Original Article

Study of Serum Iron, Calcium and Phosphorus Level in Lactating Women Compared with Non Lactating Women in Bangladesh

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Abstract

Background: *Lactation encompasses the process of milk production and secretion by mammary glands. It significantly influences the levels of serum iron, calcium, and phosphorus, thereby elevating the risks of osteoporosis, malnutrition, and other maternal complications.* ***Objective:*** *the aim of this study was to evaluate serum iron, calcium and phosphorus status in lactating women in Bangladesh.* ***Materials and method:*** *This case control study was carried out in the department of Biochemistry, Mymensingh Medical Collcge, Mymensingh, with the collaboration of the department of Gynaecology and Obstetrics, Mymensingh Medical College Hospital, Mymensingh, during the period of July 2016 to June 2017. A total of 60 apparently healthy lactating women were selected as case and 60 apparently healthy non-lactating women were selected as control. Serum iron, calcium and phosphorus levels were measured for both groups. Statistical analyses of the results were obtained by using Statistical Packages for Social Sciences (SPSS-22) software.* ***Results:*** *The mean ± SD serum calcium levels were estimated to be 7.74±0.67 mg/dL in lactating women and 8.75±0.78 mg/dL in non-lactating women respectively, and the difference was statistically significant ($p<0.05$). But the serum iron and serum phosphorus were not significantly changed between two groups.* ***Conclusion:*** *There is no significant difference in serum iron and phosphorus levels between lactating and non-lactating women but significantly decreased serum calcium level is observed in lactating women.*

Keywords: *Lactation; Serum iron; Serum calcium; Serum phosphorus.*

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Introduction

Lactation is the secretion of milk from the mammary glands to feed offspring, regulated by hormones prolactin and oxytocin. Milk production is stimulated by the delivery of the placenta, which causes a reduction in certain hormone levels.[1] Minerals are essential, non-caloric elements that play vital roles in growth and maintenance of the body, while trace elements are needed in small

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amounts for proper growth and physiological function.[2] They act as co-factors of enzymes and organizers of the molecular structure of the cell. Deficiencies or excesses of trace elements can have toxic effects.[3]

Iron is an essential element involved in numerous metabolic processes such as oxygen transport, DNA synthesis, and electron transport. It is predominantly found in hemoglobin, myoglobin, and stored as ferritin or hemosiderin in the spleen, bone marrow, and liver. Tight regulation of iron concentration is crucial due to its potential for tissue damage as it can generate free radicals. Disorders of iron metabolism range from anemia to iron overload and may even be associated with neurodegenerative diseases.[4] During lactation, serum iron concentration fluctuates to meet the demands of the infant. Hepcidin, a regulatory hormone, plays a role in maintaining iron levels during lactation. Increased levels of intestinal ferroportin 1 suggest enhanced iron absorption from the small intestine as a response to the low iron levels, preventing iron storage depletion and meeting the high iron demand during lactation.[5]

Calcium, the most abundant mineral in the body, is primarily present as hydroxyapatite in the skeleton. The remaining 2% is found in extracellular fluid and various tissues, including skeletal muscle. During lactation, serum calcium concentration decreases, with around 280 to 300 mg of calcium lost daily through breast milk. Lactating women meet this calcium requirement through temporary demineralization of their skeleton. This demineralization is not regulated by parathyroid hormone (PTH) or calcitriol but rather by parathyroid hormone-related protein (PTHrP) due to decreased estrogen levels.[6] Excessive bone resorption can lead to negative remodeling balance and bone loss when the balance between formation and resorption is disrupted. Despite its seemingly stable nature, bone undergoes dynamic turnover, which increases during lactation due to estrogen deficiency.[7]

Phosphorus is an essential mineral present in every cell of the body, making up 1% of total body weight. It is crucial for the development of bones and teeth, as well as for forming the sugar-phosphate backbone of DNA and RNA and activating catalytic proteins.[8] The majority of phosphorus is found in bones, with the remaining distributed through soft tissues. Serum phosphorus levels fluctuate during lactation to meet body demand, with no significant difference between lactating and non-lactating mothers, likely due to changes in parathyroid hormone related protein levels. Phosphorus also plays a major role in maintaining blood systemic acid balance and acts as a transport mechanism for energy. It is also a major buffer in urine due to its various forms.[9]

This study aims to observe changes in serum iron, calcium, and phosphorus levels in lactating women in Bangladesh, as lactation can impact these levels and increase the risk of anemia, osteoporosis, and degenerative changes. The findings will provide valuable insights for clinicians and update their knowledge on these levels in lactating women.

