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Full-Length Research Article

Physiological Changes in Serum Calcium, Phosphate, Vitamin D, Parathyroid Hormone and Calcitonin During Pregnancy and Lactation in Randomised Population of Zaria Women

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Summary: Pregnancy and lactation are usual but stressful physiological conditions accompanied by changes in calcium and phosphate metabolism and their regulatory hormones which may lead to calcium-related disorders in pregnant women. The aim of this study was to evaluate the changes in serum levels of calcium, phosphate, vitamin D and their regulatory hormones in pregnant and lactating women in Zaria, Nigeria. A cross-sectional descriptive study was conducted at Ahmadu Bello University Teaching Hospital, Zaria for a period of three (3) months. Blood samples were collected, anthropometric measurements (weight, height and body mass index) of 179 women were taken. Serum calcium, phosphate, vitamin D, parathyroid hormone and calcitonin were determined using standard methods. Data were presented as mean ± SD, analysis was performed using one-way ANOVA and Pearson's correlation analysis. Values were considered significant at $p \leq 0.05$. There was a significant decrease in serum calcium concentration ($p < 0.01$) during the third trimester of pregnancy and lactation. An increase in serum concentration of vitamin D, parathyroid hormone and calcitonin in the 2nd trimester and a decreased during the third trimester and lactation although not statistically significant when compared with the control. There was a negative correlation between serum calcium concentration and gestational age ($r = 0.255$) while no correlation between gestational age and serum phosphate concentration. Changes in serum calcium, vitamin D, parathyroid hormone and calcitonin during pregnancy and lactation have been demonstrated suggesting a relationship between calcium metabolism and these hormones at some stages of pregnancy.

Keywords: *Tocopherol, arsenic, stomach, ileum, colon*

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INTRODUCTION

Women during certain physiological stages such as pregnancy and lactation experience an alteration in vitamin D and calcium metabolism as the growing fetus obtains calcium from maternal stores to aid skeletal development (Kovacs, 2021). The demand for calcium during pregnancy varies from about 2-3mg/day in the 1st trimester to about 3-fold in the 3rd trimester (WHO, 2016) which is accompanied by an increase in intestinal absorption of calcium under the influence of vitamin D. This shows the importance of calcium and phosphate during pregnancy hence serum levels need to be maintained in a certain range with a complex interplay of hormones involved to maintain a balance (Fukumoto, 2014). The most important factors involved in the balance are vitamin D and parathyroid hormone (Hysaj et al.,2021). Vitamin D regulates absorption of calcium from the gastrointestinal tract as well as its excretion from kidney (Fleet, 2017). Serum phosphate level is however regulated by the absorption of phosphate in the intestine, renal phosphate management and a balance of extracellular phosphate with that in bone or intracellular fluid (Yee et al., 2021). Parathyroid hormone mainly function through conversion of vitamin D to its active metabolite (1,25-hydroxycholecalcipherol) in the kidneys, which regulates intestinal absorption, renal excretion, and exchange between the extracellular fluid and bone (Levine et al., 2014).

Studies have revealed that there is variation in the levels of serum calcium, inorganic phosphate, vitamin D and parathyroid hormone at different stages of pregnancy however there is paucity of information on the variation of these biochemical markers among pregnant women in Zaria, a major town in Northern Nigeria. Therefore, by measuring the levels of calcium, inorganic phosphate, vitamin D and their regulatory hormones at various stages of pregnancy, local reference values may be established that will contribute to the growing body of knowledge concerning calcium and phosphorus disorders during pregnancy in Nigerian women. The result of this study would establish the relationship between calcium and its regulatory hormones in pregnant women in Zaria, and its environs. This could also be beneficial in the management of pregnant women presenting with calcium associated disorders.

MATERIALS AND METHODS

Demography of Study Area: Zaria is a heterogeneous city in Kaduna State. It is inhabited by about 1,018,827 people (National Bureau of Statistics, 2015). Kaduna is located in the North-west geopolitical zone of Nigeria, Kaduna State is one of the 36 states in Nigeria with its capital in Kaduna city. Zaria occupies a portion of the high plains of Northern Nigeria, 652.6 meters above sea level and some 950 kilometers from the coast at11º031N, 7º42E.

Study Design and Study Population: This study is a cross-sectional descriptive study that was conducted between within a period of three (3) months. A total of one hundred and seventy nine (179) women attending antenatal, family planning and immunization clinics at the Ahmadu Bello University Teaching Hospital (ABUTH) Shika, Zaria, Maternal and Child Health Centre, ABUTH, Ban Zazzau, Zaria and Comprehensive Health Centre, ABUTH, Sabon Gari, Zaria were recruited for the study. A structured questionnaire about pregnancy history was administered. Blood samples were collected by a competent Laboratory Technologist at the Departments of Obstetrics and Gynaecology, ABUTH, Shika and measurements (body weight and height) were also recorded from the subjects. Body mass Index (BMI) was calculated from data collected from the anthropometric indices.

