Evaluation of Serum Calcium and Inorganic Phosphate Levels in Pregnant and Lactating Women in Enugu Metropolis

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Abstract

The importance of calcium and inorganic phosphate in pregnancy cannot be overemphasized. Their adequacy or otherwise amongst pregnant and lactating women in Enugu metropolis receiving their routine antenatal supplements was the focus of this study. Two hundred subjects (forty in each trimester; forty lactating and forty controls) were used for this study with informed consent. All subjects were within the age range of 19-40 years. Serum samples were analysed for calcium and inorganic phosphate using titrimetric and colorimetric methods respectively. Our result revealed a steady decrease in calcium from first trimester to lactating period with statistically significant values in second and third trimesters, and lactation (P<0.05) when compared with control (non-pregnant non-lactating women). Statistically significant level of inorganic phosphate (P<0.05) were observed only in the second and third trimesters. The significantly reduced level of calcium and inorganic phosphate during pregnancy and lactation (for calcium) observed in this study is indicative of inadequate calcium intake (dietary) during pregnancy or poor adherence to antenatal prescriptions. Higher provision of these elements and enlightenment on the need for supplementation within the studied metropolis is suggested to avoid the documented consequences of their deficiency to both the mother and the foetus.

Keywords: Serum, Calcium, Inorganic phosphate, Pregnancy, Lactation, Enugu

Introduction

Calcium is a divalent cation and the most abundant mineral in human body, with a total body weight of 1.9% (Nordin, 1976) of which about 99% are found in the bones and the remaining 1% in the extracellular fluid (Robertson and Marshall, 1981). Calcium absorption is vitally dependent upon vitamin D, which the most important function is to maintain serum calcium concentrations within the normal range by stimulating its absorption from the diet (Das *et al.,* 2006). During pregnancy and lactation, adequate calcium and inorganic phosphate are necessary to meet the requirement of the foetus and for the secretion in the growing infant (Barker, 1996). In addition, calcium plays vital roles as a cofactor to ATP, acts as an essential cofactor in various enzymatic conversions [which occur during blood clotting] in nerve transmission and in assisting intrinsic factor in absorption of vitamin $B_{12.}$ (Weisberg and Rhodin, 1970).The recommended daily intake of calcium for pregnant and lactating mothers is placed at 1200mg/day with a tolerable upper limit of 2500mg/day (Food and Nutrition Board, 1997). Calcium has its abundant sources for humans in foods like milk, soya beans, nuts, cheese, green leafy vegetables, and bread (Villar *et al.,* 2006)

Physiological conditions associated with life cycle changes such as growth, pregnancy and lactation are associated with increase in phosphate absorption (Kiebzam and Sacktor, 1985). Findings have shown that no major changes in either bone mass or blood inorganic phosphate have been observed during pregnancy (Sower *et al.,* 1996; Frolich *et al.,* 1991) suggesting that phosphorus supplementation is not recommended as it remains constant because of maternal adaptations to the mineral (Weiss *et al.,* 1998; Institute of Medicine, 1999); and it is abundant in many foods (NRC, 1981).

Developing infant needs calcium and phosphate for structural formation of bones and teeth, as low serum calcium during pregnancy may result in skeletal demineralised foetus (Cunningham, 2005), and in decreased bone density in the mother with consequent high risk of osteoporosis (Wu et al, 1990). It has been observed that total serum calcium declines during pregnancy reflecting lowered plasma albumin concentrations; thus, the consequent decreases in the amount bound to protein (Power et al, 1999); hence the need for calcium supplementation to maintain normal maternal serum levels and for normal mineralization of the foetus. Furthermore, low calcium intake has been implicated in the development of hypertension, colon cancer, and premenstrual syndrome (Power *et al.,* 1999), and hypocalcaemia has been shown to cause seizures in children (Balasubramanian *et al.,* 2004).

Calcium deficiency is rare in pregnancy but appears in cases of hypoparathyroidism, severe dietary inadequacy and in individuals who are unable to eat a diet rich in dairy products (Kazzi *et al.,* 1998; Stabile et al, 1995). Some factors that can impair absorption of calcium can lead to its deficiency; these include substances that can form complexes with it. Phytates which can form insoluble calcium phytate reduce calcium availability. Smoking also reduces its absorption as cadmium in cigarette smoke is a calcium antagonist. High protein diet stimulates bone calcium resorption and encourages long term bone loss (Kerstette and Allen, 1990) and high fibre diet has also been implicated in reducing serum calcium levels (De Santiago et al, 2001)

In this study, we evaluated the serum levels of calcium and inorganic phosphate in different trimesters of pregnancy and during lactation in South Eastern Nigerian women to relate the findings to some of the established knowledge about calcium and inorganic phosphate concentration during pregnancy and lactation.

