Original Article

Evaluation of Spot Urinary Calcium to Creatinine Ratio in Preeclampsia: A Cross Sectional Comparative Study

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Abstract

Background: Preeclampsia (PE) is a pregnancy related condition leading to significant mortality and morbidity. A number of renal changes are seen in preeclampsia. **Objectives:** This study was designed to determine the association of calcium to creatinine ratio in a spot sample of urine with preeclampsia. **Methodology:** This cross-sectional comparative study was conducted in the Obstetrics & Gynaecology department of Dhaka Medical College, Dhaka, Bangladesh. Pregnant women with preeclampsia without complication and women with normal pregnancy without complications were selected for the study. Calcium, creatinine, Protein and calcium to creatinine ratio was measured in a spot urine sample of all these 150 subjects. Blood was also collected from all the cases for measurement of serum calcium, creatinine and uric acid. **Results:** A total of 150 pregnant women, 75 with preeclampsia without complication and 75 with normal pregnancy without complications were selected for the study. Significant difference between PE and normal group was observed in case of mean serum calcium and uric acid concentration but mean serum creatinine did not vary significantly. Urinary creatinine concentration did not show any significant difference. But mean urinary calcium concentration in spot sample of urine was significantly lower in preeclampsia than normal pregnant woman (3.45 ± 2.61 mg/dl vs 7.39 ± 2.80 mg/dl, $p < 0.001$). Similarly mean calcium to creatinine ratio in spot sample of urine was significantly lower in the pre-eclampsia than normal group (0.06 ± 0.05 vs 0.13 ± 0.06, $p < 0.001$). Positive correlation was observed between urinary calcium/creatinine ratio and urinary calcium ($r = +0.68$, $p < 0.001$) but negative correlation ($r = -0.39$, $p < 0.001$) noted between urinary calcium/creatinine and urinary protein. **Conclusion:** Spot urinary calcium and creatinine ratio decreases in preeclampsia. So spot urinary calcium and creatinine ratio can be regarded as a predictor for preeclampsia. [Journal of National Institute of Neurosciences Bangladesh, 2017;3(1): 14-20]

Keywords: Preeclampsia; Spot; Urinary Calcium; Creatinine Ratio

Introduction

Pre-eclampsia (PE) is a pregnancy specific condition characterized by hypertension and proteinuria occurring after 20 weeks of gestation and complicates about 8 to 10% of pregnancies[1]. It is a serious complication of second half of pregnancy and leading cause of foetal

growth retardation, perinatal and maternal mortality and morbidity. All over the world PE is 3rd and 7th leading cause of maternal and perinatal mortality and morbidity respectively and 16.0% of all maternal death in UK. In developing countries Preeclampsia effect 4.4% of all deliveries and may be as high as 18% in some areas in Africa[2]. In Bangladesh it is about 10% of all deliveries[3]. PE is a multistage disease characterized by abnormal placental development leading to endothelial dysfunction and damage. There is a failure of trophoblast remodeling of uterine spiral arteries resulting in sclerotic narrowing of blood vessels, increased vascular resistance and mechanical constriction which ultimately produce placental eschaemia and infarct. This lead to release of excessive amount of secreted factor into maternal circulation producing clinical signs and symptoms of preeclampsia. The pathological lesion in utero-placental bed is necrotizing arteriopathy leading to reduced placental perfusion. In severe disease there is widespread organ dysfunction specially kidney, liver and brain. But chief target organ in kidney; hence proteinuria and hypertension are the predominant clinical features [4,5,6,7].

