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Vitamin D Status of Sudanese Children with Sickle Cell Anemia

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This was a prospective cross-sectional hospital-based study. Children with SCA age six months to 18 years who met the recruitment criteria made the study group, and sex and age- matched healthy children were the control group.

There were 64 children in the study group and 21 in the control group. Low 25 OHD, low mean serum calcium, hypocalcemia and high serum alkaline phosphatase were significantly prevalent in the study group (P.0001, P.0001, P.0001, P.003) respectively. Painful crisis, bone fracture, osteomyelitis, and anemia were not increased in these patients.

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Vitamin D Status of Sudanese Children with Sickle Cell Anemia

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Abstract- A low level of 25 hydroxy vitamin D (25 OHD) is seen more in children with sickle cell anemia (SCA) than healthy children. SCA is common in Sudan, but the status of vitamin D is unknown in Sudanese children with SCA. We aimed to determine the prevalence rate of low level of vitamin D in this population and its relation to the painful crisis, bone fracture, osteomyelitis and hemoglobin level and biochemical data.

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I. Introduction

Sickle cell anemia (SCA), a heterogeneous disorder, is characterized by the presence of Hemoglobin S where Glutamic Acid is replaced by valine at position 6 of the beta globin chain. Its clinical manifestation includes chronic hemolysis, increased susceptibility to infection and vaso-occlusive crisis (1,2). SCA is prevalent in Sudan, it ranges from 0.8%-30.4% depending on the geographical location (3).Khartoum, capital of Sudan, is a multiethnic town that contains almost all Sudanese tribes. Patients with SCA are mostly from the tribes (predominantly of African descent) that migrated from the West during the drought that struck the area in 1980 (3).

Children with sickle cell anemia are at higher risk of low 25 OHD level than healthy children. Approximately 65-100% of children with SCA were found to have vitamin D deficiency (4). Black Americans were found to have Vitamin D insufficiency more than other Americans (5, 6). This is because pigmentation probably reduces vitamin D production in the skin (7).

Vitamin D deficiency is a serum level of 25 OHD below 20 ng/ml (50 nmmol/l), insufficiency is 25 OHD level 21-29 ng/ml (52.5-72.5 nmmol/l), while severe deficiency is level between 5-10 ng/ml, and it is very severe if less than 5 ng/ml (8).

We hypothesized that Sudanese children with SCA have low plasma 25 OHD level. This study aimed to determine vitamin D status in this population, to identify risk factors associated with low vitamin D level and to determine the relation between low serum vitamin D level and the frequency of bone pain, bone fracture and osteomyelitis.

II. Patients and Method

This was a prospective Cross -sectional hospital-based study done in the outpatient clinics of a major pediatric hospital (Jafar Ibn Ouf children hospital) and a general teaching hospital (Ibrahim Malik) in Khartoum, Sudan from June 2013 to October 2013.

Study Population: Sudanese Children six months to 18 years old with a confirmed diagnosis of sickle cell anemia making the study group, age and sex- matched healthy children making the control group.

Children with liver disease, renal disease, chronic diarrhea, on vitamin D, oral calcium or had received blood transfusion in the past three months were excluded from the study. A questionnaire was used to collect data. It included: demographic data, number of admissions for painful crisis, number of blood transfusions, history of bone fracture, bone infection and 24hours dietary record. Hemoglobin level and hemoglobin electrophoresis were obtained from the patient medical records.5.5 ml of venous blood were drawn from each patient: 3.0ml were placed in a Lithium Heparin tube for 25OHD assay. Serum was separated and frozen at -20 degrees .Assay for 25OHD was performed after completion of sample collection using Tecanelisa machine (Tecan Trading AG, Switzerland). The remaining 2.5ml were put in a similar container for serum calcium, phosphate, and alkaline phosphatase measurement. These were assayed immediately using the U/V automation method using Bio system auto machine. We obtained a written informed consent from the patients or caregivers. Ethical approval was obtained from the Sudan medical specialization board and the hospitals ethical committees.

Were presented the results in the form of frequency, percentage and mean. Microsoft Excel 2007 program was used to form the graphs. Statistical tests

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were performed using Statistic Package for Social sciences (SPSS) version 19, and significance was considered at P value of < (.05).

III. Results

We enrolled 90 children in the study: 69 children with sickle cell anemia made the study group, and 21 healthy children were the control group. Five children were excluded from the study group (three were on calcium and vitamin D, and two were recently transfused with blood) leaving 64 children for analysis. All children were of the SS genotype. Patients characteristics are shown in Table (1).

