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Association between Mammographic Breast Density and Markers of Calcium Metabolism in Women: A Retrospective Observational Study

Kadınlarda Mamografik Meme Dansitesi ile Kalsiyum Metabolizması Belirteçleri Arasındaki İlişki: Retrospektif Gözlemsel Çalışma

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ABSTRACT

Aim: The aim of this study was to assess the relationship between mammographic breast density and biochemical indices of calcium metabolism, as well as femoral neck bone mineral density (BMD) in women aged 40-80 years.
Material and Methods: In this retrospective, single-center study, 598 women undergoing screening mammography between November 2020 and March 2025 were included. After applying exclusion criteria, 200 age-matched women were randomly selected and evenly stratified into four BI-RADS breast composition categories (A, B, C, and D), 50 women in each. Serum vitamin D_3, parathyroid hormone (PTH), calcium, and femoral neck t-scores were retrieved from institutional records and compared between these four categories.
Results: Vitamin D_3 levels (p=0.021) and femoral neck t-scores (p=0.041) were found to be significantly different between four categories. Post hoc analysis demonstrated significantly different vitamin D_3 concentrations in the Category D compared with categories A (p=0.021), B (p=0.032), and C (p=0.048). Femoral neck t-scores progressively decreased in Category D compared to categories A (p=0.013), B (p=0.025), and C (p=0.041). No significant differences were observed for PTH (p=0.589) or serum calcium (p=0.441).
Conclusion: Higher mammographic breast density was associated with lower vitamin D_3 concentrations and reduced femoral neck BMD. Breast composition may serve as a readily available imaging biomarker to identify women at increased risk of vitamin D deficiency and osteoporosis, and has potential integration into individualized risk stratification strategies.
Keywords: Mammography; density; calcium; metabolism; osteoporosis.

ÖZ

Amaç: Bu çalışmanın amacı, 40-80 yaş arası kadınlarda mamografi meme yoğunluğu ile kalsiyum metabolizmasının biyokimyasal göstergeleri ve femur boynu kemik mineral yoğunluğu (KMY) arasındaki ilişkiyi değerlendirmektir.
Gereç ve Yöntemler: Bu retrospektif, tek merkezli çalışmaya, Kasım 2020 ile Mart 2025 tarihleri arasında tarama mamografisi çekilen 598 kadın dahil edildi. Dışlama kriterleri uygulandıktan sonra, yaşları eşleştirilmiş 200 kadın rastgele seçildi ve her birinde 50 kadın olacak şekilde dört BI-RADS meme kompozisyon kategorisine (A, B, C ve D) eşit şekilde ayrıldı. Serum vitamin D_3, paratiroid hormonu (PTH), kalsiyum ve femur boynu t-skorları kurumsal kayıtlardan alındı ve bu dört kategori arasında karşılaştırıldı.
Bulgular: Vitamin D_3 düzeyleri (p=0,021) ve femur boynu t-skorlarının (p=0,041) dört kategori arasında anlamlı derecede farklı olduğu bulundu. Post-hoc analiz, D kategorisinde, vitamin D_3 konsantrasyonlarının A (p=0,021), B (p=0,032) ve C (p=0,048) kategorilerine kıyasla anlamlı derecede farklı olduğunu gösterdi. Femur boynu t-skorları ise D kategorisinde A (p=0,013), B (p=0,025) ve C (p=0,041) kategorilerine kıyasla giderek azaldı. PTH (p=0,589) veya serum kalsiyumu (p=0,441) için anlamlı bir fark gözlenmedi.
Sonuç: Daha yüksek mamografi meme yoğunluğu, daha düşük D_3 vitamini konsantrasyonları ve azalmış femur boynu KMY ile ilişkiliydi. Meme kompozisyonu, D vitamini eksikliği ve osteoporoz riski yüksek kadınları belirlemek için kolayca bulunabilen bir görüntüleme biyobelirteci olarak kullanılabilir ve bireyselleştirilmiş risk sınıflandırma stratejilerine entegre olma potansiyeline sahiptir.
Anahtar kelimeler: Mamografi; dansite; kalsiyum; metabolizma; osteoporoz.

