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Studies of Serum Calcium, Inorganic Phosphate and Magnesium Levels in Lactating Mothers in Owerri

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at evaluating the serum levels of Calcium, Inorganic phosphate and Magnesium together with the Body Mass Index (BMI) in lactating mothers in Owerri. A total of sixty subjects were recruited. Thirty were lactating subjects while thirty were apparently healthy individuals that served as control subjects. Whole blood (5mls) was collected by venipuncture from the subjects using sterile needles and syringes into clean and sterile plain containers. The samples were immediately centrifuged and separated. The serum samples were stored at -20°C prior to use. All reagents were commercially purchased and the manufacturers' Standard Operational Procedures were strictly followed. Serum Calcium, Inorganic phosphate and Magnesium levels were analyzed by spectrophotometric method and data was assessed using statistical packages for social sciences (SPSS) version 20.0. The results were expressed as mean and standard deviation (mean ± SD). Difference in mean values between groups was assessed by student t-test. Result with probability value of $P < 0.05$ was statistically significant. The mean ± SD values of serum Calcium, Magnesium, Inorganic phosphate and Body Mass Index (9.28 ± 0.53mg/dl, 2.24 ± 0.38mg/dl, 4.18 ± 0.33mg/dl and 25.73 ± 1.60kg/m²) were higher in lactating subjects which was

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statistically significant (P < 0.05) when compared with the control subjects (8.98 ± 0.50mg/dl, 1.98 ± 0.24mg/dl, 3.34 ± 0.37mg/dl and 24.20 ± 1.35kg/m²). There was progressive decrease between 0 – 3 months, 4 – 6 months and ≥ 7 months with the mean ± SD values of Calcium (9.43 ± 0.55, 9.29 ± 0.52 and 9.12 ± 0.53)mg/dl and Inorganic Phosphate (4.25 ± 0.36, 4.18 ± 0.36 and 4.11 ± 0.27)mg/dl, and non progressive decrease in the mean ± SD values of Magnesium (2.29 ± 0.40, 2.20 ± 0.39 and 2.24 ± 0.38)mg/dl and Body Mass Index (26.30 ± 1.77, 25.30 ± 1.70 and 25.60 ± 1.27)kg/m² which was not statistically significant (P > 0.05). The increase found was due to increased bone resorption and the decrease was due to enhanced mechanism for bone mineralization as the hormones concentrations tend to normalize in prolonged lactation.

Keywords:Calcium; inorganic phosphate; magnesium levels in lactating mothers.

1. INTRODUCTION

Lactation is the process of milk synthesis and secretion. It is characterized by enhanced episodic secretion of prolactin and oxytocin, suppression of the hypothalamus- pituitary-gonadal axis and hypoinsulinaemia [1]. Lactation is a very demanding process in terms of nutrient requirement. Efficient maternal adaptation must occur to successfully compensate for the energy and nutrient requirements of milk production and secretion. Withdrawal of estrogen and progesterone is a prerequisite for lactogenesis, because these sex steroids inhibit the lactogenic effects of prolactin [2].

Lactation leads to temporary demineralization of the maternal skeleton to meet the demands of daily loss of Calcium and phosphate in breast milk. Factors such as suckling, prolactin and the calcium receptor, control the production and release of PTHrP from the breast. This results in markedly up- regulation of bone resorption [3]. Breast milk also draws magnesium from the mother's own reserves to deliver enough to the baby. A magnesium deficient mother is at risk of a number of health disorders. Magnesium homeostasis is maintained by the intestine, the bone and the kidneys [4].

Calcium is the fifth most abundant element in the body and the most prevalent cation [5]. Calcium is found in some foods, added to others, available as a dietary supplement, and present in some medicines (such as antacids). The total body calcium depends upon the calcium absorbed from dietary intake and that lost from the body. Calcium daily dietary intake for an adult human being is about 25mmol /day (1g) of which there is a net absorption of 6 - 12mmol/day(0.25-0.5g). 99% of body calcium is stored in the skeleton while the remaining 1% of the body calcium is essential for neuromuscular excitability and cardiac muscle function [6]. The importance of calcium in lactating mothers cannot be overemphasized; therefore, knowledge of its serum level is very essential for the diagnosis of certain diseases which include Osteoporosis, Osteomalacia, rickets, Paget disease etc. Increased or decreased serum calcium level can be associated with several pathological conditions. Increased serum calcium level above the reference range results in a health condition called hypercalcemia. Also a decrease in the serum calcium level below the reference range is called hypocalcemia [7].Certain drugs and glucocorticoides, such as prednisone, can cause calcium depletion and eventually osteoporosis when they are used for months [8].

