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Prevalence of vitamin D deficiency in patients with sickle cell disease in Bahrain

Adla B Hassan[1,2], Taysir S Garadah[1,2*], Ahmed A Jaradadat[2], Abdulla Al Ajmi[1], Mohamed E Alawi[1], Reginald P Sequeira[2]

[1] Salmaniya Medical Complex, Ministry of Health, Manama, Kingdom of Bahrain.
[2] College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain
**Corresponding author E-mail: garadaht@hotmail.com*

Abstract

Background: Patients with sickle cell disease (SCD) had a low serum level of vitamin D. The prevalence and the impact of this observation in SCD patient's in Bahrain is not clear.
Aim: To assess the prevalence of vitamin D deficiency (VDD) in homozygous patients with SCD, and to evaluate the clinical and biochemical predictors of occurrence of VDD in such patients.
Methods: We evaluated the vitamin D status in 70 patients with confirmed diagnosis of SCD and compared them with an age matched control group. Serum level of vitamin D, parathormone (PTH), calcium, phosphate, alkaline phosphatase, haemoglobin (Hb), and uric acid were measured. Linear regression analysis was performed to assess the relation between PTH and vitamin D level. Multiple regression analysis was performed to assess the predictive value of gender, body mass index (BMI) >24, serum level of uric acid>400 umol/Land estimated glomerular filtration rate (eGFR) <60, for occurrence of vitamin D deficiency in patients with SCD.
Results: The mean age of the study group was 28.85 ±7.21 years compared with the control of 29.91 ±4.3 years, p=0.23. There were 40 (57%) female SCD patients compared with 39 (55%) in the control. In patients with SCD, the serum level of vitamin D was sufficient, >50 nmol/L in 4 (5%) patients, insufficient > 27.5 - ≤50 nmol/L in 21 (30%) patients, deficient >12.5 - ≤27.5 nmol/L 35 (50%) and profoundly deficient ≤12.5 nmol/L in 10(15%). In the control group, those with sufficient level of vitamin D were 53 (75%), insufficient 21(30%) deficient of 10 (15%) and none with profound deficiency, respectively. The serum level of PTH and alkaline phospahtase were significantly higher in the SCD group compared with control group. The eGFR, Hb and were significantly lower, serum level of uric acid was significantly higher ($p < 0.05$ but no significant difference between serum level of calcium or phosphate. Linear regression analysis showed inverse relation between VDD (<25 nmol/L) and PTH ($r = - 0.34$, $P<0.001$). Multiple regression analysis showed female gender, BMI>24, hyperurecemia and low eGFR were positive predictor of VDD in patients with SCD.
Conclusion: The risk of vitamin D deficiency among subjects with SCD-SS was 3.8 folds greater than control subjects; the prevalence of VDD in SCD was 95% and 25% in the control patients without SCD. On linear regression analysis, there is a significant inverse relation between serum levels of vitamin D and PTH in SCD patients. The female gender, BMI>24, hyperurecemia>400 umol/L and low eGFR<60 were positive predictor of VDD in patients with SCD.

Keywords: *Sickle cell disease, vitamin D deficiency, anemia, Bahrain.*

1 Introduction

Sickle-cell disease (SCD) or sickle-cell anemia (SCA) is an autosomal recessive genetic blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling of red blood cell (RBC) decreases the cells flexibility and results in a risk of various complications.[1]
In SCD, mutation results in the substitution of the amino acid valine for glutamic acid at position six of the beta chain leading to sickle cell hemoglobin (HbS).[2] As an autosomal recessive genetic blood disorder, the homozygous inheritance results in sickle cell disease. When HbSS is deoxygenated, it becomes relatively insoluble and forms aggregates of haemoglobin (Hb) molecules within the RBC leading to vaso-occlusive complications.
Abnormal haemoglobin can be detected by haemoglobin electrophoresis, in which various types of haemoglobin move at varying speeds. The diagnosis can be confirmed with high-performance liquid chromatography (HPLC).[3]

Sickle-cell disease may lead to various acute and chronic complications such as in SCA and SC crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, hemolytic crisis, several of which have a high mortality rate.[1]

It was reported that the treatment with hydroxyurea decreases the frequency and severity of SCD attacks by reactivating fetal haemoglobin production in place of the HbS,[4] and shown to possibly increase survival time.[5]

Recent studies report a high prevalence of vitamin D deficiency (VDD) among patients with sickle cell disease (SCD), with a rates as high as 65-100% depending on the season.[6, 7] Despite this evidence, VDD remains under-recognized and under-treated in patients with sickle cell disease.

One report showed that children with SCA are 5.3 times more likely to develop vitamin-D deficiency than healthy African-American controls.[8] It is estimated that 65% of children with SCA had severe vitamin-D deficiency (≤10 ng/mL).[6] Despite these findings, vitamin-D levels are not routinely checked or treated in patients with sickle cell anemia.

