

Vitamin D Insufficiency is a Frequent Finding in Pediatric and Adult Patients with Sickle Cell Disease and Correlates with Markers of Cell Turnover

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Abstract

Vitamin D insufficiency affects 33%-78% of children and 60-100% of adults with sickle cell disease (SCD). There are no previous reports demonstrating a correlation between vitamin D insufficiency and cell turnover in patients with SCD. We hypothesized that vitamin D insufficiency was prevalent in our SCD population (ages 0-60 years) and would correlate with reticulocyte counts in these patients. We performed a retrospective cross-sectional review of the medical records of 194 patients (ages up to 60 years) with SCD to evaluate their 25-hydroxyvitamin D levels as a function of red blood cell turnover, patient's age, number of pain crises, SCD genotype, and Hydroxyurea therapy. Vitamin D insufficiency was present in 88% of children and 96% of adults in our patient cohort. Serum 25-OH vitamin D levels correlated significantly with both age and reticulocyte counts in multiple regression analysis. Furthermore, pediatric HbSS patients had significantly lower 25-OH vitamin D levels than HbSC patients. No significant correlation was found between vitamin D levels and number of pain crises. Based on these findings, vitamin D insufficiency is a function of overall disease severity as manifested by the correlation with reticulocytosis.

Keywords: Sickle cell disease; Vitamin D deficiency; Reticulocytosis; Nutrition

Introduction

Vitamin D insufficiency is an established phenomenon in patients with Sickle Cell Disease (SCD) between 33 and 78 percent of children and between 60 and 100 percent of adults with SCD has been found to have low serum levels of 25-hydroxyvitamin D [1-7] the form that best reflects total body levels of the vitamin [8]. Although a large portion of the population has been described as having low levels of vitamin D [9,10], individuals with SCD are at higher risk for vitamin D deficiency than their healthy counterparts [1,2,7]. One research group at Children's Hospital of Philadelphia found that a significantly higher percentage of pediatric SCD patients met the criteria for vitamin D deficiency than did healthy African-American children in that city (33% vs. 9%) [1]. The same researchers also found that children with SCD as a population had substantially lower mean serum 25-OH vitamin D levels than a control population of African-American children [2]. Studies of adults with SCD have cited data from the National Health and Nutrition Examination Survey (NHANES), which reveal an overall prevalence of vitamin D deficiency in the U.S. general population of 2-6% more than tenfold lower than the prevalence in adult SCD patients [7,9]. These studies clearly demonstrate an association between vitamin D insufficiency and individuals with sickle cell disease but do not explore the causality or ramifications of this insufficiency.

The body's supply of vitamin D is synthesized in the skin, a process that requires exposure to sunlight [8]. One theory suggests that SCD patients develop vitamin D deficiency as a result of limited access to

sunlight [1,7], presumably secondary to more severe illness restricting mobility and frequent hospitalizations. To our knowledge only one study has investigated the possible link between number of vaso-occlusive crises and vitamin D deficiency, and no such correlation was found [3]. Vitamin D can also be absorbed in the small intestine from dietary sources or, more commonly, from supplements, and is stored in adipose tissue [8]. Poor nutrition and/or fat malabsorption have also been postulated to play a role in vitamin D insufficiency in SCD [2,7], although no studies evaluating these factors in vitamin D-deficient SCD patients have been published. We hypothesize that vitamin D deficiency in patients with sickle cell disease correlates with rate of red blood cell turnover, as defined by reticulocytosis, a marker of hyper hemolytic sickle cell disease.

Methods

Patients with SCD of all ages are followed by the Sickle Cell Center of Southern Louisiana at Tulane University. Vitamin D assessment (25-OH vitamin D measurement) is included during their routine, comprehensive assessments

Patient inclusion

To be included in this retrospective study, persons had to have a known diagnosis of SCD (HbSS, HbSC, and HbSβ+ or HbSβ0). Patients of all ages (up to 60 years) were included. Patients had to have a vitamin D level drawn within the 2 years prior to data collection before they were started on supplemental therapy.

