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Effective study of the prolactin on cellular communication network factor 3 at the osteoporotic lactating Iraqi women's

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Abstract: Breastfeeding is a natural biological phenomenon that usually occurs in women after childbirth due to the elevated production of the hormone prolactin, which triggers the secretion of milk from the lobules in the breast to feed the newborn. This process is generally associated with mineral, vitamin, and protein deficiencies in the mother and entails significant physiological alterations. The main objective of the study was to assess the correlation between blood prolactin hormone levels, vitamin D3, and CCN3 protein concentrations in lactating and non-lactating females. Two groups were selected for this purpose: the first included lactating females only, and the second consisted of non-lactating females. Serum samples from all participants were tested for prolactin using radioimmunoassay, vitamin D3 by chemiluminescence enzyme immunoassay, and CCN3 protein by infiltration technique. According to the quantitative analysis, lactating females had significantly higher concentrations of prolactin hormone and CCN3 compared to non-lactating females, along with a decrease in vitamin D3. To the best of the authors' knowledge, the present study reveals that the increase in prolactin hormone during lactation stimulates a protective response through the upregulation of CCN3 protein. This protein plays a crucial role in cellular signaling, enhancing bone structure, and protecting against complications such as osteoporosis, which may arise due to reduced vitamin D3 levels.

Keywords: *Bone health, Cellular communication network factor 3, Lactation, Prolactin hormone, Vitamin D3.*

1. Introduction

Lactating is a physiological state that all females go through after birth and it is also considered as important state for the babies, as the baby at this stage derives its nutrition from the mother through milk of breastfeeding [1]. One of the most important characteristics that accompany the mother at this stage is the increase in the mother's prolactin hormone. In most cases, various healthy complications begin in the lactating females, the most important of which is osteoporosis. Osteoporosis is characterized by a decrease in bone density and is formed due to the weakness of the bone matrix due to factors that enhance osteoclast cells versus osteoblastic cells [2]. The current study aims to identify the mechanism of the association between the increase in prolactin hormone in the lactating females and osteoporosis. Prolactin hormone is a hormone produced by the anterior lobe of the pituitary gland and it is a hormonal protein that targets the mammary glands in the female's breast and works to stimulate milk production from the mammary glands. During the lactating stage, the mother begins to lose most of the important elements in her body, the most important of which is vitamin D3[3].

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2. Literature Review

Lactating is a physiological condition that occurs in women after birth, where the mammary glands in the breasts begin to produce milk, which is the most important source of nutrition for the infant, via the effect the prolactin hormone. The mammary glands begin to grow during pregnancy under the influence of placental and ovarian hormones (progesterone and estrogen), which work to inhibit the production of prolactin. However, after delivery , a change occurs in the physiology of the body, as the inhibitory effect on the prolactin hormone disappears, and the hypothalamus gland begins to produce prolactin-releasing hormone, which activates the anterior lobe of the pituitary gland to produce and secrete prolactin hormone begins to target at the latter the mammary glands in the breasts, which are prepared for the prolactin effect during pregnancy , so that the mammary glands begin to produce milk for the infant [1]. Prolactin is a peptide hormone made up of peptide chains, act to enhance milk production from the mammary glands, prevent menstruation and others. On the other hand, the oxytocin hormone acts to ejaculate milk from the breast to the infant [2] See Figure 1 .

