

## A COMPARATIVE STUDY OF CALCIUM LEVELS AMONGST VARYING AGE INTERVALS AND TRIMESTERS OF PREGNANT WOMEN ATTENDING TERTIARY HOSPITAL IN PORT HARCOURT METROPOLIS, NIGERIA

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### ABSTRACT

The effect of calcium mineralization in pregnant women in Port Harcourt attending antenatal clinic was investigated. A total of 102 subjects between the ages of 18 and 40 years were recruited. Of the 102 subjects, 38 were non pregnant and were used as control while 64 of these subjects were pregnant women between the ages of 18 – 40 years. Of the 64 pregnant subjects, 23 were in their first trimester, 21 in their second trimester and 20 in their third trimester. Furthermore, age intervals 18 – 24; 25 – 32 and 33 – 40 years of the pregnant and non-pregnant subjects were determined of which control subjects had 19, 11 and 8; first trimester subjects had 8, 9 and 6; second trimester subjects had 7, 8 and 6 while third trimester subjects had 6, 8 and 6 subjects between the ages of 18 -24; 25 -32 and 33 – 40 years respectively. Serum calcium was analysed using the 0-cresolphatm complex method. Results obtained for control and pregnant women irrespective of their age intervals showed significant decrease in serum calcium level in the second and third trimester. When age interval of 18 – 24 years was considered, significant increase and decrease was observed in first trimester and third trimester respectively. When the age interval of 25 – 32 years were considered, significant decreases were seen in second and third trimesters while significant decreases in serum calcium levels were seen in first, second and third trimesters. Conclusively, the increase seen in 18-24 years first trimester could be as a result of initial judicious adherence to routine antenatal drugs including calcium. However, the significant decreases seen in the various trimesters and age intervals could be as a result of lack of balanced diet, poor adherence to antenatal drugs after initial adherence and increasing physiological demands of the growing foetus. Thus, educating and re-educating pregnant women could reduce complications during pregnancy as a result of calcium deficiency.

**KEYWORDS:** Calcium, Pregnancy, Trimester, Mineralization, Nigeria.

### INTRODUCTION

Calcium is the major mineral of the skeletal system and the most abundant cation in the human body.<sup>[1]</sup> It is involved in several functions such as cardiac potentials activity, excitability and contraction of the skeletal, cardiac and smooth muscles, a coenzyme for coagulation factor and an ultra-cellular second messenger.<sup>[1]</sup> During pregnancy, the circulating calcium in the blood helps to maintain a constant normal functioning of the nervous, musculo-skeletal and cardiovascular system of the mother and the developing foetus.<sup>[2,3]</sup> Reported complications arising as a result of reduction in the available calcium concentration in pregnancy include pre-eclampsia, premature births, excess bone loss in the mother, tetany, ricket, neural tube defects (in the infant), osteoporosis and reduced bone rigidity to withstand the weight of the baby.<sup>[2,3,4,5]</sup>

Also, during pregnancy and lactation several physiological changes in the hormones occurs<sup>[6]</sup> and calcium mineralization is greatly altered during the normal reproductive period of pregnancy and lactation.<sup>[4,7]</sup> In pregnancy, the principal maternal adjustment is an increasing parathyroid hormone secretion which maintains the serum calcium concentration in the face of falling albumin level, increasing renal excretion and placenta calcium transfer.<sup>[2]</sup> The placenta also actively transport calcium ion to the foetus, making the foetus hypercalcaemic relative to the mother which in turn stimulates calcitonin release and perhaps suppresses parathyroid hormone (PTH) secretion by the foetus.<sup>[2]</sup>

One major factors reported to affect calcium in pregnancy is the consumption of calcium-rich balanced diet.<sup>[3,8,9]</sup> In developing countries such as Nigeria were poverty, sub-optimal healthcare facilities and

accessibility or affordable rich balance diet is still a factor, lack of essential nutrients such iron, vitamins, calcium, folate, and so on during pregnancy has been reported to be a major problem in some pregnancies.<sup>[3][5][8]</sup> Inadequate calcium-rich diet among women in Nigeria has also be reported to play a critical role in maternal and foetal bone mass density defect and the prevalence of rickets among Nigerian children.<sup>[3,5]</sup>

Several studies have reported the need for the use of calcium supplementation and the consumption of calcium-rich foods during pregnancy to prevent calcium loss and improve maternal as well as neonatal bone density especially in the third trimester of pregnancy as demand for calcium peaks in the developing foetus.<sup>[10,11]</sup> However, other studies have contrary reports to this findings stating that the use of calcium supplements during pregnancy irrespective of the trimester is of no clinical relevant or importance.<sup>[2]</sup> Therefore, the aim of the research is to investigate serum calcium levels in pregnant women attending prenatal (antenatal) clinic in a tertiary hospital in Port Harcourt, metropolis taking their routine antenatal drugs.