Patient exclusion

Patients were excluded if they had received a blood transfusion within one month of assessment. Patients were not included if acutely ill (vitamin D levels were only assessed at baseline state of health). Patients prescribed vitamin D supplements were not included. Expedited IRB approval was obtained from Tulane University's Biomedical Institutional Review Board, as all data were collected retrospectively and were de-identified prior to analysis. No informed consent was required or obtained.

Vitamin D measurements

Serum testing of vitamin D was performed by an immunochemiluminometric assay (ICMA) performed on a DiaSorin LIAISON® instrument. Vitamin D levels are routinely obtained at patients' comprehensive visits (every 3-6 months). Only the initial measurement of serum 25-OH vitamin D was used in the analysis (i.e. not repeated measurements) since most patients were prescribed supplementation after the initial level was obtained in clinic.

Retrospective chart review

Patient records were accessed retrospectively to collect the following additional demographic data: patients' age, baseline laboratory data including hemoglobin, reticulocyte count, and lactate dehydrogenase (LDH). Data were also collected to assess the utilization of health care resources and clinical burden of disease over a 12-month period (either 2009 or 2010) including number of

emergency department visits, hospital admissions, and sickle cell day hospital visits with primary diagnosis of vaso-occlusive crisis. Average length of stay (LOS) for these visits was calculated. In pediatric patients (<21 years of age), it was also noted whether the patient had a history of avascular necrosis (AVN) and/or concurrent treatment with Hydroxyurea (HU).

Statistical analysis

Descriptive statistical analysis was used to characterize the patient population. ANOVA was used to assess the differences between group means (laboratory and demographic variables) and linear regression was performed to determine the significance between those variables determined to be significant in multivariate analysis. Regression analysis was used to assess each of the designated demographic variables including hemoglobin, reticulocyte count, LDH, hospital days/12 month period, number of ED visits, and genotype.

Results

Patient demographics

Data were collected on 90 pediatric patients and 104 adult patients with SCD, illustrated in Table 1. Average age of pediatric patients was 10.2 ± 5.6 years (up to 21 years), with 68% HbSS, 25% HbSC, and 7% HbSβ+. Average age of adult patients was 33.3 ± 10.5 years (21-60 years), with 71% HbSS, 19% HbSC, and 10% HbSβ+ or HbSβ0.

Variable	Pediatric Patients	Adult Patients
Number (n)	90	104
Age (years) [†]	10.2 ± 5.6	33.3 ± 10.5
Genotype		
SS	61 (68%)	74 (71%)
SC	23 (25%)	20 (19%)
SB(0 or +)	6 (7%)	10 (10%)
Vitamin D (ng/mL) [†]	17.2 ± 9.5	13.6 ± 7.9
<11	29 (32%)	51 (49%)
11-30	50 (56%)	49 (47%)
>30	11 (12%)	4 (4%)
Baseline Hgb [†]	9.2 ± 1.3	9.1 ± 1.8
Reticulocyte count (%) [†]	8.3 ± 4.9	8.2 ± 4.7**
LDH [†]	414 ± 159**	375 ± 205**
Hydroxyurea (HU)		
Yes	24 (24 HbSS)	not evaluated
No	66 (37 HbSS)	not evaluated

[†]Values for indicated fields are expressed as means ± SD. All other values reflect number of patients, with percentages in parentheses.

*n for these values are different from the total number of patients: 102 for adult reticulocyte count; 63 and 85 for LDH in pediatric and adult cohorts, respectively. Conversion factor for 25-OH vitamin D (ng/mL to nM) is 2.496.

Table 1: Patient characteristics

25-OH Vitamin D levels

Mean 25-OH vitamin D level was 17.2 ± 9.5 ng/mL (42.9 ± 23.7 nM) in the pediatric group (patients 0-21) and 13.6 ± 7.9 ng/mL (33.9 ± 19.7 nM) in the adult group (patients >21 years). 32% of pediatric patients had vitamin D deficiency, and 56% had insufficient levels of vitamin D. In adults, 49% of patients had vitamin D deficiency, and 47% had insufficient levels of vitamin D.

