Evaluation of Serum Calcium Level in Pregnant Normotensive and Pre-eclamptic/Eclamptic Women in Nnewi, Nigeria: A Case Control Study

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Background: Pre-eclampsia/Eclampsia is a major cause of maternal and perinatal morbidity and mortality worldwide and especially in the developing countries, and its etiology has been uncertain. Alteration in calcium metabolism has been implicated. Objective: The objective of this study was to compare the serum calcium levels in pregnant normotensive with pre-eclamptic/eclamptic women. Study Design: A case control study. Methodology: The following data was obtained from patients that met the inclusion criteria: age, parity and gestational age. Estimation of the serum calcium was done with a spectrophotometer. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 16. Frequency tables, student t tests and chi-square test were used where applicable. A p-value < 0.05 was considered significant. Results: The Pre-eclamptics/Eclamptics had a lower mean serum calcium level than their normotensive counterpart (1.91± 0.28mmol/L Vs 2.32± 0.21mmol, p-value= 0.02). There was no significant difference between the mean serum calcium level in the patients with Pre-eclampsia and those with eclampsia (1.89+0.29mmol/L Vs. 1.94+0.28mmol/L, P-value=0.51). There was no difference in the serum calcium levels between the two during the 2nd trimester, but there was a significant difference during the 3rd trimester. Conclusion: There was an association between lower serum calcium level and Pre-eclampsia/Eclampsia in our environment; this is more obvious during the third trimester of pregnancy. Therefore, there is need for calcium supplement, especially among those that are calcium deficient, during pregnancy in our environment to prevent pre-eclampsia.

Keywords: Preclampsia/eclampsia, Normotensive, Serum calcium level.

INTRODUCTION

Pre-eclampsia refers to a syndrome of new onset of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive women. Eclampsia is the occurrence of convulsions during pregnancy, labour or within 7 days of delivery in a patient with pre-eclampsia or pregnancy induced hypertension and not caused by epilepsy or other convulsive disorders.

Pre-eclampsia/Eclampsia is a major cause of maternal and perinatal morbidity and mortality worldwide and especially in the developing countries. In Nigeria, it causes about 11% of maternal mortality. The pathogenesis of pre-eclampsia is incompletely understood, but is clearly initiated by the presence of trophoblast. Impaired remodeling of uterine spiral arteries, reduced placental perfusion, increased production of anti-angiogenic factors, and maternal endothelial cell damage are hallmarks of the disorder.

Recent study, implicated alterations in calcium metabolism in the pathogenesis of hypertension in pregnancy. In the third trimester, calcium is deposited in the fetal skeleton at the rate of 200mg/day. In addition, urinary excretion of calcium in the third trimester is doubled. calcium deficiencies have been linked to pre-eclampsia/eclampsia. Further, hypocalcaemia, deviations in levels of 1, 25-dihydroxyvitamin D and parathyroid hormone have been shown in women with pre-eclampsia.
Calcium supplementation has been suggested to reduce the incidence of pre-eclampsia \(^5\). Hofmeyr et al\(^6\) in a systematic review of twelve studies with 15,528 women reported that calcium supplementation reduced the risk of pre-eclampsia. They suggested that the research agenda be redirected towards calcium supplementation at a community level. Similarly, in a Cochrane database systemic review 2010, calcium supplementation was found to reduce approximately by 50%, the incidence of pre-eclampsia \(^1\).

Many factors have been implicated in the pathogenesis of pre-eclampsia and it is not unlikely that the contributions of these factors to the development of pre-eclampsia may vary according to the different population characteristics and geographical locations \(^7\). On the other hand serum calcium levels in a given population can be influenced by diet, exposure to sunlight and bone mass density amongst other factors. Thus necessitating the need to determine the relationship between serum calcium levels and pre-eclamptic/eclamptic patient in different parts of the world.

There is evidence that calcium regulation may also be abnormal in pre-eclampsia/eclampsia. The researches comparing calcium levels in normal and in pregnancy complicated by pre-eclampsia/eclampsia were in developed nations. It is very important to find out in our environment the link of calcium to pre-eclampsia/eclampsia. This study is set to determine and compare the serum calcium level in normotensive and pre-eclamptic/eclamptic women.

