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Comparative Study of Urinary Calcium Levels in Women With Preeclampsia Compared to Normotensive Pregnant Women in Lagos, Nigeria

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Abstract

Background: Preeclampsia is currently defined as multisystem progressive disorder characterized by the new onset of hypertension and proteinuria or the new onset of hypertension and significant end-organ dysfunction with or without proteinuria, typically presenting in the second half of pregnancy (after twenty weeks) or postpartum in a previously normotensive woman without proteinuria and resolves within six weeks postpartum

Despite numerous theories, the aetiology of preeclampsia has not been fully elucidated. It is postulated that the rise in blood pressure is a manifestation of more than one patho-physiological condition. One of these conditions is related to abnormal renal function and probably impairment of urinary calcium excretion.

Objective: This study compared urinary calcium levels in women diagnosed with pre-eclampsia and normotensive pregnant women at the Lagos University Teaching Hospital, Lagos, Nigeria.

Methods: This was an analytical cross-sectional study that enrolled 100 preeclamptic pregnant women and their matched normotensive counterparts carried out at the antenatal clinic and the labour ward of the Lagos University Teaching Hospital. The urinary calcium level was analysed with spot urine using Orthocresolphthaleincomplexone (CPC) method (Fortress Diagnostics Limited United Kingdom, Product code BXC0291A).

Results: The mean urinary calcium levels in women with preeclampsia (2.44±1.45mmol/L) was significantly lower than that of normotensive pregnant women (4.43±1.84mmol/L) ($p < 0.001$). However, there was no significant difference in the mean urinary calcium levels in participants with mild preeclampsia (2.49±1.54mmol/l) compared to those with severe preeclampsia (2.42±1.42mmol/L), ($p = 1.000$). Conclusion: The study revealed a significantly lower level of urinary calcium in women with preeclampsia compared with that of their healthy normotensive counterparts. However, the study did not reveal any significant difference in the urinary calcium level in participants with mild and severe preeclampsia.

Keywords: Hypertension, Preeclampsia, Normotensives, Urinary calcium, proteinuria.

Introduction

Hypertensive disorders commonly complicate pregnancies worldwide [1–4]. It is found in 5-10% of pregnancies worldwide and contributes a significant proportion to both maternal and perinatal morbidity and mortality [4–6].

It has been argued to be the commonest medical disorder seen in

pregnancy [1]. Therefore, it is characterized by elevated systolic or diastolic blood pressure recorded at least twice 4-6 hours apart in pregnancy [1, 2] With subtle variations, most international societies/

guidelinesclassify hypertension during pregnancy as chronic hypertension, gestational hypertension, pre-eclampsia and eclampsia [7–9]. Just as important, preeclampsia is a multi-systemic disease with poorly understood aetiology. Its prominent features include a rise in blood pressure (BP) of greater than or equal to 140/90 mmHg with evidence of significant proteinuria in the second half of pregnancy in a previously normotensive pregnant woman without prior history of proteinuria [1, 2]. However, contrary to popular belief, recent evidence has shown that the diagnosis of preeclampsia can be made when there is hypertension with associated end-organ damage irrespective of the woman's proteinuric status [1, 7–10].

Even more important, pre-eclampsia has consistently remained one of the major causes of maternal and perinatal mortality and morbidity in both technologically advanced and developing nations [11–13]. In Nigeria, it has a prevalence of 5.5-7.6% and in terms of maternal mortality it has been speculated to be among the first three causesaccounting for about 30% of all maternal deaths [13–16]. There is a five-fold increase in perinatal mortality in pre-eclampsia with iatrogenic prematurity being the main culprit [17-19]. For this reason, it is believed that approximately 70,000 women per annum and more than 500,000 of their fetuses and newborns die as a result of preeclampsia or it's complications. This is a significant proportion and is equivalent to the loss of 1600 lives per day [10, 20, 21]. Expectedly, greater than 99% of these preventable deaths occur in maternities spread across low- and middle-income countries, especially South-east Asia and sub-Saharan Africa [1, 11, 18, 19, 22].

