Published in final edited form as:

J Health Care Poor Underserved. 2010 February ; 21(1 Suppl): 17–25. doi:10.1353/hpu.0.0238.

The “Got D’ViBE?” Study: An Inter-Institutional Project Assessing Vitamin D and Mammographic Breast Density

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Summary

A multi-institutional collaboration was forged to implement a study of the relationship between Vitamin D and breast density among medically underserved women. This effort resulted in techniques to measure vitamin D levels, breast density, and sunlight exposure. Outcomes from this collaboration may provide insight to researchers conducting similar investigations.

Keywords

Vitamin D; mammography; underinsured; methods; women’s health

The Vitamin D Behavior and Essence Study (“Got D’ViBE?”) was funded by the U54 Meharry-Vanderbilt Alliance grant to examine the role between vitamin D levels and mammographic breast density for a medically underserved population. Mammographic density is an intermediate indicator of breast cancer risk. Percent breast density is associated with number of epithelial and nonepithelial breast cells.¹ Women with increased mammographic density are more likely than women with lower densities to develop ductal carcinomas *in situ* and proliferative lesions such as atypical hyperplasia and hyperplasia without atypia.² Both atypical hyperplasia and hyperplasia without atypia are related to increased breast cancer risk. Vitamin D has chemo-preventive properties that inhibit cellular proliferation such as breast density.^{3–5} The results between breast density and vitamin D intake suggest vitamin D could reduce risk by influencing breast tissue morphology.^{6–7} The association between vitamin D and mammographic density has been examined^{8–10} showing an inverse^{11–13} and

inconclusive^{13–15} relationship. Vitamin D may influence breast tissue development through its role in negative growth regulation of breast cancer cells; however, limited data exist for this relationship in African American women, where vitamin D levels are lower due to increased melanin concentration at the skin surface.^{16–17}

The “Got D’ViBE?” study is positioned to significantly determine the relationship between vitamin D deficiency, its causal factors, and increased breast density in African American women. Vitamin D has public health benefit by identifying high risk women for routine supplementation or vitamin D deficiency screening to insure adequate concentrations. These preventative efforts may avert premature deaths or breast cancer diagnosis in African American female populations, statistically having higher mortality from this disease.¹⁸ This report describes how contributions from different disciplines and institutions were solicited and synthesized to define the research question, design the study, develop methods for data collection, and facilitate study implementation. These details may assist researchers in conducting similar investigations.

Methods of collaboration

The pilot study’s primary objective was to establish the feasibility of assembling a screening mammography cohort at Nashville General Hospital’s–Breast Health Center (NGH-BHC), a public facility serving medically indigent and underserved populations to assess the relationship of vitamin D intake, obesity, and sun exposure on breast density and ultimately breast cancer risk. The long-term goal of this study is to generate non-invasive interventions using vitamin D intake as a modifiable risk factor to lower breast density and to decrease risk of breast cancer in minority women, a population more susceptible to later-stage, advanced breast cancer. Our primary research question is to examine whether low serum vitamin D is associated with breast cancer through its association with greater breast density. Our corollary question is whether genetic polymorphisms in the vitamin D metabolism process differ by race and levels of body fat to potentially modify the association between breast density and vitamin D.

The multi-institutional collaboration consisted of 20 collaborators at 15 different sites, further detailed in Box 1. The first collaboration was forged with the NGH-BHC staff to prepare for subject recruitment by formulating recruitment methods that would facilitate study accrual without compounding the existing clinical burden at the breast center. Consensus with NGH-BHC staff was imperative for assimilating subject accrual into daily clinical operations and fulfilling the U54 study goal of targeted accrual of minority and medically underserved women.

Survey questionnaire development

Since a pre-existing questionnaire was not readily available to assess vitamin D intake through sunlight *and* diet, the next series of collaborations involved two internationally recognized breast cancer researchers in epidemiology^{19–20} and biostatistics^{21–22} at two cancer centers. With guidance from these senior investigators, a collaborative approach was used to construct survey questionnaire items on familial and personal cancer history, sun exposure and tanning behaviors, and physical activity. Sections entitled Physical Activities, Pregnancy, Mammography Adherence/History, and Smoking were modeled after the Collaborative Breast Cancer study questionnaire.²³ An interview aide, consisting of two picture methods was used to enhance recall of selected questions with visual cues.²⁴ Items were forwarded to Moffitt Cancer Center, where they were reviewed. A democratic decision-making style was utilized, in which consensus was achieved through discussions in various communication forms.

We pursued a collaboration with the Harvard School of Public Health to include a culturally sensitive food frequency questionnaire. The Harvard African American Food Frequency

Questionnaire (AAFFQ) was administered by the research staff with a “Got D’ViBE?” supplement on dietary vitamin D and supplemental vitamin D intake. We chose the AAFFQ because it is a validated instrument that quantitatively measures dietary vitamin D.

The team translated collaborated decisions into documents for institutional review board (IRB) approval. Study procedures were approved by IRBs at both Meharry Medical College (MMC) and NGH prior to implementation.

