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Clinical Significance of Low Plasma Calcium Levels in Pregnant Women with Pre-Eclampsia - A Nested Case-Control Study

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Abstract **Original Research Article**

Pre-eclampsia is one of the commonest causes of maternofoetal morbidity and mortality, it complicates 3-10% of pregnancy. The plasma levels of some biochemical parameters have been implicated in the pathogenesis of pre-eclampsia. The study aimed to compare plasma calcium levels in pre-eclampsia and control groups and assessed materno-foetal outcome. This was a nested case control study. Consecutively, consenting pregnant women were enrolled at gestational age of 18-22 weeks. They were followed up to observe those that will develop pre-eclampsia. Venous blood samples for plasma calcium estimation were obtained from subjects at recruitment and after developing pre-eclampsia. Four hundred and eighty-six (486) pregnant women were recruited out of which thirty seven patients (37) developed pre-eclampsia as cases and were matched with 37 apparently healthy controls**.** The mean plasma calcium was significantly lower in cases 1.72mmo/l (standard deviation (SD) 0.45) than control 2.08mmol/l (SD 0.39) at recruitment (P<0.001). A further reduction was recorded at diagnosis with values of 1.62mmol/l (SD 0.33) for cases and 1.99mmol/l (SD 0.18) for controls (P<0.001). There was significant statistical relationship between plasma calcium level at recruitment and degree of systolic hypertension, leg cramp in pregnancy and preterm delivery (P = 0.038, 0.02, and 0.007 respectively) as well as between plasma calcium level at diagnosis and preterm delivery (P = 0.024). In conclusion this study shows that hypocalcaemia predated pre-eclampsia and may be used to predict the development of the disease and possibility of preterm delivery.

Keywords: Pre-eclampsia, Plasma Calcium, Preterm Delivery, Leg Cramps.

INTRODUCTION

Pre-eclampsia is one of the commonest aetiologies of foetal and maternal morbidity and mortality [1]. It is characterized by onset of hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mmHg respectively on two occasions, at least 6 hours apart) and proteinuria (protein excretion of ≥ 300mg in a 24-hour urine, or a dipstick of ≥ 2+) that develop after 20 weeks of gestation [2]. The incidence of pre-eclampsia stands at 3- 10% globally [3]. The prevalence may be as high as 18% in developing countries [4]. It is known that babies born to mother with pre-eclampsia have a higher perinatal mortality, infant mortality and a 20% lower birth weights as well as more other neonatal complications [5]. Also pre-eclampsia/eclampsia still accounts for as high as 36.9% of maternal mortality in some part of Nigeria [6].

Pre-eclampsia is commonly referred to as the ‘’disease of theories’’ making its prevention and management an ongoing challenge worldwide [7]. The aetiology of this disease is poorly understood, some studies have reported that changes in levels of blood calcium in pre-eclampsia may play a role in its aetiology [8-11], while other studies failed to established a link between calcium levels and pre-eclampsia [12, 13].

Calcium is an intracellular cat ion, important for cellular metabolism such as muscle contractility, neuronal activity and cellular death. A proper balance between it and magnesium is vital to regulation of blood pressure. Calcium enables the blood vessels to contract while magnesium is required for the vessels to relax [14, 15].

Calcium plays a critical role in the functions of cardiac, vascular smooth muscle, skeletal muscle contraction as well as hormone secretion and neurotransmission [14]. It is known that the deficiency of calcium may lead to irritable nervous muscular symptoms, titanic convulsions, bleeding diasthesis and tissue exudation. Recent studies have implicated alteration in the calcium metabolism in the pathogenesis of hypertension in pregnancy [16, 17]. There are evidences consistent with a reduction in the risk of having pre-eclampsia when calcium supplement is administered [15].

Studies from different regions reported a decline in calcium levels during pregnancy [16-18]. This may be due to increase in demand for mother and growing fetus or increase renal excretion [18]. Calcium deficiency during pregnancy may cause pre-eclampsia, preterm delivery, low birth weight babies and leg cramps [14, 16, 17].