Inorganic phosphate is a form of phosphate in serum. It is a major component of hydroxy apatite in bone, thereby playing an important role in the structural support of the body and providing phosphate for extracellular and intracellular pools [9]. It can also be found in saliva, urine and muscles. The daily phosphate intake is about 30mMol, with approximately 80% being absorbed in the jejunum. The output is largely renal, with more than 90% being excreted by this route. Gastrointestinal loss of phosphate accounts for only 10% of the body's phosphate excretion [6]. Observation has shown that serum inorganic phosphate concentration is inversely proportional to blood pressure [10]. Some pathological conditions are associated with increased or decreased inorganic phosphate serum level. A condition of high serum inorganic phosphate above the reference range is called hyperphosphataemia. On the other hand, hypophosphataemia is a condition of decreased serum level of inorganic phosphate below the reference range. Variation in serum level of inorganic phosphate are associated with diseases like rickets, osteomalacia etc.

Magnesium is the second most abundant cation of the extracellular fluid. An adult human body contains approximately 25g of magnesium, with

50 - 60% present in the bones, and most of the rest in soft tissues. Less than 1% of the total magnesium is in the extracellular fluid [11]. The recommended daily allowance of magnesium for adults is about 4.5mg/kg. Magnesium influences protein and carbohydrates metabolism, plays a role in neurochemical transmission, depresses muscular excitability through its peripheral vasodilatory action. Some pathological conditions are associated with decrease in the serum level of magnesium. A condition of low serum magnesium below the reference range is called hypomagnesaemia; increase in the serum level of magnesium above the reference range is called hypermagnesaemia.

Calcium, phosphate and magnesium are electrolytes found in the human body, which rely on tight regulatory control in order to support human life and function. The kidney, intestine and bone are essential in maintaining the fine balance. Diseases affecting any of these organs, or the hormones involved in homeostasis, can disrupt the levels of each electrolyte causing symptomatic and potentially life-threatening consequences.

1.1 Aim

To evaluate the serum levels of calcium, inorganic phosphate and magnesium in lactating mothers.

2. MATERIALS AND METHOD

2.1 Study Area

The study was conducted at Imo State Specialist Hospital, Owerri.

2.2 Sample Population

A total of sixty (60) subjects who were between the ages of twenty and forty-five (20 - 45) years were recruited for this study. Thirty (30) were lactating women who had been attending Imo State Specialist Hospital Owerri. Thirty (30) were apparently healthy women who served as control subjects. The lactating women were regrouped according to duration of lactation; 0 - 3 months, 4 - 6 months and equal to or greater than 7 months.

2.3 Selection Criteria

2.3.1 Inclusive criteria

The subjects were recruited based on:

I. They were lactating women between the ages of twenty (20) to forty-five (45) years.
II. They had been attending the Obstetrics and Gynecology clinic.
III. They were apparently healthy non pregnant, nonlactating women who served as control subjects.
IV. Those whose informed consent was obtained.

2.4 Exclusive Criteria

This study excluded

I. Subjects below the age of twenty (20) years and above forty-five (45) years.
II. Subjects who were severely ill or on drugs that could affect the test results.
III. Those whose informed written consent were not obtained.

2.5 Sample Collection

The blood sample was collected by asceptical collection of 5 ml of blood through venipuncture using sterile disposable needles and syringes. It was dispensed into dry, clean centrifuge tube and allowed to clot within 2 hours at room temperature. It was then centrifuged at 1500 rpm for 10 minutes. The serum was collected and dispensed into plain bottles and was used for the analysis of Calcium, Inorganic phosphate and Magnesium.

2.6 Laboratory Procedures

All reagents were commercially purchased and the manufacturers' standard operating procedure was strictly adhered to.

2.7 Determination of Calcium

This test was done by spectrophotometric Method of Agappe Diagnostics. Catalogue Number: 11006001.

