Original Research Article

A Prospective Study of Calcium to Creatinine Ratio (CCR) and Microalbuminuria as Predictors of Pre-Eclampsia

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Abstract

Objective: To evaluate the predictive values of urinary calcium creatinine ratio and microalbumin in pregnancy for detection of patients who are going to develop pre-eclampsia. *Methods*: Spot urine samples were taken in 150 asymptomatic pregnant women between 20-28 week of gestation to determine Urinary calcium creatinine ratio and micro-albuminuria in outpatient department of TRS, hospital. The statistics was analyzed using Chi square test and t test for determine the significant association of clinical findings of pre-eclampsia to CCR and micro-albuminuria. The predictive values of CCR equal to 0.04 or < 0.04 and microalbuminuria were determined by Area under Receiver Operator Curve (ROC) for pre-eclampsia. *Results*: The Calcium to creatinine Ratio found to be a good diagnostic test for prediction of pre-eclampsia with sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of 83%, 96%, 75%, and 98% respectively. The statistical accuracy of 95% and significant p value of <0.001. The microalbuminuria is came out to be fair diagnostic test to prediction of pre-eclampsia with sensitivity, specificity, PPV and NPV of 65%, 86%, 42% and 94% respectively. *Conclusion:* CCR to be recommended as a good screening test for prediction of preeclampsia at cut off value of 0.04 in spot urine sample of all pregnant women without any symptomatology while microalbuminuria is a fair test for same at present.
Keywords: Screening test, CCR (calcium to creatinine ratio), prediction of pre-eclampsia, microalbuminuria.

INTRODUCTION

The incidence of Hypertension disease is 5 to 10% in pregnant patients, corresponding to the major cause of maternal mortality across the world [3]. The early and accurate diagnosis is single way to reduce the impact of arterial hypertension on maternal mortality with an early intervention [4]. The classical clinical manifestations of preeclampsia, i.e. high blood pressure levels (>140/90 mm Hg) after the 20th week of gestation & significant proteinuria (>300 mg in 24 h) [5]; are now considered to be a late manifestation of a disease that has been present since the first trimester of gestation. Due to the "diagnostic delay", many tests have attempted to establish the diagnosis of preeclampsia as early as possible, often even before the patient presents arterial hypertension [6].

The exact etiology remains unclear even today but due to progress in understanding the disease process along with the availability of better research tools have led to the development of numerous tests to predict preeclampsia.

In past two decades more than 100 biochemical and clinical diagnostic methods have been developed to predict preeclampsia. These tests have limitations as screening tools because of false positive results and subjective nature of result interpretation. Therefore there is immense need of a validated test for pregnant population at risk for development of preeclampsia.

MATERIALS AND METHODS

This tertiary hospital based prospective clinical study conducted in Department of Obstetrics and gynecology, TRSH, Rajpur Road, Delhi, from May 2011 to April 2012 with sample size of 150 pregnant women of 20 to 28 weeks period of gestation. After detailed interview, explanation of condition (pre-eclampsia), why going to do this test (Patient

information & Consent), single spot urine sample and analysis of sample for calcium to creatinine ratio and microalbuminuria and followed till delivery for development of pre-eclampsia. The Institutional "ETHICAL AND SCIENTIFIC COMMITTEE" approved this study. Exclusion of this study included multiple pregnancy, renal disease, molar pregnancy, hypertension, severe anemia, diabetes mellitus, and urinary tract infection.

The Mid-stream urine sample was collected in a clean sterile bottle without any preservative. After routine microscopic and dipstick examination, sample processed to analyzed microalbumin, calcium and creatinine until 7days. All sampled patients, were followed to notice for development of hypertensive disorder.

The criteria adopted for Pre-eclampsia diagnosis (systolic arterial blood pressure ≥140mm Hg and/or diastolic arterial blood pressure ≥90mm Hg with ≥300 mg/24h proteinuria) described by the National High Blood Pressure Education Program Working Group (NHBPEP), USA, in 2000 [5]. The pregnant women of study were categorized in pre-eclampsia/hypertensive disorder and remained normotensive based on above said criteria. Predictive values (by statistical analysis) of urine microalbumin and CCR between 20-28 weeks, were correlated with the development of pre-eclampsia.