The highest frequency of sickle cell disease was found in tropical regions, particularly sub-Saharan Africa, India and the Middle-East,[9] with prevalence of sickle-cell trait (HbS) of 4.2% in Saudi Arabia and 0.26% of sickle cell disease (HbSS) with the highest prevalence in the Eastern province.[10]

A population-based study from Bahrain has confirmed a high prevalence of VDD, especially in females, that shows a seasonal variation.[11] In this study we assess the prevalence of VDD in patients with SCD (HbSS) in Bahrain and evaluate the clinical predictors of VDD in such patients.

2 Materials, methods and setting

The serum level of 25-hydroxy vitamin D (25-OHD) was evaluated in 70 patients with SCD over a three months period from 1.9.2012 to 1.12.2013. All patients were clinically in a steady state and were not experiencing any acute vaso-occlusive crisis (VOC) with no hospitalization for at least 3 months prior to the clinical evaluation. Patients were enrolled in the study if they have a long history of SCD (HbSS) with documented serum electrophoresis. Patient selection was consecutive from those who attend the hematology clinics in Salmaniya Medical Complex (SMC). SMC hospital is a government public hospital of 1100 beds with catchment area of 900,000 populations. A constitutional consent form was obtained and signed by each patient before enrollment in the study.

Patients were excluded if they were aged >13 years, have chronic renal disease, other chronic pain syndromes, such as juvenile rheumatoid arthritis, chronic liver disease, recent hospitalization <3 months, history of malignancy, known malabsorption or conditions associated with poor gastrointestinal absorption, patients currently on high dose vitamin D therapy and recent blood transfusion (<30 days).

Height, weight and calculated body mass index (BMI) of each patient was recorded. History of oral medications of hydroxyurea and hospitalization for SCD crisis in the last 12 months was documented. Each patient was examined for blood pressure and heart rate, signs of heart failure such as S3 gallop, raised jugular venous pressure, tender hepatomegaly and ankle edema.

Blood samples were withdrawn for the measurements of serum level of 25-hydroxy vitamin D, hemoglobin (Hb), chemical parameters for VD regulation such as calcium, phosphorus, PTH, as well as, alkaline phosphatase as a marker for bone turnover, uric acid and estimated glomerular filtration rate (eGFR).

Vitamin D deficiency (VDD) was defined as normal with vitamin D serum level of >50 nmol/L, deficient (VDD), if serum 25OHD between >30–≤50 nmol/l; severe VDD was defined as serum 25 OHD >12.5 –≤ 30 nmol/l; and profound VDD was defined as serum 25OHD ≤12·5 nmol/l.[12] We defined vitamin D deficiency as sufficient (>50 nmol/L), insufficient (> 27.5 - ≤50 n mol/L), deficient (>12.5 - ≤27.5 nmol/L) and profoundly deficient (≤12.5 nmol/L).[12]

Serum vitamin D was measured using Ultra Performance Liquid Chromatography-interfaced with tandem Mass Spectrometry (UPLC/MS/MS) at Al Jawhara Centre. We used commercially available vitamin D kits (Chromsystems Instruments & Chemicals GmbH, Germany). Quantification of PTH in serum was performed by Enzyme-Linked Immunosorbent Assay (Creative Diagnostics, USA).

The serum level of calcium, phosphorus and alkaline phosphatase were measured using the auto-analyzer at Salmaniya Medical Complex (SMC). The control group patients were selected consecutively in the same period of time for age-matched healthy patients who were attending SMC cardiac clinics for routine electrocardiogram and turned to be normal.

3 Statistical analysis

The data was analyzed using the statistical package of social sciences (SPSS) version 17.1. All laboratory data of Hb, Vitamin D, Ca, PTH, phosphate and alkaline phosphatase, uric acid and estimated GFR are presented as mean ± SD. Unpaired student-test was used to analyze the differences between the means.
Student's t-test was applied for continuous variables and Chi-square analysis for non-continuous data. Multiple logistic regression analysis was applied to calculate the odds ratio for different clinical and biochemical variables for the occurrence of VD deficiency in patients with sickle cell disease. All reported p-values are two tailed and p-value <0.05 was regarded as significant.

4 Results

Seventy patients with SCD were evaluated. The mean age of the study group was 28.85±7.21 years and the control group 29.91 ± 4.3 years (p=0.23) with 40 (57%) and 39 (55%) female patients respectively."Table 1" shows the clinical characteristics of patients with SCD and the control group.

Table 1: Clinical characteristics and the mean ±SD of biometric values of patients with SCD and the control group.

