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Original Article

Biochemistry Section

Evaluation of Urinary Calcium Creatinine Ratio in Pre-Eclampsia

INDU PRASAD, BANDANA KUMARI, ACHAL NARAYAN ROY, PRITAM PRAKASH

ABSTRACT

Introduction: Hypertensive disorder complicates the pregnancy. The exact aetiology of hypertensive disorders of the pregnancy is still unknown and management is controversial.

Aim: To evaluate the role of urinary Calcium Creatinine Ratio (CCR) as a marker in early diagnosis of pre-eclampsia.

Materials and Methods: Urinary calcium creatinine ratio was determined in a random sample of urine in 100 patients of pre-eclampsia (Study group) and 100 normotensive pregnant (Control group) patients of gestational age 20-36 weeks. Cut off value for CCR was taken as ≤ 0.04. Comparative study was done by using chi-square test.

Results: In study group, 89% of the cases had urinary CCR ≤ 0.04. Amongst the controls only 6% of the cases had CCR ≤ 0.04. Statistically, CCR as high risk factor for early diagnosis of pre-eclampsia was significant ($p<0.001$ and 'r' = 0.169).

Conclusion: The result showed lower urinary calcium excretion and low CCR in pre-eclamptic patients than normotensive pregnant women.

Keywords: Hypertensive, Mortality, Normotensive

INTRODUCTION

Despite of so much research and changes in management, pre-eclampsia is still a leading cause of maternal morbidity and mortality in India and worldwide. About 5-7% of the pregnancies are affected by pre-eclampsia and hypertensive disorders [1]. Pre-eclampsia is a "multisystem disorder". Pathology behind pre-eclampsia is reduced perfusion of organs due to vasospasm. It is usually associated with proteinuria or oedema or both. In spite of extensive research, the mechanism how pregnancy initiates or aggravates hypertension remains unknown. It is amongst the most significant unsolved problems in obstetrics. This is the disorder of widespread vascular endothelial malfunction and vasospasm which occurs after 20 weeks of gestation and presents till 4-6 weeks postpartum.

Hypertension is diagnosed when systolic blood pressure is greater than 140 mmHg and diastolic blood pressure greater than 90 mmHg on two successive measurements done 4-6 hours apart or when systolic blood pressure is increased by 30 mmHg and diastolic blood pressure by 15 mmHg. Proteinuria is defined as excretion of 300 mg or more of protein in 24 hour urine sample or ≥1+ dipstick in a random sample of urine. Further pre-eclampsia can progress to eclampsia leading to seizures and HELLP syndrome if left untreated. It can also lead to haemorrhage and infection, resulting in significant maternal morbidity and mortality. Various predictors have been proposed e.g. roll-over test, second trimester mean arterial pressure test, serum uric acid test, angiotensinogen sensitivity test, Isometric test etc. But none of them have been proved ideal either because of high incidence of false positivity or their complexity in result interpretation. It has been found that decreased urinary excretion of calcium may be considered as a useful tool for the early diagnosis of pre-eclampsia [2]. This disorder is highly prevalent in our country in spite of great advances in obstetrics. So, the present work is focused on prevention of the disease rather than treatment. Therefore, the study was done to determine the relationship between pre-eclampsia, hypocalciuria and calcium to creatinine ratio for early prediction of pre-eclampsia in a random urine sample.

AIM

To evaluate calcium: creatinine ratio for the early diagnosis of pre-eclampsia so as to avoid maternal and fetal morbidity and mortality in the society.

MATERIALS AND METHODS

A case control study was undertaken in the Department of Biochemistry, Patna Medical College and Hospital, Patna, Bihar in the year 2010-2011.

It was carried out on a total number of 200 women of gestational age 20-36 weeks attending the antenatal clinic (outdoor), or admitted in the ward or in labour room in the Obstetrics and Gynaecology Department, Patna Medical College and Hospital, Patna, Bihar, India.

For statistical significance and comparison, these were divided into two groups.

Group I: (Study group)- Study group consists of 100 patients of pre eclampsia. These patients had the following signs present-

(1) Blood Pressure 140/90 mmHg or more.

(2) Proteinuria- Tested with Dipstick≥1+ dipstick in random sample of urine.

