



SERUM CALCIUM DYNAMICS IN PREGNANCY: NORMOTENSIVE VS. PRE-ECLAMPTIC WOMEN

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Abstract: Pre-eclampsia is a common cause of maternal and perinatal morbidity and mortality. Studies have linked the condition to hypocalcaemia. The objective of this study was to determine the ionised calcium levels in pre-eclamptic women and compare with that of their normotensive counterparts. Two hundred and eighty-four (284) women who completed the study were analyzed. Half of the participants were pre-eclamptic and the other half were normotensive. The participants in the two groups were matched for age, gravidity, and gestational age. Venous blood was obtained from the antecubital vein and analyzed for ionised serum calcium. The mean ionised calcium concentrations between the two groups were compared using the student t-test. Results show the mean ages of the participants in the pre-eclamptic and normotensive groups were 24.47 ± 6.63 and 25.19 ± 5.71 years, respectively ($p = 0.33$). Pre-eclamptic subjects had significantly lower ionised serum calcium than the normotensive pregnant women (1.03 ± 0.11 mmol/L vs. 1.14 ± 0.09 mmol/L, $p < 0.001$). There was also a significantly increased risk of hypocalcaemia amongst the pre-eclamptic pregnant women than the normotensive counterpart (OR=14.11, 95% CI: 6.16-32.35). Findings from this study suggest lower ionised calcium levels in serum and hypocalcaemia are associated indicators of preeclampsia among pregnant subjects diagnosed with the condition. Routine calcium supplementation is therefore recommended in pregnancy to reduce the risk of PE.

Keywords: Hypocalcaemia, Ionised calcium, Normotensive, Pre-eclampsia, Serum calcium

INTRODUCTION

Pre-eclampsia (PE) affects about 2.7-8.2% of all pregnancies (Abalos *et al.*, 2013), and its occurrence is seven times higher in developing nations (2.8% of live births) than in developed countries (0.4%) (Salam *et al.*, 2015). The disease accounts for 10%–15% of direct maternal deaths and 25% perinatal mortality (Say *et al.*, 2014). In Nigeria, the North eastern sub-region has one of the highest incidences of PE and contributes to the low maternal and child health indices, which is the ranked second least in the country (Kana., 2015; NDHS., 2018). A study conducted in Maiduguri found that PE was the number one cause of maternal mortality (Audu *et al.*, 2010) in the region. In addition, PE survivors have a higher risk of hypertension, coronary heart disease, and stroke (Bellamy *et al.*, 2007). Furthermore, the offspring of preeclamptic pregnancy are at increased risk of stroke and hypertension in adulthood (English *et al.*, 2015). Prevention of PE is essential in view of the grave consequences to women's

health. So far, the prevention of PE remains a challenge, largely because of poor understanding of its aetiology, low predictive value of current screening tests, and diverse presentations of the disease (Bezerra Maia *et al.*, 2012). Therefore, preventive measures must be targeted at women at high risk of developing the condition based on identified risk factors and other reliable screening tools. This is necessary for cost-effective antenatal monitoring and use of prophylactic treatment.

The role of calcium in PE was first inferred by scientists because of the low incidence of PE and Eclampsia amongst Guatemala Indians and Ethiopian women with high levels of calcium intake (Hamlin, 1962; Belizan and Villar, 1980). In addition, studies have linked PE to hypocalcaemia (Sharma, 2014; Feng, 2016; Priyanka and Rajini, 2017; Agu and Okeudo, 2018; Parvin *et al.*, 2021; Vanaja *et al.*, 2021;). Hypocalcaemia may increase blood pressure by stimulating renin release and parathyroid hormone leading to increased intracellular calcium in the vascular smooth muscle, causing vasoconstriction (Ritchie and King, 2000; Hacker *et al.*, 2012). On the other hand, increased calcium reduces parathyroid hormone release and encourages efflux of calcium from the smooth muscles resulting in uterine relaxation and a fall in blood pressure (Repke, 1994). A randomised controlled study on the effect of calcium supplementation on uteroplacental and fetoplacental blood flow in low calcium intake mothers showed a significantly lowered resistance index by Doppler in uterine and umbilical arteries amongst women on calcium supplementation compared to the control (Carroli *et al.*, 2010). However, most of the studies used total serum calcium instead of ionised active calcium.