Experimental Grouping: A total of one hundred and seventy nine (179) women involved in the study were grouped as follows: Group 1 non-pregnant women (n=15), group 2 were pregnant women in the first trimester (n=40), group three involved pregnant women in their second trimester (n=41), group four involved pregnant women in their third trimester (n=53) while group 5 involved lactating women (n=30).

Inclusion and exclusion Criteria: Healthy consenting women attending antenatal, family planning and immunization clinics at Ahmadu Bello University Teaching Hospital (ABUTH) Shika; Women and Child Centre, ABUTH, Ban Zazzau; Women Centre, ABUTH Sabon Gari, Antenatal Clinic, Sickbay, Ahmadu Bello University. Pregnant women with any disorder that affects metabolism of calcium or bone, history of endocrine, renal or liver illnesses, hypertension of pregnancy, gestational diabetes, thyroid disorders, or treatment with anti-tubercular or antiepileptic drugs in the previous 3 months were excluded from the study.

Ethical Approval Statement: Approval was obtained from the Scientific and Ethical Committee on Human Research, ABUTH, Shika, Zaria (Reference number: ABUTHZ/HREC/K30/2014) and verbal consent was obtained from all subjects.

Collection and processing of samples: Exactly 10 ml of blood sample was collected by venipuncture, transferred to clean plain serum bottles immediately and allowed to stand at room temperature (27ºC) for two hours and centrifuged at 3000 *g* for 5 minutes to obtain the serum. The separated serum was stored at -20°C in a deep freezer at the department of Chemical pathology until needed for assay.

Determination of serum calcium and inorganic phosphate level: The assay for serum calcium was done using O-cresolphthaline complexone method as described by Morin (Morin, 1974) while serum inorganic phosphate was analyzed using Fiske-Subbarow method as described by Fiske and Subbarow (Fiske and Subbarow, 1925)

Quantification of serum 1, 25 (OH) hydroxyvitamin D: Serum 1, 25(OH) hydroxyvitamin D was assayed using an enzyme-linked immunosorbent assay (ELISA) technique. The absorbance was read at 450 nm using Mindray MR-96A microplate reader (Shenzhen, China). The concentration of serum 25(OH) D in the test samples was calculated using the standard curve plotted with the different standards and their known antigen concentration.

Determination of serum parathyroid hormone and Serum Calcitonin: Serum PHT and calcitonin were assayed for using an enzyme-linked immunosorbent assay (ELISA) technique as described by Essley *et al*., 2011.

Data Analysis: Data was collected, curated, cleaned up and was presented as mean ± SD. Analysis of data was done using One Way ANOVA followed by Holm-Sidak post hoc test and level of significance was tested at $P<0.05$. Pearson's correlation analysis was done to determine the inter-relationship between the variables

RESULTS

The anthropometric indices of the subjects are as shown in Table 1. The study showed that the mean age of the control group was 29.79±5.56 years; while those in the 1st, 2nd and 3rd trimesters were 27.00±6.41, 28.17±6.11, 26.62±5.58 years, respectively. Mean age of the lactating group was 28.37±5.13 years. The mean weight of the control women was 67.40±9.49 Kg; mean weight of 1st, 2nd and 3rd trimester women were 63.39±16.91, 63.39±16.91, 68.39±13.04 Kg; while the mean weight of the lactating group was 63.13±12.82kg. Mean height of control, 1st, 2nd, 3rd trimesters and lactating groups were 159.27±9.13cm, 157.10±8.29cm, 161.78±7.12cm, 159.82±6.52cm and 160.90±7.38cm, respectively. Mean BMI was 26.56±3.12Kg/m^2, 25.32±6.98Kg/m^2, 24.58±7.06Kg/m^2, 24.00±8.35Kg/m^2 and 24.32±4.18Kg/m^2 for the control, 1st, 2nd, 3rd trimesters and lactating groups, respectively. Mean gestational ages in months were 2.71±0.49 months, 5.42±0.81 months and 7.74±0.79 months for the 1st, 2nd and 3rd trimesters, respectively.