Materials and Methods

Two hundred subjects were involved in the study, of which one hundred and twenty were pregnant (forty in each trimester); forty lactating (at first six months of postpartum) and all within the age range of 19-40 years and attended Agbani District Hospital, Balm of Gilead Maryland, University of Nigeria Teaching Hospital Ituku-Ozalla and St. Mary's Maternity Abakpa; all in Enugu metropolis.

For comparative purposes, blood samples were collected from forty non-pregnant; non-lactating women within the same age range. None of the subjects had any disorder that affected metabolism of calcium or bone, no history of endocrine, renal or liver illness, hypertension of pregnancy or gestational diabetes. None was regularly taken medications or using hormonal contraceptives. Those with any of these disorders were excluded from the study and participation was with informed consent. All the subjects were on their routine antenatal supplements.

Collection and processing of samples: 5ml of venous blood samples were collected from antecubital fossa vein (with minimum toniquet occulsion) by vein puncture with sterile needles. Collected blood samples in the syringes were gently discarded into clean plain glass tubes after removal of needle to avoid heamolysis. All the collected blood samples were allowed to clot at room temperature for two hours and then centrifuged at 3000rpm for 5 (five) minutes and the serum separated immediately to minimize the effect of red cells phosphate on phosphate level. Blood samples with any degree of haemolysis were deemed unsuitable for the study and were discarded. The separated sera were stored at -20^{o}C in a deep freezer until when needed. The samples were thawed and brought to room temperature before analysis, which was normally done within two days.

Calcium estimation: Complexometric titration method with ethylene diamine tetra-acetic acid (EDTA) by Appleton *et al*, (1959) was used. Serum, de-ionized water and standard (each 0.5ml) were placed in three universal containers labelled test, blank and standard respectively and were diluted to 5.0 ml with 1.25M KOH to ensure alkaline environment needed for the reaction. 0.25ml of calcein indicator was added to each container and mixed together; then titrated using .02N EDTA from 1.0ml graduated pipette. Colour change from yellow-green fluorescence to non-fluorescence salmon pink colour was noted and the volume of EDTA added to change the colour was also noted. Calculation: Test-blank/standard-blank x 10 = mg/100ml, Mg/100ml= Mmol/L,

Inorganic phosphate estimation: The method used was based on modified ammonium molybdate of Goldenberg *et al,* (1966). 0.2ml each of serum, standard and water were pipette into three centrifuge tubes labelled test, standard and blank respectively. 5.0ml of TCA was added to them and then centrifuged for ten (10) minutes. The supernatant was decanted carefully and completely into another clean test tube, 0.5ml of ammonium molybdate was added and then allowed to stand for 15 minutes for colour development and was read colorimetrically at 710 nm against blank. Calculation: Absorbance of test/absorbance of standard x concentration of standard x 0.58. After the estimation data were collated and statistical analysis was done using ANOVA and where significant Turkey was used for mean comparisons (P< 0.05).

Results

Table 1 shows the mean and standard deviation of the serum calcium and inorganic phosphate levels obtained from apparently healthy non-pregnant non-lactating women (control), in the trimesters and in lactation. The mean serum calcium and inorganic phosphate decreased during pregnancy when compared with control; during lactation calcium level also decreased while inorganic phosphate increased.

Table 1: Mean levels of calcium and inorganic phosphate in pregnant, lactating mothers and non-pregnant non-lactating women

Subject	N	Serum calcium (Mmol/L)	Serum inorganic Phosphate (Mmol/L)
Control	40	2.512±0.155	1.221±0.109
First trimester	40	2.460±0.154	1.207±0.142
Second trimester	40	*2.324±0.143	*1.111±0.142
Third trimester	40	*2.213±0.128	*1.085±0.135
Lactation	40	*2.180±0.112	1.197±0.128

**Statistically significant when compared with control (P<0.05)*

Table 2 showed that mean serum calcium levels have some changes throughout pregnancy and lactation. The changes were significant in 2nd and 3rd trimesters and during lactation. The mean inorganic phosphate was statistically significant at the 2nd and 3rd trimesters.