The aetiology of pre-eclampsia as of today is still largely unknown and is still been explored. That is why many authors has postulated that the elevated BP, may be a manifestation of several pathophysiological conditions [2, 23, 24]. Some of these conditions include abnormal renal function, changes in the metabolism of certain ions such as calcium, and probably impairment of urinary calcium excretion [20, 25, 26]. The widely accepted patho-physiology is that of dysfunction in the endothelium [3]. This widespread endothelial dysfunction is believed to be as early as 8th weeks of gestation. However, the manifestation of the disease may appear in the late second or early third trimester weeks after the patho-physiological process had started [20, 21, 27, 28].

Additionally, it is well known that intracellular calcium regulation plays a vital role in hypertension and literature abounds on studies of blood calcium levels during pregnancy with significant variations in total and mean serum calcium levels as pregnancy progresses [5, 6, 20, 29–32]. Conversely, not so much robust literature exists especially from a homogenous pregnant population of black women on the possible relationship between urinary calcium levels and preeclampsia. This study, therefore, compared the levels of urinary calcium excreted in preeclamptics to that of normotensive pregnant women in Lagos, Southwest Nigeria.

Materials and methods

Setting

It was an analytical cross-sectional study done at the outpatient antenatal clinic and the delivery suite Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. LUTH, located on the mainland of Lagos.

Study population

A total of 200 eligible pregnant women were recruited for the study between January 2021 and August 2021. They were accessed for inclusion criteria which include pregnant women with singleton pregnancy at 20 weeks' gestation and above with preeclampsia (cases) and their matched normotensive pregnant counterparts (comparators) who gave written informed consent. Those with a history of chronic medical or surgical conditions like hypertension, diabetes mellitus, renal failure, heart disease, chronic hypertension, urolithiasis, thyroidectomy, women with twin or high order multiples, those on over the counter medication containing calcium or those already prescribed calcium supplementation were excluded from this cross sectional study. The study population consisted of two groups matched for maternal age and gestational age. After obtaining a written informed consent, a unique identification number was assigned to each participant.

Sample size estimation

Using the findings from the study by Taufield et al, a minimum sample size of 100 participants was required in each group based on an intergroup mean difference of 40mg/ml to achieve a power of 80%, a type I error rate of 5% and a non-response rate of 20%.

Study procedures

Women who consented to be enrolled into the study were recruited. They include all pre-diagnosed women who met the inclusion criteria their age and gestational age matched healthy, normotensive counterparts as the comparator.

Group

An interviewer-administered questionnaire was used to obtain participants' information. Blood pressures were measured by the midwives using mercury sphygmomanometer according to standard protocols. Thereafter, ten milliliters of clean catch, mid-stream urine or catheter urine specimen (if catheterized) was collected from the participants into a clean universal bottle and the urine was tested for protein using urine dipstick. Based on the blood pressure, result of the urine dipstick and associated symptoms/signs, the participants were grouped into the study or comparative group and the remaining urine sample was labeled and sent immediately to LUTH Central Research Laboratory for storage and subsequent urinary calcium estimation.

Laboratory Method

The urine samples were aliquoted and transferred into cryogenic vials then stored in cryogenic box at ultra-low temperature of -80OC until laboratory analysis. The reagents with product code BXC0291A, LOT 202220, manufactured date 2020/11 and expiry date 2022/09 were all supplied ready to use by the manufacturer, Fortress Diagnostics Limited, United Kingdom. The reagents were stable until the expiry date stated by the manufacturer. A working reagent was prepared by mixing equal volumes of the buffer and chromagen and this mix was stable for 3 days at room temperature up to 25oC, or 7 days at 2-8oC. The urine samples were then diluted using 100µl of samples and 100µl of normal saline. The standard solution was prepared by mixing 25µl of the standard solution with 1000µl of the working reagent. The reagent blank was also prepared by mixing 25µl of distilled water with 1000µl of the working reagent. 25µl of the diluted urine samples was then added to 1000µl of the working reagent. This was mixed and the absorbance was then read against the prepared reagent blank after 5 to 50 minutes at a wavelength of 578nm and temperature 20-25oC.

The calcium concentration was calculated as shown below:

Calcium concentration= ($\frac{absorbance\ of\ sample}{absorbance\ of\ standard}$) x standard concentration

The kit's inter-assay imprecisions were 4.4% and 4.1% for the low control (2.20mmol/l) and high control (3.40mmol/l). The intra-assay coefficients of variation were 3.5% and 2.8% respectively for the low and high control levels, respectively. The low calcium detection limit of the assay was 0.12mmol/l and no limit to the highest detectable level was stated by the manufacturers.

