly decreased (p<0.001) in gestational hypertensive patients when compared to healthy controls. **CONCLUSION:** This study suggests that a regular evaluation of urinary calcium/creatinine ratio after 20wks of gestation may be an effective screening method for impending pre-eclampsia and may identify population at risk to be included in primary prevention programmes.

KEYWORDS: Pregnancy Induced Hypertension; Pre-Eclampsia; Urinary calcium; Urinary creatinine; Calcium/Creatinine Ratio.

INTRODUCTION: Pregnancy can be the most wonderful experience life has to offer. But it can also be dangerous. Around the world, an estimated 529,000 women die during pregnancy or childbirth.¹ Hypertension and proteinuria have long been recognized to be an important complication of pregnancy.² Pregnancy is also a state of obligate calcium loss in which the mother must provide 25 to 30gm of calcium that makes up the fetal skeleton at birth.³ Renal excretion of calcium increases during pregnancy, maximum excretion levels reached during the third trimester. Urinary excretion of calcium levels in non-pregnant women is in the range about 100 - 250 mg/day, in pregnant women the range is between 350-620 mg/day. There is a decrease in urinary calcium levels in pre-eclampsia. Decreased renal filtration and increased tubular reabsorption may result in hypocalciuria.⁴

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The mechanism of calcium homeostasis is multifactorial, involving calcium itself and other related minerals such as magnesium and phosphorous, and three calcitropic hormones-parathyroid hormone, calcitonin, and active form of vitamin D3 which is 1,25-dihydroxy cholecalciferol.⁵ Associations have been well recognized for over 25yrs between low calcium intake and the risk of hypertensive complications of pregnancy. Women with established preeclampsia have been reported to have lower urinary calcium excretion and higher PTH levels than normotensive controls. Because the placenta contributes approximately 50% of the circulating 1,25 dihydroxycholecalciferol level in pregnancy, it was postulated that, in preeclampsia, the defective placenta is unable to produce sufficient levels of 1,25 dihydroxycholecalciferol, resulting in inadequate gastrointestinal calcium absorption, low ionized calcium levels, and a secondary rise in PTH. Low calcium causes decreased threshold for excitability, increased PTH causes raised HTN.⁶ The majority of women with hypertension have diminished renal perfusion and glomerular filtration with corresponding elevated plasma creatinine. In severe cases it may be elevated two- to three- fold over normal values in non-pregnant women.⁷

With this background this study was designed to estimate the urinary calcium and creatinine in gestational hypertension patients and controls who are normotensive pregnant women from Davangere district of Karnataka.

MATERIAL AND METHODS: A total number of 100 subjects participated in the present study which included 50 gestational hypertensive patients between 24-38weeks of pregnancy. 50controls were healthy pregnant women between 24-38weeks of gestation, without any major illness. The subjects included both primi and multigravida pregnant women from Bapuji Hospital and Chigateri General Hospital, Davangere (Both attached teaching hospitals for J.J.M. Medical College, Davangere). Each gave informed consent and this study was approved by Ethical and Research Committee of J.J.M. Medical College, Davangere to use human subjects in the research study.

EXCLUSION CRITERIA: Patients with history of chronic hypertension, diabetes, renal diseases, and other chronic medical illness.

History was taken regarding age, parity, socioeconomic status, past, family, and personal history. General examination was done for blood pressure, odema and weight gain. Examination was conducted in all the cases according to the preform. Routine investigations were done in all the 100 cases.

The early morning first urine sample from all patients was collected in calcium free vials.10ml of urine was collected and 0.2ml HCL was added to prevent calcium salt precipitation. Calcium and creatinine were estimated in the collected samples. Sample was tested within 24hrs of collection. If urine was turbid then it was centrifuged at 2000rpm for 10min, and clear supernatant was used for testing.

ESTIMATION OF URINARY CALCIUM BY ORTHO-CRESOLPHTHALEIN COMPLEXONE (O-CPC)

METHOD®: PRINCIPLE: In alkaline solution, O-CPC forms a red chromophore with calcium, which is measured at a wavelength between 570-580nm. Calcium reacts directly with cresolphthalein-complexone (O-CPC) reagent containing dimethyl sulphoxide and 8-hydroxy-quinoline. The results were expressed in mg/day.

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ESTIMATION OF URINARY CREATININE BY JAFFE'S METHOD^{9,10}: PRINCIPLE: Creatinine reacts with picric acid in alkaline medium to form a reddish yellow complex, intensity of which is directly proportional to the concentration of creatinine in the specimen and can be measured at 520nm (Green filter). The results were expressed in g/day.

STATISTIC ANALYSIS: Results are expressed as mean±SD. Unpaired 't' test was used for intergroup Comparison and paired 't' test for intra group comparison. p<0.05 was considered as statistically Significant.

RESULTS:

Number of Subjects		Cases	Controls
		50	50
AGE (Years)	Mean±SD	22.4±2.9	22.3±2.8
	Range	18-28	18-32
POG (Weeks)	20-24	20(39%)	30(59%)
	25-28	24(47%)	15(29%)
	30-34	7(14%)	6(12%)
PARITY	NULLI	32(63%)	29(57%)
	MULTI	19(37%)	22(43%)

Table 1: Descriptive Information of Subjects

GROUPS		URINARY CALCIUM (mg/day)
CONTROLS	MEAN ±SD	263.0±80.3
	RANGE	110-426
CASES	MEAN±SD	86.2±19.5
	RANGE	50.5-140.3
CASES VS CONTROLS	MEAN DIFFERENCES	176.8
	t value*	15.3
	P value	<0.001, HS

Table 2: Comparison of Urinary Calcium Levels between Cases and Controls

*- unpaired t- test, HS- highly significant.

