ESTIMATION OF SERUM CALCIUM IN NORMAL PREGNANCY, PREECLAMPSIA AND ECLAMPSIA

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Summary

Estimation of serum calcium in three trimesters of pregnancy, preeclampsia and eclampsia to determine whether its alteration has any role in maintenance of normal pregnancy and occurrence of pregnancy induced hypertension (PIH). It is a prospective cross-sectional study where serum calcium level has been estimated in 148 women (15-35 years) in four different groups (Non-pregnant control, normal pregnancy in three trimesters, Preeclampsia and Eclampsia) by a chemical assay using O-Cresolphthalein complexone method. Reading was taken in a spectrophotometer & statistical analysis was done by Student's unpaired T test. **(i)** Serum calcium level in pregnancy was within a lower range compared to control group (8.42±0.04 – 8.51±0.03 in pregnancy Vs 10.05± 0.06 - 10.26±0.08 in control), progressively decreasing with increasing gestational age. **(ii)**There was significant decline in serum calcium level in preeclampsia compared to third trimester of gestation**.(iii)** Eclampsia group exhibited lowest serum calcium level**.(iv)** Difference in serum calcium level in preeclampsia and eclampsia was not statistically significant. Various studies ascertain calcium level in pathogenesis of PIH but results differ. Present study suggests significant lowering of serum calcium level in PIH; cause being multifactorial. Calcium has direct and indirect effects on smooth muscle contraction. Hypocalcaemia leading to vasoconstriction may precipitate preeclampsia and eclampsia. Study clearly indicates association of hypocalcaemia with PIH. Routine estimation of serum calcium level may be useful as diagnostic marker in high risk pregnancies and supplementation of calcium to all pregnant mothers may reduce the risk of developing preeclampsia.

Key words: *calcium, eclampsia, preeclampsia.*

Introduction

Hypertensive disorders occurring in 5-10% of total pregnancies is a major cause of maternal and foetal morbidity[1] Maternal mortality due to eclampsia varies from 2-6% and contributes to 10-15 % of total maternal deaths in India[2] Inspite of intensive research, the exact cause of pregnancy induced hypertension (PIH) still remains an enigma. Alterations in systemic haemodynamics and cellular cation metabolism like decrease in extracellular Ca^{++} and Mg^{++} has been emphasized in different studies[3,4,5] .The potential involvement of intracellular Ca^{++} in the vascular abnormalities found in PIH indicate the association of extracellular Ca^{++} metabolism with these complications. Indirect evidences obtained from several studies reveal a reduced incidence of preeclampsia in patients supplemented with oral calcium [6,7,8,9.] To define the role of Ca^{++} in the pathophysiology of preeclampsia, various workers have estimated the level of serum Ca^{++} but the results vary. With this background, the present study has been undertaken to investigate any possible changes in serum Ca^{++} in normal pregnancy and pregnancy complicated with preeclampsia and eclampsia which might throw light on the pathogenesis of the PIH.

Patients and methods:

The present study was undertaken by the Department of Pharmacology, V.S.S. Medical College, Burla and Department of Pharmacology Medical College, Kolkata. Due permission of the Institutional Ethical Committee of V.S.S. Medical College, Burla was taken. The patients who entered the study were selected from both outpatients' department and inpatients' wards of the Dept. of Obstetrics & Gynaecology V.S.S. Medical College, Burla. Written informed consent was duly obtained from the participants in their own language. The period of study was from November 2004 to June 2006. It is a prospective cross-sectional study in which serum Ca^{++} level has been estimated in four different groups of women (15-35 years of age) as specified in Table 1

Inclusion criteria

- **Hypertensive group:** Previously normotensive patients with diastolic blood pressure (DBP) ≥ 90mmHg and systolic blood pressure (SBP) ≥ 140 mmHg on two separate occasions (four hours apart) beyond 20 weeks of gestation[10]
- **Preeclamptic group:** Pregnancy induced hypertension (PIH) with concurrent proteinuria >300mg /24 hours and edema.
- **Eclamptic group:** Preeclampsia with fits in the third trimester of pregnancy.

Exclusion criteria

- Patients with history of chronic or labile hypertension or known renal disease.
- Severe prolonged diarrhea, mal-absorption syndrome, diuretic therapy, anticonvulsant therapy and mental illness.
- Patients with oral supplementation of calcium.