INTRODUCTION

Breast composition category (density) refers to the relative composition of adipose and fibroglandular tissue within the breast (1). Women with high breast density, characterized by a predominance of dense fibroglandular tissue, are at a significantly elevated risk of developing breast cancer, with evidence indicating a four- to six-fold increased risk compared to those with low-density breast tissue (2). Moreover, elevated breast density can obscure mammographic findings, complicating the early detection of malignant lesions (2). The diagnostic challenges and heightened cancer susceptibility associated with dense breast tissue underscore its pivotal role in radiological assessment (3-6). Beyond its established association with breast cancer risk, emerging evidence suggests that breast density may serve as a biomarker for systemic diseases, including potential links to metabolic and hormonal disorders (7-9).

Several studies have examined the role of breast density beyond oncological contexts (8,9). These studies have demonstrated that breast density is associated with hormonal and metabolic disorders. Based on this relationship, it has been suggested that breast density may be a biomarker for metabolic and hormonal disorders (7,8,10,11). A recent study showed that breast density is highly correlated with insulin-like growth factor (IGF) and growth hormone levels (10). In another study, Kim et al. (11) reported that breast density is associated with insulin resistance and cortisol levels. There is an increasing number of studies investigating the relationship between breast density and various metabolic disorders; however, no study has investigated the relationship between breast density and calcium metabolism disorders.

Dietary calcium is absorbed in the small intestine, facilitated by active vitamin D (1,25-dihydroxyvitamin D), which undergoes initial hydroxylation in the liver and is subsequently activated in the kidneys. The kidneys play a critical role in calcium homeostasis by regulating its reabsorption and excretion in response to parathyroid hormone (PTH). PTH enhances renal calcium reabsorption, stimulates osteoclastic activity to mobilize calcium from the bone, and promotes renal activation of vitamin D (12,13). The liver synthesizes vitamin D-binding proteins and albumin to transport calcium in the blood, as well as hydroxylation required to initiate vitamin D metabolism (14). Calcium metabolism disorders are implicated in a range of disorders, including nephrolithiasis, chronic kidney disease, and liver failure (12,15,16).

This study aimed to determine whether breast density can predict calcium metabolism disorders and related pathologies by investigating the relationship between breast density and calcium metabolism.

MATERIAL AND METHODS

Study Design

This retrospective study was conducted at the Radiology Department of Ankara Bilkent City Hospital after obtaining approval from the institutional ethics committee (28.05.2025, 2/1215). The mammography study (with craniocaudal and mediolateral projections) included 598 patients between 40 and 80 years of age, who were retrospectively evaluated between November 2020 and March 2025. Patients with genetic syndromes (3 patients), those who were breastfeeding at the time of the scan (17 patients), those with known liver disease (25 patients), known renal disease (11 patients), known skin disease (13 patients), vitamin D_3, PTH, and serum calcium values not available or obtained at the hospital's laboratory integration system (59 patients), and those who don't undergo bone mineral density (BMD) measurement (62 patients) were excluded from the study. Among the remaining 408 patients, 50 age-matched patients were randomly selected for each breast composition type (types A, B, C, and D), and four study groups were created. The flowchart of the study is presented in Figure 1.

Mammography Acquisition

Mammographic acquisitions were performed using the same mammography system, the Senographe Pristina™ (GE Healthcare), equipped with automatic parameter optimization. The mammographic images were jointly evaluated by Radiologist 1 (a board-certified radiologist (EDiR) with seven years of experience in general radiology) and Radiologist 2 (with seven years of experience in breast radiology and twenty years of experience in general radiology), who determined the mammographic breast composition types by consensus in accordance with the BI-RADS atlas (17). Four study groups were formed, each comprising 50 age-matched patients (200 patients in total) per breast composition category (Figure 2).

Data Collection

The following laboratory parameters were analyzed to evaluate calcium metabolism: Vitamin D_3, PTH, and serum calcium (corrected for albumin). Patient laboratory data were retrieved from the hospital's laboratory integration system by Radiologist 1. In addition, femoral neck t-scores from BMD for the same patients were recorded. BMD was assessed using a GE Lunar iDXA scanner (GE Healthcare Lunar, Madison, WI, USA) with the patients in the supine position. The hip region was imaged using a peak X-ray tube voltage of 100 kV. Additionally, the body mass index (BMI) values were calculated and recorded.