Materials and method

This cross-sectional study was conducted at the department of Biochemistry, Mymensingh Medical College, Mymensingh, in collaboration with the inpatient department of Obstetrics and Gynaecology at Mymensingh Medical College and Hospital, Mymensingh, during the period from July 2016 to June 2017. A total of 60 apparently healthy lactating women were selected as case and 60 apparently healthy non-lactating women were selected as control. To ensure the validity of the study, certain exclusion criteria were applied. Women with diabetes mellitus, chronic renal failure, thyroid disorders, alcoholism, those taking medications containing iron, calcium, or phosphorus within the last 6 months, as well as those with obstructive jaundice, were excluded based on their medical history and physical examination. Before the study

commenced, the selected participants were thoroughly briefed on the objectives, nature, purpose, and potential risks of all procedures involved. Written informed consent was obtained from each woman. Additionally, ethical clearance was obtained from the institutional review board's ethical committee, and the study adhered to the guidelines set by the Bangladesh Medical Research Council (BMRC). Confidentiality was assured to the participants, stating that the findings of the study would not be disclosed to any unauthorized person or authority other than for research purposes. Furthermore, the participants were informed that they had the freedom to participate and decline to answer any questions during the study. The study involved collecting data through survey questionnaires, covering various aspects such as age, height, weight, body mass index, occupation, drug history, history of recent or chronic illness, and duration of lactation. Measurements of height and weight were taken with participants wearing light clothing and without shoes. Blood pressure was measured after a 10 to 15-minute rest using standard cuffs fitted with a mercury sphygmomanometer. Body mass indexes (BMI) of the subjects were calculated using standard formula, BMI = Weight (kg)/[Height (m)]^2.

Blood sample collection: All subjects were requested for giving blood sample in the next morning without taking, any food or drink. With all aseptic precaution 5 mL o venous collected Iron median ante capital vein of the subject by a sterile disposable syringe. The collected blood samples were transferred to a dry collection was screw capped test tube immediately after removal of the needle from the syringe with gentle push to avoid hemolysis. Test tube was kept in standing position until clot formation.

Blood sample collection: Collected samples were allowed to clot for sufficient time (10-15 minutes). After clot formation, the test tubes were centrifuged at 3000 rpm for 5 minutes in a centrifuged machine. In some cases, centrifugation, serum was picked up by micro pipette gently and was kept in a screw capped glass tube for the estimation of serum iron, calcium and phosphorus. Serum iron was estimated by colorimetric method using test kit, serum calcium was determined by colorimetric method by using test kit and serum phosphorus was determined by colorimetric method using test kit. Analyses of different concentration of standard solution of serum iron, calcium and phosphorus were preformed to obtain a calibration chart.

Storage: The analytic procedure was carried out as soon as possible. Whenever there was delay in experiments, samples were stored by refrigeration at -20°c prior to the analysis, for maximum of 10 days. Control sera (both normal and abnormal), duplicate standard and duplicate were run in every batch of teat procedure.

Statistical methods

A statistical analysis was carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS 22). Quantitative variables were presented as Mean±SD and tested by the unpaired t-test. p values <0.05 were considered as statistically significant.

Results

The mean age was 29.60±4.36 years in case group and 27.80±4.0 years in control group. The mean BMI was 25.74±2.01 kg/m^2 and 26.25±2.14 kg/m^2 in case and control group respectively. The differences were not statistically significant (p >0.05) between two groups.

Table I: Biochemical characteristic of the study participants (N=120)

Biochemical parameters	Case (n=60)	Control (n=60)	p value
	Mean±SD	Mean±SD	
Serum Iron (µg/dL)	93.2±39.4	86.5±27.6	0.283^{ns}
Serum Calcium (mg/dL)	7.74±0.67	8.75±0.78	0.001^{s}
Serum Phosphorus (mg/dL)	4.10±0.63	4.28±0.79	0.170^{ns}

s= significant, ns= not significant, p value reached from unpaired t-test

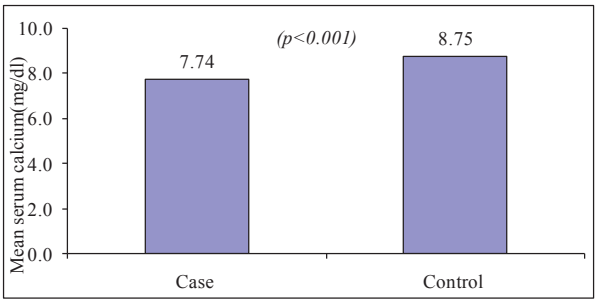

Fig. 1: Bar diagram showing significant (p<0.05) difference between mean serum calcium level of case and control

