Does Vitamin D Signaling Play a Role in Keloid Pathology?

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This abstract reports the results of Original Research. This was not a clinical trial. It was a clinical observational study. IRB approval was initially obtained from the WCG IRB on 1/13/2023, and re-approval was obtained annually until study closure on 3/31/2026 (Shriners study #OHI2201).

Running Title
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BACKGROUND

The increased incidence of keloids in populations with dark skin pigmentation suggests a putative role for vitamin D in keloid development and/or suppression. Vitamin D is made in skin in response to sunlight, with less vitamin D made in darkly pigmented skin. Vitamin D has anti-inflammatory and anti-proliferative activities that are mediated through binding to the vitamin D receptor (VDR). Upon vitamin D binding, VDR translocates to the nucleus and binds vitamin D response elements in target genes to regulate their expression. We previously identified reduced expression and decreased nuclear localization of VDR in keloid epidermis vs. normal skin, and observed reduced VDR nuclear localization in the normal skin of non-keloid patients who self-identified as Black compared to patients who self-identified as white. Additionally, we previously reported CYP24A1 overexpression in keloids compared with normal skin. CYP24A1 encodes 24 hydroxylase, which degrades the active form of vitamin D. Our previous work found that inhibition of CYP24A1 activity in keloid-derived cells could partially normalize profibrotic gene expression. Clinical studies in several countries reported lower circulating vitamin D levels in keloid patients compared with control cohorts, but a few studies reported conflicting results. We undertook a clinical observational study to better understand the relationships among skin pigmentation, vitamin D status, race/ethnicity, and scar phenotypes in a diverse pediatric patient population.

METHODS

This study was conducted with WCG IRB approval, according to the ethical standards of the Declaration of Helsinki, and with informed consent/assent at Shriners Children's Ohio. Patients scheduled for elective procedures involving excision of normal skin (e.g., breast reduction, panniculectomy), excessive scar (hypertrophic scar or burn scar), or keloid were enrolled. Skin pigmentation was measured quantitatively using a Mexameter, and self-identified race was recorded. A blood sample from each patient was collected prior to surgery to determine circulating 25-hydroxyvitamin D level, indicative of vitamin D status. VDR nuclear localization in tissue biopsies was quantified using immunohistochemistry and image analysis. Statistical analyses were performed using t test (2 groups) or One Way ANOVA (>2 groups), and correlations analyzed using Spearman Rank Order Correlation, with significance at $P<0.05$.

RESULTS

Forty seven patients were enrolled but 9 withdrew, leaving 38 patients for analysis: 18 in the non-scar "normal skin" group (2 males, 16 females; mean age 17.0 years),

13 in the "hypertrophic/burn scar" group (10 males, 3 females; mean age 15.1 years), and 7 in the keloid group (4 males, 3 females; mean age 13.6 years). Overall, patient vitamin D levels were negatively correlated with level of skin pigmentation (P=0.037). The mean vitamin D level in keloid patients was significantly lower than in non-scar, normal skin patients (18.2 vs 31.1 ng/mL, respectively; P=0.046). Further, when keloid and hypertrophic/burn scar patients were combined, their mean vitamin D level as a group (22.4 ng/mL) was significantly lower than the non-scar, normal skin patients (P=0.029). Mean VDR nuclear localization in keloid tissue samples was significantly lower than in normal skin (P=0.033), as observed previously. However, VDR localization was not different between hypertrophic/burn scars and either normal skin or keloids. Although there was a trend towards lower VDR expression in tissues from patients with low vitamin D levels, the correlation between VDR and circulating vitamin D levels was not significant due to multiple outliers. Quantitative pigmentation levels were significantly higher in keloid patients compared with normal skin or hypertrophic/burn scar groups. However, this reflects the different racial makeups of the groups: all 7 keloid patients identified as Black, whereas only 4/18 normal skin patients and 2/13 hypertrophic/burn scar patients were Black.

CONCLUSİON

This study found lower mean circulating vitamin D levels in keloid patients compared with non-scar patients, and lower VDR nuclear localization in keloid tissue compared with normal skin, in agreement with prior studies. Although there were significant differences in skin pigmentation among groups when measured quantitatively, this can be explained by racial differences among groups. Further, vitamin D levels were negatively correlated with skin pigmentation, as expected, with darker skinned patients having lower vitamin D status. Therefore, while the results are consistent with prior studies, we were unable to determine whether low vitamin D status is independently associated with keloid occurrence, or whether skin pigmentation or genetic ancestry is more important. Overall, our research suggests that vitamin D signaling may be involved in keloid pathology, although it is not yet clear whether vitamin D deficiency or low VDR levels in tissue are directly associated with risk of keloid development. However, the beneficial properties of vitamin D, and the in vitro effects previously observed with CYP24A1 inhibition, suggest a possible therapeutic role for vitamin D in keloid suppression. Future studies will investigate genomic polymorphisms related to vitamin D signaling in patients with and without keloids to determine if genetic risk factors were present in any patients in this study. `