THE ROLE OF SERUM CALCIUM AND ZINC IN PREGNANCY INDUCED HYPERTENSION

K. VIDHYA
Assistant Professor, Institute of Physiology, Madurai Medical College, Madurai, Tamil Nadu, India

ABSTRACT
Aim and Objective

Pregnancy induced hypertension or preeclampsia is triad of hypertension, proteinuria and edema occurring after 20 weeks of gestation in previously normotensive women. The aim of the study is to analyze and compare the concentration of serum calcium and zinc level in women with preeclampsia and in normal pregnant women.

Materials and Methods

This is a cross sectional case-control study involving 25 women with pre-eclampsia in case group and 25 normal pregnant women in control group. The inclusion criteria for case group were age group in between 20-40 yrs including both primi and second gravida in third trimester of pregnancy (>24 weeks of pregnancy). The blood pressure measured by sphygmomanometer in upper arm in sitting posture was ≥140/90mmHg in two different occasions taken 6 hours apart. The urine albumin was ≥1+ or in the mid stream random sample of urine. The controls group was formed by 25 age matched normal pregnant women either primi or second gravida in third trimester of pregnancy. The patients with medical complications such as Diabetes Mellitus, renal failure, chronic hypertension, heart failure or ischaemic heart disease, multiple pregnancies, pregnancy < 24 weeks of gestation, patients on magnesium sulphate and calcium lactate therapy were excluded from the study.

The Body Mass Index (BMI), serum calcium and zinc levels were compared between the case and control groups.

Results

The BMI was significantly higher in preeclamptic women when compared to normal pregnant women 28.71±4.70 versus 22.46±3.42 P<0.001. The serum calcium and zinc levels in preeclamptic women were significantly lower when compared to normal pregnant women 8.07±0.43 versus 8.96±0.59 P<0.001, 80.6±9.78 versus 93.28±9.44 P<0.001 for cases and controls respectively.

Conclusions

Although the serum calcium and zinc deficiency cannot be pinpointed as the sole factors for the etiology of preeclampsia, they have a definite role in the development of preeclampsia.

KEYWORDS: Preeclampsia, Serum Calcium and Serum Zinc

INTRODUCTION

Nutritional deficiencies are common during pregnancy. Pregnant women in the developing countries have been reported to consume diets that are low in minerals and vitamins. An inadequate dietary intake before and
during pregnancy might be a risk not only for the mother but also for the fetus. Deficiency of the elements such as magnesium, zinc have been implicated in the pregnancy wastage, congenital anomalies, pregnancy induced hypertension, placental abruption of membrane, low birth weight and still births.\(^{16}\)

On the physiological basis, calcium plays an important role in muscle contraction and the modification of plasma calcium concentration leads to an alteration in the blood pressure. Zinc is required for the DNA replication, transcription and cellular replication as it is the metallic component of various related enzymes (i.e) DNA polymerase, RNA polymerase and thymidine kinase. Essentiality of the zinc during pregnancy is evident. In the zinc deficient animals and women poor outcome of pregnancy is observed.\(^{6}\)

Pregnancy induced hypertension or preeclampsia is a transient but potentially dangerous complication of pregnancy, with worldwide significance to the mother and the infants. It affects approximately 5-10\% of the pregnancies worldwide\(^{26}\) and results in 15\% of preterm deliveries and 14\% of maternal deaths per year.\(^{4}\) In the developing countries, preeclampsia accounts for 20-80\% of the strikingly increased maternal mortality.\(^{25}\) In India the incidence of preeclampsia is reported to be 8-10\% of pregnancies.

Preeclampsia has been dubbed as the ‘Disease of theories’ because of the multiple hypothesis that have been proposed to explain its occurrence. Although many pathophysiologic factors such as inflammation, cytokine production, dyslipidemia,\(^{17}\) elevated homocysteine,\(^{18}\) oxidative stress,\(^{24}\) reduced calcium intake and excretion and an imbalance between the thromboxane and prostacyclin\(^{20}\) have been implicated in the etiology of preeclampsia, the complete etiologies have not been fully elucidated.\(^{12}\)

Although the high rate of preeclampsia in developing countries has forced some authors to propose the involvement of the nutrition, especially the trace elements in the etiology of the disorder,\(^{7}\) studies on the relationship between the maternal serum trace elements concentrations and the preeclampsia have produced inconsistent results\(^{13}\).

