Vitamin D Deficiency in Adult Sickle Cell Patients

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Abstract: Introduction: Vitamin D levels in adult black Americans with sickle cell disease (SCD) are comparatively lower than those found in the general population of black Americans. The objectives of this study were to examine the prevalence of Vitamin D deficiency (VDD) in adults with various subtypes of sickle cell disease and identify risk factors for vitamin D deficiency.

Methods: In a retrospective study serum Vitamin D25(OH)D and/or VitaminD1,25(OH)2D levels were obtained in 120 subjects with sickle cell disease. Baseline studies also included LFTs, total protein, albumin, total bilirubin, and creatinine levels. In a portion of subjects that were treated with oral ergocalciferol vitamin D levels and chemistries were obtained within 6 months of treatment. Data was statistically analyzed with Welch two sample t-tests and individual simple linear regressions (including logarithmic values) for each variable.

Results: Vitamin D25(OH)D levels were found to be significantly lower in a group of subjects with Hgb SS disease, than in a group with other subtypes of sickle cell disease. In both groups combined, significant ($p = 0.05$) and clinically suggestive negative correlations with Vitamin D25(OH)D were seen for total bilirubin and total protein, respectively. When total bilirubin and total protein levels were compared between the Hgb SS and HgbS/other groups, t-test revealed these levels were significantly higher in the Hgb SS group levels at $p < 0.001$ and $p = 0.005$, respectively.

Implications: Low total Vitamin D25(OH)D levels in adults with sickle cell disease may be a reflection of chronic inflammation and overall disease severity.

Keywords: Vitamin D deficiency■Sickle cell disease■Vitamin D binding protein■Vitamin D25(OH)D■Vitamin D1,25(OH)2D

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INTRODUCTION

Vitamin D deficiency (VDD) has been well documented in large portions of the North American population,[1−6] including a comparatively high percentage in black Americans.[1,4,7−13] Children[14−18] and adults[18−20] with sickle cell disease (SCD) are known to have a high incidence of VDD. Although the incidence of VDD is high among adult black Americans in the general U.S. population,[4,6,9,21] Vitamin D levels in adult black Americans with SCD,[19,20] are comparatively lower.[6,9,10,12]

The measurement of serum Vitamin D25(OH)D level is generally recognized as the clinical standard for evaluation of Vitamin D status in both children and adults with normal with normal liver and kidney function, and represents the primary circulating form of Vitamin D.[22] Vitamin D is obtained endogenously by conversion of 7-dehydrocholesterol to its D3 form (cholecalciferol) via ultraviolet irradiation in the skin, or by ingestion of exogenous D2 (ergocalciferol) in the diet, and transported to the liver for conversion to Vitamin D25(OH)D. Principally in the kidney, Vitamin D25(OH)D is further metabolized to Vitamin D1,25(OH)2D, which is the principal bioactive form of vitamin D.[23] Both forms are bound in the blood stream by Vitamin D binding protein (DBP)[24−29] and albumin.[28−30]

There are several hypotheses found in the literature which attempt to explain the relatively low Vitamin D levels documented in the general population of adult black Americans. These explanations include the presence of increased skin pigmentation that blocks ultraviolet light required for Vitamin D production in the skin,[12,31−33] lower dietary intake of Vitamin D,[4,12,34,35] and genetic polymorphisms in DBP,[36] which is the primary vitamin D carrier protein, binding 85−90% of total vitamin D25hydroxy in the circulation. The remaining fraction is bound by albumin (10−15%) and less than 1% is in the free form.[37]

The objectives of this study were to examine the prevalence of Vitamin D deficiency in adults with various subtypes of sickle cell disease and identify risk factors for vitamin D deficiency.

