

Assessing vitamin D and mammographic breast density in Alaskan women

Narjust Duma,¹ Ivana Croghan,²
Sarah Jenkins,³ Celine Vachon,³
Loni Neal,² Karthik Ghosh,²
Sandhya Pruthi²

¹Division of Hematology, Medical
Oncology and Palliative Care,
University of Wisconsin, Madison, WI;

²Department of Internal Medicine,
Mayo Clinic, Rochester, MN;

³Department of Health Sciences
Research, Mayo Clinic, Rochester, MN,
USA

Abstract

Vitamin D deficiency and high breast density may be associated with increased breast cancer risk. We examined a possible association between vitamin D levels and mammographic breast density in a population of Alaskan women. Patients seen in the Mayo Clinic-Alaska Native Medical Center telemedicine program from December 2014 to December 2017 were enrolled in the study. Pearson correlation was used to estimate the association between mammographic breast density and vitamin D levels. Of the 33 women enrolled, 70% of women self-identified as American Indian/Alaskan Native, 12% as White, 6% as Native Hawaiian/Pacific Islander and 12% as other. Nineteen (58%) participants were taking vitamin D supplementation. No correlation was identified between breast density and serum vitamin D levels overall (correlation = -0.03). Larger studies controlling for vitamin supplementation are needed, as this association could potentially impact breast cancer rates in populations at risk for vitamin D deficiency.

Introduction

Several studies and lines of evidence suggest that vitamin D may have antiproliferative and anticarcinogenic properties and in particular, a protective effect against breast cancer.¹ Women with higher levels of vitamin D have been reported to have a lower risk of breast cancer.² Although the exact mechanism of action is unknown, it is hypothesized that the process includes inhibition of cellular proliferation, induction of differentiation and apoptosis, and inhibition of angiogenesis in normal and malignant

breast cells.² In addition, vitamin D was reported to inhibit the synthesis of estrogens by means of suppression of aromatase conversion of androgens into active estrogens.³

Mammographic density, or the proportion of breast tissue (lactation ducts and glands) to fat tissue noted on the mammogram, has also been associated with an increase in breast cancer risk. Studies have reported that the risk of developing breast cancer is more than four times likely in an individual with 75% or greater breast density compared to women with less than 10% breast density.⁴ More recently, some but not all studies have demonstrated that serum vitamin D 25 (OH) D levels are inversely related to breast density.⁵

Alaskan populations are at the highest risk for vitamin D deficiency in the United States, due to a combination of limited sunlight exposure, darker skin pigmentation as well as recent changes in their traditional diet with an increased intake of carbohydrates and a decrease in vitamin D from natural sources like marine mammals and oily fish.⁶ Our objective was to examine the association between serum vitamin D 25 (OH) levels and mammographic breast density in a population of Alaskan women. We hypothesized that vitamin D levels were inversely associated with mammographic breast density in this population.

Materials and Methods

In 2010, the Alaska Native Medical Center (ANMC), Mayo Clinic Cancer Center and the Mayo Clinic's Breast Clinic (MCBC) established an eHealth/Telemedicine program to serve women residing in the state of Alaska who were felt to be at high risk for breast cancer. The goal of the telemedicine program was to provide comprehensive breast cancer educational programs, to guide appropriate breast cancer interventions and surveillance as well as to offer breast cancer screening and prevention recommendations to local primary care providers. The program served 299 Alaskan women from August 2011 to December 2018.

This study was approved by the ANMC and the Mayo Clinic Rochester (MCR) Institutional Review Boards (IRB), and all procedures were in accordance with the ethical standards of the IRBs and the Helsinki Declaration of 1975, as revised in 2013. Patients enrolled in the telemedicine program between ANMC and MCBC were offered the opportunity to participate in the study. Participants were required to be 18 years of age or older. The telemedicine breast clinic at the ANMC was the single

Correspondence: Narjust Duma, University of Wisconsin Carbone Cancer Center, 600 Highland Ave, Madison, WI, 53792, USA.
Tel.: +1.608.265.3837 - Fax: +1.608.265.0614.
E-mail: nduma@wisc.edu

Key words: Breast density; vitamin D; Alaskan native; telemedicine.