2.8 Procedure

Three test tubes were labeled Test (T), Standard (S) and Blank (B). 1ml of calcium reagent was pipetted into each of the tubes. To the tube labeled T, 0.01ml of the sample was added, while 0.01ml of standard and distilled water were added to the tubes labeled S and B respectively. The tubes were mixed and incubated at room temperature for 3 minutes. The absorbance of the test and standard were read at 560nm zeroing with the blank.

Calculation: $\frac{\mathbf{ODT}}{\mathbf{ODS}}$ **X** $\frac{\mathbf{10mg/dl}}{\mathbf{1}}$

Reference Range: 8.5 - 10.5mg/dl

Determination of Inorganic Phosphate

This test was done by spectrophotometric Method of Agappe Diagnostics. Catalogue Number: 11012001.

2.9 Procedure

Three test tubes were labeled Test (T), Standard (S) and Blank (B). 1ml of phosphorus reagent was pipetted into each of the tubes. To the tube labeled T, 0.02ml of the sample was added, while 0.02ml of standard and distilled water were added to the tubes labeled S and B respectively. The tubes were mixed and incubated at room temperature for 5 minutes. The absorbance of the test and standard were read at 350nm zeroing with the blank.

Calculation: $\frac{\mathbf{ODT}}{\mathbf{ODS}}$ **X** $\frac{\mathbf{5mg/dl}}{\mathbf{1}}$

2.10 Determination of Magnesium

This test was done by spectrophotometric Method according of modified by Agappe Diagnostics. Catalogue Number: 11019001.

2.11 Procedure

Three test tubes were labeled Test (T), Standard (S) and Blank (B). 1ml of magnesium reagent was pipetted into each of the tubes. To the tube labeled T, 0.01ml of the sample was added, while 0.01ml of standard and distilled water were added to the tubes labeled S and B respectively. The tubes were mixed and incubated at room temperature for 10 minutes. The absorbance of the test and standard were read at 560nm zeroing with the blank.

Calculation: $\frac{\mathbf{ODT}}{\mathbf{ODS}}$ **X** $\frac{\mathbf{2mg/dl}}{\mathbf{1}}$

2.12 Statistical Analysis

The statistical analysis was carried out using SPSS (statistical packages for social sciences). All values were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) and students T- test was used to detect the difference in the experimental variables. The test with a probability of ≤ 0.05 was considered statistically significant.

3. RESULTS

Table 1 shows the mean ± SD values of body mass index, calcium, magnesium and inorganic phosphate in lactating mothers. The mean ± SD value of BMI in lactating mothers (25.73±1.60 kg/m^2) was higher which was statistically significant (P = 0.0001) when compared with the control subjects (24.20 ± 1.35 kg/m^2).

The mean ± SD values of calcium in lactating mothers (9.28 ± 0.53mg/dl) was higher which was statistically significant (P = 0.027) when compared with the control subjects (8.98 ± 0.50mg/dl).

The mean ± SD values of magnesium in lactating mothers (2.24 ± 0.38 mg/dl) was higher which was statistically significant (P = 0.002) when compared with the control subjects (1.98 ± 0.24mg/dl).

The mean ± SD values of inorganic phosphate in lactating mothers (4.18 ± 0.33 mg/dl) was higher which was statistically significant (P = 0.001) when compared with the control subjects (3.34 ± 0.37mg/dl).

Table 2 shows the mean ± SD values of body mass index, calcium, magnesium and inorganic phosphate in lactating mothers according to duration of lactation. The mean ± SD values of BMI (26.30 ± 1.77, 25.30 ± 1.70, 25.60 ±1.27) kg/m^2 of lactating mothers decreased non – progressively which was not statistically significant (P = 0.368), as lactation progresses to ≥7months.

The mean ± SD values of calcium (9.43 ± 0.55, 9.29 ± 0.52, 9.12 ± 0.53) mg/dl of lactating mothers progressively decreased which was not statistically significant (P = 0.438), as lactation progresses to ≥7 months.

The mean ± SD values of magnesium (2.29 ± 0.40, 2.20 ± 0.39, 2.24 ± 0.38) mg/dl of lactating mothers decreased non – progressively which was not statistically significant (P =0.875), as lactation progresses to ≥7 months.

The mean ± SD values of inorganicphosphate (4.25 ± 0.36, 4.18 ± 0.36, 4.11 ± 0.27) mg/dl of lactating mothers progressively decreased which was not statistically significant (P = 0.649), as lactation progresses to ≥7 months.