Serum Levels of Calcium, Vitamin D, Parathyroid Hormone and Calcitonin: Table 2 shows the profile of mean calcium, phosphate (PO_4), vitamin D, parathyroid hormone (PTH) and calcitonin in the control, pregnant and lactating women. The mean values for serum calcium was highest in the 1st trimester, significantly reduced in the 3rd trimester and lowest in the lactating group with the highest and lowest value at 2.52 mmol/L and 2.25 mmol/L respectively. Mean serum phosphate concentration was highest in the control group 1.13±0.15 mmol/L and lowest in the 1st trimester; 1.01±0.13mmol/L. Furthermore, the mean serum vitamin D level was highest in the 2nd trimester; 46.57±20.90 µg/L and lowest in the control group; 39.29±4.51 µg/L. Mean PTH level was highest in the 2nd trimester. 45.60±17.62 pg/ml and lowest in the 1st trimester; 40.66±6.64 pg/ml. Mean calcitonin concentration was highest in the 2nd trimester 3.95±4.10 ng/L and also lowest in the 1st trimester3.05±0 ng/L.

Table 1:
Table of anthropometric indices of control, pregnant and lactating women

Parameters	Age (yrs)	Weight (kg)	Height (cm)	BMI (Kg/m^2)	Gest. Age(mths)
Control (n = 15) non-pregnant	29.79±5.56	67.40±9.49	159.27±9.13	26.56±3.12	-
1st trimester (n = 40)	27.00±6.41	63.39±16.91	157.10±8.29	25.32±6.98	2.71±0.49
2nd trimester (n = 41)	28.17±6.11	66.42±14.53	161.78±7.12	24.58±7.06	5.42±0.81
3rd trimester (n = 53)	26.62±5.58	68.39±13.04	159.82±6.52	24.00±8.35	7.74±0.79
Lactating gp (n = 30)	28.37±5.13	63.13±12.82	160.90±7.38	24.32±4.18	-

Key: n – number of women per group, Gest. Age – Mean gestational age, BMI – Body Mass Index

Table 2:
Mean (±SD) Serum concentration of calcium, phosphate, vitamin D, parathyroid hormone and calcitonin in control, pregnant and lactating women

Parameters	Control (n = 15)	1st Trim (n = 40)	2nd Trim (n =41)	3rd Trim (n =53)	Lactating Gp (n =30)
Calcium (mmol/L)	2.30±0.10	2.32±0.11	2.31±0.15	2.24±0.14**	2.21±0.17**
PO_4 (mmol/L)	1.13±0.15	1.01±0.13	1.08±0.14	1.08±0.15	1.09±0.14
Vitamin D (µg/L)	39.29±4.51	41.21±5.68	46.57±20.90	42.44±8.50	43.33±20.08
PTH (pg/ml)	40.79±8.43	40.66±6.64	45.60±17.62	41.84±10.40	44.81±12.63
Calcitonin (ng/L)	3.47±0.91	3.05±0.93	3.95±4.10	3.20±0.92	3.10±0.77

*Key: ** Represents P < 0.01 when compared to the control, Trim - Trimester, Gp - Group, PTH - parathyroid hormone*

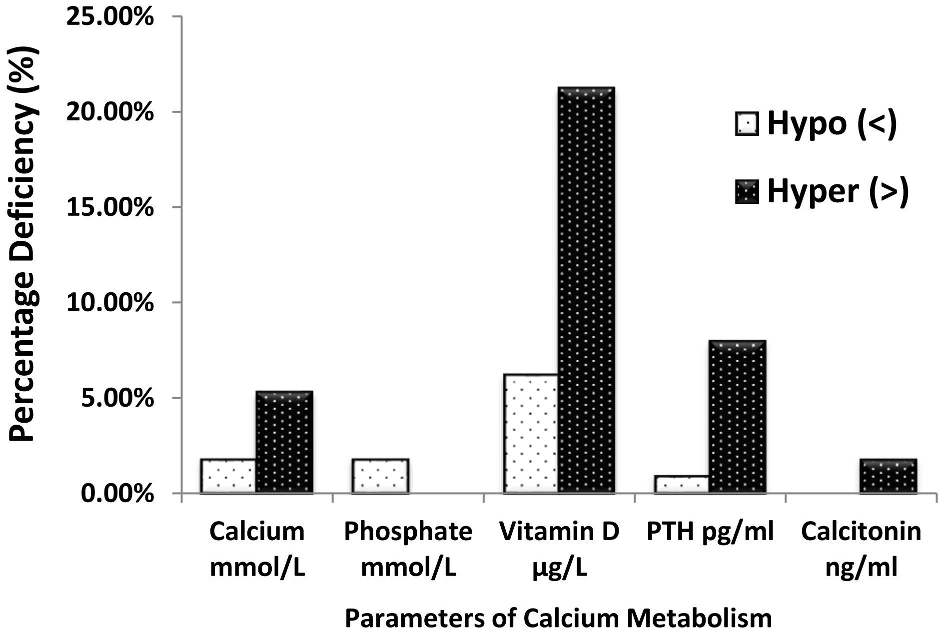

Figure 1:
Prevalence of calcium, phosphate, vitamin D, parathyroid hormone and calcitonin deficiency among pregnant women in Zaria, Nigeria