In healthy pregnant women marked glomerular hyperfiltration (IGFR) is seen above normal nongravid level by 40 to 60% primarily due to reduced plasma oncotic pressure (RPF) in glomerular capillaries, hypovolumia induced haemodilution and elevated rate of renal plasma flow (RPF) . But in PE there is variable degree of renal insufficiency and GFR and RPF both are significantly reduced[8]. One of the most important clinical problems is that pre-eclampsia and complications appear suddenly in pregnant women and unfortunately as yet no definite predictive factor is available. Several potential predictive associated factors have been proposed for preeclampsia which include markers for liver and renal function (creatinine, uric acid, ALT, AST), vascular function (thromboxane, prostaglandin, nitric oxide, cytokines, LDH), coagulation and fibrinolytic factor (platelets, fibrinogen, antithrombin 3, plasminogen activator), oxidative stress and lipid (lipoprotein, lipid peroxide, antioxidant), placental function (HCG, placental growth factor, α-FP, inhibin), calcium and calcium/creatinine ratio[9-10]. Calcium metabolism is characterized by changes during pregnancy. Calcium metabolism increases in all pregnant women possibly because of increased intestinal absorption of calcium, increased renal filtered load of calcium and increased GFR of pregnancy[11-12]. Several abnormalities of calcium metabolism has been described in preeclampsia most important being hypocalciuria i.e. reduced renal calcium excretion. It is

possibly due to reduced dietary intake of calcium, decreased absorption, increased uptake by foetus and placenta, reduced GFR, increased glomerular calcium reabsorption[13,14,15].

Conventionally, 24hrs urine is required to assess hypocalciuria which is cumbersome, subject to collection error, require good patient compliance and more than 24hrs delay in diagnosis. So patient management is delayed. Several studies revealed that spot urinary calcium/creatinine ratio is a good indicator of urinary calcium excretion (hypocalciuria) and correlates well with 24 hrs calcium excretion[16]. So estimation of spot/random urine calcium/creatinine ratio could be a simpler, quicker, more convenient and less expensive test to assess and predict pre-eclampsia. Several investigators studied spot urinary Ca/Cr ratio in PE and it was found to be reduced[1,17,18,19]. All of them suggested that spot urinary Ca/Cr ratio could be a predictive factor for pre-eclampsia. But there are some negative results also[20,21,22,23]. Inspite of these controversies several results are very promising[24,25,26]. No such work has been undertaken in Bangladesh before. So this study was done with Bangladeshi subjects to evaluate the association between spot urinary calcium and creatinine ratio and pre-eclampsia as it was required an specific, low cost, easy and quicker test to predict pre-eclampsia.

Methodology

This cross sectional comparative study was done in the Obs & Gynae department of Dhaka Medical College Hospital involving two years. A total of 150 patients, all pregnant women (75 normal pregnant and 75 with pre-eclampsia) were taken by purposive non-random sampling. Patients attending the OPD at second half of gestation and admitted into the indoor of Dhaka Medical College Hospital were the study population. The cases were selected according to inclusion and exclusion criteria and were divided into two groups-a) Group I; Patients with pre-eclampsia & B) Group II; Normal pregnant woman. Inclusion criteria for group I were-i) Pregnant woman with pre-eclampsia with gestational age between 24-40weeks (age 18-35yrs), ii) Diastolic blood pressure ≥ 90 mm Hg and Systolic blood pressure ≥ 140 mm Hg, iii) With significant proteinuria (2+ or more by dipstick method) ; and inclusion criteria for group II were, i) Normal pregnant woman of gestational age between 24-40weeks (age 18-35yrs), ii) Diastolic blood pressure < 90 mm Hg and Systolic blood pressure < 140 mm Hg, iii) without any medical or obstetric complications.   Exclusion criteria were pregnancy with complications Diabetes

