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Research Article

STATUS OF VITAMIN D IN SICKLE CELL DISEASE: A STUDY OF ONE CAPITAL AREA IN KUWAIT

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ABSTRACT

Background: Recent studies suggest that patients with sickle cell disease (SCD) have profound vitamin D deficiency (VDD). Moreover, lower levels of vitamin D can affect thyroid function which in turn affects bone formation.
Aim: To determine the prevalence of VDD in patients with homozygous SCD and to evaluate the predictors of VDD in them.
Method: Relevant data was retrieved from hospital database. Pearsons correlation and Multiple linear regression analysis was performed on these data to assess VDD in patients with SCD.
Results: The vitamin D level was remarkably lowered in SCD patients (mean 19.1±14.7 nmol/L vs. 75.9±33.5 nmol/L) when compared to controls ($p<0.05$). SCD patients had increased ALP levels (161.3±68.0 vs. 61.7±16.6, $p<0.001$), reticulocyte count (4.18±2.7 vs. 1.07±0.68, $p<0.01$) and reduced levels of TSH (1.05±0.91 vs. 1.74±1.0, $p<0.05$). No difference existed between patients of different age groups or gender.
Conclusion: A general VDD prevailed in Kuwaiti population but the risk of VDD in SCD-SS was approximately 4 folds higher than control subjects. ALP concentration inversely correlated with vitamin D levels and was the strong predictor of its deficiency.

INTRODUCTION

Ten decades earlier SCA was first detected as a genetically inherited disorder. Nevertheless, till date, finding an appropriate treatment for SCA still remains a challenge and the disorder demands a multi-disciplinary therapeutic approach (Menaa, 2013). More attempts to rapidly find treatments for decreasing mortality, morbidity and improving quality of life among patients with SCA is needed (Menaa, 2014). Despite the fact that the health care is improved, the associated episodes of the disease and progressive organ damage are still major health challenges in the management of the disease (Platt *et al*., 1994; Weatherall *et al*., 2005). These challenges are further compounded by the usually observed deficiency of vital nutrients, such as vitamin D, which has been associated with musculoskeletal health in SCA patients (Osunkwo *et al*., 2011). Numerous studies have apparently established an association between vitamin D sarcityin SCD but do not explore the consequence of this scarcity (Buison *et al*., 2004; Goodman *et al*., 2010; Rovner *et al*., 2008; Garrido *et al*., 2012). The common reasons associated with VDD in SCD are high concentration of melanin in the skin, low levels of physical activity, low food intake, reticulocytos is and highly prevalent bone resorption markers (Winters *et al*., 2014; de Oliveira *et al*., 2015). Bony alterations and bone fragility are frequent observations in SCD patients with VDD (Garrido *et al*., 2012; Arlet *et al*., 2013; Chapelon *et al*., 2009; Ozen *et al*., 2013). The most relevant clinical biomarkers for bone disease are vitamin D, alkaline phosphatase (ALP) and thyroid stimulating hormone (TSH) (de Oliveira *et al*., 2015; Arlet *et al*., 2013; Zeynep *et al*., 2016; Afonja and Boyd, 1986; Bolarin, 2001). ALP is a plasma membrane enzyme used to assess osteoblast function (Bolarin, 2001). Elevated level of alkaline phosphatase denotes severity of bone damage and is a helpful guide of progress in the management of bone pains in SCA (Afonja and Boyd, 1986). Also, high levels of serum ALP seen in patients with SCA is either because of cholestasis or bone disease (Brody, 1975). Chapelon *et al*. (2009) reported that low levels of vitamin D were seen in paediatric SCA patients whereas increased ALP levels were observed with growth (Chapelon *et al*., 2009). Acute bone disease in patients with SCA happens with osteomyelitis and vaso-occlusion whereas chronic bone disease occurs in the event of a vascular necrosis of the shoulders and hips (Damanhouri *et al*., 2015). TSH is an independent regulator of osteoblastic bone formation and osteoclastic bone resorption (Abe *et al*., 2003). Hypothyroidism, i.e. low T3 and T4 and increased TSH is

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infrequently associated with osteoporosis in SCD having VDD (Ozen *et al*., 2013; Zeynep *et al*., 2016). Previous studies showed that about 74% of children and 100% of adults with SCA suffer from 25-hydroxyvitamin D, the form that best indicator of total body levels of the vitamin [5-8, 10, 13, 21-23]. However, vitamin D status in SCD patients of Kuwait is not known. (Osunkwo *et al*., 2011; Buison *et al*., 2004; Goodman *et al*., 2010; Rovner *et al*., 2008; Winters *et al*., 2014; Chapelon *et al*., 2009; Adewoye *et al*. 2008; Lal *et al*., 2006; Rosen, 2011). We, therefore, aimed to investigate the levels of vitamin D and its predictors (age, gender, ALP, TSH, reticuloytosis) in patients with SCA in one capital area of Kuwait.