Table 1: Patients Characteristics and Mean Values of Biochemical Parameters in Patients with SCA and Control Group

Parameters	SCA n = 64	Control n = 21	P - Value
Age (Mean ± SD)	7.8±4.92	7.03±4.64	.443
Male	55 %	87.5 %	.34
Female	45 %	12.5 %	.34
25 OHD (Mean ± SD)	30±13.5	33.7±13.7	0.0001
Serum Calcium (Mean ± SD)	8.5±.82	8.8±.55	0.0001
Alkaline Phosphatase (Mean ± SD)	245.8±232.4	161.95±68.4	.03
Serum Phosphate (Mean ± SD)	4.1±.89	3.95±.53	.280
Mean Body Mass Index (BMI)	14.9±2.56	16.15±3.78	.108

The mean serum 25 OHD level was significantly lower in the study group than in the control group Table (1). Low level of 25 OHD was found in 62.5 % of the children in the study group, and 38 % of children in the control group P (0.0001). Vitamin D deficiency was present in 18.8 % of those with low vitamin D in the study group (Table 2). In the control group eight children (38 %) had a low 25 OHD level (<30 ng/dl), all were having 25 OHD insufficiency.

Table 2: 25 OHD Level in the Study and Control Group

Serum Vitamin D Level	Study Group / Frequency (%)	Control Group / Frequency %
5 - 10 ng/Dl	4 (6.3%)	0 (0%)
11 - 20 ng/Dl	8 (12.5)	0 (0%)
21 - 29 ng/Dl	28 (43.8%)	8 (38.1%)
30 - 50 ng/Dl	19 (29.6%)	13 (61.9%)
51 - 70 ng/Dl	2 (3.1%)	
71 + ng/Dl	3 (4.7%)	
Total	64 (100%)	21 (100%)

In children with SCA a low 25 OHD was present in 55% of males and 45% of females (P 0.340). The majority (80%) of children with low 25 OHD (<30 ng/dl) level in the study group were 1-12 years old, and 91.6 % (11/12) of children with 25 OHD deficiency were present within this age. The percentage of those with low vitamin D level increases with increasing age (Figure 1).

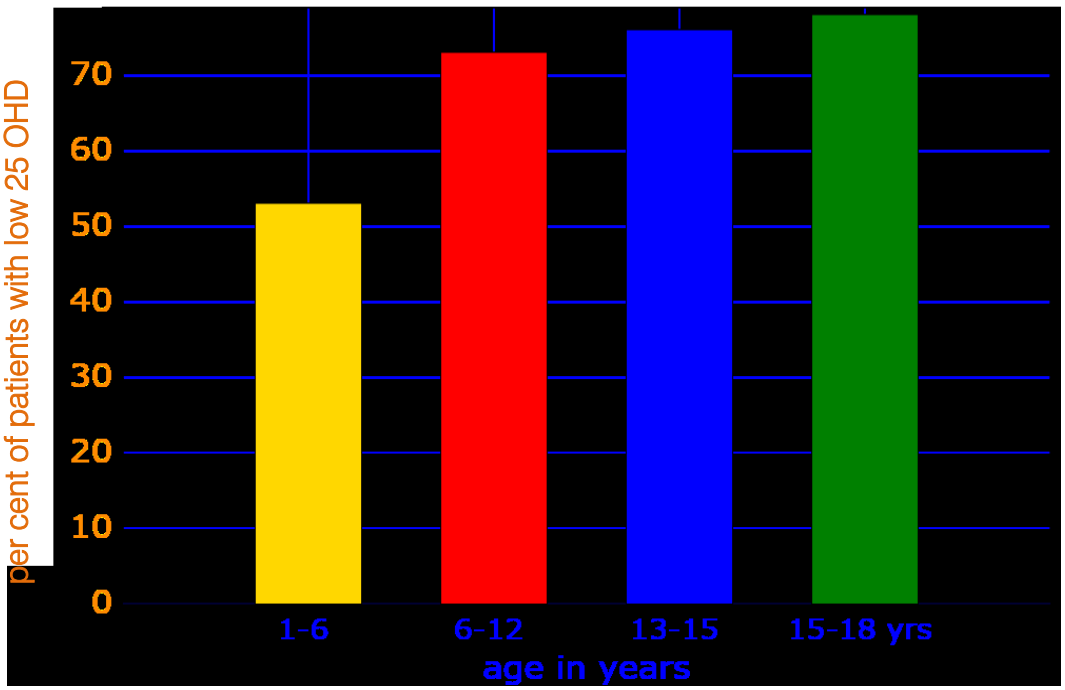

Fig. 1: Low Vitamin D Level according to Age

52 children were diagnosed to have SCA before the age of one year and 32 (61.5%) of them had low 25 OHD. Twelve children were diagnosed to have SCA after the age of one year, and 8 (66.7%) had low 25 OHD (P 0.929).