Parameters	**SCD** n = 70	**Control** n = 70	**p-value**
Mean age (years)	28.8.5∓7.21	29.91 ∓ 4.3	0.23
Female	40(57%)	39(55%)	0.76
BMI (Kg/M^2)	19.5 ∓2.4	24.04 ∓2.86	0.02
Hb (gm/dl)	of 8.9 ∓0.8	13.01 ∓1.12	0.04
SBP (mmHg)	119∓15	120∓12	0.45
HR per minute	80∓3	70∓4	0.03

BMI: body mass index; Hb: hemoglobin; SBP: systolic blood pressure; HR: heart rate.

The SCD patients compared with control group had significantly lower BMI (19.2 ± 4), low hemoglobin (10.1 ±2 gm/dl), significantly higher heart rate (81 ± 4 beat per minute) and similar systolic blood pressure in both groups. The mean values of biochemical markers in patients with SCD and the control group are shown in" table 2". The mean value of vitamin D is significantly lower of 26.8 ± 7.4 *vs.* 35.5 ± 6.5 nmol/L,P<0.01.

Table 2: The mean value ∓SD of biochemical parameters in patients with SCD and control group.

Biochemical parameters	**SCD** n = 70	**Control** n = 70	**p-value**
Vitamin D (nmol/L)	26.08∓ 7.4	52.55∓6.5	0.001
Hb (gm/dl)	9.5∓1.6	12.4∓1.9	P<0.05
Alkaline phosphatase (U/L)	190.7 ∓78	41.03 ∓12.78	P<0.01
PTH (pmol/L)	6.92∓0.6	4.13∓0.34	P<0.001,
Calcium mmol/L	2.14 ∓0.5	2.12∓0.3	P=0.36
Phosphorus (mmol/L)	1.29∓0.3	1.32∓0.2	P=0.25
Uric acid (µmol/dl)	430.3∓36	340.6∓44	P<0.05
eGFR $ml/min/M^2$	67.3∓7.8	92.5 ∓63.94	P<0.05

eGFR: estimated glomerular filtration rate; PTH: Parathormone; SCD: Sickle cell disease.

Haemoglobin level in SCD patients was significantly lower 9.5±1.6 gm/dl *vs.* 12.4, ±1.8 (p <0.05), calcium and phosphorus were slightly lower but of no statistical significance. The serum level of PTH hormone was significantly higher in SCD patients (4.5 ±1.1 *vs.* 2.5 ± 1.2 pmol/L, p< 0.05) with a significantly higher level of alkaline phosphatase compared with control of 122.4 ± 14.4 *vs.* 54.4 ± 12.5 (p<0.05).
"Table 3" showed that the SCD patients with deficient serum level of <27.5 nmol/L compared with insufficient serum level of >27.5 nmo/L had significantly high PTH and high alkaline phosphatase.
In SCD patients there were 24 (53%) in the deficient group taking hydroxyurea medication and 12 (56%) were in the insufficient group indicating the lack of influence of hydroxyurea treatment on vitamin D level.
Linear regression analysis showed inverse relation between VDD <27.5 nmol/L and PTH (r = -0.34, p<0.001). Multiple regression analysis showed female gender >18 years of age as positive predictors of VDD in SCD with odds of female

gender 2.2 (95% CI:1.8-2.6; p=0.03), BMI >24 of 1.2 (95% CI:0.8-1.6, p=0.02), hyperuricemia >400 mg/L 1.0 (95% CI:0.8-1.2, p<0.04) and low eGFR (<60) of 0.8 (95% CI: 0.7-0.9, p<0.045) .

The serum level of vitamin D in SCD patients and the control groups is depicted in "Figure 1". Patients with sufficient serum level of VD of >50 nmol/L were 4 (0. 06%) versus 53(76%) patients in the control group and those with insufficient serum level of >30- ≤50 nmol/L were 21(30%) *vs.* 7 (10%) respectively. The serum level of VD deficiency of >12.5- ≤30 nmol/L was observed in 35(50%) versus 10 (14%) in the control but profound deficiency of <12.5 nmol/L was seen in 10 (14%) in SCD patients and none in the control group.

Table 3: Vitamin D serum level in patients with SCD of < 27.5 nmol/L or more than 27.5 nmol/L.

Parameters	VD ≤27.5 N=45	VD>27.5 N=25	P value
Alkaline phosphatase (U/L)	140.5∓24	44.5∓16	<.001
PTH (pmol/L)	6.8 ∓0.8	4.2∓0.4	<0.01
Treatment with hydroxyurea	24 (53%)	14 (56%)	=0.65

