Reproductive Toxicology 57 (2015) 81–86

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Vitamin D and uterine leiomyoma among a sample of US women: Findings from NHANES, 2001–2006

Susanna D. Mitro, Ami R. Zota*

Department of Environmental and Occupational Health, Milken Institute School of Public Health at the George Washington University, Washington, DC, United States

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 5 May 2015
Accepted 26 May 2015
Available online 3 June 2015

Keywords:
Uterine leiomyoma
Fibroid
Vitamin D
Misclassification
Race
NHANES
Reproductive health

ABSTRACT

Scientific understanding of the etiology of uterine leiomyomata (UL) remains incomplete, but recent investigations have suggested an association between low Vitamin D and UL risk. In this study, we conducted a cross-sectional analysis of Vitamin D exposure, measured using serum levels of 25(OH)D (a Vitamin D metabolite), and self-reported UL diagnosis among 3590 women aged 20–54 in the National Health and Nutrition Examination Survey (NHANES 2001–2006). Multivariate logistic regression models comparing each quartile of 25(OH)D to the lowest quartile indicated no relationship between 25(OH)D and odds of UL in the whole population (P_{trend} = 0.37), or in sensitivity analyses. However, a probabilistic analysis correcting outcome misclassification indicated that insufficient 25(OH)D was associated with UL in white (Odds ratio (OR) median estimate: 2.17; 2.5, 97.5 percentiles: (1.26, 23.47)), but not black women (OR median estimate: 1.70; 2.5, 97.5 percentiles: (0.89, 3.51)), suggesting misclassification may have driven some of the null findings.

1. Introduction

Uterine leiomyomata (UL), hormonally dependent benign tumors of the uterus, are a major source of morbidity for U.S. women [1]. However, despite the high prevalence and morbidity associated with UL, scientific understanding of risk factors remains incomplete [2,3].

One of the strongest established risk factors for UL is race. Black women are more likely to develop UL, develop tumors at a younger age, develop a greater number of tumors, have more severe symptoms, and are more likely to undergo hysterectomy as a treatment when compared to white women [4–8]. UL prevalence is also associated with age [9], body mass index (BMI) [10,11], and parity [12,13]. However, the known risk factors do not fully explain the elevated risk in black women [14].

Vitamin D may be an unrecognized risk factor for UL and contribute to racial/ethnic disparities in UL. Serum levels of 25-hydroxyvitamin D (25(OH)D), a Vitamin D metabolite, have been inversely associated with various female reproductive health conditions, including infertility, polycystic ovarian syndrome, and preterm birth [15]. As a result, the possibility of an association between serum 25(OH)D and UL has been suggested. At the cellular level, treatment with biologically active $1,25(OH)_2D_3$ inhibits leiomyoma cell growth *in vitro* [16–18], and leiomyoma cells have reduced expression of the Vitamin D receptor [19]. Women with darker skin are also more likely to be Vitamin D deficient [20,21]. In one recent population-based study, around 80% of non-Hispanic black women had deficient or inadequate levels of Vitamin D, while only about 20% of white women had deficient or inadequate levels [22].

Several recent epidemiological studies suggest an inverse association between serum levels of 25(OH)D and UL prevalence. A cross-sectional study found that serum 25(OH)D was inversely associated with UL prevalence in both black and white women aged 35–49 [23]. Similarly, a second cross-sectional study reported that infertile Vitamin D-deficient women had greater than twice the odds of UL than did infertile women without deficiency [24], and a third study found that women seeking treatment for symptomatic fibroids had lower serum 25(OH)D than healthy women [25]. Finally, a genetic study reported that SNPs associated with

Abbreviations: UL, uterine leiomyomata; BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; NHANES, National Health and Nutrition Examination Survey; CDC, US Centers for Disease Control and Prevention; NCHS, National Center for Health Statistics; OR, odds ratio; AOR, adjusted odds ratio.

* Corresponding author at: Milken Institute School of Public Health, Environmental and Occupational Health, 950 New Hampshire Avenue NW 414—Floor 4, Washington, DC 20052. Tel.: +1 202 994 9289.
E-mail address: azota@gwu.edu (A.R. Zota).

http://dx.doi.org/10.1016/j.reprotox.2015.05.013
0890-6238/

Vitamin D metabolism and skin color are associated with UL in black women [26]. While these studies reduced the likelihood of misclassifying UL case status by ascertaining cases using ultrasound or prospectively ascertaining cases [4], they were subject to other methodological limitations. For example, several studies recruited cases and controls from clinical populations, and most were limited by small sample sizes or incomplete adjustment for confounders.

Although preliminary research suggests that Vitamin D may play a role in UL risk, no population-based study of Vitamin D exposure and UL has been conducted. In this cross-sectional analysis, we used data from the National Health and Nutrition Examination Survey (NHANES) to investigate the relationship between serum 25(OH)D and self-reported UL.