METHODS

In a retrospective study of serum Vitamin D25(OH)D and/or VitaminD1,25(OH)2D levels which had been randomly obtained for clinical purposes in 120 subjects with Hgb SS($n = 74$), Hgb SC ($n = 33$), HgbS/thalassemia ($n = 11$), and Hgb S/lepore ($n = 2$) were reviewed over a period of 21 months beginning in February 2010, ending in November 2011. None of the subjects were known to be taking Vitamin D supplements. Along with baseline Vitamin D25(OH)D and Vitamin D1,25(OH)2D levels, variables included liver function tests (SGPT, SGOT, Alkaline Phosphatase, total bilirubin, total protein, and albumin), and creatinine levels which coincided with baseline vitamin D levels or were obtained within 3

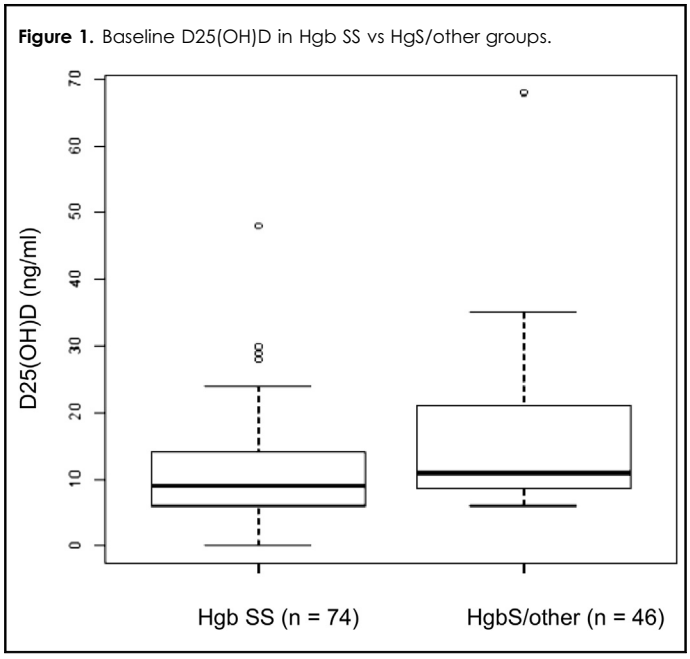


Figure 1. Baseline D25(OH)D in Hgb SS vs HgS/other groups.

months. Vitamin D levels are measured utilizing liquid chromatography−mass spectrometry (LC-MS). For purposes of this study normal ranges were 30−100 ng/ml for Vitamin D25(OH)D and 18−72 pg/ml for VitaminD1,25(OH)2D. Data includes post treatment vitamin D levels and chemistries, within 6 months, in the portion of subjects that were treated with oral ergocalciferol 50,000 units twice per week for 15 weeks. Data was statistically analyzed with Welch two sample t-tests and individual simple linear regressions (including logarithmic values) for each variable.

RESULTS & DISCUSSION

Data was divided into groups, Hgb SS and HgbS/other (SC, S/thalassemia, S/lepore). There were two subjects in the Hgb SS group with normal Vitamin D25(OH)D levels at 30 and 48. Of the HgbS/other group five subjects had normal Vitamin D25(OH)D levels at 68, 31, 33,35, and 30. All of the subjects in the HgbS/other group with normal values were HgbSC type. The mean baseline Vitamin D25(OH)D level (Figure 1) in Hgb SS subjects ($n = 74$) was 11.07, and in HgbS/other subjects was 15.86 ($n = 43$). A Welch two sample t-test yielded p-value $= 0.02$. The 95% confidence interval (CI) was 0.829−0.874 for the difference in means between the two groups.

Following treatment the mean increase in Vitamin D25(OH)D level for Hgb SS subjects ($n = 21$) was 22.74, and in HgbS/other patients was 17.00 ($n = 9$). T-test was not statistically significant. In both groups combined, individual simple linear regression for each variable was applied between liver function tests, creatinine levels, and