Table 3:
Relationship between age, gestational age, BMI and serum concentration of calcium, phosphate, parathyroid hormone, vitamin D and calcitonin

Parameters	R	P value
Body mass index and Age	0.290	<0.001
Calcium and Gestational Age	- 0.255	<0.01
Phosphate and Calcium	- 0.270	<0.001
Vitamin D and Calcium	- 0.422	<0.001
Parathyroid hormone and Calcium	- 0.448	<0.001
Parathyroid hormone and Vitamin D	0.756	<0.001
Calcitonin and Calcium	0.279	<0.001
Calcitonin and Phosphate	- 0.239	<0.001

Prevalence of Calcium, Phosphate, Vitamin D, Parathyroid Hormone and Calcitonin Deficiency: Figure 1 shows 1.76% of pregnant women with calcium below the normal range and 5.31% above the normal range in this study. Also, 1.76% of the women had phosphate levels below the normal range and none of them was hyperphosphataemic. The vitamin D concentration was low in 6.19% and high in 21.24% of the pregnant women. Furthermore, parathyroid hormone level was low in less than 1% (one per cent) of the study population with 7.97% above the normal range. Calcitonin was not below the normal range in any of the groups, but there was a 1.76% prevalence above the normal range.

Table 3 shows the correlation between age, gestational age, body mass index (BMI), and serum concentrations of calcium, phosphate, parathyroid hormone (PTH), vitamin D and calcitonin. About 20% (twenty percent) of the variables correlated significantly. Positive correlation was observed between age and BMI and parathyroid hormone and vitamin D; while negative correlation was observed between phosphate and calcium, vitamin D and calcium, parathyroid hormone and calcium and calcitonin and phosphate. These correlations were statistically significant (P < 0.001).

DISCUSSION

This study was conducted to evaluate the biochemical changes in serum concentration of calcium, phosphate, vitamin D, parathyroid hormone and calcitonin in pregnant and lactating women in Zaria.

Serum calcium has been reported to decrease during late pregnancy in humans (Ajong *et al.*, 2019). In this study, ionized calcium was measured, since it is the physiologically significant fraction of extracellular calcium. The normalcy in the level of serum calcium during the first trimester as compared to the control may probably be due to less need for calcium during the period. The drop in serum calcium by the third trimester and during lactation correlates with gestational age. This is because pregnancy and lactation cause a substantial increase in demand for calcium (Kovacs, 2021). Thus, increased intestinal calcium absorption, which more than doubles during pregnancy (Kovacs, 2021). Maternal skeleton reabsorption also helps to provide most of the calcium content of breast milk during lactation (Kovacs, 2008). This report does not correspond with Almaghamsi *et al.*, 2018 which showed that ionized calcium does not change during human pregnancy. The decreased in serum calcium concentration during the third trimester and lactation agrees with the report of Almaghamsi *et al*., 2018 who also found low calcium levels in pregnant women with preeclampsia.

The lowest value of serum calcium concentration among the pregnant women in the present study was 1.76%. This very low value could be as a result of low calcium intake of less than 500 mg/day or as a result of decreased calcium metabolism during periods of low intake. This fact is supported by the report of Madukosiri *et al* 2011 on the food intake of pregnant and lactating women in Plateau state, Nigeria. Furthermore, more women were hypercalcaemic (5.31%) and this could be as a result of calcium rich diets, such as nuts and soya bean cake supplements (tofu, *wara*) (common in Zaria and environs) which the pregnant women may have been taking. Intake of calcium supplements could cause raised concentrations of serum calcium (Institute of Medicine, 2011). This result corresponds with the report of Benali and Demouche, 2014 who observed a high prevalence (70.55%) of hypocalcaemia among pregnant women in Algeria. However, Kumar *et al.*, 2010 reported that approximately two thirds of pregnant women in India were hypocalcaemic though; none of the women had any manifest symptoms.