The possible link between plasma calcium level and pre-eclampsia, preterm delivery, low birth weight babies and leg cramps continues to be a subject of considerable debate. Most of the existing data emanated from outside sub-Saharan Africa. Hence, the need to observe the plasma calcium level early in pregnancy in our environment as it relates to the development of pre-eclampsia and its associated foeto-maternal complications.

MATERIALS AND METHODS

Study Design and Location

This was a nested case control study comparing relationship between those who developed pre-eclampsia and those who did not in Osogbo over eight months period. The study was carried out in the Departments of Obstetrics and Gynaecology of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo and Chemical Pathology of Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti.

Study Population

Apparently healthy pregnant women between 18-22 weeks of gestation receiving routine antenatal care in the hospital (LAUTECH) that satisfied the inclusion criteria were recruited into the study. All are of black race. Patients with pre-existing condition such as chronic hypertension, chronic renal disease, gestational diabetes, sickle cell anaemia, patients with multiple gestations, patients on calcium and or magnesium supplements, and patients who developed pre-eclampsia before recruitment were excluded.

Blood Sample Collection and Processing

About five milliliters (5mls) of venous blood was drawn from subjects using routine aseptic procedure of phlebotomy at recruitment and during the follow up. This was dispensed into newly opened, lithium heparin specimen bottle without obvious contamination. Each of these bottles was labelled serially for both the cases and controls. Samples were subsequently centrifuged at 3000Xg for 5 minutes and the supernatant plasma was then extracted into another plain specimen bottle. All the batches of plasma samples were kept frozen for maximum of 2 days before analysis.

Plasma calcium was measured using Ion Selective Electrode, Model ISE 6000, S/N: 04050213, SFRI sarl, Lieu-dit Berganton, 33127 St Jean d'lllac, France [19].

STATISTICAL DATA ANALYSIS

All information and results were recorded in a proforma, data obtained from the study was processed using statistical package for social science (SPSS) version 23. Frequency tables were made and results tested for statistical significance using the student t-test, chi-square test and multivariate analysis, the significant value was put at 5%

Ethical Issues

Ethical clearance for this study was obtained from the ethical review board of LAUTECH Teaching Hospital, Osogbo, Nigeria (Protocol Identification Number: LTH/EC/2017/07/292) and written informed consent was obtained from the participants.

RESULTS

Thirty seven patients (7.61%) developed pre-eclampsia out of 486 clients that were recruited for this study; the cases were carefully matched with 37 controls at similar gestational age, maternal age and parity. Majority (48%) of the study population was between ages of 20 and 29 years, majorly are of Yoruba ethnicity (90%). Most (34%) of the subjects were traders while 10% of them were unemployed, 65% were educated to tertiary level while 1.4% have no formal education. Other demographic characteristics of the study population are as shown in table 1.

There was no significant statistical difference in the mean age of the case and control groups 30.00 year (SD 5.06) and 30.08 year (SD 5.20) respectively ($P = 0.946$). Also there were no statistical differences between the body mass index (BMI) and estimated gestational age at recruitment of the study groups ($P > 0.05$). The mean systolic and diastolic blood pressure for the cases and controls at recruitment were not significantly different ($P = 0.59$ & 1.91 respectively). However at the point of diagnosis the mean systolic blood pressure was 161.08 mmHg (SD 8.0) for cases and 110.81 mmHg (SD 9.80) for the controls ($P < 0.001$) and mean diastolic blood pressure was 102.70 mmHg (SD 6.90) and 67.83 mmHg (SD 5.80) for the cases and the controls respectively ($P < 0.001$). This was significant and remained so at delivery for both

diastolic and systolic blood pressure (P < 0.001). These and other obstetrics characteristics of the study population are as shown in table 2.