Survey questionnaire training

Questionnaire training was completed at the Van-derbilt University Epidemiology Survey Core where interviewers received guidance on administering the survey questionnaire, communication strategies, probing techniques, avoiding leading questions, and controlling voice inflection so as not to bias the respondent’s answers. The collaboration with the Vanderbilt Survey Core resulted in a health literacy assessment ensuring readability ease of study instruments. Flesch Reading Ease and Flesch–Kincaid Grade Level of the questionnaire were 72.6 and 5.5, respectively, indicating that someone at a grade 5 reading level could complete the questionnaire.^{25–26}

Anthropometric measurements

Body fat is a confounder of both mammographic breast density and vitamin D; therefore body fat distribution measurements were incorporated into the protocol. To heighten precision of measurements, anthropometric training was conducted by licensed exercise physiologist at Vanderbilt University Dayani Center, a fitness center in Nashville. Team members were trained on collecting body fat distribution measures including height, weight, and body mass index (BMI, kg/m²) using a calibrated scale with height bar; waist-hip circumference and waist-to-hip ratio (WHR) using a tape measure; and a three-site Jackson and Pollock percent body fat distribution using a Lange skinfold Caliper at 1) the tricep, 2) the suprailiac, and 3) the thigh.

Sun exposure measurements

To measure objective skin color we collaborated with Konica Minolta to obtain a CM-2500c portable spectrophotometer (Konica Minolta, Ramsey, NJ). This reflectance spectrophotometer device, which operates using principles of physics for light absorption and refraction, has been used in the automotive, dentistry, textile, and cosmetic industries for true color ascertainment.²⁷ Equipped with Skin Color Analysis Software CM-SA, Version 1.1 (Konica Minolta, Ramsey, NJ), this device produces a direct and reproducible melanin index (MI), the inverse amount of back-reflected light over the visible spectrum of wavelengths estimating light absorbed, accounting for concentration of cutaneous melanin.²⁸ Higher melanin indexes indicate deeper pigmentation where melanin reflects light and blocks subcutaneous synthesis of provitamin D hormones.

Database management

In order to manage study data efficiently and import data in different formats from external sources we collaborated with Vanderbilt Institute for Clinical and Translational Research to receive training on the REDCap (Research Electronic Data Capture) system. REDCap is a secure, web-based application designed for data capture support in research investigations²⁹ through entry of report data and masking access to protected health information (PHI) to multi-site collaborators.

Our collaboration with Vanderbilt’s Clinical and Translational Research team began developing a seamless flow from data collection, coding, entry, and storage. The functionality of REDCap allowed special attention to minimizing exposure misclassification from

interviewing, coding and editing the data collection, and measurement of quantitative mammographic breast density, vitamin D levels, and percentage body fat distribution.

Biospecimen collection

We chose to work with our home institution's [Meharry Medical College's] Clinical Research Center (CRC) for in-kind phlebotomy, blood separation, and storage services. Future directions of the study call for genotyping of key genes in the vitamin D metabolism process, therefore access to CRC services were invaluable for proper processing and freezer storage of samples. Procedures for obtaining/processing blood samples and storing samples were developed by Moffitt Cancer Center and the CRC. The Sample Processing Core Director completed blood separation, after which samples were promptly stored at -80°C . The CRC performed proper packaging of biospecimens via International Air Transport Association (IATA) regulations, ensuring safe delivery of our biospecimens to collaborators for analysis.

DNA extraction

Because future plans in the study call for genotyping, we collaborated with Vanderbilt University Medical Center's DNA Resources Core for completion of DNA extractions from frozen buffy coats. This service was provided by voucher through Vanderbilt University's Clinical and Translational Research Award (CTSA). Extraction of the biospecimens allows for storage of extracted samples until enough DNA specimens are collected for cost-efficient genotyping of Vitamin D targets.

Quantitative mammographic breast density analysis

The study team acquired screening analog films from participating study subjects from NGH-BHC. We collaborated with NGH-BHC staff to prepare lists of subjects on study, so analog films would be digitized. Breast density analysis is ideally completed from digitized films, necessitating digitization of the analog films. Our collaboration with NGH-BHC and the Radiology Department provided in-house time-saving technology and perpetuated a pre-existing working relationship.

At the time of study implementation, MMC lacked the expertise to assess breast density properly. While researching validated methods to assess breast density, we encountered numerous articles by a renowned medical biophysicist on mammographic breast density.³⁰ Thus, the research team facilitated a collaboration with Sunnybrook Health Sciences Centre in Toronto, Ontario. To prepare films for breast density analysis digitized films were uploaded into the REDCap Database for assessment. Sunnybrook collaborators had limited access to the PHI in the database and downloaded only de-identified DICOM images. The digitized films were then read for both percent and absolute breast density using an interactive-thresholding method through Cumulus software, a well-validated computer-assisted program.³¹ This collaboration also precipitated the training of the Chief of Radiology at MMC in quantitative breast density Cumulus techniques for future use by MMC investigators.