Acknowledgments: A special thanks to the exceptional research staff of the Clinical Research Office within the Department of Medicine, at Mayo Clinic, as well as the staff of the Alaskan Native Medical Center and the Mayo Clinic Breast Clinic for their patience and persistence in helping to collect, compile, and organize these data. A special thanks to Fang-Fang Wu, Katrina Croghan, Bonnie Donelan Dunlap and Marilyn Sloan, for all their hard work and dedication to this study. The authors also wish to thank the study participants who participated in this clinical trial, without whom this project would not have been possible.

Contributions: ND, methodology, data analysis and drafting and editing of the manuscript; IC, conceptualization of the study, methodology, data analysis, drafting and editing of the manuscript; SJ, data curation, data analysis and figure preparation; CV and SP, conceptualization of the study, methodology, study administration, data analysis, drafting and editing of the manuscript; LN, methodology, data curation, drafting and editing of the manuscript; KG, conceptualization of the study, recruitment, drafting and editing of the manuscript. All authors contributed to revision and review of the manuscript, and approval of the final manuscript as submitted.

Funding: This study was supported in part by the University of Iowa/Mayo Clinic Lymphoma Spore (CA0972474), Mayo Clinic Cancer Center and Department of Medicine Clinical Research Office. The data entry system used was REDCap, supported in part by the Center for Clinical and Translational Science award (UL1 TR000135) from the National Center for Advancing Translational Sciences (NCATS).

Conflicts of interests: The authors declare no potential conflicts of interests.

Ethics approval and consent to participate: This study was approved by the ANMC and the Mayo Clinic Rochester (MCR) Institutional Review Boards (IRB), and all procedures were in accordance with the ethical standards of the IRBs and the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients being included in the study.

Received for publication: 27 March 2020.
Revision received: 27 July 2020.
Accepted for publication: 25 September 2020.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright: the Author(s), 2020
Licensee PAGEPress, Italy
Clinics and Practice 2020; 10:1253
doi:10.4081/cp.2020.1253

recruitment site with initiation of the study in December 2014 through December 2017. No monetary incentive was provided to study participants, but participants after consent received a blanket for their subsequent time and effort

Informed consent was obtained from all patients being included in the study (Appendix I). Blood samples were collected locally (ANMC) and serum 25 (OH) levels (25-hydroxyvitamin D2 and D3) were measured at MCR. Any and all residual blood samples were later destroyed per Occupational Safety and Health Administration (OSHA) regulations. Study participants' mammograms (digital files within a year from blood sample collection) were assessed at MCR by a blinded breast radiologist.

Medical history was obtained from medical records provided by ANMC and the participants' Breast Cancer Risk Questionnaire. This questionnaire is a standard part of the telemedicine clinical practice and includes questions on reproductive, hormonal and lifestyle risk factors, breast biopsy history, and family history. The data was managed using the REDCap® tool hosted by Mayo Clinic.

VidyoHealth® software (Vidyo, Inc.) was the encrypted e-consult telemedicine tool use for study recruitment and participation. VidyoHealth® is a scalable high-definition telemedicine product that uses the public Internet and existing general-purpose IP networks at medical facilities for doctor-patient communications.

Mammographic density estimation

Images from one screening mammogram with craniocaudal and mediolateral oblique views were obtained for all included study participants. All mammogram views were digitized on a Lumiscan 75 scanner with 12-bit grayscale depth. The pixel size was 0.130×0.130 mm² for both the 18×24-cm² and 24×30-cm² films. Percent mammographic density (dense area/total area ×100), dense area, and non-dense area were estimated using a comput-

er-assisted thresholding program previously described in several mammographic density studies.⁷ Given the similarity in density estimates from craniocaudal or mediolateral oblique views, only craniocaudal images were used for analysis. We took an average of the 2 craniocaudal views for each study participant, when available.