Group II : (Control group)- Control group consisted of 100 normotensive pregnant patients of gestational age 20-36 weeks attending the routine antenatal clinic, ward, or in labour room in the Obstetrics and Gynaecology Department, who had no risk factors for the development of pre eclampsia like; history of pregnancy induced hypertension in the past pregnancy, history of twin pregnancy in the present or past pregnancy or in the family.

Subjects with any history of hypertension, diabetes mellitus, chronic renal disease, immunological or vascular disorders were excluded.

Urine Sample Analysis

Random sample of urine was taken as specimen and analyzed by the semi-auto analyzer (colorimetric method) for the estimation of urinary calcium and urinary creatinine and ratio.

Laboratory test of urine for estimation of calcium: Urinary calcium was analysed by OCPC (O-Cresolphthalein Complex one) method colorimetrically [3].

Calcium + OCPC → Bluish- Purple Complex

Calculation:

$$\text{Urine calcium (mg/dl)} = \frac{\text{Absorbance of Test X 10 X Dilution Factor}}{\text{Absorbance of Standard}}$$

Normal urinary excretion of calcium =100-300 mg/day.

Laboratory test of Urine for Estimation of Creatinine: Urinary creatinine was estimated by Jaffe's Method (Alkaline Picrate) at 520nm [4].

Calculation:

$$\text{Urinary Creatinine in g/L} = \frac{\text{OD of Test-OD of Blank X 0.75}}{\text{OD of Standard - OD of Blank}}$$

Where, "OD" is Optical Density.

Result obtained was multiplied by 10 to convert it in mg/dl.

Normal urinary creatinine excretion =1-2 gm/day.

Reagents Used: Commercially available kits provided by Span Diagnostic Company of India were used for both the parameters.

All the collected data was reviewed and analysed for urinary calcium-creatinine ratio (CCR) in both the study and control groups.

So, the ratio is given as $\frac{\text{Urinary Calcium(mg/dl)}}{\text{Urinary Creatinine(mg/dl)}}$

Cut off for CCR is taken as ≤ 0.04 [5].

STATISTICAL ANALYSIS

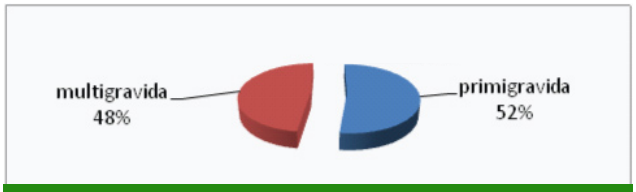
The results obtained were further analysed for SD, t- test,SEM and p value. The p value<0.001 was considered significant. Comparative study was done by using chi-square test.

RESULTS

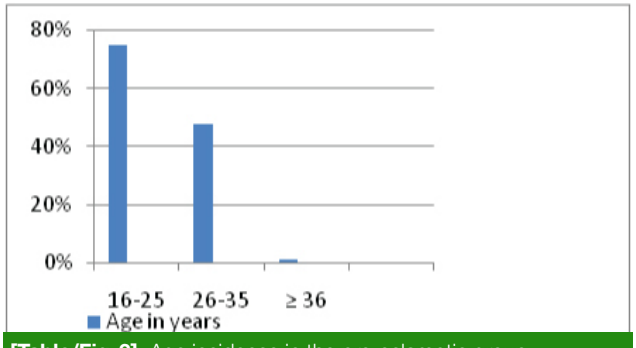
The study done was a case control study conducted on 200 pregnant women, containing 100 pre-eclamptic patients in the case and 100 normotensive pregnant women in the control group. In the study group 52% patients were primigravida [Table/Fig-1]. Most of the patients (75%) belonged to the age group of 16-25 years [Table/Fig-2].

Urinary calcium excretion in pre-eclamptic group was found to be as 4.8±1.52 mg/dl and 5.26 ± 1.91mg/dl in the age group 16-25 years and 26-36 years respectively. It was compared with urinary calcium excretion of the patients of same age interval in the control group (12.33 ± 3.58 mg/dl and 12.34 ± 2.79 mg/dl resp). This was found to be lower in pre-eclamptic group with p <0.001 [Table/Fig-3].

