fetal growth retardation and infant morbidity associated with premature delivery.[4] It is estimate that 20 to 25 percent of total prenatal mortality is caused by pregnancy-associated hypertensive disorders.[5]

Women with Preeclampsia are at increased risk for life-threatening complications like eclampsia, pulmonary oedema, cerebral hemorrhage, acute renal failure, abruptio placenta and disseminated intravascular coagulation (DIC).[6] Several authors have reported reduced urinary excretion of calcium during preeclampsia and for several weeks prior to the onset of clinically apparent disease. In addition, abnormal intracellular calcium metabolism in platelets and red blood cells has been demonstrated in women with preeclampsia as compared with normotensive pregnant women.[7]

High calcium intake is associated with increased serum calcium levels, lower parathyroid hormone concentration and a reduction in renal calcium re-absorption – all of which act to increase urinary calcium excretion – as well as with lower blood pressure and lower rate of hypertensive disorders of pregnancy.[8-11] By the lowering serum levels of parathyroid hormone, calcium supplementation could reduce intracellular calcium concentrations in vascular smooth muscle cells, diminishing their responsiveness to pressure stimuli and, therefore, lowering blood pressure.[12] One study showed that oral calcium lowered parathyroid hormone, reduced intracellular free calcium and decreased vascular endothelin production, resulting in vasodilatation and compensatory stimulation of the rennin-angiotensin system[13]. Several epidemiological studies in pregnant women and in normotensive and hypertensive subjects consistently showed an inverse relationship between calcium intake and blood pressure.[13]

Considering the above facts, this study was carried out to analyze the serum calcium level in preeclampsia as well as in healthy pregnant normotensive women. The result might stimulate further studies to find out whether dietary or pharmacological supplementation of calcium would be effective in reducing the incidence and /or severity of preeclampsia.

General objective was to compare the serum level of calcium in preeclampsia and normal pregnant women and Specific Objectives was to determine the serum calcium level in pregnant women and in patients with Pregnancy Induced Hypertension and to compare between the two groups.

Methods:

This was a case control observational study. This study was carried out in the department of Obstetrics and Gynaecology in Dhaka Medical College Hospital (DMCH). From 1st July'2016 to 31st December'2017 (18 Months). Purposive sampling method was used to select a total number of 100 participants for the study, with 50 pregnant normotensive women (control) and 50 women suffering from preeclampsia (case) included in this study.

Sample size was calculated using following formula: - n= z2pq/d2, n=the desired sample size z= the standard normal deviate usually set at 1.96 which corresponds to the 95% confidence level, p= the proportion of the target population estimated to have a particular characteristic. Here p=0.5 (assumed 50%) q = 1-p, d = degree of accuracy desired, usually set at 0.05, Calculating the above formula same size was n = 384 (estimated sample size). It was observed that 20 to 30 patients with preeclampsia comes in DMCH in each month. The duration of study is six month and the population size is roughly estimated 130. If N is less than 10,000 the required sample size was smaller. In this case final sample estimated by using the following formula. (targeted sample size),Where nf = the desired sample size, when population is less than 10,000. n = the desired sample size, when population is more than 10,000. N = the estimate of population size. Targeted sample size = 100.

Five milliliters of venous blood were collected from each of the case of preeclampsia as well as from the control subjects (normotensive pregnant women), with a disposable syringe, by peripheral venipuncture, taking full aseptic precaution. Blood was drawn for single time from each of the study subjects. From the cases and the control pregnant women, blood samples were collected during their antenatal period. In case of blood samples were allowed to clot and serum were separated by centrifugation at 300 rpm for 5-10 minutes.

Determination of serum calcium[14] was performed by colorimetric assay with endpoint determination and sample blank by Micro flow Cell Photometer AE – 100F Erma in the Department of Biochemistry, DMCH. The principle and procedure for determination of serum calcium have been shown in Appendix – III.