**METHODOLOGY**

**Study Site**

This study was conducted at Nnamdi Azikiwe University Teaching Hospital [NAUTH], Nnewi, South East Nigeria as obtaining approval from the ethical committee of the Hospital.

**Study Design**

This case control study was carried out among normotensive pregnant women (control group) and those women who had their pregnancy complicated with pre-eclampsia/eclampsia (study group).

**Study Population**

The study population included pregnant women that presented between 1\(^{st}\) January and December, 2012 for expert management. Those that fulfilled the inclusion criteria were recruited for the study. For each recruited case, the next normotensive patient that matched the biological age, parity and gestational age ranges was recruited as a control.

**Inclusion Criteria**

1. Pregnant women
2. Gestational age above 20 weeks calculated from the last menstrual period or ultrasound estimated gestational age done within the first half of pregnancy.

**Exclusion Criteria**

1. Diabetic/chronic hypertensive/chronic renal failure women
2. Women on any calcium preparation in the index pregnancy.

**Study Procedure**

The ethical clearance was obtained from the ethical committee of NAUTH. The participants that met the inclusion criteria and gave consent were recruited in the study. Recruitment started with the first pre-eclamptic/eclamptic patient and normotensive patient with similar admission criteria [age, parity and gestational age] was recruited as control. The following data were collected from each of the participants and recorded in a structured pre-designed proforma. With the patient lying down, 5mls of blood was collected without venous stasis. The specimens were single blinded on the side of the laboratory scientist. The blood was centrifuged to get the serum which was stored in the refrigerator until analysis was done and the result recorded in the proforma.

Serum calcium measurement was done using quantitative spectrophotometric method. Serum albumin level was also estimated for each sample collected and the effect of serum albumin on serum calcium was corrected by this correcting formula: Corrected serum calcium = measured serum calcium + 0.02 (40 – serum albumin) as proposed by William marshall et al\(^8\) or simply put by the addition or subtraction of 0.02 mmol/l respectively of calcium to the measured total calcium for each 1g/l albumin below or above a value of 40g/l\(^9\). This is because 50% of calcium is bound to serum albumin, total calcium concentration depends in part on it. Most analysers employ a formula to adjust for it; corrected serum calcium = 0.02 (40 – albumin) + measured calcium, where the units of albumin and calcium are g/l and mmol/l respectively \(^8\).

**Data Analysis**

Statistical analysis of the data collected was done using the SPSS version 16. Frequency tables were generated for the demographic data. Further comparative analysis was carried out using the chi-square and student-t-tests where applicable. The p-value < 0.05 was considered significant.

**RESULTS**

Table I, showed the socio-demographical characteristics of the study and the control groups. There were no significant differences in the mean age, mean parity and mean gestational age between the two groups.

Table II showed that the mean serum calcium level of the pre-eclamptic/eclamptic women was 1.91 ± 0.28mmol/l while that of the normotensive pregnant woman was 2.32 ± 0.21mmol/l. The difference was statistically significant \(P=0.02\). Although twenty-seven out of the forty five patients whose serum albumin >40g/l (hence had their serum calcium level corrected by subtracting the correction factor) were from the control group (27 vs. 18; \(p\) value=0.09), there was no statistical difference between the two groups. There was also no difference between the two group in those whose serum albumin was <40g/l and hence no correction made (3 vs. 7; \(p\) value=0.186, study and control groups respectively). Out of 65 patients that had their serum albumin < 40g/l, (and hence had their serum calcium level corrected by adding the correction factor), 39 were from the study group (39 v. 26; \(X^2=5.675;P\) value=0.017), the difference between the two groups was statistically significant. This is shown in table III.