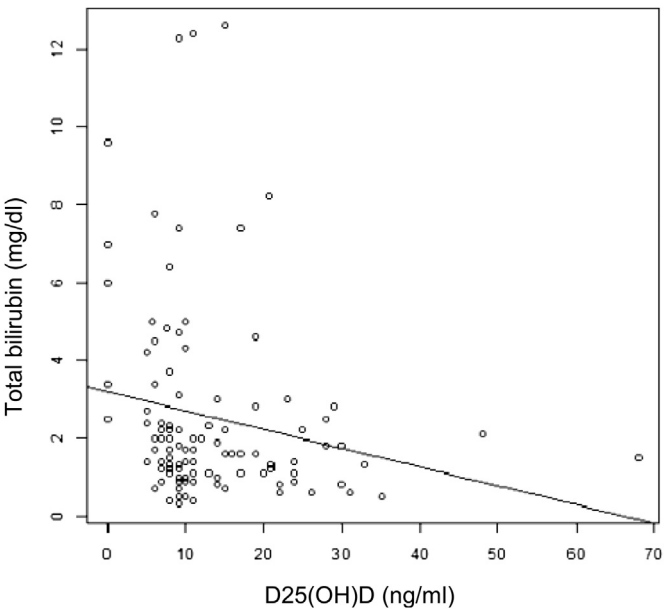


Figure 2. Simple linear regression between D25(OH)D levels and total bilirubin levels in the Hgb SS vs HgS/other groups combined ($n = 120$).

Vitamin D25(OH)D levels, with Vitamin D25(OH)D as the response variable. A significant relationship ($p = 0.05$, correlation $= -0.1892$) was seen for total bilirubin (Figure 2), and a clinically suggestive relationship ($p = 0.06$, correlation $= -0.1871$) was demonstrated for total protein (Figure 3).

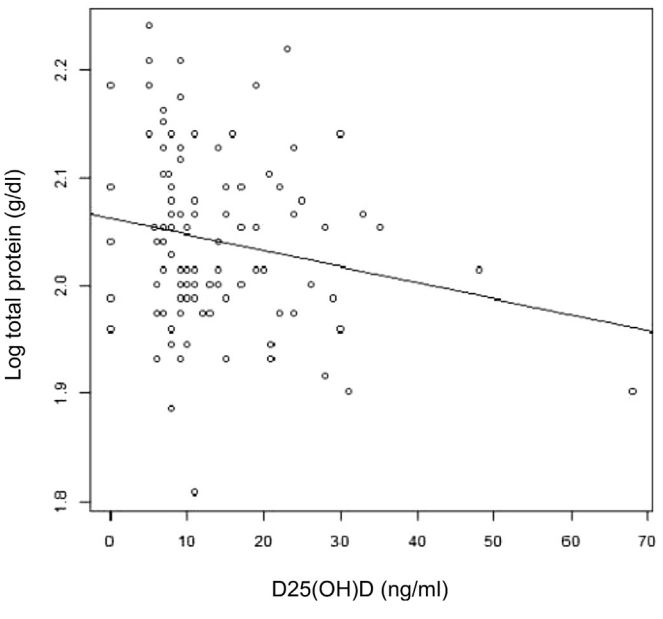


Figure 3. Simple linear regression between D25(OH)D levels and log total protein levels in the Hgb SS vs HgS/other groups combined ($n = 120$).

Figure 4. Simple linear regression between D25(OH)D levels and log total bilirubin levels in the Hgb SS group ($n = 74$).