STATISTICAL METHODS

SPSS (statistical Package for the social sciences) 17.0 used for statistical calculations. Results of study were expressed in Mean ± SD and Range values. Qualitative (non- parametric) - Chi square test used to calculate sensitivity, specificity, cutoff value and area under receiver operator curve. Quantitative- t-test used to calculate mean, standard deviation. A p value of ≤0.05 is considered as significant. CCR and micro-albuminuria predictive value calculated by Area under Receiver operator curve (ROC).

OBSERVATIONS AND RESULTS

Out of study sample 150 pregnant women was divided into two group of pre-eclampsia and normotensive with incidence of 20(13.4%) of prior group.

Majority of pregnant women fall in age group of 21-30 years (72%) with mean age of 24.2±3.97 years among normotensive and pre-eclampsia patients. The 79 (60.76%) out of 130 normotensive and 12 (60%) out of 20 preeclamptic women were nulliparous. The family history of hypertensive / pre-eclampsia found insignificant (P value 0.14) for both groups. No significant mean blood pressure differences noted among both groups at first visit of test performed while significant difference observed on the subsequent follow up visit.

Table 1: Distribution of Urine CCR in Normotensive and pre-eclamptic group

Urinary CCR	Normotensive	Pre-eclamptic
Mean ± SD	0.283±0.08	0.048±0.035
Range	0.13- 0.438	0.027-0.19
Mean difference	0.235	
P value	0.00001*	

*Highly significant

Normotensive pregnant women had urinary CCR of 0.283±0.08 (Range 0.13- 0.438) while 0.048±0.035 (Range 0.027-0.19) values in who developed pre-eclampsia with mean difference of 0.235. The p-value was highly significant (p < 0.001).

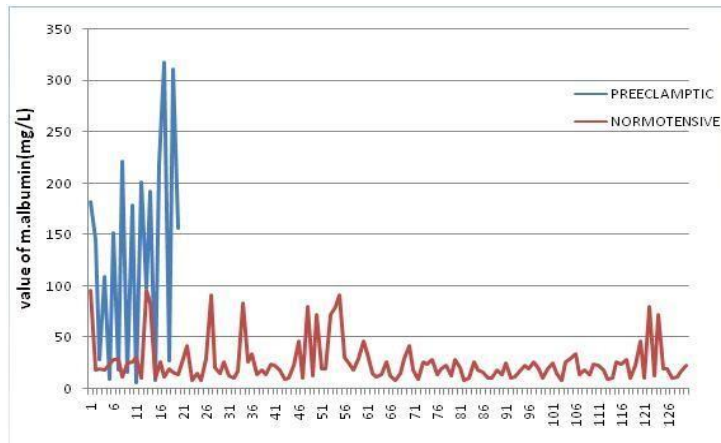### Table-2: Urinary microalbumin among normotensive and Pre-eclamptic group

Urinary Microalbumin (mg/L)	Normotensive	Pre-eclamptic
Mean ± SD	25.61±20.45	130.99±100.22
Range	8.04-96.23	6.56-317.67
Mean difference	105.38	
P value	0.00001*	

*Highly significant

The mean urinary microalbumin values is 25.61±20.45 mg/L in normotensive and 130.99±100.22 mg/L in those who developed pre-eclampsia with mean difference of 105.38mg/L. The p-value was highly significant (p < 0.001).

Graph 2: Distribution of urinary microalbumin among Normotensive and Preeclamptic pregnant women

Table 3: Distribution of pregnant women according as per positivity of test

Test used in study	Test positive	Test negative	Total
CCR (0.04)	18(12%)	132(88%)	150(100%)
Microalbuminuri(30-300mg/d)	31(20.6%)	119(79.4%)	150(100%)

The Area under curve (AUC) was used to predictive values of CCR and microalbuminuria. Numbers of patients with pre-eclampsia and without pre-eclampsia were arranged according to the value of diagnostic test. The CCR < 0.04 was taken as cut off criteria and ≥ 30-300 mg/L for microalbuminuria was considered abnormal. The cut off > 0.04 for CCR and < 30mg/L for microalbuminuria were considered normal. As per above cut off value number of true positive, true negative, false positive and false negative were segregated.

The CCR using cutoff values 0.04 had positive test results in 18(12%) pregnant women, and negative test results in 132(88%) pregnant women out of total 150. While Microalbuminuria using cutoff values 30-300mg/day, had positive test results in 31(20.6%) pregnant women, and negative test results in 119(79.4%) pregnant women out of total 150.