Statistical analysis

Percent mammographic density was estimated from mammograms obtained from these same individuals within a year from the vitamin D level assessment, and the percentages were averaged across both craniocaudal views.

Vitamin D level and breast density percentage were compared between those who were versus those who were not taking supplementation with two-sample t-tests. The Pearson correlation (r) was used to quantify the association between breast density and vitamin D levels, both overall, as well as within potential confounding subgroups (age <50 versus ≥50 years; vitamin D supplementation yes versus no; body mass index <30 versus ≥30 kg/m²), and the partial correlations adjusted for each of these were also presented. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Between December 2014 and December 2017, a total of 87 patients were approached about the study, and 48 patients consented to participate. Fifteen patients were excluded: 13 patients' breast density was not calculated in imaging within one year of their vitamin D levels, and two other patients were excluded due to lack of mammogram or vitamin D levels. The final analysis included thirty-three patients.

Study participants' median age was 53 years (range 31-79), 70% self-identified as American Indian/Alaskan Native, 12% as white, 6% as Native Hawaiian or other

Pacific Islander and 12% as other. Median weight was 73.6 kilograms (interquartile range [IQR] 67.3-87.1) with a median body-mass-index (BMI) of 31 kg/m² (IQR 26.4-34.3).

Regarding breast cancer risk factors, 23 (70%) participants were ≥12 years for their first menstrual cycle, and 20 (61%) women were post-menopausal. The median number of pregnancies was 3 (IQR 2-5), 21.5 years was the median age for their first pregnancy, and 20 women reported a history of breastfeeding (Table 1). Other breast cancer risk factors included post-menopausal hormonal supplementation reported by 23% of participants. Two women reported a personal history of deleterious *BRCA1/2* gene mutations, and 18 women had undergone a prior breast biopsy.

Fifteen women were current or former smokers (>100 cigarettes in their lifetime), 13 (39%) women noted prior exposure to second-hand tobacco smoke (≥12 months), and 19 (58%) women reported none or low (<1-2 drinks per week) alcohol consumption.

Median serum Vitamin D level was 39 ng/mL (IQR 30-52), and 9 (27%) women had low vitamin D levels (<30 ng/mL). Only one participant was found to have toxic vitamin D levels at 87 ng/mL.

In regards to breast density, the median percentage was 15.7% (IQR 8.7-27.2) with a median dense area of 23.4 cm² (IQR 16.2-36.5) and median non-dense area of 131.8 cm² (IQR 80.9-220.0). Median time from blood draw to mammogram was 132 days (IQR 55-183), and 19 (58%) participants were taking vitamin D supplementation at the time of study enrollment, with doses ranging from 400 to 50,000 units. Overall, there was no correlation between breast density percentage and vitamin D ($r = -0.03$) (Figure 1). However, there is some suggestion of a stronger correlation among those not taking vitamin D supplementation ($r = 0.39$), though the number of women in this group was small. The correlation was similar when stratified by age but was slightly higher among non-obese women (Table 1).

Table 1. Comparison of vitamin D and breast density percentage by body mass index, vitamin D supplementation (ng/mL), and age.

	Vitamin D [Median (IQR)]	Breast density percentage [Median (IQR)]
BMI <30, N=14	34.0 (24.0, 47.0)	28.9 (16.8, 34.5)
BMI ≥30, N=19	40.0 (32.0, 59.0)	9.9 (7.0, 15.7)
No vitamin D supplementation, N=14	28.0 (21.0, 37.0)	16.1 (12.2, 28.6)
Vitamin D supplementation, N=19	43.0 (38.0, 61.0)	11.4 (7.2, 27.2)
Age <50, N=13	38.0 (30.0, 43.0)	18.8 (15.7, 31.0)
Age ≥50, N=20	40.5 (28.0, 60.0)	10.7 (8.3, 18.1)

IQR, interquartile range; BMI, body mass index.

When adjusting for these potentially confounding variables, the partial correlations between Vitamin D level and breast density percentage was 0.06 (adjusted for age), 0.09 (adjusted for supplementation), and 0.11 (adjusted for BMI).