A few studies showed no difference in the serum calcium levels between women with PE and normotensive women (Kant *et al.*, 2019; Chukwunyere *et al.*, 2020). Some of the suggested explanations for these contradictions include the difference in the population studied, nutritional status of the subjects, blood sampling methods, and use of total calcium as opposed to ionised calcium, which is the free and biologically active form of calcium in the body. The majority of the body's calcium is in the bones (99%). The nonosseous calcium (1%) thus comprises protein-bound calcium (mainly albumin and globulins in the serum and calmodulin in the cells), free (or ionised) calcium, and ionic-bound calcium (complexes of phosphate, carbonate, and calcium oxalate), representing 50%, 40% and 10% of the circulating calcium respectively (Hamroun *et al.*, 2020). The ionized form of calcium plays many important roles: from its involvement in several intra- and extracellular signaling pathways to its implication in nerve impulse transmission and muscle contraction (Cheng *et al.*, 2006; Peacock, 2010). The ionised Ca is kept in a narrow reference range of 1.16-1.30 mmol/L by an integrated homeostatic mechanism involving parathyroid hormone and 1, 25- dihydroxycholecalciferol axis (Cheng *et al.*, 2006; Goltzman, 2018). Thus, methodological differences in assessing calcium levels as diagnostic tool for PE may partly contribute to the contradictory aetiology reported in literature. This study therefore was designed to compare the ionised serum calcium levels in pre-eclamptic women with that of normotensive pregnant women attending two major federal health institutions in Gombe, North-eastern Nigeria.

MATERIALS AND METHODS

The study was carried out in the Department of Obstetrics and Gynaecology, Federal Teaching Hospital and State Specialist Hospital, Gombe, Nigeria, the major referral hospitals for primary health care centres within as well as neighboring states such as Adamawa, Yobe, Bauchi and Borno States. Gombe town, has an estimated population of 319,875.

Ethical Clearance:

Ethical clearance was obtained from the research and ethics committee of Federal Teaching Hospital, Gombe (Reference No: NHREC/10/25/2018) and Gombe State Ministry of Health (Reference No:

MOH/ADM/S/658/VOL.11/67). Only women that gave informed written consent were recruited for the study. The information obtained were treated with the utmost confidentiality and used for research purposes only.

Sample Size and Sampling Method

The sample size for the study was calculated using Fisher's formula with a prevalence of 2.36% (Geidam *et al* 2008). With a type 1 error of 0.05, power of 80%, and 10% attrition rate, the minimum sample size for each group of the study was calculate as 161 hence the total sample size for the study was 322. The subjects for this study were selected using the purposive non-probability sampling method. Pregnant women at or above the gestational age of 20 weeks with singleton fetuses were selected as cases on presentation to the hospitals. Pregnant women with multiple pregnancies, chronic medical conditions such as hypertension, diabetes mellitus, nephritis, chronic renal disease, sickle-cell anaemia, thyroid disorders, and patients on calcium supplements were excluded from the study. Pregnant women diagnosed with PE who met the inclusion criteria were designated as preeclamptic women while normotensive women matched for maternal age, gravidity, and gestational age, were recruited as control.

Study Design

A pre-tested structured proforma was used to obtain relevant socio-demographic data from selected participants. For preliminary diagnosis of PE, screening for hypertension and urinalysis was conducted for each participant (Brown *et al.*, 2018). Other tests as well as ionised calcium concentration were determined as briefly presented below.

Hypertension

To diagnose for hypertension, the blood pressure was measured with a liquid crystal mercury sphygmomanometer (Accoson, Harlow, England), with the patient sitting in a chair and forearm placed on a table (so that it is positioned at about heart level) or lying supine on a couch. Patients were allowed to rest for 5-10 minutes before blood measurements were done. The systolic and diastolic readings were taken at Korotkoff sounds I and V, respectively. A reading of 140/90 mmHg and above, at least 4 hours apart or a single reading of 160/110 and above, were diagnostic of hypertension. A repeat measurement was recorded after 4 – 6 hours to ensure consistency in blood pressure measurement.

Urinalysis

Urinalysis was done using Medi-Test Combi 2 Dipstick (Macherey-Nagel, Duren, Germany) to detect presence of proteins in urine (proteinuria). A Midstream clean catch urine specimen was collected in a universal sterile container. The Combi 2 dipstick was immersed into the urine for 1-2 seconds, removed, and the changes observed after 30 seconds were compared with standard chart as provided by manufacturer. The colour change corresponding to the protein content was noted. The presence of protein "++" or more on the urinalysis dipstick was adjudged significant proteinuria (Brown *et al.*, 2018).