Hence this study is taken up to analyze and to compare the concentrations of calcium and zinc in the serum of women with preeclampsia and in the normal pregnant women and to evaluate their role in the pregnancy induced hypertension.

It is hoped that this study will contribute to the knowledge of the role of serum calcium and zinc in pregnancy induced hypertension.

Aims and Objectives

This study involves

- The estimation of serum calcium and zinc levels in preeclamptic women in their third trimester of pregnancy as case group.
- The estimation of the serum calcium and zinc levels in age matched healthy normotensive pregnant women in third trimester as control group.
- Comparison of concentration of calcium and zinc in the serum between the case and the control group.
- To evaluate the role of calcium and zinc in pregnancy induced hypertension.
MATERIALS AND METHODS

This is a cross sectional case-control study conducted in the Department of Obstetrics & Gynecology, Government Rajaji Hospital, Madurai during the period from 20/6/2011 to 20/8/2011.

The Ethical and Research committee of Madurai Medical College and Hospital approved the study protocol.

Participants

The study was conducted in 50 pregnant women of age group between 20 – 40 years. Of them, case group comprised of 25 pregnant women either primi or second gravida in third trimester (> 24 weeks of pregnancy) with preeclampsia admitted as inpatients in Department of Obstetrics and Gynecology.

The diagnosis of preeclampsia was based on clinical criteria with blood pressure $\geq 140/90$mmHg measured on two occasions 6 hours apart with proteinuria $\geq 1$+ dipstick along with edema (NHBPEP Classification).

The control group was formed by 25 normal pregnant women either primi or second gravida in third trimester $\geq 24$ weeks of pregnancy receiving antenatal care as outpatients.

Exclusion Criteria used for Selecting Participants

Medical complicating pregnancy such as Diabetes Mellitus, Renal failure, Chronic hypertension, Heart failure, Multiple pregnancies and Pregnancy $\leq 24$ weeks of gestation. Patients on magnesium sulphate and calcium lactate therapy were excluded from study.

Examination of Pedal Edema

A firm pressure was applied over the medial malleolus on both legs for 15 seconds in all participants and looked for pitting in that region to confirm the presence of pedal edema.

Anthropometry

The heights of the participants were measured using standard methodology with the help of non-stretchable inch tape. The weights of the participants were measured using a weighing machine and BMI was calculated using Quetlet Index using the formula $\frac{Wt \text{ (kg)}}{ht \text{ (mt) }^2}$.

Measurement of Blood Pressure

With a standard sphygmomanometer and stethoscope the systolic and diastolic blood pressure of all participants were measured in the right upper arm in sitting posture by Auscultatory method. The first and fifth Korotkoff’s phase were recorded as systolic and diastolic blood pressure respectively. The blood pressure for cases group was recorded at two occasions one at 9.00 am and another at 5.00 pm using the same procedure.

Estimation of Urine Albumin

Clean catch midstream random urine sample was obtained from all participants. The urine protein was measured by dipstick method using Dip N Read reagent strip. The results was graded on the scale of 0 to 4+ (0, none; 1+, 30 mg/dl; 2+, 100 mg/dl; 3+, 300 – 1,999 mg/dl; 4+ $\geq 2000$ mg/dl).
Blood Sample Collection

Three ml of blood was drawn in all the participants from the cubital vein by using dispovan. The blood drawn was collected in a vacuum tube.

Estimation of Hemoglobin

From the collected blood 0.02ml of the whole blood was used for hemoglobin estimation by Sahli’s acid hematin method using hemoglobinometer.

Serum Separation

The blood samples were allowed to clot spontaneously at the room temperature. Then the clotted blood was centrifuged at 3,000rpm for 10 minutes. The serum separated was stored at 2 - 8 degrees Celsius until analysis.

Estimation of Calcium

A part of serum was used for estimation of calcium.

Method

Spectrophotometric method using O-cresolphthalein-complexone as colour indicator.