Discussion

We attempted to determine an association between vitamin D levels and mammographic breast density as a risk factor for breast cancer. In our cohort, no significant association was observed. One possible confounder was that many of our study participants were taking vitamin D supplementation at the time of study enrollment. The patients evaluated in the telemedicine program were at high risk for breast cancer with many of them having a breast biopsy in the past, suggesting they were already connected to the healthcare system. The Alaska Department of Health and Social Services recommends vitamin D supplementation to patients with limited sunlight exposure and insufficient dietary intake as well as screening of pregnant women for vitamin D deficiency as advised by the American Congress of Obstetricians and Gynecologists.⁸ Thirty women in our study reported at least one pregnancy; vitamin D deficiency may have been diagnosed during prenatal evaluation with subsequent initiation of vitamin D supplementation.

One of the challenges associated with conducting studies involving vitamin D include the absence of standardized cut-off points for levels that confer vitamin D sufficiency. This limits the ability to draft global recommendations regarding vitamin D testing and supplementation. Although serum 25-OH vitamin D concentration is considered the best available indicator of a person's vitamin D level currently, it is not clear how reliable this biomarker is for determining optimal vitamin D levels in understudied populations such as American Indian/Alaskan Native, Black, Hispanic and young women.⁹

Mammographic breast density is a well-established risk factor for breast cancer. Mammographic breast density is defined as the percentage of fibroglandular tissue in the breast (non-radiolucent in mammographic imaging).⁴ Breast density is not a static characteristic; it decreases with age and can be affected by the use of postmenopausal hormones.⁴ Many studies have tried to identify possible protective factors against breast cancer, vitamin D being one of these. An inverse association between vitamin D intake and the risk of breast cancer was reported in both pre- and post-

menopausal women.¹⁰ However, this was not confirmed by a pooled analysis from prospective studies where 25 (OH) vitamin D levels were measured before diagnosis.¹¹

While our findings did not yield an association between breast density and vitamin D levels, further studies controlling for age, BMI, vitamin D supplementation, and inclusion of patients in more remote areas of Alaska and other populations at high risk for vitamin D deficiency (*i.e.*, Institutionalized, African American and Hispanic patients) should be considered. Vitamin D supplementation can be easily provided to patients living in remote areas and may offer other benefits beyond a possible decreased breast cancer risk in patients with severe vitamin D deficiency (<20 ng/mL).¹² However, it is important to mention that several trials of vitamin D supplementation in adults without vitamin D deficiency, have failed to demonstrate a reduction in cardiovascular or cancer mortality.^{13,14}

Conclusions

Our study has several limitations. First, our study population may not be representative of the Alaskan women as a whole, as the sampling methods were limited in size (33 participants), geographic scope, and other potentially important environmental and demographic characteristics. Next, we failed to control for vitamin D supplementation or other medications that may accelerate the metabolism of vitamin D (*i.e.*, phenytoin). Additionally, there are likely additional factors such as diet, socioeconomic status and other variables (racial/ethnic background, prior diagnosis of vitamin D deficiency) that contributed to the variability of our study participants' vitamin D levels and breast density and should be accounted as confounders in our study.

In summary, in our cohort, we did not observe an association between vitamin D levels and mammographic breast density. Several confounders were identified,