Procedure

- The study population was arranged according to gestation period, gravidity and hypertensive complications of pregnancy.
- Brief history of the subjects was taken and relevant general examinations, laboratory reports of haemoglobin, urine for albumin were recorded.
- From each subject, two ml of venous blood was collected either during outpatients attendance or admission to the indoor ward before starting any medication. The blood samples were placed in glass test tubes and allowed to clot at room temperature. The supernatant fluid was centrifuged for 15 minutes and the serum thus separated was utilized for estimation of calcium.
- Serum Ca^{++} was estimated by a chemical assay using O-Cresolphthalein complexone method [11, 12], and the reading was taken in a spectrophotometer.
- Serum Ca^{++} level in normal pregnant women in third trimester, preeclampsia and eclampsia patients were compared with non pregnant control group.
- Statistical analysis was done by Student's unpaired T test, (sample size < 30). When the observations were more than 30, the same method was used to calculate Z value instead of 't'. p - value was determined from the t-table.

Results

Table I
The Grouping Pattern Of The Study Population Arranged According To Age, Gestation Period And Gravidity.

Variables	Group I Non-pregnant women (n=20)	Group II Normal pregnant women in different trimesters			Group III Pregnant women with pre eclampsia (n=40)	Group IV Pregnant women with eclampsia (n=24)
		1st (n=18)	2nd (n=20)	3rd (n=26)		
Age (years) Mean± SE	27.95±1.42	21.7±1.04	22.6±0.98	24.61±0.95	23.7±0.65	21.87±0.75
Gestational age(weeks)	-	10±0.4	20.2±0.67	35.5±0.7	28.5±0.62	36.2±0.65
Gravidity (%)						
G1	-	55.5	50	42.3	47.5	66.6
G2	-	22.2	20	30.7	35	20.8
G3& above	-	22.2	30	26.9	17.5	12 .5

Total study population consists of 148 subjects (15-35 years) who belonged to four groups as follows:

Group I- Non pregnant control (n=20),
Group II- Women with normal pregnancy in 3 trimesters (n=64),
Group III- Preeclamptic patients (n=40)
Group IV-Eclamptic patients (n=24).

Table -2
Age wise Distribution of Serum Calcium (mg %) Level in Non Pregnant Control Cases

Age (years)	No of cases	Serum Ca^{++} (mg%)	
		Mean± SE	P value
Upto 20	3	10.05± 0.06	<0.1
21-30	11	10.44±0.023	
Above 30	6	10.26±0.08	<0.1

P value of upto 20 *vs*. above 30 = < 0.1

Serum Ca^{++} level in control group lies within the normal range and there is no significant difference between the different age groups.

Table- 3
Age and Trimester wise distribution of Serum Calcium (mg %) Level in Normal Pregnancy

Age (years)	Serum Ca^{++} level (mg%)								
	Normal Pregnancy								
	1st. trimester			2nd. trimester			3rd. trimester		
	No. of cases	Mean± SE	P value	No. of cases	Mean± SE	P value	No. of cases	Mean± SE	P value
Upto 20	11	9.26±0.02	<0.1	8	8.9±0.03	<0.1	8	8.42±0.04	<0.1
21-30	5	9.3±0.02	<0.1	10	8.92±0.02	<0.1	15	8.49±0.02	<0.1
Above 30	2	9.33±0.09		2	8.94±0.06		3	8.51±0.03	
Total	18	9.3±0.01		20	8.92±0.01		26	8.48±0.01	

P= Age wise comparison
P of upto 20 vs> 30
= < 0.1 (1st. Trimester)
= < 0.1 (2nd. Trimester)
= < 0.1 (3rd. Trimester)

P1= Trimester wise comparison
1st. vs 2nd. trimester r= < 0.001***
2nd.vs 3rd. trimester = < 0.001***
1st.vs 3rd. trimester = < 0.001***

Serum Ca^{++} level in pregnancy was within a lower range in comparison to control group (8.42±0.04–8.51±0.03 in pregnancy Vs 10.05± 0.06- 10.26±0.08in control). The level progressively decreased with increase in gestational age. The fall in serum Ca^{++} was highly significant between the three trimesters of pregnancy. Serum Ca^{++} distributed in age wise manner in pregnant women did not show any significant difference.

Most consistent observation in the third trimester of pregnancy was the significant decline in serum Ca^{++} level in comparison to the non pregnant control for all the three age groups. There was a significant decline in serum Ca^{++} level in preeclampsia patients in comparison to the third trimester of gestation, in all the age wise arranged sub populations with a highly significant fall in all the age groups. Patients with eclampsia had lowest serum calcium amongst all the four study groups. However the difference in serum calcium level in preeclampsia and eclampsia was not statistically significant.

