Impact of Parenteral Compared to Oral Vitamin D3 (25-OH Cholecalciferol) Therapy on the Bone Pain Frequency and Serum Level of VD in Adult Patients with Homozygous Sickle Cell Anemia

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Background: Bone pain frequency and optimal methods of vitamin D (VD) administration in adult patients with sickle cell anemia (SCA) are unclear.

Objective: To assess bone pain frequency and level of VD in adult SCA patients after vitamin D medication.

Setting: Salmaniya Medical Complex, Bahrain.

Design: A Prospective Controlled Trial.

Method: The study was performed from 1 January 2013 to 31 December 2014. Sixty-nine SCA patients were studied and compared with an age and gender-matched control group. Bone pain frequency was assessed using Visual Analogue Scale (VAS). Measurement of serum level of VD, parathormone (PTH), calcium and alkaline phosphatase (ALP) at baseline, one and three months after treatment. Vitamin D Deficiency (VDD) was defined as <50 nmol/L. The mean difference of biochemical and clinical parameters was compared using paired Student t-test.

Result: Fifty-one (74%) patients from the study group and 14 (20.3%) patients from the control group had VDD. Twenty-six (37.7%) patients were treated with IM injection of 600,000 IU once and 25 (36.2%) were treated with oral capsule of 50,000 IU weekly. Patients on IM treatment had pain frequency of 56 episodes per month before treatment, which was reduced to 43 (P<0.05) after one month; further reduction to 34 episodes (P<0.01) was achieved after three months. Patients on oral medication had pain frequency of 57 episodes per month before treatment, which reduced to 50 episodes after one month (P<0.05) and 40 after 3 months (P<0.01).

Vitamin D level increased to 54.15±2.73 in one month compared to 19.55±9.63 nmol/ml (P<0.05) before treatment. Patients on oral medication had VD increment of 31.64±4.44 compared to 22.11±9.46 nmol/ml (P<0.05) after one month and 53.69±2.37 nmol/ml after three months (P<0.001).

Conclusion: Frequency of bone pain was reduced significantly in adult SCA patients with VDD after one month of treatment of vitamin D3 injection with normalization of serum level.

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Sickle Cell Anemia (SCA) is an autosomal recessive genetic blood disease characterized by sickle shaped RBCs, which decreases the flexibility and results in hemolytic anemia and other complications. SCA is characterized by synthesis of hemoglobin S (HbS) and the substitution of the amino acid in position six of the beta chain. The homozygous form of SCA (HbSS) results from the inheritance of two sickle cell genes[1,2]. SCA is characterized by recurrent episodes of hemolytic anemia, vaso-occlusive crises that end with hypoperfusion and organ dysfunction due to obstruction of microvascular system and destruction of red blood cells[3]. The incidence of SCA in the Arabian Peninsula is 1.2% to 2.6%[4,5].

SCA patients may have acute bone involvement due to vaso-occlusive crisis or acute osteomyelitis. In addition, it could lead to chronic debilitating diseases such as osteonecrosis, osteopenia and growth impairment[6,7]. Bone involvement could lead to high risk of morbidity and affect the quality of life[8,9].

Osteopenia and osteonecrosis, although may be asymptomatic, could result in bone fracture, vertebral collapse and deformities that require analgesic, mechanical and surgical support[10,11].

Vitamin D is essential for the normal absorption of calcium and for maintaining calcium homeostasis. Vitamin D deficiency

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results in rickets, osteomalacia and bony changes in the spine. Sickle cell disease patients are at an increased risk of vitamin D deficiency[12]. In one study, 91% percent of children with SCA had VDD (25-OHD) with serum levels of <20 μg/L[13].

Neither the clinical impact of vitamin D deficiency on the frequency of chronic bone pain syndrome nor the clinical response after correction of VDD with vitamin D medications have been previously evaluated in adult SCA patients.

The aim of this study is to assess bone pain frequency and level of VD in adult SCA patients after vitamin D medication.

METHOD

Sixty-nine patients with homozygous SCA (HbSS) were included in the study. Patients were selected consecutively from the hematology clinic from 1 January 2013 to 31 December 2014. Another 69 patients, age and gender matching the first group, with no evidence of SCA were evaluated as a control group and were attending hematology clinic for bone pain.

The diagnosis of homozygous SCA (HbSS) was made by solubility screening test for sickling and confirmed by high-performance liquid chromatography (LPLC). Informed consent was obtained from all patients.