## MATERIALS AND METHOD

### 1.1 Materials

Materials used include spectrophotometer, automatic pipette and serum samples from subjects. Calcium reagent was purchased from Randox Diagnostics United Kingdom. The 0-cresolphatlin complex method was used for the analysis of serum calcium. The procedure used for the assay was as instructed by the kit manufacturer.

### 2.2 Study area and participants description

Pregnant women in Port Harcourt attending antenatal clinic in Braith Waite Memorial Hospital was investigated. A total of 102 subjects between the ages of 18 and 40 years were recruited. Of the 102 subjects, 38 were nonpregnant and were used as control (reacted negative to pregnancy test) while 64 of these subjects were pregnant women. Of the 64 pregnant subjects, 23 were in their first trimester, 21 in their second trimester and 20 in their third trimester. Furthermore, age intervals 18 – 24; 25 – 32 and 33 – 40 years of the pregnant and non-pregnant subjects were determined of which control subjects had 19, 11 and 8; first trimester subjects had 8, 9 and 6; second trimester had 7, 8 and 6 while third trimester subjects had 6, 8 and 6 subjects between the ages of 18 -24; 25 -32 and 33 – 40 years respectively. The study protocol was explained carefully to each of them and consents were given to participate in the study.

### 2.3 Sample collection and preparation

5ml of whole blood specimen was collected into a plain specimen container. The specimens were allowed to clot and later retracted and centrifuged at 3000rpm for 5 minutes to obtain serum. The serum specimens were then transferred to another labeled plain bottle using disposable Pasteur pipette.

### 2.4 Statistical Analysis

Data obtained were statistical analysed using Graphpad prism 5.03 version. Mean, standard deviation (SD) and one-way ANOVA using multiple turkey comparative test was used to compare control and the various trimester of pregnancy. Statistical significant was seen at  $p < 0.05$

## RESULTS

When overall control and overall pregnant women at various trimesters were compared, the result obtained had  $1.93 \pm 0.14$ ;  $1.93 \pm 0.25$ ;  $1.68 \pm 0.14$  and  $1.67 \pm 0.15$  for overall control, 1<sup>st</sup> trimester, 2<sup>nd</sup> trimester and 3<sup>rd</sup> trimester respectively. The results showed significant reduction in serum calcium concentration in 2<sup>nd</sup> and 3<sup>rd</sup> trimesters of pregnancy when compared to 1<sup>st</sup> trimester pregnancy and control subjects (table 3.1; figure 3.1). When age intervals were considered, subjects within the interval of 18 -24 years had  $1.86 \pm 0.11$ ;  $2.08 \pm 0.18$ ;  $1.70 \pm 0.16$  and  $1.68 \pm 0.12$  for control, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester respectively. The results obtained indicated significant increase in calcium level among 1<sup>st</sup> trimester pregnant subjects while 3<sup>rd</sup> trimester showed significant decrease compared to control. Significant decreases were also seen 2<sup>nd</sup> and 3<sup>rd</sup> trimester compared to 1<sup>st</sup> trimester (table 3.2; figure 3.2).

Age interval of 25 -32 years had  $1.96 \pm 0.12$ ;  $1.91 \pm 0.23$ ;  $1.68 \pm 0.15$  and  $1.65 \pm 0.15$  for control, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester respectively. The results obtained indicated significant decreases in calcium level among 2<sup>nd</sup> and 3<sup>rd</sup> trimester pregnant subjects when compared to control subjects and 1<sup>st</sup> trimester pregnant subjects (table 3.3; figure 3.3). Finally, age interval of 33 -40 years had  $2.05 \pm 0.16$ ;  $1.77 \pm 0.27$ ;  $1.65 \pm 0.10$  and  $1.68 \pm 0.18$  for control, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester respectively. The results obtained indicated significant decreases in calcium level among 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester pregnant subjects when compared to control subjects (table 3.4; figure 3.4). The analysis of variance (ANOVA) among the different age intervals at the different trimesters showed no significant difference. However, a significant decrease was seen in 33 -40 years age interval when compared to 18 – 24 years age interval in the 1<sup>st</sup> trimester of pregnancy (table 3.5; figure 3.5).