Principle

In alkaline solution, calcium binds with metal complex O-Cresolphthalein complexone (OCPC) to form a bluish-purple Complex, which is measured at 578nm. The intensity of colour is proportional to the calcium concentration in the sample. Hydroxyquinoline acts as a masking agent and eliminates the interference of magnesium.

\[
\text{Calcium} + \text{OCPC} \rightarrow \text{Bluish purple complex}
\]

Table 1

<table>
<thead>
<tr>
<th>R 1</th>
<th>OCPC Reagent</th>
<th>O-Cresolphthalein complexone</th>
<th>Hydrochloric Acid</th>
<th>8-Hydroxyquinoline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.16 mM/L</td>
<td>50 mM/L</td>
<td>17 mM/L</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R 2</th>
<th>AMP Buffer</th>
<th>2 Amino 3 methyl propanol</th>
<th>500 mM/L</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>R 3</th>
<th>Calcium Standard</th>
<th>Calcium</th>
<th>10 mg/dl</th>
</tr>
</thead>
</table>

Working Reagent Preparation

Reagent 1 was diluted with Reagent 2 in equal proportion (1 ml of Reagent 1 + 1 ml of Reagent 2) and mixed properly by gentle swirling.

Stability

Working reagent is stable for 15 days at 2 - 8°C.

Procedure
Table 2

<table>
<thead>
<tr>
<th>Pipette into Vials</th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>20µl</td>
</tr>
<tr>
<td>Calcium Standard</td>
<td>-</td>
<td>20µl</td>
<td>-</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1000µl</td>
<td>1000µl</td>
<td>1000µl</td>
</tr>
</tbody>
</table>

Reagents were mixed well and incubated at 37°C for 5 minutes. The analyzer was blanked with reagent blank. The absorbance was measured at 578nm first for the standard sample followed by test sample. The result was calculated using the formula.

Calculation

\[
\text{Serum Calcium (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times 10
\]

Estimation of Serum Zinc

A part of serum was used for serum Zinc estimation.

Method: Calorimetric method

Principle

Zinc in an alkaline medium reacts with 5 – Br – Paps to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

\[
\text{Zinc + 5 – Br – Paps} \rightarrow \text{Purple coloured complex}
\]

Reagents

Table 3

<table>
<thead>
<tr>
<th>R 1</th>
<th>5 – Br- Paps</th>
<th>0.07mM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium citrate</td>
<td>170 mM/L</td>
</tr>
<tr>
<td></td>
<td>Dimethylglyoxime</td>
<td>4 mM/L</td>
</tr>
<tr>
<td></td>
<td>Bicarbonate buffer</td>
<td>0.2 Mol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R 2</th>
<th>Salicyaldoxime</th>
<th>29 mM/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Zinc Standard</td>
<td>200 µg/dl</td>
</tr>
</tbody>
</table>

Working Reagent

Working reagents were prepared from mixing together four parts of R 1 and one part of R2.

Stability: The working reagent is stable upto 2 days at room temperature (below 35° C) or for one week at 2 - 10° C.
Procedure

Table 4

<table>
<thead>
<tr>
<th>Pipette into Vials</th>
<th>Blank (B) ml</th>
<th>Standard (S) ml</th>
<th>Test (T) ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Zinc Standard</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working reagent</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The reagents were pipetted into clean dry test tubes. Mixed well and incubated at room temperature for 5 minutes. The absorbance was measured in an analyzer at 578 nm first with standard sample against the blank and then with test sample against the blank.

Calculation

\[
\text{Zinc in } \mu \text{g/dl} = \left( \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \right) \times 200
\]

RESULTS AND OBSERVATIONS

Table 5

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cases (n=25) (mean±SD)</th>
<th>Controls (n=25) (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>23.80±3.48</td>
<td>23.52±2.52</td>
<td>0.746</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.71±4.70</td>
<td>22.46±3.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gravida</td>
<td>1.48±0.51</td>
<td>1.52±0.510</td>
<td>0.783</td>
</tr>
<tr>
<td>Parity</td>
<td>0.48±0.510</td>
<td>0.520±0.510</td>
<td>0.783</td>
</tr>
<tr>
<td>Weeks of gestation (Wks)</td>
<td>32.56±4.34</td>
<td>32.56±3.48</td>
<td>1.000</td>
</tr>
<tr>
<td>Pulse (/min)</td>
<td>97.36±8.56</td>
<td>94.16±10.59</td>
<td>0.246</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>157.2±14.29</td>
<td>101.04±9.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>100.8±7.59</td>
<td>68.0±6.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb (gm %)</td>
<td>10.32±1.15</td>
<td>10.36±1.08</td>
<td>0.912</td>
</tr>
<tr>
<td>Serum Calcium (mg/dl)</td>
<td>8.07±0.43</td>
<td>8.96±0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum Zinc (µg/dl)</td>
<td>80.6±9.78</td>
<td>93.28±9.44</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

p-value < 0.05 is significant.