Statistical Analysis

The data distribution was evaluated for normality using the Kolmogorov-Smirnov test. Descriptive statistics were presented as median, 25^{th}-75^{th} percentile, minimum, and maximum. BI-RADS breast density categories were compared using the Kruskal-Wallis test in terms of calcium metabolism markers (vitamin D_3, PTH, serum calcium level,

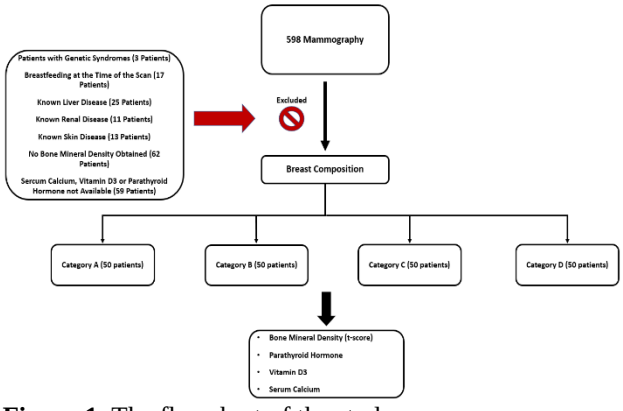


Figure 1. The flowchart of the study

and femoral neck t-scores). Where significant differences emerged, post-hoc pairwise comparisons were performed using Dunn's test with Bonferroni correction to account for multiple testing. Statistical significance was set at $p<0.05$. Statistical analyses were performed using IBM SPSS Statistics, Version 28.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 200 patients were included in this study. The median age of the patients was 54 (range, 40-80) years. There was no statistically significant difference between the breast composition categories in terms of age (p=0.811). The median BMI value was 28.9 (range, 23.1-34.9) kg/m^2 for Category A, 28.2 (range, 22.6-34.1) kg/m^2 for Category B, 27.4 (range, 22.0-33.3) kg/m^2 for Category C, and 26.9 (range, 21.7-32.8) kg/m^2 for Category D. The overall difference in BMI across categories was not statistically significant (p=0.063).

A statistically significant difference was observed in vitamin D_3 levels among categories (p=0.021). Median vitamin D_3 concentration was 31.5 (range, 13-99) nmol/L in Category A, while it was 53.0 (range, 13-98) nmol/L in Category B, 63.5 (range, 14-106) nmol/L in Category C, and 53.0 (range, 12-86) nmol/L in Category D. Post hoc analysis demonstrated significantly higher vitamin D_3 levels in Category D compared with Category A (p=0.021) and Category B (p=0.032), and higher levels in Category C compared with Category D (p=0.048).

No statistically significant differences were found among breast composition categories for PTH (p=0.589) or serum calcium levels (p=0.441). Median PTH values ranged between 49 (range, 23-94) ng/L and 58 (range, 22-165) ng/L across the four breast composition categories, while the calcium levels ranged from 9.4 (range, 8.3-10.1) mg/dL to 9.7 (range, 8.6-10.6) mg/dL (Table 1).

A statistically significant difference was also found in femoral neck t-scores among the categories (p=0.041). Median t-scores were found as -1.0 (range, -2.6 to -1.6) in Category A, -1.2 (range, -2.0 to -2.8) in Category B, and -1.1 (range, -2.0 to -1.0) in Category C, while it was found -1.8 (range, -2.0 to -0.8) in Category D. Post hoc test results indicated significantly lower t-scores in Category D compared with the categories A (p=0.013), B (p=0.025), and C (p=0.041).

Overall, as the glandular component of the breast increased from Category A to D, vitamin D_3 levels tended to decrease