The data was collected by direct visits and examinations of the patients. Data collecting tools are-

1) History taking.
2) Clinical examination and relevant biochemical tests.
3) Close monitoring and record of different parameters of hypertensive and normotensive pregnant women.

The purpose of the study was explained to all included subjects in the local language. Age was estimated in nearest full years as stated by the patients. Occupation was divided into- Housewife/student/service/others.

Socioeconomic status was categorized as low/mid/rich. Spot record of blood pressure was done in the sample groups within 24 to 28 weeks of gestations. 5ml of venous blood was aseptically collected by veni-puncture from median cubital vein and was sent to the laboratory for measurement of calcium level in the serum.

Age, Parity, Socioeconomic status, occupation, Blood pressure, Proteinuria, Perinatal outcome, Maternal outcome were the Variable measures.

Before starting the study, permission was taken from Ethical committee. The study was a non-interventional procedure. Written consent was taken from each subject. Complication that may arise (e.g. infection) during veni puncture was tried to avoid by using full aseptic precaution and sterile needle. Confidentiality was strictly maintained.

Using appropriate computer software program, data was processed. Statistical analysis was done by appropriate statistical study. A P value <0.05 was taken as minimum level of significance.

Results:

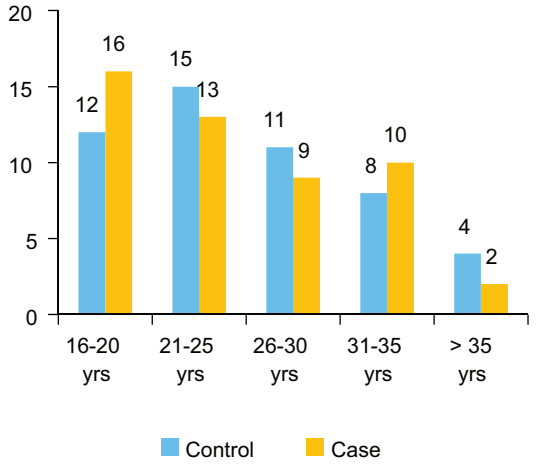

Figure 1 Shows that among the control 30% was belongs to 21-25 years age group and among the cases 32% was belongs to 16-20 years age group. In case of control mean (SD) age was 23.11± 5.9 years and for the case group mean age was (SD±) 24.5±6.3 years.

Table I shows that out of 50 control women, 22 (44%) were primigravida and 28 (56%) were multigravida, whereas in the 50 preeclamptic women (case), 35 (70%) were primigravida and 15 (30%) multigravida. Significant higher number of women were primigravida in the case group (P<0.05).

Table-I

Gravidity Distribution of the study subjects

Gravidity	Control (N=50)		Case (N=50)		P
	No.	%	No.	%	value[a]
Primi	22	(44.0)	35	(70.0)	**<0.05s**
Multi	28	(56.0)	15	(30.0)	

a = Chi – square test (χ^2)
s = Significant

Table II shows that statistically there was no significant difference between the mean (SD±) gestational age of control (35.40±2.67years) and case (34.57±3.19 years).

Table II

Gestational age of the study subjects

Group	n	Range(weeks)	Mean±SD	P value[a]
Control	50	29.0-40.0	35.40±2.67	**>0.30 ns**
Case	50	28.0-40.0	34.57±3.19	

a = Unpaired Student's t test

ns = Not significant

Table III shows that family history of hypertension was present in 14 (28%) women of control group compared to 18 (36%) of case group. The distribution was not statistically significant.

Table III

Family history of hypertension of the study subjects

Family history	Control (N=50)		Case (N=50)		P
of Hypertension	No.	%	No.	%	value[a]
Present	14	(28.0)	18	(36.0)	>0.10 ns
Absent	36	(36.0)	32	(64.0)	

a = Chi – square test (χ^2)
ns = Not significant

Table IV shows antenatal care was regular in 34(68%) vs 4(08%), irregular in 7 (14%) vs 10(20%) and none in 9 (18%) vs 36(62%) control and case group, respectively. Significantly higher number of women of case group had no antenatal care during their present pregnancy than that of control.