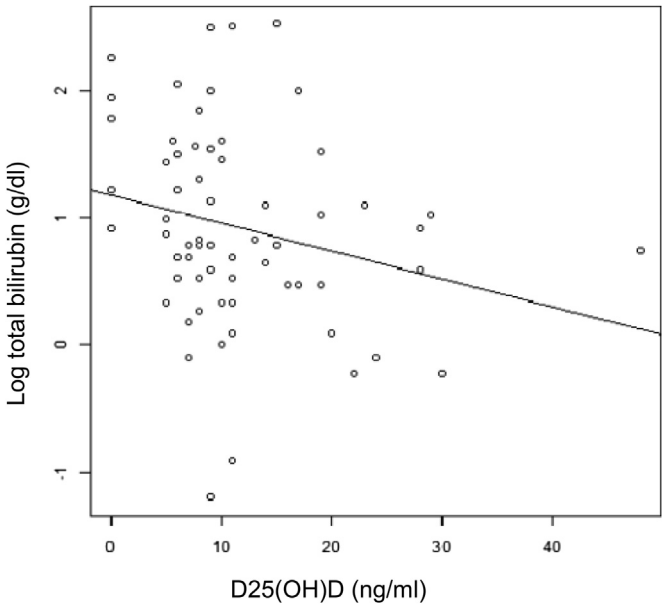
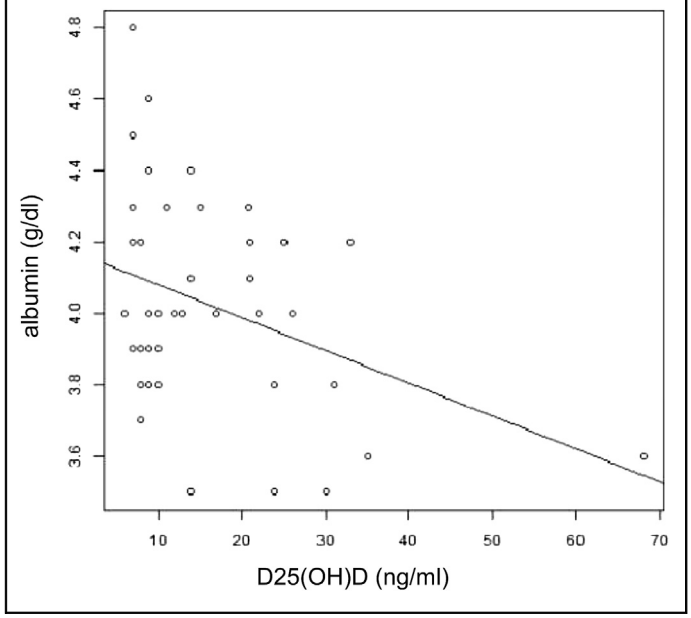


Figure 5. Simple linear regression between D25(OH)D levels and albumin levels in the Hgb S/other group ($n = 46$).



The mean baseline Vitamin D1,25(OH)2D level in Hgb SS subjects ($n = 23$) was 42.88, and in HgbS/other subjects was 45.48 ($n = 16$). T-test yielded no statistically significant difference between the two groups. There was insufficient data for analysis of post treatment Vitamin D1,25(OH)2D levels. In both groups combined, individual simple linear regression for each variable was applied between liver function tests, creatinine levels, and Vitamin D1,25(OH)2D levels, with Vitamin D1,25(OH)2D as the response variable. No statistically significant relationships were demonstrated.

In the Hgb SS group alone, simple individual linear regression was applied to each variable (Figure 4), with Vitamin D25(OH)D as the response variable did not reveal any significant relationships. The closest variable to statistical significance was total bilirubin at $p = 0.06$, with correlation of −0.2396 (Figure 4).

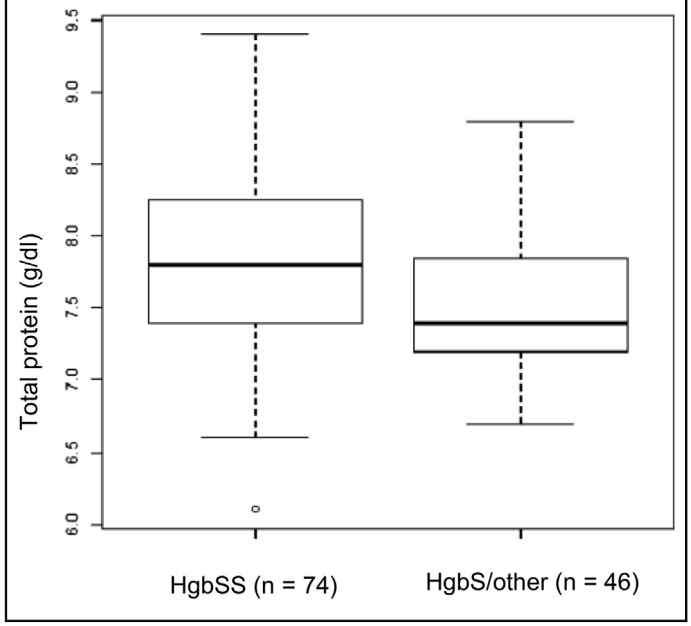
When individual linear regression for each was applied to the HgbS/other group alone (Figure 5), with Vitamin D25(OH)D as the response variable, albumin was statistically significant ($p = 0.01$, correlation = −0.3491).