In the study group, the mean serum calcium was significantly lower in patients with SCA than control (8.5 ± 0.82 mg/dl vs. 8.8 ± 0.55 mg/dl P.0001). Hypocalcemia (Serum calcium below 8.6 mg/dl) occurred in 30 children in the study group (43.4%), and none in the control group, and it developed more in children with low 25 OHD than in those with normal 25 OHD (70% vs. 8.3%) (P0.0001) Table (3).

Table 3: Serum Calcium and its Relation to 25 OHD Level in the Study Group

Serum Calcium	25 OHD Deficiency	%	25 OHD Insufficiency	%	Normal 25 OHD	%	Total	%
<8.6	11	91.7	17	60.7	2	8.3	30	46.9
8.6-10.3	1	8.3	10	35.7	22	91.7	33	51.6
10.4	-	-	1	3.6	-	-	1	1.5
Total	12	100	28	100.0	24	100%	64	100.0

Frequent painful crises (≥ Five attacks per year) were experienced by 60 % of children with low serum calcium compared to 48.8 % of those with normal calcium (P 0.368). The mean serum alkaline phosphatase level was significantly higher in those with low 25 OHD level than those with a normal level (460 IU vs. 237 IU P.0.0001). An elevated serum alkaline phosphatase (>320 IU) was more frequent in children with low 25 ODH than in those with normal level (62.5%% vs. 12.5% P value 0.030). High serum alkaline phosphatase level was not associated with frequent painful crises (37.5% vs. 25%P 0.883).

The mean serum phosphate in the study group was comparable to that in the control group (4.1 ± 0.89 meg/l vs. 3.95 ± 0.53meg/l P 0.28). Hypophosphatemia (serum phosphate level <2.5 mg/dl) was found in 9 (14.1%) children with low 25 OHD but none in those with normal 25 OHD (p 0.280). All hypophosphatemic children were hypocalcemic.

Low 25 OHD level in the study group was not associated with lower hemoglobin level, the need for blood transfusion, more painful crises, increased bone fractures or osteomyelitis (Table 4).

Table 4: Clinical Complications and its Relation to 25 OHD Level

Number	Study Group With Low 25 OHD	Study Group With Normal 25 OHD	P Value	Control Group	P Value
Painful Crisis per Patient per Year	1	0.95	0.135	0	-
Number of Hospital Admission for Pain per Year / Patient	3.3	2.6	0.453	0	-
Fractures	2**	2*		2	0.179
Osteomyelitis	2	0	NS	0	NS
Mean Hemoglobin (G/Dl	6.9	7.6	0.132	0	0
Number of Blood Transfusion	2.1	2.7	0.446	0	0

**: Caused by osteomyelitis.

*: Caused by a fall from one- meter height.

There were more children with BMI below the 3rd percentile in the study group than the control group (67.2% vs 33.3% P 0.003). Within the study group, there was no difference in the BMI between those with low and normal 25 OHD level (62.5% vs. 75%) (P 0.108) Table (5).

Table 5: Body Mass Index (BMI) in Relation to 25 OHD Level in the Study Group

BMI Percentile	25 OHD Deficiency (5-20 ng/Dl)	%	25 OHD Insufficiency (21-29 ng/Dl)	%	Normal 25 OHD (50-70 ng/Dl)	%	Total	Overall %
<3rd	9	75.0	16	57.1	18	75	43	67.1
At 10th	2	16.7	3	10.7	0	0	5	7.8
At 25th	1	8.3	5	17.9	4	16.7	10	15.6
At 50th	0	0	4	14.2	2	8.3	6	9.3
At 75th	0	0	0	0	0	0	0	0
Total	12	100.0	28	100.0	24	100.0	64	100.0

Dietary intake of fish, meat, and milk, was comparable in the study group and the control group and within the study group (P value 0.117, 0.108) respectively.

In the study group, sun exposure for more than 15 minutes a day was comparable in those with low and normal 25 OHD (87.5% vs. 87.5 % P 0.163).