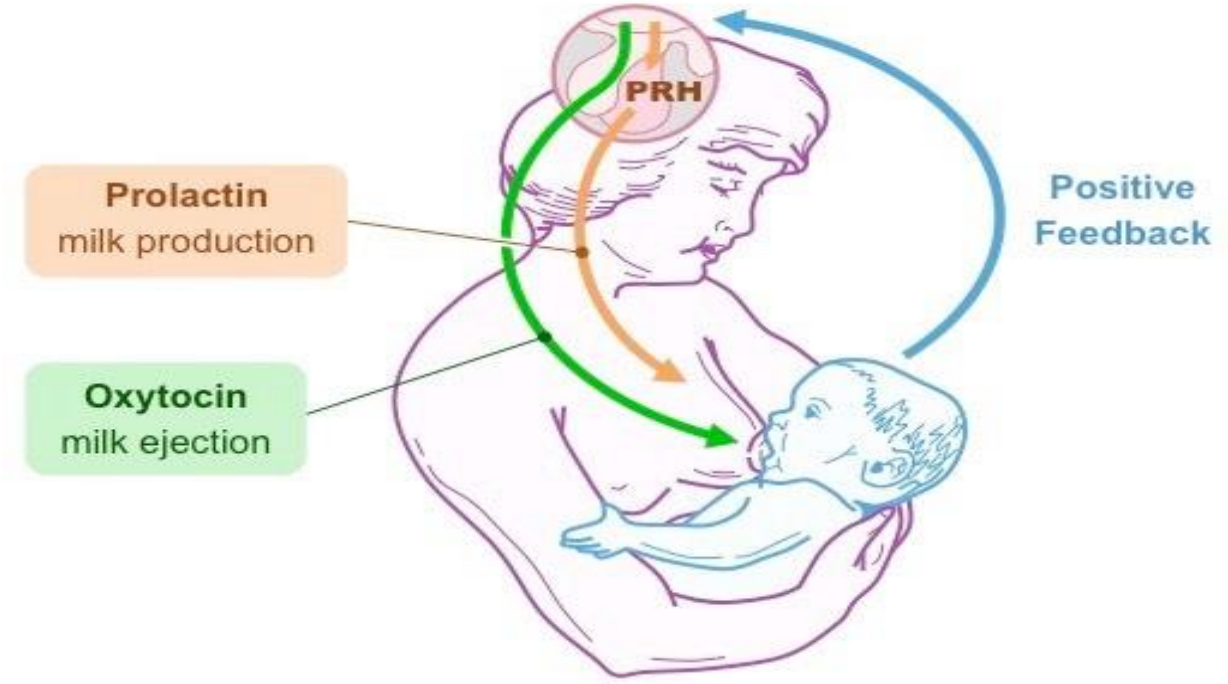

Figure 1.
Prolactin and oxytocin hormones secretion mechanism (PRH: Prolactin).
Source: Jacob and Pita [2].

Cellular Communication Network Factor 3 (CCN3) is a protein structure produced by the CCN3 gene in neurons of chondrocytes, smooth muscle and endotheliocytes, and belongs to the CCN3 protein family. CCN3 is a small protein and rich in cysteine [3]. CCN3 has multiple and important functions in the human body, the most important of which is the arrangement of the extracellular matrix in bones, cartilage, blood vessels, cancer and others. CCN3 activity depends on the presence of calcium ions in signaling pathways that require calcium. In addition, CCN3 interacts with several proteins in the cell like integrin, fibulin-1C, S100A4 and Notch to achieve its function, whether intracellular or extracellular. CCN3 protein effectively strength the bone matrix by activating certain proteins that are involved in this process , such as bone morphogenetic protein 4 (BMP-4) and others has the same function in strengthening and rebuilding bone tissue [4]

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Vitamin D3 is a derivative of steroid compounds that are fat soluble and has two sources, exogenous through food or nutritional supplements and endogenous through the conversion of the subcutaneous 7-Dehydrocholesterol to vitamin D3 [5]. Vitamin D3 has metabolic stages in the body until it is converted to the active form, as it passes through the liver and kidneys to become effective. It also needs special receptors to start its effectiveness. See figure 2.