**Table 3.1: Comparative analysis of overall control subjects and overall pregnant subjects at various trimester between the ages of 18 – 40 years.**

Parameter	Control (non-pregnant)	1 <sup>st</sup> trimester (pregnancy)	2 <sup>nd</sup> trimester (pregnancy)	3 <sup>rd</sup> trimester (pregnancy)	P value	F value	Remark
Calcium (mmol/L)	$1.93 \pm 0.14^a$	$1.93 \pm 0.25^{ac}$	$1.68 \pm 0.14^{bde}$	$1.67 \pm 0.15^{bde}$	<0.0001	18.16	S

**Table 3.2: Comparative analysis of control subjects and pregnant subjects at various trimester between the ages of 18 - 24 years.**

Parameter	Control (non-pregnant)	1 <sup>st</sup> trimester (pregnancy)	2 <sup>nd</sup> trimester (pregnancy)	3 <sup>rd</sup> trimester (pregnancy)	P value	F value	Remark
Calcium (mmol/L)	1.86±0.11 <sup>a</sup>	2.08±0.18 <sup>bc</sup>	1.70±0.16 <sup>ade</sup>	1.68±0.12 <sup>bde</sup>	<0.0001	13.08	S

**Table 3.3: Comparative analysis of control subjects and pregnant subjects at various trimester between the ages of 25 – 32 years.**

Parameter	Control (non-pregnant)	1 <sup>st</sup> trimester (pregnancy)	2 <sup>nd</sup> trimester (pregnancy)	3 <sup>rd</sup> trimester (pregnancy)	P value	F value	Remark
Calcium (mmol/L)	1.96±0.12 <sup>a</sup>	1.91±0.23 <sup>ac</sup>	1.68±0.15 <sup>bde</sup>	1.65±0.15 <sup>bde</sup>	0.0003	8.227	S

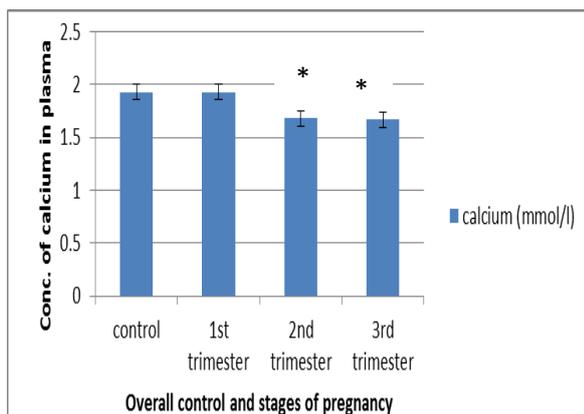
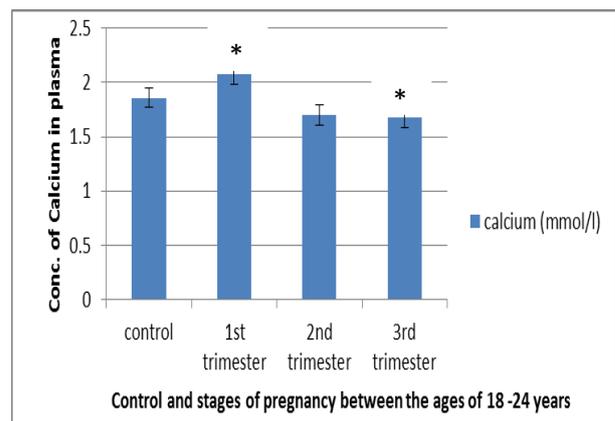
**Table 3.4: Comparative analysis of control subjects and pregnant subjects at various trimester between the ages of 33 -40 years.**