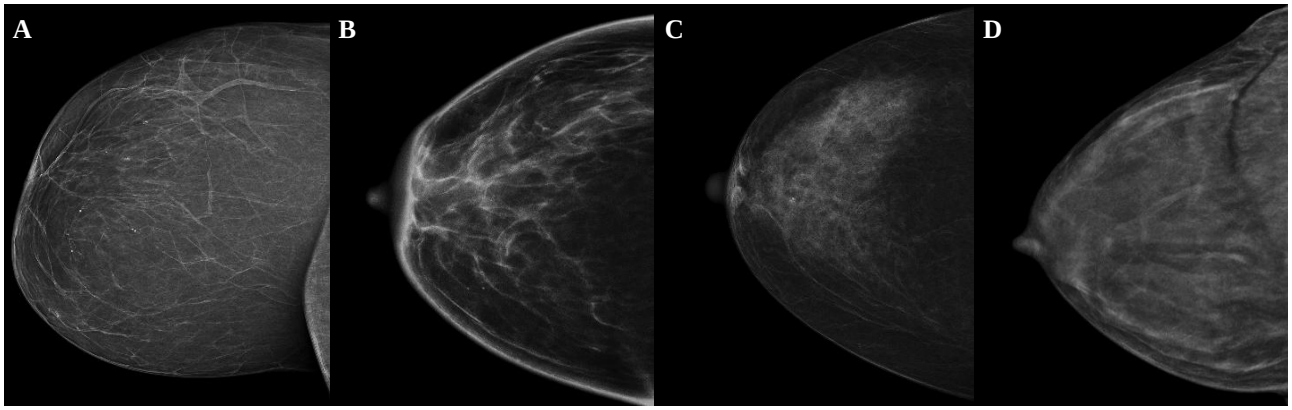


Figure 2. Mammography examples of breast composition types for **A)** category A, breast is almost entirely made up of fatty tissue, **B)** category B, breast is mainly made up of fatty tissue, with some small areas of fibroglandular (dense) tissue, **C)** category C, breast has a mix of fatty and fibroglandular (dense) tissue, which may obscure small masses, and **D)** category D, breast is mainly made up of fibroglandular tissue and are extremely dense, which may obscure even large mases

Table 1. Descriptive statistics and overall comparisons by breast composition categories

	Category A	Category B	Category C	Category D	p
Body Mass Index (kg/m^2)	28.9 (27.3 – 30.7) [23.1 – 34.9]	28.2 (26.9 – 29.6) [22.6 – 34.1]	27.4 (26.1 – 28.7) [22.0 – 33.3]	26.9 (25.7 – 28.1) [21.7 – 32.8]	0.063
Vitamin D_3 (nmol/L)	31.5 (24.0 – 55.5) [13.0 – 99.0]	53.0 (26.5 – 64.0) [13.0 – 98.0]	63.5 (45.2 – 70.2) [14.0 – 106.0]	53.0 (43.0 – 76.2) [12.0 – 86.0]	**0.021**
PTH (ng/L)	55.0 (47.0 – 71.8) [29.0 – 158.0]	58.0 (31.5 – 71.0) [22.0 – 165.0]	50.0 (40.0 – 68.0) [18.0 – 89.0]	49.0 (40.5 – 64.5) [23.0 – 94.0]	0.589
Serum calcium (mg/dL)	9.6 (9.2 – 9.7) [8.2 – 10.7]	9.7 (9.2 – 9.9) [8.6 – 10.6]	9.7 (9.4 – 9.9) [8.6 – 10.1]	9.4 (9.2 – 9.6) [8.3 – 10.1]	0.441
t-score (femoral neck)	-1.0 (-1.4 – -0.4) [-2.6 – -1.6]	-1.2 (-1.7 – -0.7) [-2.0 – -2.8]	-1.1 (-1.3 – -1.0) [-2.0 – -1.0]	-1.8 (-2.0 – -1.2) [-2.0 – -0.8]	**0.041**

PTH: parathyroid hormone, data are reported as median (25^{th}-75^{th} percentile) [minimum-maximum], post hoc pairwise comparisons according to the Bonferroni-adjusted Dunn test; for Vitamin D_3: A vs B: p=0.811, A vs C: p=0.532, A vs D: p=0.021, B vs C: p=0.886, B vs D: p=0.032, C vs D: p=0.048; for t-score (femoral neck): A vs B: p=0.046, A vs C: p=0.035, A vs D: p=0.013, B vs C: p=0.048, B vs D: p=0.025, C vs D: p=0.041

while femoral neck t-scores declined significantly, suggesting an inverse trend between dense breast composition and bone mineral parameters (Table 1).

DISCUSSION

This study identifies a significant association between lower serum vitamin D_3 concentrations and higher mammographic breast density in women aged 40-80. Furthermore, serum calcium and PTH levels did not differ significantly across the breast density categories. By systematically examining the interplay between breast density and biochemical markers of calcium metabolism, specifically serum vitamin D_3, calcium, and PTH, across a well-characterized cohort, this study represents one of the first large-scale analyses of these relationships. These findings offer important new insights into the metabolic factors that are related to calcium metabolism that may underlie variations in breast density.