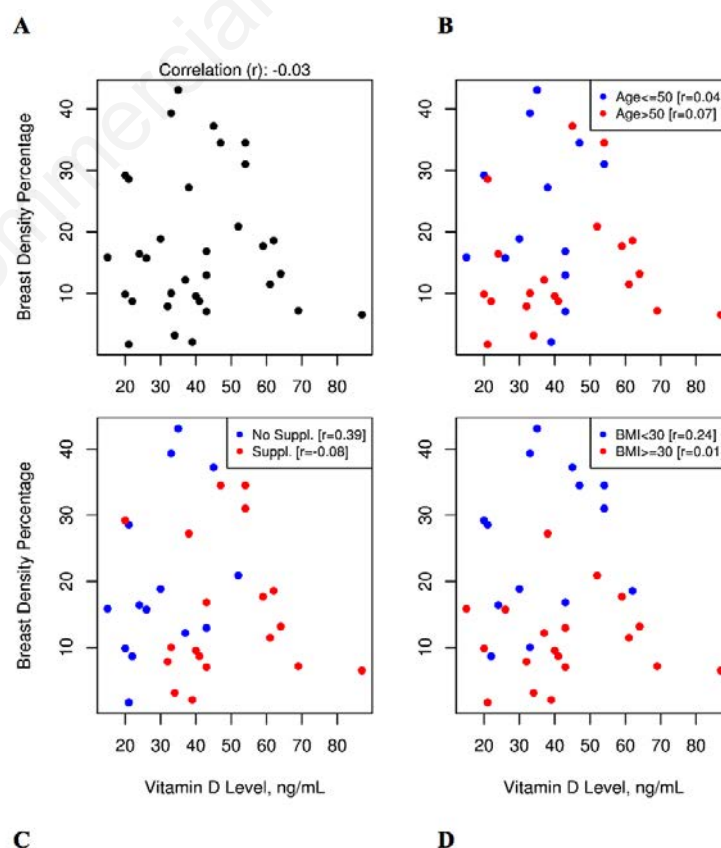


Figure 1. The Pearson correlation between breast density and vitamin D levels, (A) overall, (B) divided by age <50 years and >50 years, (C) history of vitamin D supplementation versus no vitamin D supplementation, (D) divided by participants' body mass index (BMI).

including vitamin D supplementation, age, and BMI. Further studies are necessary to determine if the previously observed association between mammographic breast density and vitamin D levels applies to other populations at high risk for vitamin D deficiency. The use of telemedicine has revolutionized the practice of medicine over the past years; our study provides an example for future studies which attempt to recruit and offer clinical trials to patients in remote geographic areas.

References

1. Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1991-7.
2. Garland CF, Gorham ED, Mohr SB, et al. 2007. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol* 2007;103:708-11.
3. Krishnan AV, Swami S, Feldman D. Vitamin D and breast cancer: inhibition of estrogen synthesis and signaling. *J Steroid Biochem Mol Biol* 2010;121:343-8.
4. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. *J Natl Cancer Inst* 1995;87:670-5.
5. Bérubé S, Diorio C, Mâsse B, et al. Vitamin D and calcium intakes from food or supplements and mammographic breast density. *Cancer Epidemiol Biomarkers Prev* 2005;14:1653-9.
6. Sharma S, Barr AB, Macdonald HM, et al. Vitamin D deficiency and disease risk among aboriginal Arctic populations. *Nutr Rev* 2011;69:468-78.
7. Ghosh K, Brandt KR, Sellers TA, et al. Association of mammographic density with the pathology of subsequent breast cancer among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2008;17:872-9.
8. Pachoe M. A brief overview on Vitamin D for Alaska health care providers. *State of Alaska Epidemiol Rep* 2017;3. Available from: http://www.epi.alaska.gov/bulletins/docs/rr2017_3.pdf Accessed: March 20, 2020.
9. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. *Am J Clin Nutr* 2008;88:507S-10S.
10. Chen P, Hu P, Xie D, et al. Meta-analysis of vitamin D, calcium and the prevention of breast cancer. *Breast Cancer Res Treat* 2010;121:469-77.
11. Yin L, Grandi N, Raum E, et al. Meta-analysis: serum vitamin D and breast cancer risk. *Eur J Cancer* 2010;46:2196-205.
12. Chowdhury R, Kunutsor S, Vitezova A, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ* 2014;348:g1903.
13. Manson JE, Cook NR, Lee I-M, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med* 2019;380:33-44.
14. Scragg R, Stewart AW, Waayer D, et al. Effect of monthly high-dose vitamin D supplementation on cardiovascular disease in the vitamin D assessment study: a randomized clinical trial. *JAMA Cardiol* 2017;2:608-16.