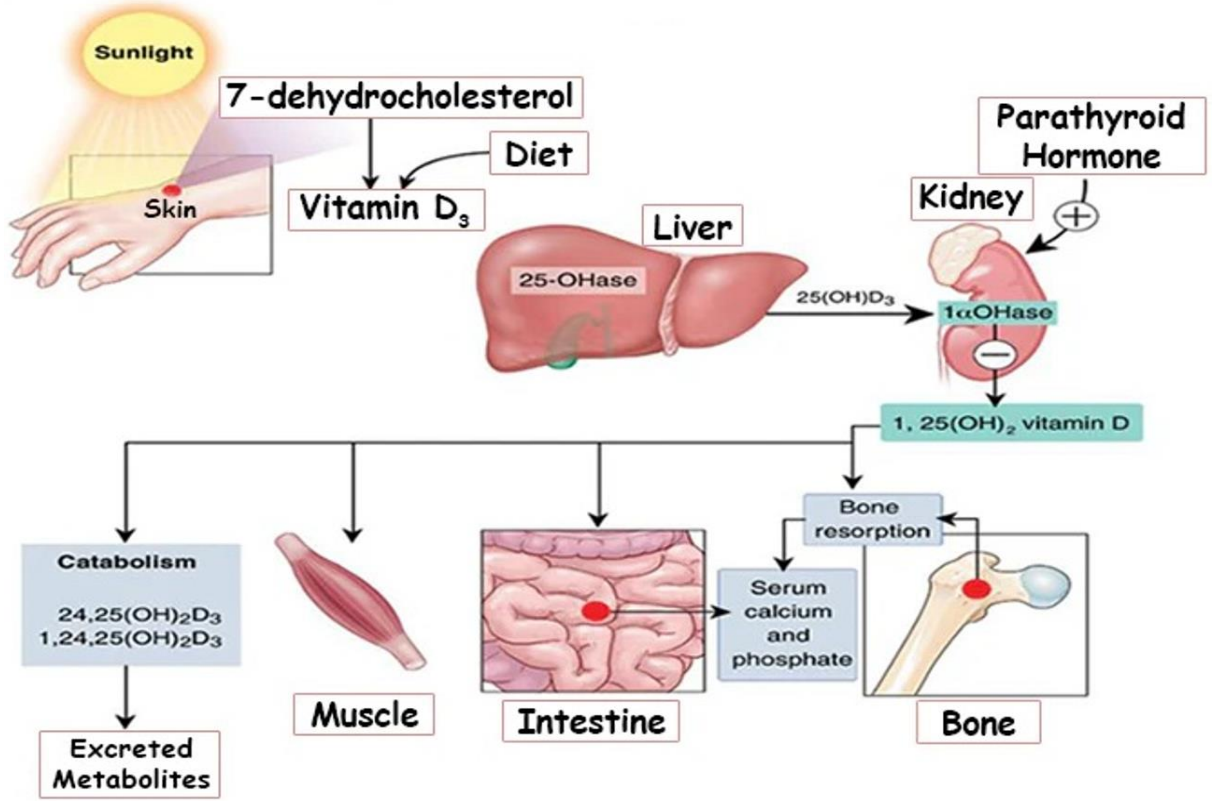

Figure 2.
Vitamin D3 metabolism.

Vitamin D3 has multiple functions for the body, formation of bone matrix is the most crucial one . Building the bone matrix requires the presence of vitamin D3 because it increases the absorption of calcium in the intestine. In the case of vitamin D3 deficiency, the bone tissue begins to resorption and weakness, which lower bone density and is referred as osteoporosis. Osteoporosis often appears in lactating women for physiological and lifestyle reasons [6].

This study aims to contribute to clarifying the effect of prolactin hormone on bone density in Iraqi lactating women through its effect on CCN3 protein, which it act to strengthen the bone matrix.

3. Material and Methods

3.1. Study Design

The following two groups of Iraqi women are included in this study:

- 25 postpartum women with joint bone pain , aged 35-45 years participated in this study . These individuals were diagnosed with osteoporosis based on the ACR classification criteria for 2022 [7].
- 25 comparative group consisted of 25 of non-breast feeding women between the ages 35-45 years.

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Clinical manifestations that are in line with the ACR guidelines for the first group of participants were demonstrated by episodes of osteoporosis in the present study. Convenience samples of all the participants were enlisted from various gynecological clinics in Baghdad, Iraq during May to July 2024 once they gave their voluntary informed consent from answered structured questionnaires. This study followed ethical considerations in accordance with the Helsinki Declaration of 2000. Also, this study was agreed from Ethical Accreditation Committee for Research / Iraq.

3.2. Sample Collection and Preparation

Venous blood specimens were taken from all bodies. An aliquot of each serum sample was collected by centrifugation and then stored at 80°C for further analysis. Some of the types of biomarkers measured included prolactin hormone, Vitamin D3, CCN 3, osteocalcin, RANKL/OPG, C reactive protein, malondialdehyde, total antioxidant capacity interleukin-6 and tumor necrosis factor alpha.

3.3. Measurement of Biomarkers

The study employed advanced biochemical techniques to measure biomarkers using specialized kits. The details of the kits used for prolactin hormone, vitamin D3, and CCN3 are shown in Table 1. The additional biomarkers were measured using commercial ELISA kits according to the manufacturer's protocols.

Table 1.
Biomarker Kits.

Lot Number	Kit Company Name	Biochemical Principle of Test	Biomarker Name
RE52131	IBL International GmbH/Germany	Solid-phase enzyme-linked immunosorbent assay (ELISA), sandwich principle	Prolactin hormone
VESHB09E	Boditech Med Inc./Korea	Fluorescence immunoassay (FIA)	Vitamin D3
SK00265-01	Aviscera Bioscience/USA	Quantitative sandwich ELISA	CCN3

Additional biomarkers (osteocalcin, RANKL/OPG ratio, CRP, MDA, TAC, IL-6, TNF-α) were quantified using ELISA kits from Abcam (UK), following the standard sandwich ELISA protocol, with sensitivity as per the manufacturer's specifications.