Statistical analysis

Data was entered into an excel spreadsheet initially, it was later imported and analyzed using statistical package for the social sciences version 29; (SPSS) Armonk, NY: IBM Corp. The categorical variables were presented as percentage and frequency tables. With regards to continuous variables, test of normality was also done Kolmogorov-Smirnov test. Those that were normally distributed were presented as mean (± standard deviation), while others presented as median and interquartile range. The student's independent t- test was used to compare mean of normally distributed continuous variables, while Mann Whitney U test was used to compare the median of non-normally distributed variables between the preeclamptic and normotensive participants. One Way Analysis of Variance (ANOVA) and the Kruskal Wallis test were used to assess the differences in the mean and median urinary calcium levels respectively across the study groups of normotensives, mild and severe preeclamptic participants. Furthermore, a Post hoc Bonferroni test was done to determine the pairwise difference between severity of pre-eclampsia. A two-tailed test of hypothesis was assumed, and the level of statistical significance was set at $p < 0.05$.

Ethical Consideration

The ethical principle of Helsinki was obeyed throughout the study. The approval of this study was given by the Health Research Ethics Committee in our facility. The approval number is ADM/DCST/HREC/APP/3518.

Results

All in all, two hundred women will enrolled in this study, 100 (50%) were diagnosed with preeclampsia and 100 (50%) were healthy pregnant women matched for age and gestational age.

The mean participants' ageand gestational age of the preeclamptic study participants was not significantly different to that of the normotensive comparative participants (Table 1).

From Table 2, it is evident that the mean urinary calcium levels were significantly lower in preeclamptic (2.44± 1.45 mmol/l) than in than in normotensive pregnancy (4.43± 1.84 mmol/l).

Table 3 shows that there was a statistically significant difference in the urinary calcium levels amongst the normotensive, mild and severe preeclamptic participants ($p < 0.001$).

In Table 4, a pairwise comparison of urinary calcium levels among preeclamptic subgroups and normotensives by post hoc Bonferroni test revealed that there were statistically significant differences in the mean urinary calcium levels between women with mild preeclampsia and that of normotensive pregnant women (2.49 ± 1.54 mmol/L vs 4.43 ± 1.85 mmol/L, $p < 0.001$) and also that of women with preeclampsia with severe features and normotensive pregnant women (2.42 ± 1.42 mmol/L vs 4.43

Table 1 Socio Demographics and Obstetrics Characteristics of the Respondents

Characteristics	**Preeclampsia N=100**	**Normotensive N=100**	**Total (%) N=200**	**Statistics**
	Frequency n (%)	**Frequency n (%)**		**P-value**
Age (years)				
20-25	7(7.0)	9(9.0)	16(8.0)	0.927^
26-30	26(26.0)	25(25.0)	51(25.5)	
31-35	32(32.0)	34(34.0)	66(33.0)	
>35	35(35.0)	32(32.0)	67(33.5)	
Mean Age ±SD	32.2±5.5	31.7±5.6	32.0±5.5	0.531#
Booking Status				
Booked	32(32.0)	100(100.0)	132(66.0)	<0.001Δ
Unbooted	68(68.0)	0(0.0)	68(34.0)	
Parity (median, range)	1(0-7)	1(0-5)		0.804*
0	26(26.0)	25(25.0)	51(25.5)	0.599^
1	26(26.0)	29(29.0)	55(27.5)	
2	26(26.0)	27(27.0)	53(26.5)	
>3	22(22.0)	19(19.0)	41(20.5)	
Gestational age (weeks)				
<34	55(55.0)	51(51.0)	106(53.0)	0.389^
34-37	28(28.0)	24(24.0)	52(26.0)	
>37	17(17.0)	25(25.0)	42(21.0)	
Mean Gestational age ± SD	32.9±4.5	33.2±4.6	33.0±4.5	0.628#
Educational Qualification				
None	0(0.0)	1(1.0)	1(0.5)	0.016 Δ
Primary	6(6.0)	1(1.0)	7(3.5)	
Secondary	46(46.0)	33(33.0)	79(39.5)	
>secondary/ Tertiary	48(48.0)	65(65.0)	113(56.5)	
Marital Status				
Married	99(99.0)	99(99.0)	198(99.0)	1.000 Δ
Single	1(1.0)	1(1.0)	2(1.0)	
Occupational status				
Professional	4(4.0)	12(12.0)	16(8.0)	0.147 Δ
Semi-skilled	20(20.0)	22(22.0)	42(21.0)	
Skilled	18(18.0)	12(12.0)	30(15.0)	
Housewife/ unskilled	58(58.0)	54(54.0)	112(56.0)	
Symptoms at Presentation				
Yes	62(62.0)	0(0.0)	62(31.0)	<0.001 Δ
No	38(38.0)	100(100.0)	138(69.0)	
Proteinuria				
Nil	0(0.0)	100(100.0)	100(50.0)	<0.001 Δ
1+	0(0.0)	0(0.0)	0(0.0)	
2+	80(80.0)	0(0.0)	80(40.0)	
3+	18(18.0)	0(0.0)	18(9.0)	
4+	2(2.0)	0(0.0)	2(1.0)	
SBP (mmHg) (mean ± SD)	162.0 ± 15.8	115.8 ± 8.2	138.9 ± 26.3	<0.001#
DBP (mmHg) (mean ± SD)	102.8 ± 10.9	69.8 ± 6.2	86.3 ± 18.8	p<0.001#