Mellitus, Renal sufficiency, urinary Tract infection, Liver Disease, Essential Hypertension, Heart\disease, Thyroid Disease, and Renal Stone. Pre-eclampsia was defined as 1) pregnant patient with Diastolic Blood pressure ≥90mm Hg and Systolic blood pressure ≥ 140mm of Hg, 2) Significant proteinuria: 2+ pr more by Dipstick test of two random clean catch urine specimen collected at 04 hours interval, 1st in the morning and 2nd 04 hours later. Variables utilized were age (years), gestational age (weeks), Parity, gravidity, socio-economic condition, previous history of pre-eclampsia, history of pre-eclampsia in the family, occupation, pulse, systolic blood pressure , diastolic blood pressure, oedema, proteinuria, uterine height, foetal movement, foetal heart rate, height, weight, basal metabolic index, serum calcium, serum creatinine, serum uric acid, urinary calcium, urinary creatinine, urinary protein, urinary calcium/creatinine ratio. After patient selection clinical examination was done and clinical data were collected and recorded according to data sheet. Questionnaires and checklist were also utilized. A morning random urine sample of all the patients was collected and proteinuria detected by dipstick method. After all aseptic precaution 05 ml of venous blood was taken and sent to laboratory to determine serum calcium, creatinine & uric acid. Urine was sent to the laboratory to determine calcium, creatinine, protein and calcium/creatinine ratio. A second urine sample was collected after 04 hrs to detect proteinuria again by dipstick method. Statistical analysis by SPSS version 12.0. Chi-square test,'t' test, Fisher's Exact test and correlation (r) tests were done. Level of significance was expressed as P value. P value <0.05 was considered as significant.

Results

Regarding the demographic variables, mean age ± SD of the preeclampsia and normal pregnant group were 27.57± 4.88 yrs and 26.22± 3.30 yrs respectively. There was no statistically significant difference (p=0.05). but in case of gestational age, mean ± sd value were 30.96± 3.50 and 28.49±4.29 weeks respectively and differed significantly (p<0.001). When age group was evaluated maximum no of preeclampsia pts were observed in age groups below 30 yrs (65.32%) and below 35 weeks of gestational age (89.33%). No significant difference was observed regarding occupation and socioeconomic status. Comparison of gravidity between the two groups revealed no significant difference ($\chi2$=.718, p >0.05). Primigravidity was noted in 30(40%) cases of preeclampsia and 25(33.33%) cases of normal pregnant patients. Regarding parity 44% patients of preeclampsia and 37% normal pregnant were nullipara; but 36% of both groups were primipara (Table 1).

No significant difference was observed in respect to pulse rate (p>0.05). but systolic (151±9.01 vs 106 ± 9.5, p<0.001)and diatolic blood pressure (98.27±6.80 vs 69±7.4,p<0.001) showed a great variation between the preeclampsia and normal pregnant groups. Distribution of different grades of oedema revealed highly significant difference between two groups ($\chi 2$=125.69,p<0.001). grade II oedema was the highest (51.7%) in preeclampsia patients. Grade III oedema seen in 11% of preeclampsia patients. Mean heights of the two groups were identical but highly significant difference was noted between preeclampsia and normal pregnant group in case of weight (63.13 ±3.26 vs 52.96± 5.29,p<0.001) and body mass index (27.43± 1.54 vs 23.57± 1.54,p<0.001). Uterine height did not show any variation between preeclampsia and normal pregnant patients (29.42± 3.20 vs 28.37 ± 4.37, p>0.05). similarly foetal heart rate and foetal movement did not show statistical significant difference. History of past preeclampsia was observed in 12% (9/75) of preeclampsia patients and 2.7% (2/75)

Table 1: Table showing demographic variables

Variables		Cases		Chi-square Value	P
		Preeclampsia (75)	Normal pregnancy (75)		
Gravidity	Primi	30 (40%)	25 (33.33%)		
	Multi	45(60%)	50 (66.7%)	0.718	0.49
Occupational status	HW	71	66		
	Job	3	4	2.86	0.24
	Business	1	5		
Socio-economic Condition	Poor	16	18		
	Middle	57	57	1.75	0.50
	Rich	2	0		
P/H/O	(+)	9	2		
pre-eclampsia	(-)	66	73	3.8	0.056