In table IV, the mean serum calcium in pre-eclamptic patient was 1.89 ± 0.29mmol/l while that in the eclamptic patent was 1.94 ± 0.28mmol/l. There was no significant difference between the mean serum calcium level in Pre-eclamptic and eclamptic patients.
Table I. Sociodemographic Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study group n=60</th>
<th>Control group n=60</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>30.45 ± 4.83 (SD)</td>
<td>30.65 ± 4.65 (SD)</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean parity</td>
<td>1.83 ± 2.0(SD)</td>
<td>1.81 ± 1.84</td>
<td>P=0.51</td>
</tr>
<tr>
<td>Mean gestational age</td>
<td>33.48 ± 4.9 (SD)</td>
<td>34.33 ± 4.61 (SD)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Table II. Comparison of Serum Calcium Levels in Study and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group n=60</th>
<th>Control group n=60</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Calcium mmol/L</td>
<td>1.91 ± 0.28 [SD]</td>
<td>2.32 ± 0.2 [SD]</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table III. The distribution based on the level of serum albumin (and corrections made for serum Calcium level)

<table>
<thead>
<tr>
<th>Serum albumin (g/l)</th>
<th>study group (%)</th>
<th>control (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;40g/l</td>
<td>18 (30)</td>
<td>27 (45)</td>
<td>0.090</td>
</tr>
<tr>
<td>=40g/l</td>
<td>3 (5)</td>
<td>7 (11.67)</td>
<td>0.186</td>
</tr>
<tr>
<td>&lt;40g/l</td>
<td>39 (65)</td>
<td>26 (43.33)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Table IV. Comparison Of Mean Serum Calcium Level Between Pre-Eclamptic And Eclamptic Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-eclampsia n=47</th>
<th>Eclampsia n=13</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Serum calcium mmol/L</td>
<td>1.89± 0.2</td>
<td>1.94 ± 0.28</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table V. Mean Serum Calcium by Trimesters

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Study group</th>
<th>Control group</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Trimester</td>
<td>n=13</td>
<td>n=13</td>
<td>P-value</td>
</tr>
<tr>
<td>Serum calcium [mmol/L]</td>
<td>1.94 ± 0.24</td>
<td>2.40 ± 0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>n=47</td>
<td>n=47</td>
<td>P-value</td>
</tr>
<tr>
<td>Serum calcium [mmol/L]</td>
<td>1.90 ± 0.31</td>
<td>2.29 ± 0.19</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Chi-square (47 vs 13)=38.212, p-value 0.000.

Table V showed a comparative analysis of the mean serum calcium levels of the two groups according to the trimesters. In both the study and the control groups, no significant difference was observed in the mean serum calcium level in the second trimester [1.94±0.14mmol/l Vs 2.40±0.24mmol/l, P-value 0.09]. In the third trimester, the mean serum calcium was 1.90 ± 0.31mmol/l for the study group and 2.29 ± 0.19mmol/l for the control group, P value=0.001. More cases occurred in the third trimester (47 vs. 13, p-value=0.000) than in the 2nd trimester.

DISCUSSION

Pre-eclampsia/eclampsia stands out as a major cause of maternal and perinatal mortality and morbidity in both the developed and developing world. However, the exact aetiology of the disease still remains unknown. There are many theories, but all that is known for sure is that its mediator originates in the placenta and is believed to be a woman's immunological reaction to the fetus and placenta. Normal pregnancy involves a number of physiological changes in the maternal system that affect calcium metabolism resulting in the lowering of maternal serum calcium levels. However, several reports have shown that calcium regulation is abnormal in pre-eclampsia/eclampsia.