MATERIALS AND METHODS

Study Design

This retrospective study was carried out in the Haematology Laboratory Department, Amiri Hospital, Kuwait, for a period of one year from month 2015 till month 2015.Twenty-eight SCA patients of both sexes (15 males and 13 females) aged between 0 years and 70 years were included in the study. Twelve matching controls (4 males and 8 females) served as control groups. The patients and controls were divided into two groups: paediatric group, which includes newborn till 21 years old, adult group, which includes subjects from 22 till 70 years. Patient medical history was retrieved from the hospital's medical records department, and each patient was examined by a haematologist. Data collected from patient's records included age, gender, vitamin D (25-OH) levels, serum alkaline phosphatase (ALP), serum thyroid stimulating hormone (TSH), reticulocyte count and percentages of haemoglobin (Hb) sub-types. This study was ethically approved by the Research Committee in the Kuwait Ministry of Health.

Exclusion Criteria

Patients were excluded if they were diagnosed with HbAS or other form of genotypes apart from HbSS, were prescribed vitamin D supplements, had acute complications or clinical crises within three months from data collection, had other comorbid disease such as hepatitis, cancer, human immunodeficiency virus (HIV), metabolic bone disease and endocrine dysfunction.

Reference Values

The reference ranges for various parameters used in this study are:

For vitamin D

Vitamin D deficiency: 25(OH) D<50 nmol/L
Vitamin D insufficiency: 25(OH) D between 50 nmol/L and 75 nmol/L
Vitamin D sufficiency: 25(OH) D>75 nmol/L
Vitamin D critically high: 25(OH) D>250nmol/L

For ALP:

Low ALP: <42 IU/L

Normal ALP: between 42 IU/L and 98 IU/L

High ALP: >98 IU/L

3) For TSH:

Low TSH: <0.5uIU/ml
Normal TSH: within 0.5 uIU/ml and 5.0 uIU/ml
High TSH: >5 uIU/ml

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Science Software (SPSS) version 21 (Chicago, IL). Descriptive statistics were used for demographic data and baseline characteristics of the patients. Qualitative variables were described by frequency and percentage and analyzed by Fisher's exact, chi-square. Quantitative variables were described by mean and SD of the mean for parametric analysis such as Student's t test. Bivariate analysis using Pearson was performed to find the correlation of vitamin D deficiency in SCD patients with various parameters. Multiple linear regression analyses was performed on only those variables which had p value <0.1 in bivariate analysis. Statistical significance was set at $P \leq 0.05$.

RESULTS

Characteristics of the study population

The current study included a total of 40 subjects. Of them, 28 were homozygous (HbSS) SCD patients and 12 were matched controls. Patient and control group comprised both adults (n=26, range: 22-70 years) and children (n=14, range: 0-21 years). The mean age of the pediatric SCD patients were18.1±2.9 years and for adult SCD patients were 35.4±10.9 years. The gender distribution of patient was 1:1(15 male: 13 female). The characteristics and laboratory findings of the study population are shown in Table 1.

25-OH Vitamin D status in studied population

The mean 25-OH vitamin D levels in the studied population (n=40) was 35.9±33.9 nmol/L indicating a general prevalence of VDD in Kuwaiti population. Females tend to have higher vitamin D levels compared to men although this difference was not statistically significant (41.03±38.5 vs. 30.42± 28.03, p=0.3).VDD was equally prevalent in paediatric and adult population (30.5±31.1nmol/L vs. 38.9±35.5nmol/L, p=0.4).Similarly, no significant correlation was also observed between vitamin D status and age (Pearsons's coefficient = 0.139, p=0.3) The vitamin D level was remarkably lowered in SCD patients (mean 19.1±14.7 nmol/L vs.75.9±33.5nmol/L)when compared to controls (p<0.05) (Figure 1A). However, the vitamin D levels were comparable within patient between both the age groups (p=0.6, Table1) and both the genders (male18.1±8.9 vs. female 20.2±19.7, p=0.7).

Biochemical analysis

The ALP levels were considerably high in SCD patients as compared to controls (161.3±68.0 vs. 61.7±16.6, p<0.001) (Figure 1B).Further, ALP levels tend to be high in female SCD patients than males but the difference was not statistically significant (p=0.19, Table 1). Between paediatric and adult patients, ALP was comparable (p=0.4, Table 1).