Table 2: Physical and Clinical Variables

Variables	Mean ± SD		Test Value	P value
	PE (n = 75)	Normal(n=75)		
Age	27.57 ± 4.88	26.22 ± 3.30	1.97(t)	0.05
Mean Gestational age	30.96 ± 3.50	28.49 ± 4.29	3.05(t)	*0.0001
Pulse Rate	84.48 ± 6.99	84.51 ± 4.50	0.2(t)	0.97
Systolic Blood Pressure	151.67 ± 9.01	106.00 ± 9.58	30.05(t)	*0.0001
Diastolic Blood Pressure	98.27 ± 6.80	69.60 ± 7.43	24.63(t)	*0.0001
Oedema(Grade)	23(Gr I)	7(Gr I)		
	38(Gr II)	0(GrII)	125.69($\chi 2$)	*0.0001
	8(Gr III)	0(GrIII)		
Height	151.78 ± 3.55	150.12 ± 4.16	2.63 (t)	*0.009
Weight	63.13 ± 3.26	52.96 ± 5.29	14.16(t)	*0.0001
BMI	27.43 ± 1.54	23.57 ± 1.90	13.63(t)	*0.0001
Uterine Height	137.73 ± 3.24	137.82 ± 3.12	.17(t)	0.095
Foetal Heart Rate	29.42 ± 3.20	28.37 ± 4.37	1.68(t)	0.85

*Significant

of normal pregnancy (p>0.05). History of preeclampsia in family was not observed in any subject of the two group (Table 2).

Serum calcium concentration of preeclampsia group (8.28±.42) and normal pregnancy (8.42±.39) varied significantly (p<0.05). On the other hand serum creatinine concentration didn't show significant difference (1.13±.27 vs 1.05± .21, p>0.05). Significantly higher serum Uric acid concentration was observed in preeclampsia patients (6.76± 1.99 vs 3.86± .75, p<0.01). Regarding urinary biochemical in the present study, we estimated spot urinary calcium concentration (mg/100ml), spot urinary creatinine concentration (mg/100ml) and spot urinary protein concentration (mg/100ml). From these results we calculated spot urinary calcium/creatinine (mg per 100 ml/mg per 100ml) ratio. We found that women with preeclampsia didn't differ significantly from normal pregnant patients in respect of urinary creatinine concentration (60.45±32.13 vs 53.11 ± 20.17, p>0.05). But the results were significant in case of spot urinary calcium and protein concentration. Women with preeclampsia showed reduced excretion of calcium in comparison to normal pregnant women when spot urinary calcium concentration was evaluated (3.45 ±2.61 vs 7.39± 2.80, p<0.001). In the present study we compared spot urinary calcium to creatinine ratio of normal pregnant woman and pre-eclampsia and the result was highly significant. The ratio reduced in preeclampsia patient in comparison to normal pregnant (.06±.05 vs .13±.06, p<0.001). A correlation analysis was done between spot urinary calcium/creatinine ratio and other variables. It correlated significantly and positively with spot urinary calcium concentration

(r=0.64, p<0.001) and correlated negatively with spot urinary protein concentration (r=0.28, p<0.01) (Table 3).

Table 3: Biochemical variables among the Study Population

Variables	Mean ± sd		't' Test Value	P value
	PE(n = 75)	Normal(n= 75)		
Serum Calcium	8.28 ± .42	8.42 ± .39	2.08	*0.039
Serum Creatinine	1.13 ± .27	1.05 ± .21	1.92	0.056
Serum Uric Acid	6.76 ± 1.99	3.87 ± .75	11.74	**0.0001
Urinary Calcium	3.45 ± 2.61	7.39 ± 2.80	8.89	*0.0001
Urinary Creatinine	60.45 ± 32.13	53.11 ± 20.17	1.67	0.09
Urinary Protein	302.82 ± 16.28	246.31 ± 3.10	10.07	**0.0001
Urinary Ca/Cr	.06 ± 0.05	0.13 ± 0.06	7.31	*0.0001

*Significant

Discussion

This cross sectional comparative study was conducted in the department of obstetrics and Gynaecology, Dhaka Medical College Hospital including both outdoor and indoor. During the two years of study period total 150 patients were enrolled and divided into two groups; preeclampsia and normal pregnancy, each comprising of 75 subjects.