2. Methods

We used data from three cycles of NHANES (2001–2002, 2003–2004, 2005–2006) to examine the association between serum 25(OH)D and self-reported UL. NHANES is an ongoing series of cross-sectional national surveys conducted by the US Centers for Disease Control and Prevention (CDC), including both physical exams and questionnaires. Due to the probability-based sampling methods used, NHANES data are representative of the non-institutionalized population of the U.S. [27]. All participants provided written informed consent, and the National Center for Health Statistics (NCHS) obtained institutional review board approval to conduct the surveys [27].

2.1. Study population

NHANES 2001–2006 included the examination of 30,070 individuals [27]. Serum 25(OH)D was measured in 27,266 participants. Only women aged 20–54 years were eligible to contribute data on UL diagnosis ($n = 4953$). We first removed individuals who were missing information about BMI ($n = 306$) and individuals who self-identified as a race or ethnicity other than non-Hispanic white, non-Hispanic Black, or Mexican American ($n = 464$). We then removed those who did not provide any reproductive health data ($n = 405$) or were missing parity data ($n = 445$), reducing the eligible population to 3785 individuals. Finally, we removed individuals who reported not knowing whether they had been diagnosed with UL ($n = 11$) or were missing serum 25(OH)D ($n = 184$). The final study population was 3590 individuals. Women excluded from the study population due to missing data were significantly younger, less likely to be white, and less likely to have sufficient Vitamin D (not shown).

2.2. Vitamin D analysis

Serum 25(OH)D was measured in NHANES participants aged 1 year and older [28]. Vials were stored at −20 °C between collection and analysis. Analytical methods for serum 25(OH)D measurements are described extensively in Yetley et al., 2010 [29]. Briefly, hydroxylated metabolites including 25(OH)D were extracted from serum samples using acetonitrile, and the treated sample was assayed using the DiaSorin radioimmunoassay kit, an equilibrium radioimmunoassay procedure with an antibody that has specificity to 25(OH)D [30]. Measured 25(OH)D values below 5 ng/mL or greater than 70 ng/mL were verified by reassay. Values less than 5 ng/mL (the lowest standard) were recorded as 3 ng/mL ($n = 17$, 0.47% of the study population). Blinded split replicate samples were sent to the National Center for Environmental Health laboratory at the CDC [29], and samples with a coefficient of variation greater than 10% were also reassayed. The sensitivity of the assay is at or below 1.5 ng/mL [30]. Information on the number of samples below sensitivity was not available from NHANES.

2.3. UL ascertainment

UL diagnosis was assessed using the following question in the reproductive health questionnaire, “Has a doctor or other health care professional ever told you that you had uterine fibroids?” Women who answered yes were recorded as having a UL diagnosis.

2.4. Statistical analysis

Serum 25(OH)D (ng/mL) levels were categorized as deficient, insufficient, or sufficient, and then compared between women with and without reported UL diagnosis using chi-squared tests. To assess the odds of UL by serum OH(25)D level, multiple logistic regression was used. Serum 25(OH)D was log transformed for continuous analyses, and was additionally assessed by quartiles. Data on season, parity, BMI, age, race/ethnicity, history of hysterectomy or oophorectomy, current pregnancy status, age at menarche, last menstrual period, and menopausal status were obtained from NHANES. Models were adjusted for risk factors determined *a priori*: age (years), race/ethnicity (White, Black, Mexican American), parity (continuous), BMI (continuous), season (November–April or May–October), and average daily physical activity (Mainly sit, Walk a lot, Carry light loads or climb stairs, Heavy work). Models additionally adjusting for current pregnancy (yes or no) and age at menarche (years) did not result in substantially altered results, and those covariates were excluded in final analyses. We did not adjust for oral contraceptives because they may be a consequence of the outcome [31].

2.5. Sensitivity analysis

Several sensitivity analyses were also done. Models were run without including physical activity because of concerns that adjusting for physical activity constitutes an over-adjustment for confounding [23]. Analyses were conducted in a population including only premenopausal women (excluding women who had undergone hysterectomy or oophorectomy, or had not had periods for 12 months due to menopause), and separately in each racial/ethnic group. To increase the sensitivity of the outcome measure, the population was limited to women younger than 35. To increase the precision of the exposure measure, we conducted an analysis excluding women missing supplement data or who reported using Vitamin D supplements. Finally, we repeated all analyses using a binary version of serum 25(OH)D (sufficient (>20 ng/mL) versus insufficient (0–20 ng/mL)), based on published nutritional guidelines [32]. All analyses were conducted using SAS 9.3 (Cary, NC) adjusting for the clustered sampling design and the NHANES sample population weights. A (two-sided) P-value <0.05 was considered statistically significant.

To quantify the potential magnitude of effect of the misclassification of undiagnosed women in our analysis, we stratified by race and used a probabilistic sensitivity analysis to model the odds of UL that would have been observed among women with Vitamin D insufficiency in the absence of misclassification, based on race-specific sensitivity and specificity values [33]. We modeled sensitivity as 23–32% for whites and 34–58% for blacks, and modeled specificity as 86–96% in both races, based on Myers et al., 2012 [34]. We ran the model 1000 times for each race, incorporating both systematic and random error in the simulation, to produce probabilistic estimates of the odds ratio (OR) [33].