Table 1. Demographics and laboratory findings of the study population

Variable	SCD		Controls	
Number of subjects	Pediatric (10)	Adult (18)	Pediatric (4)	Adult (8)
Age (years)	18.1±2.9	35.4±10.9	13.5±5.6	42.5±10.4
Gender				
Male	6	9	2	2
Female	4	9	2	6
Hb sub-types				
HbA%	-	-	97.7±0.1	97.9±0.3
HbA_2%	2.4±1.4	2.8±1.4	2.3±0.1	2.0±0.4
HbS%	80.9±6.4	73.3±14.4	-	-
HbF%	16.6±6.83	19.7±13.7	<1	<1
Vit. D (nmol/L)	17.4±13.4	20.1±15.7	63.3±40.41	81.4±30.6
% Deficient (<50 nmol/L)	100	94.4	25	12.5
%Insufficient (50-75 nmol/L)	-	5.5	50	25
% Sufficient (>75 nmol/L)	-	-	25	62.5
ALP (IU/L)	148.8±53.4	176.9±75.4	58.8±8.4	63.3±19.8
% low (<42 IU/L)	-	5.5	-	-
% within range (42-98 IU/L)	-	16.7	100	12.5
%high (>98 IU/L)	100	77.8	-	87.5
TSH (ulu/ml)	1.4±0.9	1.5±0.6	2.1±1.1	1.8±0.9
Reticulocyte count (%)	5.6±2.9	4.5±2.3	1.5±0.8	0.9±0.9

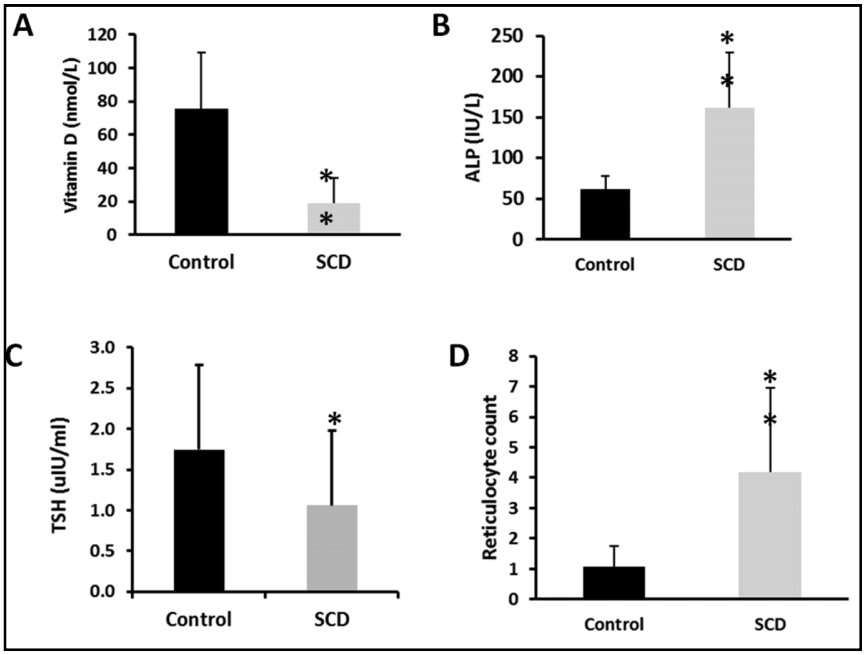

Figure 1. Laboratory findings in SCD patients. Comparison of vitamin D (A), ALP (B), TSH (C) and reticulocytes (D) in SCD patients with healthy controls. *p<0.05, **p<0.001

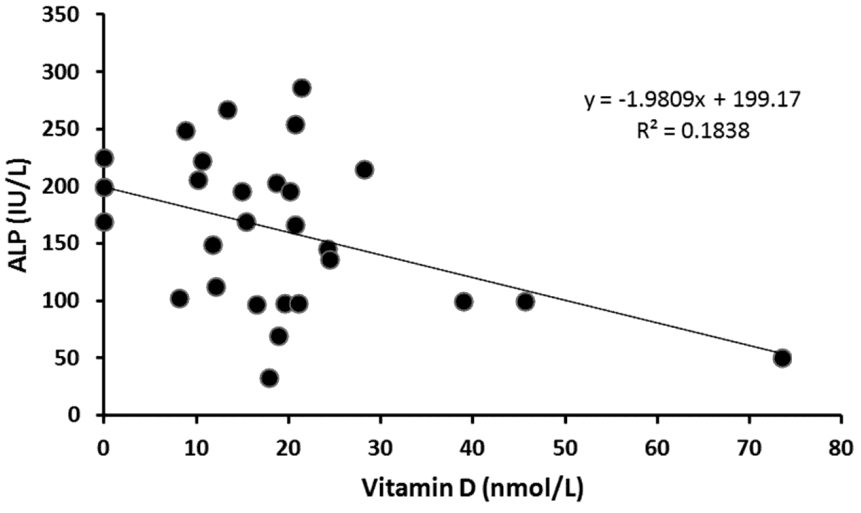

Figure 2. Inverse correlation of vitamin D with ALP in SCD patients (Pearson's correlation coefficient = -0.429, p<0.05)