Table 3 shows comparison of plasma calcium levels in cases and controls at recruitment and at diagnosis both of which were significant (P < 0.001). There exists a significant relationship between recruitment plasma calcium and preterm birth (P = 0.007) as shown in table 4, this difference remained significant after a multivariate analysis was performed to control for pre-eclampsia (P = 0.029). Table 5 presents significant relationship between low plasma calcium at diagnosis and preterm delivery (P = 0.024), however this difference became insignificant when a multivariate analysis was performed to control for pre-eclampsia (P = 0.316). Figure 1 illustrates trend in blood pressure in both group from recruitment to delivery.

This study also established a significant relationship between plasma calcium level at recruitment and degree of systolic hypertension on one hand and the development of leg cramp in pregnancy on the other. Twenty two (84.6%) out of 26 patients with low calcium level at recruitment later developed severe systolic hypertension as against 4 (44.4%) out of 9 patients with normal calcium level at recruitment (P = 0.038). Also 24 (57.1%) out of 42 patients who developed leg cramp had low calcium level at recruitment as opposed to 3 (13.0%) of the 23 patients with normal calcium level at recruitment that developed leg cramp in pregnancy (P = 0.02).

Table-1: Socio-demographic Characteristics of the Study Groups

Variables	Patients (%)	Control (%)
Age (years)		
<20	1 (2.7)	1 (2.7)
20-29	18 (48.7)	18 (48.7)
30-39	16(43.2)	16 (43.2)
40 and above	2 (5.4)	2 (5.4)
Total	37(100)	37(100)
Parity		
Nullipara	14 (37.8)	14 (37.8)
Para 1	18 (48.7)	18 (48.7)
Multipara	5 (13.5)	5 13.5)
Total	37	37
BMI		
<18.5	1 (2.7)	1 (2.7)
18.5-24.9	15 (40.5)	18 (48.7)
25.0-29.9	12 (32.4)	10 (27.)
30-34.9	6 (16.2)	6 (16.2)
35.0-39.9	3 (8.2)	2 (5.4)
Total	37(100)	37(100)
Delivery EGA		
<36	25 (67.6)	4 (10.8)
37 and above	12 (32.4)	33 (89.2)
Total	37(100)	37(100)

BMI = Body mass index; EGA = Estimated gestational age (weeks)

Table 2: The Demographic and Obstetrics Characteristics of the Study Population

Variables	Patients (n=37) Mean (SD)	Control (n=37) Mean (SD)	't' value	P value
Age (years)	30.00 (5.06)	30.08 (5.20)	-0.68	0.946
Parity	1.13 (1.13)	1.32 (1.08)	-0.73	0.465
BMI (kg/m^2)	27.2 (4.50)	25.2 (5.32)	1.75	0.84
Gestational Age (Recruitment)	20.37 (1.67)	20.10 (1.64)	0.70	0.486
Social Class	2.10 (0.73)	2.4 (0.86)	-1.73	0.87
Recruitment Systolic Bp (mmHg)	106.48 (11.15)	105.18 (9.36)	0.54	0.59
Recruitment Diastolic BP (mmHg)	64.05 (6.43)	66.10 (6.94)	-1.32	1.91
Diagnosis/control systolic BP	161.08 (8.00)	110.81 (9.80)	24.02	0.000
Diagnosis/control diastolic BP	102.70 (6.90)	67.83 (5.80)	23.40	0.000
Delivery systolic BP	144.32 (11.43)	122.10 (6.80)	10.14	0.000
Delivery diastolic BP	90.27 (7.60)	74.32 (6.0)	9.97	0.000

SD = Standard deviation; BMI = Body mass index; Kg/m^2 = kilogram per meter square; BP = Blood pressure; mmHg = Millimeter of mercury

Table-3: Comparison of Plasma Calcium Levels in Case and Control Groups

Parameters	Patients (n=37) Mean (SD)	Control (n=37) Mean (SD)	't' value	P value
Ca (mmol/l) at recruitment	1.72 (0.45)	2.08 (0.390	-3.684	0.000
Ca (mmol/l) at Diagnosis/control	1.62 (0.33)	1.99 (0.18)	-5.79	0.000

Ca= Calcium; mmol/)l = millimole per litre; SD = Standard Deviation

Table-4: Relationship between Plasma Calcium Level at Recruitment and Fetal Outcome