Patients with SCA were excluded if they had a history of advanced renal or hepatic failure, blood transfusion in the last four weeks and/or were taking vitamin D and calcium medication.

Medical history of each patient was documented. Weight, height and body mass index (BMI) were documented. Patients were examined clinically for joint and peripheral bone tenderness.

Patients were provided a booklet for recording the frequency of bone or chest pain daily and marking the severity of pain using a visual analogue scale. The five grades and their color coding are as follows:

Grade 1: White (1/10 to 2/10)
Grade 2: Green (3/10 to 4/10)
Grade 3: Yellow (5/10 to 6/10)
Grade 4: Orange (7/10 to 8/10)
Grade 5: Red (9/10 to 10/10)

The severity of pain was defined as follows: 1 - mild (white or green), 2 - moderate (yellow), 3 - severe (red or orange). The frequency and severity of bone pain were tabulated daily, weekly and summed monthly for three months.

Serum level of vitamin 25 (OH) D, (D3) was considered normal if the level was ≥50 to 170 nmol/L. Vitamin D level was measured at baseline for all patients. VDD was considered insufficient if serum 25 (OH) D was between ≥27.5 to <50 nmol/L, and deficient VDD if the level was at <27.5 nmol/L.

Patients with vitamin D3 deficiency of <50 nmol/L were given either IM injection of vitamin D of 600,000 IU, 26 (37.7%) or with oral capsule of 50,000 IU once weekly for three months, 25 (36.2%).

The following blood tests were performed: vitamin D (25-OH cholecalciferol), calcium, hemoglobin (Hb), reticulocyte count, Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and Parathyroid Hormone (PHT) at baseline, one month and three months' interval.

Vitamin D3 level was tested using commercially available automated Chemiluminescence Micro-particle Immuno-Assay (CMIA) kits. Quantification of parathormone level was performed using Enzyme–Linked Immunosorbent Assay (ELISA kits). The serum level of calcium, phosphorus and alkaline phosphatase were measured using an auto-analyzer.

Data analysis was performed using SPSS version 20.11. Data was presented as the mean ± SD; paired Student t-test was performed to assess the mean difference between variables in the two groups.

Continuous variables were assessed using Student t-test and Chi-square was used for non-continuous data. P-value was considered significant at level of <0.05.

RESULT

Sixty-nine adult patients with SCA were enrolled with a mean age of 28.9 ± 8.9 years; forty-six (66.7%) were females. Fifty-one (73.9%) patients from the SCA group and 14 (20.3%) patients from the control group had VDD with serum level <50 nmol/L. The mean age of patients with VDD in the SCA group was 27.52 ± 8.7 years (range 18-45), 17 (24.6%) patients had insufficient VDD and 34 (49.3%) patients were deficient. In the control group, six (8.7%) patients had insufficient VDD and eight (11.6%) patients were deficient.

Table 1 shows the biometric data of adult patients with SCA compared to the control group. Patients with SCA had a significantly lower level of hemoglobin 8.3±1.2 gm/dl and vitamin D3 of 22.8±7.3 nmol/L, serum calcium of 2.1±0.04 mmol/L and BMI of 22.5±2.3. Furthermore, there were significantly high levels of reticulocyte counts of 5.4±0.72, PTH of 6.1±0.4 pmol/L and ALP of 315.2±15.9 U/L.

Table 1: Biometric Data of Patients with SCA Compared to the Control Group

	Sickle Cell Anemia (N=69)	Control (N=69)	P-value
Age	27.52±8.33	26.9±9.55	0.67
Female	46 (66.6%)	45 (65.2%)	0.59
BMI	22.5±2.3	28.8±3.8	<0.01
Vitamin D3 (nmol/L)	22.8±7.3	67±16.9	<0.01
Parathormone (pmol/L)	6.1±0.4	4.2±0.6	<0.01
Calcium (mmol/L)	2.1±0.04	2.2±0.03	<0.05
LDH (U/L)	345±76.9	220±45.6	<0.05
Hb (gm/dl)	8.3±1.2	12.6±1.8	<0.01
Reticulocyte count (%)	of 5.4±0.72	2.1±0.82	<0.01
ALP (U/L)	315.2±15.9	101.7±12.9	<0.01
ALT (U/L)	82.3±8.9	41.8±7.8	<0.01

Data presented as **Mean ± Standard Deviation**
Hb: Hemoglobin **SCA:** Sickle Cell Anaemia
LDH: Lactate Dehydrogenase **ALP:** Alkaline Phosphatase
BMI: Body Mass Index

Table 2 shows the serial data of serum level of vitamin D3 after one and three months of treatment with oral or intramuscular VD. Patients treated with IM injection had a mean serum level of 19.55±9.63, 53.97±1.36 and 54.15±2.73 nmol/L at baseline, one month and three months respectively; patients on oral vitamin therapy had VD level of 22.11±9.46, 31.64±4.44 and 53.69±2.37 nmol/L at baseline, one month and three months respectively. Normalization of serum level after one month was observed in patients treated with IM injection but not with oral therapy, albeit both treatment modalities had normalized VD level after three months.