TSH hormone levels were reduced in SCD patients compared to controls (1.05±0.91 vs. 1.74±1.0, $p<0.05$) (Figure 1C). Among patients, the TSH levels were comparable between children and adults ($p=0.5$, Table 1) and tended to be higher for females than males ($p=0.1$, Table 1). Reticulocytosis was increased in SCD patients than controls (4.18±2.7 vs. 1.07±0.68, $p<0.01$) (Figure 1C). In patients, the reticulocyte count was similar between adult and children ($p=0.9$, Table 1) as well as between male and female ($p=0.05$, Table 1).

Association of Vitamin D deficiency with various factors

Bivariate analysis of vitamin D in SCD patients showed significant correlation with only ALP levels ($p<0.05$) (Figure 2). No correlation of vitamin D was observed with age, gender, TSH and reticulocyte count in patient. Multiple linear regression further confirmed that low vitamin D levels in SCD patients was significantly associated with high ALP levels ($p=0.02$).

DISCUSSION

This is the first study to assess the association of bone markers (ALP and TSH) with vitamin D status in SCD patients. Bone diseases are commonly reported in SCD patients and have been increasingly associated with hypovitamin D state of the patients (Buison *et al*., 2004; Arlet *et al*., 2013; Chapelon *et al*., 2009; Adewoye *et al*., 2008).Association of ALP, a marker for bone turn over, with low vitamin D status in SCD patients is however not clear. While some studies have shown increased levels of ALP in SCD patients with reduced vitamin D, others have failed to show it (Ozen *et al*., 2013; Zeynep *et al*., 2016). Further, direct correlation of ALP with VDD is lacking. In the present study, hypo-vitamin D status in SCD patients strongly correlates with high ALP levels. Although TSH levels were considerably lower in SCD patients, no significant correlation with vitamin D was observed. Endocrine dysfunctions are frequently observed in SCD patients (Zeynep *et al*., 2016). Abberent thyroid function tests have also been reported in patients with SCD (Parshad *et al*., 1989). In the study of Parshad *et al*. (1989) it was reported that male patients had hypothyroidism reflected by significantly lower endogenous T3 and higher TSH levels. The etiology of thyroid dysfunction in SCD is unclear. Autopsy reports suggest transfusional hemosiderosis and cellular damage to the thyroid gland to be the cause of thyroid dysfunction in SCD patients (Steinberg *et al*., 2009). Contrarily, in the present study TSH was found remarkably low in SCD patients. Male as well as female and children as well as adult patients had lower TSH. However, the TSH values were within the normal reference range suggesting TSH insufficiency in patients. Pituitary- or hypothalamic-dysfunction can cause TSH insufficiency or it can be of idiopathic origin (Basu *et al*., 2005). This needs to be investigated in SCD patients. Also, further studies in large cohort are required to confirm these findings. Elevated reticulocyte count is frequently observed in SCD patients (paediatric and adult) and is associated with disease severity (hemolysis) and VDD (Winters *et al*., 2014). Increased bone marrow activity due to high hemolysisin SCD patients might prevent sufficient vitamin D absorption leading to vitamin deficiency. We observed, an increased reticulocytosis in SCD patients with low 25-(OH) vitamin D indicating increased hemolysis in the patient group. Nonetheless, it was not associated with low vitamin D levels suggesting high reticulocytosis is not contributing to low vitamin D status in SCD patients. The major limitation of study is retrospective design due to which all the parameters affecting or affected by vitamin D could not be analysed. Further, the sample size is relatively small, thus limiting the statistical power of our findings.

Conclusion

In conclusion, ALP is an independent predictor of low vitamin D status of SCD patients. Insufficient TSH levels in the patient group suggest presence of pituitary-hypothalamic dysfunction which can be an indirect effect of hypovitamin D status, but might also contribute to the severity of bone disorders in these patients, thus complicating management of bone disease inpatients. Kuwaiti physicians should consider this when treating SCD patients.

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