IV. Discussion

This is the first study in Sudan that determined vitamin D status in Sudanese children with sickle cell anemia .Like other studies we found a high prevalence of low 25 OHD level (62.5%) with 70% of them being deficient. Twelve studies reported vitamin D status in children and adolescent with sickle cell anemia (4, 9-20), four of them had used a definition similar to ours (10-13) Low vitamin D was present in 80-98% of children included in three of these studies and 81.5-100 % of them were deficient (10, 11, 13). These rates are higher than ours. The prevalence rate reported from Madrid, Spain (12), a sunny country like Sudan, is comparable to ours. We could explain this finding by the fact that our children had good sun exposure despite they had dark skin color: a factor that influences vitamin D synthesis. (21).

Males were more likely to have low 25OHD level than females although this was not statistically significant, a finding similar to that reported by Mohammed et al. (15) Low 25OHD level was present from the age of one year throughout childhood. Its frequency increased with increasing age. A similar observation was reported in normal children (22) as well as children with SCD (4, 9, 12). However, this effect of age on vitamin D status was not observed in studies from Kuwait and Saudi Arabia (10, 15).

We observed a tendency towards low serum calcium in the study group. A similar tendency was reported before in children and adults with sickle cell disease (15, 23- 26). This tendency was observed in the absence of low serum albumin (23). Hypocalcaemia was reported in 14 % of Saudi patients with sickle cell disease: in the same study low vitamin D was present in 12% of patients. We observed hypocalcaemia in almost half of our patients and the majority of them were those with low 25 OHD. Suggested causes of hypocalcemia include an increased activity of calcium magnesium ATPase (24, 27, 28), reduced intestinal calcium absorption, and impaired vitamin D synthesis .(15) Low dietary intake of vitamin D was found to be significantly associated with lower serum vitamin D levels in both healthy children and children with HbSS. (4) However, this is not the case in this study as adequate sun exposure and adequate intake of diet rich in vitamin D was seen in our patients. This is similar to what was reported by others (15).

Low serum 25OHD causes high level of ALP (29). In this study low 25OHD level was found to be associated with significantly high ALP and low calcium level. High alkaline phosphatase was reported in Kuwaiti children who had SCA and 25 OHD deficiencies (10). However, normal calcium and alkaline phosphatase level (13) or lack of association between ALP and 25 OHD level (12) were reported in children with SCA. Serum ALP in SCA may be elevated due to bone destruction and vaso-occlusive crisis (VOC) and it is considered a sensitive marker of bone turn over (30). This is unlikely to be the case in this study as those patients with low 25OHD were in their steady state and had no more painful crisis than those with normal level of 25 OHD. Furthermore painful crises were found not to affect serum calcium level (23).

The status of serum phosphate in children with SCD was reported by few studies (12, 23, 31, 32). Elevated levels were reported by two studies (23, 31). One of these studies suggested resistance to the phosphaturic effect of fibroblast growth factor 23 (FGF23) to be the cause. (31) Low serum phosphate level was reported by Al-harbi et al and that was attributed to elevated level of parathyroid growth hormone (PTH) (32). An inverse correlation between PTH and phosphorous level was observed by Garrido et al (12). In our study the serum phosphate level was normal in the majority of patients: however, 14% were hypophosphatemic. We did not measure PTH in this study but others had reported high level of the hormone when vitamin D or serum calcium were low (15) and all our patients with hypophosphatemia had hypocalcaemia. Thus, elevated levels of PTH could be the cause of low phosphate level in our patients. Therefore, in children with SCA elevated serum ALP, hypophosphatemia or hypocalcemia can be taken as a marker of low 25 OHD during steady state condition.

The significance of low 25 OHD and if there is a pathological association is hard to know. Our result did not suggest an association with increased painful episodes, as indicated by the number of pain episodes per year and number of hospital admissions due to pain. Similarly, two retrospective studies from America and the United Kingdom failed to demonstrate increased painful episodes with low 25 OHD (9, 33). Furthermore, Jackson etal did not find an increased rate of acute painful crisis or acute chest syndrome in 64% of their patients despite the presence of severe vitamin D deficiency (<10 ng/ml) (25). But, Adegoke et al. and Lee et al. found a possible association between low serum vitamin D levels and increased frequency of acute pain episodes (34, 35). In a randomized controlled trial, six weeks of a high oral dose of vitamin D in children and adolescent with SCA and low vitamin D level reduced the number of pain days per week irrespective of baseline 25 OHD levels (16).