Similarly, urinary creatinine excretion in the pregnant pre-eclamptic women was found to be as125.69 ± 14.56 mg/dl



[Table/Fig-1]: Distribution of patients in study group according to gravida.



[Table/Fig-2]: Age incidence in the pre-eclamptic group.

Group	Age Interval (Years)	N	Urinary Calcium Excretion (mg/dl)			t	p
			Mean	SD	SEM		
Study	16-25	5	4.8	1.52	0.17	16.11	< 0.001
Control	16-25	48	12.33	3.58	0.51		
Study	26-36	25	5.26	1.91	0.39	11.25	<0.001
Control	26-36	52	12.34	2.79	0.38		

[Table/Fig-3]: Urinary calcium excretion in different age groups.

Group	Age Interval (Years)	N	Urinary Calcium Excretion (mg/dl)			t	p
			Mean	SD	SEM		
Study	16-25	75	125.69	14.56	1.69	3.4	< 0.001
Control	16-25	48	133.80	12	1.73		
Study	26-36	25	127.62	14.69	1.98	4.4	<0.001
Control	26-36	52	134.83	12.52	2.2		

[Table/Fig-4]: Urinary creatinine excretion in different age groups.

Group	N	Urinary Calcium Excretion(mg/dl)			t	p
		mean	SD	SEM		
Study	100	4.91	1.62	0.16	21.07	<0.001
Control	100	12.43	3.18	0.31		

[Table/Fig-5]: Urinary calcium excretion in pre-eclamptic group.

Group	N	Urinary Calcium Excretion(mg/dl)			t	p
		mean	SD	SEM		
Study	100	120.53	14.31	14.31	5	<0.01
Control	100	130.64	12.50	12.5		

[Table/Fig-6]: Urinary calcium excretion in pre-eclamptic group.

Group	Urinary Calcium		Urinary Creatinine		Co-relation Co-efficient (r)
	Mean	SD	Mean	SD	
Study (100)	4.91	1.62	120.53	14.31	0.169
Control (100)	12.43	3.18	130.18	12.50	

[Table/Fig-7]: Co-relation between calcium and creatinine excretion. p= 0.058

Group (N)	CCR≤0.004	CCR>0.004
Study (100)	89%	11.0%
Control (100)	6%	94.0%

[Table/Fig-8]: Distrubution of patients on the basis of CCR. Chi square (X^2) = 138.13, p < 0.001

and 127.62 ± 14.69 mg/dl respectively in the age group 16-25 years and 26-36 years. It was found to be lower than that in the controls (133.80 ±12 and 134.83 ± 12.52) mg/dl.This value was again significant with p<0.001[Table/Fig-4].

In pre-eclamptic group mean urinary calcium was 4.91 mg/dl which was lower than the mean value of control group (12.43 mg/dl) [Table/Fig-5].

Mean urinary creatinine in the pre-eclamptic group and normotensive group were 120.53 mg/dl and 130.64 mg/dl respectivley [Table/Fig-6].

The correlation coefficient 'r' was equal to 0.169 between both study and control groups [Table/Fig-7]. Amongst the study group 89% of the patients had CCR ≤ 0.004 [Table/Fig-8]. Chi square (χ^2) value was 138.13 showing that ratio is highly significant (p<0.001).

DISCUSSION

The present study showed that 89% of the pre-eclamptic women had CCR ≤0.004. When calculated statistically, it was found that CCR alone can be taken as high risk factor for pre-eclampsia. The cut off value (CCR ≤ 0.04) was similar with that of various studies [1,5-9]. 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. Hence, a definite relationship was found between low calcium creatinine ratio and development of pre-eclampsia.

Result of the present study corresponds with the result of Gaurang K et al., [1], Mittal Shilpa et al., [10], Kazemi AFN et al., [11] Taufield et al., [12], Tufan Bilgin et al., [13], Segovia BL et al., [14], Ingec M et al., [15], Halhali A et al., [16], Dasgupta Mandira et al., [17], Sirohiwal Daya et al.,[18], Donovan et al., [19], Sheela CN et al., [7]. They estimated that calcium excretion in pre-eclampsia was significantly lower than in normal pregnant women. The result obtained in the present study is more or less similar to the finding noted above. During normal pregnancy, body physiologically compensates for increased calcium demand by either increasing the intestinal absorption or by decreasing the loss by renal mechanisms. But changes in the renal function

may lead to increased reabsorption of calcium by the distal tubules of kidney [20]. These mechanisms, thus, lead to hypocalciuria in women destined to develop pre-eclampsia.