Table 2: Serum Level of VD in Patients with SCA before Treatment, One and Three Months after Intramuscular or Oral Vitamin D3 (N=51)

Vitamin D Serum Level	Baseline	One Month after Treatment	P-value	Three Months after Treatment	P-value
IM treatment (nmol) [N=26]	19.55±9.63	54.15±2.73	P<0.05	53.97±1.36	P<0.001
Oral treatment (nmol/L) [N=25]	22.11±9.46	31.64±4.44	P<0.05	53.69±2.37	P<0.001

Data presented as **Mean ± SD**
VD: vitamin D3, cholecalciferol **IM:** intramuscular
SCA: sickle cell anemia **VDD:** vitamin D 3 deficiency

Patients had a visual analogue scale for daily recording of bone pain episodes. The frequency of bone pain was tabulated and summed up over one-month intervals. The variation of pain frequency was also assessed regarding response to the vitamin D treatment.

Figure 1 displays the frequency of bone pain episodes on intramuscular injection, which was reduced by 23.2% compared to the baseline (43 episodes after one month compared to 56), P<0.01, with further reduction of 39.2% after three months (34 episodes compared to 56 at baseline), P<0.01.

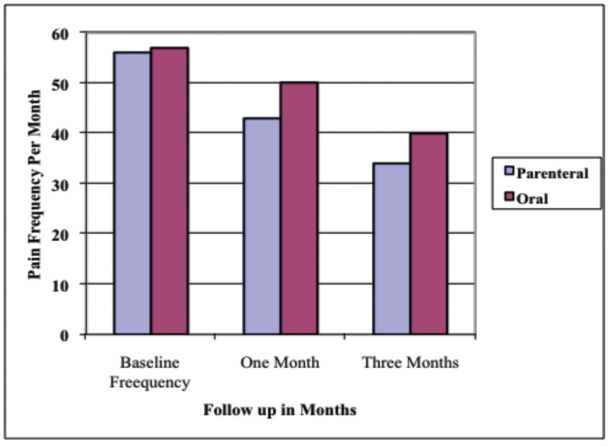

Figure 1: Bone Pain Episodes per Month in Patients with SCA and VDD in Response to Parenteral and Oral VD Medications in One and Three Months' Interval (N=51)

Patients with oral medications had a reduction in pain frequency of 12.2% after one month (50 episodes compared to 57 at baseline), P<0.05, and a further reduction of 29.8% (40 compared to 57 at baseline), P<0.05, after three months.

Eleven (15.9%) patients with pain episodes of mild intensity at baseline increased to 23 (65.2%) patients after three months' treatment. Similarly, twenty-three (33.3%) patients with severe pain intensity at baseline reduced to 8 (11.6%) patients after three months. Sixteen (23.2%) patients with moderate pain intensity at baseline increased to 20 (28.9%) after three months. Therefore, it was observed that the treatment of patients with VDD had a remarkable reduction of pain severity over three months.

Table 3 shows the serum level of parathyroid hormone (PTH) and alkaline phosphatase (ALP) at baseline with a significant reduction of serum level after three months of treatment compared to baseline. The level of serum calcium showed significant increment compared to baseline. Hemoglobin level and reticulocyte count did not show any significant changes after vitamin D treatment.

Table 3: Serum Level of Hematological and Hormonal Variables at Baseline and Three Months after Medication with VD, Data Presented as the Mean ± SD

	Baseline	3 Months after Treatment	P-value
PTH pmol/L	6.4±0.66	4.7±0.37	<0.005
ALP	315.2±15.9	151.7±12.9	<0.01
Calcium in mmol/L	2.10±0.06	2.22±0.10	<0.01
Hb	8.2±0.57	8.4±0.54	0.893
Reticulocyte count	5.5±0.72	5.37±0.71	0.766

Hb: Hemoglobin **ALP:** Alkaline Phosphatase
PTH: Parathormone

DISCUSSION

Fifty-one patients with SCA had VDD, a prevalence of 73.9% and serum level of vitamin D <50 nmol/L. In general, the prevalence of VDD in SCA varies between 65% and 100% depending on the season and geographical area[14,15].