Determination of Serum Ionized Calcium Levels Five millilitres of venous blood samples were obtained from the antecubital vein of the participants, without stasis and under strict asepsis. The samples were collected into plain serum bottles and transported immediately to the laboratory in ice-packs to minimise haemolysis. Samples were allowed to clot spontaneously in the specimen bottles. Serum was aspirated from the layer just above the cells and aliquot amount was put in a cryovial bottle and mounted on an Ion selective electrode analyzer (GENUS biotech Inc. Shenzhen, China) to measure the un-bound free calcium ions by comparing against two standard calcium solutions (A and B) as provided by the manufacturers through their vendors (Numbs Diagnostics) in Nigeria. The ionised serum calcium values were read directly from the analyzer.

Statistical Analysis

Data were analysed using IBM Statistical Package for Social sciences (SPSS) for windows version 20.0 (IBM, Armonk, NY, USA 2012). The socio-demographic data are presented as percentages. Other replicate determinations for the two study groups are presented as mean $\pm$ SD while unpaired 2-tailed student-t-test was used to compare means. Odd ratio was used to test for association between hypocalcaemia and pre-eclampsia with $P < 0.05$ considered statistically significant.

RESULTS

Table 1 presents socio-demographic data of participants for this study. A total of 322 women were recruited for the study out of which 284 (88.20%) that is 142 pre-eclamptic and 142 normotensive women completed the study., the age range of participants was between 16 to 40 years, with the majority (49.30%) being in the 20-29 years age group. No difference ($p > 0.05$) was observed in the mean age (24.47 ± 6.63 versus 25.19 ± 5.71 years), parity (2.13 ± 2.50 versus 2.32 ± 2.11), and gestational age (35.15 weeks ± 3.948 versus 35.39 weeks ± 4.41) of the preeclamptic women compared to the normotensive control. Similarly, there was no statistically significant ($p > 0.05$) difference in the age ranges and proportion of primigravidas amongst the pre-eclamptic women compared to the normotensive control. Majority of the participants were housewives out of whom 80.28% were pre-eclamptic. Similarly, 104 participants received education up to the secondary level with 45.07% representing normotensive subjects.

As presented in Table 2, the mean systolic and diastolic blood pressures of the pre-eclamptic patients were 158.94 ± 18.17 mmHg, and 103.38 ± 12.71 mmHg, respectively. In the pregnant normotensive group, the mean systolic and diastolic blood pressures were 105.13 ± 13.41 mmHg and 67.31 ± 9.12 mmHg, respectively. All pre-eclamptic patients tested positive for proteinuria, while none of the controls had proteinuria. Results presented in Table 3 show the mean ionised calcium levels were significantly lower ($p < 0.001$) in pre-eclamptic patients (1.03 ± 0.11 mmol/L) compared to normotensive controls (1.14 ± 0.09 mmol/L). The odds ratio association (Table 4) suggests there is an increased risk of hypocalcaemia amongst the pre-eclamptic subjects than the normotensive women.

DISCUSSION

The mean age of participants in this study was 24.83 ± 6.19 years which is attributable to high rate of early marriages in the study area. Moreover, Ugwu et al. (2011) and Adewolu (2013) in a similar study reported mean ages of 24.5 ± 2.9 years in Enugu and in Benin City, South East and South South regions of Nigeria respectively. Our study showed significantly lower ionised calcium amongst preeclamptic women compared to the normotensive group. An imbalance in calcium homeostasis in the body has been linked to PE. Ionised calcium, the active circulating form, is usually maintained within a narrow range. It is regulated by two main hormones (parathyroid hormone (PTH) and calcitonin), prohormone (vitamin D and three organs (bone, kidney and small intestine) through complex feedback loops (Cheng et al., 2006; Goltzman et al., 2018). Its measurement gives a better status of circulating calcium levels in patients than assessing total calcium levels. Gebreyohannes et al. (2021), in their study on the association of dietary calcium intake, showed that low serum levels of ionized calcium have a stronger link to PE than low total serum calcium. Women with low serum ionised calcium are almost eight times (OR 7.5, 95% CI 2.39–23.61) more likely to develop PE compared to women with normal serum ionised calcium, while women with low total serum calcium level are three times (OR 3.0, 95% CI 1.02–9.37) more likely to develop PE than women with normal serum calcium levels. Because of the association of diet with serum calcium, sufficient dietary intake of calcium is required to maintain homeostatic serum ionised calcium levels (Ross, 2011).

Recent studies have indicated high poverty levels and maternal malnutrition in northeastern Nigeria (Uthman, 2009; NDHS., 2018; Jaiyeola and Choga, 2021). This may contribute to the high incidence of PE in this region since many families may not be taking adequate amounts of dietary calcium.