Fetal outcome	Low calcium level	Normal calcium level	High calcium level	Pearson Chi-square	P value
Preterm	23	4	2	9.947*	0.007
Term	19	19	7		
Total	42	23	9		
Low birth weight	14	3	2	3.27 †	0.195
Normal birth weight	28	20	7		
Total	42	23	9		
Live birth	41	22	9	0.503	0.778
Still birth	1	1	0		
Total	42	23	9		
SCBU Admission	7	2	1	0.829	0.661
No SCBU Admission	34	20	8		
Total	41	22	9		

** = likelihood ratio (10.5); † =likelihood ratio (3.49) Normal calcium level (2.05-2.43mmol/L); SCBU = Special Care Baby Unit*

Table-5: Relationship between Plasma Calcium Level at Diagnosis and Fetal Outcome

Fetal outcome	Low calcium level	Normal calcium level	Pearson Chi-square	P value
Preterm	26	3	5.06 *	0.024
Term	30	15		
Total	56	18		
Low birth weight	17	2	2.644 †	0.104
Normal birth weight	39	16		
Total	56	18		
Live birth	54	18	0.661	0.416
Still birth	2	0		
Total	56	18		
SCBU Admission	9	1	1.394	0.238
No SCBU Admission	45	17		
Total	54	18		

** = likelihood ratio (5.53); † = likelihood ratio (2.99) Normal calcium level (2.05-2.43mmol/L; SCBU = Special Care Baby Unit*

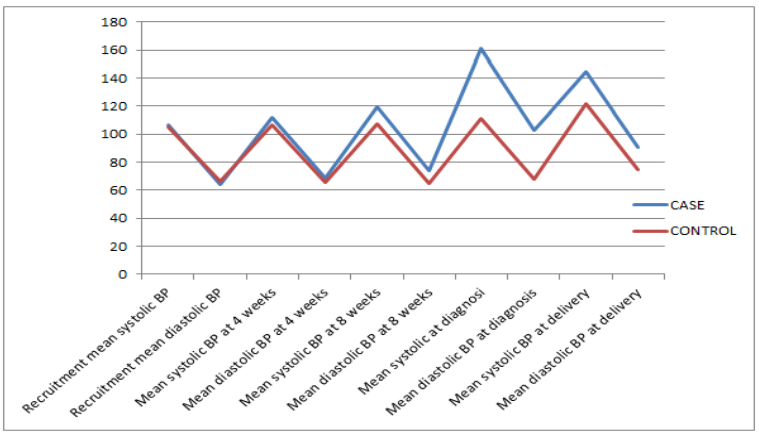

Fig-1: Blood Pressure Trends in Case and Control Groups from Recruitment to Delivery

DISCUSSION

This study was conducted to established relationship between plasma calcium levels in pregnancy and development of pre-eclampsia and other foeto-maternal outcome. Similar to findings in other studies [13, 17] our study showed no statistically significant relationship between maternal age and pre-eclampsia but contradicted the findings by Macdonald-wallis *et al.* [20] which was a very large longitudinal cohort study with sample size of 11,651 as compared to our sample size. The majority of patient in this study were of low parity in both study groups and this is similar to finding of some studies in the literature [13, 16, 17]. In this case our study further reaffirmed the known fact that pre-eclampsia is common among women of low parity especially the primigravidae. However history of previous pregnancies was not taken into consideration in those multiparous women that developed pre-eclampsia in this study, history of pre-eclampsia is a known a risk factor. Women with history of pre-eclampsia have increased risk of having pre-eclampsia in subsequent pregnancies especially those with long inter-pregnancy interval [21].

The mean gestational age at recruitment for case and control were similar, this ensured good comparison between the two groups. Studies have suggested gradual reduction in calcium levels as pregnancy advances [16-18], likewise; the systolic and diastolic blood pressure at recruitment for the two groups was not significant. There was no significant difference between the BMI of the case and the control groups in this study. This is in consonant with the findings of other studies [13, 14] and it makes our comparative analysis unbiased. However Poorolajal and Jenabi [22] found significant relationship between BMI and pre-eclampsia in their study which was a meta-analysis as against our nested case control study.