When total protein levels were compared between the Hgb SS (mean = 7.862) and Hgbs/other (mean = 7.532) groups (Figure 6), t-test revealed a significant difference at $p = 0.005$. The 95% CI was 0.102−0.557 for the difference in means between the two groups.

When total bilirubin levels were compared between the Hgb SS (mean = 3.368) and HgbS/other (mean = 1.295) t-test (Figure 7) revealed a statistically significant difference at $p < 0.001$ with a 95% CI of 1.278−2.868 for the difference in means. When albumin levels were compared between the Hgb SS (mean = 4.052) and HgbS/other (mean = 4.02) groups, t-test revealed no significant difference.

Total Vitamin D25(OH)D levels among adults with sickle cell disease in this study were comparatively lower than those found in the general population of adult black Americans, as seen previously in the literature. This

Figure 6. Baseline total protein levels (g/dl) Hgb SS vs HgS/other groups.



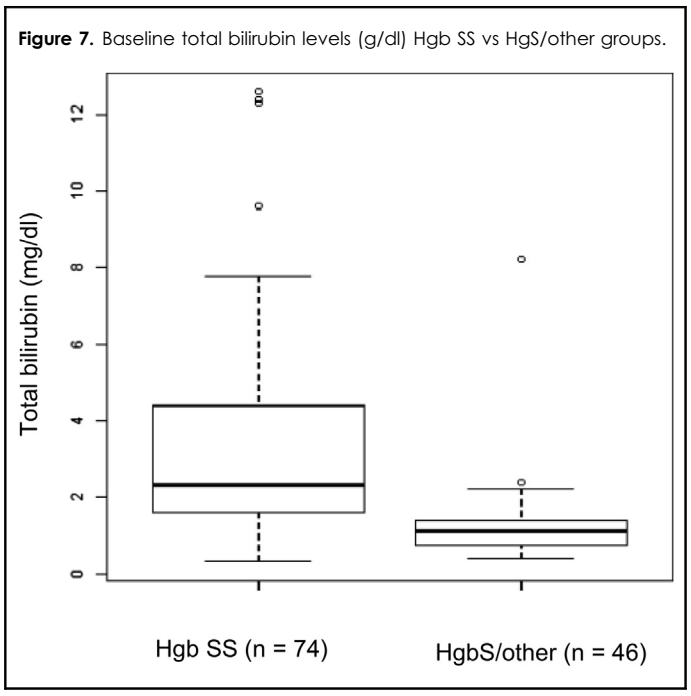


Figure 7. Baseline total bilirubin levels (g/dl) Hgb SS vs HgS/other groups.

relationship is not unique to black Americans. Tayo et al (2014) analyzed total D25(OH)D levels in 20 Jamaicans and 50 West Africans with sickle cell disease. They found that mean levels were 37% and 39% lower than in controls, respectively, and postulated that metabolic demands on the liver in sickle cell disease may reduce its capacity to synthesize DBP.[38]

The patients in this study with Hgb SS disease had significantly lower baseline Vitamin D25(OH)D levels compared to those as a group with Hgb SC, Hgb/thal, and HgbS/Lepore disease. There were only two (2.7%) subjects in the Hgb SS group with normal Vitamin D25(OH)D levels, and there were five (10.9%) in the HgbS/other group. Total bilirubin levels and total protein levels were both significantly higher in the Hgb SS subjects when compared to Hgbs/other subjects. Vitamin D25(OH)D levels in Hgb SS and HgbS/other subjects combined were shown to have a significant inverse correlation with total bilirubin levels ($p = 0.05$) and clinically suggestive inverse correlation with total protein levels ($p = 0.06$). Total bilirubin was also nearly significant as an inverse correlate to Vitamin D25(OH)D levels in Hgb SS subjects alone ($p = 0.06$).