 # Student's t-test; Δ Fischer's test; ^ Pearson's Chi-square,
**Mann Whitney U test,*
SBP- systolic BP, DBP- Diastolic BP.

Table 2 Comparison of Urinary Calcium Levels between Respondents

Variable	Measure of central tendency	Preeclampsia N=100	Normotensive N=100	Total N=200	P-value
Urinary Calcium (mmol/L)	Mean (SD)	2.44 (1.45)	4.43 (1.84)	3.44 (1.93)	<0.001#
	Median (IQR)	2.26 (2.06-2.46)	3.91 (3.19-5.31)	2.71 (2.22-4.17)	<0.001*

*SD: Standard deviation. *Mann Whitney U test. # Student's t-test*

Table 3 One-way analysis of variance in mean Urinary calcium levels across the study groups

Variable	Measure of central tendency	Normotensives N=100	Preeclampsia (mild) N=30	Preeclampsia (severe) N=70	Statistics	P-value
Urinary Calcium (mmol/l)	Mean (SD)	4.43 (1.85)	2.49 (1.54)	2.42 (1.42)	◊=35.836	p<0.001
	Median (IQR)	3.91 (3.19-5.31)	2.28 (2.11-2.48)	2.24 (2.02-2.45)	ŏ=108.862	p<0.001

ŏKruskal Wallis test ◊ANOVA

Table 4 Pairwise Comparison of Urinary Calcium Levels among Preeclamptics and Normotensives by Post hoc Bonferroni Test

Preeclampsia status	Normotensive (N=100)	Mild preeclampsia (N=30)
Mild preeclampsia (N=30)	< 0.001$	
Preeclampsia with severe features (N=70)	< 0.001$	1.000$

$ p-value for Bonferroni pear wise comparison

± 1.85 mmol/L, p < 0.001). However, there was no observed statistical difference between the urinary calcium levels in pre-diagnosed women with either mild or severe preeclampsia (2.49 ± 1.54 mmol/L vs 2.42 ± 1.42 mmol/L), respectively. (P-value =1.0).

Discussion

This study compared the levels of urinary calcium excreted in women diagnosed with preeclampsia and normotensive pregnant women using Orthocresolphthaleincomplexone (CPC) method which is an accurate, inexpensive, less time-consuming and the recommended field method of calcium estimation. We found that the mean urinary calcium level among preeclamptic women was significantly lower than that of normotensive pregnant women. However, further sub analysis showed that there is so significant difference in urinary calcium level in women with mild or severe preeclampsia.

The finding of significantly lower levels of mean urinary calcium levels in preeclamptic women than in normotensive pregnant women is in agreement with the findings by Pal and colleagues who also reported in their study that pregnant women diagnosed with preeclampsia excrete lesser amount of calcium in urine when compared with healthy normotensive pregnant women at the same gestational age at diagnosis [32]. Although they suggested different mechanisms to explain the reason behind the hypocalciuria in women with preeclampsia, the most plausible in their explanation was that of changes in glomerular filtration rate in preeclampsia, increased requirement of calcium by the pregnant hypertensive women resulting in increased intestinal absorption and increased calcium uptake by the developing fetus with or without changes in calcium reabsorption in the renal tubules.