3.4. Statistical Analysis

Comparative analysis between the lactating and non-lactating groups was performed using the t-test. Differences were considered statistically significant when $p < 0.05$. Statistical variables analyzed included the mean, standard deviation (SD), and p-values for each biomarker. The analysis was extended to include oxidative stress and inflammatory markers to enhance the scope of the study. Data analysis was conducted using SPSS software version 29 (2022) [8].

4. Results

The research reveals significant differences in biomarker levels between lactating and non-lactating women. The results, analyzed using the t-test, show higher levels of prolactin hormone and CCN3 protein in lactating women compared to non-lactating, while vitamin D3 levels were notably lower in the lactating group. Display all these findings in Table 2 and Figure 3.

Table 2.
Prolactin Hormone, Vitamin D3, and CCN3 Protein Levels in Lactating and Non-Lactating Women.

Biomarker	Lactating Women (Mean ± SD)	Non-Lactating Women (Mean ± SD)	p-value
Prolactin Hormone (ng/ml)	30.4 ± 2.1	19.5 ± 1.7	0.01
Vitamin D3 (ng/ml)	9.9 ± 3.2	17.1 ± 2.4	0.009
CCN3 Protein (pg/ml)	41.6 ± 2.9	33.5 ± 1.8	0.01

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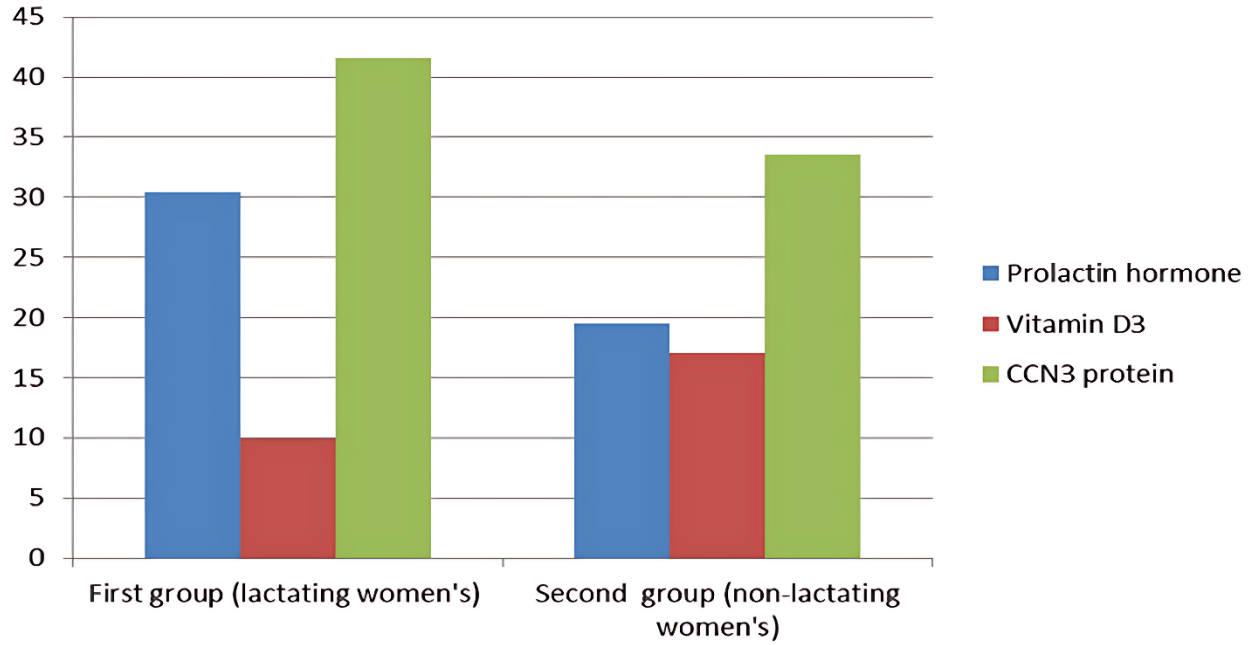

Figure 3.
Comparison between First group (lactating women's) and Second group (non-lactating women's) according to prolactin hormone, vitamin D3 and CCN3 protein level

There are notable differences between the groups in markers of inflammation and bone turnover . The current study also demonstrated that the concentrations of osteocalcin was higher in non-lactating women and bone turn-over was more marked in these women, whereas RANKL/OPG ratio and the concentration of C-reactive protein (CRP) was higher in lactating women. We can find these findings in Table 3.

Table 3.
Comparison of Bone Turnover and Inflammatory Markers.