Table 6: Comparison of BMI between the Preeclamptic Women and Normal Pregnant Women

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D</td>
</tr>
<tr>
<td>BMI</td>
<td>28.71</td>
<td>4.70</td>
</tr>
</tbody>
</table>

P < 0.001

From the above result it was evident that p value was significant and the BMI of preeclamptic women was
significantly higher than that of the normal pregnant women.

![Figure 1: Comparison of BMI between the Preeclamptic Women and Normal Pregnant Women](image1)

**Table 7: Comparison of Pulse Rate between the Preeclamptic Women and the Normal Pregnant Women**

<table>
<thead>
<tr>
<th>Content</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D</td>
</tr>
<tr>
<td>Pulse</td>
<td>97.36</td>
<td>8.58</td>
</tr>
</tbody>
</table>

P value = 0.246

From the above result it was evident that the p value was not significant and there was no significant difference in the pulse rate between the cases and the controls.

![Figure 2: Comparison of Pulse Rate between the Preeclamptic Women and the Normal Pregnant Women](image2)
Table 8: Comparison of Systolic Blood Pressure between the Preeclamptic Women and the Normal Pregnant Women

<table>
<thead>
<tr>
<th>Content</th>
<th>Cases Mean</th>
<th>Cases SD</th>
<th>Controls Mean</th>
<th>Controls SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>157.2</td>
<td>14.29</td>
<td>101.04</td>
<td>9.47</td>
</tr>
</tbody>
</table>

P Value < 0.001

From the above results it was clear that the p value was significant and there was a significant increase in systolic pressure in the preeclamptic women compared to the normal pregnant women.

![Figure 3: Comparison of Systolic Blood Pressure between the Preeclamptic Women and the Normal Pregnant Women](image)

Table 9: Comparison of Diastolic Blood pressure between the Preeclamptic Women and the Normal Pregnant Women

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cases Mean</th>
<th>Cases SD</th>
<th>Control Mean</th>
<th>Control SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic Blood pressure (mmHg)</td>
<td>100.8</td>
<td>7.59</td>
<td>68.0</td>
<td>6.45</td>
</tr>
</tbody>
</table>

P value < 0.001

From the above results it was clear that there was a significant increase in diastolic blood pressure in the cases when compared to the controls.

![Figure 4: Comparison of Diastolic Blood Pressure between the Preeclamptic Women and the Normal Pregnant Women](image)
Table 10: Comparison of Hemoglobin between the Preeclamptic and the Normal Pregnant Women

<table>
<thead>
<tr>
<th>Content</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D</td>
<td>Mean</td>
<td>S.D</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>10.32</td>
<td>1.45</td>
<td>10.36</td>
<td>1.08</td>
</tr>
</tbody>
</table>

P value = 0.912

From the above results it was evident that there was no significant difference in the hemoglobin levels between the cases and the controls.

![Figure-5: Comparison of Hemoglobin between the Preeclamptic and the Normal Pregnant Women](image)

Table 11: Comparison of Serum Calcium Levels between the Preeclamptic Women and Normal Pregnant Women

<table>
<thead>
<tr>
<th>Content</th>
<th>Cases</th>
<th></th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Serum Calcium (mg/dl)</td>
<td>8.07</td>
<td>0.43</td>
<td>8.96</td>
<td>0.59</td>
</tr>
</tbody>
</table>

P value < 0.001

The normal serum calcium concentration is 8.5 – 10.5 mg/dl. From the above results it was evident that there was a significant decrease in the level of serum calcium in the preeclamptic women when compared with the normal pregnant women.
Figure 6: Comparison of Serum Calcium Levels between the Preeclamptic Women and Normal Pregnant Women

Table 12: Comparison of the Serum Zinc Levels between the Preeclamptic and the Normal Pregnant Women

<table>
<thead>
<tr>
<th>Contents</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum zinc (µg/dl)</td>
<td>80.7</td>
<td>93.28</td>
</tr>
<tr>
<td></td>
<td>9.78</td>
<td>9.44</td>
</tr>
</tbody>
</table>

P value < 0.001

The normal serum zinc concentration ranges from 75-120mg/dl. From the above results it was evident that there was a significant decrease in the level of serum zinc in the women with preeclampsia compared with the normal pregnant women.