Sickle cell disease has been characterized as a chronic inflammatory condition in children and adults.[39–43] Ischei (1979) found total protein in children with Hgb SS disease to be significantly elevated when compared to healthy controls and that it increases with age.[44] Adu et al (2012) attributed a relative hyperproteinemia in patients with Hgb SS disease, when compared to AS and AA subjects, to hyperglobulinemia.[45] Pandey et al (2012) noted significantly higher levels of total protein and total bilirubin in patients with Hgb SS disease than in those with hgb S/thalassemia disease.[46] Significantly elevated biomarkers for inflammation have been seen in Hgb SS subjects when compared to those with Hgb SC.[41] Similarly, in this study the Vitamin D25(OH)D levels in Hgb SS subjects were significantly lower than in the HgbS/other group, total bilirubin and total protein were significantly higher. Thus, If hyperproteinemia and hyperbilirubinemia are interpreted as an indication of overall disease severity and chronic inflammation, there is an association between low levels of total Vitamin D25(OH)D and evidence of chronic inflammation among the various sickle cell populations in this study.

There are other examples in the literature that point more specifically to a relationship between total Vitamin D levels, inflammation, and disease severity. In a study of systemic inflammatory (SIRS) following orthopedic surgery, Waldron et al (2013) concluded that serum Vitamin D25(OH)D declines during SIRS, is a negative acute phase reactant which has implications for acute and chronic diseases, and its deficiency may be a consequence rather than a cause of chronic inflammatory diseases.[47] Winters et al (2014) demonstrated significantly lower D25(OH)D levels in children with Hgb SS disease than in children with Hgb SC disease. Additionally, they found that higher reticulocyte counts correlated significantly with lower levels of D25(OH)D adults and children, and suggested that D25(OH)D deficiency is a function of overall disease severity.[18]

A high incidence of renal impairment in patients with sickle cell disease may contribute to low D25(OH)D levels by hindering the its metabolism to active form.[48] In a study of 300 adult subjects with sickle cell disease, Guasch et al (2006) showed that 70% of adults with Hgb SS disease and 40% with other sickling disorders had some degree of glomerular involvement, as manifested by albuminuria, and incidence increases with age. Renal insufficiency defined by glomerular filtration rate (GFR) less than 90 cc/min was present in 21% of the subjects.[49] Estimated GFR of less than 60 cc/min was found to be a predictor of D25(OH)D deficiency by Adla et al (2013) in a population of 70 adolescent and adult (mean age 28.85 ± 7.21 years) patients with sickle cell disease.[50] GFR was not included as a variable in this study, but there was no significant relationship between serum creatinine values and D25(OH)D levels.

It has been reported that 75% of black Americans have some degree of lactose maldigestion,[51] and avoidance of foods high in Vitamin D may be partially responsible for the high incidence of deficiency seen in this population.[52]

Concurrently, a large discrepancy in Vitamin D intake exists between white and black Americans, with blacks having lower intake, and is a difference that widens with age as intake by blacks declines further.[6,34,35] Kawchak et al (2008) showed a decline in adequacy of Vitamin D intake associated with age in children with sickle cell disease.[53] Winters et al (2014) confirmed a significant negative correlation between Vitamin D25(OH)D levels and age in a black American population of pediatric and adult SCD patients.[18] Varying degrees of anorexia due to frequently recurrent illness and hospitalization,[54] insufficient time outside in the sun,[15] and avoidance of time in direct sunlight in an effort to avoid dehydration[20] may contribute to reduced intake.

There is some speculation that the level of increased bilirubin secondary to hemolysis in sickle cell disease may lead to bile sludging and reduced secretion of bile salts into the intestinal lumen, thereby resulting in malabsorption of fat and fat soluble vitamins.[55] In this study the Hgb SS group had significantly higher levels of total bilirubin compared to the HgbS/other group, and there was a nearly significant ($p = 0.06$) inverse correlation between total bilirubin and D25(OH)D in the Hgb SS group. Svarch et al (2001) found evidence of chronic intestinal malabsorption in a group of 31 Cuban patients with sickle cell disease, including a flat lactose absorption curve compared to a control group. Jejunal biopsies revealing a significant decrease in villous height compared to controls, and lymphoblastic infiltration in the lamina propria were suggestive of a subclinical malabsorption disorder.[56]