Table 3 showed a negatively low degree of correlation between first, third trimesters and lactation phosphate; while others showed positively low degree of correlation.

Discussion

Pregnancy and lactation have been observed to induce dynamic changes in calcium metabolism. Adequate calcium and inorganic phosphate are necessary for protection of maternal bone density and teeth; and reduce the risk of pregnancy induced

Table 2: Comparisons of mean values of calcium and inorganic phosphate between the controls, the trimesters of pregnancy and during lactation

Subject	Serum calcium (Mmol/L) P-value (Sig.)	Serum inorganic phosphate (Mmol/L) P-value (Sig.)
Control vs.1st trimester	0.438	0.990
Controlvs.2nd trimester	0.001	0.002
Controlvs.3rd trimester	0.001	0.001
Control vs. Lactation	0.001	0.926

(P<0.05) shows significant value of mean difference.

Table 3: Comparisons of serum calcium and inorganic phosphate mean values between first, second and third trimesters of pregnancy and lactation

Subject	Serum calcium (Mmol/L) P-value	Serum inorganic phosphate (Mmol/L) P-value (Sig.)
1st vs. 2nd trimester	0.001	0.011
1st vs. 3rd trimester	0.001	0.001
1st vs. Lactation	0.001	0.997
2nd vs. 3rd trimester	0.004	0.909
2nd tri. vs. Lactation	0.001	0.031
3rd tri. vs. Lactation	0.818	0.002

(P<0.05) indicates significant values of mean difference

hypertension and pre-eclampsia (Sukonpan and Phupong, 2005). This is also important in mineralization during the foetal bone formation (Pitkin, 1985). Findings have shown a downward trend of serum calcium levels in pregnancy from first trimester to third trimester. This trend has been attributed to several factors including the decrease in serum albumin that accompanies hemodilution in pregnancy (Power et al 1999). Research has also shown that intestinal absorption of calcium is doubled during pregnancy from, as early as 12 weeks of gestation, reflecting the mineral's importance and high need for its appropriate dietary intake and supplementation.

Our study did not show any statistical significant difference in serum calcium between the non pregnant women and first trimester of pregnancy; this may probably be due to less need of calcium during the period. Statistical significant differences were, however, recorded when non pregnant, non lactating women were compared with second and third trimesters as well as during lactation (P<0.05). This observation is in line with the work of Laskey *et al.* (1998) that reported increased need of calcium in late pregnancy and during lactation. It is evident that the rate of transfer of calcium into the developing foetus or infant exceeds the maternal absorption (bioavailable calcium), thereby altering the maternal calcium homeostasis.

Serum inorganic phosphate levels also showed statistical significant decreases in second and third trimesters as compared to non pregnant, non lactating (P<0.05). This is in agreement with Roy *et al.* (1979) who reported that phosphate concentrations generally fall during the 29 to 32 weeks of pregnancy. This view and our data contradict some findings, which indicated that phosphate levels during pregnancy remain constant (Sower et al, 1996; Frolich et al, 1991). However, there was no significant difference in inorganic phosphate level during lactation when compared to non-lactating non-pregnant rather a rapid increase in inorganic phosphate level was observed during lactation. This is in consonant with the work of Kametas *et al.* (2003) which showed that serum phosphate levels are within the non-pregnant range; possibly because of increased renal reabsorption and skeletal resorption during lactation.

Our data support the view that there is a decreasing trend in serum calcium level during pregnancy from first trimester reaching nadir at the third trimester. This was made manifest in the statistical significant differences between the non-pregnant, non-lactating, and second trimester, third trimester and during lactation. Though supplementation of calcium (as calcium lactate) has been a practice during pregnancy, as part of the routine medication, the significantly reduced calcium level observed in the second and third trimesters and during lactation in this study could either be due to inadequate dietary intake or poor adherence to anti-natal supplementation.

In conclusion therefore, this study further emphasized the much needed attention towards adequate dieting and supplementation of calcium and inorganic phosphate in pregnancy and during lactation to avoid the documented deleterious effects of their deficiency or reduced levels to both mother and foetus.

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