The possible link between calcium levels in pregnancy and development of pre-eclampsia, leg cramp and preterm delivery continues to be controversial. Some studies have established relationships [8-11, 14-17], while findings from some other studies are opposite, they did not establish relationship between plasma calcium level and pre-eclampsia [12, 13].

The mean plasma calcium level at the point of recruitment in our study was low for those patients that eventually developed pre-eclampsia while that of the control groups were normal, this was statistically significant (P<0.001), and similar to the finding of Ositadinma *et al*. [17] in that the mean serum calcium level in second trimester in the pre-eclampsia / eclampsia groups in their study was low compared to that of the control group. However, the difference in their study was not statistically significant; this may be as a result of small sample size recruited in second trimester. Their study recruited thirteen patients while the present study recruited thirty seven patients.

Further reduction in the mean plasma calcium level was observed in both groups at the point of diagnosis, but more pronounced in the pre-eclampsia group and was statistically significant (P<0.001). This agrees with the findings of other investigators [8, 14, 17] but differs from the Korle – Bu study that shows no significant difference between women with pre-eclampsia and apparently healthy pregnant women [13] The reason for this disparity may be due to regional differences in the two study sites, study designs (nested case control vs. comparative cross sectional study) and also the sample size in our study was higher.

Plasma calcium level at recruitment shows significant relationships with severity of systolic hypertension (P < 0.05) which was similar to the findings by Ephraim *et al*. [16] but not with diastolic hypertension (P > 0.05) which contradict the findings of Ephraim *et al*. [16]. Our study was a nested case control study streamlined to women that developed pre-eclampsia only, as compared to that of Ephraim study which was a case control study that considered other forms of gestational hypertensive disorders. We also established a significant relationship between development of leg cramps later in pregnancy and plasma calcium level at recruitment (P = 0.02). Although results from previous studies including a Cochrane database of systematic review shows that calcium supplements have no significant effect on leg cramps in pregnancy [23, 24], further researches are needed in this regard to further ascertain this, as socio-economic, environmental, cultural and dietary factors may pose a significant difference.

Similarly plasma calcium level at recruitment shows significant statistical relationship with preterm delivery (P = 0.007), this difference remained significant even after a multivariate analysis was performed to control for pre-eclampsia (P = 0.029), this was similar to the findings by Santorelli *et al*. [25]. There are evidences in a study that suggest calcium supplementation in high doses (minimum of 1g per day) prevents preterm delivery especially in women from region with low calcium intake [26]. Plasma level of calcium at diagnosis showed statistical relationship with preterm delivery (P = 0.024) which was in contrast to the findings of Demirturk *et al.* [27], they found no association between maternal plasma calcium level and preterm delivery in their comparative study. The significance in our study however disappeared after a multivariate analysis to control for pre-eclampsia

(P = 0.316). This may suggest that the preterm delivery was associated with intervention in the management of pre-eclampsia of which the current definitive solution is delivery of the baby irrespective of the gestational age once there are indications.

The strength of our study include the longitudinal nature of the research, baseline plasma calcium were assessed before the cases developed pre-eclampsia and clearly shows that low plasma calcium levels predate pre-eclampsia and that preterm delivery is significantly related to plasma calcium levels in second trimester than plasma calcium level at delivery. The limitation of this study includes the fact that it was hospital based study with its inherent self selection bias; the result might be different if it was a community based research. Also the study was conducted in a single facility which might limit generalization of its findings. Despite the limitations, the research adds important information to available data on relationship between low plasma calcium levels and development of pre-eclampsia and preterm delivery.

CONCLUSION

In conclusion Hypocalcaemia predated pre-eclampsia and may be used to predict the development of the disease and possibility of preterm delivery. Measurement of this biomarker early in pregnancy may be advocated for prevention of pre-eclampsia and its associated maternal and fetal complications.

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