Our results align with and extend those of previous studies that have explored the links between breast density, vitamin D_3, and its metabolic pathways (18-23). It is well known that vitamin D signaling via the vitamin D receptor exerts antiproliferative, pro-differentiation, and pro-apoptotic effects in mammary epithelium and breast tumors, with additional influences on autophagy and epithelial-mesenchymal transition programs (18). Furthermore, crosstalk with the IGF axis provides a plausible pathway: vitamin D analogues suppress IGF-1 receptor signaling and increase IGF-binding proteins (e.g., IGFBP-3), thereby lowering bioavailable IGF-1 and downstream mitogenic drive in breast cells (19,20). Consistent with this biology, our findings suggest that the relationship between vitamin D/calcium exposure and mammographic breast density is secondary to this IGF-linked mechanism. Changes in breast cells with IGF signals may affect the fibroglandular tissue density relative to the fat tissue. Also, they showed that regular intake of vitamin D supplements is also related to lower density, with the effect being most pronounced in premenopausal women (21). Similarly, Diorio et al. (22) reported a stronger negative association between vitamin D and calcium intake and breast density among premenopausal women with higher IGF-1 or IGFBP-3 levels, again suggesting an interaction between calcium metabolism, breast density, and growth factor pathways. Maskarinec et al. (24) showed that in premenopausal women, the IGF-1/IGFBP-3 ratio was positively associated with breast density, whereas IGFBP-3 was inversely associated. Further supporting this, a cross-sectional study by Izzo et al. (25) found higher plasma IGF-1 levels and IGF-1/IGFBP-3 ratios in women with dense breasts, but no significant association was found in postmenopausal women after adjusting for confounders. These observations suggest that the roles of growth factors and metabolic regulators are more pronounced before menopause, possibly due to their interaction with endogenous estrogens.

Although previous studies have not demonstrated a relationship between vitamin D and breast density in premenopausal women, the relationship between vitamin D_3 and breast density in postmenopausal women is controversial. Sprague et al. (23) found no association between circulating vitamin D_3, PTH, IGF-1, IGFBP-3, or calcium and breast density after adjustment for BMI in postmenopausal women. In contrast, a laboratory study has suggested that vitamin D may exert a chemopreventive effect on breast tissue, but this didn't demonstrate a significant relationship with breast density in the postmenopausal population (24).

The lack of association between calcium or PTH and breast density in our study is consistent with prior studies. Bérubé et al. (26) did not find an association between calcium intake and breast density in postmenopausal women, and Vachon et al. (27) reported that only modest dietary calcium intake influences overall. The findings of this study have important clinical implications. Because breast density is routinely assessed during mammography, it may serve as a practical and non-invasive biomarker for identifying women at an increased risk of vitamin D deficiency and osteoporosis. Early identification can facilitate timely interventions, such as vitamin D supplementation and bone health screening, thereby potentially improving patient outcomes.

This study has several limitations. First, the menopausal status (including time since menopause and use of hormone therapy), seasonal variation in vitamin D (timing of blood sampling across seasons), and sunlight exposure (e.g., time spent outdoors, clothing/sunscreen habits, latitude/UV index) that may affect calcium metabolism were not evaluated. In addition, potentially relevant factors such as vitamin D supplementation, dietary intake, physical activity, and genetic predisposition were not comprehensively evaluated. Although individuals with known metabolic diseases were excluded, undiagnosed or subclinical conditions could still have influenced the findings. Second, serum vitamin D was assessed at a single time point, which is susceptible to intra-individual variability and seasonal fluctuation, and laboratory/assay-related variability was not examined. Third, breast composition may sometimes be subjective, and it was categorized without any inter- or intraobserver reproducibility analysis being performed for density classification, introducing potential misclassification bias. Finally, subgroup analyses, particularly in postmenopausal women, may have been underpowered to detect subtle associations, and potential non-linear or interaction effects (e.g., menopausal status) were not modeled.

Prospective, multicenter studies are warranted with standardized, season-balanced blood sampling; systematic collection of BMI, menopausal status and therapy, sunlight exposure metrics (validated questionnaires or device-based estimates), diet/supplementation, and physical activity; with intra-interobserver consistency evaluation; and longitudinal vitamin D assessments to capture temporal variation to support our findings. Incorporating multivariable modeling and interaction terms will better account for confounding and clarify the independent relationship between breast density and vitamin D status.

CONCLUSION

Lower vitamin D_3 levels and BMD are associated with breast density in women, whereas no such relationship was observed with serum calcium or PTH. This suggests that women with high breast density may be at greater risk for vitamin D deficiency and osteoporosis. Breast density may be a new imaging biomarker for risk classification of osteoporosis and vitamin D deficiency.

Ethics Committee Approval: The study was approved by the Medical Research Ethics Committee of Ankara Bilkent City Hospital (28.05.2025, 2/1215).

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