While others noted decrease of urinary creatinine in pre-eclampsia, Mittal Shilpa et al., [10], Kazemi AFN et al., [11] and Moni SY et al., [21] observed increase in urinary creatinine in pre-eclamptic patients.

Sera from normotensive pregnant women and pre- eclamptic women exert distinct changes on cellular metabolism of calcium (Ca^{2+}). There is increase in the intracellular calcium metabolism in normal vascular smooth muscle cells (VSMC) [22]. Normal pregnant sera amplify, whereas pre-eclamptic sera blunt the voltage dependent calcium channels (VDCC).

Atallah et al., suggested the physiological basis behind the development of pre-eclampsia in patients with low calcium intake. They said low intake of calcium stimulates PTH production, which increases the level of intracellular calcium. This causes VSMC contraction leading to hypertension. Thus, calcium supplementation in turn would reduce the level of intracellular calcium and relax the vessels [23]. Some cases have reported that pre-eclampsia exerts severe vasospasm which may cause intrinsic renal changes resulting in severe fall in GFR. This might be responsible for hypocalciuria in pre-eclampsia [17].

Chi square has been used to find significant association (183.13). This finding corresponds with that of Sheela CN et al., [7].

Patrick J Saudan et al., suggested primary or secondary disturbances of renal calcium handling in pre-eclampsia leads to its decreased excretion in urine. Though they found decreased excretion of urinary calcium in pre-eclampsia but lacked the sufficient sensitivity of the test to use it as tool for early diagnosis [24]. Izumi et al., found a limited value of CCR in prediction of preeclampsia [25]. Though the present study calculated the predictive value of CCR but couldn't calculate the sensitivity and specificity.

Estimation of calcium creatinine ratio in a random sample of urine is a simple test. Moreover, the test can be performed easily, hence ensuring patient compliance. It has a good predictive value, hence, justifies the cost. It can therefore be recommended as a screening tool for pre-eclampsia after 20 weeks of gestation in the pregnant women during their antenatal visit. Pre-eclampsia and gestational hypertension is the significant causes of both fetal and maternal morbidity and mortality.

CONCLUSION

Many tests to predict pre-eclampsia are coming up. So, the present study is intended to identify at risk patient and a selection criteria for primary prevention. In subjects, 89% of the cases had urinary CCR ≤ 0.04. The Correlation Coefficient (r), Chi square value showed a significant association. On statistical analysis, it was found that when CCR alone was taken as high risk factor for the early diagnosis of pre-eclampsia, it was highly significant $p < 0.001$.

When high risk factors and urinary calcium creatinine ratio (CCR) were combined then 70% chance of developing pre-eclampsia was found. So, this test forms a satisfactory basis for the early diagnosis of pre-eclampsia. The present study showed lower urinary calcium excretion and CCR in pre-eclamptic than normotensive pregnant women.

Therefore, a single random urinary CCR may be an effective tool for the early diagnosis of pre-eclampsia and may identify population of greatest risk to be included in primary prevention programmes. So, early therapeutic use of calcium may significantly reduce the morbidity and mortality in patients of pre-eclampsia.

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AUTHOR(S):
1. Dr. Indu Prasad
2. Dr. Bandana Kumari
3. Dr. Achal Narayan Roy
4. Dr. Pritam Prakash

PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Biochemistry, IGIMS Patna, India.
2. Senior Resident, Department of Biochemistry, IGIMS Patna, India.
3. Professor and Head, Department of Biochemistry, PMCH, Patna, India.
4. Assistant Professor, Department of Biochemistry, NMCH, Patna, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Indu Prasad,
B-303, Luv Kush Apartment, Siddharth Nagar, Jagdeopath, Bailey Road, Patna-800014, India.
E-mail: drindupd@gmail.com

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