This study also revealed an increase in serum phosphate concentration from the 1st to the 3rd trimester; though this increase was not statistically significant. The observed increase in serum phosphate level could possibly be due to an increase in macromolecular metabolism during pregnancy (Correia-Branco *et al.,* 2020) since phosphorus plays a vital role in intermediary metabolism of carbohydrates and is also an important component of physiological substances such as organic phosphate esters, phospholipids, nucleic acids and nucleotides. The increase in serum phosphate concentration in this study agrees with the report of Kametas *et al.,* 2003 which showed that serum phosphate levels are within the non-pregnant range possibly. However, Møller *et al.*, 2013 reported no significant changes in the serum phosphate of pregnant women during the various trimester whereas, Ikekpeazu *et al,* 2010 reported a significant decrease in serum inorganic phosphate levels in the second and third trimesters as compared to the non-pregnant, non-lactating group. The population of pregnant women in this study showed a prevalence of 1.76% hypophosphataemia and none was observed to have hyperphosphataemia.

In the present study, vitamin D concentration was highest in the 2nd trimester. But the 3rd trimester showed a lower vitamin D concentration than the 1st trimester. The increase in Vitamin D concentration in the second trimester could be due to negative feedback stimulation from decreased calcium levels which was also reported during the second trimester and probably due to an increase in the synthesis of vitamin D which occurs in the maternal kidneys, maternal decidua, placenta, and fetus (Kovacs, 2016). The higher vitamin D levels observed in this study during the 2nd trimester of pregnancy corresponds with the finding of Møller *et al*, 2013. However, the hypovitaminosis

D observed as compared to the control in this study was 6.19% while the hypervitaminosis D was 21%. This is in contrast to the 29% vitamin D deficiency reported by Gbadegesin *et al,* 2017 in a study of pregnant women in Lagos, Nigeria.

In this study, parathyroid hormone (PTH) levels were higher in the 1st trimester and 2nd trimester, and lower in the 3rd trimester. This decrease in PHT in the 3rd trimester could be due to direct inhibition of PTH production either by previously high concentrations of 1,25-dihydroxyvitamin D or by augmented intestinal absorption of calcium Holt, 2013. This finding agrees with earlier studies (Madar *et al.* 2009, Marwaha *et al.,* 2011, Roth *et al.,* 2013 and Schoenmakers *et al.,* 2013). Furthermore, there was a prevalence of 0.88% hypoparathyroidism and 7.97% hyperparathyroidism.

In this study, serum calcitonin levels were increased in the 2nd trimester, but decreased by the 3rd trimester. There was also a slight decrease in serum calcitonin concentration in the lactating group. The increase in calcitonin level during the 2nd trimester may be attributed to increased production by the C-cells of the thyroid gland and additional sources of expression that may contribute to the circulating level of calcitonin, such as mammary tissue, placenta, and pituitary tissue (Kovacs, 2016). The slight increase in serum calcitonin concentration observed in the lactating group could be attributed to its possible physiological role in protecting the maternal skeleton during pregnancy and lactation (Kovacs, 2016). There was no hypocalcitoninaemia observed in this study, but there was a 1.76% prevalence of hypercalcitoninaemia. This finding contradicts the findings of Møller *et al.,* 2013 who found no change in the level of calcitonin; during the three trimesters of pregnancy.

There was strong negative correlation between vitamin D and calcium as well as vitamin D and phosphate. This suggests that as vitamin D level increases, calcium and phosphate concentrations were decreasing.. Also, the proliferation of the lactiferous ducts, lobules of the breasts and foetal calcium accretion This negative correlation between vitamin D and calcium could be as a result of the diversion of essential elements such as sodium, potassium and calcium to the site of growth and synthesis for growth to take place (especially during the 3rd trimester of pregnancy and the lactation period), causes an increase in calcium demands during this phase. As a result of this increase in demand, there is a negative feedback triggered causing a decrease in the blood serum concentration of calcium and the subsequent increase in vitamin D synthesis, intestinal absorption and reduced renal excretion. This corresponded with the report of Kirby *et al.,* 2013 who demonstrated that serum vitamin D concentrations could increase up to seven times during the course of a normal pregnancy in rats and mice, and this corresponds to the time of rapid foetal mineral accretion (in the third trimester of pregnancy) which could lead to reduced serum calcium levels.

Parathyroid hormone showed a significant negative correlation with calcium. This finding corroborates with the report of Gillies *et al.,* 2015 who reported that high calcium in the diet caused a lower serum PTH concentration. Some animal models have shown that pregnant mice have very low PTH levels (Kirby *et al.,* 2013), these maybe as a result of negative feedback mechanisms.

In conclusion, changes in serum calcium, vitamin D, parathyroid hormone as well as calcitonin during pregnancy and lactation have been demonstrated suggesting a relationship between calcium metabolism and these regulatory hormones at some stages of pregnancy. These variations observed may be critical to the developmental processes during pregnancy.

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