Correlation of vitamin D levels with age

In this cohort of pediatric SCD patients, we demonstrated a negative correlation between serum 25-OH vitamin D levels and age ($p < 0.0001$). These data confirm the findings of previous studies showing that vitamin D levels trend downward with increasing age [1,2,5] when the same analysis was applied to our adult population, the opposite trend was observed-younger patients had lower 25-OH vitamin D levels than older adults ($p = 0.024$)-a relationship which has not previously been observed [7] based on the R^2 values of the analysis, age accounts for 22.3% of the variability in vitamin D levels in children with SCD and for only 4% of the variability in vitamin D levels in adults with SCD.

Correlation of vitamin D levels with red blood cell turnover (reticulocytosis)

The level of vitamin D insufficiency correlated significantly with increased reticulocytosis, used as a marker of cell turnover in both the pediatric SCD population ($p = 0.006$) and in the adult SCD population ($p = 0.014$), as shown in Figure 1. More specifically, patients with higher reticulocyte counts tended to have lower levels of 25-OH vitamin D; vitamin D levels did not correlate with baseline hemoglobin in either pediatric or adult SCD patients ($p = 0.538$ and 0.971 , respectively).

Correlation of vitamin D levels with SCD genotype (pediatric patients)

Children with SS genotype ($n = 61$) had significantly lower 25-OH vitamin D levels than children of SC genotype ($n = 23$). The same was not true of adult patients, for whom 25-OH vitamin D levels were indistinguishable in SS and SC groups ($n = 74$ and $n = 20$, respectively). When correction was made for reticulocyte count in pediatric patients, the significance of the correlation between vitamin D levels and sickle cell genotype was maintained, suggesting that the link between vitamin D levels and SCD genotype is not merely attributable to increased red blood cell turnover.

Relationship between vitamin D levels and healthcare utilization

We evaluated the healthcare utilization in our patient population using the number of emergency room visits, sickle cell day hospital (SCDH) visits, and the number of hospital admissions as well as average length of stay over a given calendar year. Number of hospital visits analyzed in conjunction with vitamin D status, showed no

correlation between vitamin D levels and either frequency of healthcare encounters for pain or average length of stay per encounter in the adult population (all p -values ≥ 0.4). Likewise, no association was made between vitamin D levels and frequency of healthcare encounters for vaso-occlusive crisis in pediatric patients (p -values ≥ 0.4).

Hydroxyurea

Hydroxyurea (HU) has been studied extensively as a disease-modifying agent in sickle cell disease. It is known to increase levels of Fetalhemoglobin (HbF), to decrease leukocyte and reticulocyte counts, and likely to decrease the "stickiness" of red blood cells and the vascular endothelium [11]. This translates clinically into overall decreases in healthcare encounters for vaso-occlusive crises, decreased lengths of stay during hospitalization, and decreased utilization of opioid analgesics, for those patients who are maintained on HU [12,13]. Despite these advantages, when serum 25-OH vitamin D levels in pediatric patients who were prescribed HU ($n = 24$, all HbSS) were compared with levels in HbSS patients who were not on HU ($n = 37$), there was no statistically significant difference in the mean 25-OH vitamin D levels between the two groups ($p = 0.333$).

Discussion

To the authors' knowledge, this is the only study to assess vitamin D deficiency across the age spectrum in sickle cell disease. Several previous articles have presented the high prevalence of vitamin D deficiency in SCD; however, this was the first paper to evaluate the role of cell turnover in predicting vitamin D deficiency. Reticulocyte count provides a measure of red blood cell turnover in sickle cell patients, and can provide physicians with information about both the degree of sickling in a patient and the capacity of the bone marrow to compensate for loss of erythrocytes. Recent evidence suggests that reticulocyte count can be a predictor of long-term outcomes in sickle cell disease, as well. Several studies have validated reticulocytosis as a marker for both pulmonary hypertension and cerebrovascular accident [14-17]. However, this is the first study to identify the association of high hemolysis with vitamin D insufficiency. This finding is useful moving forward in determining the cause of vitamin D deficiency in this patient population. It appears from this study that the increased activity of the bone marrow in high hemolytic patients with SCD may prevent sufficient vitamin D absorption.