Seventeen (24.6%) VDD patients had VD insufficiency and 29 (42%) patients had VD deficiency; that finding is dissimilar to a previous report by Arlet, who found that 46% patients had VD deficiency and 46% had VD insufficiency in SCA patients[16].

Goodman et al found that 28% of SCA patients with VDD of less than <30 nmo/L and 60% had a serum level of <10nmol/L[17]. A recent study of children with SCA found that the mean age was 4.84.3± years and the mean serum 25(OH)D level was 21.50±13.14 ng/ml; fifty-six percent of the children had levels of 25(OH) vitamin D of <20 ng/ml, whereas 79 had level less than 30 ng/ml and 18% had less than 11 ng/ml. Secondary hyperparathyroidism was observed in 25% of children[18].

The intensity of chronic bone pain was severe in 24 (39.6%) patients before treatment and reduced to 8 (15.6%) patients after three months of treatment; this is similar to other studies of vitamin D supplements[19-21].

The prevalence of VDD in females in this study was high (58.8%) which is similar to other study where the prevalence was 71%[22].

The low level of VDD among Bahraini patients with SCA of 74% is high and mostly of multifactorial origin. Al-Hadad et al found that males with VDD could be due to wearing the traditional Arab garments which covers the whole body including the scarf head cover and the use of thick and black garments for females, both of which reduce ultraviolet rays penetration to the skin[23]. In addition, the use of veils among females had been documented in 87% and found to be a significant and independent factor associated with vitamin D deficiency. Further, dark skin, excess heat and humidity in Bahrain inhibit people from direct exposure to the sun, which reduces cutaneous vitamin D synthesis.

Studies by Rodan et al and Boonen et al reported that patients with SCA and VDD had high levels of parathormone (PTH) and alkaline phosphatase and low calcium as markers of high bone turnover, and could mostly be secondary to low calcium levels as part of VDD[24,25]. In another study by Cashman et al, there was significantly higher PTH in patients with serum level VDD of <42 nmol/L compared to VD level of >72 nmol/L[26].

Furthermore, patients with SCA in our study showed low BMI with a mean of 22.5±2.3. This finding is similar to a study by Erkal et al, where a lower BMI was observed in SCA with VDD and regarded as a positive predictive marker[27].

Patients with VDD after treatment with intramuscular vitamin D3 responded better with regards to normalization of vitamin D3 serum level in one month, contrary to oral medication that required three months for vitamin D3 level to normalize.

The normalization of VD after one month of parenteral therapy compared to oral supplements may be explained by the fact that patients with SCA had poor absorption of Vitamin D and calcium compared with good bioavailability of parenteral administration[28].

Afro-American patients absorb dietary calcium better compared to Caucasians with optimal bone and kidney calcium-retaining. There may be metabolic and racial differences that could decrease vitamin D level among Caucasians. These observations suggest that African-Americans may require less dietary calcium than Caucasians[29].

Burn et al found that a single intramuscular dose of 15mg (600,000) IU injection of 25-OH cholecalciferol was effective in initiating and sustaining healing of osteomalacia for at least six months[30]. In addition, it was observed that blood level increased from 11ng/ml at baseline to 30ng/ml at six months and 31ng/ml in one year.

The frequency of bone pain episodes improved after IM injection treatment compared to oral therapy. The frequency of bone pain episodes with IM VD was reduced from 56 per month to 43 after one month of treatment and 34 after three months, while the oral therapy reduced the frequency to 50 at one month of treatment and 40 at three months respectively. Such finding is explained by the suboptimal normalization of Vitamin D3 level on oral compared to IM injection after one month.

Although it is clear that vitamin D treatment reduced bone pain frequency and intensity and concurred with vitamin D serum level normalization, there are several other confounding factors that may affect the clinical and biochemical outcomes. A further larger study may be warranted in such group of patients.

CONCLUSION

The frequency of bone pain was reduced significantly in adult patients with SCA and VDD after one month of treatment of vitamin D3 parenterally or orally with further reduction at three months' interval. However, the parenteral VD injection normalizes serum level (>50 nmol/ml) after one-month of treatment, while the oral supplement normalizes at three months.

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