Furthermore, although Agarwal and co-workers in their study were able to establish that there is an association between low urinary calcium levels and urinary calcium to creatinine ratio in preeclampsia, there sample size was not sufficient to draw a definitive conclusion. Currently, not so much studies exist in literature that evaluated the relationship between urinary calcium and preeclampsia [33]. Other possible explanation for these findings in our study may be related to the decreased dietary intake of calcium, decreased intestinal absorption of calcium, increased calcium uptake by the fetus and placenta or due to increased distal tubular reabsorption of calcium, which is likely to be independent of sodium reabsorption, as implied by several studies that were able to demonstrate reduced fractional excretion of calcium in patients with preeclampsia [25–28].

However, the finding of our study is different from that by Tejaswi et al who in their prospective cohort study of 100 pregnant women between 20–28 weeks gestation, aged less than 35 years found no significant difference in the mean urinary calcium levels, but reported that urinary calcium creatinine ratio is a good predictor of preeclampsia [33]. The average mean arterial blood pressure at entry into the study also did not differ significantly between those that subsequently developed preeclampsia and remained normotensive. This may have influenced the ability of the study to detect any difference in urinary calcium excretion.

Prajapati in a prospective study also found that urinary calcium excretion was not significantly different between groups of normotensives, preeclamptic and pregnancy- induced hypertensive patients [35]. This study involved a relatively large sample size of with calcium estimation done using spot urine samples. However, unlike our study, participants were recruited between 20-30 weeks gestation but only 16 women out of the total sample population of 456 women developed preeclampsia [35]. Hence, this may also have affected the ability of the study to detect a difference.

Further analysis revealed that there was a statistically significant difference between urinary calcium levels in normotensive participants and participants with mild as well as preeclampsia with severe features. However, no significant difference existed between the urinary calcium levels in

participants with mild preeclampsia and those with preeclampsia and severe features. The clinical implication of this finding is that urinary calcium excretion may be a reliable tool for determining the occurrence of preeclampsia but not the severity. This finding mirrors the study of Anandpara et al, who also did not show any significant difference in urinary calcium excretion between participants with mild preeclampsia and those with severe features [35].

Our study finding of no significant association between the severity of preeclampsia and urinary calcium level may be due to the fact that preeclampsia is a continuous spectrum of progressive multisystemic disease with the possibility of having a severe disease e.g. end-organ dysfunction even in the presence of perceived mild features like mild hypertension or absence of proteinuria [1]. Another possible reason for this finding is that the criteria for the determination of the severity of preeclampsia as defined by various clinical practice guidelines (CPGs) is froth with a lot of inconsistencies [1]. Some CPGs; for instance, define severe preeclampsia as the occurrence of the disease at less than 34 weeks' gestation, hence a severe disease in a particular CPG may be considered a mild disease in another CPG [33].

This observed association of low urinary calcium levels in preeclamptic women may mean that urinary calcium level may play a role in the identification, but, not in the categorization of preeclampsia and it may also not be a marker of severity of preeclampsia. However, whether the hypocalciuria is a cause or a consequence of preeclampsia still remains to be clarified.

However, there is need for further robust studies involving larger population of preeclamptic women to investigate any association between pre-eclampsia, and calcium excretion in the urine.

The strength is attributed to its relatively large sample size in a homogenous population of pregnant women which is sufficient to detect effect and increase the reliability of the obtained results. However, we are limited by the fact that the study is a one centre cross-sectional study which may not be able to show cause effect relationship between low urinary calcium and preeclampsia in the entire Sub-Saharan Africa.

Conclusions

This study revealed a significantly lower level of urinary calcium among preeclamptic participants in comparison with their healthy normotensive counterparts. However, there was no significant difference in the urinary calcium level in participants with mild and severe preeclampsia. The finding of low levels of urinary calcium in preeclamptic participants suggests that urinary calcium excretion may have a role in the aetiopathogenesis of preeclampsia. Considering the cost effectiveness and wide availability of tools for urinary calcium estimation, it holds a promising future for early diagnosis of preeclampsia.

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