GROUPS		URINARY CREATININE (gm/day)
CONTROLS	MEAN ±SD	0.95±0.16
	RANGE	0.8-1.6
CASES	MEAN±SD	1.21±0.37
	RANGE	0.80-1.88
CASES VS CONTROLS	MEAN DIFFERENCES	0.26
	t value*	4.63
	P value	<0.001, HS

Table 3: Comparison of Urinary Creatinine Levels Between Cases And Controls

*- unpaired t- test, HS- highly significant

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DISCUSSION: In our study, the mean value of urinary calcium in controls were 263 ± 80.3 mg/day when compared to gestational hypertensive cases who had mean value of 86.2 ± 19.5 mg/day. In the present study it was found that the mean urinary calcium levels were lower in gestational hypertensive patients when compared to normotensive pregnant patients and was statistically significant with p-value < 0.001 .

In majority of pre eclamptic women, there is mild to moderately diminished glomerular filtration which appears to result from a reduced plasma volume. Intrinsic renal changes caused by severe vasospasm may cause severe fall in GFR in some cases. This might be responsible for hypocalciuria in pre-eclampsia.¹¹ In normal pregnancy there is physiological hypercalciuria due to an increased GFR, however the fall in calcium excretion in pre-eclampsia 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 at the cellular level.¹¹

Normally there is an increase in the level of intracellular calcium in pregnancy. This effect is exaggerated in preeclampsia due to a significant increase in the membranous calcium content. Normotensive pregnant women and pre-eclamptic women exert distinct changes on cellular Ca^{2+} metabolism in normal vascular smooth muscle cells (VSMC). In preeclampsia, the voltage dependent calcium channels (VDCC) are blunted that is their action is decreased where as in normal pregnancy action of these channels are increased. This is responsible for increase in intracellular calcium levels in pre-eclampsia.¹¹

Normally, these calcium ions are released from intracellular source in response to agonist-stimulated production of inositol tri-phosphate. In pre-eclampsia there is an inhibitory effect on inositol tri phosphate generation or a resistance to the effect of inositol tri-phosphate. Hence release of intracellular calcium is decreased. Moreover, the agonist-induced release of calcium from internal stores triggers a capacitative influx of extracellular Ca^{2+} across the plasma membrane. But due to the reduced calcium release in preeclampsia, there is an inhibition of calcium influx as well. Thus there is increase intracellular Ca^{2+} and decrease in extracellular or plasma levels of Ca^{2+} which may lead to the release of parathormone causing increased reabsorption of calcium in kidneys leading to decreased urinary excretion of calcium.¹¹

Hence they suggested that, hypocalciuria in preeclampsia is independent of renal function and reflects a complex alteration in calcium homeostasis at the cellular level and can be used for predicting preeclampsia.¹¹ It was also seen that an increase in circulating calcium produces vasodilation and a decrease causes vasoconstriction. Also hypocalciuria, would decrease stabilization of cellular membrane decreasing its threshold for excitability, which may be responsible for the convulsions seen in eclampsia.¹² Some other studies have suggested that the hypocalciuria seen in patients with hypertensive disorders of pregnancy may be due to increased distal tubular reabsorption of calcium, though cause for increased reabsorption is not known.¹³ However, there are few studies suggest that hypocalciuria may be due to decrease in glomerular filtration rate seen in these patients and also tubular reabsorption was reduced because the fractional excretion of calcium was decreased and PTH levels were normal, hence they attributed hypocalciuria to changes in kidney function.¹³

In our study, the mean values of urinary creatinine found in the controls were 0.95 ± 0.16 gm/day while in the cases it was 1.21 ± 0.37 gm/day. The urinary creatinine levels in the cases were significantly higher when compared to controls and was statistically significant ($p < 0.001$) between cases and controls.

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Pregnancy induced hypertension is known to be associated with a deterioration in renal function and increased creatinine concentration have been demonstrated in women with pregnancy induced hypertension.

Previous studies have assessed the use of urinary calcium/creatinine ratio as clinical tools for predicting which gestational hypertensive women were at risk of developing pre-eclampsia, but yielded different results.¹⁴

In the present study the urinary calcium/ creatinine ratio was lower in women with gestational hypertension when compared to normotensive pregnant women. The cut-off value taken was 0.16. Gestational hypertensive patients had a ratio less than 0.16 where as normal pregnant women had a ratio more than 0.16. This was statistically significant with a p-value less than 0.001.

CONCLUSION: The study shows a close relationship between urinary calcium as well as creatinine excretion in the gestational hypertensive patients. The current study supports that the levels of urinary calcium are significantly decreased in gestational hypertensive patients compared to normotensive pregnant women, whereas urinary creatinine are increased in gestational hypertensive patients compared to normotensive pregnant women. It is also emphasized from the study that a regular evaluation of urinary calcium/creatinine ratio after 20 weeks of gestation may be an effective screening method for impending pre-eclampsia and may identify population at greater risk to be included in primary prevention programmes.

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AUTHORS:

1. Swapna V. S.
2. Triveni Jambale
3. Jayaprakash Murthy D.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Biochemistry, Viswabharathi Medical College, Kurnool, Andhra Pradesh, India.
2. Assistant Professor, Department of Biochemistry, Gadag Institute of Medical Sciences, Gadag, Karnataka, India.
3. Professor, Department of Biochemistry, Oxford Medical College, Bangalore, Karnataka, India.

FINANCIAL OR OTHER**COMPETING INTERESTS:** None**NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. Swapna V. S,
W/o. Dr. B. Lakshman kumar,
40/362, Vasavi Homes, Flat no -502,
Vidyanagar, Kurnool-518001,
Andhra Pradesh.
E-mail: drswapnavs07@gmail.com

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