The overall incidence of bone fracture in this study (6.3%) is not different from that in the control group. If we excluded those in whom fractures were

associated with osteomyelitis then the incidence will be comparable to that reported in healthy British children (36). Reports regarding the prevalence of bone fractures in children with SCA are limited. Bone fractures were reported in 18.8% of Egyptian children, adolescent and young adults with SCA (37). Fung et al. reported a prevalence rate of 12.5% of bone fractures in children with SCA age 12-18 years, with falls and recreational sport being the commonest predisposing factors (38). Four cases, including two with pathological fractures, were reported from Spain (12). One pathological vertebral fracture was detected in 97 Omani children with SCA(39). The French Study Group on sickle cell disease reported the acute clinical events in 299 homozygous sickle cell patients (age 10.1+/-5.8 yrs.): there was no single case of bone fracture despite the presence of osteomyelitis in 12% of the cohort(40).

A slight decrease in bone mineral density (BMD) was reported in children with SCA (12, 37, 19). Low BMD was not found to be associated with vitamin D deficiency(12,36) or calcium and ALP level (38) .It is probably due to an abnormal bone formation (19).The relationship between low BMD and bone fractures was not evaluated in two studies that reported low BMD and bone fracture (12,37). In African American children (non-SCD) with fracture of the forearm, 25 OHD insufficiency was present in 59% of them, but all of them had normal BMD (41). BMD was not measured in this study. Despite a high prevalence rate of low vitamin D among our patients, the rate of bone fracture was similar to that in healthy children. Therefore, vitamin D insufficiency does not seem to predispose Sudanese children with SCA to bone fracture.

Staphylococcus aureus, salmonella, Pseudomonas aeruginosa, Escherichia coli (E. coli) and klebsiella pneumonae are causative organisms of osteomyelitis in children (42,43).Vitamin D3 was shown to have an inhibitory activity, in vitro, on strains of Streptococcus pyogenes, staphylococcus aureus, Klebsiella pneumoniae, E. coli and other bacteria (44). Gram-positive bacteria, meningococcal disease, invasive pneumococcal disease, and group A streptococcal disease are more common when vitamin D levels are low (45). However, the prevalence rate of osteomyelitis reported in this study (3.1%) is lower than rate reported in the literature (12%). (40). This means low 25 OHD did not predispose Sudanese children with SCA to osteomyelitis.

Vitamin D was found to have a positive effect on erythropoiesis. Therefore vitamin D deficiency is expected to limit erythropoiesis and to increase anemia in patients with anemia of chronic diseases. (46) In this study the were no differences in the mean hemoglobin level or requirement of blood transfusion in patients with low level of 25 OHD compared to vitamin D sufficient patients. Winters et al. found no correlation of baseline hemoglobin level and 25 OHD level in either pediatric or adult patients (9). Busse et al. supplemented 50 sickle cell disease patients aged 0 to 21years, who were vitamin D deficient, with vitamin. They studied the association between time- dependent 25 OHD level, hemoglobin concentration, and reticulocyte percentage over time in days. This did not improve the anemia: in fact a reduction in hemoglobin with reticulocytosis was observed with increasing 25-OHD suggesting hemolysis (47). A recent study had shown that Serum 25-hydroxyvitamin D correlated with biomarkers of hemolysis in SCD (48). Kaitlyn et al. from Canada found that a 1 g/L increase in hemoglobin concentration was associated with a 0.4 (95% CI: 0.1- 0.8) nmol/L increase in mean serum 25OHD concentration (P = 0.01) (49). Since patients with SCA are prone to low levels of 25 OHD and thus they are likely to receive vitamin D supplementation further studies are needed in this area to determine its safety.

High BMI was reported to be associated with low vitamin D level in normal children and adolescent (50, 51). A similar finding was also observed in young adults with SCA (52). Children with SCA are known to have low BMI {53). We had a similar finding in this study. However, we failed to demonstrate any difference in BMI between those with low and normal vitamin D level in the study group.

V. Conclusion

A high prevalence rate of vitamin D insufficiency or deficiency in Sudanese children with SCA was found. We could not identify the cause but it is probably multifactorial. Living in a country with enough sun light throughout the year does not guarantee adequate level of vitamin D. Low levels of serum calcium and phosphorus and high alkaline phosphatase can be used as marker of low vitamin D level. Despite low vitamin D level bone fractures were not increased.

Study Limitations: Most of the obtained clinical data were recall data which might had led to over or underestimation of the results. The number of children in the control group was small with possibility of overestimation of the prevalence rate of low vitamin D in the study group.

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