Vitamin D measurement

At grant submission, there was not a local laboratory reliable for quantitative measurement of vitamin D; therefore, our collaborator at Medical University of South Carolina (MUSC) facilitated collaboration with Heartland Assays in Iowa to analyze Vitamin D concentrations in blood serum. Stored serum samples from the CRC's -80°C freezer were packaged by certified Saf-T-Pak handler and sent for analysis using radioimmunoassay (DiaSorin, Stillwater, MN). This process is an FDA-approved chemiluminescence immunoassay (CLIA)³² to quantify levels of bio-available vitamin D found in serum.

Vitamin D analysis results were sent from Heartland Assays to our biochemistry and molecular biology collaborator, who is a prominent vitamin D scientist^{33–35} at MUSC. Vitamin D level results are interpreted at MUSC and classified into deficient, insufficient, and sufficient vitamin D levels.

Discussion

This paper documents multi-collaborative methods and the significance of efforts to address complex interactions among biological and behavioral processes relating to breast morphology and vitamin D intake contributing to breast cancer disparities. Our study strives to bridge the gap in limited data existing concerning the relationship of vitamin D to breast density in African American women, an understudied population predisposed to vitamin D deficiency and more advanced breast cancer stages. A remarkable feature is the collaborative nature of this study's design and implementation. Twenty multidisciplinary investigators at 15 sites brought a diverse range of experience and expertise in epidemiologic, translational, and methodologic issues to address the research questions of this study. Collaborative development of these extensive data collection materials required ongoing availability, teamwork, communication, and commitment to a democratic process. This collaborative approach was more time-consuming than other approaches. However, it facilitated a sense of ownership and commitment among investigators increasing involvement in developing instruments that address vitamin D deficiency and breast density. As a result, these materials are more comprehensive, and are enhanced in both depth and breadth.

Acknowledgments

Toni Lewis and research funded by National Cancer Institute grant number U54 CA091408-06. Research support provided by the Clinical Research Center of Meharry Medical College, Grant P20RR011792 from the National Institutes of Health and RCMI Clinical Research Infrastructure Initiative and National Cancer Institute grant number U54 RR026140; REDCap, Vanderbilt University Medical Center DNA Resources Core, and CTSA Grant 1 UL1 RR024975 from the National Center for Research Resources and National Institutes of Health; and Imaging Research, Sunnybrook Health Sciences Centre, Ontario, Canada. The authors would also like to thank the "Got D'ViBE?" Study interviewers and participants for their support of this study.

Notes

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Box 1**STUDY ORGANIZATION BY COLLABORATING ROLE IN THE “GOT D’VIBE?” STUDY**

Institution and Group Name	Role	
Sunnybrook Health Sciences Centre, Imaging Research Group	1	Completion of breast density analysis from uploaded DICOM images
University of Iowa, Heartland Assays	1	Completion of Vitamin D Assays from serum using
Medical University of South Carolina, Department of Neonatology, Pediatric Nutritional Services	1	Interpretation of Vitamin D levels
Vanderbilt University, Epidemiology Survey Core	1	Provided training on administration of questionnaire instrument during interview
	2	Aided in ensuring readability ease of study instruments
Harvard School of Public Health Channing Laboratories	1	Coded FFQ raw data
	2	Performed nutrient analysis
Meharry Medical College, Clinical Research Center	1	Provided in-kind phlebotomist to complete blood collection
	2	Performed blood spinning procedures to separate buffy coat, plasma, and serum
	3	Provided storage for separated bloods in 280°C freezer
	4	Aided in packaging of biospecimens using Saf-T-Pak regulations
Vanderbilt University Center for Human Genetics Research, DNA Core	1	Performed DNA extraction from buffy coat samples
Vanderbilt University, Department of Radiology	1	Scanned and digitized films to CD for FTP upload
Moffitt Cancer Center, Risk Assessment, Detection & Intervention	1	Provided external input on questionnaire development and implementation
	2	Advised on scientific issues as requested by “Got D’ViBE?” Study Team
Konica Minolta	1	Trained research staff on measurement of sun exposure via skin color and melanin index using reflectance spectroscopy
Vanderbilt Dayani Center, Director of Exercise Testing Lab, Exercise Physiologist	1	Trained research staff on performing reliable and valid body fat distribution measures, body mass index (BMI) Waist-to-Hip Ratio (WHR), skinfold three site body fat distribution measures
Vanderbilt University, Department of Biostatistics	1	Provided external input on questionnaire development and implementation
	2	Advised on scientific issues as requested by “Got D’ViBE?” Study team
Meharry Medical College/ Nashville General Hospital Breast Health Center	1	Facilitated recruitment of patients from the Nashville General Hospital-Breast Health Center
	2	Provided analog films for scanning and digitizing
Nashville General Hospital, Department of Radiology	1	Scanned and digitized films to CD for FTP upload

Institution and Group Name		Role
Vanderbilt University,	1	Creation of REDCap database
Vanderbilt Institute for Clinical and Translational Research	2	Training on maintenance and use of REDCap database

REDCap = Research Electronic Data Capture