Table 1. The Mean ± SD Values of Body Mass Index, Calcium, Magnesium and Inorganic Phosphate in Lactating Mothers of the Study Population

Parameters	Lactating subjects (N = 30)	Control subjects (N = 30)	P-Value
BMI (kg/m²)	25.73 ± 1.60*	24.20 ± 1.35	0.0001
Calcium (mg/dl)	9.28 ± 0.53*	8.98 ± 0.50	0.027
Magnesium (mg/dl)	2.24 ± 0.38*	1.98 ± 0.24	0.002
Phosphate (mg/dl)	4.18 ± 0.33*	3.34 ± 0.37	0.001

Key::Statistically significant when compared with the mean ± SD values of the control subjects (P-value < 0.05), N: Sample Size, BMI: Body Mass Index*

Table 2. The Mean ± SD Values of Body Mass Index, Calcium, Magnesium and Inorganic Phosphate in Lactating Women According to the Duration of Lactation of the Study Population

Parameters	0-3 months (N=10)	4-6 months (N=10)	≥7months (N=10)	F-value	P-value
BMI(kg/m^2)	26.30 ± 1.77	25.30 ± 1.70	25.60 ± 1.27	1.036	0.368
Calcium(mg/dl)	9.43 ± 0. 55	9.29 ±0.52	9.12 ± 0.53	0.851	0.438
Magnesium (mg/dl)	2.29 ± 0.40	2.20 ± 0.39	2.24 ± 0.38	0.134	0.875
InorganicPhosphate(mg/dl)	4.25 ± 0.36	4.18 ± 0.36	4.11 ± 0.27	0.440	0.649

KEY: N – Sample size.

4. DISCUSSION

In this study, result from table 1 showed that there was a statistically significant increase (P = 0.027) in the mean ± SD values of serum calcium (9.28 ± 0.53 mg/dl), in lactating mothers when compared with the control subjects (8.98 ± 0.50mg/dl). This was due to increased bone resorption and reduced renal calcium excretion during lactation. This is in agreement with the studies of [12] which observed an increased serum calcium levels in lactation due to activated osteoclastic bone resorption. Also, the result from table 2 showed that there was a progressive decrease which was statistically non- significant (P = 0.438) in the mean ± SD values of serum calcium in lactating mothers as lactation progresses between 0 – 3 months (9.43 ± 0.55 mg/dl), 4 – 6 months (9.29 ± 0.52mg/dl) and ≥ 7 months (9.12 ± 0.53 mg/dl). This was due to prolactin and estradiol concentrations which tend to normalize as lactation progresses. This agrees with the studies of [13] which noted a lower mineral content of breast milk at 6 months compared with 3 months postpartum and a daily maternal mineral loss which may be greater when lactation extends to 6 months and beyond.

The result from table 1 showed that there was a statistically significant increase (P = 0.001) in the mean ± SD values of serum Inorganic phosphate (4.18 ± 0.33 mg/dl) in lactating mothers when compared with the control subjects (3.34 ± 0.37 mg/dl). Also, result from table 2 showed that there was a progressive statistically non-significant decrease (P = 0.649) in the mean ± SD values of serum Inorganic phosphate in lactating mothers as lactation progresses between 0 - 3 months (4.25 ± 0.36 mg/dl), 4 - 6 months (4.18 ± 0.36 mg/dl) and ≥ 7 months (4.11± 0.27 mg/dl). This was due to the concentration of the hormones prolactin and estradiol which tend to normalize as lactation progresses. This equally agrees with the studies of [13] and could be explained by the work of [14] which observed a negative bone mineral balance during the interval of greatest milk production, despite ingestion of supplemental calcium and phosphorous; with balance returning only at the period of lessened milk production.

The result from table 1 showed that there was a statistically significant increase (P = 0.002) in the mean ± SD values of serum magnesium (2.24 ± 0.38 mg/dl) in lactating mothers when compared with the control subjects (1.98 ± 0.24 mg/d). The increase was due to increased maternal skeletal resorption to meet the demands of daily loss of these minerals in breast milk. This is in agreement with the studies of [3] which noted a temporary demineralization of the maternal skeleton to meet the demands of daily loss of bone minerals in breast milk. The result from table 2 showed that there was a non- progressive statistically non – significant decrease (P = 0.875) in the mean ± SD values of serum magnesium in lactating mothers as lactation progresses between 0 – 3 months (2.29 ± 0.40 mg/dl), 4 – 6

months (2.20 ± 0.39 mg/dl) and ≥ 7 months (2.24 ± 0.38 mg/dl). This was due to prolactin and estradiol concentrations which tend to normalize as lactation progresses. This is in agreement with the findings of [13] which observed a daily maternal mineral loss which may be greater when lactation extends to 6 months and beyond.