Table 4: Association of Calcium Creatinine ratio (CCR) with pre-eclampsia

CCR	Normotensive	Pre-eclampsia	Total
Test Positive (<0.04)	3(2%)	15(10%)	18(12%)
Test Negative (>0.04)	127(84.6%)	5(3.4%)	132(88%)
Total	130(86.6%)	20(13.4%)	150(100%)

18(12%) out of total 150, were CCR positive (<0.04) and 132(88%) were CCR negative (>0.04). Among pre-eclampsia patients, 15(10%) were test positive and remaining 5(3.4%) were test negative. 3(2%) Normotensive were test positive and 127(84.6%) were test negative. 15(75%) pre-eclampsia patients had test positive for CCR (<0.04).

Table 5: Association of Microalbuminuria and pre-eclampsia

Microalbuminuria	Normotensive N (%)	Pre-eclampsia N (%)	Total N (%)
Test Positive(>30-300mg/L)	18 (12%)	13 (8.6%)	31 (20.6%)
Test Negative(<30mg/L)	112 (74.6%)	7 (4.6%)	119 (79.2%)
Total	130 (87.7%)	20 (13.3%)	150 (100%)

The 31(20.6%) were positive for microalbuminuria including 13 pre-eclamptic and 119(79.4%) were tested negative for microalbuminuria) including 7 pre-eclamptic. 18 (12%) Normotensive were test positive and 112 (74.6%) were test negative.

Table 6: Results of statistical analysis

Test	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	Accuracy (%)	P value
CCR (0.04)	83	96	75	98	95	0.0001*
Microalbuminuria(30-300mg/L)	65	86	42	94	83	0.0001*

* Highly significant p value

The CCR of (<0.04) has sensitivity of 83%, specificity of 96%, positive predictive value of 75%, negative predictive value of 98%, with diagnostic accuracy of 95% and highly significant (<0.0001) P value. Microalbuminuria has sensitivity of 65%, specificity of 86%, positive predictive value of 42%, negative predictive value of 94%, with diagnostic accuracy of 83% and highly significant (<0.0001) P value.

DISCUSSION

The basic and predominant pathological changes in pre-eclampsia is endothelial dysfunction, which sets in as early as 8-18 weeks of gestation, however, the signs and symptoms appear in the late mid trimester. To arrest the disease process in the initial stages of settlement or to prevent complications especially in women predisposed to pre-eclampsia [8]. Proteinuria and alterations/reduction in calcium excretion are common features of various forms of hypertension and renal disorders [1]. This tertiary hospital based prospective clinical study is designed to find out the validity of urinary microalbumin and CCR, and to identify high risk population for the development of pregnancy hypertension. By early diagnosis, patients can be benefited from intense observation and aggressive treatment.

The present hospital based prospective study is comprising 150 pregnant women. Single spot urine test was done during their first visit to antenatal clinic between 20 and 28 weeks of gestation. Rodriguez H M [1] tested their patients between 24 and 34 weeks, Kazeroni T et al., [2] at 20 to 24 weeks, Sheela CN, Beena S R [7] at 20 to 24 weeks. Daya Sirohiwal et al., [9] conducted their study between 20- 28 weeks.

The prevalence of hypertension is 6 to 8% in young women of child bearing age, but increases as advancing age. The pre-eclampsia incidence is reportedly increased in < 21 yr. and > 35 yr of age [10]. Majority (65%) of pre-eclampsia women age was 21-30years followed by 30% women <20years and 0.5% above 31years in present study.

The incidence of pre- eclampsia is 13-20% in primiparous women. In our study 12 (60%) Preeclamptic women out of 20 are nulliparous. In 1924 Husselman showed that primi gravida were eight times more likely to develop eclampsia than multigravida. The study of Kar J et al., [5] showed, nulliparity with decreased CCR at high risk for developing pre-eclampsia.

Pre-eclampsia is seen more commonly in primiparous with no significant family history and similar was also found in our study. After reviewing from literature and found that there is a familial tendency to the development of both pre-eclampsia and eclampsia. Suzuki Y et al., [3] 1992 reported in their study that CCR was low in pregnant women of PIH without family history of hypertension. Women with past history of pre-eclampsia are at a risk for development of pre-eclampsia. In study done by Lie et al., [11], mothers with pre- eclampsia in first pregnancy were found to have 13.1% risk of recurrence in next pregnancy and same was found in out with incidence of 15%. The present study showed significant lower urinary CCR in pre-eclampsia women as compared to normotensive group.