Regarding the demographic variables, mean age ± SD of the preeclampsia and normal pregnant group were 27.57±4.88 yrs and 26.22±3.30 yrs respectively. There was no statistically significant difference (p=0.05). But

in case of gestational age, mean ± SD value were 30.96± 3.50 and 28.49±4.29 weeks respectively and differed significantly (p<0.001). When age group was evaluated maximum no of preeclampsia pts were observed in age groups below 30 years (65.32%) and below 35 weeks of gestational age (89.33%). No significant difference was observed regarding occupation and socioeconomic status. Comparison of gravidity between the two groups revealed no significant difference (χ^2=.718, p >0.05). Primigravidity was noted in 30 (40%) cases of preeclampsia and 25 (33.33%) cases of normal pregnant patients. These results correlate well with the study outcome by Qublan et al[9] and Soudan et al[22]. Regarding parity 44% patients of preeclampsia and 37% normal pregnant were nullipara; but 36% of both groups were primipara.

Regarding clinical variables no significant difference was observed in respect to pulse rate (p>0.05). But systolic (151±9.01 vs 106 ± 9.5, p<0.001) and diastolic blood pressure (98.27±6.80 vs 69±7.4,p<0.001) showed a great variation between the preeclampsia and normal pregnant groups. Distribution of different grades of oedema revealed highly significant difference between two groups (χ^2=125.69,p<0.001). Grade II oedema was the highest (51.7%) in preeclampsia patients. Grade III oedema seen in 11% of preeclampsia patients. Mean heights of the two groups were identical but highly significant difference was noted between preeclampsia and normal pregnant group in case of weight (63.13 ±3.26 vs 52.96± 5.29,p<0.001) and body mass index (27.43± 1.54 vs 23.57± 1.54,p<0.001). Uterine height did not show any variation between preeclampsia and normal pregnant patients (29.42± 3.20 vs 28.37 ± 4.37, p>0.05). Similarly foetal heart rate and foetal movement did not show statistical difference. History of past preeclampsia was observed in 12% (9/75) of preeclampsia patients and 2.7% (2/75) of normal pregnancy (p>0.05). History of preeclampsia in family was not observed in any subject of the two groups.

Biochemical variables: Serum calcium concentration of preeclampsia group (8.28±.42) and normal pregnancy (8.42±.39) varied significantly (p<0.05). On the other hand serum creatinine concentration didn't show significant difference (1.13±.27 vs 1.05± .21, p>0.05). Studies of Saudan et al[22] supported our results of serum calcium but not that of serum creatinine. This variation in serum creatinine concentration from other studies is probably due to the exclusion criteria in the present study, patients of preeclampsia with complications (for example renal failure) were excluded from the study. Significantly higher serum

Uric acid concentration was observed in preeclampsia patients (6.76± 1.99 vs 3.86± .75, p<0.01). Previous Research works by Saudan et al[22] and Qublan et al[9] had similar results indicating possibilities of serum uric acid to be considered as an important factor for preeclampsia.