There was an inverse association between calcium deficiency and the incidence of pre-eclampsia/eclampsia. This is similar to the results obtained by Belizan et al, Nasar et al, Idougun et al, Soemin et al, Sulamin et al and Seely et al. However, this is contradictory to some studies which reported no difference between the two groups. Trumbo et al reported that the relationship between calcium and risk of pregnancy-induced hypertension and pre-eclampsia is highly unlikely, inconsistent and inconclusive. The possible explanation for these opposing findings in the literature is uncertain. However, it is known that certain factors affect the result of the estimation of calcium in serum. These include albumin levels, method of collecting samples, postural changes of patients during sample collection and method of analysis. The negative results of the other studies may also be caused by underlying chronic hypertension or renal disease during pregnancy. The difference in the serum calcium values obtained in different studies may be due to the difference in the population studied.
In our study, the effect of variations in serum albumin levels on serum calcium was corrected by the using William et al formula (serum calcium + 0.02(40 – serum albumin) or simply put, by addition or subtraction of 0.02 mmol/l respectively to the measured total calcium for each 1g/l albumin below or above a value of 40g/l. Venous stasis is an important artefactual cause of hypercalcaemia. Venous stasis increases calcium concentration by ultra filtration. It is known that postural changes [example from horizontal to vertical position] can cause false elevation in measured total blood calcium. Our patients were not allowed to change position during sample collection. Some of the studies with opposing findings used the quantitative colorimetric method rather than our spectrophotometric method which is more sensitive and gives better result in measurement of serum calcium.

It is interesting to observe that thirty nine out of the sixty-five patients that had their serum albumin < 40g/l and hence had their serum calcium corrected by adding the correction factor were from the study group (39 v. 26; p value = 0.017). This may be explained by the loss of albumin in urine (albuminuria) among the patients with pre-eclampsia, causing reduction in serum albumin. There was no statistical difference between the two groups with regards to the proportion that either had their serum albumin > 40g/l or equal to 40g/l.

There was no significant difference in the mean serum calcium in the pre-eclamptic patients compared to eclamptic patients. This is not surprising as the pathogenesis of the disease is the same. This study also illustrated that the mean serum calcium level in both the study and control groups were lower in the 3rd trimester of pregnancy than in the 2nd trimester. The low values in the 3rd trimester are probably due to the increased demand by the growing fetus. Similarly, the mean serum calcium level in study group during the 2nd trimester was lower than the control, though these were not statistically significant. The mean serum calcium in the study group in the 3rd trimester was lower than the control group. This may explain why preeclampsia/eclampsia occurred more in the third trimester than in the 2nd trimester in this study (47 vs 13, p-value 0.000).

The exact role of the association of low serum calcium levels and Pre-eclampsia/Eclampsia is uncertain. Does low serum calcium level precede the development of pre-eclampsia or is it a sequel to pre-eclampsia? One theory postulated that low serum calcium stimulates parathyroid hormone or renin release, thereby increasing intracellular calcium in vascular smooth muscle, leading to vasoconstriction and thus a rise in blood pressure. An increased level of intracellular calcium in pre-eclampsia has been documented. Other workers have suggested that low calcium influences the production of other vasoactive agents such as nitric oxide, prostacycline and angiotensin. It was also postulated that, defective placenta in pre-eclampsia/eclampsia is unable to produce sufficient levels of 1,25-(OH)2 D, resulting in inadequate calcium absorption, low ionized calcium levels, and a secondary rise in PTH and a decrease in serum 1,25-dihydroxyvitamin D concentration.

The most compelling evidence that alteration in calcium metabolism may be a factor in pre-eclampsia/eclampsia derives from clinical trials of calcium supplementation during pregnancy, as reported by WHO. A Cochrane systematic review of 13 RCTs involving a total of 15,730 women, investigated the effects of routine [daily] supplementation with at least 1g of calcium when used for preventing pre-eclampsia and related problems. For all women, irrespective of the baseline risk of developing pre-eclampsia and calcium intake status, calcium supplementation more than halved the risk of pre-eclampsia when compared to placebo. This risk reduction was 41% for women at low risk of developing pre-eclampsia whereas the largest risk reduction [78%] was recorded among those at high risk of hypertensive disorders.

CONCLUSION

There was an association between lower serum calcium level and Pre-eclampsia/Eclampsia in our environment; this is more obvious during the third trimester of pregnancy. Therefore, calcium consumption/supplement should be encouraged, especially among those with calcium deficiency, during the second and third trimester of pregnancy to prevent pre-eclampsia.

REFERENCES

18. Fatemeh V,Sedigheh A,Masoud S, Habibolla E. Serum Calcium and Magnesium in Pre-eclamptic and Normal Pregnancies.