Discussion

Lactation encompasses the process of milk production and secretion by mammary glands. It significantly influences the levels of serum iron, calcium, and phosphorus, thereby elevating the risks of osteoporosis, malnutrition, and other maternal complications. Maternal iron storage, iron transport proteins (FPNI and Cp), and the regulatory molecule hepcidin play crucial roles in nutrient supply to the offspring, as evident in previous studies.[10] Despite the obligatory transfer of iron into breast milk, maternal iron status is not adversely affected. Contrary to the general belief of lactating women being in a negative nutritional balance, the iron status of lactating women in our population remains under-evaluated. Iron transfer to the fetus may rely on maternal iron reserves, and iron deficiency has been identified as a prevalent issue among lactating women.[11] Adequate social and nutritional support for lactating mothers is essential as recommended by the WHO Expert Consultative Council in 2001.[12] Various factors such as low socioeconomic conditions, dietary inadequacies, disease or medication use, renal insufficiency, changes in stem cell physiology, malabsorption, polypharmacy, and genetics can impact the bioavailability of micronutrients. Iron, being a reactive metal ion, can lead to cellular damage through the production of highly reactive oxygen-derived free radicals. The reduction of iron from ferric (Fe^{3+}) to ferrous (Fe^{2+}) states plays a significant role in the process of lipid peroxidation. As iron concentration increases, it can accumulate in the liver and ferritin, along with iron storage proteins, may serve as a source of iron for promoting superoxide-dependent lipid peroxidation.[13] In this study, the mean ± SD serum iron levels were estimated to be 93.2±39.4 µg/dL and 86.5±27.6 µg/dL and in the case and control groups, respectively. This findings indicate a non-significant (p>0.05) increase in serum iron levels among lactating women compared to healthy reproductive women. Notably, this study results are consistent with several previous studies.[5,14-17] These findings contribute to the existing body of knowledge and support the understanding of serum iron dynamics in lactating women compared to non-lactating women.

In this study, the mean ± SD serum calcium levels were estimated to be 7.74±0.67 mg/dL in case and 8.75±0.78 mg/dL in control. A significant decrease (p<0.001) in mean serum calcium level was observed in case group compared to control group. These findings are consistent with previous studies by Kovacs & Fuleihan[6], De Santiago et al.[18], and Cross et al.[19], providing further support for the correlation between lactation and decreased serum calcium levels. During breastfeeding, there is a significant transfer of minerals from mother to child, which constitutes a substantial portion of the mother's mineral intake, particularly when dietary intake is inadequate. The high calcium demand for fetal skeletal growth can lead to reduced serum calcium levels in lactating mothers.[20] However, it is important to note that conflicting findings have been reported. Some studies, such as those by Kovacs & Kronenberg[9] and Heringhausen & Montgomery[21], have reported higher serum calcium levels in lactating women. During lactation, the combined effect of PTHrP and estrogen deficiency can increase skeletal resorption, decrease renal calcium losses, elevate blood calcium levels, and direct calcium into breast milk. Low levels of calcium in lactating women can contribute to osteoporosis, muscle

cramps, miscarriage, joint pain, and bone deformities.[22]

This study investigated the phosphorus levels in lactating and non-lactating women in Bangladesh. It was observed that the mean±SD of serum phosphorus levels in the lactating group was found to be 4.10±0.63 mg/dL, while in the non-lactating group it was 4.28±0.79 mg/dL. Upon comparing these two groups, a non-significant decrease in the mean value of serum phosphorus ($p>0.05$) was observed among lactating women compared to apparently healthy reproductive women. This finding aligns with the conclusions drawn by previous studies conducted by Heringhausen & Montgomery[21] and Naylor et al.[23] These studies similarly reported no significant disparities in serum phosphorus levels between lactating and non-lactating women. Therefore, the present study findings corroborate and support the existing body of research on this topic.

Several studies, including Kovacs & Fuleihan[6], Kent et al.[24], King et al.[25], and Lippuner et al.[26] have reported elevated phosphorus levels in lactating women, which contrasts with the current study findings. These studies suggest that the increased reabsorption of phosphorus by the kidneys, elevated intake from the diet, and skeletal resorption, combined with reduced renal excretion influenced by PTHrP, may contribute to higher phosphorus levels during lactation. Phosphorus plays a crucial role in the normal metabolism of various compounds, and low levels can lead to osteomalacia, osteoporosis, joint pain, stiffness, and lack of appetite.

Conclusion

This study was undertaken to assess the serum iron, calcium, and phosphorus levels in lactating women in Bangladesh. The study findings revealed that the serum iron and phosphorus levels were relatively similar between lactating and non-lactating women. However, a notable and statistically significant decrease in serum calcium levels was observed among lactating women. This highlights the importance of monitoring calcium levels and ensuring adequate intake during lactation to support the physiological demands of milk production.

Limitations

The sample size was small and other biochemical parameters were not evaluated as well as done in a selected hospital from Mymensingh with short duration, which may not be adequate to represent the total population. Therefore, a large scale prospective study with the application of more modern sophisticated technology may be planed to find out the relationship of these biochemical variables with lactating women.

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