Regarding urinary biochemical in the present study we estimated spot urinary calcium concentration (mg/100ml), spot urinary creatinine concentration (mg/100ml) and spot urinary protein concentration (mg/100ml). From these results we calculated spot urinary calcium/creatinine (mg per 100 ml/mg per 100ml) ratio. We found that women with preeclampsia didn't differ significantly from normal pregnant patients in respect of urinary creatinine concentration (60.45±32.13 vs 53.11 ± 20.17, p>0.05). But the results were significant in case of spot urinary calcium and protein concentration. Women with preeclampsia showed reduced excretion of calcium in comparison to normal pregnant women when spot urinary calcium concentration was evaluated (3.45 ±2.61 vs 7.39± 2.80, p<0.001). This is in accordance with the observations made by previous authors. Taufield et al[27] from the study of 40 women in third trimester, were the first to conclude that pre-eclampsia is associated with hypocalciurea, probably due to increased tubular reabsorption of calcium and decrease in glomerular filtration rate. Huikeshoven and Zuiderhoudt[16] also studied urinary calcium excretion in 41 women and found a significant decrease. Sanches-Ramos et al[31] confirmed previous finding of altered urinary calcium excretion in pregnant woman with pre-eclampsia. Vural et al[32] conducted a case control study with 59 patients and concluded that hypocalciuria may be an important feature of pre-eclampsia. Malas et al[28], Hwang et al[33] and Bilgin et al[25] from their studies concluded that hypocalciuria is an important feature of pre-eclampsia and 24 hrs urinary calcium excretion or calcium/creatinine ratio in random urine samples is a reliable index of pre-eclampsia. Results of the present study are consistent with previous ones.

In the present study we compared spot urinary calcium to creatinine ratio of normal pregnant woman and pre-eclampsia and the result was highly significant. The ratio reduced in preeclampsia patient in comparison to normal pregnant (.06±.05 vs .13±.06, p<0.001). These results correlate very well with several previous studies. Kazerooni et al1 conducted a similar study with 102 patients and result was in favor of significantly lowered calcium/creatinine ratio in preeclampsia. Similar were the outcome of research work by Szmidt-Adjide et al[13] who also revealed results in favor of the present proposition in a case

control study consisting of 47 preeclampsia women and 50 controls. Rodriguez et al[19] suggested that calcium/creatinine ratio may be useful in predicting preeclampsia and evaluated the calcium creatinine ratio in 88 normotensive pregnant women. The patients were between 24-34 weeks gestation and first morning urinary samples were tested. Similar findings by Ritz et al[26] demonstrated a predictive nature of spot urine calcium/ creatinine ratio in preeclampsia. Previous studies[29] showed that there is a good correlation between 24 hours urinary calcium excretion (mg/24 hrs) and calcium to creatinine ratio of a single voided morning urine sample (mg/dl). Gokce et al[30] also demonstrated strong correlation between spot urine calcium/creatinine ratio and 24 hours urinary calcium estimation in adults and concluded that estimation of calcium/creatinine in a single voided random urine specimen is a simple, cost effective and reliable method of estimating total urinary calcium excretion. This observation has also been made by Huikeshoven and Zuijderhovdt[16] and they also showed that this correlation was better than the one between the calcium concentration of a single voided sample and 24 hour calcium concentration. From these result we can come to the conclusion that spot urinary calcium creatinine ratio is a very useful indicator of hypocalciuria. It is very important to point out that hypocalciuria in observed in many studies with preeclampsia patients[27,30-32]. Thus spot urinary calcium/creatinine ratio can be considered to be a very important and valuable predictor in the care and study of pregnant patients with preeclampsia. We also compared spot urinary protein of both groups which show highly significant variation.

A correlation analysis was done between spot urinary calcium/creatinine ratio and other variables. It correlated significantly and positively with spot urinary calcium concentration (r=0.64, p<0.001) and correlated negatively with spot urinary protein concentration (r=0.28, p<0.01).

Conclusion

The results of the present and several other studies reveal that reduced urinary calcium excretion or hypocalciuria may be an important feature of preeclampsia. It requires 24 hrs urine calcium estimation which is cumbersome, time consuming, costly and non compliant. But it has been observed that spot urine calcium/creatinine ratio decreases in preeclampsia and significantly correlate with urinary calcium excretion. So spot urinary calcium to creatinine ratio measurement can be a very good alternative to hypocalciuria measurement. Spot urinary calcium to creatinine ratio can be a good predictor of preeclampsia.

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