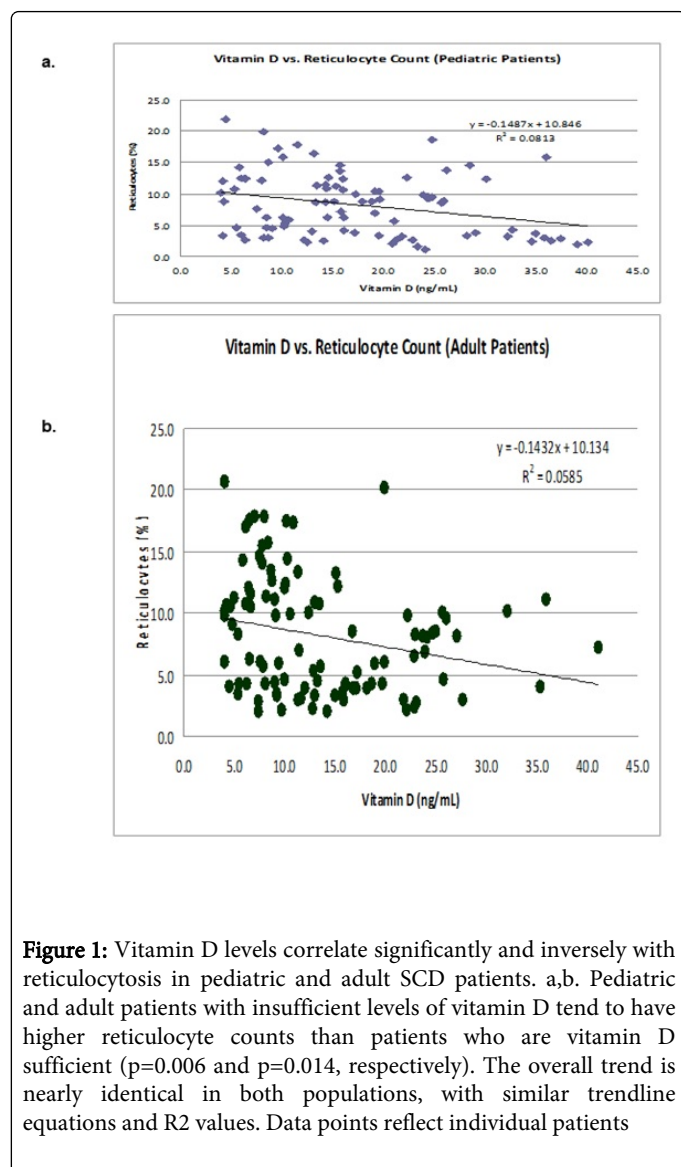


Figure 1: Vitamin D levels correlate significantly and inversely with reticulocytosis in pediatric and adult SCD patients. a,b. Pediatric and adult patients with insufficient levels of vitamin D tend to have higher reticulocyte counts than patients who are vitamin D sufficient ($p=0.006$ and $p=0.014$, respectively). The overall trend is nearly identical in both populations, with similar trendline equations and R^2 values. Data points reflect individual patients

Prevalence of vitamin D insufficiency in this cohort

For this analysis, vitamin D deficiency was defined as 25-OH vitamin D <11 ng/ml (<27.5 nM) and vitamin D insufficiency defined as 11-30 ng/ml (27.5-75 nM). These cutoffs have been used in a previous study of vitamin D levels in SCD patients [1], and were chosen based on population cohort studies of vitamin D in bone health [18,19]. All patients noted to have vitamin D insufficiency or deficiency were treated with weekly vitamin D supplementation (50,000 IU) per week until levels were sufficient. They were then maintained on 1,000 IU daily.