The result from table 1 showed that there was a statistically significant increase (P = 0.0001) in the mean ± SD values of the Body Mass Index (BMI) of the lactating mothers (25.73 ± 1.60 kg/m²) when compared with the control subjects (24.20 ± 1.35 kg/m²). This was due to part of the obligatory weight gained during pregnancy. This is in agreement with the studies of [15] which observed an obligatory pregnancy weight gain. Result from table 2 showed a non - progressive statistically non-significant decrease (P = 0.368) in the mean ± SD values of Body Mass Index (BMI) of lactating mothers as lactation progresses between 0 – 3 months (26.30 ± 1.77 kg/m²), 4 – 6 months (25.30 ± 1.70 kg/m²) and ≥ 7 months (25.60 ± 1.27 kg/m²). This was due to the caloric expenditures required for lactation. This is in agreement with the findings of [16] which noted that breastfeeding may promote postpartum weight loss, due to the caloric expenditures required for lactation.

Lactation is accompanied with a number of physiological changes which include increased levels of prolactin and parathyroid hormone – related peptide (PTHrP) with low levels of parathyroid hormone (PTH) and estrogen for the first several months of breastfeeding (estrogen may also remain low as long as lactation lasts in some women). The period of these changes correspond with the period of neonatal higher mineral requirement and increased suckling which stimulate PTHrP release, spikes prolactin production and lowers estrogen levels. The lowered estrogen (bone mineralization enhancer) levels results to increased bone demineralization. Increased bone demineralization occurs during the first 6 months postpartum, during which period the maternal mean serum mineral (Calcium, Inorganic phosphate and Magnesium) levels were increased. As lactation progresses longer than 6 months, suckling reduces with the introduction of solid food from which the neonate absorbs a fraction of the needed minerals especially calcium. The concentrations of prolactin and estradiol also tend to normalize. Thus, the decrease in the mean maternal serum levels of calcium, inorganic phosphate and magnesium, though the decrease was statistically non-significant as maternal bone resorption continues all through the period of lactation. This reduces maternal bone mass and could lead to impaired bone health. These findings was supported by the studies of [17], in which they found that PTHrP levels correlate negatively with Parathyroid Hormone (PTH) and positively with ionized calcium levels of lactating women. Ma, [12] also observed that PTHrP levels correlate with loss of bone mineral density during lactation. This was in agreement with the studies of [18,19], in which they observed that longer breastfeeding reduces maternal mineral density even more. However, this was not in agreement with the studies of [20], where they observed no significant differences in the number of fractures and the length of breastfeeding period in a - six - year follow up study. Kalkwarf*etal*., [21] also observed that fragility fractures of the spine and other sites rarely occur during lactation and may result from the normal physiological resorption of the skeleton during lactation, combined with effects of low bone mass or skeletal resorption that may have occurred during pregnancy. Inadequate consumption of the dietary sources of these bone minerals could pose a serious problem during pregnancy and lactation. Since the richest sources of these minerals especially calcium (which are cheese and milk) may not be within the reach of most women within reproductive age; and their supplements not significant (as only a small percentage of women take them); this study would suggest the enhancement of serum calcium by the effect of sunlight through Vitamin D. this is in order to build adequate bone mass before peak bone formation is reached so as to avoid impaired bone health due to pregnancy and lactation [22-24].

5. CONCLUSION

In conclusion, this study found out that there was decreased serum levels of calcium, inorganic phosphate and magnesium in prolonged lactation; which may be due to enhanced and efficient mechanism for bone mineralization as the hormones especially prolactin and estrogen levels tend to normalize.

ETHICAL APPROVAL AND CONSENT

A letter of introduction was obtained from the Head of Department, Medical Laboratory Science of Imo State University. The letter was submitted to the ethical committee of the hospital. An ethical approval was obtained for the

collection of blood samples from lactating mothers attending the Obstetrics and Gynecology clinic and appropriate dates fixed for sample collection. Written informed consent along with questionnaires was obtained from all the participants in the study.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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