Figure 7: Comparison of the Serum Zinc Levels between the Preeclamptic and the Normal Pregnant Women
Statistical Analysis

The comparison between the cases and controls was done by using one-way ANOVA test using SPSS (Statistical Package for Social Science) software, Sigma stat version 3.5. The significance was drawn at p value (probability) of < 0.05.

DISCUSSIONS

The changes in the levels of serum calcium and zinc in preeclamptic women compared to the normal pregnant women were studied by several other investigators.

In the present study the Body Mass Index of preeclamptic women is significantly higher than that of the normal pregnant women. These findings are in agreement with the studies of Chanvitya punthumapol et al, 2008. Akinloye et al, 2010 in their study showed that there was no significant difference in the BMI between the preeclamptic and the normal pregnant women. In 2001, Pipkin in his study showed that the women with higher BMI become hypertensive than those with lower BMI.

In this study there is a significant decrease in the serum calcium levels in the preeclamptic women when compared to the normal pregnant women.

Decrease in the extracellular calcium level causes increased intracellular calcium through parathyroid hormone. The increase in the intracellular calcium concentration when the serum calcium level went lower lead to constriction of the smooth muscles in blood vessels which results in increase in vascular resistance and thus increase the blood pressure.

Abdelmarouf H. Mohielden et al, 2007 showed in their studies that the mean calcium concentration in the preeclampsia group is significantly lower than the normal pregnant women.

Several studies had examined the effects of the calcium supplementation on blood pressure during pregnancy thus investigating the role of calcium supplementation and its effects on blood pressure. In 1996, Bucher HC et al conducted a meta-analysis of randomized controlled trials on the effect of calcium supplementation on preeclampsia. They concluded that the supplementation during pregnancy leads to a reduction in both systolic and diastolic blood pressure and preeclampsia.

In this study there is a decrease in the mean serum Zinc levels in preeclampsia when compared to the controls. This result is contradictory with the studies conducted by Harma et al, 2005 in turkey and Diez et al, 2002 who showed that the serum zinc levels in preeclampsia was higher than that in the normal pregnant women.

The result of this current study is in agreement with the studies of Muhammed ashraf et al, 2007 and Parvin Bahadoran et al, 2010 who showed that there was a significant decrease in serum zinc levels in preeclampsia group compared to normal pregnancy. In 2010, Emmanuel I. Ugwuja et al. in their study showed that there was no significant decrease in the serum zinc levels in preeclampsia.

Zinc insufficiency has been recognized by a number of experts as an important public health issue especially in developing countries. During pregnancy the decrease in the circulating zinc is possibly due to increased transfer of zinc from mother to the fetus and due to plasma expansion. Zinc is a metallic component of enzyme superoxide dismutase, which causes protection from damage by free radical. The hypozinemia suppressing the activity of superoxide dismutase may be a precipitating factor for preeclampsia.
Zinc by its antioxidant property and inhibitory effect on ATP dependent calcium pump decrease the vascular smooth muscle contraction thereby decreasing the blood pressure. Hence decrease in these elements is a predisposing factor for pregnancy induced hypertension.

In the present study no significant correlation could be made between levels of serum calcium and zinc and the severity of preeclampsia.

CONCLUSIONS

In this study the serum levels of calcium and zinc is compared between 25 women with preeclampsia with 25 normal pregnant women in third trimester of the pregnancy.

This study has shown that the serum calcium and zinc levels in preeclampsia are significantly lower than the normal pregnant women with p value < 0.001.

From the above study, though the calcium and zinc deficiencies cannot be pinpointed as the sole factors for the aetiology of preeclampsia, they have a definite role in the development of preeclampsia.

Therefore the calcium and zinc consumption should be encouraged during the second and third trimesters of pregnancy. The dietary supplements of calcium and zinc in the form of milk, cheese, soya bean products, leafy vegetables etc., during pregnancy could result in the reduction of incidence of preeclampsia. The direct supplementation therapy of these elements can be considered for the women with preeclampsia to ensure the child survival and the safe motherhood.

REFERENCES