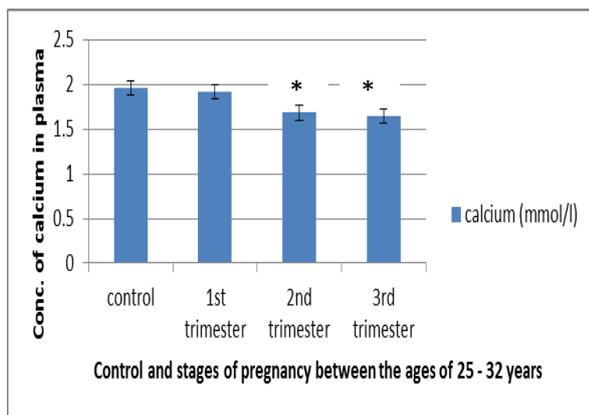
Parameter	Control (non-pregnant)	1 <sup>st</sup> trimester (pregnancy)	2 <sup>nd</sup> trimester (pregnancy)	3 <sup>rd</sup> trimester (pregnancy)	P value	F value	Remark
Calcium (mmol/L)	2.05±0.16 <sup>a</sup>	1.77±0.27 <sup>bc</sup>	1.65±0.10 <sup>bcc</sup>	1.68±0.18 <sup>bcc</sup>	0.0018	6.995	S

**Table 3.5: ANOVA of Calcium levels at age intervals in the various trimester of pregnancy.**

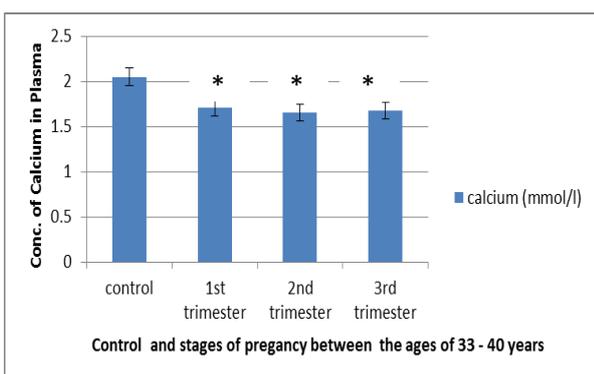
Age interval (years)	Calcium levels (mmol/L) at the various ages of Pregnancy		
	1 <sup>st</sup> trimester	2 <sup>nd</sup> trimester	3 <sup>rd</sup> trimester
18 - 24	2.08±0.18 <sup>a</sup>	1.70±0.16 <sup>a</sup>	1.68±0.12 <sup>a</sup>
25 – 32	1.91±0.23 <sup>ac</sup>	1.68±0.15 <sup>ab</sup>	1.65±0.15 <sup>ab</sup>
33 – 40	1.77±0.27 <sup>bc</sup>	1.65±0.10 <sup>ab</sup>	1.68±0.18 <sup>ab</sup>
Pvalue	0.056	0.8930	0.8927
Fvalue	3.324	0.1969	0.1143
Remark	NS	NS	NS

<sup>a</sup>Not significantly different from control, <sup>b</sup>Significantly different from <sup>a</sup>, <sup>c</sup>significantly different from <sup>d</sup>, <sup>e</sup>2<sup>nd</sup> trimester not significantly different from 3<sup>rd</sup> trimester

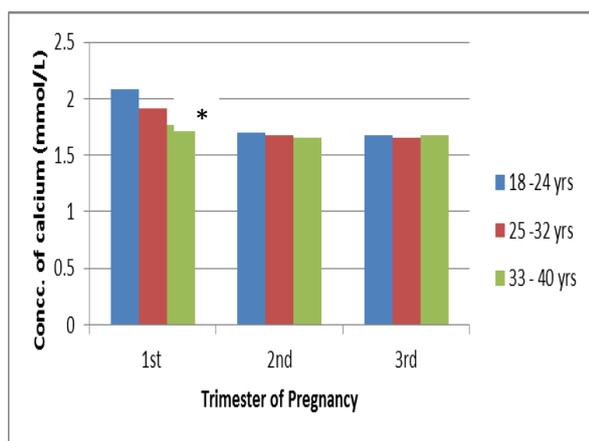
**Figure 3.1: Plot of calcium levels in control and various trimesters in pregnancy. \*significantly from control subjects.****Figure 3.2: Plot of calcium levels in control and various trimesters in pregnancy between the ages of 18 -24 years. \*significantly from control subjects.**



**Figure 3.3: Plot of calcium levels in control and various trimesters in pregnancy between the ages of 25 -32 years. \*significantly from control subjects.**