Table IV

Status of antenatal care of the study subjects

Antenatal care	Control (N=50)		Case (N=50)		P
	No.	%	No.	%	value[a]
Regular	34	(68.0)	04	(08.0)	<0.05 s
Irregular	07	(14.0)	10	(20.0)	
None	9	(18.0)	36	(72.0)	

a = Chi-square test (χ^2)
s = Significant

Table V shows proteinuria was found nil in all 50 (100%) women of control group. In the case group, level of Proteinuria was 2+ in 23 (46%), followed by 3+ 18 (36%) and 1+ in 9 (18%). Status of Proteinuria was found to be significantly high among women of case group than that of control.

Table V

Status of Proteinuria of the study subjects

Proteinuria	Control (N=50)		Case (N=50)		P
	No.	%	No.	%	value[a]
0 (0.1 g/L)	50	(100.0)	00	(00.0)	<0.01s
1+ (0.3 g/L)	00	(00.0)	09	(18.0)	
2+ (1.0 g/L)	00	(00.0)	23	(46.0)	
3+ (3.0 g/L)	00	(00.0)	18	(36.0)	

a = Chi – square test (χ^2)
s = Significant

Table VI shows both systolic (159.0 9.08 vs 110.9 7.05 mmHg) and diastolic (103.80 7.39 vs 74.9 5.01 mmHg) blood pressure were significantly very high among cases in comparison to control (P<0.001).

Table VI

Status of blood pressure among the study subjects

Group	n	Range (mmHg)	Mean±SD (mmHg)	P value[a]
Systolic blood pressure				
Control	50	100.0-120.0	110.9±7.05	<0.001s
Case	50	140.0-200.0	159.0±9.08	
Diastolic blood pressure				
Control	50	70.0-80.0	74.9±5.01	<0.001s
Case	50	90.0-120.0	103.80±7.39	

a = Unpaired Student's t test
s = Significant

Table VII shows that mean (SD) serum calcium level in control group was 9.40 .66 (range 8.1-10.6) mg/dl and in case group was 6.57 .87 (range 5.6-8.0) mg/dl. Mean serum calcium level was found to be significantly low in women of case group in comparison to control (P<0.01).

Table VII

Serum calcium level of the study subjects

Group	n	Range (mg/dl)	Mean±SD (mg/dl)	P value[a]
Control	50	8.1-10.6	9.40±.66	<0.01s
Case	50	5.6-8.0	6.57±.87	

a = Unpaired Student's t test
s = Significant

Discussion:

Calcium homeostasis is an important aspect of maternal and fetal physiology during gestation, since fetal bone mineralization requires adaptive adjustments in maternal calcium regulation. In the third trimester, calcium is deposited in the fetal skeleton at the rate of 200 mg/day. In addition, women double their urinary excretion of calcium in the third trimester. Recent studies have implicated alterations in calcium metabolism in the pathogenesis of hypertension during pregnancy. Deficiencies in calcium intake have been linked to Preeclampsia/Eclamsia. A hypocalciuria and deviations of levels of 1,25 – dihydroxy vitamin D and parathyroid hormone have been shown in women with Preeclampsia.This study was carried out to determine the levels of serum calcium in control (apparently healthy) and case (Preeclamptic) pregnant women as an effect of serum calcium for the development of Preeclampsia. About the parity, we have found that significantly higher number of women were primigravida than that of multigravida (P<0.05) (Table –I). This finding is consistent with the findings of Dutta[15], in which he has found the incidence of Preeclampsia in primigravida is about 10 percent and in multigravidas' 5 percent. The gestational age was studied from 28 to 40 weeks both in control and Preeclamptic women. No significant difference was found between the mean (±SD) gestational age of control than that of the Preeclamptic women (P>0.30 ns) (Table – II). No such study had been identified so far. About family history of hypertension, no significant development of preeclampsia was found (P>0.10 ns) (Table – III). This is consistent with the findings of Prenila[16], in which the author has identified it as a predisposing factor to develop Preeclampsia, but she has not mentioned