Healthcare utilization

Vaso-occlusive crisis is the primary reason for which patients of all ages with SCD are admitted to the hospital. Patients who are frequently admitted may be more sedentary or more likely to engage in indoor activities. Without sunlight, vitamin D cannot be synthesized in the skin, and the individual is left without adequate

stores of the vitamin [8]. However, the amount of healthcare utilization including total number of days spent inpatient did not correlate with vitamin D insufficiency. In the pediatric cohort, the degree of vitamin D insufficiency did correlate significantly with average length of stay in the hospital ($p=0.006$). However, these patients also tend to be more hemolytic. Thus, given the demonstrated correlations between low serum 25-OH vitamin D and reticulocytosis and SCD genotype, the relationship between the disease process and vitamin D status is likely to be far more complex.

Vitamin D and bone health

In the past, vitamin D has been studied primarily in the context of bone health. Osteopenia is well established in the context of sickle cell disease [4,5]. Bone density scans are not routinely obtained on asymptomatic sickle cell patients in our practice; therefore, as this study was retrospective in design, we have no data on the prevalence of osteopenia in this patient cohort, nor can we comment on a correlation between osteopenia and vitamin D levels in this population. However, despite the 88% of pediatric patients in this study with vitamin D insufficiency, we note <5% of children with SCD who have previously documented Avascular Necrosis (AVN).

Limitations

There are several limitations to this study. The sample size is relatively small, thus limiting the statistical power of our findings. Due to the retrospective nature of the study, we also lacked a control population for the prevalence of vitamin D insufficiency in our community. However, a study of healthy preschool-aged African-American children from low-income families in Atlanta, GA found that 26% had 25-OH vitamin D levels less than 20 ng/mL (50 nM) [20] compared to 46% of our patients less than 5 years old with similar 25-OH vitamin D levels. A study out of Boston, MA reported a 36% prevalence of vitamin D deficiency-defined as 25-OH vitamin D levels <=15 ng/mL (<=37.4 nM)-in healthy African American adolescents ages 11-18 years [21]. By comparison, 61% of patients aged 11-18 years old in our cohort had 25-OH vitamin D levels <=15 ng/mL (<=37.4 nM), despite residing at lower latitudes than the Boston study subjects. Therefore, it is reasonable to conclude that African-American children and adolescents with SCD are at increased risk for vitamin D deficiency than their healthy counterparts. Good control data for vitamin D levels in adult SCD patients is not as forthcoming. Data from National Health and Nutrition Examination Surveys (NHANES) suggest that between 14 and 44% of African-American adults have 25-OH vitamin D levels less than 10 ng/mL (25 nM), while only 2-6% are vitamin D sufficient (25-OH vitamin D >30 ng/mL, or >75 nM) [9]. These percentiles closely match those from the adult SCD patients in the current study. However, a significant number of African-American individuals included in the NHANES data likely have sickle cell disease, making it impossible to utilize this data as a true historical control.

Conclusions

This study suggests that vitamin D insufficiency in patients with sickle cell disease may be related to the disease process itself. Specifically, the data support our hypothesis that low vitamin D levels correlate with reticulocytosis in SCD patients. It should be emphasized that the data presented here is merely correlative. Any cause-effect relationships that exist between inadequate levels of vitamin D and various measures of sickle cell disease severity must be identified

through prospective interventional studies. Further, the clinical ramifications of this association, as well as the potential impact of vitamin D replacement on clinical outcomes of SCD, are topics for future study. Further investigations will also focus on dietary issues in SCD patients and on the possibility of differential vitamin D metabolism in these individuals. Through improved understanding of the causes and effects of vitamin D deficiency and insufficiency in the context of sickle cell disease, we can begin to predict whether vitamin D replacement as an adjunct therapy could improve the quality of life of SCD patients.

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