Discussion

The common hypertensive disorders of pregnancy include preeclampsia and eclampsia which is peculiar only to human pregnancy. Preeclampsia is characterized by development of hypertension (Diastolic blood pressure ≥ 90mmHg & systolic blood pressure ≥ 140 mmHg on two separate occasions, four hours apart) proteinuria (>300mg /24 hours) and edema after 12 hours of bed rest, beyond 20 weeks of gestation in previously normotensive patients. Eclampsia is its severe form complicated with fits and coma in third trimester of pregnancy[10]. It is very essential to understand the pathological changes occurring in the biochemical infrastructure in these conditions in order to find out the best possible ways to guide pregnancy and obstetric management. During pregnancy there is widespread adjustment in plasma level of some essential ions like calcium which is normally maintained in a delicate manner. There are a number of studies where the level of Ca^{++} has been ascertained, but the results differ widely, two of the studies[13, 14,] have reported a fall in serum Ca^{++} level.

Table- 4

Serum calcium (mg %) level in non pregnant control, normal pregnancy in 3rd. Trimester, pre eclampsia & eclampsia

Age (years)	Serum Ca^{++} level (mg%)										
	Non pregnant control		Normal Pregnancy in3rd. trimester			Pre eclampsia			Eclampsia		
	No. of cases	Mean ± SE	No. of cases	Mean ± SE	P^1	No. of cases	Mean ± SE	P^2	No. of cases	Mean ± SE	P^3
Upto 20	3	10.05 ± 0.06	8	8.42± 0.04	<0.001***	22	7.76± 0.06	<0.001***	16	7.61± 0.07	< 0.1
21-30	11	10.44 ± 0.23	15	8.49± 0.02	<0.001***	14	7.84± 0.03	<0.001***	07	7.65± 0.11	< 0.1
Above 30	6	10.26 ± 0.08	3	8.51± 0.03	<0.001***	04	7.9±0 .04	<0.001***	01	7.72	< 0.1
Total	20	10.33 ±0.13	26	8.48± 0.01	<0.001***	40	7.8± 0.06	<0.001***	24	7.68± 0.04	< 0.1

p^4 = upto 20 years=< 0.001***, 21-30 years= < 0.001***, more than 30 years =< 0.001***
p^1 – non pregnant vs normal pregnancy in 3rd. trimester
p^2 - normal pregnancy in 3rd. trimester vs pre eclampsia
p^3 - pre eclampsia vs eclampsia
p^4 - normal pregnancy in 3rd. trimester vs eclampsia

This gradual decrease was parallel to that of albumin which is the main Ca^{++} binding protein carrying about half of the total serum calcium. Some observed no difference in serum Ca^{++} level in pregnant and non-pregnant women[15]. This study was done to estimate the serum Ca^{++} level in the three trimesters of pregnancy as well as in preeclampsia and eclampsia to find out whether alterations in serum calcium level has any role in the maintenance of normal pregnancy and occurrence of PIH. The total serum Ca^{++} declined progressively and significantly throughout pregnancy with the advancement of gestational age. This is consistent with the findings of various workers [16, 17]. A similar significant fall in serum calcium in preeclampsia and eclampsia in comparison to third trimester of pregnancy was observed which suggests that PIH is invariably associated with significant lowering of serum Ca^{++} [5,18.] The cause of this hypocalcaemia may be multifactorial. Physiological changes in pregnancy itself may lead to hypocalcaemia **(i)** There is increased plasma volume in second and third trimester of pregnancy and **(ii)** relative early increase in glomerular filtration rate which leads to increased urinary loss of calcium [19] **(iii)** Another reason of calcium loss is obligatory transfer of calcium (30 mg approx.) to the foetus from the maternal system[20] **(iv)** Finally, increased maternal level of estrogen, other steroids and calcitonin may be involved in inhibiting parathormone induced bone resorption and thus further lowering of serum calcium [20]. Two randomized controlled trials show calcium supplementation significantly reduced blood pressure[21] which indicates an inverse relation between calcium intake and PIH. It was observed by some workers that risk of PIH is reduced with increasing dietary calcium intake [22] Similarly, a reduced incidence of preeclampsia was found in patients supplemented with oral calcium [8, 9]. Preeclampsia is associated with an exaggerated response to vasoactive hormones [23]

In addition to possible direct effect on smooth muscle contraction, calcium may also have an indirect effect by modifying responsiveness to or regulating synthesis of a number of vasoactive agents such as angiotensin II, endothelin, prostacyclin, nitric oxide[4]. This study clearly indicates the association of hypocalcaemia with PIH. Routine supplementation of calcium to all pregnant mothers definitely reduces the risk of developing preeclampsia.

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