As previously noted the measurement of serum Vitamin D25(OH)D level has conventionally been recognized as the clinical standard for evaluation Vitamin D status. Vitamin D25(OH)D has been called a prohormone,[29,57,58] as well as a "pre-hormone"[59] and is the immediate metabolic precursor to Vitamin D1,25(OH)2D, the active form of Vitamin D. Sutton and McDonald (2003) characterized Vitamin D25(OH)D as a "secosteroid precursor" and Vitamin D1,25(OH)2D as its "hormonal form".[60] The "free-hormone hypothesis" credits the unbound or free fraction of hormones with biologic activity, versus the protein bound portions in the in the circulation,[13,61] and is likely to apply to the free, unbound form of Vitamin D25(OH)D.[29,61]

Vitamin D25(OH)D and its metabolites show preferential binding to DBP, but the potential exists for binding to albumin as a major secondary carrier because of its abundance in the circulation.[28,29] Bikle et al (1986) concluded that the concentration of free Vitamin D25(OH)D in serum is independent of changes in the concentrations of DBP and albumin.[37] In a separate study Bikle et al (1986) demonstrated that patients with liver disease had low total Vitamin D25(OH)D levels that correlated with low DBP and albumin levels, but most had normal free Vitamin D25(OH)D levels.[62] Schwartz et al (2014) found that patients with cirrhosis had lower levels of free Vitamin D25(OH)D compared to black Americans except where DBP and albumin levels were lower in those with cirrhosis. Despite lower DBP levels than other racial/ethnic groups, free Vitamin D25(OH)D were not affected in black Americans.[13] There was no significant difference between the mean albumin levels of the Hgb SS and HgbS/other groups in this study. However, higher albumin levels showed a statistically significant correlation with lower D25(OH)D levels in the HgbS/other subjects alone.

Similar findings have been confirmed regarding levels of total and free Vitamin D1,25(OH)2D. Like Vitamin D25(OH)D, DBP is the principle carrier of total Vitamin D1,25(OH)2D in serum and albumin as the secondary carrier in patients with low levels of DBP.[28] Bikle et al (1984) showed that free Vitamin D1,25(OH)2D were well maintained in subjects with cirrhosis and reduced DBP levels, and that accurate levels of free Vitamin D1,25(OH)2D cannot be inferred by measuring total Vitamin D1,25(OH)2D and DBP levels in subjects with various pathophysiologic conditions.[63] Zella et al (2008) concluded that DBP in mice is an important binder of total Vitamin D1,25(OH)2D3 in the circulation, but that it does not affect the biologically active reserve of free Vitamin D1,25(OH)2D3.[64] Despite abnormally low mean baseline levels of D25(OH)D among both groups in this study, levels of Vitamin D1,25(OH)2D, the principal bioactive form of vitamin D, were well within normal levels in both groups and there was no significant or suggestive difference between the two groups.

Garrett-Mayer et al (2012) showed that differences in Vitamin D25(OH)D levels between black and white men could be eliminated by treating black men with Vitamin D3 4000 IU daily for one year. They suggested that black men may be able to convert Vitamin D3 more rapidly into Vitamin D25(OH)D more rapidly than white men.[9] Following oral Vitamin D2 50,000 units twice per week for 15 weeks, the Vitamin D25(OH)D levels in the Hgb SS and HgbS/other subjects both remained below normal in this study, and there was no significant difference in increase between the two groups. Compliance was not confirmed.

IMPLICATIONS

This study raises the question of whether or not total Vitamin D25(OH)D, vs the unbound form, is a clinically accurate measure of functional Vitamin D stores in black Americans, especially in those with sickle cell disease. Additionally, low total Vitamin D25(OH)D levels in sickle cell disease may be a reflection of chronic inflammation

and overall disease severity in this population. We would agree with Barclay (2013) that a new definition for VDD may be needed for blacks,[65] and with Nolan (2015) that the threshold for defining adequate Vitamin D levels in Caucasians may not be appropriate for black Americans with sickle cell disease.[48] Comprehensive studies are needed in the sickle cell population that include analysis of the relationships between total and free forms of Vitamin D, variations in quantity and binding affinity of DBP, and biomarkers for inflammation related disease severity.

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