**Figure 3.4: Plot of calcium levels in control and various trimesters in pregnancy between the ages of 33 -40 years. \*significantly from control subjects.**



**Figure 3.5: Plot of calcium levels at age interval in the various trimesters of pregnancy.\*significantly from 1<sup>st</sup> trimester subjects.**

## DISCUSSION

This study was aimed at assessing the level of calcium in pregnant women attending antenatal in Port Harcourt metropolis, Nigeria. From the result obtained when overall control and overall pregnant women were compared, no significant reduction in plasma calcium levels were observed in the first trimester compared to their respective controls in the overall subject as well as

in the age intervals of 25-32 years (table 3.1 and 3.3). However, there were significant decreases in the second and third trimester of pregnancy. The non-significant differences observed in the first trimester could be as a result of habitual prenatal routine drugs and calcium-rich diet as advised in prenatal clinics especially for the adolescent women. However, significant decreases observed in the second and third trimester of pregnancy could be due to behavioral pattern among these pregnant women not adhering to the routine antenatal drugs (calcium inclusive) especially after complying for the first trimesters and increased calcium demands from the growing foetus. This finding agrees with the reports of.<sup>[2,9]</sup> The decrease in calcium level in second and third trimester when compared to first trimester could be as a result of increased physiological demand from the developing foetus, non-adherence to routine drugs or a fall in the compliance level in taking routine drugs or possibly poor nutritional diet. This finding is in agreement with the reports of.<sup>[3,5]</sup> When the age interval of 18 -24 years were considered, there was significant increase of calcium in first trimester pregnancy among these groups compared to the control group within the same age interval. This finding is contrary to the report of.<sup>[12,13]</sup> They reported significant calcium loss or decrease in plasma calcium in early pregnancies. The increase seen among this group could also be tied to the fact that the degree of foetal demand of calcium at this stage of pregnancy is reduced compared to the 2<sup>nd</sup> and 3<sup>rd</sup> trimesters. In addition, the habitual adherence to calcium supplements given during this stage of pregnancy could also cause a difference. Our findings concur with the reports of.<sup>[3,9,14]</sup> They reported that the use of calcium supplements and calcium-rich foods tends to prevent calcium loss in adolescent pregnant women as well as the maintenance of maternal and neonatal bone density. When the age intervals of 33 – 40 years were considered, significant decreases were seen in all the trimesters (table 3.4). The decrease in the trimester especially the second and third trimester could be attributed to increases calcium requirement in foetal development and poor calcium intake in form of supplements or calcium-rich foods. Our finding is also in line with the reports of.<sup>[11,15,16]</sup>

The ANOVA of age intervals at the various trimesters indicated no significant difference among the age intervals except in the 1<sup>st</sup> trimester were significant reduction in calcium levels in the age intervals of 33 – 40 years compared to the age interval of 18 -24 years (table 3.5; figure 3.5). This finding is contrary to the reports of.<sup>[9,17]</sup> They reported a probable reduction in calcium level among adolescent mothers compared to adult mothers in early pregnancy. However, the reduction seen in the adult mothers (33- 40 years) could also be as a result of non-compliance in the intake of routine prenatal drugs which is quite common among adult mothers with more than one pregnancy.

## CONCLUSION

We would like to conclude that from this study, adolescent women that probably adhere to their routine drugs in the first trimester, tends to maintain plasma calcium levels. However, it was also established that the level of calcium decreases in pregnant women irrespective of the age intervals due to the high demand by the foetus for their development and failure to habitually adhere to prenatal drugs given to supplement nutritional requirements during pregnancy. It will also be of interest to state that poverty, poor nutritional status and sub-optimal healthcare during pregnancy have also contributed to the falling level of calcium in pregnancy especially in developing countries like Nigeria.

## RECOMMENDATION

It is imperative therefore, for pregnant women whether adolescent or adult women to be monitored regularly for calcium levels and foods rich in calcium and other supplements should be given duly during pregnancy so as to meet up the daily requirement of calcium. In addition, governmental policies should be put in place to improve on the sub-optimal healthcare facilities even in rural regions, to meet the demands of pregnancy especially in developing countries.

## Limitation of Study

The limitation associated with this study was that vitamin D in the plasma of these pregnant subjects was not estimated to ascertain the influence of vitamin D in the absorption of calcium via the intestine.

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