Biomarker	Lactating Women (Mean ± SD)	Non-Lactating Women (Mean ± SD)	*p*-value
Osteocalcin (ng/ml)	9.7 ± 1.8	15.4 ± 2.3	0.02
RANKL/OPG Ratio	3.8 ± 0.7	2.1 ± 0.5	0.01
C-Reactive Protein (CRP) (mg/dL)	0.18 ± 0.05	0.12 ± 0.04	0.03

From another aspect, and according to higher MDA and lower TAC concentration , the lactating women underwent oxidation stress in comparison to the control group . Furthermore , inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were significantly elevated in the lactating group compared to the non-lactating group. These results are summarized in Table 4.

Table 4.
Oxidative Stress and Inflammatory Markers in Lactating and Non-Lactating Women.

Biomarker	Lactating Women (Mean ± SD)	Non-Lactating Women (Mean ± SD)	*p*-value
Malondialdehyde (MDA) (µmol/L)	5.3 ± 1.2	3.8 ± 1.1	0.01
Total Antioxidant Capacity (TAC) (mmol/L)	1.1 ± 0.3	1.5 ± 0.4	0.04
Interleukin-6 (IL-6) (pg/mL)	12.2 ± 3.5	8.7 ± 2.8	0.02
Tumor Necrosis Factor-alpha (TNF-α) (pg/mL)	18.4 ± 4.7	13.6 ± 3.9	0.03

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5. Discussion

Lactating is a physiological state that all females go through after birth and it is also considered as important state for the babies, as the baby at this stage derives its nutrition from the mother through milk of breastfeeding [9]. One of the most important characteristics that accompany the mother at this stage is the increase in the mother's prolactin hormone. In most cases, various healthy complications begin in the lactating females, the most important of which is osteoporosis. Osteoporosis is characterized by a decrease in bone density and is formed due to the weakness of the bone matrix due to factors that enhance osteoclast cells versus osteoblastic cells [10]. The current study aims to identify the mechanism of the association between the increase in prolactin hormone in the lactating females and osteoporosis. Prolactin hormone is a hormone produced by the anterior lobe of the pituitary gland and it is a hormonal protein that targets the mammary glands in the female's breast and works to stimulate milk production from the mammary glands. During the lactating stage, the mother begins to lose most of the important elements in her body, the most important of which is vitamin D3 [11, 12].

Vitamin D3 is derived from steroid compounds and works effectively to enhance bone building by supporting osteoblast with calcium by increasing its absorption from the intestines. Any deficiency in the level of vitamin D3 leads to a weakening of the bone matrix and causes osteoporosis. Therefore, the body begins to react to correct these metabolic pathways, and among these reactions is the increase in CCN3 protein, which is a protein that supports the strengthening of the cellular matrix intracellular and extracellular to protect the bone [6]. CCN3 protein is produced by most cells, the most important of which are neurons of chondrocytes, and aims to enhance and strengthen the cellular matrix in most tissues of the body, the most important of which is bone tissue. The body begins to increase the secretion of CCN3 to protect the body from the health problems that the lactating mother goes through, and the most important of these health problems is the loss of bone density and causing osteoporosis. As the loss of bone density can stimulate the brain to send cellular signals to stimulate the production of CCN3 protein, which works to support cellular signals by stimulating it to produce BMP-4 protein, which works on the mechanism of strengthening the bone [4, 13]. Our current study showed that there is a physiological increase in the prolactin hormone in lactating females, which in turn can stimulate brain cells to enhance the production of CCN3 protein by directly affecting the prolactin hormone on the brain. On the other hand, the prolactin hormone works to produce milk for the baby, which is rich in vitamins, minerals and proteins important for the baby at the expense of the lactating mother, which makes the lactating mother exposed to osteoporosis disorder because the mother will lose vitamin D3 with the milk. Causing osteoporosis also has the ability to enhance the secretion of CCN3 protein in order to protect the body from health problems for the lactating mother's body resulting from breastfeeding. The results of our study with the increase in prolactin hormone accompanied by a decrease in vitamin D3 in lactating females are consistent with Ryan and Kovacs [14] who proved the same results [14]. On the other hand, our study showed that the prolactin hormone was elevated along with the CCN3 protein in lactating females, which is consistent with Babey, et al. [15] who showed the same results [15].

6. Conclusion

This study shed light on the pivotal role of the physiological increase in prolactin hormone levels in lactating women and its associated effect on CCN3 protein levels. The elevated CCN3 protein acts as a protective mechanism, mitigating potential complications of lactation, particularly osteoporosis. This interplay suggests that CCN3 protein enhances cellular communication and contributes to bone tissue preservation and regeneration in response to increased prolactin and decreased vitamin D3 levels observed during lactation.

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Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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