whether it is significant or not. There is a significant relation of antenatal care with the development of Preeclampsia. In our study, we have found significantly higher number of women of preeclampsia has no antenatal care during their present pregnancy than that of control (P<0.05s) (Table – IV). This is consistent with the finding of Arulkumaran and Biswas[17], in that they have found women with no risk factors prior to pregnancy become 'at risk' if they develop obstetric disorders such as ante- partum hemorrhage, pregnancy – induced hypertension or diabetes. Hence, not only initial surveillance but routine antenatal care is important to identify those at risk. The level of proteinuria in control and Preeclamptic pregnant women and status of Proteinuria was found to be significantly high among women of case group than that of control (P<0.01s) (Table – V). This is consistent with the findings of Campbell and Lees[18], in which they have identified relatively specific relation of proteinuria with Preeclampsia. The elevation of systolic and diatolic blood pressure were significantly high among cases in comparison to control (P<0.001s) (Table – VI). This is consistent with the finding of Roberts and Redman1, in which they have found in preeclampsia that there is rise of blood pressure of >15 mmHg diastolic and >30mmHg systolic from measurement in early pregnancy or to >140/ 90 mmHg in late pregnancy if no early reading is available. Main study was to find out the level of low serum calcium on Preeclampsia. The study had shown that serum calcium level was significantly low in Preeclampsia than that of control pregnant women (P<0.01s) (Table – VII). This is consistent with the finding of Hojo and August[19], in which they have found that calcium deficiency is associated with preeclampsia. Beliza et al.[20] have identified that calcium supplemented group showed a significant decrease of diastolic blood pressure, calcium intake seems to be inversely correlated with the incidence of Eclampsia and a reduction pregnancy – induced hypertension with calcium supplementation. This low serum calcium found in our study may be due to intake of diet low in calcium as the Preeclamptic women belonged to low socioeconomic classes.[21] Aruna Patel, Brijesh Singh, Arun Patel, Manoj Sharma had also found that Mild PIH is associated with significant hypocalcaemia compared to normotensive pregnant patient. Also severe PIH is associated with significant change compared to normotensive pregnancies. So, their study advocates the value of serum Ca++ a marker of pregnancy complicated by hypertension or severe pre-eclampasia.[22] In the study of Indumati V, Kodliwadmath M V and Sheela M K - a significant decrease in serum total and ionized calcium levels was seen in the normal primigravida cases as compared to the non-pregnant controls, with a further highly signifcant decrease in the PIH cases as compared to the normal pregnancy cases. This indicates an association between calcium defciency and PIH.[23] Chanvitya Punthumapol and Boonsri Kittichotpanich had also found low serum calcium level in preeclamptic patients than normal pregnant women.[24]

Conclusion:

There is a significant association between hypocalcemia and preeclampsia, which is not usually found in normotensive pregnant women. We should give routinely calcium supplementation to the Preeclamptic patients in our country at the recommended dose that is 2gm per day and ensure that they are taking it properly by regular antenatal checkup and monitoring blood pressure. This will help us to evaluate the rationality of using calcium in prevention of Preeclampsia. We have to use calcium in preconceptional period which can help to prevent preeclampsia or other hypertensive disorders in pregnancy and will reduce maternal and perinatal mortality and morbidity. More studies have to done in this respect.

Limitations of the study:

Sample size was small. Hospital stay is only for a short period due to various reasons including shortage of beds. So, all the maternal and perinatal outcome could not be estimated.

Source of funding: self

Conflict of interest: None declared

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