Table 1: Sociodemographic characteristics of pre-eclamptic and normotensive women attending Federal Teaching Hospital and State Specialist Hospital, Gombe

Pre-eclamptic Normotensive			Total (%)	χ ²	P value
Characteristics	(%)	(%)			
Age group (years)					
<20	38(26.76)	35(24.64)	73 (25.70)	0.16	0.68
20 – 29	66 (46.48)	74 (52.11)	140 (49.30)	0.89	0.34
30 – 39	35 (24.65)	30 (21.13)	65 (22.89)	0.49	0.48
≥40	3 (2.11)	3 (2.11)	6 (2.11)	0.00	1.00
Occupation					
Civil servants	7 (4.93)	11 (7.75)	18 (6.34)	0.94	0.33
Traders	8 (5.63)	1 (0.70)	9 (3.17)	5.60	0.02
Teachers	6 (4.23)	9 (6.34)	15 (5.28)	0.63	0.42
Housewives	114 (80.28)	104 (73.24)	218 (76.76)	1.97	0.16
Others	7 (4.93)	17 (11.97)	24 (8.45)	4.55	0.03
Educational Status					
None	54 (38.03)	29 (20.42)	83 (29.23)	10.64	0.001
Primary	30 (21.13)	11(7.75)	41 (14.44)	10.29	0.001
Secondary	40 (28.17)	64 (45.07)	104 (36.62)	8.74	0.003
Tertiary	18 (12.67)	38 (26.76)	56 (19.72)	8.89	0.003
Gravidity					
Primigravida	58 (40.85)	55 (38.73)	113 (39.79)	0.13	0.71
Multigravida	84 (59.15)	87 (61.27)	171 (60.21)	0.13	0.72

Table 2: Blood pressure and urinalysis of pre-eclamptic and normotensive women attending Federal Teaching Hospital and State Specialist Hospital, Gombe

Parameter	Pre-eclampsia	Normotensives	t-test	p-value
Mean systolic blood pressure (mmHg)	158.94± 18.17	105.13± 13.41	28.43	<0.001
Mean diastolic blood pressure (mmHg)	103.38 ± 12.71	67.31±9.12	27.56	<0.001
Proteinuria	+	-		

+ = positive; - = negative

Table 3: Comparison of ionized serum calcium levels in Pre-eclamptic and Normotensive women attending Federal Teaching Hospital and State Specialist Hospital, Gombe

Parameter	Pre-eclamptic	Normotensive	t-test	p-value
Ionised serum calcium (mmol/L)	1.03 ± 0.11	1.14 ±0.09	10.12	< 0.001

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Table 4: Association between hypocalcaemia and pre-eclampsia in pre-eclamptic and normotensive subjects attending Federal

Teaching Hospital and State Specialist Hospital, Gombe

Patient Category	Normocalcaemia	Hypocalcaemia	Total	Odds ratio	95% CI
Pre-eclamptic	7(4.93)	135(95.07)	142(100)		
Normotensive	60(42.25)	82(57.75)	142(100)	14.11	6.16-32.35
Total	67(23.59)	217(76.41)	284(100)		

CI = confidence interval

Furthermore, studies conducted in the region revealed high prevalence of hypocalcaemia amongst pregnant women that extends into postpartum period (Sanchez *et al.*, 1997; Bukar *et al.*, 2010; Bako *et al.*, 2021). Findings in this study supports earlier reports on hypocalcemia as discussed and the calculated odds ratio of 14.11 (95% CI: 6.16-32.35) in association with blood pressure indicate an increased risk of hypocalcaemia amongst pre-eclamptic women in Gombe. According to Ajong *et al.* (2019), pregnant women with normal systolic blood pressure were significantly less likely to have hypocalcaemia than their counterparts with higher systolic blood pressures (OR = 0.41, CI: 0.18–0.89). These further supports the observation that adequate screening for serum ionized calcium levels could be an early indicator and necessary preventive intervention in pregnant women at risk of developing PE. The main limitation of this study is that subjects were mainly urban dwellers and findings may not necessarily represent residents in rural communities. Another limitation is that nutritional status of participating subjects was not assessed during the conduct of this study.

CONCLUSION

This study showed that ionised calcium levels was significantly low in pregnant women with PE compared to the normotensive control group and a strong association exists between hypocalcemia and women diagnosed with PE. This finding supports the hypothesis that hypocalcaemia may be involved in the aetiopathogenesis of PE. Calcium supplementation is recommended for pregnant women at risk of PE in the study area as a costeffective method for preventing the disease.

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