The Area under Receiver operator curve (ROC) was used to calculate predictive cut-off value of CCR and which was calculated 0.04. The normotensive pregnant had urinary CCR of 0.283±0.08, with range from 0.13 to 0.438 and in pre-eclampsia had 0.048±0.035 with range from 0.027 to 0.19 and mean difference of 0.235 between both groups. The statistical analysis revealed significant (p < 0.001) p value. These findings are similar to the studies of Hellen Rodriguez M et al., [1], Das Gupta mandira et al., Ozcan T et al., [4], Patricia A devine et al., [6], Kar J et al., [5], Suzuki Y et.al3, Kazerooni T et al., [2].

This present study analyzed, that 18(12%) Out of total 150, were CCR positive (<0.04) and 132(88%) were CCR negative (>0.04). Among pre-eclampsia patients, 15(10%) were test positive and remaining 5(3.4%) were test negative. 3(2%) Normotensive were test positive and 127(84.6%) were test negative. 15(75%) pre-eclampsia patients had test positive for CCR (<0.04). In our study, with a cut off value of 0.04, the CCR has sensitivity of 83%, specificity of 96%, positive predictive value of 75%, negative predictive value of 98%, and diagnostic accuracy of 95%. There result are similar to Rodriguez H M [1] and co-workers in 1988 also estimated the CCR cut value and at 0.04 they analyzed a sensitivity of 70%, specificity of 95%, PPV of 64%, NPV of 96% for prediction of pre-eclampsia.

Sheela CN, Beena SR, Mhaskar Arun et al., (2011) [7] studied CCR in a spot urine sample in 200 asymptomatic pregnant women between 20-24 weeks of gestation for prediction of preeclampsia. They suggested that CCR at 0.04 in spot urine sample being a good test for prediction of pre-eclampsia can be recommended as a screening test in all asymptomatic pregnant women, for pre-eclampsia.

Single spot urine sample was taken in our study; this is similar to study done by Ozcan T et al., [4] and Kazerooni T et al., [2], who performed spot urine test. Thus it can replace the conventional 24 hr urine collection which is more cumbersome. The above mentioned authors had conclusion in their studies that decreased urinary CCR in single spot sample has a significant association with development of preeclampsia and it can be used as screening test.

In the present study, mean urinary microalbumin values in Normotensive, was 25.61 ± 20.45 mg/L, ranging from 8.04 to 96.23 and in preeclampsia was 130.99 ± 100.22 mg/L, ranging from 6.56 to 317.67. The mean values were found to be significantly higher in preeclampsia patients. Statistical analysis showed significant p-value was ($p < 0.001$). The predictive cut-off value of micro albuminuria was calculated by using Area under Receiver operator curve (ROC) and it was >30-300mg/L. Microalbuminuria at cut off value of (>30-300mg/L) found to have sensitivity 65%, specificity 86%, positive predictive value 42%, negative predictive value 94%, and diagnostic accuracy of 83% for prediction of pre-eclampsia. Microalbuminuria in a range up to 300mg/d is present in pregnant women well before the onset of overt pre-eclampsia. This can be a good test for identifying such women.

Overall the CCR was found a reliable and better predictor of pre- eclampsia. Microalbuminuria is considered as good test. The major limitation of our study was that the number of affected cases was small (n=20). These findings should therefore be interpreted with caution, so conduction of study at large scale required.

CONCLUSION

The only successful treatment for pre-eclampsia is delivery as no definitive preventive strategies have been identified. However, several of the recent observations based on these biochemical tests provide stimuli for the development of novel therapies for future application as mentioned above.

A single estimation of CCR of less than or equal to 0.04, , in asymptomatic pregnant women urine spot sample between 20-28 weeks of gestation is a good predictor of future development of pre-eclampsia and therefore recommended as a screening test for all pregnant women as it is justifies the cost effectiveness. Microalbuminuria, is a fair test for prediction of pre-eclampsia and cannot be recommended as a screening test at present.

Measurement of spot urinary CCR and microalbumin is non-invasive, inexpensive, and easy to carry out. Their use as early predictors will help to identify pregnant females at high risk of pre-eclampsia and prompt the initiation of education and prophylactic interventions (i.e. primary prevention, e.g. close prenatal care, calcium supplementation, low-dose aspirin), thus minimizing the severity of pregnancy induced hypertension.

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