PTH: parathormone

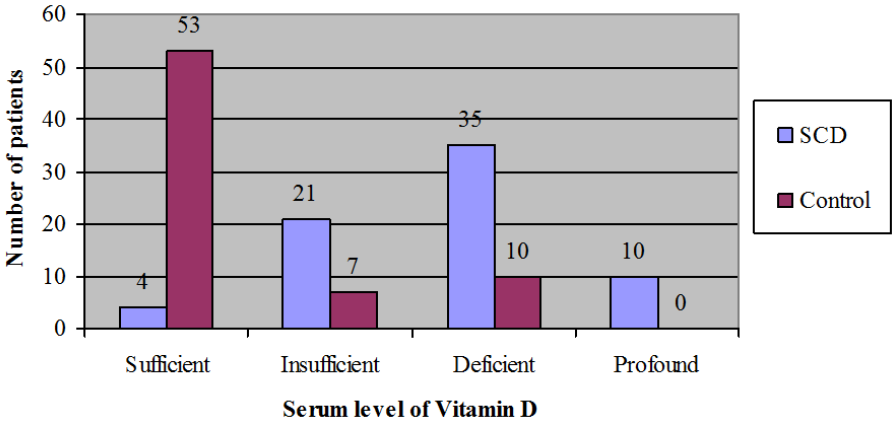

Fig. 1: The status of serum level vitamin D in patients with and without sickle cell disease.

5 Discussion

The study evaluated the prevalence of vitamin D deficiency in 70 patients with SCD. The prevalence of low serum level of vitamin D ≤50 nmol/L was (94%) in total. Thirty percent of patients had vitamin D deficiency of 27.5 -50 nmol/L and 64% had severe deficiency of <27.5 nmol/L. This finding is in keeping with two recent reports about VDD in patients with SCD. First report by Goodman *et al.*,[13] studied 148 patients with SCD and found 98% of patients had low (suboptimal) serum level (<30 ng/ml) and 60% had severe VD deficiency of <10 ng/ml. The second study by Arlet *et al.*[14] was conducted on 56 patients with SCD found that VDD was detected in 26 (46%) patients with severe deficiency and another 26 (46%) with insufficiency.

Rovner *et al.*, in their study reported 61 patients with homozygous SS sickle cell disease; vitamin D status was compared with healthy African-American children living in the same geographic area. Ninety-three percent of subjects with SCD-SS and 90% of healthy subjects had vitamin D insufficiency [25-hydroxy vitamin D <30 mg/mL]. The risk of vitamin D deficiency among subjects with SCD-SS was 5.3 (95% CI: 2.5, 8.2) times greater than control subjects.[15]

Erkal *et al.*, studied 994 patients with general bone pain regarding the prevalence of VDD and concluded that 75% had VDD of <50 nmol/L. Female with VDD <25 nmol/L were 30% compared with male 19%. The most positive predictor of VDD was the female gender, high body mass index, and the lack of sun exposure, living at high altitude and wearing scarf.[16]

The very low level of VD in SCD is ubiquitous and it seems of multifactorial origin. It may be of cultural, behavioral and environmental factors all together. The level of high PTH and high level of alkaline phosphatase in VD deficient patients with SCD indicate that this finding mostly secondary to low serum calcium due to VD deficiency. On regression analysis there was a significant inverse relationship between PTH serum level and the level of VDD in this study. Such a finding was also shown previously by Boonen *et al*.,[17] and Rodan *et al.*[18]

Cashman *et al*, had conducted a study on healthy adolescents, and concluded that individuals with VD level >74.1 nmol/L had a significantly lower PTH and normal bone turnover markers compared with those of low VD status <46.4 nmol/L with higher PTH.[19]

Recent report showed that high BMI and female gender were positive predictors of VDD in SCD patients. In this study female gender, BMI>24, hyperurecemia>400 umol/L and low eGFR<60 were positive predictor.

In the control group the mean value of VDD was of 52 nmol/L, (n=70) compared with 26 nmol/L in SCD patients. Those who had VDD were of 24 % versus 94% in SCD patients. The majority of control patients had a normal serum level of VD of >50 nmol, n=53 and only 4 patients had normal level in SCD group.[20]
The low status of serum level of VD in the control group was surprising and may need further evaluation and follow up to assess the seasonal impact, cultural differences, nutritional factors and exposure to sunlight.
There are other important findings in this study such as the significant high heart rate in SCD patients compared with control patients. The positive predictive value of high BMI of vitamin D deficiency is in agreement with another report of Saintonge S et al, where an inverse relation was found between the High BMI and the VD.[21] The significance of low Hb in patients with SCD may be explained partly by the state of chronic low hemoglobin due to long-standing hypoxia.

6 Limitations of the study

The study focused on the prevalence of vitamin D in SCD patients in a single centre. There was no clinical evaluation and correlation for the presence or absence of muscle pain. Thus, further study is required to clarify the clinical outcomes.

7 Conclusion

The risk of vitamin D deficiency among subjects with SCD-SS was 3.9 times greater than the control subjects. The prevalence of VDD in SCD was 94% and 24% in the control patients without SCD. The mean serum level of PTH was significantly greater in SCD patients with the marker of bone turnover. PTH had an inverse linear relationship to serum level of vitamin D.

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